Dominicin, a Cyclic Octapeptide, and Laughine, a Bromopyrrole Alkaloid, Isolated from the Caribbean Marine Sponge *Eurypon laughlini*

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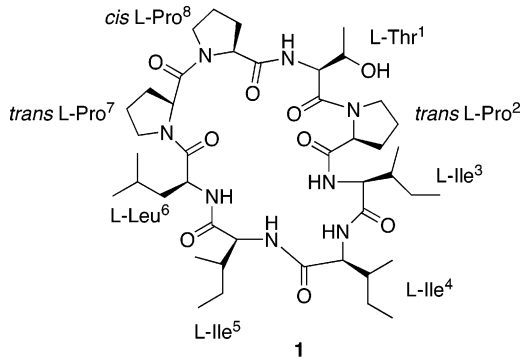
Dominicin, a new cyclic peptide, and the new bromopyrrole alkaloid laughine (**4**) have been isolated from the marine sponge *Eurypon laughlini* collected in Dominica. The structures of **1** and **4** were determined by a combination of spectroscopic analysis and chemical degradation. Single-crystal X-ray diffraction analysis confirmed the structure of dominicin (**1**).

Sponges continue to be the single richest marine source of structurally novel secondary metabolites.[1] As part of an ongoing search for new natural products with potential anticancer activity, we have screened a library of marine sponge extracts in a cell-based assay designed to detect antimitotic agents.[2] Crude extracts of the marine sponge *Eurypon laughlini* (Diaz, Alvarez & Van Soest, 1987) (order Poecilosclerida, family Raspailiidae) collected in the Caribbean showed promising activity in the assay. Bioassay-guided fractionation of the *E. laughlini* extract led to the isolation of the new cyclic octapeptide dominicin (**1**), the known alkaloids keramadine (**2**)[3] and agelongine (**3**),[4] and the novel alkaloid laughine (**4**). The structures of the new metabolites **1** and **4** are described below.



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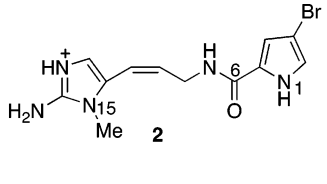
Results and Discussion

Eurypon laughlini was harvested by hand using scuba on the sand flats off Rollo Head, south of Portsmouth, Dominica. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. Thawed sponge specimens were repeatedly extracted with MeOH, and the extracts were combined, concentrated in vacuo, and partitioned between H_2O and EtOAc. The EtOAc-soluble materials were subsequently partitioned between 4:1 MeOH/H_2O and hexanes. Bioassay-guided fractionation of the MeOH/H_2O-soluble components via Sephadex LH-20 and flash reversed-phase column chromatography followed by reversed-phase HPLC gave pure samples of the cyclic octapeptide dominicin (**1**) and the alkaloids keramadine (**2**),[3] agelongine (**3**),[4] and laughine (**4**). The final HPLC step in the purification resulted in loss of the antimitotic activity, and as a result, the bioactive compounds in the extract have not yet been identified.



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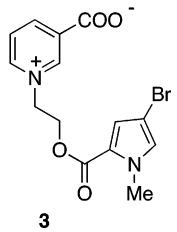

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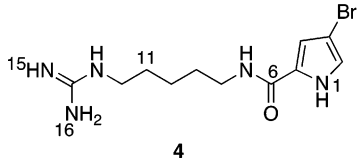

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Dominicin (**1**) was obtained as optically active crystals that gave a $[M + H]^+$ ion in the HRFABMS at *m/z* 845.5512, appropriate for a molecular formula of $C_{43}H_{72}N_8O_9$ (calcd for $C_{43}H_{73}N_8O_9$, 845.5505), requiring 12 sites of unsaturation. The crystals of **1** gave a sharp, well-resolved peak when analyzed by reversed-phase HPLC using a variety of solvent systems and a single, clean molecular ion in the HRFABMS. Despite these indications of purity, many of the resonances in the ^1H and ^{13}C NMR spectra were doubled, tripled, or broadened when the spectra were recorded in a variety of solvents (e.g., MeOH-d_4, DMSO-d_6, MeCN-d_3), resulting in poor resolution that precluded structural studies. Attempts to simplify the NMR spectra by heating (to 50 °C) or adjusting the pH by addition of TFA or Et_3N did not alleviate the problem. Eventually it was found that acceptable NMR spectra, with only a single set of well-resolved resonances, could be obtained using C_5D_5N as the solvent.

