

Tupuseleiamides and Basiliskamides, New Acyldipeptides and Antifungal Polyketides Produced in Culture by a *Bacillus laterosporus* Isolate Obtained from a Tropical Marine Habitat

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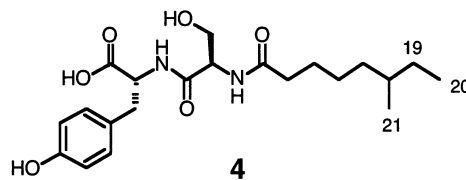
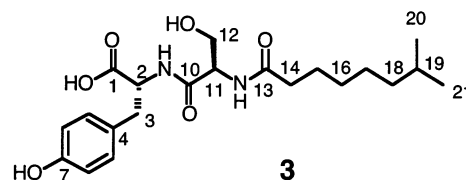
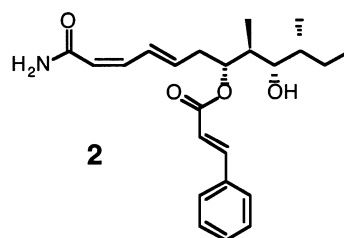
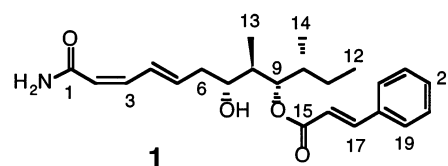
Laboratory cultures of PNG 276, a *Bacillus laterosporus* isolate obtained from coastal waters off Papua New Guinea, have been shown to produce the novel metabolites basiliskamide A (**1**), basiliskamide B (**2**), tupuseleiamide A (**3**), and tupuseleiamide B (**4**). The structures of **1** to **4** were elucidated by analysis of spectroscopic data and chemical degradation. Basiliskamides A (**1**) and B (**2**) show potent in vitro anti-*Candida* activity.

The widespread occurrence of antibiotic-resistant human pathogens and the paucity of effective antifungal drugs have created an urgent need for new antimicrobial agents.^{1,2} Microorganisms isolated from marine habitats represent a potentially rich and relatively unexplored resource for the discovery of new antibiotics.^{3–5} We have previously reported that the bacterium PNG-276, obtained from the tropical waters off the coast of Papua New Guinea and tentatively identified as *Bacillus laterosporus*, produces the cyclic decapeptide antibiotics loloatins A–D^{6,7} and the linear cationic peptide antibiotic bogorol A⁸ in laboratory culture. Crude organic extracts obtained from PNG-276 cultures also show potent inhibition of *Candida albicans* and *Escherichia coli*. Further chemical investigations of PNG276 cultures grown both on solid agar and in liquid broth in an attempt to identify the compounds responsible for the remaining biological activity exhibited by the culture extracts have resulted in the isolation of the novel antifungal metabolites basiliskamides A (**1**) and B (**2**) and two new acyldipeptides, tupuseleiamides A (**3**) and B (**4**), whose structures are described below.

Results and Discussion

PNG-276 was first grown for 5 days as confluent lawns on trays of solid tryptic soy agar supplemented with NaCl to a final concentration of 1%. The cultures were harvested by gently scraping the bacterial cells from the agar surface, and the cell mass was exhaustively extracted with MeOH. Bioassay-guided fractionation of the antifungal MeOH crude extract using Sephadex LH20 and reversed-phase open column chromatography and HPLC gave pure samples of basiliskamides A (**1**) and B (**2**).

Basiliskamide A (**1**) was isolated as a clear solid that gave a $[M + H]^+$ ion at m/z 386.2336 in the high-resolution FABMS appropriate for the molecular formula of $C_{23}H_{31}NO_4$. The ^{13}C NMR spectrum of **1** (Table 1) showed only 21 well-resolved resonances, indicating that there was an element of symmetry in the molecule. Resonances in the 1H NMR spectrum (Table 1) of basiliskamide A were all well dispersed, which facilitated the interpretation of the



COSY, HMQC, and HMBC spectra and the subsequent identification of the two major substructures A and B (Figure 1).

