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Ohmyungsamycins A and B: Cytotoxic and Antimicrobial Cyclic Peptides Produced by *Streptomyces* sp. from a Volcanic Island

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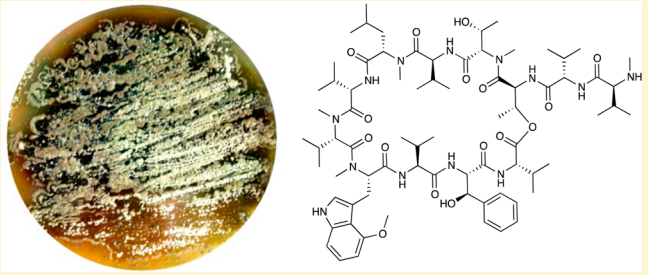
Supporting Information

ABSTRACT: Ohmyungsamycins A and B (**1** and **2**), which are new cyclic peptides, were isolated from a marine bacterial strain belonging to the *Streptomyces* genus collected from a sand beach on Jeju, a volcanic island in the Republic of Korea. Based on the interpretation of the NMR, UV, and IR spectroscopic and MS data, the planar structures of **1** and **2** were elucidated as cyclic depsipeptides bearing unusual amino acid units, including *N*-methyl-4-methoxytrytophan, *β*-hydroxyphenylalanine, and *N*,*N*-dimethylvaline. The absolute configurations of the *α*-carbons of the amino acid residues were determined using the advanced Marfey's method. The configurations of the additional stereogenic centers at the *β*-carbons of the threonine, *N*-methylthreonine, and *β*-hydroxyphenylalanine units were assigned by GITC (2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate) derivatization and the modified Mosher's method. We have developed a new method utilizing PGME (phenylglycine methyl ester) derivatization coupled with chromatographic analysis to determine the absolute configuration of *N*,*N*-dimethylvaline. Our first successful establishment of the absolute configuration of *N*,*N*-dimethylvaline using PGME will provide a general and convenient analytical method for determining the absolute configurations of amino acids with fully substituted amine groups. Ohmyungsamycins A and B showed significant inhibitory activities against diverse cancer cells as well as antibacterial effects.



Bioactive secondary metabolites from marine organisms are now generally considered to be promising and developable drug sources.[1] Since the approval of zinconotide from a tropical cone snail as an analgesic agent in the United States in 2004, several secondary metabolites from marine invertebrates and their synthetic analogues, such as ecteinascidin-743 and halichondrin, have been marketed for clinical use.[1] In addition to marine macroorganisms, marine microorganisms have been more recently spotlighted as chemical treasures.[2,3] A statistical overview of marine natural products between 1985 and 2008 clearly demonstrates that the pace of discovery of bioactive secondary metabolites from marine microbes has accelerated over the last 15 years.[4] Leading the drug candidates discovered from marine microbes is salinosporamide A, a potent proteasome inhibitor from the obligate marine actinomycete *Salinispora tropica*, which has advanced to phase II clinical trials for the treatment of myeloma patients.[5]

Investigations of unique environments that have rarely been studied are necessary to efficiently discover new bioactive compounds.[6] Jeju Island is a volcanic island in the subtropical region of the Republic of Korea. Its volcanic bedrocks, which are rich in basalt and trachyte, provide feldspar and mafic minerals and form distinctive seashores that differ from common quartz-rich sand beaches[7] and that have the potential to harbor unique microorganisms that genetically encode new bioactive compounds. During our recent studies of microorganisms collected around Jeju Island, we discovered a new lasso peptide, sungsanpin, from a deep-sea *Streptomyces* sp.[8] and new polyene polyols, separacenes A–D, from a seashore *Streptomyces* sp.[9] Our continued isolation and chemical screening of marine actinomycetes led to the discovery of a *Streptomyces* strain (SNJ042, most closely related to *Streptomyces cheonanensis*) that was isolated from Shinyang Beach on Jeju Island; this strain produces new cyclic peptides, ohmyungsamycins A and B (**1** and **2**). The ohmyungsamycins were found to be structurally unique due to the incorporation of unusual amino acids such as *N*-methyl-4-methoxytryptophan, *β*-hydroxyphenylalanine, and *N*,*N*-dimethylvaline. Here, we report the spectroscopic structural elucidation, the determination of absolute configuration, including the application of a newly developed chromatographic

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Table 1. NMR Data for Ohmyungsamycin A (1) in Pyridine-d_5[a]

	C/H	δ_C, type	δ_H, mult (J, Hz)		C/H	δ_C, type	δ_H, mult (J, Hz)
Val-1	1	174.3, C		Val-6	39	173.2, C	
	2	58.3, CH	4.70, dd (8.5, 8.5)		40	54.3, CH	4.96, m[b]
	2-NH		9.28, d (8.5)		40-NH		9.39, d (9.0)
	3	32.8, CH	2.23, m		41	31.9, CH	2.40, m
	4	19.3, CH_3	1.12, m[b]		42	19.0, CH_3	1.14, m[b]
	5	18.6, CH_3	0.97, m[b]		43	19.7, CH_3	1.03, d (6.0)
β-hydroxy-Phe-2	6	172.7, C		*N*-Me-Leu-7	44	171.7, C	
	7	60.0, CH	5.49, dd (8.0, 2.5)		45	54.8, CH	5.65, dd (8.0)
	7-NH		9.66, d (8.0)		46a	38.9, CH_2	1.74, m
	8	73.2, CH	5.94, d (2.5)		46b		1.58, m
	9	143.0, C			47	25.1, CH	1.43, m
	10	127.0, CH	7.68, d (7.5)		48	23.1, CH_3	0.67, d (6.0)
	11	128.6, CH	7.41, dd (7.5, 7.5)		49	21.9, CH_3	0.67, d (6.0)
	12	127.5, CH	7.27, d (7.5)		50	31.3, CH_3	3.52, s
	13	128.6, CH	7.41, dd (7.5, 7.5)	Val-8	51	173.5, C	
	14	127.0, CH	7.68, d (7.5)		52	55.6, CH	5.29, dd (8.5, 8.5)
Val-3	15	174.5, C			52-NH		8.06, d (8.5)
	16	58.3, CH	5.41, dd (8.5, 8.5)		53	31.0, CH	2.57, m
	16-NH		8.02, d (8.5)		54	19.0, CH_3	1.20, d (7.0)
	17	33.1, CH	2.66, m		55	19.7, CH_3	1.16, d (7.0)
	18	19.8, CH_3	1.36, d (6.5)	*N*-Me-Thr-9	56	170.6, C	
	19	20.0, CH_3	1.20, d (7.5)		57	62.4, CH	5.61, d (3.5)
N-Me-4-methoxy-Trp-4	20	169.9, C			58	66.5, CH	5.05, m[b]
	21	70.6, CH	4.59, dd (11.0, 4.5)		59	20.5, CH_3	1.34, d (6.5)
	22a	27.1, CH_2	4.45, dd (13.5, 4.5)		60	34.5, CH_3	3.68, s
	22b		4.32, dd (13.5, 11.0)	Thr-10	61	171.4, C	
	23	112.9, C			62	52.5, CH	5.86, dd (8.5, 2.0)
	24	124.3, CH	7.19, m[b]		62-NH		10.02, d (8.5)
	24-NH		11.80, d (1.5)		63	69.4, CH	6.04, qd (6.5, 2.0)
	25	139.5, C			64	16.8, CH_3	1.52, d (6.5)
	26	105.8, CH	7.30, m	Val-11	65	173.4, C	
	27	122.7, CH	7.27, m		66	57.9, CH	5.24, dd (9.5, 7.5)
	28	99.5, CH	6.67, d (7.0)		66-NH		8.65, d (9.5)
	29	154.9, C			67	31.8, CH	2.26, m
	30	55.4, CH_3	3.83, s		68	19.8, CH_3	0.96, m[b]
	31	118.4, C			69	18.8, CH_3	0.96, m[b]
	32	40.8, CH_3	2.51, s	*N*-Me-Val-12	70	174.2, C	
N-Me-Val-5	33	169.4, C			71	71.4, CH	3.11, d (6.0)
	34	70.9, CH	3.19, m		72	32.2, CH	2.18, m
	35	29.0, CH	3.03, m		73	19.1, CH_3	1.09, m
	36	21.9, CH_3	1.22, d (7.0)		74	19.1, CH_3	1.09, m
	37	19.7, CH_3	1.02, m[b]		75	35.9, CH_3	2.54, s
	38	39.9, CH_3	3.18, s				