The ^{13}C/DEPT/HMQC NMR spectra for **1** recorded in C_5D_5N identified 43 carbon resonances (9 × CH_3, 13 × CH_2, 13 × CH, 8 × C), in agreement with the MS data. General features of both the ^1H and ^{13}C NMR spectra (Table 1 and Supporting Information) suggested **1** was a peptide. Eight ^{13}C NMR resonances had chemical shifts appropriate for amide carbonyls (δ 175.8, 173.1, 172.1, 171.6, 171.1, 170.7, 170.5, 169.8) and eight resonances had chemical shifts appropriate for amino acid α-carbons (δ 62.2, 61.6, 61.4,

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Table 1. NMR Data for Dominicin (**1**) Recorded in C_5D_5N

	δ_H[a]	δ_C[b]	HMBC[c]		δ_H[a]	δ_C[b]	HMBC[c]
Thr1				Ile5			
α	5.10 (br s)	59.9	Thr1: γC*H*$_3$, N*H*	α	4.28 (br s)	62.2	Ile5: γ_A*H*, βC*H*$_3$
β	4.69−4.71	66.2	Thr1: α*H*, γC*H*$_3$, N*H*	β	2.11 (m)	36.1	Ile5: γ_A*H*, βC*H*$_3$, δC*H*$_3$
γCH$_3$	1.53 (d, *J* = 6.4 Hz)	19.4	Thr1: β*H*	γ_A	1.33 (m)	26.1	Ile5: βC*H*$_3$, δC*H*$_3$
*C*O		169.8	Thr1: α*H*, β*H*, Pro2: δ_B*H*	γ_B	1.67−1.71		
N*H*	8.91 (d, *J* = 7.9 Hz)			δCH$_3$	0.69 (bt, *J* = 6.9 Hz)	11.6	Ile5: γ_A*H*
O*H*	5.90 (br s)			βCH$_3$	1.07 (d, *J* = 6.9 Hz)	16.0	
Pro2				*C*O		170.7	Leu6: αH, N*H*
α	4.69−4.71	61.6	Pro2: β_A*H*, β_B*H*, γ_A*H*, δ_A*H*, δ_B*H*	N*H*	9.28 (br s)[d]		
β_A	1.97 (m)	29.6	Pro2: γ_A*H*, δ_A*H*, δ_B*H*	Leu6			
β_B	2.09 (m)			α	5.32 (m)	49.5	Leu6: β_A*H*, β_B*H*, γ*H*, N*H*
γ_A	1.47 (m)	25.5	Pro2: β_A*H*, β_B*H*, δ_A*H*, δ_B*H*	β_A	1.65 (m)	41.7	Leu6: αH, γ*H*, γ_AC*H*$_3$, γ_BC*H*$_3$
γ_B	1.67−1.71			β_B	2.05 (m)		
δ_A	3.62 (m)	47.8	Pro2: β_A*H*, β_B*H*, γ_A*H*	γ	1.92 (m)	24.9	Leu6: αH, β_A*H*, β_B*H*, γ_AC*H*$_3$, γ_BC*H*$_3$
