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Cyclic Hexapeptides from the Deep South China Sea-Derived *Streptomyces scopuliridis* SCSIO ZJ46 Active Against Pathogenic Gram-Positive Bacteria

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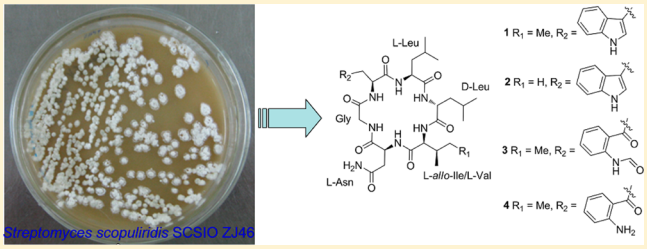
Supporting Information

ABSTRACT: Three new cyclohexapeptides, desotamides B–D (**2**–**4**), and the known desotamide (**1**) were isolated from marine microbe *Streptomyces scopuliridis* SCSIO ZJ46. The sequences and absolute configurations of **2**–**4** were elucidated on the basis of high-resolution spectroscopic data, Marfey's method, and chiral-phase HPLC data. Desotamide C (**3**) contains a unique *N*-formyl-kynurenine residue, whereas **4** lacks formylation at the same site. Compounds **1** and **2** displayed notable antibacterial activities against strains of *Streptococcus pnuemoniae*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus epidermidis* (MRSE), and structure activity relationship studies revealed the indispensability of the Trp component for antibacterial activity within this new scaffold.



Natural-product-derived antibiotics have played, and continue to play, a pivotal role in the treatment of bacterial infections and diseases. However, the emergence of drug-resistance among human pathogenic bacteria has proven to be an increasingly important problem calling for the development of new therapeutic approaches.[1−3] Resistance to currently employed antibacterial drugs has been encountered with increasing frequency over the course of the last few decades, spurring new efforts to identify new drug candidates able to circumvent or ablate bacterial mechanisms of resistance. Cyclic peptides, for instance, with a broad spectrum of biological activities such as tyrocidine A, vancomycin, and daptomycin are clinically important antibacterial drugs. Compared to their linear counterparts, cyclic peptides display different cell distribution and membrane penetration properties and are resistant to proteases.[4,5] Terrestrially derived natural products have proven highly effective and well-known antibacterial drugs in the 20th century. However, more recent efforts have revealed that marine-derived products also possess exciting potential as new antibacterial agents.[6−8] In our efforts to screen novel antibacterial components from the South China Sea-derived actinomycetes, we have discovered novel antibacterial cyclic peptides such as marthiapeptide A,[9] from the deep-sea-derived *Marinactinospora thermotolerans* SCSIO 00652, and marformycins A–F, from the deep-sea-derived *Streptomyces drozdowiczii* SCSIO 10141.[10] Our ongoing investigations into another deep-sea-derived actinomycete, *Streptomyces scopuliridis* SCSIO ZJ46, whose fermentation extract was found to inhibit pathogenic *Streptococcus pnuemoniae* and methicillin-resistant *Staphylococcus epidermidis* (MRSE), led to the isolation of desotamide (**1**), along with three new cyclic hexapeptides, desotamides B–D (**2**–**4**). Here we report the isolation, structure elucidation, and cytotoxic/antibacterial activities of the new cyclohexapeptides **2**–**4**.

RESULTS AND DISCUSSION

Streptomyces scopuliridis SCSIO ZJ46 was isolated from a sediment sample collected in the South China Sea at a depth of 3536 m. An 8 L scale fermentation, extraction and subsequent isolation following silica gel chromatography and further purification by HPLC yielded compounds **1**–**4**. Compound **1**, the predominant product, was isolated as an amorphous powder. Its molecular formula, $C_{35}H_{52}N_8O_7$, was determined upon analysis of the HRESIMS peak at *m*/*z* 697.4028 $[M + H]^+$, requiring 14 degrees of unsaturation. Following detailed analysis of the ^1H, ^{13}C, COSY, HSQC, and HMBC NMR spectroscopic data for **1** (Supporting Information, Table S1), it was deduced that **1** is a hexapeptide with an assigned amino acid sequence of cyclo-(-Trp-Gly-Asn-Ile-Leu-Leu-). A subsequent literature survey revealed **1** to be identical to the known compound desotamide.[11] Furthermore, the application