[a] ^{1}H and ^{13}C data were recorded at 900 and 225 MHz, respectively. [b] Overlapped signals.

analysis using phenylglycine methyl ester (PGME) derivatives for *N*,*N*-dimethylvaline, and the biological activities of the ohmyungsamycins.

RESULTS AND DISCUSSION

The *Streptomyces* strain SNJ042 was isolated from a sediment sample collected from Shinyang Beach, Jeju Island. Strain SNJ042 was cultivated in a seawater-based liquid medium, and its production of secondary metabolites was chemically analyzed by LC/MS. In our chemical analysis, we detected that SNJ042 produced a major compound with the UV spectra indicating an indole moiety (λ_{max} at 203 and 281 nm). Our dereplication process based on its indole signature and molecular ion (m/z at 1458.8) indicated that this secondary metabolite is possibly unknown. This initial evaluation prompted us to scale up the culture, isolate the compound, and elucidate the structure. During our scale-up process, we discovered two new cyclic peptides, ohmyungsamycins A and B.

Ohmyungsamycin A (**1**) was isolated as a white powder, and the molecular formula was established as $C_{75}H_{119}N_{13}O_{16}$ by HRFABMS data coupled with ^{1}H and ^{13}C NMR spectra (Table 1). The ^{1}H NMR spectrum showed seven downfield amide proton signals at δ_H 10.1−8.0 and five *N*-methyl groups at δ_H 3.7−2.5, indicating an amino acid-based structure bearing 12 residues. The ^{13}C NMR spectrum consistently displayed 12 amide or ester carbonyl signals at δ_C 175−169. In addition to these peptide features, ohmyungsamycin A showed unusual signals in the ^{1}H, ^{13}C, and HSQC NMR spectra that were not associated with standard amino acids. The methyl group at δ_C 55.4 - δ_H 3.83 is clearly a signal resulting from a methoxy group.

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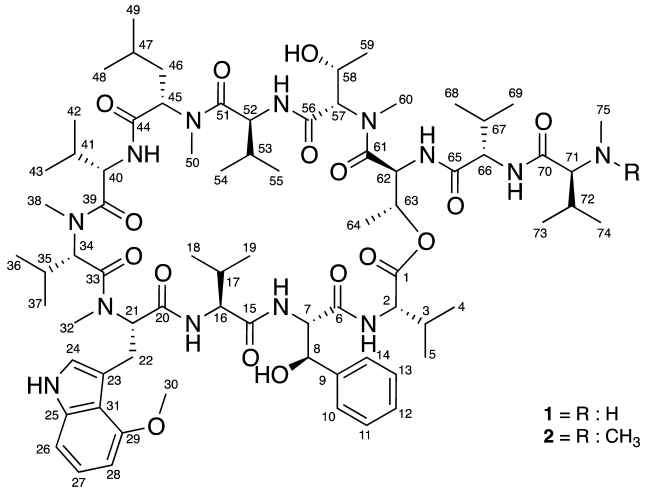
The oxygenated methine correlation at δ_C 73.2 - δ_H 5.94 also suggested the presence of additional oxidation modifying a typical amino acid unit.



Further analysis of the COSY, TOCSY, HSQC, and HMBC NMR spectra enabled the straightforward identification of 10 standard amino acids, including five valines, two *N*-methylvalines, one threonine, one *N*-methylthreonine, and one *N*-methylleucine. One of the remaining two amino acid units was *β*-hydroxyphenylalanine. The COSY and TOCSY correlations of 7-NH (δ_H 9.66), H-7 (δ_H 5.49), and H-8 (δ_H 5.94) and a HMBC correlation from H-7 to C-6 (δ_C 172.7) established that the backbone of the amino acid bears an oxygenated *β*-carbon (C-8; δ_C 73.2). The HMBC correlation from H-8 to the aromatic carbons C-9 (δ_C 143.0), C-10 (δ_C 127.0), and C-14 (δ_C 127.0) revealed that this unit is an aromatic residue. The ^{1}H−^{1}H couplings between the aromatic protons H-10 to H-14, along with their coupling constants (J = 7.5 Hz), established this as a six-membered aromatic ring and thus identified this unusual residue as *β*-hydroxyphenylalanine (Figure 1a). The last amino

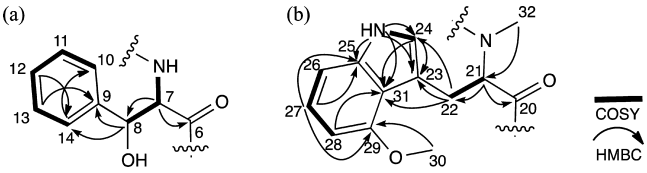


Figure 1. Structure elucidation of the unusual amino acids in **1** on the basis of COSY and HMBC NMR spectra: (a) *β*-hydroxyphenylalanine; (b) *N*-methyl-4-methyoxytryptophan.