A broad three-proton 1H NMR resonance at δ 7.41–7.40 (H-20/22, H-21), which showed HMQC correlations to carbon resonances at δ 130.4 (C-21) and 128.9 (C-20/22), along with a broad two-proton 1H NMR resonance at δ 7.71 (H-19/23), which showed a HMQC correlation to a carbon resonance at δ 128.4 (C-19/23), were all assigned to a monosubstituted phenyl ring, which accounted for the element of symmetry required by the ^{13}C NMR data. A pair of one-proton doublets at δ 7.65 (H-17) and 6.61 (H-16) in the 1H NMR, which showed COSY correlations to each other, were assigned to a vinyl group. HMBC correlations

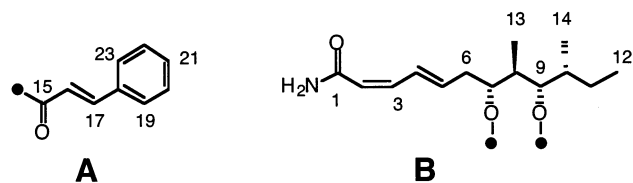
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Table 1. NMR Data for Basiliskamides A (**1**) and B (**2**) Recorded at 500 MHz (^1H) and 100 MHz (^{13}C) in $\text{DMSO}-d_6$

C#	basiliskamide A (1)		basiliskamide B (2)	
	^1H	^{13}C	^1H	^{13}C
1		167.4		167.4
2	5.55 (d, $J = 11$ Hz)	119.3	5.57 (d, $J = 11$ Hz)	119.9
3	6.31 (dd, $J = 11$, 11 Hz)	140.5	6.33 (dd, $J = 11$, 11 Hz)	140.0
4	7.40 (dd, $J = 11$, 15 Hz)	128.2	7.51 (dd, $J = 15$, 11 Hz)	128.8
5	5.91 (dt, $J = 15$, 7 Hz)	140.5	5.87 (dt, $J = 15$, 7 Hz)	138.0
6	2.28 (m)	34.7	2.53 (m)	31.8
6'	1.99 (m)		2.36 (m)	
7	3.49 (m)	69.6	5.40 (dt, $J = 10.5$, 3 Hz)	74.0
8	2.06 (m)	40.7	1.92 (m)	39.4
9	4.92 (dd, $J = 9.5$, 2 Hz)	76.3	3.26 (m)	73.0
10	1.67 (m)	35.5	1.40 (m)	36.3
11	1.25 (m)	26.4	1.38 (m)	26.5
11'	1.11 (m)		1.21 (m)	
12	0.87 (t, $J = 7.5$ Hz)	10.1	0.85 (t, $J = 7$ Hz)	11.8
13	0.84 (d, $J = 7$ Hz)	11.6	0.83 (d, $J = 7$ Hz)	10.7
14	0.90 (d, $J = 7$ Hz)	12.8	0.74 (d, $J = 7$ Hz)	12.1
15		166.0		165.5
16	6.61 (d, $J = 16$ Hz)	118.0	6.59 (d, $J = 16$ Hz)	118.5
17	7.65 (d, $J = 16$ Hz)	144.6	7.60 (d, $J = 16$ Hz)	144.1
18		134.0		134.0
19/23	7.71 (m)	128.4	7.70 (m)	128.2
20/22	7.41 (m)	128.9	7.40 (m)	129.0
21	7.40 (m)	130.4	7.40 (m)	130.2
OH	4.57 (d, $J = 5$ Hz)		4.48 (m)	
NH ₂	7.31, 6.83 (s)		7.34, 6.86 (s)	

**Figure 1.** Substructures of basiliskamide A (**1**).

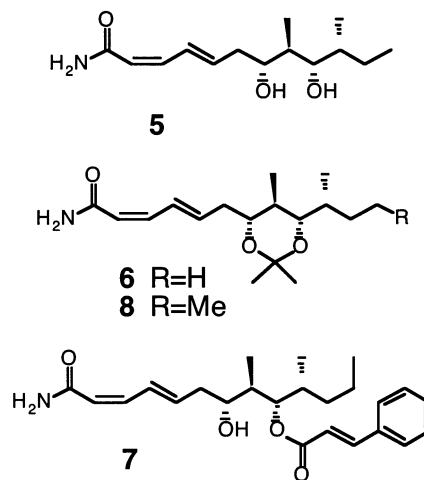
observed between the vinyl resonance at δ 7.65 (H-17) and the phenyl carbon resonance at δ 128.4 (C-19/-23) showed that the vinyl group was the single substituent on the phenyl ring. Additional HMBC correlations observed between both the vinyl proton resonances at δ 7.65 (H-17) and 6.61 (H-16) and a carbon resonance at δ 166.0 (C-15) showed that the phenyl and vinyl fragments were part of a cinnamoyl residue **A**. The vinyl protons had a vicinal scalar coupling of 16 Hz, demonstrating the cinnamoyl residue had the *E* configuration.