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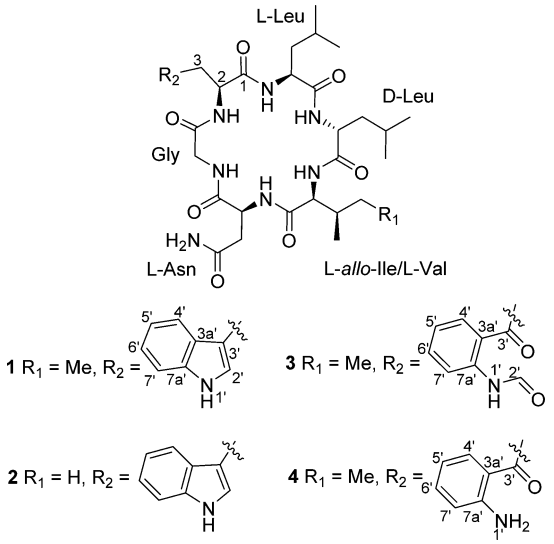

of Marfey's analysis and chiral-phase HPLC of the acid hydrolysate of **1** supported the absolute configuration as depicted.

Compound **2** was isolated as a white amorphous powder with a molecular formula of $C_{34}H_{50}N_8O_7$ as determined by HRESIMS; the MS data for **2** indicated the absence of one CH_2 unit relative to compound **1**. The HRESIMS/MS analysis of the protonated molecule (Figures S3, S4) of **2** revealed the loss of a 99 amu fragment rather than the 113 amu fragment noted upon identical analysis of **1**; replacement of the Ile in **1** with a Val in **2** seemed likely. Detailed comparisons of the ^{1}H and ^{13}C NMR spectroscopic data of **2** with those of **1** revealed their structural similarity (Table 1), with the exception of a methylene unit (δ_H 1.27, m; 1.18, m; and δ_C 25.5) in **1** that was absent in **2**. Moreover, the ^{13}C NMR signals of two Ile methyls in **1** were shifted downfield from δ_C 14.3 and 11.3 to δ_C 19.0 and 17.3, respectively, in **2**. Additionally, the methine signal of Ile in **1** was shifted from δ_C 35.1 to δ_C 28.6 in **2**, suggesting once more that the Ile in **1** is replaced with a valine in compound **2**. The presence in **2** of the Val residue and other amino acids shared with compound **1** was confirmed by detailed analyses of COSY, HSQC, and HMBC correlations (Figure 1). The absolute configurations of the amino acids in **2** were determined by application of Marfey's HPLC method (for determination of the L-Trp, L-Asn, L-Val, L-Leu, and D-Leu residues) and careful comparisons of ^{1}H and ^{13}C NMR data with those of **1**. Ultimately, compound **2** was established as cyclo-(-L-Trp-Gly-L-Asn-L-Val-D-Leu-L-Leu-) and was named desotamide B.