acid was assigned as *N*-methyl-4-methoxytryptophan. The COSY correlation between H-21 (δ_H 4.59) and H$_2$-22 (δ_H 4.45, 4.32) connected the *α*-carbon (C-21; δ_C 70.6) and *β*-carbon (C-22; δ_C 27.1) of the amino acid unit. The HMBC correlation from H$_2$-22 to C-23 (δ_C 112.9), C-24 (δ_C 124.3), and C-31 (δ_C 118.4) indicated that this unit had an aromatic group in the side chain. The proton−proton couplings in the COSY and TOCSY NMR spectra between H-26 (δ_H 7.30) and H-27 (δ_H 7.27) and between H-27 and H-28 (δ_H 6.67, J = 7.0 Hz) allowed us to construct a partial structure of a six-membered aromatic ring. The strong long-range heteronuclear couplings from H-27 and H-24 to C-25 (δ_C 139.5), from H-28 and H-24 to C-31, and from H-27 to C-29 (δ_C 154.9), along with the COSY correlation between 24-NH (δ_H 11.80) and H-24, indicated the presence of an indole ring in the side chain. The methoxy group was assigned at C-29 on the basis of the HMBC correlation from H$_3$-30 to C-29. Lastly, further HMBC couplings from the *N*-methyl group at C-32 to C-21 and from H-21 to the C-20 carbonyl carbon (δ_C 169.9) established this residue as an *N*-methyl-4-methoxytryptophan unit (Figure 1b).

The connectivity of the 12 identified amino acid units was established on the basis of the HMBC correlations (Figure 2). First, the 2-NH (δ_H 9.28) amide proton of Val-1 correlated with the carbonyl carbon (C-6; δ_C 172.7) of *β*-hydroxy-Phe-2 in the HMBC NMR spectrum, establishing the linkage between Val-1 to *β*-hydroxy-Phe-2. The NH (7-NH; δ_H 9.66) of *β*-hydroxy-Phe-2 clearly showed a heteronuclear correlation to C-15 (the amide carbon of Val-3; δ_C 174.5), thus establishing the connectivity between *β*-hydroxy-Phe-2 and Val-3. The next amino acid was deduced as *N*-methyl-4-methoxy-Trp-4 on the basis of the HMBC correlation from 16-NH (δ_H 8.02) to C-20 (the carbonyl carbon of *N*-methyl-4-methoxy-Trp-4; δ_C 169.9). The long-range ^{1}H−^{13}C coupling from H$_3$-32 (*N*-methyl of *N*-methyl-4-methoxy-Trp-4; δ_H 2.51) to C-21 (*α*-carbon of *N*-methyl-4-methoxy-Trp-4; δ_C 70.6) and C-33 (the carbonyl carbon of *N*-methyl-Val-5; δ_C 169.4) connected this residue to *N*-methyl-Val-5. The *N*-methyl protons (H$_3$-38; δ_H 3.18) of *N*-methyl-Val-5 displayed an HMBC correlation with C-39 (the carbonyl carbon of Val-6; δ_C 173.2), thereby establishing the sequence from *N*-methyl-Val-5 to Val-6. Val-6 was then connected to *N*-methyl-Leu-7 on the basis of the HMBC coupling of 40-NH (δ_H 9.39) with C-44 (δ_C 171.7). The *N*-methyl group of *N*-methyl-Leu-7 displayed strong correlations to C-45 (δ_C 54.8) and C-51 (δ_C 173.5), thus confirming the extension of the peptide chain to Val-8. The NH of Val-8 at δ_H 8.06 coupled with the carbonyl carbon of *N*-methyl-Thr-9 (C-56; δ_C 170.6), which connected the *N*-methyl-Thr-9 unit to Val-8. Further analysis of the HMBC spectrum enabled us to establish the connectivity between *N*-methyl-Thr-9 and Thr-10 on the basis of the heteronuclear coupling of H$_3$-60 (δ_H 3.68) and C-61 (δ_C 171.4). The next residue was assigned as Val-11 on the basis of the HMBC correlation from the NH proton (δ_H 10.02) of Thr-10 to the amide carbon (C-65) of Val-11 at δ_C 173.4. The last unit of the peptide, *N*-methyl-Val-12, was then connected by the long-range coupling of 66-NH (δ_H 8.65) and C-70 (δ_C 174.2).

The elucidated structure thus far bore a phenyl ring, an indole ring, and 12 carbonyl groups, which accounted for 22 of the 23 degrees of unsaturation deduced from the molecular formula. Therefore, we deduced that the last double bond equivalent must be a result of the presence of an additional ring. Careful analysis of the HMBC spectrum revealed a correlation from H-63 (*β*-proton of Thr-10; δ_H 6.04) to C-1 (the carbonyl carbon of Val-1; δ_C 174.3), thus indicating a macrolide tethered through Thr-10 and Val-1. The planar structure of ohmyungsamycin A (**1**) was thus defined as a cyclic depsipeptide composed of 12 amino acid residues.

Ohmyungsamycin B (**2**) was purified as an amorphous white powder, and the molecular formula was determined to be $C_{76}H_{121}N_{13}O_{16}$ through analysis of the HRFABMS data and the ^{1}H and ^{13}C NMR spectra (Table 2). The molecular formula of **2** possesses one more carbon and two more proton atoms than that of **1**, which indicates that ohmyungsamycin B is an analogue of **1** with slight modifications. The ^{1}H and ^{13}C NMR spectra of **2** had very similar features compared to those of **1**, as expected. The only difference was an additional *N*-methyl group at δ_H 2.54 and δ_C 35.9. Further analysis of the COSY, TOCSY, HSQC, and

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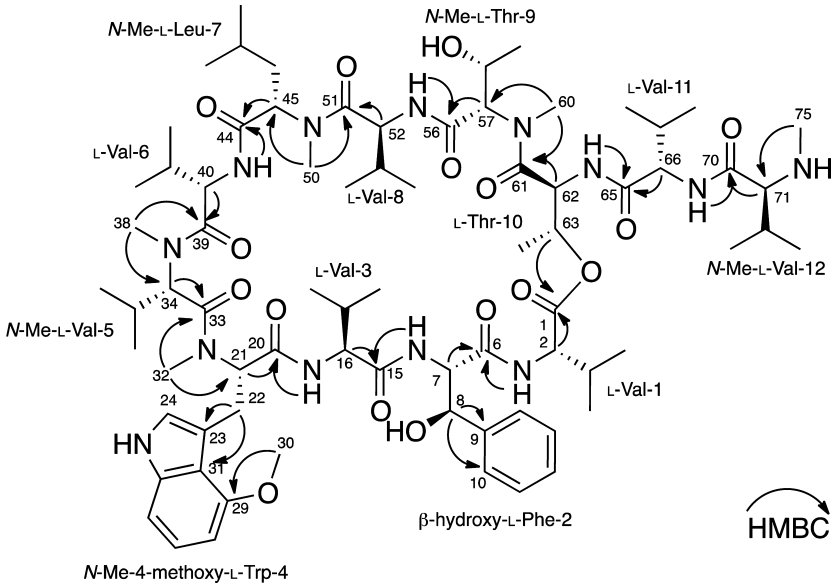

Figure 2. Key HMBC correlations for the determination of the planar structure of ohmyungsamycin A (**1**).