Analysis of COSY, HMQC, and HMBC data (Table 1) collected for basiliskamide A routinely identified substructure **B**, including the positions of the $\Delta^{2,3}$ and $\Delta^{4,5}$ olefins, the methyl branches at C-8 and C-10, and the presence of -OR substituents at C-7 and C-9. HMBC correlations observed between both δ 5.55 (H-2) and 6.31 (H-3) and a carbon resonance at δ 167.4 (C-1) showed that C-2 was attached to a carbonyl carbon. Only one nitrogen and two hydrogen atoms remained unaccounted for by the cinnamoyl **A** and the linear **B** fragments, suggesting that the C-1 carbonyl was a primary amide. A pair of broad one-proton resonances at δ 7.31 and 6.83, which showed COSY correlations to each other but did not show HMQC correlations to carbon resonances, were assigned to the primary amide NH protons. The NH resonance at δ 6.83 showed a HMBC correlation to δ 119.3 (C-2), confirming the presence of the primary amide at the terminus of the linear fragment **B**. A COSY correlation observed between

an OH proton resonance at δ 4.57 and a resonance at δ 3.49 (H-7) showed that there was an alcohol functionality at H-7, and therefore, the cinnamoyl fragment had to be attached to the linear carbon chain via an ester linkage at C-9. A HMBC correlation observed between the methine resonance at δ 4.92 (H-9) and the cinnamoyl carbonyl resonance at δ 166.0 (C-15) confirmed the presence of the ester linkage and completed the constitution of basiliskamide A (**1**).

The configurations of the $\Delta^{2,3}$ and $\Delta^{4,5}$ olefins were assigned on the basis of their vicinal coupling constants and confirmed by a series of NOE experiments. H-2 and H-3 had a vicinal coupling constant of 11 Hz typical of *Z* olefins, while H-4 and H-5 had a 15 Hz vicinal coupling typical of *E* olefins. Irradiation of the H-3 resonance at δ 6.31 induced a NOE in the H-2 resonance at δ 5.55, in agreement with the *Z* configuration for the $\Delta^{2,3}$ olefin, and irradiation of the H-5 resonance (δ 5.91) induced a NOE in the H-3 resonance (δ 6.31), consistent with the *E* configuration for the $\Delta^{4,5}$ olefin.

An empirical method developed by Rychnovsky was used to establish the relative stereochemistry of the chiral centers at C-7 and C-9 in basiliskamide A (**1**). Diisobutylaluminum hydride (DIBAL) reduction of the ester bond of **1** yielded the corresponding 1,3-diol **5**. Reaction of diol **5** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate gave the acetonide derivative **6**. Analysis of the



HMQC data for **6** showed that the acetonide methyl carbon resonances had chemical shifts of 19.8 and 30.4 ppm, typical of acetonides formed from *syn*-1,3-diols.⁹ Further analysis of the ^1H NMR data for the acetonide **6** revealed that the dioxane ring existed in a chair conformation, with the C-6 and C-10 carbons equatorial. Irradiation of the H-10 resonance simplified the H-9 resonance to a doublet, facilitating the assignment of a vicinal coupling constant of 9.5 Hz between H-9 and H-8. This indicated that H-8 and H-9 were both axial, and therefore, the C-13 methyl was equatorial and the relative stereochemistries at C-7, C-8, and C-9 are as shown in **1**. The *S* configuration of the secondary alcohol at C-7 in basiliskamide A was established using Ohtani's method of Mosher ester analysis (for C-7 MTPA ester of **1**: ($\Delta\delta_S - \delta_R$) H-3 (+0.16), H-5 (+0.21), H-8 (-0.09), H-10 (-0.06)).¹⁰ The configuration of C-10 in **1** was not determined experimentally. However, basiliskamide A is a homologue of the known compound YM-47522 (**7**), and the absolute configuration of C-10 in **7** has been determined to be *R* by synthesis.^{11,12} Since the other chiral centers in **1** and **7** have identical configurations (7*S*, 8*S*, 9*R*), it was assumed that basiliskamide A (**1**) also has the

Table 2. NMR Data for Tupuseleiamides A (**3**) and B (**4**) Recorded at 500 MHz (¹H) and 100 MHz (¹³C) in DMSO-*d*₆

