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Verrucamides A−D, Antibacterial Cyclopeptides from *Myrothecium verrucaria*

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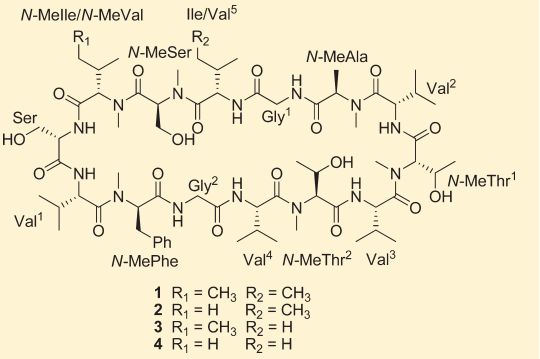
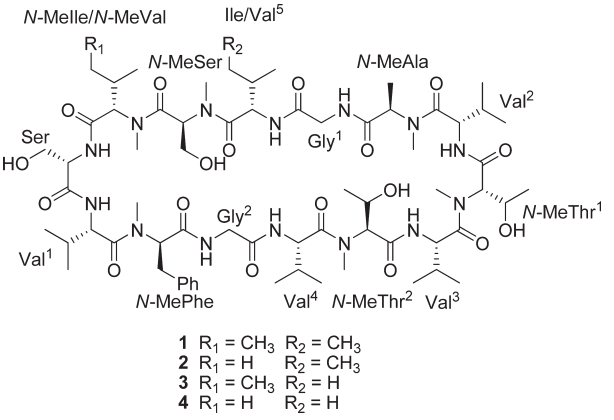
Supporting Information

ABSTRACT: Four new cyclic tetradecapeptides, verrucamides A−D (**1**−**4**), have been isolated from the solid-substrate fermentation culture of the ascomycete fungus *Myrothecium verrucaria*. The structures of these compounds, each featuring six *N*-methylated amino acid residues, were elucidated primarily by NMR and MS methods. The absolute configurations of **1**−**4** were assigned by application of Marfey's method on their acid hydrolysates. Compounds **1**−**4** showed antimicrobial activity against the Gram-positive bacterium *Staphylococcus aureus*.



Fungi are well-known producers of bioactive cyclopeptides and cyclodepsipeptides.[1−4] Noteworthy examples include cyclosporin A, an immunosuppressant isolated from *Tolypocladium inflatum*;[5] the destruxins, anti-insectan metabolites isolated from the insect pathogenic fungus *Metarrhizium anisopliae*;[6] omphalotin, a potent nematicidal agent from *Omphalotus olearius*;[7] and the echinocandins, antifungal lipopeptides isolated from *Glarea lozoyensis*, *Coleophoma empetri*, and *Aspergillus nidulans* var. *echinulatus*,[8] from which the antifungal drug Cancidas has been successfully developed.[9]

Myrothecium verrucaria is a fungal pathogen that attacks important crop plants and weeds,[10−12] with some isolates reported as potential biocontrol agents against weeds.[12−16] Chemical studies of *M. verrucaria* have afforded a variety of bioactive secondary metabolites, such as trichothecene sesquiterpenoids,[2] antimicrobial diterpenoids,[17−20] and a cytostatic pyrrole.[21] During an ongoing search for new antimicrobial natural products, the fungus *M. verrucaria* (XZ04-18-2) isolated from a soil sample that was collected in Linzhi, Tibet, People's Republic of China, was subjected to chemical investigation. An EtOAc extract of its solid-substrate fermentation culture showed significant antibacterial activity against *Staphylococcus aureus* Col (CGMCC 1.2465). Bioassay-guided fractionation of the extract led to the isolation of four new cyclic tetradecapeptides, which we named verrucamides A−D (**1**−**4**). Details of the isolation, structure elucidation, and antibacterial activity of these compounds are reported herein.



