Theopapuamide, a Cyclic Depsipeptide from a Papua New Guinea Lithistid Sponge *Theonella swinhoei*

Anokha S. Ratnayake,[†] Tim S. Bugni,[†] Xidong Feng,[‡] Mary Kay Harper,[†] Jack J. Skalicky,[§] Kaleem A. Mohammed,[†] Cynthia D. Andjelic,[⊥] Louis R. Barrows,[⊥] and Chris M. Ireland*[,†]

Department of Medicinal Chemistry, Department of Biochemistry, and Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112, and Wyeth Research, 401 North Middletown Road, Pearl River, New York 10965

Received May 25, 2006

Theopapuamide (**1**), a new cytotoxic peptide, has been isolated from the lithistid sponge *Theonella swinhoei* from Papua New Guinea. The structure was established by analysis of NMR, mass spectrometry, and chemical methods. The undecapeptide (**1**) contains several unusual amino acid residues, of which the occurrence of β-methoxyasparagine and 4-amino-5-methyl-2,3,5-trihydroxyhexanoic acid (Amtha) is unprecedented in natural peptides. Compound **1** also contains an amide-linked fatty acid moiety, 3-hydroxy-2,4,6-trimethyloctanoic acid (Htoa). Theopapuamide (**1**) was cytotoxic against CEM-TART and HCT-116 cell lines, with EC_{50} values of 0.5 and 0.9 μM, respectively.

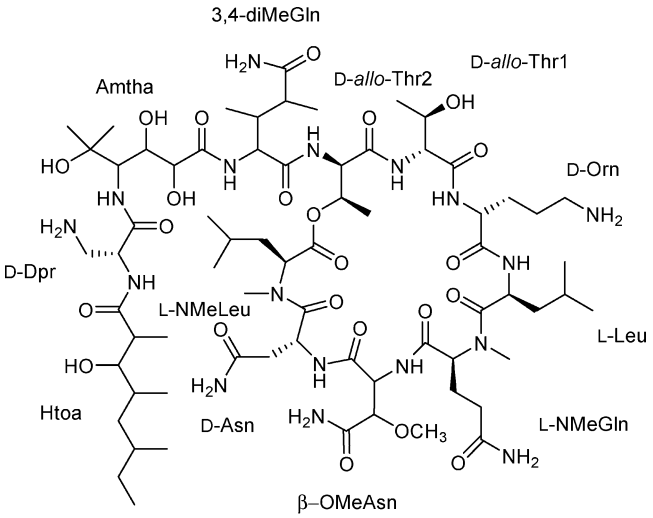
Marine sponges have proven to be a significant source of biologically active cyclic peptides and depsipeptides.[1] Among these sponge depsipeptides, callipeltin A (from a New Caledonian sponge *Callipelta* sp.),[2] neamphamide A (from a Papua New Guinea sponge *Neamphius huxleyi*),[3] and papuamides A and B (from Papua New Guinea sponges *Theonella mirabilis* and *Theonella swinhoei*)[4] are well known for their potent HIV-inhibitory activity and their structurally unique features incorporating several modified amino acid residues. For instance, the atypical amino acid residues 3,4-dimethyl-L-glutamine and β-methoxytyrosine are common to all of the above-mentioned marine depsipeptides but to date have not been described elsewhere. The rarity of these atypical amino acid residues has inspired their chemical synthesis.[5] The structural diversity found among lithistid sponge metabolites (genus *Theonella* and *Callipelta*) has been attributed to symbiotic microorganisms.[1]

As part of our continuing studies on *Theonella swinhoei* from Papua New Guinea,[6] the aqueous CH_3CN extract of the sponge was analyzed and proved active in an in vitro anti-HIV assay. Fractionation of the active extract resulted in the isolation of a new cyclic depsipeptide, theopapuamide (**1**). This paper describes the isolation, structure elucidation, and stereochemical analysis of theopapuamide (**1**).

Results and Discussion

The crude aqueous CH_3CN extract of *T. swinhoei* (family Theonellidae) was concentrated under vacuum and fractionated by C18 flash column chromatography. Further purification on Diaion HP-20 resin followed by CN-HPLC afforded the new cyclic undecapeptide theopapuamide (**1**, 15.8 mg, 3.95×10^{-3} % yield wet wt) as an off-white amorphous solid ($[\alpha]^{25}_D$ −3.0, *c* 0.86, MeOH).