	C#	tupuseleiamide A (3)		tupuseleiamide B (4)	
		¹ H	¹³ C	¹ H	¹³ C
Tyr	COOH	12.61 (br s)		12.68 (br s)	
	1		172.6		172.5 (br)
	2	4.34 (m)	53.6	4.32 (m)	53.5
	3	2.89 (m)	35.9	2.90 (m)	35.6
	3'	2.80 (m)		2.80 (m)	
	4		127.1		127.0
	5,9	6.96 (d, <i>J</i> = 8.0 Hz)	130.1	6.96 (d, <i>J</i> = 8.0 Hz)	130.0
	6,8	6.62 (d, <i>J</i> = 8.0 Hz)	114.9	6.62 (d, <i>J</i> = 8.0 Hz)	114.8
	7		155.9		155.4
	OH	9.14 (s)		9.20 (s)	
	NH	7.77 (d, <i>J</i> = 6.5 Hz)		7.84 (d, <i>J</i> = 7.5 Hz)	
Ser	10		170.0		179.9
	11	4.30 (m)	54.8	4.30 (m)	54.7
	12	3.52 (m)	61.6	3.53 (m)	61.5
	12'	3.49 (m)		3.48 (m)	
	OH	4.75 (m)		4.80 (m)	
	NH	7.75 (d, <i>J</i> = 7.4 Hz)		7.81 (d, <i>J</i> = 8.0 Hz)	
<i>N</i> -acyl group	13		172.3		172.2
	14	2.10 (t, <i>J</i> = 8.0 Hz)	35.1	2.09 (t, <i>J</i> = 8.0 Hz)	35.1
	15	1.46 (m)	25.2	1.44 (m)	25.4
	16	1.20 (m)	28.9	1.26 (m, <i>J</i> = 1.19 Hz)	26.0
	17	1.23 (m)	26.1	1.27 (m)	28.7
	18	1.13 (m)	38.3	1.03 (m)	35.9
	19	1.48 (m)	27.3	1.26 (m)	33.4
	20	0.83 (d, <i>J</i> = 6.6 Hz)	22.4	0.81 (t, <i>J</i> = 7.0 Hz)	11.0
	21	0.83 (d, <i>J</i> = 6.6 Hz)	22.4	0.80 (d, <i>J</i> = 6.2 Hz)	18.9

R configuration at C-10. The observation that the H-9/H-10 vicinal coupling constant is ~2 Hz both in the acetonide **6** prepared from basiliskamide A (**1**) and in the corresponding acetonide (**8**)¹¹ prepared from YM-47522 (**7**) is consistent with identical *R* configurations at C-10 in both **1** and **7**.

Basiliskamide B (**2**) was isolated as a clear solid that gave a [M + H]⁺ ion at *m/z* 386.2336 in the high-resolution FABMS appropriate for the molecular formula of C₂₃H₃₁NO₄, identical to the formula of basiliskamide A (**3**). Analysis of the 1D and 2D NMR data obtained for **2** (Table 1) showed that it was simply a regioisomer of **1**, in which the cinnamoyl ester was at C-7 instead of C-9. Basiliskamides A (**1**) and B (**2**) were both converted to the same diol **5** by DIBAL reduction, demonstrating that both molecules had identical configurations.

Next, liquid cultures of PNG-276 were examined in an attempt to identify the *E. coli* active components produced by the culture. The liquid broth from cultures of PNG-276 was lyophilized to half its volume, neutralized with strong acid, and extracted with EtOAc. The organic extract was loaded onto a reversed-phase Sep-Pak and subjected to a five-step gradient elution from 1:1 MeOH/H₂O to 100% MeOH. The 4:1 MeOH/H₂O fraction was concentrated to dryness in vacuo and further purified by reversed-phase HPLC, eluting with 2:3 MeCN/H₂O (0.2% TFA) to give tupuseleiamides A (**3**) and B (**4**). Neither of the tupuseleiamides showed activity in our antibiotic assays, and the *E. coli* active compounds, which were not isolated in sufficient quantities to permit structure elucidation, are still under investigation.

Tupuseleiamide A (**3**) was isolated as an optically active colorless solid that gave a HRFABMS [M + H]⁺ peak at *m/z* 409.2342, corresponding to a molecular formula of C₂₁H₃₂N₂O₆, requiring seven degrees of unsaturation. Inspection of the ¹H, ¹³C, and HMQC NMR spectra (Table 2) revealed signals that were suggestive of a peptide, and chiral GC analysis of the pentafluoropropionyl amide isopropyl ester (PFPA-IPA) derivatized amino acids liberated from **3** by 6 N HCl hydrolysis revealed the presence of D-tyrosine and D-serine.

