Papuamides A−D, HIV-Inhibitory and Cytotoxic Depsipeptides from the Sponges *Theonella mirabilis* and *Theonella swinhoei* Collected in Papua New Guinea

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Abstract: The novel cyclic depsipeptides papuamides A (**1**), B (**2**), C (**3**), and D (**4**) have been isolated from Papua New Guinea collections of the sponges *Theonella mirabilis* and *Theonella swinhoei*. Their structures were determined by a combination of spectroscopic analysis and chemical degradation and derivatization studies. In addition to glycine, alanine, and threonine, these peptides contain a number of unusual amino acids including 3,4-dimethylglutamine, β-methoxytyrosine, 3-methoxyalanine, and 2,3-diaminobutanoic acid or 2-amino-2-butenoic acid residues. Papuamides A−D (**1**−**4**) are also the first marine-derived peptides reported to contain 3-hydroxyleucine and homoproline residues. These peptides also contain a previously undescribed 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid moiety N-linked to a terminal glycine residue. Papuamides A (**1**) and B (**2**) inhibited the infection of human T-lymphoblastoid cells by HIV-1_{RF} in vitro with an EC_{50} of approximately 4 ng/mL. Compound **1** was also cytotoxic against a panel of human cancer cell lines with a mean IC_{50} of 75 ng/mL.

Sponges in the genus *Theonella* (order Lithistida) have been a prolific source of structurally diverse, biologically active peptides.[1] Recent examples of *Theonella* peptides include polytheonamides,[2] cyclotheonamides,[3] theonellapeptolides,[4] theonellamides,[5] theonegramides,[6] keramamides,[7] mozamides,[8] mutoporins,[9] microsclerodermins,[10] cupolamide,[11] oriamide,[12] and cyclolithistide A.[13] The biosynthetic origin of compounds isolated from *Theonella* sponges has often been attributed to symbiotic bacteria or microalgae. Recently, the peptide metabolite theopalauamide[14] was found localized in eubacteria that live symbiotically within *Theonella swinhoei*. Many *Theonella*-derived peptides demonstrate potent cytotoxicity,[2,5,11,12] thrombin inhibition,[3] phosphatase inhibition,[9] protease inhibition,[3] or antifungal properties.[5,6,10,13,14] However, there have not been any previous reports of peptides from *Theonella* that inhibit the human immunodeficiency virus (HIV). The only sponge-

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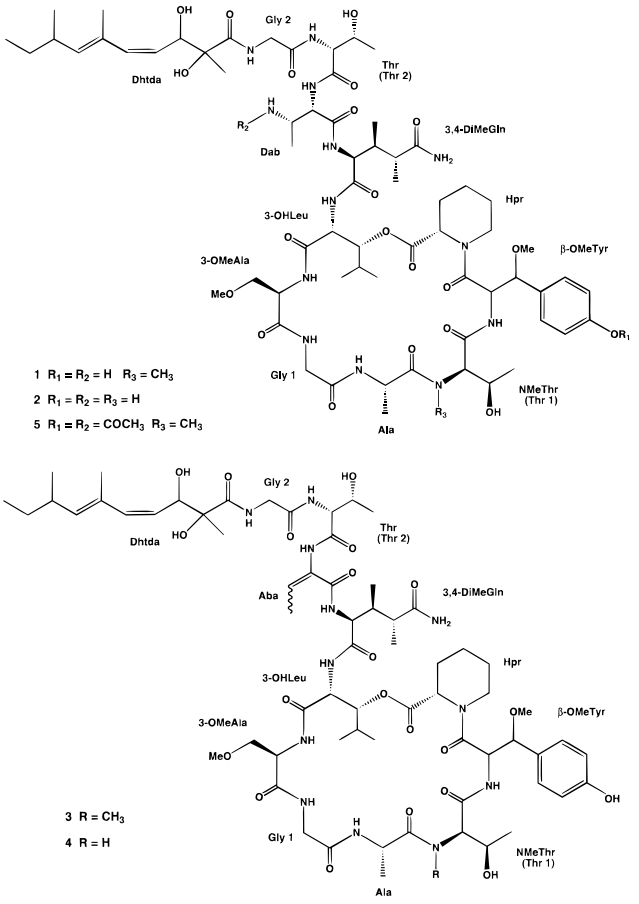
derived, nonribosomal peptides previously reported to have anti-HIV activity are the callipeltins,[15] which were isolated from the Lithistid sponge *Callipelta* sp. We report herein the isolation and structure determination of papuamides A−D (**1**-**4**), HIV-inhibitory and cytotoxic depsipeptides isolated from Papua New Guinea collections of *Theonella mirabilis* and *Theonella swinhoei*.



The extracts on which the present investigations were based were from independent collections of *T. mirabilis* and *T. swinhoei* made along the north coast of Papua New Guinea by a contractor to the U.S. National Cancer Institute (NCI) and by staff of the University of British Columbia (UBC), respectively. In both cases, sponge samples were frozen immediately after collection. Chemical investigations of *T. mirabilis* were initiated at the NCI following observations that both the aqueous and organic extracts exhibited activity in the NCI's primary in vitro anti-HIV screen.[16] The UBC group, which has an ongoing program to study cytotoxic metabolites from marine sponges,[17] initiated its investigations based on observations that extracts of *T. swinhoei* demonstrated potent in vitro cytotoxicity against a panel of human cancer cell lines. Independent bioassay-guided fraction efforts at NCI and UBC employed a combination of solvent/solvent partitioning, reversed-phase flash chromatography, gel permeation on Sephadex LH-20, and C_{18} reversed-phase HPLC separations. The anti-HIV activity of *T. mirabilis* was tracked to a mixture of peptides consisting primarily of papuamides A (**1**) and B (**2**)[18] while the cytotoxic activity of *T. swinhoei* was traced to papuamides A−D (**1**−**4**).

Papuamide A (**1**), the most abundant peptide in both *Theonella* samples, was isolated as an optically active amorphous glass. A molecular formula of $C_{66}H_{105}N_{13}O_{21}$ was established for **1** by high-resolution FABMS. Papuamide A (**1**) gave a sharp, well-resolved peak when analyzed by reversed-phase HPLC using a variety of solvent systems, and it gave a single clean molecular ion in the HRFABMS. However, many of the resonances in the ^{13}C and ^{1}H NMR spectra of **1** were doubled or tripled. For example, in both DMSO-d_6 and MeOH-d_4, the resonance ultimately attributed to H3 and H5 of the tyrosine residue in **1** appeared as three doublets in the ratio 4:2:1. Other NMR solvents, including pyridine-d_5, DMF-d_7, acetone-d_6, and a wide variety of combined solvent systems all provided spectra with different peak ratios or broadened, poorly resolved resonances that were not suitable for structural studies. Attempts to simplify the NMR spectra by heating (50 °C) or adjusting the pH by addition of TFA, pyridine, or Et_3N did not alleviate the problem. Eventually it was found that acceptable spectra with only a single set of well-resolved resonances could be obtained using solvent suppression and a mixture of CD_3CN-H_2O (4:1) as the NMR solvent. Complete NMR spectral assignments (Table 1) and the structural elucidation of **1** utilized NMR data sets collected at three different concentrations, 3.0, 15.6 and 27.5 mM, in CD_3CN-H_2O (4:1). The doubling and tripling of resonances observed in the NMR spectra of papuamide A (**1**) was attributed to the existence of slow conformational equilibria.

The peptide nature of **1** was apparent from a series of exchangeable amide NH protons between δ 7.0 and δ 9.5 that were coupled to either α-methine protons or α-methylene protons between δ 3.7 and δ 5.3. In addition, 13 ^{13}C NMR resonances observed between δ 181.0 and δ 169.0 had chemical shifts appropriate for amide or ester carbonyls. A diacetate derivative **5** was produced by sequential treatment of **1** with Ac_2O in MeOH followed by Ac_2O in pyridine. Compound **5** also provided a single set of well-dispersed proton resonances in the CD_3CN-H_2O (4:1) solvent mixture. Extensive NMR analyses of compounds **1** and **5**, including data from COSY, TOCSY, ROESY, HSQC, HMBC, and HSQC-TOCSY experiments, established the presence of alanine, threonine, and two glycine residues. Seven uncommon amino acids were also elucidated: homoproline (Hpr), *β*-methoxytyrosine (*β*-OMeTyr), *N*-methylthreonine (NMeThr), 3-methoxyalanine (3-OMeAla), 3-hydroxyleucine (3-OHLeu), 3,4-dimethylglutamine (3,4-DiMeGln), and 2,3-diaminobutanoic acid (Dab). In addition, an amide-linked 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid (Dhtda) moiety was elucidated. COSY and TOCSY data helped establish the proton spin systems in the Dhtda residue. The C4−C5 olefin was assigned a *Z* geometry on the basis of coupling between H4 and H5 of approximately 11 Hz, while ROESY correlations between H5 and H7 and between the C6 Me group and both H3 and H8 established the C6−C7 double bond as *E*. The presence of a hydroxyl substituent at C2 was revealed by its ^{13}C chemical shift of δ 78.53, while substitution at C2 with a methyl group was evident from HMBC

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(18) Subsequent mass spectrometric analysis of HPLC side fractions from *T. mirabilis* revealed MH^+ molecular ions at *m/z* 1400 and 1386 which are consistent with the co-occurrence of papuamides C (**3**) and D (**4**), respectively, as trace constituents of *T. mirabilis*.