For compound **1**, a molecular formula of $C_{69}H_{123}N_{17}O_{23}$ was established on the basis of the divalent molecular ion $[M + 2H]^{2+}$ observed at *m*/*z* 779.9563 by HRESI-FTMS. The protonated molecular ion at *m*/*z* 1558.9053 $[M + H]^+$ in the HRESI-FTMS was in agreement with a molecular weight of 1557.8980 ($\Delta = -0.2$ mmu) for the neutral compound **1**. The molecular formula suggested 17 units of unsaturation. Subsequent examination of 1D spectra of **1** reflected characteristic features of a peptide, such as an abundance of exchangeable N−H protons (δ_H 6.27−9.05) and carbonyl signals



Theopapuamide (1)

(δ_C 169.9−179.9) in the ^1H and ^{13}C NMR spectra, respectively. However, the interpretation of NMR spectra of **1** was hampered by the existence of multiple conformations in several of the common NMR solvents (MeOH-d_4, CD_3CN−DMSO-d_6, and DMSO-d_6). Attempts to improve resolution by employing acetone-d_6 and DMF-d_7 as solvents failed due to poor solubility of **1**. Moreover, efforts to achieve a favorable conformational ratio by addition of LiCl[7] (0.5−4 equiv, CD_3CN−DMSO-d_6, 25 °C) were also ineffective. Gratifyingly, one major conformation, with improved resolution, was observed using a mixture of CD_3CN-H_2O (4:1) at 25 °C.

The aliphatic nature of the peptide was supported by the absence of any sp^2-hybridized carbon resonances in the region between 110 and 150 ppm, as well as localization of resonances in the upfield region of the ^{13}C NMR spectrum of **1**. Due to the extensive overlap of aliphatic resonances in **1**, the assembly of individual amino acid residues required combined analysis of $^1H-^1H$-COSY, gHSQC, gHSQC-TOCSY, 1D-TOCSY, and z-DIPSI-tocsy[8] spectra. In conjunction with NMR analysis, the gross structure elucidation of **1** was guided by standard amino acid analysis,[9] which revealed molar concentrations of ~1:2:1 for Asx, Thr, and Leu, respectively. The presence of two N-methylated amino acid residues was suggested on the basis of the characteristic ^1H and ^{13}C chemical shifts of the N-methyl groups at δ_H 2.81 (δ_C 30.8) and 2.88 (δ_C 31.8). HMBC correlations were used to identify these N-methylated amino acids as NMeLeu and NMeGln, respectively. Additionally,

* To whom correspondence should be addressed. Tel: (801) 581-8305. Fax: (801) 585-6208. E-mail: cireland@pharm.utah.edu.
[†] Department of Medicinal Chemistry, University of Utah.
[‡] Wyeth Research.
[§] Department of Biochemistry, University of Utah.
[⊥] Department of Pharmacology and Toxicology, University of Utah.

10.1021/np060229d CCC: $33.50
Published on Web 11/10/2006

the presence of a methoxy-bearing amino acid residue was suggested by the characteristic ^{1}H and ^{13}C chemical shifts of the O-methyl group at δ_H 3.34 (δ_C 60.3). On the basis of HMBC correlations, the methoxy-bearing amino acid residue was identified as β-OMeAsn. An ester-linked threonine residue was suggested from a typical ~1.0 ppm downfield shift of the β-hydroxymethine proton (δ_H 5.55). A subsequent ^{15}N-HSQC experiment showed four pairs of signals for the primary amide protons of 3,4-diMeGln, Asn, β-OMeAsn, and NMeGln residues. However, the ^{1}H−^{15}N-correlations for the primary amino protons of Orn and Dpr could not be observed under the given experimental conditions [CD_3CN-H_2O (4:1), 25 °C].

The connectivity between amino acid residues was established on the basis of careful analysis of HMBC, WATERGATE-NOESY, and WATERGATE-ROESY[10] spectra. Further inspection of HMBC data supported formation of an eight-residue ring moiety in **1** via an ester linkage between the C-3-hydroxyl group of Thr2 (δ_{H-3} 5.55) and the C-1-carboxylic acid of NMeLeu (δ_{C-1} 171.5). Additionally, a NOESY cross-peak between Thr2 (δ_{H-3} 5.55) and NMeLeu (δ_{H-2} 5.07) was in agreement with such a macrocyclic ring formation. The constituents of the macrocycle, identified on the basis of sequential α-NH/NH and α-NH/NCH_3 NOESY correlations, consisted of NMeLeu (δ_{CH3} 2.81), Asn (δ_H 8.18), β-OMeAsn (δ_H 6.69), NMeGln (δ_{CH3} 2.88), Leu (δ_H 7.20), Orn (δ_H 8.00), and two residues of Thr (Thr1 δ_H 8.25, Thr2 δ_H 8.89). Furthermore, on the basis of HMBC and NOESY data, the α-NH (δ_H 8.89) of the ester-linked Thr was attached to a four-residue sequence of 3,4-diMeGln, 4-amino-5-methyl-2,3,5-trihydroxyhexanoic acid (Amtha), 2,3-diaminopropionic acid (Dpr), and 3-hydroxy-2,4,6-trimethyloctanoic acid (Htoa), forming the linear portion of the peptide. Of the component amino acid residues identified in undecapeptide **1**, five of the residues were also described in both callipeltin A and neamphamide A (3,4-diMeGln, Thr (×2), Leu, and NMeGln).[2,3] A Htoa dimer, bourgeanic acid, has been isolated from several *Ramalina* sp. lichens.[11]

