Tauramamide, a Lipopeptide Antibiotic Produced in Culture by *Brevibacillus laterosporus* Isolated from a Marine Habitat: Structure Elucidation and Synthesis

Kelsey Desjardine,[†] Alban Pereira,[†] Helen Wright,[†] Teatulohi Matainaho,[‡] Michael Kelly,[§] and Raymond J. Andersen*[,†]

Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada V6T 1Z1, Discipline of Pharmacology, University of Papua New Guinea, N.C.D., Papua New Guinea, and Ocean Pharmaceuticals, Inc., 4156 H Street Road, Blaine, Washington 98230

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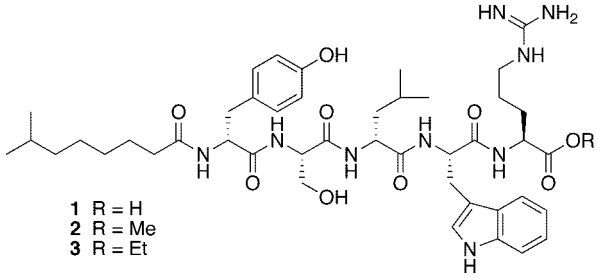
Tauramamide (**1**), a new lipopeptide antibiotic, is produced by cultures of the marine bacterial isolate *Brevibacillus laterosporus* PNG276 obtained from Papua New Guinea. Tauramamide was isolated as its methyl and ethyl esters **2** and **3**, whose structures were elucidated by analysis of NMR, MS, and chemical degradation data. A total synthesis of tauramamide (**1**) and tauramamide ethyl ester (**3**) confirmed the structure proposed from spectroscopic analysis and provided the natural product for antimicrobial testing. Tauramamide (**1**) and ethyl ester **3** show potent and relatively selective inhibition of pathogenic *Enterococcus* sp.

Microorganisms isolated from marine habitats are proving to be an extremely rich source of novel bioactive secondary metabolites.[1] As part of an ongoing program aimed at discovering new antibiotics,[2] we have been exploring crude extracts obtained from laboratory cultures of a marine isolate of *Brevibacillus laterosporus* (PNG-276). To date, we have reported three structurally unrelated families of antibiotics, the loloatins,[3] the bogorols,[4] and the basiliskamides,[5] that are produced by PNG-276. Several fractions from the chromatographic separation of the PNG-276 cell extract, which did not contain members of the known families, showed strong antibacterial activity. Investigation of one of these sets of fractions has resulted in the identification of tauramamide (**1**), a new lipopeptide antibiotic.[6] The details of the isolation and structure elucidation of tauramamide methyl ester (**2**), the total synthesis of tauramamide (**1**), and the antibacterial activities of the free acid **1** and its ethyl ester **3** are presented below.



Results and Discussion

The marine bacterial isolate PNG-276, identified as *Brevibacillus laterosporus* by the analysis of 16S RNA, was obtained from tissues of an unidentified tube worm collected off the coast of Loloata Island, Papua New Guinea. Crude MeOH extracts of *B. laterosporus* cells harvested from cultures grown as lawns on solid agar showed broad-spectrum antibiotic activity against the human pathogens MRSA, VRE, *Mycobacterium tuberculosis*, *Candida albicans*, and *Escherichia coli*. Bioassay-guided fractionation of the cell extracts employing sequential application of Sephadex LH20 chromatography, reversed-phase flash chromatography, and reversed-phase HPLC led to the isolation of pure tauramamide methyl ester (**2**) as a pale yellow oil (7.4 mg, 0.02% dry cell weight).

Methyl ester **2** gave a $[M + H]^+$ ion at *m/z* 878.5181 in the HRESI-TOFMS, consistent with a molecular formula of $C_{45}H_{67}N_9O_9$ (calcd for $C_{45}H_{68}N_9O_9$, 878.5140), requiring 17 sites of unsaturation. Preliminary examination of the ^1H and ^{13}C NMR data recorded for **2** (Supporting Information) revealed that the molecule was a peptide. Detailed analysis of the COSY, HSQC, HMBC, and TOCSY data identified arginine, tryptophan, leucine, tyrosine, and serine residues (Table 1). Five aliphatic methylene resonances between δ 1.07 and 1.36, a methine resonance at δ 1.44, a pair of isochronous methyl doublets at δ 0.83, and a methylene resonance at δ 2.01 in the ^1H NMR spectrum were tentatively assigned to a 7-methyloctanoyl fragment on the basis of COSY, HSQC, and HMBC correlations (Table 1). A methyl resonance at δ 3.62, which showed a HMBC correlation to a carbon resonance at δ 172.1, was assigned to a methyl ester.