Table 1. ^{1}H and ^{13}C NMR Assignments for Papuamide A (**1**)[a]

	δ					
	^{13}C	^{1}H	mult (*J*, Hz)	TOCSY	HMBC[b]	ROESY
			Homoproline (**Hpr**)			
CO	170.33					
2	54.76	4.94	d (3.9)	1.15, 1.35, 1.63, 2.10	20.55, 26.06, 170.33	1.35, 1.63, 2.10, 4.07
3	26.06	1.67	m	1.15, 1.35		1.15, 1.35
		2.10	m	1.15, 1.67		1.63, 4.94
4	20.55	1.15	m	1.67, 1.69, 2.10		1.35, 1.67
		1.63	m	1.15, 1.35, 1.69, 2.10		1.35, 2.10, 4.94
5	24.82	1.35	m	1.15, 1.67, 1.69, 2.10		1.15, 1.67, 4.07, 4.24 4.94, 5.24
		1.69	m	1.15, 2.10, 3.14, 4.07		4.07
6	44.24	3.14	m	1.67, 2.10, 4.07	54.76	4.07
		4.07	br d (11.7)	1.67, 2.10, 3.14		1.35, 1.69, 3.14, 4.24
			β-Methoxytyrosine (***β*-OMeTyr**)			
CO	173.22					
α	52.07	5.24	t (9.3)	4.24, 7.76	84.23, 168.75, 173.22	1.35, 3.03, 4.07, 4.24 4.32, 7.20, 7.76
β	84.38	4.24	d (9.3)	5.24, 7.76	52.07, 56.81, 130.21, 173.22	4.07, 5.24, 7.20, 7.76
β-OMe	56.81	3.03	s		84.23	5.24, 7.20
1	128.88					
2, 6	130.21(2)	7.20	d (8.3)	6.76	84.23, 115.74, 130.21, 157.94	0.48, 3.03, 4.24, 5.24 6.76, 7.76
3, 5	115.74(2)	6.76	d (8.3)	7.20	115.74, 128.88, 157.94	0.48, 7.20
4	157.94					
NH		7.76	d (9.3)	4.24, 5.24	168.75	0.48, 3.87, 4.24, 4.32 5.24, 7.20
			N-Methylthreonine (**NMeThr**)			
1	168.75					
2	64.11	4.32	m	0.48, 3.87	19.64, 63.66, 168.75	0.48, 3.87, 5.24, 7.76
3	63.66	3.87	d (6.4)	0.48, 4.32	19.64, 30.91, 64.11	0.48, 3.06, 4.32, 7.76
4	19.64	0.48	d (5.9)	3.87, 4.32	64.11	3.87, 4.32, 6.76, 7.20 7.76
N-Me	30.91	3.06	s		64.11, 174.41	3.87, 4.49
			Alanine (**Ala**)			
1	174.41					
2	47.73	4.49	m	1.36, 7.12	15.39, 174.41	1.36, 3.06, 7.12
3	15.39	1.36	d (10.7)	4.49, 7.12	47.73, 174.41	4.49, 7.12
NH		7.12	d (5.9)	1.36, 4.49	15.39, 47.73, 171.04	1.36, 4.49, 8.27
			Glycine 1 (**Gly 1**)			
1	171.04					
2	43.05	3.72	br d (15.6)	3.89, 8.27	171.04	3.89, 8.27
		3.89	br d (15.6)	3.72, 8.27	171.04	3.72, 8.27
NH		8.27	br s	3.72, 3.89	172.11	3.72, 3.83, 3.89, 4.18 7.12
			3-Methoxyalanine (**3-OMeAla**)			
1	172.11					
2	55.94	4.18	d (3.9)	3.60, 3.83, 7.93	71.12, 172.11	3.60, 3.83, 7.93, 8.27
3	71.12	3.60	br d (16.9)	3.83, 4.18, 7.93	172.11	3.34, 4.18
		3.83	br d (16.9)	3.60, 4.18, 7.93	172.11	3.34, 4.18, 8.27
3-OMe	59.27	3.34	s		71.12	3.60, 3.83, 4.93
NH		7.93	br s	3.60, 3.83, 4.18	172.07	4.18, 4.26
			3-Hydroxyleucine (**3-OHLeu**)			
1	172.07					
2	53.63	4.93	m	0.83, 5.23, 8.96		0.83, 0.98, 1.95, 3.34 5.23
3	78.54	5.23	d (10.7)	0.84, 1.95, 4.93, 8.96	170.33	0.84, 1.95, 4.93
4	28.54	1.95	m	0.83, 0.84, 5.23	18.85, 78.54	0.83, 0.84, 4.93, 5.23 8.96
5	18.44	0.84	d (6.4)	0.83, 1.95, 5.23	18.85, 78.54	1.95, 5.23
5N	18.85	0.83	d (6.4)	0.84, 1.95, 4.93	18.44, 78.54	1.95, 4.93
NH		8.96	br s	4.93, 5.23		1.95, 4.26
			3,4-Dimethylglutamine (**3,4-DiMeGln**)			
1	174.14					
2	58.67	4.26	d (7.8)	0.98, 2.12, 8.69	37.52, 174.14	0.98, 2.12, 2.67, 7.93 8.96
3	37.52	2.12	m	0.98, 4.26	179.04	0.98, 1.11, 2.67, 4.26 8.69

Table 1 (Continued)

	δ					
	^{13}C	^{1}H	mult (*J*, Hz)	TOCSY	HMBC[b]	ROESY
			3,4-Dimethylglutamine (**3,4-DiMeGln**)			
3-Me	12.75	0.98	d (6.4)	2.12, 2.67, 4.26	58.67	2.12, 2.67, 4.26, 4.93
4	40.64	2.67	m	0.98, 1.11	12.75, 179.04	0.98, 1.11, 2.12, 4.26
						7.10, 8.69
4-Me	15.08	1.11	d (6.8)	2.67	179.04	2.12, 2.67
5	179.04					
2-NH		8.69	br s	4.26, 4.65	171.60	2.12, 2.67
5-NH		6.58	br s	7.10		7.10
		7.10	br s	6.58		1.35, 2.67, 6.58
			2,3-Diaminobutanoic acid (**Dab**)			
1	171.60					
2	55.27	4.65	br s	1.28, 8.69	48.77, 171.60	
					173.11	
3	48.77	3.96	m	1.28	55.27	
4	15.27	1.28	br s	3.96, 4.65	48.77, 55.27	
2-NH		8.69			173.11	
			Threonine (**Thr**)			
1	173.11					
2	59.66	4.44	d (5.9)	1.12, 4.31, 7.83	173.11	1.12, 4.31, 7.83
3	67.83	4.31	m	1.12, 4.44, 7.83	19.58	1.12, 4.44
4	19.58	1.12	d (9.3)	4.31, 4.44, 7.83	59.66, 67.83	4.31, 4.44, 7.83
NH		7.83	d (8.3)	1.12, 4.31, 4.44	172.20	1.12, 4.44, 8.31
			Glycine (**Gly 2**)			
1	172.20					
2	43.54	3.82	dd (16.6, 6.8)	4.12, 8.31	177.83	4.12, 8.31
		4.12	dd (16.6, 6.3)	3.82, 8.31	172.20, 177.83	3.82, 8.31
NH		8.31	br s	3.82, 4.12	177.83	3.82, 4.12, 7.83
			2,3-Dihydroxy-2,6,8-trimethyl-4,6-decadienoic Acid (**Dhtda**)			
1	177.83					
2	78.53					
2-Me	22.14	1.20	s		72.68, 177.83	4.80, 5.48
3	72.68	4.80	d (10.3)	5.48, 6.10	124.93, 138.51	1.20, 1.75, 5.22, 5.48
						6.10
4	124.93	5.48	dd (10.3, 2.9)	4.80, 6.10	131.45	1.20, 4.80, 6.10
5	138.51	6.10	dd (11.7, 2.9)	4.80, 5.48	16.57, 72.68, 139.10	1.75, 4.80, 5.22, 5.48
6	131.45					
6-Me	16.57	1.75	br s	5.22	131.45, 138.51	2.32, 4.80, 6.10
					139.10	
7	139.10	5.22	d (9.5)	0.92, 1.21, 1.34, 1.75	16.57, 20.34, 30.54	0.92, 1.21, 2.32, 4.80
				2.32	34.67, 138.51	6.10
8	34.67	2.32	m	0.82, 0.92, 1.21, 1.34	20.69, 131.45	0.82, 0.92, 1.21, 1.34
				5.22		1.75, 5.22
8-Me	20.69	0.92	d (6.4)	1.21, 1.34, 2.32, 5.22	30.54, 34.67, 139.10	1.34, 2.32, 5.22
9	30.54	1.21	m	0.82, 0.92, 1.34, 2.32	34.67	0.82, 0.92, 2.32, 5.22
		1.34	m	0.82, 0.92, 1.21, 2.32	139.10	0.82, 0.92, 2.32
				5.22		
10	12.00	0.82	t (6.4)	1.21, 1.34, 2.32	30.54, 34.67	1.21, 1.34, 2.32