Routine analysis of the 1D and 2D NMR data identified the ¹H and ¹³C resonances corresponding to the tyrosine and serine residues (Table 2). A 7-methyloctanoyl fragment, which accounted for the remaining site of unsaturation, was also routinely identified from analysis of the COSY, HMQC, and HMBC data (Table 2). The ¹H NMR spectrum contained a broad singlet at δ 12.61, which showed no HMQC correlations to carbon resonances. This resonance was assigned to a carboxylic acid OH.

The constitution of the acyldipeptide **3** was established by HMBC correlations. The tyrosine NH resonance at δ 7.77 showed a HMBC correlation to the carbonyl resonance of the serine residue at δ 170.0, and the serine NH resonance at δ 7.75 showed a correlation to the 7-methyloctanoyl carbonyl resonance at δ 172.3 to give the sequence shown in **3**.

Tupuseleiamide B (**4**) was isolated as an optically active colorless solid that gave a HRFABMS [M + Na]⁺ peak at *m/z* 431.2157, corresponding to a molecular formula of C₂₁H₃₂N₂O₆. Analysis of the 1D and 2D NMR data (Table 2) for tupuseleiamide B (**4**) showed that it differed from tupuseleiamide A (**3**) only in the methyloctanoyl fragment. The chemical shift-equivalent methyl doublets at δ 0.83 in the ¹H NMR spectrum of **3**, assigned to the *iso* terminus of the methyloctanoyl residue, were replaced by a triplet at δ 0.81 and a doublet at δ 0.80 in the ¹H NMR spectrum of **4**. Analysis of the COSY, HMQC, and HMBC NMR data obtained for tupuseleiamide B showed that the methyloctanoyl fragment had the *anteiso* structure shown in **4**. The configuration at C-18 was not determined. Tupuseleiamides A (**3**) and B (**4**) are novel acyldipeptides in which the amino acids both have the nonprotein D configuration.

MICs for the basiliskamides against *C. albicans* and *Aspergillus fumigatus* were determined using a standard agar dilution assay in order to provide a comparison with the published values for the homologue YM-45722 (**7**).¹³ The observed MIC values were as follows: *C. albicans* [basiliskamide A (**1**), 1.0 μg/mL; basiliskamide B (**2**), 3.1 μg/mL; YM-45722 (**7**), 25 μg/mL], and *A. fumigatus* [basiliskamide A (**1**), 2.5 μg/mL; basiliskamide B (**2**), 5.0 μg/mL; YM-45722 (**7**), >50 μg/mL]. A macrobroth dilution assay

was used to compare the activity of basiliskamide A (**1**) with amphotericin B against seven fresh clinical isolates of *C. albicans*. Both compounds had identical MICs of 0.5 $\mu\text{g/mL}$ against each of the seven isolates.

The *in vitro* cytotoxicity of basiliskamide A (**1**) was also compared to amphotericin B. Human diploid fibroblast cells (foreskin) were exposed to varied concentrations of the two compounds in RPMI 1640 tissue culture medium and observed for cytopathic effects. Basiliskamide A produced minimal cytotoxicity at a concentration of 100 $\mu\text{g/mL}$, the highest concentration tested, and no cytopathic effect at lower concentrations. Amphotericin B produced cytopathic effects at a concentration of only 12.5 $\mu\text{g/mL}$, and the cells were destroyed at a concentration of 100 $\mu\text{g/mL}$.

Basiliskamides A (**1**) and B (**2**) are new antifungal metabolites that show potent activity against *C. albicans*. When tested against a small panel of recent clinical isolates of *C. albicans*, basiliskamide A (**1**) showed *in vitro* activity comparable to amphotericin B. Basiliskamide A also showed at least 4-fold less cytotoxicity for normal human fibroblast cells than amphotericin B. The linear polyketide chain in basiliskamide A (**1**) is one methylene carbon shorter than the corresponding polyketide chain in the known *Bacillus* metabolite YM-45722 (**7**).^{11,13} Biogenetically this can be rationalized by incorporation of a propionate starter unit in **7** and an acetate starter unit in **1**. Interestingly, the loss of one carbon from the polyketide chain in **1** appears to result in a significant increase in activity against both *C. albicans* and *A. fumigatus* relative to **7**.