RESULTS AND DISCUSSION

Verrucamide A (**1**) was obtained as a white powder with a molecular formula of $C_{68}H_{114}N_{14}O_{18}$ (19 degrees of unsaturation), determined by HRESIMS. Analysis of its ^{1}H and ^{13}C NMR (Table 1) and HSQC data revealed eight amide *N*-H protons (δ_H 6.85−8.38), 21 methyl groups including six *N*-methyls, seven methylenes (two of which are oxygenated), 20 methines (14 of which are heteroatom-bonded), one monosubstituted phenyl ring, and 14 carboxylic carbons (δ_C

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Table 1. NMR Spectroscopic Data of Verrucamide A (1) in DMSO-d_6

unit	pos.	δ_C,[a] mult.	δ_H[b] (J in Hz)	HMBC (H → C)	ROESY
N-MePhe	CO	169.6, qC			
	α	66.5, CH	4.04, dd (11, 4.0)	CO, CO (Val[1])	*N*-Me, 2/6
	β	34.0, CH_2	3.02, dd (14, 11)	α, 1, 2/6	2/6
			3.38, m[c]	α, 1, 2/6	
	1	138.9, qC			
	2/6	129.3, CH	7.21, m	*β*, 4, 2/6	α, *β*
	3/5	128.3, CH	7.27, m	1, 3/5	
	4	126.3, CH	7.21, m	2/6	
	N-Me	39.4, CH_3	2.74, s	α, CO (Val[1])	α, α (Val[1])
Val[1]	CO	172.1, qC			
	α	53.3, CH	4.48, dd (9.0, 8.0)	CO, *β*, *γ*, *γ*′, CO (Ser)	*N*-Me (*N*-MePhe)
	β	30.5, CH	1.90, m	α, *γ*, *γ*′	
	γ	19.6, CH_3	0.80, m[c]	α, *β*, *γ*′	
	γ′	17.7, CH_3	0.82, m[c]	α, *β*, *γ*	
	*N*H		8.38, d (9.0)	α, CO (Ser)	α (Ser)
Ser	CO	169.8, qC			
	α	55.1, CH	4.55, dt (6.5, 6.5)	CO, *β*	*N*H (Val[1])
	β	60.9, CH_2	3.45, m[c]		
			3.40, m[c]		
	*N*H		7.94, d (6.5)	α, CO (*N*-MeIle)	α (*N*-MeIle)
N-MeIle	CO	170.9, qC			
	α	58.5, CH	4.80, m[c]	CO, *β*, *γ*, *N*-Me,	
CO (*N*-MeSer)	*N*-Me, *N*H (Ser)				
	β	34.0, CH	1.91, m	*β*-Me	
	β-Me	15.1, CH_3	0.93, m[c]	α, *β*, *γ*	
	γ	25.6, CH_2	1.22, m		
			0.92, m[c]		
	δ	11.2, CH_3	0.75, m[c]	*β*, *γ*	
	N-Me	31.9, CH_3	3.12, s	α, CO (*N*-MeSer)	α
N-MeSer	CO	169.7, qC			
	α	53.7, CH	6.35, dd (8.5, 4.5)	CO, *β*, *N*-Me	
	β	58.0, CH_2	3.58, m	α	*N*-Me
			3.32, m[c]		
	N-Me	29.8, CH_3	2.89, s	α, CO (Ile)	*β*, *β* (Ile), *β*-Me (Ile)
Ile	CO	171.9, qC			
	α	52.5, CH	4.87, m[c]	CO, *β*, *γ*, CO (Gly[1])	
	β	37.1, CH	1.82, m	α	*N*-Me (*N*-MeSer)
	β-Me	15.2, CH_3	0.86, m[c]	α, *β*, *γ*	*N*-Me (*N*-MeSer)
	γ	23.1, CH_2	1.55, m	α, *β*, *β*-Me, *δ*	
			1.20, m	α, *β*, *β*-Me, *δ*	
	δ	10.8, CH_3	0.75, m[c]	*β*, *γ*	
	*N*H		7.81, d (9.0)	CO (Gly[1])	
Gly[1]	CO	168.1, qC			
	α	43.1, CH_2	3.73, dd (17, 4.5)	CO	
			3.16, m[c]	CO	
	*N*H		7.95, t (5.0, 4.5)	α	α (*N*-MeAla)
N-MeAla	CO	170.3, qC			
	α	59.8, CH	3.67, q (6.5)	CO, *β*, *N*-Me, CO (Val[2])	*N*-Me, *N*H (Gly[1])
	β	12.6, CH_3	1.25, d (6.5)	CO, α	
	N-Me	37.6, CH_3	3.20, s	α, CO (Val[2])	α, α (Val[2])
Val[2]	CO	170.9, qC			
	α	51.9, CH	4.72, m[c]	CO, *β*, *γ*, *γ*′	*N*-Me (*N*-MeAla)
	β	31.5, CH	1.82, m	α, *γ*, *γ*′	
	γ	16.6, CH_3	0.80, m[c]	α, *β*, *γ*′	
	γ′	17.1, CH_3	0.76, m[c]	α, *β*, *γ*	
	*N*H		8.29, d (9.0)	α, CO (*N*-MeThr[1])	α (*N*-MeThr[1])