The amino acid sequence of tauramamide methyl ester (**2**) was determined by analysis of ROESY data. ROESY correlations observed between the Arg NH (δ 8.39) and the Trp αH (δ 4.53), between the Trp NH (δ 8.14) and the Leu αH (δ 4.20), between the Leu NH (δ 7.75) and the Ser αH (δ 4.22), between the Ser NH (δ 8.03) and the Tyr αH (δ 4.41), and between the Tyr NH (δ 7.96) and the C-2 methylene protons of the 7-methyloctanoyl residue (δ 4.22) demonstrated that the Tyr residue was the N-terminus of the pentapeptide Tyr-Ser-Leu-Trp-Arg, and it was N-acylated with a 7-methyloctanoyl fragment. The methyl ester functionality indicated by the NMR data had to be on the C-terminal Arg residue, leading to the proposed linear lipopeptide structure **2** for tauramamide methyl ester, which accounted for all of the atoms and the 17 sites of unsaturation indicated by the HRMS analysis. Hydrolysis of **2** with aqueous HCl followed by Marfey's analysis[7] of the amino acids revealed that Tyr and Leu had the D configuration, while Arg, Trp, and Ser were L as shown.

It was possible that the methyl ester in **2** was an artifact of the isolation procedure because MeOH had been used to extract the *B. laterosporus* cells. In order to determine if the methyl ester was natural or an isolation artifact, a second batch of *B. laterosporus* cells was extracted with EtOH, and MeOH was not used at any point in the purification. In this case, tauramamide ethyl ester **3** was isolated from the cell extract, and there was no detectable trace of the methyl ester **2**, demonstrating that the free carboxylic acid **1** must be the natural product. All attempts to convert the small amounts of the ester **2** obtained from the *B. laterosporus* cultures to the natural product **1** failed to generate sufficient amounts of

* To whom correspondence should be addressed. Tel: 604 822 4511. Fax: 604 822 6091. E-mail: randersn@interchange.ubc.ca.
[†] Chemistry and EOS, UBC.
[‡] UPNG.
[§] Ocean Pharmaceuticals.

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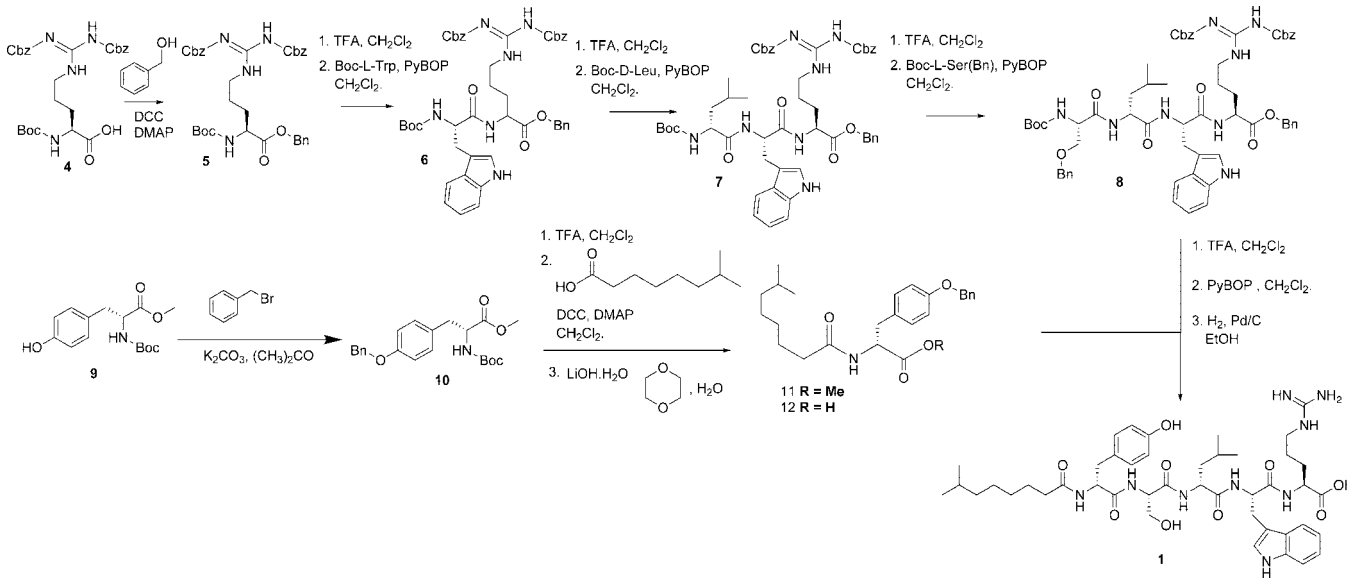
Table 1. NMR Data for Tauramamide Methyl Ester (**2**), Recorded at 500 MHz (^{1}H) and 100 MHz (^{13}C) in DMSO-d_6