Experimental Section

General Experimental Procedures. Specific rotations were determined using a Jasco P-1010 polarimeter with a sodium lamp (589 nm). UV spectra were recorded with a Carey UV-vis spectrophotometer. IR spectra were recorded with a Perkin-Elmer 16000 series FTIR. NMR spectra were recorded on AMX500 and AV400 Bruker spectrometers. Chemical shifts are quoted in parts per million (ppm), δ , and are referenced to the deuterated solvent used (DMSO- d_6 δ_{H} 2.49, δ_{C} 34.9; CDCl_3 δ_{H} 7.26, δ_{C} 77.0). Coupling constants (J) are reported in hertz. Size exclusion chromatography was performed using Sephadex LH-20 gel permeation beads. Reversed-phase column chromatography was performed using either a 10 or 2 g Waters Sep-Pak. Normal-phase column chromatography was performed using 230–400 mesh silica gel 60. HPLC separations were performed with a Whatman Partisil 10 ODS-3 column.

PNG-276. The marine bacterial isolate PNG-276, tentatively identified as *B. laterosporus* by the analysis of cellular fatty acids and 16S RNA, was obtained from the tissues of an unidentified tube worm collected at –15 m off the coast of Loloata Island, Papua New Guinea. PNG-276 has been cryopreserved and deposited in the marine microbial collections at UBC and SeaTek. The basiliskamides and tupuseliamides were named after marine geographic features in the region of Loloata Island.

Basiliskamides: Culture Conditions and Isolation. PNG-276 was grown in moderate scale culture as confluent lawns for 5 days at 16 °C on trays of solid tryptic soy agar supplemented with NaCl to a final concentration of 1%. The cultures were harvested by gently scraping the cells from the agar surface.

PNG-276 cells (21.5 g, dry weight) were extracted with MeOH over a period of 6 days. The combined methanolic extracts were concentrated *in vacuo* and partitioned between EtOAc (3 \times 100 mL) and H₂O/MeOH (10:1, 200 mL). The EtOAc extract was dried over anhydrous Na₂SO₄, filtered, and reduced to dryness *in vacuo* (6.5 g). The extract was then chromatographed, in two parts, on Sephadex LH-20 (100% MeOH) to give 226 mg of a fraction containing a UV absorbing

compound. This fraction was subsequently subjected to a step gradient (1:1 MeOH/H₂O to 100% MeOH) on a reversed-phase Waters 10 g Sep-Pak. The UV-absorbing fraction (82 mg) was further separated into crude basiliskamide A (**1**) and crude basiliskamide B (**2**) (28 mg total) by a normal-phase silica gel column (4:1 EtOAc/CH₂Cl₂). Final purification by reversed-phase HPLC (7:3 MeOH/H₂O) yielded pure basiliskamides A (**1**, 14 mg) and B (**2**, 9 mg) as clear solids.

Basiliskamide A (1): clear solid; $[\alpha]_{\text{D}}^{25}$ (MeOH) –78°; UV (MeOH) λ_{max} 262 nm (ϵ 41 000); IR (film) ν_{max} 3348, 3205, 2966, 2934, 1705, 1697, 1635, 1595, 1450 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; HRFABMS $[\text{M} + \text{H}]^+$ m/z 386.2336 (calcd for C₂₃H₃₂NO₄, 386.2331).

Basiliskamide B (2): clear solid; $[\alpha]_{\text{D}}^{25}$ (MeOH) –12°; UV (MeOH) λ_{max} 262 nm (ϵ 43 000); IR (film) ν_{max} 3348, 3205, 2962, 2926, 1702, 1664, 1637, 1595, 1450 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; HREIMS $[\text{M}]^+$ m/z 385.2253 (calcd for C₂₃H₃₁NO₄, 385.2253).

Reduction of Basiliskamides to 1,3-Diol (5). To a stirred solution (–78 °C) of basiliskamide A (**1**) (4.7 mg, 12 μmol) in 1 mL of THF was added 4 equiv of diisobutylaluminum hydride (DIBAL-H) (1 M in hexane). The reaction was stirred for a further 18 h, then diluted with EtOAc (3 mL) and quenched by the addition of 2 mL of NH₄Cl(aq), stirring until the reaction mixture turned cloudy (10 min). The mixture was extracted three times with EtOAc, and the combined organics were reduced to dryness *in vacuo*. Preparative normal-phase TLC (100% EtOAc) followed by reversed-phase HPLC (7:3 MeOH/H₂O) gave 2 mg of **5** (64%): ¹H NMR (500 MHz, DMSO- d_6) δ 7.44 (1H, dd, J = 15 Hz, 11 Hz), 7.35 (1H, br s, NH), 6.86 (1H, br s, NH), 6.36 (1H, dd, J = 11 Hz), 6.02 (1H, dt, J = 15 Hz, 7 Hz), 5.57 (1H, d, J = 11 Hz), 4.48 (1H, d, J = 5.4 Hz, OH), 4.69 (1H, d, J = 4.4 Hz, OH), 3.80 (1H, m), 3.23 (1H, m), 2.26 (1H, m), 2.07 (1H, m), 1.61 (1H, m), 1.36 (1H, m), 1.35 (1H, m), 1.18 (1H, m), 0.84 (3H, t, J = 7 Hz), 0.72 (3H, d, J = 7 Hz), 0.66 (3H, d, J = 7 Hz); HRFABMS $[\text{M} + \text{H}]^+$ m/z 256.1921 (calcd for C₁₄H₂₆NO₃, 256.1913).