δ_B	3.77 (m)			γ_ACH$_3$	0.99 (d, *J* = 7.4 Hz)	22.9	Leu6: β_A*H*, β_B*H*, γ_BC*H*$_3$,
*C*O		173.1	Pro2: α*H*, β_A*H*, β_B*H*, Ile3: α*H*, N*H*	γ_BCH$_3$	1.01 (d, *J* = 7.2 Hz)	22.6	Leu6: β_A*H*, β_B*H*, γ*H*, γ_AC*H*$_3$
Ile3				*C*O		171.1	Leu6: αH, β_A*H*, β_B*H*
α	4.62 (t, *J* = 8.6 Hz)	58.4	Ile3: β*H*, γ_A*H*, γ_B*H*, βC*H*$_3$, N*H*	N*H*	8.44 (d, *J* = 8.7 Hz)		
β	2.23 (m)	35.1	Ile3: α*H*, γ_A*H*, γ_B*H*, βC*H*$_3$, δC*H*$_3$, N*H*	Pro7			
γ_A	1.27 (m)	25.9	Ile3: α*H*, β*H*, βC*H*$_3$, δC*H*$_3$	α	5.10 (br s)	60.1	Pro7: β_B*H*, γ_A*H*, γ_B*H*, δ_A*H* and/or δ_B*H*
γ_B	1.67−1.71			β_A	1.71−1.67	28.3	Pro7: αH, γ_B*H*, δ_A*H* and/or δ_B*H*
δCH$_3$	0.85 (t, *J* = 7.4 Hz)	10.6	Ile3: β*H*, γ_A*H*	β_B	1.82 (m)		
βCH$_3$	1.12 (d, *J* = 6.8 Hz)	16.2	Ile3: α*H*, β*H*, γ_B*H*	γ_A	1.75	25.1	Pro7: αH, β_B*H*, δ_A*H* and/or δ_B*H*
*C*O		175.8	Ile3: α*H*	γ_B	2.09 (m)		
N*H*	8.00 (d, *J* = 8.6 Hz)			δ_A	3.91 (m)	48.0	Pro7: β_A*H* and/or γ_A*H*
Ile4				δ_B	3.91 (m)		
α	4.68	59.7	Ile4: βC*H*$_3$	*C*O		170.5	Pro7: β_A*H*, β_B*H*, Pro8: α*H*, δ_B*H*
β	2.61 (br s)	36.0	Ile4: γ_A*H*, γ_B*H*, βC*H*$_3$, δC*H*$_3$	Pro8			
γ_A	1.27 (m)	25.8	Ile4: βC*H*$_3$, δC*H*$_3$	α	4.69−4.71	61.4	Pro8: β_A*H*, β_B*H*, γ_A*H* and/or γ_B*H*, δ_A*H*
γ_B	1.67−1.71			β_A	1.80 (m)	30.2	Pro8: αH, γ_A*H* &/or γ_B*H*, δ_A*H*
δCH$_3$	0.90 (t, *J* = 7.3 Hz)	12.2		β_B	2.82 (m)		
βCH$_3$	1.09 (d, *J* = 6.9 Hz)	17.0		γ_A	1.67−1.71	22.2	Pro8: αH, β_A*H*, β_B*H*, δ_A*H*, δ_B*H*
*C*O		171.6	Ile4: α*H*	γ_B	1.67−1.71		
N*H*	7.14 (br s)[d]			δ_A	3.53 (m)	46.4	Pro8: αH, β_B*H*, γ_A*H* and/or γ_B*H*
				δ_B	3.71 (m)		
				*C*O		172.1	Pro8: α*H*, β_A*H*, β_B*H*, Thr1: α*H*, N*H*