[a] Spectra were recorded in CD_3CN-H_2O (4:1) at 500 MHz for ^{1}H and 125 MHz for ^{13}C and referenced to the residual solvent signal of CD_3CN (δ_H 1.93, δ_C 1.30). [b] Optimized for $J = 3.5$ and $J = 8.5$ Hz. Carbons correlated to proton resonances in the ^{1}H column.

correlations observed between the methyl protons (δ 1.20) and both C1 (δ 177.83) and C3 (δ 72.68). To the best of our knowledge, the Dhtda group is an unprecedented structural moiety. The only previous reports of the amino acid residues β-OMeTyr and 2,3-DiMeGln have been as constituents of the callipeltins.[15] Papuamide A (**1**) also represents the first marine-derived peptide to contain 3-OHLeu and Hpr residues.

The sequence of the amino acids, the location of the decadienoic acid derivative, and the macrocyclic structure of papuamide A (**1**) were established by detailed interpretation of HMBC and ROESY experiments with compound **1** and its diacetate derivative **5.** HMBC correlations (Figure 1) observed for papuamide A (**1**) defined most of the amino acid connectivities; however, assignments of the 3-OHLeu and the C-terminal Hpr residues were possible due to correlations that were only observed with the diacetate **5**. Inter-amino acid ROESY correlations (Figure 2) helped confirm the amino acid sequence. Again, several key confirmatory ROESY correlations were only seen with compound **5**. A HMBC correlation between the N-terminal Gly-2 NH (δ 8.31) and the carbonyl (δ 177.83) of the Dhtda moiety established that Dhtda was amide-linked to Gly-2. The downfield chemical shift of the H3 carbinol proton (δ 5.23) of 3-OHLeu suggested that the hydroxyl group was part of a macrocyclic lactone. A HMBC correlation between H3 and the Hpr carbonyl carbon (δ 170.33) confirmed cyclization via ester bond formation between the C-terminal Hpr residue and the hydroxyl group of 3-OHLeu.

Several chemical degradations were undertaken to generate small fragments of papuamide A (**1**) to help confirm the overall structural assignment. First, papuamide A (**1**) was treated with aqueous Et_3N (5%) at 49 °C for 16 h. Removal of the reagents in vacuo and fractionation of the reaction mixture via reversed-

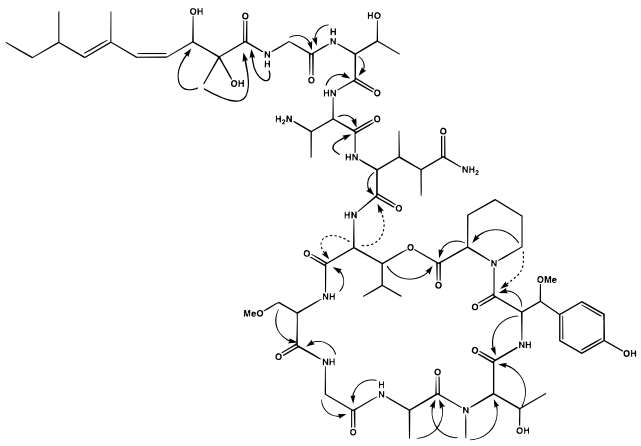


Figure 1. Key papuamide A (**1**) HMBC correlations. Dashed arrows indicate correlations only observed in diacetate **5**.

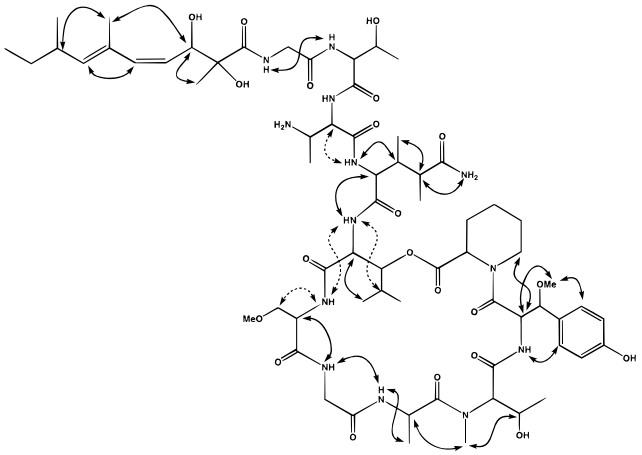
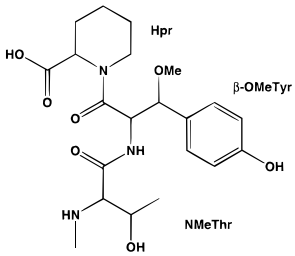


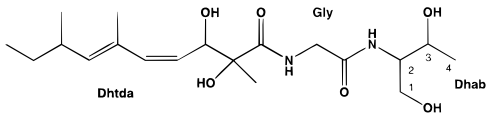
Figure 2. Key papuamide A (**1**) ROESY correlations. Dashed arrows indicate correlations only observed in diacetate **5**.

phase HPLC gave tripeptide **6** in moderate yield. Tripeptide **6** was obtained as an optically active clear oil that gave a $[M + H]^+$ ion in the HRFABMS at *m*/*z* 438.2235 appropriate for a molecular formula of $C_{21}H_{32}N_3O_7$. Analysis of the ^1H, ^{13}C, HMQC, and HMBC NMR data for **6** showed that it contained Hpr, *β*-OMeTyr, and NMeThr residues. The doubling of resonances observed in the NMR spectra of papuamide A (**1**) was also apparent in the NMR spectra of the tripeptide **6**. HMBC correlations observed in compound **6** among the *β*-OMeTyr Hα resonance at *δ* 5.19, the *β*-OMeTyr NH resonance at *δ* 9.17, the NMeThr H2 resonance at *δ* 3.27, and the NMeThr carbonyl resonance at *δ* 164.5 demonstrated an amide bond between the *β*-OMeTyr amino nitrogen and the carbonyl of the NMeThr residue. In addition, the *β*-OMeTyr Hα proton resonance at *δ* 5.19, the *β*-OMeTyr H*β* proton resonance at *δ* 4.31, and the Hpr H2 proton resonance at *δ* 5.14 all showed HMBC correlations to an amide carbonyl resonance at *δ* 169.6, demonstrating the presence of an amide bond between the Hpr tertiary amino nitrogen and the *β*-OMeTyr carbonyl. The Hpr H2 proton resonance at *δ* 5.14 showed an HMBC correlation to a carbonyl resonance at *δ* 172.0 that was assigned to the free terminal carboxylic acid. ROESY and NOE data confirmed the connectivities assigned by the HMBC data for tripeptide **6**.