Compound **3** was obtained as a minor product and as a white amorphous powder. HRESIMS analyses revealed **3** to have the molecular formula $C_{35}H_{52}N_8O_9$ with two more oxygen atoms than found in compound **1**. The characteristic UV absorption bands for the Trp residue in **1** at 220 and 281 nm were shifted to 230, 260, and 324 nm in **3**, indicating a clear change in Trp characteristics and environment between **1** and **3**. The ^{1}H NMR spectroscopic data for **3** resembled those of **1**, except that all ^{1}H NMR signals for the Trp residue in **1** were significantly shifted downfield in **3** (Table 1). Further analyses of the ^{13}C NMR data for **3** revealed that two of the eight aromatic carbon signals of the Trp residue in **1** were shifted to δ_C 200.2 and 161.1, respectively, corresponding to two carbonyl carbon signals in **3**. These data indicated that the C2′/C3′ double bond of the indole moiety in Trp residue was oxidatively cleaved giving rise to an *N*-formyl-kynurenine (NFK) residue. Analysis of the HSQC, COSY, and HMBC spectra supported this conclusion (Figure 1). Thus, the Trp residue in **1** was replaced by the NFK residue in **3**. Analysis of other COSY, HSQC, and HMBC NMR data for **3** revealed the same structural elements present in **1**, barring the NFK for Trp swap (Figure 1). The amino acid sequence for **3** was also supported by HRESIMS/MS data analysis of the protonated molecule (Figures S5, S6). Acid hydrolysis of **3** converted the NFK residue to the corresponding kynurenine (Kyn) residue which, upon chiral-phase HPLC analysis and comparisons to L-Kyn and D-Kyn standards, enabled us to assign the NFK residue in **3** as the L-isomer. The application of Marfey's analysis, chiral-phase HPLC of the acid hydrolysate of **3**, and ^{1}H and ^{13}C NMR data comparisons of **3** with those of **1** (Figures S9, S10) revealed that the amino acids shared between **3** and **1** all have the same absolute configurations. Consequently, the structure of **3** was determined to be cyclo-(-L-NFK-Gly-L-Asn-L-Ile-D-Leu-L-Leu-) and was named desotamide C.

Compound **4** was isolated as a minor product with a molecular formula of $C_{34}H_{52}N_8O_8$, as determined by HRESIMS. The MS data revealed that **4** possesses one CO unit less than compound **3**. Moreover, the ^{1}H and ^{13}C NMR spectroscopic data for **4** were very similar to those of **3**. However, the two proton signals at δ_H 11.01 (H-1′, s) and 8.45 (H-2′, s) in **3** failed to appear in the ^{1}H spectra of **4**. In turn, a two proton singlet at δ_H 7.05 (2H, H-1′, brs) was noted in ^{1}H NMR spectrum for **4**. Additionally, the carbon signal at δ_C 161.1, attributable to the formyl moiety of **3**, was absent in the spectra of **4** (Table 1). These data indicate that the formyl group of the NFK residue in **3** was absent in **4** thus rendering a Kyn residue instead of the NFK of **3**. Accordingly, the planar structure of **4** was elucidated to be cyclo-(-Kyn-Gly-Asn-Ile-Leu-Leu-) on the basis of COSY, HSQC, and HMBC spectroscopic data sets (Figure 1); the ordering of amino acids is supported by HRESIMS/MS data of the protonated molecule (Figures S7, S8). The acid hydrolysate of **4** afforded identical chromatographic results relative to the hydrolysate of **3** upon chiral-phase HPLC analysis. As a result, the absolute configuration of Kyn in **4** was assigned as L-Kyn (Figure S10). Examination of the Marfey's HPLC profiles, chiral-phase HPLC profiles of the acid hydrolysate (Figures S9, S10), and comparisons of the ^{1}H and ^{13}C NMR data with those of **1**−**3** revealed that **4** has amino acid residues identical to those found in **3**. The structure, including absolute configuration, of **4** was thereby determined to be cyclo-(-L-Kyn-Gly-L-Asn-L-Ile-D-Leu-L-Leu-), and was accordingly named desotamide D.

The isolation of **1** as the major metabolite, and of **2**−**4** as minor products from *S. scopuliridis* SCSIO ZJ46, provides significant clues regarding their biosynthetic relationship. Many microorganisms produce a pair of secondary metabolites containing Ile and Val moieties, respectively. This stems from the promiscuity of the adenylation domain in selecting amino acid building blocks during nonribosomal peptide assembly. For instance, methylpendolmycin and pendolmycin are both produced by *M. thermotolerans* SCSIO 00652 and are attributed to adenylation domain promiscuity during NRPS processing.[12,13] Biosynthetically, postassembly line oxidation of the Trp C-2′/C-3′ olefin in **1** by a Trp-2, 3-dioxygenase within the genome of *S. scopuliridis* SCSIO ZJ46 explains the formation of **3**. Consequently, the generation of **4** may well be the result of NFK deformylation in **3**.