Concurrent attempts to sequence the peptide by Edman degradation[12] were unsuccessful, signifying a concealed or chemically modified N-terminus in **1**. Additionally, theopapuamide (**1**) was resistant to digestion by a variety of proteases (trypsin, thermolysin, and pepsin)[13] likely due to collective effects of N-methylated amino acid residues, peptide bonds involving D-amino acids, and the cyclic nature of **1**. Initial attempts to clarify the amino acid sequence by MS/MS (ESI and MALDI) analysis of the intact cyclic depsipeptide (**1**) were not informative and yielded random cleavage products. Subsequently, the ester linkage in theopapuamide (**1**) was subjected to base hydrolysis (1 N KOH, rt, 2 h) to generate the acyclic peptide **2** [*m*/*z* 1576.9 $(M + H)^+$]. As anticipated, the acyclic peptide **2** was amenable to tandem MS approach.[14] Accordingly, successful sequence analysis of **2** was carried out on the basis of fragment ion spectra generated by SORI-CID ESI-FTMS/MS (Figure 1).

The absolute configurations of the amino acid constituents of theopapuamide (**1**) were determined by acid hydrolysis of **1** (6 N HCl, 110 °C, 12 h), followed by chiral HPLC analysis of the hydrolysate and RP-HPLC analysis of FDAA derivatives.[15] By chiral HPLC [Chirex phase 3126(D), *i*PrOH−2 mM $CuSO_4$ (5:95)], diagnostic peaks were observed for L-NMeLeu, D-Asp, L-Leu, L-NMeGlu, D-Orn, and D-Dpr residues. The chrial HPLC analysis failed to give sufficient separation of the L/ L-*allo*-Thr standards [D/L-*allo*-Thr (6.3)], but ruled out the presence of D/L-Thr in the hydrolysate of **1**. Subsequently, the acid hydrolysate of **1** was derivatized with FDAA and analyzed by RP-HPLC, which allowed for the assignment of D-configuration for the two *allo*-Thr residues. To facilitate configurational analysis of the remaining stereocenters in **1**, an X-ray crystallographic study was attempted. Unfortunately, under a variety of conditions, theopapuamide (**1**) yielded only fine crystals that were unsuitable for X-ray diffraction studies.

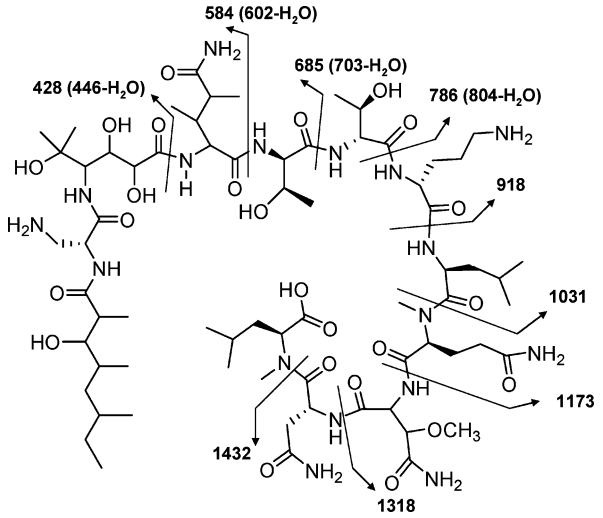


Figure 1. SORI-CID MS/MS fragmentations of acyclic peptide **2**.