The second chemical degradation reaction involved treating papuamide A (**1**) with excess $NaBH_4$ in aqueous 2-propanol at room temperature. Purification of the crude reaction product via reversed-phase HPLC gave the previously identified tripeptide **6** and the new degradation product **7**, both in very low



6



7

yield. Compound **7** was obtained as a clear oil that gave a $[M + Na]^+$ ion at *m*/*z* 409.2324 in the HRFABMS appropriate for a molecular formula of $C_{19}H_{34}N_2O_6$. Detailed analysis of the ^1H, ^{13}C, HMQC, and HMBC NMR data showed that **7** contained a Gly residue, the 13-carbon Dhtda fragment previously identified in papuamide A (**1**), and a 1,3-dihydroxy-2-aminobutane (Dhab) moiety. Doubling of some resonances was also apparent in the ^1H NMR spectra of **7**. HMBC correlations observed from the Dhtha C2-Me singlet at *δ* 1.08 and the Gly α-methylene proton resonances at *δ* 3.75−3.65 to a carbonyl resonance at *δ* 175.4 confirmed that the Dhtda fragment was attached via an amide bond to the Gly amino nitrogen. The Gly NH (*δ* 7.29) and the Gly α-methylene (*δ* 3.75−3.65) resonances also showed HMBC correlations to a carbonyl at *δ* 168.9, confirming the existence of an amide bond between the amino nitrogen of the Dhab moiety and the Gly carbonyl. ROESY correlations confirmed the amide linkages identified via these HMBC data. Isolation and identification of peptide fragments **6** and **7** helped confirm the overall amino acid sequence of papuamide A (**1**).

The absolute stereochemistries of the amino acid constituents of papuamide A (**1**) were determined following acid hydrolysis (6 N HCl, 108 °C, 16 h) of the parent peptide. Commercially available standards of alanine, threonine, 3-methoxyalanine, and homoproline were available, while synthetic samples of 3-hydroxyleucine,[19] 2,3-diaminobutyric acid,[20,21] and *N*-methylthreonine[22] were analyzed. Stereochemical assignments were made using a combination of GC−MS and LC−MS techniques to compare the amino acids from papuamide A (**1**) with appropriate standards. Treatment of the acid hydrolysate of **1** with Marfey's reagent[23] and LC−MS analysis of the resulting 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide derivatives established the presence of L-alanine, L-threonine, L-*N*-methylthreonine, D-methoxyalanine, and L-homoproline. LC−MS analysis of the amino acid derivatives generated by reaction of the hydrolysate with 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) confirmed these assignments. Treatment of the hydrolysis mixture with acetyl chloride in 2-propanol followed

(19) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, A. B., III. *J. Am. Chem. Soc.* **1996**, *118*, 3584−3590.

(20) Schmidt, U.; Mundinger, K.; Riedl, B.; Haas, G.; Lau, R. *Synthesis* **1992**, 1201−1202.

(21) (2*S*,3*S*)-Diaminobutyric acid for comparative purposes was also obtained from the acid hydrolysate of aciculitin B. Bewley, C. A.; He, H.; Williams, D. H.; Faulkner, D. J. *J. Am. Chem. Soc.* **1996**, *118*, 4314−4321.

(22) Beulshausen, T.; Groth, U.; Schöllkopf, U. *Liebigs Ann. Chem.* **1992**, 523−526.

(23) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

by trifluoroacetic anhydride in CH_2Cl_2 gave a mixture of trifluoroacetyl isopropyl ester derivatives which were analyzed by GC−MS using a Chirasil-L-Val capillary column. Comparison with appropriate standards confirmed the presence of (2*R*,3*R*)-3-hydroxyleucine and (2*S*,3*R*)-diaminobutanoic acid. Comparison by GC−MS of the trifluoroacetyl isopropyl esters and LC−MS of both the FDAA and GITC derivatives of 3,4-dimethylglutamic acid in the acid hydrolysate of papuamide A (**1**) with those of the same derivatives from the hydrolysate of authentic callipeltin A[15] showed that the 3,4-dimethylglutamic acid derived from these two peptides was indistinguishable. In addition, GC−MS and LC−MS analysis of the 3,4-dimethylpyroglutamic acid, which also forms under these hydrolysis conditions,[15] indicated that the 3,4-DiMeGln residue in papuamide A (**1**) is identical to that in callipeltin A. This allowed assignment of its configuration as (3*S*,4*R*)-dimethyl-L-glutamine. It was not possible to isolate or characterize the β-methoxytyrosine and 2,3-dihydroxy-2,6,8-trimethyldeca-(4*E*,6*Z*)-dienoic acid moieties in the acid hydrolysate of **1**, and thus their stereochemistries were not assigned. Attempts to make appropriate derivatives of compound **1**, compound **2**, and the diacetate **5** were also unsuccessful due to decomposition of the starting materials.

The molecular formula of papuamide B (**2**) was established as $C_{65}H_{103}N_{13}O_{21}$ by HRFABMS and confirmed by extensive NMR analyses. It only differed from the molecular formula of papuamide A (**1**) by the loss of CH_2. The ^{1}H and ^{13}C NMR spectral data of **2** were also very similar to those recorded for **1**. However, it was possible to obtain well-resolved spectra of **2** in a variety of NMR solvents including CD_3OH. Detailed interpretation of 1-D and 2-D NMR data sets allowed the complete spectral assignment (Table 2) for papuamide B (**2**). While the presence of two OMe groups was evident, appropriate NMR resonances for an *N*-methyl group were lacking in **2**. This suggested that the NMeThr in papuamide A (**1**) was simply replaced with a Thr residue in papuamide B (**2**). Amino acid analysis by GC−MS of the acid hydrolysate of **2** confirmed the presence of 2 equiv of L-threonine. The structure of compound **2** was ultimately elucidated through a similar series of NMR experiments and by LC−MS and GC−MS characterization of its constituent amino acids, as described above for papuamide A (**1**). Aside from substitution of Thr for the NMeThr found in **1**, the amino acid composition, sequence, stereochemistries, point of lactonization, and attachment of the Dhtda group in papuamide (**2**) were identical to those in papuamide A (**1**).

Papuamides C (**3**) and D (**4**) were obtained as optically active colorless glasses whose HRFABMS data were consistent with molecular formulas of $C_{66}H_{103}N_{12}O_{21}$ and $C_{65}H_{101}N_{12}O_{21}$, respectively. Comparison of the ^{1}H, ^{13}C, HMQC, and HMBC NMR data obtained for **3** and **4** with those of **1** and **2** indicated that the two compounds were closely related to papuamides A (**1**) and B (**2**)**.** The relationship between papuamides C (**3**) and D (**4**) was the same as that of **1** and **2** in that papuamide C (**3**) was *N*-methylated at the amino nitrogen of threonine, while papuamide D (**4**) was not.

The^{1}H NMR spectra of papuamides C (**3**) and D (**4**) were very poorly resolved; however, the HMBC and COSY data collected for papuamide D (**4**) proved to be useful in elucidating the gross structures. Preliminary analysis of the NMR data revealed that the structures of papuamides C (**3**) and D (**4**) differed from those of **1** and **2** at the Dab residue. NMR resonances for the C2/H2 α-methine and the C3/H3 β-methine of the Dab residue in **1** and **2** were not apparent in the spectra of **3** and **4**. Instead there were resonances appropriate for a 2-amino-2-butenoic acid (Aba) residue. Thus, the Aba olefinic methyl resonance at δ 1.66 (H4) was correlated in the COSY spectrum of **4** to an olefinic proton at δ 6.67 (H3). This olefinic proton resonance was in turn correlated in the HMQC spectrum to a carbon resonance at δ 135.4 (C3), and the Aba olefinic methyl resonance at δ 1.66 was correlated to C2 (δ 129.3) and C3 (δ 135.4) in the HMBC spectrum. Additional HMBC correlations were observed between the Aba carbonyl resonance at δ 166.7 (C1) and both the 3,4-DiMeGln NH resonance at δ 8.00 and the Aba H3 resonance at δ 6.67. These latter HMBC correlations confirmed the amide linkage between the amino nitrogen of the 3,4-DiMeGln residue and the Aba carbonyl. The Aba NH resonance of papuamide D (**4**) at δ 9.10 and the Thr H2 resonance at δ 4.44 both showed HMBC correlations to a carbonyl resonance in the region δ 172.5−172.7. This set of HMBC correlations was consistent with an amide bond between the Aba α-amino nitrogen and the Thr carbonyl. On the basis of the NMR and MS evidence, papuamides C and D were assigned the structures **3** and **4**, respectively. Confirmation of the proposed structures for **3** and **4** came from conversion of papuamide A (**1**) to papuamide C (**3**) via Hofmann elimination using excess MeI in the presence of K_2CO_3. Unfortunately, ROESY, NOESY, or difference NOE data failed to provide unambiguous proof for the configuration of the Aba olefins in papuamide C (**3**) or D (**4**). Acid hydrolysis and amino acid analysis by a combination of GC−MS and LC−MS techniques, as described above, revealed that the absolute stereochemistries of the amino acid constituents of papuamides C (**3**) and D (**4**) were identical to those found in papuamides A (**1**) and B (**4**).