	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
OMe			Ser		
	51.8	3.62 s, 3H	1		
Arg			2	55.1	4.22 m
1	172.1		3	61.7	H2 3.47 m
					H2′ 3.52 m
2	51.3	4.29 m	3-OH		4.83 bs
3	27.5	H2 1.67 m	NH		8.03 bs
		H2′ 1.77 m			
4	25.0	1.52 m, 2H	Tyr		
5	40.6	3.11 m, 2H	1		
6	156.7		2	54.5	4.41 bs
2-NH		8.39 bd (7.0)	3	36.5	H2 2.64 m
					H2′ 2.85 m
5-NH		7.50 bs	1′	127.8	
Trp			2′, 6′	130.0	7.02 d (7.8), 2H
1			3′, 5′	114.7	6.62 d (7.8), 2H
2	53.1	4.53 bs	4′	155.7	
3	27.8	H2 2.89 m	4′-OH		9.13 s
		H2′ 3.15 m			
3′	109.8		NH		7.96 bs
3a′	127.1		methyloctanoyl		
4′	118.4	7.60 d (7.7)	1		
5′	118.0	6.96 dd (7.7, 7.2)	2	35.1	2.01 m, 2H
6′	120.7	7.04 bd (7.2)	3	25.1	1.36 m, 2H
7′	111.1	7.30 d (7.8)	4	28.7	1.07 m, 2H
7a′	136.0		5	26.5	1.14 m, 2H
2′	123.9	7.11 bs	6	38.0	1.07 m, 2H
2-NH		8.14 bs	7	27.0	1.44 bm (6.7)
1′-NH		10.76 bs	8	22.5	0.83 d (6.7), 3H
Leu			8′	22.5	0.83 d (6.7), 3H
1					
2	51.7	4.20 m			
3	40.5	1.08 m, 2H			
4	23.7	1.25			
5	22.8	0.71 d (6.3), 3H			
5′	21.5	0.68 d (6.3), 3H			
NH		7.75 bs			

Scheme 1. Synthesis of Tauramamide (**1**)



the natural product for biological testing. Therefore, we undertook a total synthesis of tauramamide (**1**) in order to confirm the proposed structure and to generate sufficient quantities of compound for antimicrobial evaluation.

The synthesis of tauramamide (**1**) started with the commercially available N-protected arginine derivative **4**, which was converted to its benzyl ester **5** by reaction with benzyl alcohol, DCC, and DMAP (Scheme 1). Deprotection of the α-amino nitrogen by treatment of **5** with TFA, followed by three cycles of standard PyBOP-activated peptide coupling with Boc-L-Trp, Boc-D-Leu, and Boc-L-Ser(Bn) in sequence, generated the protected tetrapeptide **8**. The phenolic hydroxyl in the Tyr methyl ester **9** was protected by treatment with benzyl bromide and potassium carbonate in acetone to give **10**. Exposure of the amino group in **10** by reaction with TFA, followed by DCC-mediated amide formation with 7-methyloctanoic acid, and subsequent LiOH-catalyzed ester hydrolysis gave acid **12**. Treatment of the protected tetrapeptide **8** with TFA followed by PyBOP-mediated amide coupling with the acid **12** gave

Table 2. Antimicrobial Activity (MICs in μg/mL) of Synthetic Tauramamide (**1**) and Tauramamide Ethyl Ester (**3**)

bioasay	pathogen	tauramamide (**1**)	tauramamide ethyl ester (**3**)
Alamar Blue	MRSA	ppt[a]	25
	C. albicans	ppt[a]	200
	Enterococcus sp.	0.1	0.1
agar inclusion	MRSA	200	9.4
	C. albicans	50	75

[a] The compound precipitated from solution at higher concentrations and was not active at lower concentrations where it was soluble.

protected tauramamide. Hydrogenolysis using Pd/C in EtOH removed the Bn and Cbz protecting groups to liberate tauramamide (**1**), which was purified by reversed-phase HPLC to give a pure sample. Esterification of **1** with EtOH and HCl gave ethyl ester **3**, which was identical by HPLC, MS, and NMR comparison with the material extracted from *B. laterosporus* cells with EtOH, confirming the structure proposed for the natural product **1**.