Treatment of basiliskamide B (**2**) as described above generated diol **5**, identical by spectroscopic and chromatographic comparison with **5** obtained from **1**.

Formation of Acetonide (6). To a stirred solution of 1.5 mg of diol **5** (5.9 μmol) in 0.5 mL of 2,2-dimethoxypropane was added pyridinium *p*-toluenesulfonate (5 wt % of diol **5**). The reaction mixture was stirred and heated at 60 °C for 1 h. The reaction mixture was filtered through silica (100% EtOAc), and the solvents were removed *in vacuo*. Reversed-phase HPLC (4:1 MeOH/H₂O) yielded 1 mg of **6** (58%): ¹H NMR (400 MHz, DMSO- d_6) δ 7.46 (1H, dd, J = 15 Hz, 11 Hz, H-4), 7.35 (1H, br s, NH), 6.85 (1H, br s, NH), 6.37 (1H, dd, J = 11 Hz, 11 Hz, H-3), 5.94 (1H, dt, J = 15 Hz, 7 Hz, H-5), 5.58 (1H, d, J = 11 Hz, H-2), 3.57 (1H, m, H-7), 3.48 (1H, dd, J = 9.5 Hz, 2 Hz, H-9), 2.44 (1H, m, H-6), 2.19 (1H, m, H-6'), 1.54 (1H, m, H-10), 1.36 (3H, s, Me-17), 1.33 (1H, m, H-8), 1.30 (1H, m, H-11), 1.25 (1H, m, H-11'), 1.23 (3H, s, Me-16), 0.83 (3H, t, J = 7 Hz, Me-12), 0.75 (3H, d, J = 7 Hz, Me-13), 0.71 (3H, d, J = 7 Hz, Me-14); ¹³C NMR (DMSO- d_6 , 100 MHz) δ 167.8 (C-1), 140.6 (C-3), 138.5 (C-5), 128.9 (C-4), 120.2 (C-2), 97.6 (C-15), 74.9 (C-9), 74.0 (C-7), 36.5 (C-6), 35.1 (C-8), 34.6 (C-10), 30.4 (C-16), 26.7 (C-11), 19.8 (C-17), 12.7 (C-13), 12.1 (C-12), 11.6 (C-14); HRFABMS $[\text{M} + \text{H}]^+$ m/z 296.2220 (calcd for C₁₇H₃₀NO₃, 296.2257).

Reaction of Basiliskamide A (1) with (R)-MTPA Acid Chloride. To a stirred solution of **1** (1.5 mg, 4.0 μmol) in 0.5 mL of dry CH₂Cl₂ were added a few crystals of DMAP, a drop of triethylamine, and (R)-MTPA acid chloride (4 mg, 16.0 mmol). The solution was stirred for 16 h. The removal of solvent *in vacuo*, preparative reversed-phase TLC (100% MeOH), followed by reversed-phase HPLC (4:1 MeOH/H₂O) gave the (R)-MTPA ester of basiliskamide A (0.8 mg, 33%): ¹H NMR (500 MHz, DMSO- d_6) δ 7.72 (3H, br envelope), 7.43 (9H, br envelope), 7.35 (1H, br s), 6.84 (1H, br s), 6.70 (1H, d, J = 16 Hz), 6.20 (1H, dd, J = 11 Hz, 11 Hz), 5.58 (2H, m), 5.17 (1H, m), 4.98 (1H, m), 3.43 (3H, s), 2.60 (1H, m), 2.26 (2H, br m), 1.72 (1H, m), 1.29 (2H, br m), 1.17 (1H, m), 0.95 (3H, d, J = 7 Hz), 0.93 (3H, d, J = 7 Hz), 0.88 (3H, t, J = 7

Hz); HRFABMS $[M + H]^+$ m/z 602.2715 (calcd for $C_{33}H_{39}NO_6F_3$, 602.2729).