Papuamide A (**1**) and B (**2**) were evaluated in a tetrazolium-based assay[24] that measures the ability of test compounds to protect CEM-SS T-cell cultures from infection by HIV-1_{RF}. Increasing concentrations of compound **1** or **2** were added to cell cultures coincident with the addition of HIV. At day six post-infection, cultures treated with papuamide A (**1**) exhibited a concentration-dependent increase in cellular viability, indicating an inhibition of productive infection relative to control cultures, with an EC_{50} (50% effective concentration) of 3.6 ng/mL. A cytotoxic IC_{50} (concentration inhibiting growth or viability of uninfected control cells by 50%) was measured for **1** at 74 ng/mL. The HIV-inhibitory and cytotoxic activities of papuamide B (**2**) in this assay were virtually identical to those observed for papuamide A (**1**). Papuamide A (**1**) also produced a characteristic cytotoxicity profile when tested against a panel of human cancer cell lines, revealing a relative sensitivity of multi-drug-resistant cell lines and a relative resistance of leukemia cell lines. The mean IC_{50} of papuamide A (**1**) against the human cancer cell line panel was 75 ng/mL.

Experimental Section

General Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were obtained from neat samples on KCl disks in a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were recorded on a Varian Unity INOVA-500 NMR spectrometer. NMR spectra were referenced to residual signals from the NMR solvent. High-resolution fast atom bombardment (HRFAB), electron impact, and chemical ionization mass spectra were acquired on a JEOL SX102 mass spectrometer operated at an accelerating voltage of 10 kV and 1000 resolution. Mass spectral data were acquired by a JEOL XMS data system. Electrospray mass spectra were acquired on a Hewlett-Packard HP1100 integrated LC−MS system.

(24) Gulakowski, R. J.; McMahon, J. B.; Staley, P. G.; Moran, R. A.; Boyd, M. R. *J. Virol. Methods* **1991**, *33*, 87−100.

Table 2. ^{1}H and ^{13}C NMR Assignments for Papuamide B (**2**)[a]

	δ					
	^{13}C	^{1}H	mult (*J*, Hz)	TOCSY	HMBC[b]	ROESY
				Homoproline (**Hpr**)		
CO	171.33					
2	53.33	5.26	d (7.8)	1.56, 1.70, 1.72, 2.10 3.30, 4.02	21.35, 27.45, 171.33	1.72, 2.10
3	27.45	1.70	m	2.10, 5.26		
		2.10	m	1.56, 1.70, 3.30, 4.02 5.26		1.50, 1.70, 1.72, 5.26
4	21.35	1.56	m	1.72, 2.10, 4.02, 5.26		
		1.72	m	1.56, 1.70, 3.30, 4.02 5.26		1.50, 2.10, 4.02, 5.26
5	26.24	1.50	m	3.30		1.72, 2.10, 3.30, 4.02
		1.70	m	1.72, 2.10, 3.30, 4.02		2.10, 3.30
6	44.47	3.30	m	1.50, 1.70, 1.72, 2.10 4.02, 5.26	53.33	1.50, 1.70, 4.02
		4.02	m	1.70, 1.72, 2.10, 3.30 5.26		1.50, 1.72, 3.30, 4.37
				β-Methoxytyrosine (***β*-OMeTyr**)		
CO	171.07					
α	53.50	5.18	t (9.4)	4.37, 7.98	84.74	3.10, 4.02, 4.37, 7.18 7.98
β	84.74	4.37	d (9.8)	5.18, 7.98	53.50, 56.91, 130.83	3.10, 4.02, 5.18, 7.18 7.98
β-OMe	56.91	3.10	s			4.37, 5.18, 7.18
1	129.31					
2, 6	130.83(2)	7.18	d (8.3)	6.75	84.74, 116.25 130.83, 158.91	0.56, 3.10, 4.37, 5.18 6.75
3, 5	116.25(2)	6.75	d (8.3)	7.18	116.25, 129.31 158.91	7.18
4	158.91					
NH		7.98	d (9.3)	4.37, 5.18		3.98, 4.37, 5.18
				N-Methylthreonine (**NMeThr**)		
1	170.98					
2	60.18	3.98	m	0.56, 3.78, 7.81	67.92, 170.98 174.42	0.56, 7.72, 7.81, 7.98
3	67.92	3.78	m	0.56, 3.98, 7.81		0.56, 7.72, 7.81
4	19.54	0.56	d (6.4)	3.78, 3.98	60.18, 67.92	3.78, 3.98, 7.18
NH	7.81		d (9.3)	3.78, 3.98		1.42, 3.78, 3.98, 4.29
				Alanine **(Ala)**		
1	174.42					
2	50.75	4.29	q (6.8)	1.42, 7.72	18.00, 174.42	1.42, 7.72, 7.81
3	18.00	1.42	d (7.3)	4.29, 7.72	50.75, 174.42	4.29, 7.72, 7.81
NH		7.72	d (7.3)	1.42, 4.29		1.42, 3.78, 3.98, 4.29
				Glycine 1 (**Gly 1**)		
1	172.06					
2	43.85	3.70	m	4.02, 8.51	172.06	4.02, 8.51
		4.02	m	3.70, 8.51	172.06	3.38, 3.70, 8.51
NH		8.51	t (5.9)	3.70, 4.02		3.70, 4.02, 4.42 7.12
				3-Methoxyalanine (**3-OMeAla**)		
1	172.44					
2	55.41	4.42	m	3.75, 3.81, 8.21	172.44	3.75, 3.81, 8.21, 8.50
3	71.88	3.75	m	3.81, 4.42, 8.21		4.42, 8.21
		3.81	m	3.75, 4.42, 8.21		4.42, 8.21
3-OMe	59.31	3.38	s		71.88	4.02
NH		8.21	d (6.8)	3.75, 3.81, 4.42	172.44	0.92, 3.75, 3.81, 4.42 4.98, 5.26, 8.78
				3-Hydroxyleucine (**3-OHLeu**)		
1	172.74					
2	55.75	4.98	d (4.4)	0.93, 1.98, 5.26, 8.78	172.74	0.93, 1.98, 5.26, 8.21
3	79.41	5.26	d (7.8)	4.98, 8.78	18.97, 19.39, 171.33	0.92, 0.93, 4.98, 8.21
4	30.06	1.98	m	0.92, 0.93, 4.98	18.97, 19.39, 79.41	0.92, 0.93, 4.98, 8.78
5	18.97	0.92	d (6.4)	1.98	79.41	1.98, 5.26, 8.21, 8.78
5N	19.39	0.93	d (6.4)	1.98, 4.98	79.41	1.98, 4.98, 5.26, 8.78
NH		8.78	d (4.4)	4.98, 5.26		0.92, 0.93, 1.10, 1.98 4.20, 8.21
				3,4-Dimethylglutamine (**3,4-DiMeGln**)		
1	174.13					
2	58.68	4.20	d (9.8)	1.10, 2.28, 9.54	37.50	2.28, 8.78
3	37.50	2.28	m	1.10, 2.66, 4.20, 9.54	14.66, 118.14	1.10, 1.22, 2.66, 4.20 9.54

Table 2 (Continued)