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz. [c] Proton resonances correlated to the carbon resonance listed in the δ_C column. [d] Assignments for NH protons of Ile4 and Ile5 are interchangeable.

60.1, 59.9, 59.7, 58.4, 49.5), consistent with an octapeptide. Detailed analysis of the COSY, HMQC, and HMBC data (Table 1) revealed that dominicin (**1**) contained threonine, leucine, three isoleucine, and three proline residues. Hydrolysis of peptide **1** with 6 N HCl, followed by Marfey's HPLC[5] and chiral GC[6] analyses of the amino acids in the hydrolysate, confirmed the presence of these four amino acids and established that all eight amino acid residues had the L configuration. The eight amino acid residues accounted for all of the atoms in the molecular formula of dominicin (**1**) and 11 of the sites of unsaturation. Furthermore, there was no evidence for terminal amino or carboxylic acid functionalities, and therefore, dominicin (**1**) was presumed to be a cyclic peptide.

The amino acid sequence in **1** was established by analysis of HMBC (Table 1) and 1D-NOESY data. HMBC correlations observed between the Thr1 CO (δ 169.8) and the Pro2 αH (δ 4.70), and between the Pro2 CO (δ 173.1) and the Ile3 NH (δ 8.00), identified amide linkages between the Thr1 CO and the Pro2 N and between the Pro2 CO and the Ile3 N. Additional HMBC correlations between the Thr1 NH (δ 8.91) and αH (δ 5.10) resonances and the Pro8 CO (δ 172.1), and between the Pro8 αH (δ 4.70) and Pro7 CO (δ 170.5) resonances, identified amide bonds between the Thr1 N and Pro8 CO and between the Pro8 N and Pro7 CO. 1D-NOESY correlations observed between the α-H of Leu6 (δ 5.32) and the Pro7 δ-methylene resonance at δ 3.91 demonstrated the presence of an amide bond between the Leu6 CO and the Pro7 N. The above correlations established the partial structure −CO-Ile3-Pro2-Thr1-Pro8-Pro7-Leu6-NH−, and the two remaining residues, Ile4 and Ile5, had to link Ile3 and Leu6 to complete the cyclic octapeptide structure **1**. A single-crystal X-ray diffraction analysis of dominicin (**1**) confirmed the structure assigned by spectroscopic analysis, as shown in Figure 1.

The X-ray diffraction analysis showed that in the crystals of dominicin (**1**) investigated the Pro2 and Pro7 peptide bonds were *trans* and the Pro8 peptide bond was *cis* (Figure

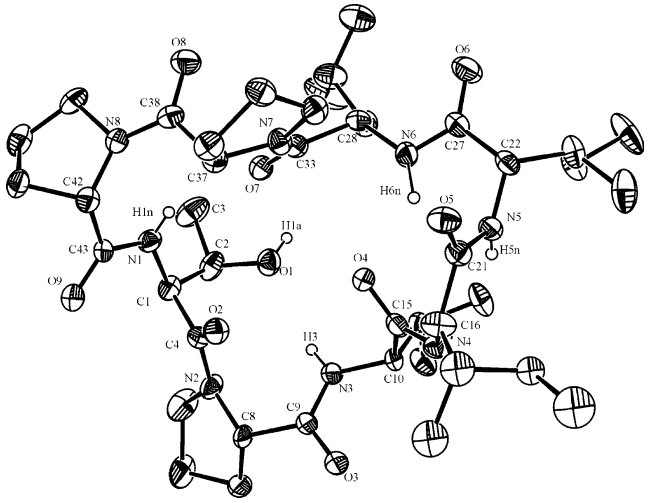

Figure 1. ORTEP diagram of dominicin (**1**).[13]

1). The chemical shifts of the proline γ carbons and the chemical shift difference of the proline β and γ carbons (Pro2 δ Cγ 25.5 ppm, $\Delta\delta_{C\beta-C\gamma} = 4.1$ ppm; Pro7 δ Cγ 25.1 ppm, $\Delta\delta_{C\beta-C\gamma} = 3.2$ ppm; Pro8 δ Cγ 22.2 ppm, $\Delta\delta_{C\beta-C\gamma} = 8.0$ ppm) in the ^{13}C NMR spectrum of **1** recorded in C_5D_5N confirmed that the proline peptide bonds had the same conformations in pyridine as in the solid state.[7−9]

The structures of the known alkaloids keramadine (**2**)[3] and agelongine (**3**)[4] were confirmed by comparison of their spectroscopic data with the literature values. Laughine (**4**) was isolated as a pale yellow oil that gave a $[M + H]^+$ ion at *m/z* 316.0779/318.0760 in the HRESIMS, consistent with a molecular formula of $C_{11}H_{18}N_5OBr$ (calcd for $C_{11}H_{19}N_5OBr$, 316.0773/318.0753), requiring 5 sites of unsaturation. A number of similarities were observed in the NMR data obtained for laughine (**4**) and the co-occurring alkaloid keramadine (**2**)[3] (Table 2). For example, it was apparent that both compounds contained a 4-bromopyrrole fragment attached to a carbonyl at C-2. COSY, HMQC, and HMBC data (Table 2) readily identified a saturated linear five-carbon fragment in **4** that was attached to nitrogen atoms bearing protons at both ends of the chain. HMBC correlations were observed from both NH-7 (δ 8.05) and H-8 (δ 3.19) to the C-6 amide carbonyl resonance at δ 159.5, demonstrating that the linear five-carbon fragment was linked at one end to the pyrrole through an amide bond. The remaining atoms (1 × C; 2 × N, 3 × H) and the final site of unsaturation could be accommodated by a guanidine functionality, which had to be attached to the other terminus of the linear five-carbon fragment. An HMBC correlation observed from H-12 (δ 3.07) to a carbon resonance at δ 156.6 (C-14) confirmed the presence of the guanidine and its attachment to C-12, to complete the structure of **4**. Laughine (**4**) is a new member of the oroidin family of alkaloids. It has the amino imidazole fragment normally present in the oroidins, such as keramidine (**2**), replaced by a linear alkyl guanidine fragment. The alkyl guanidine fragment found in laughine (**4**) can formally arise from cleavage of the C-11/N-15 bond in the standard oroidin skeleton.