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Table 1. Continued

unit	pos.	δ_C,[a] mult.	δ_H[b] (J in Hz)	HMBC (H → C)	ROESY
N-MeThr[1]	CO	169.3, qC			
	α	61.2, CH	5.41, d (9.5)	CO, *β*, *γ*, *N*-Me,	
CO (Val[3])	*γ*, *N*H (Val[2])				
	β	63.1, CH	3.89, m[c]	α	
	γ	19.3, CH_3	0.94, d (6.5)	α, *β*	α
	N-Me	30.5, CH_3	3.14, s	α, CO (Val[3])	α (Val[3])
Val[3]	CO	171.6, qC			
	α	52.7, CH	4.78, m[c]	CO, *β*, *γ*, *γ*′	*N*-Me (*N*-MeThr[1])
	β	30.0, CH	1.90, m	α, *γ*, *γ*′	
	γ	18.8, CH_3	0.90, m[c]	α, *β*, *γ*′	
	γ′	19.1, CH_3	0.82, m[c]	α, *β*, *γ*	
	*N*H		6.85, d (9.5)	CO (*N*-MeThr[2])	α (*N*-MeThr[2])
N-MeThr[2]	CO	169.1, qC			
	α	62.8, CH	4.72, m[c]	CO, *β*, *γ*, CO (Val[4])	*N*H (Val[3])
	β	63.2, CH	3.89, m[c]		
	γ	20.9, CH_3	0.84, m[c]	α, *β*	
	N-Me	31.5, CH_3	3.23, s	α, CO (Val[4])	α (Val[4])
Val[4]	CO	173.1, qC			
	α	53.1, CH	4.74, m[c]	CO, *β*, *γ*, *γ*′, CO (Gly[2])	*N*-Me (*N*-MeThr[2])
	β	31.0, CH	2.12, m	α, *γ*, *γ*′	
	γ	18.3, CH_3	0.88, m[c]	α, *γ*′	
	γ′	18.6, CH_3	0.80, m[c]	α, *β*, *γ*	
	*N*H		7.48, d (9.0)	CO (Gly[2])	
Gly[2]	CO	168.3, qC			
	α	42.8, CH_2	3.89, m[c]	CO	
			3.24, m[c]	CO, CO (*N*-MePhe)	
	*N*H		7.66, t (6.0, 5.0)	α, CO (*N*-MePhe)	

[a] Recorded at 150 MHz. [b] Recorded at 500 MHz. [c] Mutiplicity due to overlapping.

168.1−173.1). These data accounted for 18 of the 19 unsaturations and were indicative of a peptidic structure, suggesting that **1** is a monocyclic peptide. Interpretation of ^{1}H−^{1}H COSY, TOCSY, HSQC, and HMBC data of **1** established the amino acid residues isoleucine (Ile), serine (Ser), glycine (Gly; 2×), and valine (Val; 4×), together with five *N*-methylated units, *N*-methylalanine (*N*-MeAla), *N*-methylisoleucine (*N*-MeIle), *N*-methylphenylalanine (*N*-MePhe), *N*-methylserine (*N*-MeSer), and *N*-methylthreonine (*N*-MeThr; 2×).