	δ					
	^{13}C	^{1}H	mult (*J*, Hz)	TOCSY	HMBC[b]	ROESY
			3,4-Dimethylglutamine (**3,4-DiMeGln**)			
3-Me	15.98	1.10	d (6.8)	2.28, 4.20	37.50, 43.93, 58.68	2.28, 2.66, 7.60, 8.78
4	43.93	2.66	dq (7.3, 2.9)	1.22, 2.28	15.98, 37.50, 181.14	1.10, 1.22, 2.28, 7.60, 9.54
4-Me	14.66	1.22	d (7.3)	2.66	37.50, 43.93, 181.14	2.28, 2.66
5	181.14					
2-NH		9.54	br s	2.28, 4.20		2.28, 2.66, 3.80, 4.52
5-NH		7.00	br s		43.93	7.60
		7.60	br s			1.10, 2.66, 7.00
			2,3-Diaminobutanoic Acid (**Dab**)			
1	171.87[c]					
2	55.72	4.52	d (7.3)	1.31, 3.80, 8.51		9.54
3	49.15	3.80	m	1.31, 4.52, 8.51	55.72	8.51, 9.54
4	15.80	1.31	d (6.8)	3.80, 4.52	49.15, 55.72	3.80
2-NH		8.51	d (8.3)	3.80, 4.52		3.80, 4.45
			Threonine (**Thr**)			
1	174.22[c]					
2	60.02	4.45	m	1.19, 4.24, 7.91		1.19, 4.24, 7.91, 8.51
3	68.39	4.24	dd (6.4, 3.4)	1.19, 4.45, 7.91		1.19, 4.45, 7.91
4	20.06	1.19	d (6.4)	4.24, 4.45	60.02, 68.39	4.24, 4.45, 7.91
NH		7.91	d (7.8)	4.24, 4.45	172.16	1.19, 4.06, 4.24, 4.45
			Glycine (**Gly 2**)			
1	172.16					
2	43.84	3.86	d (17.1)	4.06, 8.50	172.16, 179.33	4.06
		4.06	d (17.1)	3.86, 8.50	172.16, 179.33	3.86, 7.91, 8.50
NH		8.50	t (5.9)	3.86, 4.02	179.33	1.26, 4.06
			2,3-Dihydroxy-2,6,8-trimethyl-4,6-decadienoic Acid (**Dhtda**)			
1	179.33					
2	78.72					
2-Me	22.74	1.26	s		73.08, 78.72, 179.33	5.49, 8.51
3	73.08	4.79	d (10.3)	5.49, 6.16	125.93, 139.07	5.49
4	125.93	5.49	t (10.3)	4.79, 6.16	132.17	1.26, 1.80, 4.79, 6.16
5	139.07	6.16		4.79, 5.49	17.00, 73.08, 139.67	1.80, 5.26, 5.49
6	132.17					
6-Me	17.00	1.80	br s	5.26	132.17, 139.07	2.36, 5.26, 5.49
					139.67	
7	139.67	5.26	d (7.8)	0.86, 0.97, 1.28, 1.39	17.00, 31.45, 35.63	1.80, 6.16
				1.80, 2.36	139.07	
8	35.63	2.36	m	0.86, 0.97, 1.28, 1.39	21.07, 139.67	0.97, 1.28, 1.39, 1.80
				5.26		
8-Me	21.07	0.97	d (6.8)	0.86, 1.28, 1.39, 2.36	31.45, 35.63, 139.67	1.39, 2.36
				5.26		
9	31.45	1.28	m	0.86, 0.97, 2.36, 5.26	12.49, 21.07, 35.63	0.86, 2.36
					139.67	
		1.39	dd (11.3, 7.3)	0.86, 0.97, 2.36, 5.26	12.49, 21.07, 35.63	0.86, 0.97, 2.36
					139.67	
10	12.49	0.86	t (7.8)	0.97, 1.28, 1.39, 2.36	31.45, 35.63	1.28, 1.39

[a] Spectra were recorded in CD_3OH at 500 MHz for ^{1}H and 125 MHz for ^{13}C and referenced to the residual solvent signal (δ_H 3.30, δ_C 49.00). [b] Optimized for $J = 3.5$ and $J = 8.5$ Hz. Carbons correlated to proton resonances in the ^{1}H column. [c] Assignments are interchangeable.

Collection, Extraction, and Purification. Specimens of *T. mirabilis*[25] were collected in 1993 from a depth of −10 m near Madang Harbor on the north coast of Papua New Guinea and frozen immediately after collection. Frozen sponge (1359 g wet wt) was mixed with dry ice and ground to a fine powder. Extraction with H_2O and lyophilization of the resulting solution provided 57 g of aqueous extract. The sponge material was then freeze-dried and extracted with MeOH−CH_2Cl_2 (1:1) and 100% MeOH to give 2.5 g of organic extract. A 32 g portion of the aqueous extract was separated by vacuum liquid chromatography on wide-pore (275 Å) C_4 packing eluted with an aqueous−MeOH step gradient. The material that eluted with H_2O−MeOH (2:1) (308 mg) was fractionated on Sephadex LH-20 with MeOH−H_2O (7:3) to give 93 mg of a peptide rich sample. Final purification was achieved by reversed-phase C_{18} HPLC using a linear gradient with increasing concentrations of CH_3CN in H_2O (0.05% trifluoroacetic acid, vol/vol) to give 12 mg of papuamide A (**1**) and 5 mg of papuamide B (**2**). A portion of the organic extract (1.5 g) was separated by a modified Kupchan solvent−solvent partition protocol.[26] The aqueous-soluble fraction from this protocol (793 mg) was applied to a C_{18} vacuum flash chromatography column and eluted with increasing concentrations of MeOH in H_2O. Fractions eluted with MeOH−H_2O (1:1) and 100% MeOH were combined and purified by Sephadex LH-20 and C_{18} HPLC, in a manner similar to that described above for the aqueous extract, to give an additional 5 mg of **1** and 3 mg of **2**.

Specimens of *T. swinhoei* (Gray, 1867)[25] were collected at a depth of −10 m from vertical walls northeast of Kranket Island, Madang Lagoon, Papua New Guinea, in January 1994. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. A voucher sample of the sponge has been deposited in the Zoological Museum at the University of Amsterdam. Approximately 1 kg of lyophilized sponge was cut into small pieces and immersed in and subsequently extracted repeatedly with MeOH (3 × 300 mL). The combined MeOH extracts

(25) Taxonomic identifications were made by R. Van Soest.

(26) Grode, S. H.; James, T. R.; Cardellina, J. H., II; Onan, K. D. *J. Org. Chem.* **1983**, *48*, 5203−5207.

(27) Bokesch, H. R.; Groweiss, A.; McKee, T. C.; Boyd, M. R. *J. Nat. Prod.* in press.

were concentrated in vacuo and then partitioned between H_2O (500 mL) and EtOAc (3 × 150 mL). The H_2O extract was evaporated to dryness in vacuo to yield 23.45 g of a red-orange amorphous solid as fraction A. The dried EtOAc-soluble portion of the original crude MeOH extract was taken up in 4:1 MeOH−H_2O (466 mL) and extracted repeatedly with CH_2Cl_2 (4 × 60 mL). The combined CH_2Cl_2 extracts were concentrated to yield a red oil designated fraction C, and the MeOH−H_2O-soluble portion was evaporated to give fraction B.

Fractionation of the aqueous extract, fraction A, by sequential application of (i) C_{18} reversed-phase flash chromatography (gradient elution: H_2O to MeOH), (ii) Sephadex LH-20 (eluent: MeOH), (iii) C_{18} reversed-phase HPLC (eluent: 2:3 CH_3CN−aqueous 0.05% TFA), and (iv) C_{18} reversed-phase HPLC (eluent: 42.5:57.5 CH_3CN−aqueous 0.05% TFA) gave pure papuamide A (**1**) (57 mg) and papuamide B (**2**) (6.4 mg) as clear glasses. Separation of the CH_2Cl_2 extract, fraction C, via sequential application of (i) C_{18} reversed-phase flash chromatography (gradient elution: 1:1 MeOH−H_2O to MeOH), (ii) Sephadex LH-20 chromatography (eluent: MeOH), and (iii) C_{18} reversed-phase HPLC (eluent: 42.5:57.5 CH_3CN−aqueous 0.05% TFA) gave pure papuamide C (**3**) (25 mg) and papuamide D (**4**) (16 mg) as clear glasses.

Papuamide A (1). Amorphous glass; $[\alpha]^{25}_D = +12.0°$ (*c* 3.47, MeOH); UV [MeOH] λ_{max} 227 (ϵ 22 800), 274 (ϵ 1400) nm; [MeOH + NaOH] λ_{max} 233 (ϵ 18 500) 285 (ϵ 1900) nm; IR (neat, KCl) 3360, 2930, 1748, 1652, 1456, 1203 cm^{-1}; HRFABMS of a CsI-doped sample provided *m*/*z* 1548.6549 $(M + Cs)^+$, calculated 1548.6602 for $C_{66}H_{105}N_{13}O_{21}Cs$; ^1H NMR see Table 1; ^{13}C NMR see Table 1.

Papuamide B (2). Amorphous glass; $[\alpha]^{25}_D = +12.9°$ (*c* 0.13, MeOH); UV [MeOH] λ_{max} 225 (ϵ 24 000), 274 (ϵ 1900) nm; HRFABMS of a CsI-doped sample provided *m*/*z* 1534.6486 $(M + Cs)^+$, calculated 1534.6445 for $C_{65}H_{103}N_{13}O_{21}Cs$; ^1H NMR see Table 2; ^{13}C NMR see Table 2.