HMBC spectra revealed that the last unit of ohmyungsamycin A, *N*-methyl-Val-12, was replaced with *N*,*N*-dimethyl-Val-12.

Because ohmyungsamycins A and B are composed of α-amino acids, the advanced Marfey's method using FDAA (1-fluoro-2,4-dinitrophenyl-5-alanine amide) was applied to determine the absolute configurations of the amino acid α-carbons.[10] Acid hydrolysis of **1** was performed for 1 h to minimize degradation of the fragile amino acids such as β-hydroxyphenylalanine and *N*-methyl-4-methoxytryptophan.[11] LC/MS analyses of the L- and D-FDAA derivatives allowed us to determine that the absolute configurations of all α-carbons found in **1** are *S* (L).

Because threonine has an additional stereogenic center at the β-carbon, its configuration in **1** was established through comparison of the retention time of its GITC (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate) derivative with the retention times of the GITC derivatives of authentic standards of L-*allo*-Thr and L-Thr as well as standards of *N*-methyl-L-*allo*-Thr and *N*-methyl-L-Thr.[12] The LC/MS analysis of the GITC derivatives established that ohmyungsamycin A possesses L-Thr and *N*-methyl-L-Thr and not the *allo*-forms.

Although the absolute configurations of common amino acid residues were readily assigned via the advanced Marfey's method, β-hydroxyphenylalanine bears one additional stereogenic center at the β-carbon (C-8), whose absolute configuration could not be determined by the advanced Marfey's method or GITC derivatization. Thus, we applied the modified Mosher's method to determine the absolute configuration of this chiral center.[13] *S*- and *R*-MTPA esters (**3** and **4**) at C-8 were obtained through derivatization of **1** with *R*- and *S*-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl). The ^{1}H chemical shifts around the β-carbon of β-hydroxyphenylalanine were determined from the ^{1}H and COSY NMR spectra, and $\Delta\delta_{S-R}$ values were calculated. On the basis of the consistent distribution of $\Delta\delta_{S-R}$ values, the absolute configuration of the β-carbon of β-hydroxyphenylalanine (C-8) was assigned as *R* (Figure 3).

Ohmyungsamycin B (**2**) incorporates *N*,*N*-dimethylvaline, which does not contain an NH to react with FDAA or GITC for the determination of its absolute configuration. We therefore developed a new method utilizing phenylglycine methyl ester (PGME) derivatization of the carboxylic acid side of *N*,*N*-dimethylvaline and subsequent chromatographic analysis by LC/MS. PGME was originally developed as an anisotropic reagent with which to generate diastereomers for determining the absolute configuration of chiral carboxylic acids on the basis of ^{1}H NMR spectroscopic analysis in a manner analogous to the method employing MTPA for secondary alcohols.[14] Instead of NMR analysis, which requires the preparative separation of esterified products, a chromatographic application using MTPA esters has been reported for determining the absolute configuration of 2-hydroxyisoleucic acid.[15] Thus, we developed a new method for chromatographic assignment of the absolute configuration of the *S*- and *R*-PGME adducts of *N*,*N*-dimethylvaline in **2** by LC/MS analysis (Figure 4a).

After acid hydrolysis, the free amino acids were coupled with *S*- and *R*-PGME. *S*- and *R*-PGME derivatives (**5** and **6**) of *N*,*N*-dimethylvaline were clearly separated during our LC/MS analysis (Figure 4b). The absolute configuration of *N*,*N*-dimethylvaline in **2** was determined to be L (*S*) by comparison of the retention times of its PGME derivatives with those of authentic *N*,*N*-dimethyl-L- and D-valine-PGME adducts (Figure 4b). The remaining stereogenic centers of **2** were deduced to be identical to those in **1** on the basis of the high similarity of the ^{1}H and ^{13}C chemical shifts, the ^{1}H−^{1}H coupling constants, the optical rotation values, and the CD spectra (see the Supporting Information).

After establishing the absolute configuration of **1** completely, we analyzed trans-annular ROESY correlations of **1**. The ^{1}H NMR spectrum of **1** actually displayed an array of minor signals (5:1 major to minor ratio), indicating that this compound has another conformer in the solution caused by the amide bond heterogeneity. The assignment of ^{1}H NMR chemical shifts of the conformer and the careful analysis of trans-annular ROESY correlations of both conformers revealed that the main differences between the two conformers reside mainly in the orientations of the *N*-methyl groups at C-38 and C-60 in *N*-Me-Val-5 and *N*-Me-Thr-9. In the major conformer, H_3-38 are positioned on the outside of the macrolactone ring and H_3-60 are oriented toward the inside of the ring. However, in the minor conformer, the orientations of these methyl groups are opposite,

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Table 2. NMR data for Ohmyungsamycin B (2) in Pyridine-d_5[a]