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 HMz) Spectroscopic Data of Desotamides B–D (2–4) in DMSO-d_6

	2		3		4	
pos.	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
	Trp		NFK		Kyn	
1	170.8, C		170.3, C		171.6, C	
2	55.2, CH	4.34, m[a]	50.2, CH	4.63, m[a]	50.9, CH	4.58, m[a]
3	27.1, CH_2	3.15, dd (14.5, 4.5)	41.2, CH_2	3.55, dd (17.0, 8.5)	40.5, CH_2	3.45, dd (17.0, 8.0)
		2.98, dd (14.5, 10.0)		3.42, m[a]		3.19, dd (17.0, 5.0)
2-NH		8.32, d (7.5)		8.51, brs		8.38, d (8.0)
1′(NH)		10.82, s		11.01, s		7.05, brs
2′	123.4, CH	7.15, s	161.1, CH	8.45, s		
3′	109.9, C		200.2, C		198.8, C	
3′a	126.9, C		124.0, C		116.8, C	
4′	117.9, CH	7.51, d (7.5)	130.4, CH	8.00, d (8.0)	131.8, CH	7.65, d (8.0)
5′	118.2, CH	6.98, t (7.5)	123.2, CH	7.24, t (7.5)	115.5, CH	6.53, t (7.5)
6′	120.7, CH	7.06, t (7.0)	133.9, CH	7.61, t (8.0)	135.3, CH	7.23, t (8.0)
7′	111.2, CH	7.33, d (8.0)	121.0, CH	8.41, d (8.0)	117.7, CH	6.70, d (8.0)
7′a	136.0, C		138.0, C		151.6, C	
	Gly		Gly		Gly	
1	169.0, C		169.2		170.3, C	
2	43.1, CH_2	3.90, dd (16.0, 6.0)	43.3, CH_2	3.77, d (15.0)	44.0, CH_2	3.82, dd (15.0, 5.0)
		3.35, dd (16.0, 5.0)		3.42, m[a]		3.33, dd (15.0, 5.0)
NH		7.89, t (5.0)		8.20, m[a]		8.14, t (5.0)
	Asn		Asn		Asn	
1	170.8, C		171.0, C		171.6, C	
2	49.3, CH	4.52, dd (13.5, 6.0)	49.2, CH	4.60, m[a]	49.9, CH	4.56, m[a]
3	36.6, CH_2	2.78, dd (16.0, 5.5)	37.3, CH_2	2.64, dd (16.0, 7.0)	37.7, CH_2	2.62, d (7.0)
		2.64, dd (16.0, 5.5)		2.55, m[a]		2.51, m[a]
4	171.8, C		171.4, C		172.9, C	
2-NH		7.70, d (7.0)		8.00, d (8.0)		7.72, d (8.0)
NH2		7.58, s		7.46, s		7.54, s
		7.05, s		6.96, s		6.96, s
	Val		Ile		Ile	
1	170.5, C		170.6, C		172.1, C	
2	58.8, CH	4.01, dd (7.0, 5.5)	56.5, CH	4.19, dd (8.0, 5.5)	56.8, C	4.17, dd (8.0, 4.5)
3	28.6, CH	2.19, m	35.0, CH	2.01, m	35.8, CH	1.95, m
4	17.3, CH_3	0.86, d (7.0)	25.5, CH_2	1.24, m	26.3, CH_2	1.15, m
				1.15, m		
5			11.2, CH_3	0.81, t (7.0)	12.0, CH_3	0.76, t (7.5)
3-Me	19.0, CH_3	0.86, d (7.0)	14.4, CH_3	0.82, d (8.0)	15.0, CH_3	0.76, d (7.0)
NH		8.26, d (7.0)		8.20, m[a]		8.22, d (8.0)
	Leu1		Leu1		Leu1	
1	172.9, C		172.4, C		173.9, C	
2	51.5, CH	4.37, m[a]	51.6, CH	4.34, m[a]	52.4, CH	4.28, m[a]
3	39.5, CH_2	1.45, m	39.7, CH_2	1.46, m	40.5, CH_2	1.42, m[a]
4	24.0, CH	1.55, m	24.0, CH	1.53, m	24.7, CH	1.48, m
5	22.0, CH_3	0.83, d (5.0)	21.8, CH_3	0.82, d (5.0)	22.7, CH_3	0.79, d (5.5)
4-Me	22.2, CH_3	0.85, d (4.0)	22.0, CH_3	0.85, d (5.0)	22.9, CH_3	0.79, d (5.5)
NH		8.18, d (5.5)		8.13, brs		8.27, d (6.0)
	Leu2		Leu2		Leu2	
1	171.5, C		171.4, C		172.5, C	
2	50.6, CH	4.37, m[a]	50.9, CH	4.39, m[a]	51.7, CH	4.31, m[a]
3	41.2, CH_2	1.55, m	40.8, CH_2	1.54, m	41.6, CH_2	1.47, m
		1.45, m				
4	24.3, CH	1.43, m	24.2, CH	1.47, m	24.9, CH	1.42, m[a]
5	22.4, CH_3	0.89, d (6.5)	22.4, CH_3	0.90, d (5.5)	23.0, CH_3	0.83, d (5.5)
4-Me	22.5, CH_3	0.90, d (6.5)	22.5, CH_3	0.90, d (5.5)	23.1, CH_3	0.84, d (5.5)
NH		7.71, d (7.0)		7.77, brs		7.59, d (8.0)