Papuamide C (3). Clear glass; $[\alpha]^{25}_D = +4.4°$ (*c* 1.14, MeOH); UV [4:1 CH_3CN−H_2O] λ_{max} 229 (ϵ 18 470), 276 (ϵ 2722) nm; HRFABMS provided *m*/*z* 1399.7361 $(M + H)^+$, calculated 1399.7361 for $C_{66}H_{103}N_{12}O_{21}$, and *m*/*z* 1421.7211 $(M + Na)^+$, calculated 1421.7181 for $C_{66}H_{102}N_{12}O_{21}Na$; ^1H NMR (500 MHz, 4:1 CD_3CN−H_2O) δ **Hpr** H-2, 5.0 (1H); H-3, 1.61 (1H) and 2.07 (1H); H-4, 1.65 (2H); H-5, 1.30 (1H) and 1.65 (1H); H-6, 3.06 (1H) and 4.03 (1H); ***β*-OMeTyr** H-α, 5.25 (1H); H-*β*, 4.22, (1H); OMe on C-*β*, 2.99 (3H); H-2 and H-6, 7.17 (2H); H-3 and H-5, 6.74 (2H); NH, 7.67 (1H); **NMeThr** H-2, 4.30 (1H); H-3, 3.82 (1H); H-4, 0.39 (3H); *N*-Me, 3.01 (3H); **Ala** H-2, 4.44 (1H); H-3, 1.39 (3H); NH, 7.02 (1H); **Gly 1** H-2, 3.44 (1H) and 4.08 (1H); NH, 8.32 (1H); **3-OMeAla** H-2, 4.11 (1H); H-3, 3.62 (1H) and 3.78 (1H); OMe on C-3, 3.29 (3H); NH, 7.96 (1H); **3-OHLeu** H-2, 5.00 (1H); H-3, 5.25 (1H); H-4, 1.90 (1H); H-5, 0.84 (3H); H-6, 0.77 (3H); NH 8.65 (1H); **3,4-DiMeGln** H-2, 4.28 (1H); H-3, 2.07 (1H); Me on C-3, 0.95 (3H); H-4, 2.56 (1H); Me on C-4, 1.08 (3H); NH on C-2, 8.07 (1H); **Aba** H-3, 6.72 (1H); H-4, 1.65 (3H); NH, 9.05 (1H); **Thr** H-2, 4.47 (1H); H-3, 4.29 (1H); H-4, 1.16 (3H); NH, 7.82 (1H); **Gly 2** H-2, 3.78 (1H) and 3.98 (1H); NH, 8.32 (1H); **Dhtda** Me on C-2, 1.19 (3H); H-3, 4.79 (1H); H-4, 5.46 (1H); H-5, 6.10 (1H); Me on C-6, 1.73 (3H); H-7, 5.20 (1H); H-8, 2.31 (1H); Me on C-8, 0.89 (3H); H-9, 1.16 (1H) and 1.32 (1H); H-10, 0.77 (3H). Most protons appeared as two resonances and in general were very broad and poorly resolved; hence, only the chemical shift is given. Assignments and some chemical shifts based on HMQC, HMBC, and COSY data. ^{13}C NMR (125 MHz, 4:1 CD_3CN−H_2O) δ **Hpr** CO, 170.8; C-2, 54.0; C-3, 26.6; C-4, 21.1; C-5, 25.3; C-6, 44.6; ***β*-OMeTyr** CO, 171.8−172.8; C-α, 52.3; C-*β*, 84.8; OMe on C-*β*, 57.1; C-1, 129.1; C-2 and C-6, 130.8; C-3 and C-5, 116.2; C-4, 158.1; **NMeThr** C-1, 169.3; C-2, 64.2; C-3, 64.0; C-4, 19.7; *N*-Me, 31.3; **Ala** C-1, 174.5; C-2, 48.4; C-3, 15.6; **Gly 1** C-1, 171.8−172.8; C-2, 42.8; **3-OMeAla** C-1, 171.8−172.8; C-2, 57.6; C-3, 71.2; OMe on C-3, 59.4; **3-OHLeu** C-1, 171.8−172.8; C-2, 55.9; C-3, 80.1; C-4, 28.8; C-5, 19.7; C-6, 18.7; **3,4-DiMeGln** C-1, 175.3; C-2, 58.9; C-3, 37.6; Me on C-3, 13.1; C-4, 41.1; Me on C-4, 15.4; C-5, 179.6; **Aba** C-1, 166.8; C-2, 129.5; C-3, 135.2; C-4, 13.6; **Thr** C-1, 171.8−172.8; C-2, 59.9; C-3, 69.0; C-4, 19.9; **Gly 2** C-1, 171.8−172.8; C-2, 43.8; **Dhtda** C-1, 178.6; C-2, 78.9; Me on C-2, 22.4; C-3, 73.0; C-4, 125.0; C-5, 139.1; C-6, 131.8; Me on C-6, 16.9; C-7, 139.8; C-8, 35.0; Me on C-8, 20.9; C-9, 40.0; C-10, 12.5. Many of the carbons appeared as multiple resonances. Assignments based on HMQC, HMBC, COSY, and ROESY data.

Papuamide D (4). Clear glass; $[\alpha]^{25}_D = +16.1°$ (*c* 0.68, MeOH); UV [4:1 CH_3CN−H_2O] λ_{max} 226 (ϵ 18 490), 274 (ϵ 2272) nm; HRFABMS provided *m*/*z* 1385.7157 $(M + H)^+$, calculated 1385.7204 for $C_{65}H_{101}N_{12}O_{21}$, and *m*/*z* 1407.7029 $(M + Na)^+$, calculated 1407.7024 for $C_{65}H_{100}N_{12}O_{21}Na$; ^1H NMR (500 MHz, 4:1 CD_3CN−H_2O) δ **Hpr** H-2, 5.07 (1H); H-3, 1.57 (1H) and 2.11 (1H); H-4, 1.63 (2H); H-5, 1.27 (1H) and 1.66 (1H); H-6, 3.10 (1H) and 4.05 (1H); ***β*-OMeTyr** H-α, 5.18 (1H); H-*β*, 4.26 (1H); OMe on C-*β*, 3.02 (3H); H-2 and H-6, 7.16 (2H); H-3 and H-5, 6.74 (2H); NH, 7.76 (1H); **Thr 1** H-2, 3.81 (1H), H-3, 3.66 (1H); H-4, 0.45 (3H); NH, 7.86; **Ala** H-2, 4.17 (1H); H-3, 1.38 (3H); NH, 7.14; **Gly 1** H-2, 3.48 (1H) and 4.08 (1H); NH, 8.23 (1H); **3-OMeAla** H-2, 4.17 (1H); H-3, 3.61 (1H) and 3.73 (1H); OMe on C-3, 3.28 (3H); NH, 7.96 (1H); **3-OHLeu** H-2, 4.99 (1H); H-3, 5.23 (1H); H-4, 1.90 (1H); H-5, 0.82 (3H); H-6, 0.77 (3H); NH, 8.57 (1H); **3,4-DiMeGln** H-2, 4.25 (1H); H-3, 2.03 (1H); Me on C-3, 0.92 (3H); H-4, 2.50 (1H); Me on C-4, 1.05 (3H); NH on C-2, 8.00 (1H); **Aba** H-3, 6.67 (1H); H-4, 1.66 (3H); NH, 9.10 (1H); **Thr 2** H-2, 4.44 (1H); H-3, 4.27 (1H); H-4, 1.15 (3H); NH, 7.83 (1H); **Gly 2** H-2, 3.79 (1H) and 4.05 (1H); NH, 8.32 (1H); **Dhtda** Me on C-2, 1.18 (3H); H-3, 4.77 (1H); H-4, 5.45 (1H); H-5, 6.10 (1H); Me on C-6, 1.72 (3H); H-7, 5.18 (1H); H-8, 2.30 (1H); Me on C-8, 0.88 (3H); H-9, 1.15 (1H) and 1.31 (1H); H-10, 0.77 (3H); ^{13}C NMR (125 MHz, 4:1 CD_3CN−H_2O) δ **Hpr** CO, 170.7; C-2, 53.9; C-3, 26.7; C-4, 21.4; C-5, 25.5; C-6, 44.7; ***β*-OMeTyr** CO, 171.9; C-α, 52.9; C-*β*, 84.3; OMe on C-*β*, 57.3; C-1, 129.3; C-2 and C-6, 130.4; C-3 and C-5, 116.3; C-4, 157.9; **Thr 1** C-1, 170.7; C-2, 60.8; C-3, 66.9; C-4, 19.2; **Ala** C-1, 174.9; C-2, 50.3; C-3, 17.4; **Gly 1** C-1, 172.0; C-2, 42.9; **3-OMeAla** C-1, 172.6; C-2, 57.1; C-3, 71.1; OMe on C-3, 59.3; **3-OHLeu** C-1, 172.7; C-2, 55.6; C-3, 79.8; C-4, 28.9; C-5, 19.2; C-6, 18.9; **3,4-DiMeGln** C-1, 175.0; C-2, 58.7; C-3, 37.8; Me on C-3, 13.1; C-4, 41.1; Me on C-4, 15.5; C-5, 179.8; **Aba** C-1, 166.7; C-2, 129.3; C-3, 135.4; C-4, 13.6; **Thr 2** C-1, 172.7; C-3, 59.9; C-3, 69.0; C-4, 19.8; **Gly 2** C-1, 172.5; C-2, 43.7; **Dhtda** C-1, 178.6; C-2, 78.8; Me on C-2, 22.4; C-3, 73.0; C-4, 124.8; C-5, 139.2; C-6, 131.8; Me on C-6, 16.9; C-7, 139.8; C-8, 35.0; Me on C-8, 20.9; C-9, 30.9; C-10, 12.4. Many of the carbons appeared as multiple resonances. Assignments based on HMQC, HMBC, COSY, and ROESY data.