	C/H	δ_C, type	δ_H, mult (J, Hz)		C/H	δ_C, type	δ_H, mult (J, Hz)
Val-1	1	174.3, C		Val-6	39	173.2, C	
	2	58.3, CH	4.70, dd (8.5, 8.5)		40	54.3, CH	4.96, m[b]
	2-NH		9.26, d (8.5)		40-NH		9.39, d (9.0)
	3	32.8, CH	2.23, m		41	31.9, CH	2.40, m
	4	19.3, CH_3	1.12, m[b]		42	19.0, CH_3	1.14, m[b]
	5	18.6, CH_3	0.97, m[b]		43	19.7, CH_3	1.03, d (6.0)
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	7-NH		9.66, d (8.0)		46a	38.9, CH_2	1.74, m
	8	73.2, CH	5.94, d (2.5)		46b		1.58, m
	9	143.0, C			47	25.1, CH	1.43, m
	10	127.0, CH	7.68, d (7.5)		48	23.1, CH_3	0.67, d (6.0)
	11	128.6, CH	7.41, dd (7.5, 7.5)		49	21.9, CH_3	0.67, d (6.0)
	12	127.5, CH	7.27, d (7.5)		50	31.3, CH_3	3.52, s
	13	128.6, CH	7.41, dd (7.5, 7.5)	Val-8	51	173.5, C	
	14	127.0, CH	7.68, d (7.5)		52	55.6, CH	5.29, dd (8.5, 8.5)
Val-3	15	174.5, C			52-NH		8.06, d (8.5)
	16	58.3,CH	5.41, dd (8.5, 8.5)		53	31.0, CH	2.57, m
	16-NH		8.02, d (8.5)		54	19.0, CH_3	1.20, d (7.0)
	17	33.1, CH	2.66, m		55	19.7, CH_3	1.16, d (7.0)
	18	19.8, CH_3	1.36, d (6.5)	*N*-Me-Thr-9	56	170.6, C	
	19	20.0, CH_3	1.20, d (7.5)		57	62.4, CH	5.61, d (3.5)
N-Me-4-methoxy-Trp-4	20	169.9, C			58	66.5, CH	5.07, m[b]
	21	70.6, CH	4.59, dd (11.0, 4.5)		59	20.5, CH_3	1.34, d (6.5)
	22a	27.1, CH_2	4.45, dd (13.5, 4.5)		60	34.5, CH_3	3.68, s
	22b		4.32, dd (13.5, 11.0)	Thr-10	61	171.4, C	
	23	112.9, C			62	52.5, CH	5.86, dd (8.5, 2.0)
	24	124.3, CH	7.19, m[b]		62-NH		10.17, d (8.5)
	24-NH		11.80, d (1.5)		63	69.4, CH	6.04, qd (6.5, 2.0)
	25	139.5, C			64	16.8, CH_3	1.52, d (6.5)
	26	105.8, CH	7.30, m	Val-11	65	173.4, C	
	27	122.7, CH	7.27, m		66	57.9, CH	5.24, dd (9.5, 7.5)
	28	99.5, CH	6.67, d (7.0)		66-NH		8.65, d (9.5)
	29	154.9, C			67	27.6, CH	2.05, m
	30	55.4, CH_3	3.83, s		68	19.8, CH_3	0.95, m[b]
	31	118.4, C			69	18.8, CH_3	0.95, m[b]
	32	40.8, CH_3	2.51, s	*N*,*N*-diMe-Val-12	70	174.2, C	
N-Me-Val-5	33	169.4, C			71	71.5, CH	3.08, d (6.0)
	34	70.9, CH	3.19, m		72	32.2, CH	2.16, m
	35	29.0, CH	3.03, m		73	19.1, CH_3	1.11, m
	36	21.9, CH_3	1.22, d (7.0)		74	19.1, CH_3	1.11, m
	37	19.7, CH_3	1.02, m[b]		75	35.9, CH_3	2.54, s
	38	39.9, CH_3	3.18, s		76	35.9, CH_3	2.54, s

[a] ^{1}H and ^{13}C data were recorded at 900 and 225 MHz, respectively. [b] Overlapped signals.

generating a different set of trans-annular ROESY correlations (see Table S4 and Figure S23 in the Supporting Information).

Ohmyungsamycins A and B (**1** and **2**) are structurally most similar to the cyclic peptides isolated from *Nonomuraea* sp., a terrestrial actinomycete, reported in a patent.[16] However, the ohmyungsamycins have different numbers of amino acid residues, and the absolute configurations of the cyclic peptides from *Nonomuraea* sp., particularly the β-carbons of β-hydroxyphenylalanine, threonine, and *N*-methylthreonine, and the α-carbon of *N*,*N*-dimethylvaline were not rigorously determined.

To evaluate the effects of ohmyungsamycin A and B on the growth of various human cancer cells, the growth inhibitory potential was determined using a colorimetric sulforhodamine B (SRB) protein dye staining method.[17] As summarized in Table 3, ohmyungsamycin A (**1**) exhibited potent antiproliferative effects against HCT-116, A549, SNU-638, MDA-MB-231, and SK-HEP-1 cells, with IC_{50} values ranging from 359 to 816 nM. In particular, ohmyungsamycin A was 78 times more potent against the human colon cancer cell line HCT-116 than the positive control compound, etoposide. However, ohmyungsamycin B (**2**) displayed weak cytotoxicity against the tested cancer cells, with IC_{50} values of 12.4 to 16.8 μM. In the case of normal lung epithelial (MRC-5) cells, ohmyungsamycins A and B (**1** and **2**) both showed virtually no cytotoxicity (IC_{50} > 40 μM), indicating that these compounds exhibit relatively selective antiproliferative activity against cancer cells compared to normal cells (see Table 3 and Tables S2 and S3, Supporting Information). This difference in the antiproliferation activity against cancer cells compared to normal cells by the compounds may be associated

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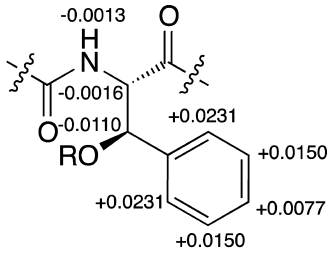

3: R = *S*-MTPA
4: R = *R*-MTPA

Figure 3. $\Delta\delta_{S\text{-}R}$ values in ppm around the β-carbon of β-hydroxyphenylalanine (C-8) for *S*- and *R*-MTPA esters (**3** and **4**) of **1** in pyridine-d_5.

with the consequence of genetic background or of the biologically various characteristics between cancer and normal cells. However, the exact molecular mechanism behind the selectivity should be further investigated.

Ohmyungsamycin A (**1**) also displayed significant inhibitory activity against both Gram-positive and Gram-negative bacteria, including *Bacillus subtilis*, *Kocuria rhizophila*, and *Proteus hauseri* (MIC = 1.07−4.28 μM) (Table 4). However, ohmyungsamycin A did not inhibit *Staphylococcus aureus*, *Salmonella enterica*, or *Escherichia coli*. Ohmyungsamycin B (**2**) was less active than **1**, exhibiting MIC values of 8.50 to 34.0 μM against *B. subtilis*, *K. rhizophila*, and *P. hauseri*. Ohmyungsamycin A (**1**) is much more potent than ohmyungsamycin B (**2**) with regard to its cytotoxicity and antibacterial activity. Thus, the presence of the additional *N*-methyl group at the terminus of **2** could lead to a significant decrease the biological activity of the ohmyungsamycins.

In summary, a chemical study of marine actinomycete strains from a volcanic island sand beach led to the discovery of ohmyungsamycins A and B, which bear unusual amino acids, including β-hydroxyphenylalanine, *N*-methyl-4-methoxytryptophan, and *N*,*N*-dimethylvaline. We developed a new chromatographic method using PGME derivatization to determine the absolute configuration of *N*,*N*-dimethylvaline. Our new method will serve as a general and convenient analytical procedure for determining the absolute configurations of amino acids that are fully substituted at the amine position. The structural uniqueness and significant biological activities of the ohmyungsamycins indicate that the chemical and biological investigation of unique environments, including volcanic islands, can be an effective strategy for discovering new biologically active compounds.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded in MeOH at 589 with a 1-cm cell. UV spectra were measured with UV−Vis spectrometer from 200 to 600 nm in a 1-cm cuvette. IR spectra were recorded with 4 cm^{-1} resolution. ^1H NMR (900 MHz) and ^{13}C NMR (225 MHz) spectral data were obtained on a 900-MHz NMR spectrometer. Low-resolution LC/MS data were obtained on an HPLC coupled to a quadrupole mass spectrometer. High-resolution fast-atom bombardment (HR-FAB) mass spectrometry data were recorded with high-resolution mass spectrometer.