In summary, theopapuamide (**1**) was cytotoxic against CEM-TART and HCT-116 cell lines with EC_{50} values of 0.5 and 0.9 μM, respectively. Theopapuamide (**1**) contains a high degree of D-amino acids and N-methylated amino acids along with several other modified amino acid residues of nonribosomal origin. Theopapuamide (**1**), neamphamide A, and callipeltins share the same basic structural skeleton of a seven- or eight-residue ring moiety formed by cyclization through a β-hydroxyl group of a Thr residue and a polysubstituted side chain linked to the amino terminus of a 3,4-diMeGln residue. Since the 3,4-diMeGln residue is conserved in all of the above-mentioned HIV-inibitory marine depsipeptides (callipeltin A, neamphamide A, and papuamides A,B), it has been postulated by Acevedo et al.[5c] that the 3,4-diMeGln residue may play a role in their biological activity. However, theopapuamide (**1**), which also carries the atypical 3,4-diMeGln residue, failed to show any appreciable HIV activity. The major difference between theopapuamide (**1**) and related HIV-inhibitory peptides is the absence of a β-methoxytyrosine residue in **1**. Therefore, this work indicates the potential significance of the β-methoxytyrosine residue for biological activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were acquired in spectroscopy grade methanol using a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded on a JASCO FT/IR-420 spectrometer. NMR spectra were recorded on Varian INOVA spectrometers operating at 500/600 MHz and 125/150 MHz, respectively. Chemical shifts are reported in ppm and were referenced to residual acetonitrile (δ_C 118.69; δ_H 1.49) in CD_3CN-water (4:1). Low-resolution mass spectra were obtained using a PE Sciex API III mass spectrometer operating in the ESI mode. High-resolution ESIMS analyses were performed on a Bruker (Billerica, MA) APEXII FTICR mass spectrometer equipped with an actively shielded 9.4 T superconducting magnet (Magnex Scientific Ltd., UK), an external Bruker APOLLO ESI source, and a Synrad 50W CO_2 CW laser.[14] Nanoelectrospray was employed. Typically, a 5 μL sample was loaded into the nanoelectrospray tip (New Objective, Woburn, MA) and a high voltage, about 1000 V, was applied between the nanoelectrospray tip and the capillary. Mass spectra were internally/externally calibrated using HP tuning mix. In the FTMS/MS experiments, the precursor ions were isolated using correlated sweep and then dissociated using a sustained off-resonance irradiation with collision-induced dissociation (SORI-CID). Automated amino acid analysis was performed on a Hitachi L8800 amino acid analyzer. Reversed-phase flash column chromatography was performed on Bakerbond C18, 40 μm, preparative LC packing. HPLC was performed on either a Beckman System Gold or an Agilent 1100 series instrument (diode array detector). For chiral

HPLC, a Phenomenex Chirex phase 3126 (D) (250 × 4.6 mm) column was utilized. All solvents were HPLC grade, purchased from Fisher Scientific. Amino acid standards were purchased from Aldrich or Fisher Scientific.

Biological Material. The marine sponge *Theonella swinhoei* Gray (family Theonellidae) was collected by hand using scuba from Milne Bay, Papua New Guinea (10°21.55′ S, 150°44.70′ E) in 2001. A voucher specimen of the sample (PNG01-5-051) is held at the University of Utah.

Extraction and Isolation. The frozen sponge (400 g wet wt) was cut into small pieces and soaked in CH_3CN-H_2O (1:1 v/v, 3 × 1000 mL). The aqueous CH_3CN extract was evaporated to dryness under vacuum, and the resulting crude material was applied onto a C18 column pre-equilibrated with aqueous CH_3CN (~90% H_2O). The

Table 1. ^1H and ^{13}C NMR (500/125 MHz) Data for Theopapuamide (**1**), in CD_3CN-H_2O (4:1)