The amino acid sequence of verrucamide A (**1**) was proposed using the HMBC correlations (Figure 1) from relevant *N*-H or *N*-CH_3 protons to neighboring carboxylic carbons (except for that from Gly[1] *N*H to *N*-MeAla CO, which was not observed in the HMBC spectrum). Specifically, the Val[4] *N*-H (δ_H 7.48) signal showed an HMBC correlation to the amide carbon signal of Gly[2] (δ_C 168.3), indicating that it is acylated by Gly[2]. The *N*-H of Gly[2] (δ_H 7.66), the *N*-CH_3 of *N*-MePhe (δ_H 2.74), the *N*-H of Val[1] (δ_H 8.38), and the *N*-H of Ser (δ_H 7.94) signals were individually correlated to the carboxylic carbon signals of *N*-MePhe (δ_C 169.6), Val[1] (δ_C 172.1), Ser (δ_C 169.8), and *N*-MeIle (δ_C 170.9) residues, establishing a partial sequence of *N*-MeIle−Ser−Val[1]−*N*-MePhe−Gly[2]−Val[4] for **1**. The remaining partial sequences, Gly[1]−Ile−*N*-MeSer and *N*-MeThr[2]−Val[3]−*N*-MeThr[1]−Val[2]−*N*-MeAla, were also established in a similar fashion. In the HMBC spectrum, the *N*-CH_3 signal of *N*-MeIle (δ_H 3.12) showed a cross-peak to the amide carbon signal of *N*-MeSer (δ_C 169.7), and the *N*-CH_3 signal of *N*-MeThr[2] (δ_H 3.23) showed a cross-peak to the amide carbon signal of Val[4] (δ_C 173.1). These correlations enabled assignment of the linear sequence for **1**. Although no further sequence-relevant HMBC correlations were observed from either the *N*-H or the α-protons of Gly[1] to the carboxylic carbon of *N*-MeAla (δ_C 170.3), Gly[1] has to be acylated by *N*-MeAla to complete a cyclic structure for **1** on the basis of unsaturation requirement. Such a linkage was supported by a ROESY correlation between the *N*-H proton of Gly[1] (δ_H 7.95) and the α-proton of *N*-MeAla (δ_H 3.67) (Figure 1).

Marfey's method[22] was applied to assign the absolute configurations of the amino acid residues resulting from acid hydrolysis of verrucamide A (**1**). The 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) derivatives of the acid hydrolysate of **1** and the authentic D- and L-amino acids were subjected to HPLC-MS analysis. The absolute configurations of all amino acid residues in **1** except for *N*-MeSer were established by comparison of their HPLC retention times and molecular weights with those of corresponding authentic D- and L-standards. Upon analysis, the amino acid residues Ser, Ile, *N*-MeIle, *N*-MeThr, and Val were determined to have the L-configuration, whereas the *N*-MeAla and *N*-MePhe units were deduced to have the D-configuration (Table S1; Supporting Information). However, attempts to determine the absolute configuration of the *N*-MeSer residue using Marfey's method were unsuccessful. Considering ROESY correlations between the *N*-CH_3 signal of *N*-MeSer (δ_H 2.89) and

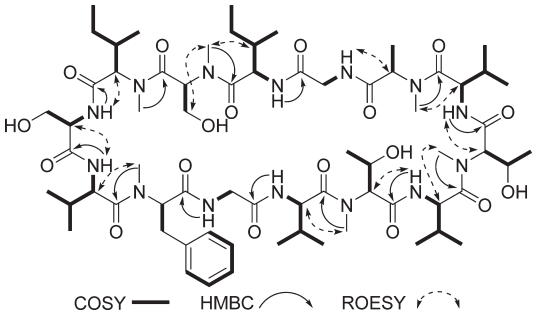

Figure 1. Selected 2D NMR correlations for **1**.

Table 2. Antibacterial Activity of 1−4 against *S. aureus*

compound	IC_{50} (μg/mL)	MIC (μg/mL)
1	3.59 ± 0.08	10.0
2	5.31 ± 0.04	20.0
3	4.45 ± 0.28	10.0
4	9.09 ± 0.19	40.0
ampicillin	0.09 ± 0.01	0.625

the β-proton signals of *N*-MeSer (δ_H 3.58) and Ile (δ_H 1.82), the absolute configuration of the *N*-MeSer residue was tentatively assigned as L. Therefore, the structure of verrucamide A was established as shown.

The molecular formula of verrucamide B (**2**) was determined to be $C_{67}H_{112}N_{14}O_{18}$ (19 degrees of unsaturation) by HRESIMS, 14 mass units less than that of **1**. Analysis of its ^1H and ^{13}C NMR spectroscopic data (Table S2; Supporting Information) revealed nearly identical structural features to those found in **1**, except that the *N*-MeIle residue was replaced by a *N*-MeVal unit, which was confirmed by relevant $^1H-^1H$ COSY and HMBC correlations. The absolute configurations of the Ser, Ile, *N*-MeSer, *N*-MeThr, *N*-MeVal, and Val residues in **2** were all assigned as L, and those of the *N*-MePhe and *N*-MeAla were assigned as D (Table S1; Supporting Information) using Marfey's method.