Isolation of the Strain SNJ042 and Phylogenetic Analysis. Marine sediments were collected from Shinyang Beach, Jeju Island, Republic of Korea, in 2010. The sediment (1 g) was diluted in 30 mL of sterilized artificial seawater, and the suspension was heated in a water bath for 5 min (55 °C). Two hundred microliters of the solution was spread onto agar plates containing various media. Strain SNJ042 was isolated from A6 isolation medium (18 g/L agar containing 3.4% seawater and 5 μg/mL of polymyxin B sulfate). The strain was deposited in the Korea Collection for Type Cultures (accession no. KCTC18240P). The 16S rDNA (1469 bp) of SNJ042 was sequenced for phylogenetic analysis. On the basis of the 16S rDNA sequences of SNJ042 and type strains of related *Streptomyces* spp., strain SNJ042 is most closely related to *Streptomyces cheonanensis* (97% similarity, see the Supporting Information, Figure S20).

Cultivation and Extraction. Strain SNJ042 was cultivated on solid YEME medium (4 g of yeast extract, 10 g of malt extract, 4 g of glucose, and 18 g of agar per 1 L of sterilized 3.4% seawater) at 25 °C. The bacterium was transferred to liquid A1+C medium (10 g of starch, 4 g of yeast, 2 g of peptone, and 1 g of $CaCO_3$ per 1 L of sterilized 3.4% seawater) in a 100-mL Erlenmeyer flask containing 50 mL of the medium. After incubation at 30 °C with shaking at 200 rpm for 3 days, 5 mL of the liquid culture was inoculated to each 1 L of A1+C in 2.8-L Fernbach flasks. The culture was cultivated at 30 °C with shaking at 200 rpm for 6 days. The entire culture (84 L) was extracted twice with 126 L of distilled EtOAc using a separation funnel. After anhydrous sodium sulfate was added to remove residual water, the organic phase was concentrated in vacuo to yield 9.8 g of dry extract.

Isolation of Ohmyungsamycins A and B (1 and 2). A portion of the dry crude extract (2.3 g) was resuspended with Celite in MeOH and dried *in vacuo* to generate Celite-adsorbed extract. The Celite-adsorbed extract was loaded onto 2 g of prepacked C_{18} Sepak resin. The extract was fractionated by elution with a step gradient composed of water and MeOH. Ohmyungsamycins A and B (**1** and **2**) eluted in the 80% MeOH fraction. To obtain pure ohmyungsamycins A and B, the material from the 80% MeOH fraction was purified by semipreparative reversed-phase HPLC (Kromasil, C_{18}, 10 × 250 mm, flow rate: 2 mL/min, detection: UV 280 nm, gradient solvent system: 70−85% aqueous MeOH over 50 min). Ohmyungsamycins A and B eluted at 35.1 and 35.3 min, respectively. The entire procedure was repeated six times to furnish 14.5 mg of **1** and 4.6 mg of **2**.

*Ohmyungsamycin A (**1**):* white powder; $[\alpha]_D^{25}$ −42 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.42), 281 (2.83) nm; CD (MeOH) ($\Delta\varepsilon$) 211 (−36.6); IR (neat) ν_{max} 3297, 2963, 1640, 1535, 1509, 1201 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) see Table 1; HRFABMS *m*/*z* 1458.8983 $[M + H]^+$ (calcd for $C_{75}H_{120}N_{13}O_{16}$, 1458.8976).

*Ohmyungsamycin B (**2**):* white powder; $[\alpha]_D^{25}$ −39 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.43), 281 (2.81) nm; CD (MeOH) ($\Delta\varepsilon$) 212 (−45.6); IR (neat) ν_{max} 3297, 2963, 1640, 1531, 1509, 1199 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) see Table 2; HRFABMS *m*/*z* 1472.9106 $[M + H]^+$ (calcd for $C_{76}H_{122}N_{13}O_{16}$, 1472.9133).

Determination of the Absolute Configurations of α-Carbons of the Amino Acid Residues in Ohmyungsamycin A (1). One milligram of ohmyungsamycin (**1**) was hydrolyzed in 0.5 mL of 6 N HCl at 115 °C for 1 h, and the reaction mixture was subsequently cooled in ice−water for 3 min. The reaction solvent was evaporated *in vacuo*, and residual HCl was completely removed by the addition of 0.5 mL of water and removal of the solvent three times. The hydrolysate was lyophilized to complete dryness for 24 h. The hydrolysate from the reaction containing the free amino acids was divided into two portions, and each portion was transferred into an 8 mL vial. The hydrolysate was then dissolved in 100 μL of 1 N $NaHCO_3$. Either 50 μL of 10 mg/mL L-FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) or 50 μL of D-FDAA in acetone was added to each of the two vials containing the dissolved free amino acids. The reaction mixtures were incubated at 80 °C for 3 min. A 50-μL aliquot of 2 N HCl was added to neutralize the reaction, and 300 μL of aqueous 50% CH_3CN was added to the vials. A 20-μL aliquot of each reaction mixture was analyzed by LC-MS using a gradient solvent system (20 to 60% CH_3CN containing 0.1% formic acid over 40 min, C_{18} reversed-phase column: 100 × 4.6 mm, detection: UV 340 nm). L-FDAA derivatives eluted before D-FDAA derivatives for all amino acid residues in the hydrolysate. Thus, the absolute configurations of all of the amino acid residues in **1** were determined to be L (see Table S1, Supporting Information).

Determination of the Absolute Configuration of the β-Carbon of β-Hydroxyphenylalanine. The absolute configuration of