Table 2 shows the antimicrobial activity of tauramamide (**1**) and tauramamide ethyl ester (**3**). Both compounds show potent (MICs 0.1 μg/mL) and relatively selective activity against the important Gram-positive human pathogen *Enterococcus* sp. The ethyl ester **3** shows weaker activity against MRSA, but neither compound is appreciably active against *C. albicans*. Tauramamide (**1**) is a new lipopeptide antibiotic that contains two D amino acids and is acylated at the N-terminus, which are both hallmarks of a non-ribosomal peptide synthase biosynthetic origin.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a JASCO J-1010 polarimeter equipped with a halogen lamp (589 nm) and a 10 mm microcell. NMR spectra were recorded on Bruker Avance 400, Bruker AMX 500, and Bruker Avance 600 (equipped with a cryoprobe) spectrometers at 400, 500, and 600 MHz, respectively, in $CDCl_3$ and DMSO-d_6. Chemical shifts are given in δ (ppm) with the residual $CDCl_3$ solvent peak referenced to δ_H 7.24 and δ_C 77.0 and the residual DMSO solvent peak referenced to δ_H 2.49 and δ_C 39.5 as the internal reference. ESIMS spectra were obtained with Kratos MS-50, Micromass LCT, and Bruker Esquire-LC mass spectrometers. Si gel (Silicycle, 230–400 mesh) and Sephadex LH20 were used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm and Whatman, MKC18F 60 A) were used for TLC analysis. A Waters 1500 Series pump system equipped with a Waters 2487 dual λ absorbance detector and a CSC-Inertsil 150A/ODS2 column was used for HPLC. All reagents were purchased in analytical or higher grade. Dry CH_2Cl_2 was prepared by distillation from CaH.

Culture Conditions for PNG 276. PNG-276 was grown as lawns on 21 pans of solid tryptic soy agar (24 × 37 × 0.5 cm) supplemented with NaCl to a final concentration of 1%. Cultures were grown at room temperature for 5 days before live cells were scraped from the solid agar surface and lyophilized.

Isolation of Tauramamide Methyl Ester (2). Lyophilized cells (~30 g) were exhaustively extracted with MeOH (3 × 250 mL). The combined MeOH extract was concentrated *in vacuo* to yield a brown gum (3.8 g), which was partitioned between H_2O and EtOAc. The EtOAc-soluble portion (1.0 g) was fractionated via sequential Sephadex LH-20 chromatographies, the first eluted with MeOH and the second eluted with EtOAc/MeOH/H_2O (20:5:2) yielding fractions that were pooled according to bioactivity, NMR, and TLC characteristics [**2**: R_f = 0.7 on normal-phase Si gel (eluent: 4:1:1 *n*-BuOH/MeOH/H_2O, visualized with ceric sulfate/phosphomolybdic acid dip)].

The most active fraction (140.9 mg) was subjected to reversed-phase C-18 chromatography (Waters 2 g Sep-Pak) using step gradient elution from H_2O to MeOH to CH_2Cl_2 to give 92.8 mg of active material. This material was fractionated by isocratic reversed-phase HPLC (40% MeCN/H_2O + 0.1% TFA) to give pure tauramamide methyl ester (**2**; 7.4 mg, 0.02% dry cell weight).

Tauramamide methyl ester (2): pale yellow glass; $[\alpha]^{25}_D$ −14.6 (*c* 0.6, MeOH); for NMR data, see Table 1; HRESIMS $[M + H]^+$ *m/z* 878.5181 (calcd for $C_{45}H_{67}N_9O_9$, 878.5185).

Acid Hydrolysis of Tauramamide Methyl Ester (2). Ester **2** (0.8 mg, 0.9 mol) was added to 0.5 mL of 6 N HCl and sealed in a screw-top vial, and the mixture was heated to 108 °C with stirring for 16 h. After cooling, the mixture was evaporated under N_2 to remove HCl. A second hydrolysis for Trp analysis was performed using ester **2** (0.2 mg, 0.2 umol) under identical conditions, but heating for 1 h instead of 16 h.

Preparation of FDAA Derivative Standards. Amino acid standards (2.0 mmol) were dissolved in H_2O (40 mL), and to this solution was added Marfey's reagent (*N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide, 62.5 mM in 60 mL of Me_2CO), followed by $NaHCO_3$ (1 M, 20 mL). The mixture was heated for 1 h at 43 °C, after which HCl (2 N, 10 mL) was added. An additional aliquot of Me_2CO (50 mL) was added to solubilize the samples.