Experimental Section

General Experimental Procedures. Melting points were taken using a Fisher-Johns apparatus, and the reported values are uncorrected. Optical rotations were measured using a Jasco P-1010 spectrophotometer. UV spectra were recorded with a Waters 2487 dual λ absorbance detector. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-500 and AM-400 spectrometers. ^{1}H chemical shifts are referenced to the residual C_5D_5N and DMSO-d_6 signals (δ 8.71, 7.55, 7.19, 3.30, and 2.49 ppm, respectively), and ^{13}C chemical shifts are referenced to the C_5D_5N and DMSO-d_6 solvent peaks (δ 149.9, 135.5, 123.5, 49.0, and 39.5 ppm, respectively). Low- and high-resolution FABMS were recorded on a Kratos Concept II HQ mass spectrometer with xenon as the bombarding gas and a thioglycerol sample matrix, and low- and high-resolution ESI-QIT-MS were recorded on a Bruker-Hewlett-Packard 1100 Esquire-LC system mass spectrometer. Merck Type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography. Waters 2 g Sep-Pak's were used for reversed-phase flash chromatography. Reversed-phase HPLC purifications and HPLC analysis of amino acid derivatives were performed on a Waters 600E System Controller liquid chromatograph attached to a Waters 996 photodiode array detector. All solvents used for HPLC were Fisher HPLC grade.

Chiral capillary GC analyses of amino acid derivatives were carried out on a Hewlett-Packard 5880A gas chromatograph using a Chirasil-Val column (0.25 mm × 50 m, Alltech, Deerfield, IL) and helium as the carrier gas (flow rate: 1 mL/min; split ratio: 40:1). The program rate for the amino acid derivatives was 90° (4 min) to 220° (27.5 min) at 4°/min. The other conditions were as follows: injector temperature 250°; detector temperature 275°; makeup gas N_2 (30 mL/min).

Single-crystal X-ray diffraction measurements were made on a Rigaku/ADSC diffractometer with graphite-monochromated Mo Kα radiation. Crystals were mounted on a glass fiber. The data were collected at a temperature of -100.0 ± 0.1 °C to a maximum 2θ value of 50.1°. Data were collected in a series of ϕ and ω scans in 0.50° oscillations with a 57.0 s exposure. The crystal-to-detector distance was 38.79 mm.[10]

Animal Material. Specimens of *Eurypon laughlini* were collected by hand using scuba at a depth of 10−15 m on sand flats off Rollo Head, 5 km south of Portsmouth, Dominica. Freshly collected sponge was frozen on site and transported to Vancouver frozen over dry ice. A voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR. 17185).

Extraction of *E. laughlini* and Isolation of Dominicin (1), Keramadine (2), Agelongine (3), and Laughline (4). A sample of sponge (2.6 kg) was cut into small pieces and immersed in and subsequently extracted repeatedly with MeOH (4 × 4 L) at room temperature. The combined MeOH extracts were concentrated in vacuo, and the resultant brown gum was partitioned between EtOAc (4 × 250 mL) and H_2O (1 L). The combined EtOAc extracts were evaporated to dryness to give 6.3 g of brown oil that was partitioned between hexanes (3 × 175 mL) and 4:1 MeOH/H_2O (700 mL). The MeOH/H_2O fraction was evaporated to dryness to give 3.7 g of an amorphous brown solid, which was chromatographed on Sephadex LH-20 eluting with 4:1 MeOH/CH_2Cl_2 to give one fraction (264.4 mg) exhibiting antimitotic activity against MCF7 breast cancer cells ($IC_{50} < 6.25$ μg/mL) and a later eluting fraction (543.4 mg) having no biological activity.