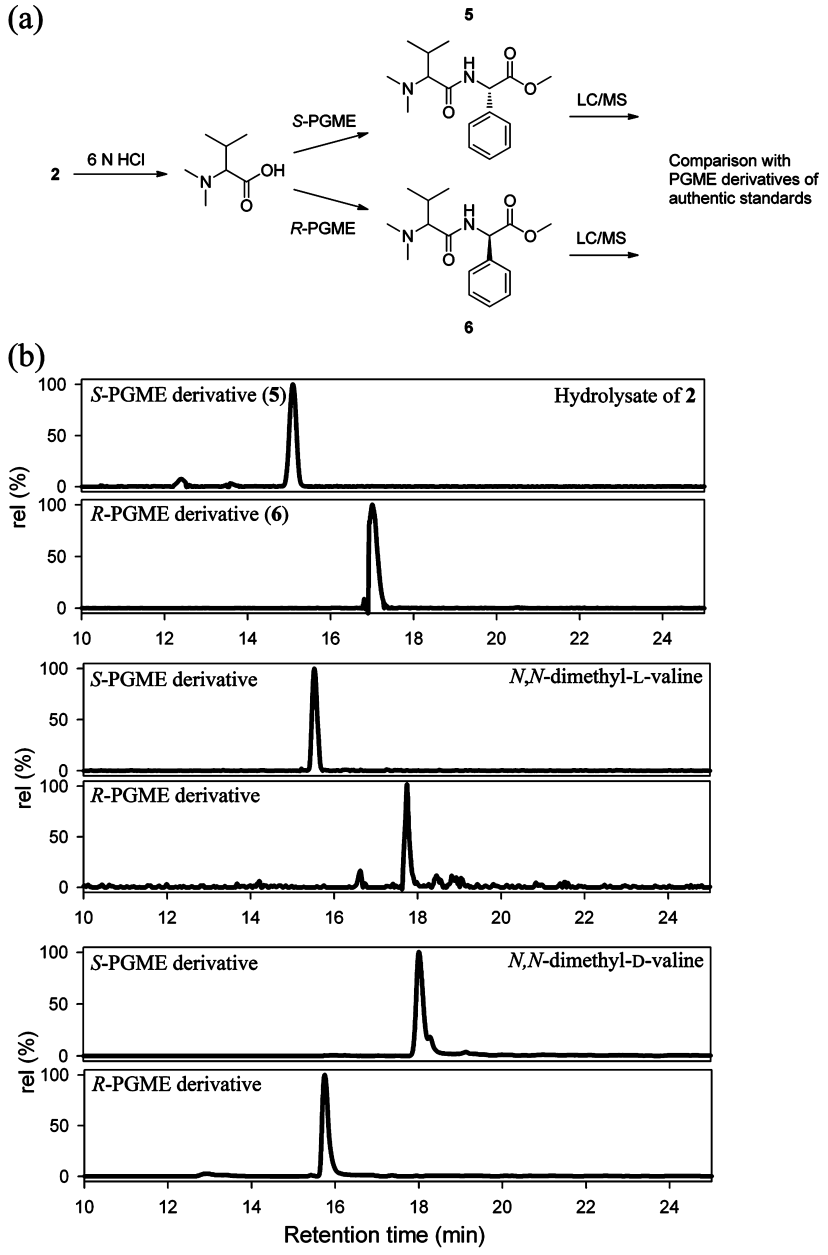

Figure 4. Chromatographic determination of the absolute configuration of *N*,*N*-dimethylvaline in **2** by PGME derivatization. (a) Procedure for the chemical reaction with PGME. (b) LC/MS analysis (ion extraction for M.W. 292) of *S*- and *R*-PGME derivatives of the hydrolysate of **2** (top), *N*,*N*-dimethyl-L-valine (middle), and *N*,*N*-dimethyl-D-valine (bottom).

the stereogenic center at the β-carbon of β-hydroxyphenylalanine was determined using the modified Mosher's method with *R*- and *S*-α-methoxy-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl). Ohmyungsamycin A (**1**, 2 mg, dried under high vacuum for 24 h) was dissolved in 1 mL of distilled pyridine after a catalytic amount of 4-dimethylaminopyridine (DMAP) was added. The reaction mixture was stirred for 10 min. Fifty microliters of *R*-MTPA chloride was carefully added to the reaction vial. After 2 h, 50 μL of MeOH was added to the vial to quench the reaction. *S*-MTPA ester (**3**) was purified by semipreparative HPLC (Kromasil, C_{18}, 10 × 250 mm, gradient solvent system: 30−100% aqueous MeOH over 50 min, t_R 32 min). The *R*-MTPA ester (**4**) was obtained using the same procedure (t_R: 32 min).

S-MTPA ester ***3****:* ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 11.80 (1H, br s), 10.29 (1H, d, *J* = 8.5), 9.75 (1H, d, *J* = 8.5), 9.38 (1H, d, *J* = 9.0), 9.20 (1H, d, *J* = 8.5), 8.57 (1H, d, *J* = 9.5), 8.09 (1H, d, *J* = 8.5), 8.02 (1H, d, *J* = 9.0), 7.92 (2H, d, *J* = 7.0), 7.62 (2H, d, *J* = 8.0), 7.44−7.24 (9H, m), 6.67 (1H, d, *J* = 7.0), 6.63 (1H, br s), 6.40 (1H, m), 6.00 (1H, m), 5.86 (2H, m), 5.72 (1H, m), 5.65 (1H, m), 5.45 (1H, m), 5.39 (1H, dd, *J* = 9.5, 9.5), 5.27 (1H, dd, *J* = 7.5, 7.5), 5.21 (1H, m), 4.65 (1H, dd, *J* = 8.0, 8.0), 4.58 (1H, m), 4.45 (1H, dd, *J* = 13.5, 4.5), 4.32 (1H, dd, *J* = 13.5, 11.5), 3.81 (3H, s), 3.69 (3H, s), 3.67 (1H, d, *J* = 8.0), 3.55 (3H, s), 3.38 (3H, s), 3.20 (1H, d, *J* = 8.0), 3.17 (3H, s), 3.03 (1H, m), 2.66 (1H, m), 2.53 (1H, m), 2.49 (3H, s), 2.48 (3H, s), 2.37 (1H, m), 2.25−2.17 (3H, m), 1.73 (1H, m), 1.63 (1H, m), 1.47 (3H, d, *J* = 6.5), 1.46 (1H, m), 1.35 (3H, d, *J* = 6.5), 1.29 (3H, d, *J* = 6.5), 1.22−1.15 (12H, m), 1.12 (3H, d, *J* = 6.5), 1.09−1.05 (9H, m), 1.01 (6H, m), 0.93 (9H, m), 0.69 (6H, *J* = 7.0). The molecular formula of **3** was confirmed as $C_{85}H_{126}F_3N_{13}O_{18}$ by ESIMS analysis ($[M + H]^+$ at *m*/*z* 1674).

R-MTPA ester ***4****:* ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 11.85 (1H, br s), 10.36 (1H, d, *J* = 8.5), 9.75 (1H, d, *J* = 8.5), 9.37 (1H, d, *J* = 9.0), 9.19 (1H, d, *J* = 8.5), 8.60 (1H, m), 8.02 (1H, d, *J* = 8.5), 7.93 (1H, d, *J* = 9.0), 7.87 (2H, d, *J* = 7.0), 7.62 (2H, d, *J* = 8.0), 7.44−7.24 (9H, m), 6.67 (1H, d, *J* = 7.0), 6.56 (1H, br s), 6.44 (1H, m), 6.01 (1H, m), 5.83 (2H, d, 2.5), 5.72 (1H, m), 5.67 (1H, m), 5.46 (1H, m), 5.39 (1H, dd, *J* = 9.5, 9.5), 5.27 (1H, m), 5.22 (1H, m), 4.65 (1H, dd, *J* = 8.0, 8.0), 4.58 (1H, dd, *J* = 11.0, 4.5), 4.44 (1H, dd, *J* = 13.5, 4.5), 4.34 (1H, m), 3.81 (3H, s), 3.69

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Table 3. Inhibitory Effects of Ohmyungsamycins A and B (1 and 2) on the Proliferation of Human Cancer Cells and Normal Cells

		IC_{50} (μM)		
cell line	classification	ohmyungsamycin A	ohmyungsamycin B	etoposide[a]
HCT-116	colon cancer cells	0.359	12.4	28.1
A549	lung cancer cells	0.551	15.6	1.36
SNU-638	stomach cancer cells	0.532	13.5	1.37
MDA-MB-231	breast cancer cells	0.688	12.7	19.0
SK-HEP-1	liver cancer cells	0.816	16.8	5.04
MRC-5	lung normal cells	>40	>40	36.2

[a]Etoposide was used as a positive control.