no.	δ_C	δ_H	mult. (*J* in Hz)	HMBC (H → C)	wgnoesy[a,b]
			N-Methylleucine (NMeLeu)		
1	171.5				
2	55.3	5.07	dd (12.1,4.4)	171.5, 30.8	2.81, 1.76, 1.52, 1.39, 0.76, 5.55
3	36.9	1.76	m		2.81, 5.07
		1.52			2.81, 5.07
4	25.4	1.39	m		2.81, 5.07
4-CH_3	23.4	0.87	d (6.6)		
5	21.3	0.76	d (6.6)		2.81, 5.07, 2.69
N-CH_3	30.8	2.81	s	172.2, 55.3	0.76, 1.39, 1.76, 1.52, 8.18
			Asparagine (Asn)		
1	172.2				
2	48.0	5.20	m	37.7	8.18
3	37.7	2.69	dd (15.3,8.9)	174.9, 48.0	6.27, 8.18
		2.41	dd (15.3,4.4)	174.9, 172.2, 48.0	8.18
4	174.9				
4-NH_2		6.27	m		2.69
		7.04			
NH		8.18	d (9.0)	169.9	6.69, 5.20, 4.89, 2.81, 2.69, 2.41
			β-Methoxyasparagine (*β*-OMeAsn)		
1	169.9				
2	56.2	4.89	dd (9.0,1.7)	169.9, 80.5, 171.8	8.18, 4.41, 3.34
3	80.5	4.41	d (1.7)	173.4, 169.9, 60.3, 56.2	6.85, 7.19, 6.69, 4.89, 3.34
3-OCH_3	60.3	3.34	s	80.5	4.89, 6.69, 5.19
4	173.4				
4-NH_2		6.85	m	80.5	4.41
		7.19			4.41
NH		6.69	d (9.0)	171.8	8.18, 2.88, 5.19, 4.89, 2.01
			N-Methylglutamine (NMeGln)		
1	171.8				
2	57.8	5.19	m	171.8	6.69, 2.88
3	22.9	2.32	m		
		1.78			2.88
4	31.2	2.18	m		2.88
		2.01		22.9	6.91, 2.88
5	175.1				
5-NH_2		6.42	m		
		6.91			2.01
N-CH_3	31.8	2.88	s	177.7, 57.8	7.20, 5.19, 2.18, 6.69, 2.01, 1.78, 2.32
			Leucine (Leu)		
1	177.7				
2	50.7	4.55	dd (8.8, 3.0)		7.20, 0.95
3	39.0	2.00	m		7.20
		1.51			7.20
4	25.5	1.83	m		7.20
4-CH_3	23.6	1.00	d (6.6)	39.0	
5	20.9	0.95	d (6.6)	39.0	2.88, 7.20, 4.55
NH		7.20		174.1, 50.7	8.00, 4.55, 1.83, 2.00, 2.88, 1.51
			Ornithine (Orn)		
1	174.1				
2	52.0	4.54	m		8.00, 1.38, 1.97, 1.64,1.53
3	27.7	1.97	m		8.00, 4.54
		1.38			8.00, 4.54
4	24.4	1.64	m		8.00, 2.85
		1.53			8.00, 2.85
5	40.1	2.85	m		1.53, 1.64
5-NH_2		na			
NH		8.00	d (9.7)	171.2	4.54, 1.53, 1.64, 8.25, 1.38, 7.20, 1.97, 3.92c
			Threonine-1 (Thr1)		
1	171.2				
2	63.5	3.92	m	171.2	4.29, 1.24, 8.25, 8.00
3	67.2	4.29	m	171.2	3.92
3-OH		na			
4	19.8	1.24	d (6.6)	67.2, 63.5	3.92
NH		8.25	d (8.8)		3.92, 8.89, 8.00

Table 1. Continued

no.	δ_C	δ_H	mult. (*J* in Hz)	HMBC (H → C)	wgnoesy[a,b]
			Threonine-2 (Thr2)		
1	172.5				
2	55.7	5.15	d (3.3)		8.89, 5.55
3	71.2	5.55	dd (6.6, 3.3)	171.5	5.15, 1.17, 8.89, 5.07
4	14.3	1.17	d (6.6)	71.2, 55.7	
NH		8.89	d (10.0)	175.3	8.25, 5.55, 5.15, 4,22, 1.17
			3,4-Dimethylglutamine (3,4-diMeGln)		
1	175.3				
2	58.7	4.22	dd (9.9,5.5)	37.1, 175.3	9.05, 8.89, 7.07, 6.55
3	37.1	2.17	m		9.05
3-CH_3	14.3	1.04	d (6.7)	58.7, 42.0, 37.1	2.64
4	42.0	2.64	m		9.05, 7.07, 2.17, 1.13, 1.04, 6.55
4-CH_3	14.7	1.13	d (6.6)	179.9, 42.0, 37.1	2.64
5	179.9				
5-NH_2		7.07	m		2.64
		6.55			2.64
NH		9.05	br	176.2	4.22, 2.64, 2.17, 3.86
			4-Amino-5-methyl-2,3,5-trihydroxyhexanoic Acid (Amtha)		
1	176.2				
2	71.2	4.12	d (8.3)	72.6, 176.2	1.23, 1.09, 3.86
2-OH		na			
3	72.6	3.86	dd (8.3,3.2)	71.2, 56.4	9.05, 7.79, 4.12
3-OH		na			
4	56.4	3.96	m	74.5	7.79, 1.09, 1.23
4-NH		7.79	d (10.0)	171.3	3.86, 4.68
5	74.5				
5-CH_3	27.1	1.09	brs	74.5, 56.4	4.12, 3.96
5-OH		na			
6	27.6	1.23	brs	74.5, 56.4	3.96, 4.12
			2,3-Diaminopropionic Acid (Dpr)		
1	171.3				
2	52.2	4.68	brdd (7.1,6.9)	41.0	7.79, 8.39
3	41.0	3.48	m	52.2	4.68, 3.31
		3.31		52.2	4.68
3-NH_2		na			
NH		8.39	brd (8.0)		2.57, 4.68
			3-Hydroxy-2,4,6-trimethyloctanoic Acid (Htoa)		
1	179.6				
2	45.1	2.57	m	13.8, 179.6	8.39, 0.99, 0.90
2-CH_3	13.8	0.99	d (6.7)	179.6, 79.4, 45.1	1.72, 3.51, 2.57
3	79.4	3.51	brd (10.5,1.4)	13.8, 17.6	2.57, 1.72, 0.90, 0.99
3-OH		na			
4	32.2	1.72	m		3.51, 1.37
4-CH_3	17.6	0.90	d (6.6)	79.4, 32.2	2.57
5	36.2	1.18	m	17.6	2.57, 1.72[c]
		0.99		17.6	1.37[c]
6	32.2	1.37	m	30.5, 11.4, 19.4	3.51[c], 1.72[c]
6-CH_3	19.4	0.80	d (6.7)	30.5, 32.2	1.37[c], 1.72[c]
7	30.5	1.28	m	11.4, 19.4, 32.2	
		1.09		11.4, 19.4, 32.2	
8	11.4	0.83	d (7.3)	30.5, 32.2	