The antimitotic active material was further fractionated using reversed-phase silica gel flash chromatography employing a step gradient from 1:1 MeOH/H_2O to MeOH, with a final CH_2Cl_2 wash. A 30.8 mg fraction, eluting with 7:3 MeOH/H_2O, showed antimitotic activity at 1.6 μg/mL. Pure dominicin (**1**) (8.0 mg) was obtained from this mixture via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm 25 × 0.94 cm column, with 1:1 MeCN/H_2O as eluent. Dominicin was inactive, and only weak antimitotic activity was observed for all the other fractions collected off the HPLC.

The biologically inactive fraction was further purified using reversed-phase silica gel flash chromatography, employing a step gradient from 1:1 MeOH/H_2O to MeOH, with a final CH_2Cl_2 wash. A 151.9 mg fraction, eluting with 1:1 MeOH/H_2O, was further purified via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm 25 × 0.94 cm column, with 1:4 MeCN/(0.05% TFA/H_2O) as eluent. This was followed by reversed-phase HPLC using the same column but with 9:11

Table 2. NMR Data for Laughine (**4**) and Keramadine (**2**) Recorded in DMSO-d_6

	laughine (**4**)				keramadine (**2**)	
	δ_H[a]	δ_C[b]	COSY[c]	HMBC[d]	δ_H[a]	δ_C[b]
1	11.75 (bs)		H2, H4		11.83 (bs)	
2	6.95 (bs)	120.9	H1, H4		6.98 (bs)	121.3
3		94.8		H2		94.9
4	6.82 (bs)	111.2	H1, H2		6.84 (bs)	111.5
5		127.0		H2, H4		126.6
6		159.5		H7, H8		159.5
7	8.05 (t, J = 5.4 Hz)		H8		8.42 (t, J = 5.6 Hz)	
8	3.19 (m)	38.2	H7, H9	H9, H10	4.01 (bt, J = 5.6 Hz)	37.8
9	1.48 (m)	28.9	H8, H10	H8, H11	5.85 (dt, J = 11.5, 5.6 Hz)	133.4
10	1.29 (m)	23.5	H9, H11	H8, H9, H11, H12	6.25 (bd, J = 11.5 Hz)	113.8
11	1.48 (m)	28.1	H10, H12	H9, H12		123.7
12	3.07 (m)	40.7	H11, H13	H11	7.09 (bs)	111.9
13	7.46 (bs)		H12		12.45 (bs)	
14		156.6		H12		146.4
16					7.73 (s)	
15*Me*					3.38 (s)	29.2

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz. [c] Proton resonances correlated to the proton resonance listed in the δ_H column. [d] Proton resonances correlated to the carbon resonance listed in the δ_C column. Experiments optimized for both 2 and 8 Hz.

MeOH/(0.05% TFA/H_2O) as eluent to give one fraction consisting of a mixture of keramadine (**2**) and laughine (**4**), and a fraction containing pure agelongine (**3**) (4.2 mg). Pure **2** (2.4 mg) and **4** (1.0 mg) were obtained after an additional reversed-phase HPLC chromatographic step using a Alltech Econosil C-18 5 μm column with 3:2 MeOH/(0.05% TFA/H_2O) as eluent.

Dominicin (1): clear prisms; mp 168−171 °C; $[\alpha]^{25}_D$ −118.7° (*c* 7.8, MeOH); ^{1}H and ^{13}C NMR, see Table 1; positive ion HRFABMS $[M + H]^+$*m/z* 845.5512 (calcd for $C_{43}H_{73}N_8O_9$, 845.5505) and $[M + Na]^+$ *m/z* 867.5352 (calcd for $C_{43}H_{72}N_8O_9$-Na, 867.5325).