[a]Resonances overlapped.

Prior to this study, desotamide (1) was subjected to *E. coli* bacterial RNA polymerase inhibitory assays and failed to display any inhibitory activity.[9] In this study, compounds **1**–**4** were evaluated for their antimicrobial activities against *Staphylococcus*

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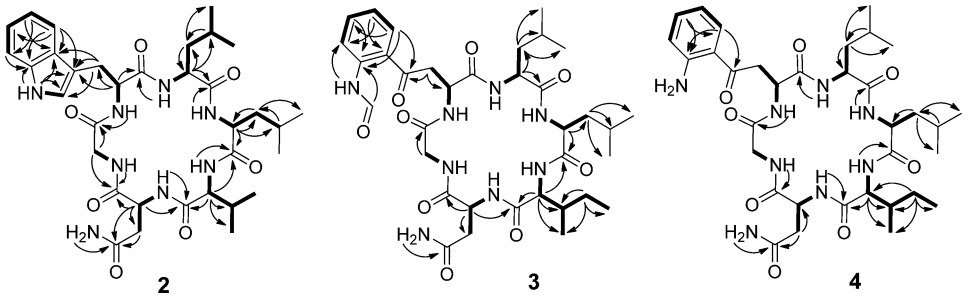

Figure 1. COSY (bold line) and selected HMBC (arrow line) correlations of compounds **2**–**4**.

aureus ATCC 29213, *Klebsiella pneumoniae* ATCC 13883, *Streptococcus pneumoniae* NCTC 7466, methicillin-resistant *Staphylococcus aureus* shhs-A1 (MRSA, a clinical isolate), and methicillin-resistant *S. epidermidis* shhs-E1 (MRSE, a clinical isolate) (Table 2). Notably, both **1** and new compound **2** showed similar antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* NCTC 7466 and MRSE shhs-E1 with MIC values of 16.0, 12.5, 32.0 μg/mL, respectively. In addition, compounds **1**–**4** lacked cytotoxicities (IC_{50} > 100 μM) against four human tumor cell lines SF-268, MCF-7, NCI-H460, and HepG-2, which is good for antibacterial drug development.

Table 2. Antibacterial Activities (MIC, μg/mL) of Compounds 1–4

	1	2	3	4	vancomycin
Staphylococcus aureus ATCC 29213	16.0	16.0	>128.0	>128.0	1.0
Klebsiella pneumoniae ATCC 13883	>128	>128.0	>128.0	>128.0	>128.0
Streptococcus pneumiae NCTC 7466	12.5	12.5	>100	>100	0.37
MRSE (clinical isolate shhs-E1)	32.0	32.0	>128	>128	1.0
MRSA (clinical isolate shhs-A1)	>128	>128	>128	>128	1.0