Marfey's Analysis of Tauramamide Methyl Ester (2). The acid hydrolysate of ester **2** was treated in the same fashion as the amino acid standards described above. HPLC analyses of derivatized amino acids were performed using an Alltech Econosil C18 HPLC column eluted with the following solutions. *Solution A*: prepared by adding Et_3N (28 mL, 0.2 mol) to H_2O (3.57 L) while stirring; to this mixture was added H_3PO_4 dropwise until the mixture was pH 3.0; MeCN (400 mL) was then added, resulting in a mixture of 9:1 triethylammonium phosphate (50 mM, ph 3.0)/MeCN. *Solution B*: HPLC grade MeCN. The HPLC analysis employed a linear gradient from 100% solution A to 60% solution A/40% solution B over 40 min at a flow rate of 1 mL/min, with UV detection at 210 nm.

Isolation of Tauramamide Ethyl Ester (3). PNG-276 was cultured and the cells were collected as described above. Extraction of the cells and fractionation of the crude extract proceeded as described above for the isolation of **2** except that EtOH was substituted for MeOH. This extraction yielded tauramamide A ethyl ester (**3**; 4.1 mg, 0.015% dry cell weight).

Tauramamide ethyl ester (3): pale yellow glass; $[\alpha]^{25}_D$ −31.9 (*c* 0.8, MeOH); for 1D and 2D NMR data, see the Supporting Information; HRESIMS $[M + H]^+$ *m/z* 892.5294 (calcd for $C_{46}H_{70}N_9O_9$, 892.5297).

Boc-Arg(Cbz)$_2$-OBn (5). To a solution of Boc-Arg(Cbz)$_2$-OH (**4**) (2.5 g, 4.6 mmol) and benzyl alcohol (0.71 mL, 6.9 mmol) in CH_2Cl_2 (50 mL) at 0 °C were added DCC (1.04 g, 5.06 mmol) and DMAP (0.06 g, 0.46 mmol), and the resulting mixture was stirred overnight. The solution was poured into water and extracted with CH_2Cl_2 (30 mL × 3). The organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by Sephadex LH20 column chromatography (eluent: 100% MeOH) afforded **5** as a colorless solid (2.91 g, 97%). **5**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.36 (1H, s), 9.12 (1H, s), 7.30–7.10 (15H, m), 5.30 (1H, d, J = 8.3 Hz), 5.10–4.90 (6H, m), 4.24 (1H, m), 3.83 (2H, m), 1.85–1.70 (4H, m), 1.31 (9H, s); HRESIMS *m/z* 655.2743 (calcd for $C_{34}H_{40}N_4O_8Na$, 655.2744).

Boc-Ser(Bn)-D-Leu-Trp-Arg(Cbz)$_2$-OBn (8). A solution of Boc-Arg(Cbz)$_2$-OBn (**5**) (1.87 g, 2.9 mmol, 1 equiv) in CH_2Cl_2 (25 mL) was treated with TFA (3 mL, 40.3 mmol, 14 equiv) and stirred for 4 h, whereupon it was concentrated *in vacuo* to a pale red oil. This oil was dissolved in CH_2Cl_2 (20 mL) and added to a solution of Boc-Trp-OH (0.82 g, 2.7 mmol, 0.90 equiv) and PyBOP (1.69 g, 3.24 mmol, 1.10 equiv) in CH_2Cl_2 (10 mL), followed by addition of DIEA (1.55 mL, 8.90 mmol, 3 equiv). After stirring overnight at 25 °C, saturated NH_4Cl was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography on Si gel (80% EtOAc/hexanes) afforded **6** as a white, amorphous solid (1.87 g, 85%). The procedure above was repeated successively with Boc-D-Leu-OH (0.48 g, 2.07 mmol) and Boc-Ser(Bn)-OH (0.46 g, 1.57 mmol) to afford Boc-D-Leu-Trp-Arg(Cbz)$_2$-OBn (**7**) (1.93 g, 76%) and Boc-Ser(Bn)-D-Leu-Trp-Arg(Cbz)$_2$-OBn (**8**) (1.75 g, 81%), respectively.

Tetrapeptide 8: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.40 (1H, s), 9.28 (1H, s), 9.00 (1H, s), 7.26–6.90 (30H, m), 5.65 (1H, m), 5.20–4.90 (6H, m), 4.80 (1H, dd, J = 6.3, 7.4 Hz), 4.55–4.40 (2H, m), 4.40–4.25 (2H, m), 3.95–3.80 (2H, m), 3.67 (1H, m), 3.50 (1H, dd, J = 6.1, 2.8 Hz), 3.31 (1H, dd, J = 6.3, 8.7 Hz), 3.15 (1H, dd, J = 5.7, 8.7 Hz), 1.85–1.75 (1H, m), 1.75–1.60 (2H, m), 1.60–1.35 (4H, m), 1.41 (9H, s), 0.80 (6H, m); HRESIMS *m/z* 1131.5162 (calcd for $C_{61}H_{72}N_8O_{12}Na$, 1131.5167).