Reaction of Basiliskamide A (1) with (S)-MTPA Acid Chloride. A solution of **1** (1.5 mg) in 0.5 mL of dry CH_2Cl_2 was treated as above, but with the (S)-MTPA acid chloride (4 mg, 16.0 mmol) to give the (S)-MTPA ester of basiliskamide A (0.4 mg, 17%): 1H NMR (500 MHz, $DMSO-d_6$) δ 7.72 (3H, br envelope), 7.54 (1H, m), 7.43 (8H, br envelope), 7.38 (1H, br s), 6.91 (1H, br s), 6.72 (1H, d, $J = 16$ Hz), 6.36 (1H, dd, $J = 11$ Hz, 11 Hz), 5.78 (1H, m), 5.64 (1H, d, $J = 11$ Hz), 5.13 (1H, m), 4.94 (1H, m), 3.42 (3H, s), 2.63 (1H, br m), 2.35 (1H, m), 2.17 (1H, m), 1.65 (1H, m), 1.27 (2H, br m), 1.15 (1H, m), 0.89 (3H, d, $J = 7$ Hz), 0.87 (3H, t, $J = 7$ Hz), 0.70 (3H, d, $J = 7$ Hz); HRFABMS $[M + H]^+$ m/z 602.2735 (calcd for $C_{33}H_{39}NO_6F_3$, 602.2730).

Tupuseleiamides: Culture Conditions and Isolation. Multiple 100 mL cultures (tryptic soy broth with 1% NaCl) were inoculated with 500 mL of a 1.0 McFarland standardized bacterial suspension of PNG-276 in 0.85% saline. The cultures were then grown for an additional 6–7 days at 22 °C on a shaker.

Liquid culture broth (800 mL) of PNG-276 was lyophilized to half its volume, neutralized with strong acid, and extracted with EtOAc. The organic extract (1.3 g) was loaded onto a reversed-phase Sep-Pak (10 g) and subjected to a five-step gradient elution from 1:1 MeOH/H₂O to 100% MeOH. The 4:1 MeOH/H₂O fraction was concentrated to dryness in vacuo (88 mg) and further purified by reversed-phase HPLC, eluting with 2:3 MeCN/H₂O (0.2% TFA) to give tupuseleiamides A (**3**, 2.0 mg) and B (**4**, 0.3 mg).

Tupuseleiamide A (3): clear solid; $[\alpha]_D -78^\circ$ (MeOH); UV (MeOH) γ_{max} (ϵ) 262 nm (41 000); IR (film) ν_{max} 3449, 1728, 1660, 1614, 1516, 1222 cm^{-1} ; 1H and ^{13}C NMR data, see Table 2; HRFABMS $[M + H]^+$ m/z 409.2342 (calcd for $C_{21}H_{33}N_2O_6$, 409.2339).

Tupuseleiamide B (4): clear solid; $[\alpha]_D -78^\circ$ (MeOH); IR (film) ν_{max} 3449, 1717, 1660, 1620, 1517, 1224 cm^{-1} ; UV (MeOH) γ_{max} (ϵ) 262 nm (41 000); 1H and ^{13}C NMR data, see Table 2; HRFABMS $[M + Na]^+$ m/z 431.2157 (calcd for $C_{21}H_{32}N_2O_6Na$, 431.2158).

Total Acid Hydrolysis and GC Analysis. In a screw-top vial, tupuseleiamide A (**3**) (0.5 mg) was dissolved in 6 N HCl (1 mL) and heated at 110 °C for 16 h. The HCl was removed

under a stream of N_2 gas. *i*-PrOH/HCl (250 mL) was added to the reaction vial and heated to 110 °C for a further 45 min followed by solvent removal under a stream of N_2 gas. Dichloromethane (250 mL) and pentafluoropropionyl anhydride (100 μ L) were added to the reaction vial and heated to 110 °C for 15 min. Excess reagent was removed under N_2 gas and the derivative was redissolved in dichloromethane (200 mL). Standards were prepared in the same fashion using optically pure l-amino acids and their racemic mixtures. The amino acid standards and the hydrolysates were analyzed on a 25 m chiralsil-Val Heliflex column with FID detection using the following conditions: He carrier, detector temp 90 °C, initial time 4 min, program rate 4 °C/min, final oven temp 200 °C, final time 27.5 min.

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