The emergence of drug-resistant bacteria represents a tremendous threat to global public health.[1] *S. epidermidis* and *S. aureus* have become two of the most prevalent causes of nosocomial infections, whereas *Streptococcus pneumoniae* is a pathogen causing invasive diseases like sepsis, meningitis, and pneumonia.[2] These are just a handful of microbes for which the development of drug resistance has been well-documented and which present a clinically significant challenge. The discovery of antibacterial activities against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* and MRSE for compounds **1** and **2** may provide a new template for antibacterial drug discovery. In a complementary fashion, the antibacterial activities of compounds **1**–**4** reveal the Trp moiety to be essential, thereby highlighting a critical structural element to this advancing antibacterial scaffold. These studies therefore illuminate new opportunities for the use of established compounds in addition to showcasing a new antibacterial drug scaffold on which can be built new leads.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with an MCP-500 polarimeter (Anton Paar). UV spectra were measured with a UV-2600 spectrometer (Shimadzu). IR spectra were obtained using an IRAffinity-1 spectrophotometer (Shimadzu). NMR spectra were acquired with an Avance 500 spectrometer (Bruker) at 500 MHz for the ^1H nucleus and 125 MHz for the ^{13}C nucleus. Low-resolution and high-resolution mass spectrometric data were determined using an amaZon SL ion trap mass spectrometer (Bruker) and MaXis quadrupole-time-of-flight mass spectrometer (Bruker), respectively. Column chromatography (CC) was carried out on silica gel (100–200 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd.). RP HPLC was performed using LC3000 solvent delivery modules equipped with a smartline UV detector 2550 (Knauer) and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm).

Bacterial Materials. Strain SCSIO ZJ46, collected in the South China Sea at E 120°0.250′ and N 20°22.971′, was isolated from a sediment sample at depth 3536 m. It was identified as *Streptomyces scopuliridis* SCSIO ZJ46 on the basis of morphological characteristics[14] and 16S rRNA gene sequence analysis by comparison with other sequences in the GenBank database. The DNA sequence has been deposited in GenBank (accession no. KJ784514). The strain is preserved at the RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences and at the China General Microbiological Culture Collection Center (CGMCC) with the no. CGMCC 7862.

Fermentation and Extraction. The strain *Streptomyces scopuliridis* SCSIO ZJ46 was placed onto modified ISP-4 agar plates and incubated at 28 °C. The mycelium was inoculated into each of the 250 mL Erlenmeyer flasks containing 50 mL of MAM2ab medium (0.5% soybean flour, 0.5% soluble starch, 0.2% yeast extract, 0.2% peptone, 2% glucose, 3% crude sea salt (Guangdong salt industry group copportion, Guangzhou, China), 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.4% NaCl, 0.2% $CaCO_3$, pH 7.2.) and were incubated at 28 °C on rotary shakers (200 rpm). Following fermentation, each of the seed cultures was aseptically transferred to 1 L Erlenmeyer flasks containing 200 mL of MAM2ab medium. Flasks were incubated at 28 °C on rotary shakers (200 rpm) for 8 days. About 8 L of terminal fermentation culture was centrifuged (3600 rpm, 10 min) to generate the supernatant and mycelium. The supernatant was extracted with equal volumes of butanone three times; the extract solutions were combined and then concentrated under reduced pressure to give an extract S (extracted from the supernatant). Similarly, the mycelium cake was repeatedly extracted with 1 L of acetone to get an extract M (extract from the mycelium). The residue of extracts S (1.25 g) and M (61.28 g) were finally combined according to the HPLC-PDA analysis.

Isolation. The residue was subjected to silica gel CC using gradient elution with a $CHCl_3$/MeOH mixture (200/0, 196/4, 190/10, 184/16, 180/20, 160/40, 100/100, 0/200 v/v) to give eight fractions (A1–A8), respectively. Fractions A4–A7 were subjected to silica gel CC with $CHCl_3$/MeOH mixtures (147/3, 144/6, 141/9, 138/12, 135/15, 132/18, 129/21, 126/24 v/v) to afford fractions B1–B8. Fractions B4–B6 were further purified by preparative RP HPLC eluting with a linear gradient from 45% to 68% B (A: H_2O, B: CH_3CN) over the course of 20 min at 260 nm 8.0 mL/min, which gave compounds **1** (278.3 mg) and **2** (25.2 mg) at 17.0 and 14.9 min, respectively. The accumulated minor compounds **3** (4.5 mg) and **4** (3.6 mg) were noted with retention times of 13.8 and 15.3 min and isolated by repetitive RP HPLC under the same conditions used to isolate **1** and **2**.