Boc-D-Tyr(Bn)-OMe (10). A solution of Boc-D-Tyr-OMe (**9**) (2.0 g, 6.8 mmol), K_2CO_3 (1.4 g, 10.1 equiv), and BnBr (1 mL, 8.1 mmol) in acetone was refluxed for 3 h and then stirred overnight at 25 °C. After filtration and solvent evaporation, column chromatography on

Si gel (30% EtOAc/hexanes) afforded **10** as an amorphous, white solid (2.60 g, 99%). **10**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.20 (5H, m), 6.94 (2H, d, J = 5.6), 6.81 (2H, d, J = 5.7), 4.94 (2H, s), 4.89 (1H, d, J = 5.0 Hz), 4.45 (1H, dd, J = 4.4 Hz), 3.61 (3H, s), 2.95 (1H, dd, J = 3.64, 9.2 Hz), 2.91 (1H, dd, J = 3.6, 9.0 Hz), 1.33 (9H, s); HRESIMS *m/z* 408.1790 (calcd for $C_{22}H_{27}NO_5Na$, 408.1787).

$(CH_3)_2CH_2(CH_2)_5$CONH-D-Tyr(Bn)-OMe (11). A solution of Boc-D-Tyr(Bn)-OMe (**10**) (1.50 g, 3.9 mmol) in CH_2Cl_2 (35 mL) was treated with TFA (2 mL, 27 mmol) and stirred for 4 h, whereupon it was concentrated *in vacuo* to a colorless oil. This oil was dissolved in CH_2Cl_2 (10 mL) and added to a solution of 7-methyloctanoic acid (0.62 g, 3.9 mmol) and PyBOP (2.0 g, 3.8 mmol) in CH_2Cl_2 (40 mL), followed by addition of DIEA (2.0 mL, 12 mmol). After stirring overnight at 25 °C, saturated NH_4Cl was added and the resulting solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography on Si gel (80% EtOAc/hexanes) afforded **11** as a yellowish powder (1.67 g, 88%). **11**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.15 (5H, m), 6.98 (2H, d, J = 8.5), 6.80 (2H, d, J = 8.5), 6.71 (1H, d, J = 8.0), 4.87 (2H, s), 4.79 (1H, dd, J = 6.8, 7.2 Hz), 3.57 (3H, s), 3.01 (1H, dd, J = 5.6, 14.0 Hz), 2.89 (1H, dd, J = 7.0, 14.0 Hz), 2.10 (2H, t, J = 7.6 Hz), 1.51 (2H, quintet, J = 7.96 Hz), 1.43 (1H, septet, J = 6.5 Hz), 1.18 (4H, m), 1.08 (2H, quintet, J = 6.8 Hz), 0.80 (6H, d, J = 6.5 Hz); HRESIMS *m/z* 426.2645 (calcd for $C_{26}H_{36}NO_4$, 426.2644).