Table 4. Inhibitory Activities of Ohmyungsamycins A and B (1 and 2) against Bacterial Strains

	MIC (μM)		
strain	ohmyungsamycin A	ohmyungsamycin B	ampicillin
Staphylococcus aureus ATCC6538p	not active	not active	1.14
Bacillus subtilis ATCC6633	4.28	34.0	1.14
Kocuria rhizophila NBRC12708	1.07	8.50	1.14
Proteus hauseri NBRC3851	2.14	17.0	2.28
Salmonella enterica ATCC14028	not active	not active	1.14
Escherichia coli ATCC35270	not active	not active	8.87

(3H, s), 3.61 (1H, m), 3.54 (3H, s), 3.29 (3H, s), 3.19 (1H, d, J = 8.5), 3.17 (3H, s), 3.02 (1H, m), 2.66 (1H, m), 2.50 (6H, s), 2.37 (1H, m), 2.18−2.11 (4H, m), 1.73 (1H, m), 1.63 (1H, m), 1.50 (3H, m), 1.44 (1H, m), 1.34−1.17 (21H, m), 1.14−1.05 (9H, m), 1.01−0.84 (15H, m), 0.67 (3H, J = 7.0), 0.66 (3H, J = 7.0). The molecular formula of **4** was confirmed as $C_{85}H_{126}F_3N_{13}O_{18}$ by ESIMS analysis ($[M + H]^+$ at m/z 1674).

Determination of the Absolute Configurations of the β-Carbons of Threonine and *N*-Methylthreonine (GITC Derivatization). Two milligrams of authentic standards of *N*-methyl-L-*allo*-Thr and *N*-methyl-L-Thr were each dissolved in 2 mL of water. Then, 200 μL of 6% triethylamine and 200 μL of 1% GITC in acetone were added, and the reaction mixture was stirred at room temperature for 15 min. Next, 100 μL of 5% acetic acid was added to neutralize the reaction mixture. The product was analyzed by LC/MS with a gradient solvent system (10% to 100% CH_3CN containing 0.1% formic acid over 20 min, C_{18} reversed-phase HPLC column: 250 × 4.6 mm). The GITC derivatives of *N*-methyl-L-*allo*-Thr and *N*-methyl-L-Thr eluted at 15.9 and 15.1 min, respectively. The hydrolysate of **1** was also derivatized with GITC using an identical method. The GITC derivative of the hydrolysate of **1** eluted at 15.1 min in the LC/MS analysis, which indicated that the unit in **1** is *N*-methyl-L-Thr, not *N*-methyl-L-*allo*-Thr. The same procedure was performed for L-Thr and L-*allo*-Thr. The GITC derivatives of allo-L-Thr and L-Thr eluted at 15.5 and 15.8 min, respectively, and the retention time of the GITC derivative of L-Thr from the hydrolysate of **1** was 15.8, indicating that the threonine residue in **1** is L-Thr.

Determination of the Absolute Configuration of *N,N*-Dimethylvaline. Two milligrams of ohmyungsamycin B (**2**) was hydrolyzed and dried under high vacuum for 24 h. The hydrolysate was dissolved in 2 mL of tetrahydrofuran (THF) and was split equally into two vials. The vials were treated with 6 mg of *S*-PGME or *R*-PGME, and 7 mg of ethyl-(*N,N*-dimethylamino)propylcarbodiimide hydrochloride (EDC) was added to each vial. After the reaction mixtures were stirred at 25 °C for 6 h, *S*- and *R*-PGME amide products ($[M + H]^+$ m/z at 293) were analyzed with LC/MS (Phenomenex, C_{18}, 250 × 4.6 mm, gradient system: 10−100% aqueous CH_3CN over 40 min). *S*- and *R*-PGME amide products eluted at 15.5 and 17.7 min, respectively. The absolute configuration of *N,N*-dimethylvaline in **2** was determined to be L through comparison of the retention times of the *S*- and *R*-PGME amide products with those of the authentic standards of *N,N*-dimethyl- L- and D-valine.

Cell Culture. Human lung cancer (A549), colon cancer (HCT116), stomach cancer (SNU638), breast cancer (MDA-MB-231), liver cancer (SK-HEP-1), and lung fibroblast (MRC-5) cells were provided by the Korean Cell Line Bank (Seoul, Korea). Cells were cultured in medium (DMEM for MDA-MB-231 and SK-HEP-1 cells; RPMI 1640 medium for A549, HCT116, and SNU638 cells; MEM for MRC-5 cells) supplemented with 10% heat-inactivated FBS and antibiotics−antimycotic solution (100 units/mL penicillin G sodium, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B). Cells were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

Cell Proliferation Assay. Cells (3.5×10^3 cells/mL for HCT-116 and SK-HEP-1; 4×10^3 cells/mL for A549; 5×10^3 cells/mL for MDA-MB-231, and SNU-638; 7×10^3 cells/mL for MRC-5 cells) were treated with various concentrations of ohmyungsamycin A and B for 3 days. After treatment, cells were fixed with 10% TCA solution, and cell viability was determined using the sulforhodamine B (SRB) assay. The results were expressed as percentages relative to solvent-treated control incubations, and IC_{50} values were calculated using nonlinear regression analysis (percent survival versus concentration).

Antibacterial Assay. Six bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Kocouria rhizophila*, *Proteus hauseri*, *Salmonella enterica*, and *Escherichia coli*) were used for antibacterial activity assays. Bacteria were grown overnight in Luria−Bertani (LB) broth at 37 °C. Cells were harvested by centrifugation and washed twice with sterile distilled water. Ohmyungsamycins A and B (**1** and **2**) were dissolved in 100% dimethyl sulfoxide (10 mg/mL) and diluted in 96-well plates with m Plate Count Broth to prepare serial 2-fold dilutions in the range of 100 to 0.39 μg/mL. Test bacteria were added to each well (1×10^5 cells/mL) and incubated for 16 h at 37 °C. The minimum inhibitory concentration (MIC) values were determined as the lowest concentration of test compounds that inhibited bacterial growth. Ampicillin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of **1** and **2**, detailed Marfey's analysis results, and identification of strain SNJ042. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): The patent application for the ohmyungsamycins has been submitted.

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