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Desotamide (1). white amorphous powder; $[\alpha]_{25}^{D}$ −13.2 (*c* 0.36, MeOH) [lit. $[\alpha]_{25}^{D}$ −6.7 (*c* 0.35, MeOH)].[11]

Desotamide B (2). white amorphous powder; $[\alpha]_{25}^{D}$ −11.9 (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.58), 281 (3.68) nm; IR (ATR) ν_{max} 3260, 1632, 1523 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, Table 1; (+)− HRESIMS *m*/*z* 683.3879 $[M + H]^+$ (calcd for $C_{34}H_{51}N_8O_7$, 683.3875) and 705.3699 $[M + Na]^+$ (calcd for $C_{34}H_{50}N_8NaO_7$, 705.3695).

Desotamide C (3). white amorphous powder; $[\alpha]_{25}^{D}$ −32.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.42), 260 (4.03), 324 (3.56) nm; IR (ATR) ν_{max} 3271, 1632, 1524 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, Table 1; (+)-HRESIMS *m*/*z* $[M + H]^+$ calcd for $C_{35}H_{53}N_8O_9$, 729.3930; found, 729.3926; $[M + Na]^+$ calcd for $C_{35}H_{52}N_8NaO_9$, 751.3749; found, 751.3742.

Desotamide D (4). white amorphous powder; $[\alpha]_{25}^{D}$ −24.2 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.29), 257 (3.64), 367 (3.50) nm; IR (ATR) ν_{max} 3260, 1632, 1531 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, Table 1; (+)-HRESIMS *m*/*z* $[M + H]^+$ calcd for $C_{34}H_{53}N_8O_8$, 701.3981; found, 701.3952; $[M + Na]^+$ calcd for $C_{34}H_{52}N_8NaO_8$, 723.3800; found, 723.3764.

Hydrolysis of Compounds 1−4 and HPLC Analysis by Marfey's Method and Chiral-phase HPLC. Compounds **1** (1.50 mg), **2** (1.23 mg), **3** (0.97 mg), **4** (0.68 mg) were each dissolved in 6 N HCl (1 mL), sealed and heated at 110 °C for 18 h. After cooling to room temperature, the hydrolysates were dried under reduced pressure and redissolved into 100 μL of H_2O and divided into two equal parts. One half of each hydrolysate was added to 1 N $NaHCO_3$ (25 μL) and reacted with 50−100 μL of 1% (w/v) 1-fluoro-2, 4-dinitrophenyl-5-L-alaninamide (FDAA) for 1 h at 40 °C. To the mixture was added 1 N HCl (25 μL) to neutralize and terminate the reaction. MeOH was then added to the quenched reaction to afford a total volume of 500 μL; 10 μL of each hydrolysate derivatization reaction was used for HPLC analysis using a Phenomenex ODS column (150 × 4.6 mm, 5 μm) with a gradient from 10% to 70% solvent B (CH_3CN/H_2O/TFA, A: 15/85/0.1, B 90/10/0.1) over the course of 40 min and UV detection at 340 nm at a flow rate of 1 mL/min. Similarly, 10 μL of the standard amino acids in H_2O (10 μM) was added to 1 N $NaHCO_3$ (5 μL) and each mixture was treated with 1% (w/v) FDAA (20 μL) for 1 h at 40 °C. Derivatization reactions were terminated with 1 N HCl (5 μL) and diluted to a total volume of 500 μL with MeOH. Of these standard amino acid derivatization reactions, 10 μL was subjected to HPLC analysis and used as structural standards in the elucidation of structures **1**−**4** (Figures S9a and S9b). For each hydrolysate of **1**−**4**, the half of each reaction not subjected to derivatization was diluted to 200 μL with water, and 10−20 μL of each hydrolysate was used for chiral-phase HPLC analysis using an MCI GEL CRS10W column (Mitsubishi, 50 × 4.6 mm, 3 μm) eluted with 2 mM $CuSO_4$ aqueous solution at a flow rate of 0.5 mL/min and employing UV detection at 254 nm. Amino acid standards were used under these conditions also and served as important control/standards by which structural information about **1**−**4** could be ascertained (Figure S10).[15]