$(CH_3)_2CH_2(CH_2)_5$CONH-D-Tyr(Bn)-Ser(Bn)-D-Leu-Trp-Arg(Cbz)$_2$-OBn (13). A solution of $(CH_3)_2CH_2(CH_2)_5$CONH-D-Tyr(Bn)-OMe (**11**) (0.717 g, 1.68 mmol) and LiOH·H_2O (0.705 g, 16.8 mmol) in 1,4-dioxane/H_2O (150 mL, 2:1) was stirred at 25 °C. After 1 h, TLC (80% EtOAc/hexanes) showed an absence of starting material. Solvents were evaporated, and the resulting residue was dissolved in H_2O, acidified, and extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid (acid **12**, 0.68 g, 98%). In a separate reaction, Boc-Ser(Bn)-D-Leu-Trp-Arg(Cbz)$_2$-OBn (**8**) (1.42 g, 1.3 mmol) in CH_2Cl_2 (50 mL) was treated with TFA (3 mL, 40.3 mmol) and stirred for 5 h, whereupon it was concentrated *in vacuo* to a red oil. This oil was dissolved in CH_2Cl_2 (20 mL) and added to a solution of acid **12** (0.58 g, 1.41 mmol) and PyBOP (0.73 g, 1.41 mmol) in CH_2Cl_2 (30 mL), followed by addition of DIEA (0.66 mL, 3.84 mmol). After stirring 16 h at 25 °C, the reaction was quenched with saturated NH_4Cl, and 1 M HCl was used to neutralize the aqueous layer. CH_2Cl_2 (3 × 30 mL) extractions were performed; the organic extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography on Si gel (80% EtOAc/hexanes) afforded **13** as a pale yellow solid (1.45 g, 81%). **13**: ^{1}H NMR (DMSO, 600 MHz) δ 10.74 (1H, s), 9.16 (1H, s, broad), 8.52, (1H, d, J = 6.9 Hz), 8.15, (1H, d, J = 8.0 Hz), 8.13, (1H, d, J = 8.6 Hz), 8.00 (2H, d, J = 6.9 Hz), 7.58 (1H, d, J = 7.7 Hz), 7.60–6.80 (25H, m), 7.13 (3H, m), 7.09 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 7.1, 7.1 Hz), 6.93 (1H, dd, J = 7.4, 7.4), 6.85 (3H, m), 5.20 (2H, m), 5.05–5.00 (6H, m), 4.55 (3H, m), 4.29 (2H, m), 3.90 (1H, m), 3.85 (1H, m), 3.61–3.45 (4H, m), 3.07 (1H, m), 2.85 (1H, m), 2.65 (1H, m), 1.98 (2H, m), 1.70 (1H, m), 1.43 (3H, m), 1.32 (4H, m), 1.25–1.00 (10H, m), 0.79 (3H, d, J = 6.2 Hz), 0.78 (3H, d, J = 6.3 Hz), 0.62 (6H, d, J = 5.5 Hz); HRESIMS *m/z* 1402.7125 (calcd for $C_{81}H_{96}N_9O_{13}$, 1402.7128).

Tauramamide (1). A solution of $(CH_3)_2CH_2(CH_2)_5$CONH-D-Tyr(Bn)-Ser(Bn)-D-Leu-Trp-Arg(Cbz)$_2$-OBn (**13**) (0.21 g, 0.15 mmol) and 10% Pd/C, wet Degussa type E101NE/W, was stirred in the presence of H_2 at 25 °C and 20 atm, until TLC (RP, 90% CH_3CN/H_2O) showed the absence of starting material (around 1 week). Catalyst filtration, followed by solvent evaporation and reversed-phase column chromatography (90% CH_3CN/H_2O), afforded a colorless solid residue. Further purification by reversed-phase HPLC (50% CH_3CN/H_2O, 0.1% TFA) yielded pure tauramamide (**1**) (0.132 g, 81%): $[\alpha]^{25}_D$ –51.8 (*c* 0.9, MeOH); ^{1}H NMR (DMSO, 600 MHz) δ 12.69 (1H, s, br), 10.76 (1H, d, J = 1.5 Hz), 9.14 (1H, s), 8.32 (1H, d, J = 7.8 Hz), 8.14 (1H, d, J = 8.3 Hz), 8.03 (1H, d, J = 7.8 Hz), 7.99 (1H, d, J = 7.7 Hz), 7.75 (1H, d, J = 7.8 Hz), 7.62 (1H, d, J = 7.8 Hz), 7.48 (1H, dd, J = 5.8, 5.8 Hz), 7.30 (1H, d, J = 8.0 Hz), 7.10 (1H, d, J = 1.9 Hz), 7.03 (1H, dd, J = 7.1, 7.1 Hz), 7.02 (1H, d, J = 8.3 Hz), 7.02 (1H, d, J = 8.3 Hz), 6.95 (1H, dd, J = 7.4, 7.4 Hz), 6.61 (1H, d, J = 8.4 Hz), 6.61 (1H, d, J = 8.4 Hz), 4.82 (1H, s, broad), 4.54 (1H, m), 4.42 (1H, m), 4.23 (1H, m), 4.22 (1H, m), 4.21 (1H, m), 3.51 (1H, m), 3.44 (1H, m), 3.16 (1H, m), 3.12 (2H, m), 2.88 (1H, m), 2.86 (1H, m), 2.62 (1H, dd, J = 10.2, 14.0 Hz), 2.00 (2H, m), 1.79 (1H, m), 1.64 (1H, m), 1.54 (1H, m), 1.45 (1H, m), 1.35 (2H, m), 1.35 (1H, m), 1.24 (1H, m), 1.17 (1H, m), 1.15 (1H, m), 1.14 (1H, m), 1.09 (2H, m), 1.08 (1H, m), 1.07 (1H, m), 1.06 (1H, m), 0.826 (3H, d, J = 6.8 Hz), 0.823 (3H, d, J = 6.8 Hz), 0.68 (3H, d, J = 6.8 Hz), 0.66 (3H, d, J = 6.5 Hz); HRESIMS *m/z* 864.4981 (calcd for $C_{44}H_{66}N_9O_9$, 864.4984).