Laughine (4): pale yellow oil; UV (MeOH) λ_{max} 206 (ϵ 3799), 265 (ϵ 4016) nm; ^{1}H and ^{13}C NMR, see Table 2; positive ion HRESIMS $[M + H]^+$*m/z* 316.0779/318.0760 (calcd for $C_{11}H_{19}N_5$-OBr, 316.0773/318.0753).

Hydrolysis of Dominicin (1). Purified dominicin **1** (2.0 mg, 2.3 μmol) was hydrolyzed in 0.5 mL of 6 N HCl (freshly distilled, constant boiling HCl) at 108 °C with stirring for 16 h in a threaded Pyrex tube sealed with a Teflon screw cap. The cooled reaction mixture was evaporated to dryness, and traces of HCl were removed from the residual hydrolysate by repeated evaporation from H_2O (3 × 0.4 mL). The resultant hydrolysate mixture obtained was split into two equal portions.

Derivatization of Amino Acids with Marfey's Reagent and HPLC Analysis.[4] To a 0.5 mL vial containing 2.0 μmol of pure amino acid standards in 40 μL of H_2O was added 2.8 μmol of *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alanine amide (FDAA) in 80.0 μL of acetone followed by 20 μL of 1 N $NaHCO_3$. The mixture was heated for 1 h at 40 °C. After cooling to room temperature, 10 μL of 2 N HCl was added and the resulting solution was filtered through a 4.5 μm filter and stored in the dark until HPLC analysis.

One-half of the hydrolysate mixture from dominicin (**1**) was dissolved in 176 μL of H_2O, and to this was added 12.3 μmol of FDAA in 352 μL of acetone followed by 98 μL of 1 N $NaHCO_3$. The derivatrization reaction was carried out and worked up as described above. A 2 μL aliquot of the resulting mixture of FDAA derivatives was analyzed by reversed-phase HPLC. An Alltech Econosil C-18 5 μ column with a linear gradient of (A) 9:1 triethylammonium phosphate (50 mM, pH 3.0)/MeCN and (B) MeCN, with 0% B at start to 40% B over 55 min (flow rate 1 mL/min), was used to separate the FDAA derivatives with UV detection at 340 nm. Each peak in the chromatographic trace was identified by comparing its retention time and photodiode array UV spectrum with that of the FDAA derivative of the pure amino acid standard and by co-injection. In all cases a peak at 43.67 min was observed which was attributed to excess FDAA. Retention times (min) are given in parentheses: L-Thr (33.95), L-*allo*-Thr (34.26), D-*allo*-Thr (35.82), D-Thr (38.17), L-Pro (40.92), D-Pro (42.76), L-Ile (54.04), L-*allo*-Ile (54.04), L-Leu (55.04), D-Ile (59.61), D-*allo*-Ile (59.61), D-Leu (60.02).

PFP-IPA Derivatization of Amino Acids and Chiral GC Analysis.[5] Acetyl chloride (1.25 mL) was slowly added to 2-propanol in an ice bath. The resulting isopropyl acetate solution (250 μL) was distributed among half of the dried acid hydrolysate of dominicin (**1**) and 1−2 mg of the pure dry amino acid standard, each in a 5 mL screw-capped vial. The vials were heated to 110 °C for 45 min. Excess reagent was removed under a stream of N_2. After cooling the vials in an ice bath, CH_2Cl_2 (250 μL) and pentafluoropropyl anhydride (100 μL) were added and the vials heated to 110 °C for 15 min. Excess reagent was evaporated under dry N_2, and CH_2Cl_2 (200 μL) was added to each vial. A 2 μL aliquot of each sample was analyzed by chiral GC.

Through a combination of both Marfey's HPLC[4] and chiral GC analysis[5] it was possible to determine the complete configuration of the isoleucine in the hydrolysate mixture of dominicin (**1**) as L (in Marfey's HPLC method the L- and L-allo-isoleucine are unresolvable, as are the D- and d-allo-isoleucine, and in the GC method the L-allo and D-isoleucine are unresolvable).

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Supporting Information Available: 1D ^{1}H and ^{13}C NMR spectra for dominicin and laughine. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 258283). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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