Verrucamide C (**3**) was obtained as a white powder. HRESIMS data for **3** gave the same molecular formula $C_{67}H_{112}N_{14}O_{18}$ as **2**. Analysis of its NMR data (Table S3; Supporting Information) indicated that **3** has the same amino acid sequence as **1**, but differs in having a Val^5 unit instead of an Ile residue, which was supported by $^1H-^1H$ COSY and HMBC correlations. The absolute configuration of the Val^5 unit in **3** was assigned as L, while those of all other amino acid residues were assigned the same configurations as in **1** using Marfey's method (Table S1; Supporting Information).

Verrucamide D (**4**) gave a pseudomolecular ion $[M + Na]^+$ peak, consistent with a molecular formula of $C_{66}H_{110}N_{14}O_{18}$ (19 degrees of unsaturation). This formula is 28 mass units less than that of **1**, suggesting that **4** is a homologue of **1** with two less methylenes. Interpretation of the 1D and 2D NMR data of **4** (Table S4; Supporting Information) indicated that the Ile and *N*-MeIle units in **1** are replaced by Val^5 and *N*-MeVal, respectively. The Val^5 and *N*-MeVal residues in **4** were both determined to have the L-configuration (Table S1; Supporting Information).

Compounds **1**−**4** were tested for antibacterial activity against the Gram-positive bacterium *S. aureus* Col (CGMCC 1.2465) (Table 2), showing IC_{50} and MIC values of 3.59 to 9.09 and 10.0 to 40.0 μg/mL, respectively, while the positive control ampicillin showed IC_{50} and MIC values of 0.09 and 0.625 μg/mL, respectively.

Although *N*-methylated cyclopeptides and cyclodepsipeptides have been encountered frequently as fungal secondary metabolites,[23−27] verrucamides A−D (**1**−**4**) differ markedly from the known cyclopeptides by virtue of the presence of two D-configured amino acid residues, *N*-MeAla and *N*-MePhe, as well as the relatively rare *N*-MeSer unit, which has been previously reported from *Streptomyces* sp. Tü 6075.[28] In addition, compounds **1**−**4** feature six *N*-methylated amino acid units, which is relatively uncommon for the cyclopeptides isolated from terrestrial fungi.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury 500 and 600 spectrometers using solvent signals (DMSO-d_6; δ_H 2.49/δ_C 39.5) as references. The HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using a Bruker APEX III 7.0 T spectrometer. HPLC-MS analyses were performed on an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument equipped with a 1200 HPLC system and an electrospray ionization (ESI) source. The fragmentor and capillary voltages were kept at 125 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 10 psi, respectively. All MS experiments were performed in positive ion mode. Full-scan spectra were acquired over a scan range of m/z 100−1000 at 1.03 spectra/s.

Fungal Material. The culture of *M. verrucaria* was isolated from a soil sample collected in Linzhi, Tibet, in July 2004. The isolate was identified by one of the authors (X.L.) based on morphology and sequence (Genbank Accession No. JF812340) analysis of the ITS region of the rDNA and assigned the accession number XZ04-18-2 in X.L's culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions, 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5, and the flasks were sterilized by autoclave. Three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Fermentation was carried out in 10 Fernbach flasks (500 mL), each containing 150 mL of liquid media (2% maltose, 6% dextrin, 0.7% peptone, 0.75% cotton-seed meal, 0.25% $MgSO_4 \cdot 7H_2O$, 0.25% $CaCO_3$, 0.1% $FeSO_4 \cdot 7H_2O$, and 0.001% $ZnSO_4 \cdot 7H_2O$; final pH 6.0) and 30 g of vermiculite. Each flask was inoculated with 15 mL of the seed culture and incubated at 25 °C under static conditions for 40 days.

Extraction and Isolation. The fermented material (1.5 L) was freeze-dried and extracted with EtOAc (4 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (1.9 g), which was fractionated by silica gel VLC using petroleum

ether−EtOAc−MeOH gradient elution. The fraction (600 mg) eluted with 15−100% MeOH was chromatographed on a silica gel column (2.5 × 30 cm) eluting with CH_2Cl_2−MeOH. The fraction (100 mg) eluted with 5:1 CH_2Cl_2−MeOH was separated by Sephadex LH-20 column chromatography (CC) eluting with 1:1 $CHCl_3$−MeOH. The resulting subfractions were combined and further purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 55−80% MeCN in H_2O for 45 min; 2 mL/min) to afford **1** (6.0 mg, t_R 34.94 min), **2** (6.0 mg, t_R 29.00 min), **3** (3.5 mg, t_R 27.35 min), and **4** (3.5 mg, t_R 22.55 min).