Tauramamide ethyl ester (3). A solution of tauramamide (**1**) (0.063 g, 0.073 mmol) and HCl (1 mL of 2 M HCl in diethyl ether, 2 mmol) in EtOH was stirred at 25 °C until TLC spots for starting material (RP, 90% CH_3CN/H_2O) disappeared (around 5 days). Solvent evaporation, followed by reversed-phase HPLC (50% CH_3CN/H_2O, 0.1% TFA), yielded pure tauramamide ethyl ester (**3**) (0.0559 g, 86%): $[\alpha]^{25}_D$ –4.7 (*c* 1.85, MeOH); ^{1}H NMR (DMSO, 600 MHz) δ 10.77 (1H, d, J = 1.6 Hz), 9.14 (1H, s), 8.43 (1H, d, J = 7.4 Hz), 8.16 (1H, d, J = 8.3 Hz), 8.04 (1H, d, J = 7.7 Hz), 7.98 (1H, d, J = 7.7 Hz), 7.75 (1H, d, J = 7.7 Hz), 7.62 (1H, d, J = 7.7 Hz), 7.48 (1H, dd, J = 5.5, 5.6 Hz), 7.30 (1H, d, J = 8.0 Hz), 7.10 (1H, d, J = 2.2 Hz), 7.04 (1H, dd, J = 7.2, 7.5 Hz), 7.02 (1H, d, J = 8.6 Hz), 7.02 (1H, d, J = 8.6 Hz), 6.96 (1H, dd, J = 7.2, 7.5 Hz), 6.61 (1H, d, J = 8.6 Hz), 6.61 (1H, d, J = 8.6 Hz), 4.84 (1H, dd, J = 5.2, 5.5 Hz), 4.54 (1H, m), 4.42 (1H, m), 4.25 (1H, m), 4.24 (1H, m), 4.23 (1H, m), 4.09 (2H, m), 3.51 (1H, m), 3.45 (1H, m), 3.15 (1H, dd, J = 3.8, 14.6 Hz), 3.11 (2H, m), 2.88 (1H, m), 2.84 (1H, m), 2.62 (1H, dd, J = 10.0, 13.8 Hz), 2.00 (2H, m), 1.77 (1H, m), 1.66 (1H, m), 1.53 (2H, m), 1.45 (1H, m), 1.35 (2H, m), 1.26 (1H, m), 1.18 (3H, t, J = 7.2 Hz), 1.14 (2H, m), 1.08 (2H, m), 1.08 (2H, m), 1.06 (2H, m), 0.824 (3H, d, J = 6.7 Hz), 0.822 (3H, d, J = 6.6 Hz), 0.70 (3H, d, J = 6.6 Hz), 0.67 (3H, d, J = 6.6 Hz); HRESIMS *m/z* 892.5295 (calcd for $C_{46}H_{70}N_9O_9$, 892.5297).

Antibacterial Assays. Culture media for bacterial strains: Tryptic soya agar (TSA+) or broth (TSB+) with 5 g/L NaCl; for *C. albicans* Sabouraud agar (Sab) or TSB+.

Alamar Blue Bioassays. This assay was run as a 1:2 serial dilution bioassay. The compound to be tested was dissolved in solvent (DMSO or EtOH). Alamar Blue (AB), an oxidation–reduction indicator, undergoes colorimetric change in response to cellular metabolic reduction. If the compound has antimicrobial activity (i.e., pathogen is killed and/or inhibited), then the AB remains blue/purple. If the pathogen is not killed and is able to grow, then the AB is oxidized and changes to bright pink.

Agar Extract Inclusion Bioassay. The compounds were dissolved in solvent (DMSO or EtOH) and incorporated into Mueller Hinton agar cooled to 50 °C. Agar was then serially diluted 1:2 with additional cooled agar and aliquoted into Petri plates. Several pathogens (each ~2.5 × 10^5 cells/mL inoculum concentration) were spotted onto the agar surface. Plates were incubated overnight at 37 °C, and then the plates were checked for inhibition of pathogen growth to determine MIC.

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Supporting Information Available: NMR spectra for **1**, **2**, and **3**; tables of NMR data for **1** and **3**, ^{13}C NMR data for synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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