[a] 2D [^{1}H,^{1}H] NOESY with watergate water suppression, using a 200 ms mixing time. [b]Data recorded at 600 MHz. [c]Correlation based on a WATERGATE-ROESY experiment using a 50 ms mixing time, at 600 MHz. na: Not assigned.

column was eluted with a step gradient of CH_3CN-H_2O (0−100% CH_3CN) containing 0.05% TFA. The 20% CH_3CN eluate was chromatographed on Diaion HP-20 resin using an aqueous acetone solvent gradient of 20%−100% acetone to yield eight fractions. Fractions two through five were combined and further purified on HPLC (Phenomenex Luna 5 μm CN, 250 × 10 mm, flow rate 2.5 mL/min, detection 210 and 230 nm) using a gradient of 10%−100% CH_3CN in H_2O (0.05% TFA) over 30 min to yield four fractions. The third fraction was rechromatographed on a C8-HPLC column (ZORBAX Eclipse XDB 5 μm, 150 × 4.6 mm, flow rate 0.5 mL/min, detection 210 and 230 nm) using a gradient of 50%−100% MeOH in H_2O (0.05% TFA). Pure theopapuamide (**1**) was obtained as an off-white, amorphous solid (15.8 mg, 3.95×10^{-3}% yield wet wt).

Theopapuamide (1): off-white, amorphous solid, $[\alpha]^{25}_D$ −3.0 (*c* 0.86, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (3.28), 210 (4.12) nm; IR (film) ν_{max} 3305, 2915, 2850, 1650, 1525, 1450, 1195, 1130 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESI-FTMS *m*/*z* 1558.90536 (M + H)$^+$ (calcd for $C_{69}H_{124}N_{17}O_{23}$, 1558.90505).

Base Hydrolysis of Theopapuamide (1). Approximately 0.5 mg of **1** was treated with 1 N KOH (200 μL) at rt for 2 h. The reaction mixture was diluted by adding ice, neutralized with 0.5 N HCl, and extracted with *n*-BuOH (2 × 3 mL). The *n*-BuOH layer was evaporated to dryness, and an aliquot was analyzed by LRESIMS [*m*/*z* 1576.9 (M + H)$^+$].

Determination of Absolute Configuration. (a) Acid Hydrolysis. Theopapuamide (**1**), 100 μg, was dissolved in degassed 6 N HCl (250 μL) and heated in a sealed glass vial at 110 °C for 12 h. The solvent was removed in vacuo, and the residue was analyzed by HPLC.