*Verrucamide A (**1**):* white powder; $[\alpha]^{25}_D$ −254 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.99) nm; IR (neat) ν_{max} 3301 (br), 2965, 2934, 2876, 1627, 1531, 1411, 1342, 1278, 1099 cm^{-1}; ^1H and ^{13}C NMR, HMBC, and ROESY data see Table 1; HRESIMS *m/z* 1437.8351 (calcd for $C_{68}H_{114}N_{14}O_{18}Na$, 1437.8333).

*Verrucamide B (**2**):* white powder; $[\alpha]^{25}_D$ −168 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.97) nm ; IR (neat) ν_{max} 3298 (br), 2965, 2934, 2876, 1624, 1529, 1410, 1341, 1282, 1096 cm^{-1}; ^1H and ^{13}C NMR, HMBC, and ROESY data see Table S2 (Supporting Information); HRESIMS *m/z* 1423.8141 (calcd for $C_{67}H_{112}N_{14}O_{18}Na$, 1423.8171).

*Verrucamide C (**3**):* white powder; $[\alpha]^{25}_D$ −152 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.95) nm; IR (neat) ν_{max} 3296 (br), 3063, 2965, 2934, 2876, 1622, 1536, 1413, 1342, 1218, 1100 cm^{-1}; ^1H and ^{13}C NMR, HMBC, and ROESY data see Table S3 (Supporting Information); HRESIMS *m/z* 1423.8194 (calcd for $C_{67}H_{112}N_{14}O_{18}Na$, 1423.8171).

*Verrucamide D (**4**):* white powder; $[\alpha]^{25}_D$ −222 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.94) nm; IR (neat) ν_{max} 3298 (br), 3063, 2965, 2934, 2875, 1628, 1534, 1411, 1342, 1218, 1099 cm^{-1}; ^1H and ^{13}C NMR, HMBC, and ROESY data see Table S4 (Supporting Information); HRESIMS *m/z* 1409.7985 (calcd for $C_{66}H_{110}N_{14}O_{18}Na$, 1409.8015).

Absolute Configuration of Compounds 1−4 (ref 22). Separate solutions of compounds **1**−**4** (0.5 mg each) in 6 N HCl (1.0 mL) were heated at 110 °C for 24 h. Upon removal of excess HCl under vacuum, the hydrolysates were placed in a 1 mL reaction vial and treated with a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA; 150 μL) in acetone, followed by 1.0 N $NaHCO_3$ (40 μL). The reaction mixtures were heated at 45 °C for 1.5 h, cooled to room temperature, and acidified with 2.0 N HCl (20 μL). Similarly, the standard L- and D-amino acids were derivatized separately. The derivatives of the hydrolysates and the standard amino acids were subjected to HPLC-ESIMS analysis (Kromasil C_{18} column; 5 μm, 4.6 × 250 mm; 1.0 mL/min) at 35 °C using the following gradient program: solvent A, H_2O (0.1% HCOOH); solvent B, MeCN; linear gradient, 15−45% of B in A over 50 min with UV detection at 340 nm. The retention times and ESIMS data for FDAA derivatives of the hydrolysates and the standard amino acids are summarized in Table S1 (Supporting Information).

Antibacterial Assay (ref 29). The antibacterial assay was conducted in triplicate following the National Center for Clinical Laboratory Standards (NCCLS) recommendation.[30] The bacterial strain, *Staphylococcus aureus* Col (CGMCC 1.2465), was grown on Mueller-Hinton broth (MHB). The targeted microbe (3−4 colonies) was cultured in broth (37 °C for 24 h), and the final suspension of bacteria (in MHB medium) was 10^6 cells/mL. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test organism was added to each well, achieving a final volume of 200 μL (ampicillin was used as the positive control). After incubation at 37 °C for 24 h, the absorbance at 595 nm was measured with a microplate reader (TECAN). The inhibition was calculated and plotted versus test concentrations to afford the IC_{50}, whereas the MIC was defined as the lowest concentration that completely inhibited the growth of the test organism.[31]

■ ASSOCIATED CONTENT

Supporting Information. NMR data of **2**−**4**, HPLC-ESIMS data for FDAA derivatives of the acid hydrolysates of **1**−**4** and the standard amino acids, and ^1H and ^{13}C NMR spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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