Antibacterial Activities and Cytotoxicity Assay. The antibacterial activities of compounds **1**−**4** were assessed using sequential 2-fold serial dilutions of each agent in MH broth according to the previously reported and a standard methods provided by the Clinical and Laboratory Standards Institute (CLSI).[9,16] Vancomycin was used as a control. The cell growth inhibitory activities of compounds **1**−**4** against human cell lines SF-268, MCF-7, NCI-H460, and HepG-2 were tested using the previously published methods.[15,17]

■ ASSOCIATED CONTENT

Supporting Information

Spectra of ESIMS/MS, 1D and 2D NMR, Marfey's method analyses, and chiral-phase HPLC analysis for compounds **1**-**4** were available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Arias, C. A.; Murray, B. E. *N. Engl. J. Med.* **2009**, *360*, 439−443.

(2) Bogaert, D.; de Groot, R.; Hermans, P. W. M. *Lancet Infect. Dis.* **2004**, *4*, 144−154.

(3) Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. *Clin. Infect. Dis.* **2009**, *48*, 1−12.

(4) Pomilio, A. B.; Battista, M. E.; Vitale, A. A. *Curr. Org. Chem.* **2006**, *10*, 2075−2121.

(5) Dartois, V.; Sanchez-Quesada, J.; Cabezas, E.; Chi, E.; Dubbelde, C.; Dunn, C.; Granja, J.; Gritzen, C.; Weinberger, D.; Ghadiri, M. R.; Parr, T. R. *Antimicrob. Agents Chemother.* **2005**, *49*, 3302−3310.

(6) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311−335.

(7) Laport, M. S.; Santos, O. C. S.; Muricy, G. *Curr. Pharm. Biotechnol.* **2009**, *10*, 86−105.

(8) Mayer, A. M. S.; Rodriguez, A. D.; Taglialatela-Scafati, O.; Fusetani, N. *Mar. Drugs* **2013**, *11*, 2510−2573.

(9) Zhou, X.; Huang, H.; Chen, Y.; Tan, J.; Song, Y.; Zou, J.; Tian, X.; Hua, Y.; Ju, J. *J. Nat. Prod.* **2012**, *75*, 2251−2255.

(10) Zhou, X.; Huang, H.; Li, J.; Song, Y.; Jiang, R.; Liu, J.; Zhang, S.; Hua, Y.; Ju, J. *Tetrahedron* **2014**, DOI: 10.1016/j.tet.2014.02.007.

(11) Miao, S. C.; Anstee, M. R.; LaMarco, K.; Matthew, J.; Huang, L. H. T.; Brasseur, M. M. *J. Nat. Prod.* **1997**, *60*, 858−861.

(12) Huang, H.; Yao, Y.; He, Z.; Yang, T.; Ma, J.; Tian, X.; Li, Y.; Huang, C.; Chen, X.; Li, W.; Zhang, S.; Zhang, C.; Ju, J. *J. Nat. Prod.* **2011**, *74*, 2122−2127.

(13) Ma, J.; Zuo, D.; Song, Y.; Huang, H.; Yao, Y.; Li, W.; Zhang, C.; Ju, J. *ChemBioChem* **2012**, *13*, 547−552.

(14) Kämpfer, P. In *Bergey's Manual of Systematic Bacteriology*; Goodfellow, M. et al., Eds; Springer: New York, 2012; Vol. *5*, pp 1446−1804.

(15) Chen, Z.; Song, Y.; Chen, Y.; Huang, H.; Zhang, W.; Ju, J. *J. Nat. Prod.* **2012**, *75*, 1215−1219.

(16) CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 8th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, 2009.

(17) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.