(b) Chiral HPLC Analysis. The acid hydrolysate of **1** (aliquot of 10 μL) was analyzed by chiral HPLC on a Phenomenex D-penicillamine column [Chirex phase 3126 (D) (250 × 4.6 mm)]. The identities of amino acids of the acid hydrolysate were confirmed by comparison of their retention times with those of authentic standards using HPLC under the following conditions: mobile phase, *i*PrOH−2 mM $CuSO_4$ (5:95); flow rate, 1.0 mL/min; detection, UV 254 nm; retention times of the standards (min): L-NMeGlu (52.3), D-NMeGlu (24.1), L-Asp (20.0), D-Asp (26.9), L-Leu (18.5), D-Leu (29.0), L-NMeLeu (15.0), L-Dpr (5.8), D-Dpr (6.5), D-Orn (3.7), L-Orn (3.5), D/L-*allo*-Thr (6.3), D-Thr (5.8),

L-Thr (5.2); retention times of the component amino acids of the hydrolysate (min): L-NMeGlu (52.4), D-Asp (26.8), L-Leu (18.5), L-NMeLeu (14.9), D-Dpr (6.5), D-Orn (3.7).

(c) HPLC Analysis of Marfey's (L-FDAA) Derivatives. To the peptide hydrolysate (20 μg in 20 μL of H_2O) was added 6% triethylamine in H_2O (10 μL) followed by a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-alanine amide in acetone (L-FDAA, 20 μL). The mixture was heated at 50 °C in an oil bath for 1 h, quenched with 5% acetic acid (10 μL), and dried in vacuo. The residue was dissolved in H_2O (40 μL), and aliquots (5−10 μL) were analyzed by C18-HPLC. The D- and L-amino acid standards (1 mg/mL, 50 μL) were derivatized in a similar manner, and the retention times were compared with those of the component amino acids of the peptide hydrolysate. C18-HPLC analysis conditions: column, Phenomenex 5 μm, 250 × 4.6 mm; mobile phase, a gradient of 10%−50% CH_3CN in H_2O (0.05% TFA) over 30 min; flow rate, 1.0 mL/min; detection, UV 340 nm; retention times of authentic L-FDAA-*allo*-Thr (min): L-*allo*-Thr (19.8) and D-*allo*-Thr (20.6); retention times of the L-FDAA-*allo*-Thr of the hydrolysate (min): D-*allo*-Thr (20.6).

Assay for T Cell Viability. CEM-TART cells were maintained in 80% RPMI medium, 20% fetal bovine serum supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B in a humidified incubator at 37 °C, 5% CO_2. A 200 μL amount of cells was seeded in 96-well microtiter plates at a final concentration of 30 000 cells/well. Cells were administered 3.3 ng/mL to 5 μg/mL (dissolved in DMSO) of compound, each dose in quadruplicate. After 72 h, 11 μL of MTT (5 mg/mL) was added to each well, and plates were incubated for an additional 4 h. The medium was aspirated, 100 μL of DMSO added to the cells to solubilize purple formazan product, and the absorbance read at 570 nm using a plate reader (Bio-Rad). Average absorbance for each set of compound-treated wells was compared to the average absorbance of the control wells to determine the fractional survival at any particular drug concentration. The effective concentration 50 (EC_{50}) is defined as the drug concentration that yielded a fractional survival of 50%. EC_{50} values reported are the average of three independent experiments and were determined using GraphPad Prism. The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM-TART from Drs. Herbert Chen, Terence Boyle, Michael Malim, Bryan Cullen, and H. Kim Lyerly.

Cell Culture and MTT Assay.[16] The cancer cell line HCT-116 (human colon tumor) was grown at 37 °C, 5% CO_2 in McCoy's 5a medium containing 10% fetal bovine serum, penicillin (50 IU/mL), streptomycin (50 μg/mL), and 2 mM L-glutamine. Cells were plated at a density of 5000 cells/well in a 96-well plate. Twenty-four hours after plating, they were exposed to medium containing compound at a concentration of 0.1 to 100 μM for 48 h at 37 °C. After exposure of cells to compounds, 100 μL of fresh culture medium containing MTT at a final concentration of 0.3 mg/mL was added to each well and incubated for 3 h at 37 °C. Formazan crystals were solubilized in 100 μL of DMSO. The absorbance of each well was measured by a microplate reader (Multi-skan Labsystems) at 570 nm. The percentage cytotoxicity was calculated by comparison of the A_{570} reading from treated versus control cells.

Acknowledgment. The authors wish to acknowledge the government of Papua New Guinea and PNG BioNET for permits to collect the sponge. We thank P. Krishna, University of Utah Mass Spectrometry and Proteomics Core Facility, for performing the LRESIMS experiments. The following NIH and NSF grants funded NMR instrumentation: RR06262, RR13030, DBI-0002806. This project was funded by NIH grant CA 36622.

Supporting Information Available: ^1H and ^{13}C NMR spectra and selected 2D spectra (gHSQC, DQFCOSY, ^{15}N-HSQC, WATERGATE-NOESY, WATERGATE-ROESY, and 2D-TOCSY) of theopapuamide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Bewley, C. A.; Faulkner, D. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 2162−2178. (b) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.

(2) Zampella, A.; D'Auria, M. V.; Paloma, L. G.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202−6209.

(3) Oku, N.; Gustafson, K. R.; Cartner, L. K.; Wilson, J. A.; Shigematsu, N.; Hess, S.; Pannell, L. K.; Boyd, M. R.; McMahon, J. B. *J. Nat. Prod.* **2004**, *67*, 1407−1411.

(4) Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, de Silva, E. D.; Lassota, P.; Allen, T. M.; Soest, R. V.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899−5909.

(5) (a) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *Tetrahedron Assym.* **2001**, *12*, 1353−1358. (b) Liang, B.; Carroll, J.; Joullié, M. M. *Org. Lett.* **2000**, *2*, 4157−4160. (c) Acevedo, C. M.; Kogut, E. F.; Lipton, M. A. *Tetrahedron* **2001**, *57*, 6353−6359. (d) Çalimsiz, S.; Lipton, M. A. *J. Org. Chem.* **2005**, *70*, 6218−6221. (e) Thoen, J. C.; Ramos-M.; Lipton, M. A. *Org. Lett.* **2002**, *4*, 4455−4458. (f) Zampella, A.; D'Orsi, R.; Sepe, V.; Casapullo, A.; Monti, M. C.; D'Auria, M. V. *Org. Lett.* **2005**, *7*, 3585−3588. (g) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *J. Org. Chem.* **2002**, *67*, 9210−9215.

(6) Ratnayake, A. S.; Davis, R. A.; Harper, M. K.; Veltri, C. A.; Andjelic, C. D.; Barrows, L. R.; Ireland, C. M. *J. Nat. Prod.* **2005**, *68*, 104−107.

(7) (a) Köck, M.; Kessler, H.; Seebach, D.; Thaler, A. *J. Am. Chem. Soc.* **1992**, *114*, 2676−2686. (b) Kessler, H.; Hehlein, W.; Schuck, R. *J. Am. Chem. Soc.* **1982**, *104*, 4534−4540.

(8) 2D [^1H,^1H] TOCSY using a z-DIPSI-2 isotropic mixing scheme. Cavanagh, J.; Rance, M. *J. Magn. Reson.* **1992**, *96*, 670−678.

(9) Standard amino acid analysis, 5.7 N HCl, 110 °C, 16 h; concentrations of the amino acids were quantified using a Hitachi model L8800 analyzer.

(10) 2D [^1H,^1H] NOESY with watergate water suppression. Piotto, M.; Saudek, V.; Sklenáf, V. *J. Biomol. NMR* **1992**, *2*, 661−665.

(11) (a) Bodo, B.; Hebrard, P.; Molho, L.; Molho, D. *Tetrahedron Lett.* **1973**, *18*, 1631−1634. (b) White, J. D.; Johnson, A. T. *J. Org. Chem.* **1994**, *59*, 3347−3358.

(12) (a) Edman, P. *Acta Chem. Scand.* **1950**, *4*, 283−293. (b) Yarwood, A. In *Protein Sequencing, a Practical Approach*; Findlay, J. B. C., Geisow, M. J. Eds.; IRL Press: Oxford, 1989; Chapter 6, pp 119−145.

(13) Trypsin digestion, 37 °C, pH 7.9, 2 days; thermolysin digestion, 37 °C, pH 8.1, 1−2 days; pepsin digestion, 37 °C, pH 1.5, 2 days.

(14) (a) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Kruppa, G.; Feng, X.; Lotvin, J. A.; Siegel, M. M. *Anal. Chem.* **2003**, *75*, 2730−2739. (b) Palmblad, M.; Håkansson, K.; Håkansson, P.; Feng, X.; Cooper, H. J.; Giannakopulos, A. E.; Green, P. S.; Derrick, P. J. *Eur. J. Mass Spectrom.* **2000**, *6*, 267−275.

(15) (a) Bhushan, R.; Brückner, H. *Amino Acids* **2004**, *27*, 231−247. (b) B'Hymer, C.; Bayon-M, M.; Caruso, J. A. *J. Sep. Sci.* **2003**, *26*, 7−19. (c) Hess, S.; Gustafson, K. R.; Milanowski, D. J.; Alvira, E.; Lipton, M. A.; Pannell, L. K. *J. Chromatogr. A* **2004**, *1035*, 211−219.

(16) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

NP060229D