Preparation of Diacetate 5. A MeOH solution containing 7 mg of papuamide A (**1**) was treated with 200 μL of acetic anhydride for 1 h. Removal of the solvent under a stream of N_2 provided the *N*-acetate derivative which was dissolved in anhydrous pyridine and reacted with 200 μL of acetic anhydride. After 3 h the solvent was removed in vacuo and the residue purified by C_{18} HPLC eluted with H_2O for 10 min followed by a gradient of increasing concentration of CH_3CN in H_2O (30% CH_3CN to 70% CH_3CN over 15 min). Diacetate **5** was the principal reaction product (2 mg), eluting with a retention time of 18.5 min. HRFABMS of a CsI-doped sample provided *m*/*z* 1632.6776 $(M + Cs)^+$, calculated 1632.6813 for $C_{70}H_{109}N_{13}O_{23}Cs$; ^1H NMR (500 MHz, 4:1 CD_3CN−H_2O) δ **Hpr** H-2, 5.02 (1H, d, $J = 3.9$ Hz); H-3, 1.66 (1H, m) and 2.07 (1H, m); H-4, 1.15 (1H, m) and 1.69 (1H, m); H-5, 1.35 (1H, m) and 1.66 (1H, m); H-6, 3.13 (1H, dt, $J = 14.2$ and 5.4 Hz); ***β*-OMeTyr** H-α, 5.26 (1H, t, $J = 9.3$ Hz); H-*β*, 4.34 (1H, d, $J = 9.3$ Hz); OMe on C-*β*, 3.03 (3H, s); NH, 7.71 (1H, d, $J = 9.3$ Hz); H-2 and H-6, 7.05 (2H, d, $J = 8.8$ Hz); H-3 and H-5, 7.40 (2H, d, $J = 8.8$ Hz); OAc on C-4, 2.22 (3H, s); **NMeThr** H-2, 4.32 (1H, d, $J = 6.8$ Hz); H-3, 3.82 (1H, m); H-4, 0.40 (3H, d, $J = 6.8$ Hz); *N*-Me, 3.01 (3H, s); **Ala** H-2, 4.47 (1H, quint, $J = 6.3$ Hz), H-3, 1.40 (3H, d, $J = 7.3$ Hz); NH, 7.06 (1H, d, $J = 5.9$ Hz); **Gly 1** H-2, 3.78 (1H, dd, $J = 7.8$ and 4.9 Hz) and 4.13 (1H, dd, $J = 7.8$ and 3.4 Hz); NH, 8.41 (1H, dd, $J = 4.9$ and 3.4 Hz); **3-OMeAla** H-2, 4.14 (1H, dt, $J = 3.9$ and 3.4 Hz); H-3, 3.69 (1H, dd, $J = 10.8$ and 3.4 Hz) and 3.79 (1H, dd, $J = 10.8$ and 3.4 Hz); OMe on C-3, 3.32 (3H, s); NH, 8.04 (1H, d, $J = 3.9$ Hz); **3-OHLeu** H-2, 5.15 (1H, t, $J = 10.3$ Hz); H-3, 5.32 (1H, dd, $J = 10.3$ and 2.0 Hz); H-4, 1.91 (1H, m); H-5, 0.79 (3H, d, $J = 6.4$ Hz); H-6, 0.85 (3H, d, $J = 6.4$ Hz); NH, 8.37 (1H, d, $J = 10.3$ Hz); **3,4-DiMeGln** H-2, 4.31 (1H, dd, $J = 5.4$ and 3.4 Hz); H-3, 2.10 (1H, m); Me on C-3, 0.98 (3H, d, $J = 6.8$ Hz); H-4, 2.60 (1H, dq, $J = 7.3$ and 3.4 Hz); Me on C-4, 1.14 (3H, d, $J = 7.3$ Hz); C-2 NH, 8.58 (1H, d, $J = 5.4$ Hz); C-5 NH, 6.46 (1H, br s) and 6.95 (1H, br s); **Dab** H-2,

4.39 (1H, dd, $J = 6.8$ and 3.9 Hz); H-3, 4.27 (1H, m); H-4, 1.16 (3H, d, $J = 7.3$ Hz); C-2 NH, 7.67 (1H, d, $J = 7.3$ Hz); C-3 NH, 7.58 (1H, d, $J = 8.3$ Hz); *N*-Ac on C-3, 1.85 (3H, s); **Thr** H-2, 4.38 (1H, dd, $J = 6.8$ and 3.7 Hz); H-3, 4.20, (1H, dq, $J = 6.4$ and 3.7 Hz); H-4, 1.08 (1H, d, $J = 6.4$ Hz); NH, 8.12 (1H, d, $J = 6.8$ Hz); **Gly 2** H-2, 3.80 (1H, dd, $J = 8.3$ and 5.9 Hz) and 4.09 (1H, dd, $J = 8.3$ and 5.9 Hz); NH, 8.27 (1H, t, $J = 5.9$ Hz); **Dhtda** Me on C-2, 1.18 (3H, d, $J = 2.9$ Hz); H-3, 4.79 (1H, d, $J = 10.3$ Hz); H-4, 5.42 (1H, dt, $J = 10.3$ and 2.9 Hz); H-5, 6.11 (1H, dt, $J = 11.7$ and 3.4 Hz); Me on C-6, 1.74 (3H, d, $J = 3.4$ Hz); H-7, 5.21 (1H, d, $J = 9.8$ Hz); H-8, 2.31 (1H, m); Me on C-8, 0.91 (3H, d, $J = 6.8$ Hz); H-9, 1.20 (1H, m) and 1.33 (1H, m); H-10, 0.81 (3H, t, $J = 7.8$ Hz); ^{13}C NMR (125 MHZ, 4:1 CD_3-$CN-H_2O$), chemical shifts and assignments were deduced from HSQC and HMBC correlations, δ **Hpr** CO, 170.8; C-2, 53.4; C-3, 26.4; C-4, 20.8; C-5, 25.4; C-6, 44.1; ***β*-OMeTyr** CO, 172.2; C-α, 51.9; C-*β*, 84.2; OMe on C-*β*, 57.2; C-1, 136.0; C-2 and C-6, 130.4; C-3 and C-5, 122.7; C-4, 151.5; OAc on C-4, 171.2 and 20.7; **NMeThr** C-1, 169.4; C-2, 63.8; C-3, 63.3; C-4, 19.7; *N*-Me, 30.8; **Ala** C-1, 174.4; C-2, 48.2; C-3, 15.1: **Gly 1** C-1, 171.5; C-2, 42.9: **3-OMeAla** C-1, not observed; C-2, 57.3; C-3, 70.7; OMe on C-3, 59.1; **3-OHLeu** C-1, 172.9; C-2, 55.5; C-3, 79.7; C-4, 28.5; C-5, 18.2; C-6, 19.2; **3,4-DiMeGln** C-1, 174.7; C-2, 58.2; C-3, 37.9; Me on C-3, 13.1; C-4, 41.4; Me on C-4, 15.1; C-5, 179.6; **Dab** C-1, 171.6; C-2, 59.0; C-3, 47.6; C-4, 16.1; **Thr** C-1, 172.3; C-2, 59.4; C-3, 68.1; C-4, 19.2; **Gly 2** C-1, 172.1; C-2, 43.5; **Dhtda** C-1, 178.3; C-2, not observed; Me on C-2, 21.8; C-3, 72.2; C-4, 125.3; C-5, 138.8; C-6, 131.8; Me on C-6, 16.6; C-7, 139.3; C-8, 34.7; Me on C-8, 20.2; C-9, 30.6; C-10, 12.0.

Hydrolysis of Papuamide A (1) with Triethylamine. A solution of papuamide A (**1**) (20.3 mg) in 2 mL of 5% aqueous triethylamine was heated at 49 °C for 16 h with stirring. The solution was evaporated to dryness in vacuo and the reaction mixture chromatographed by C_{18} reversed-phase HPLC using a linear gradient of (A) 0.05% aqueous TFA and (B) CH_3CN with 0% B at start to 100% B over 120 min to give pure tripeptide **6** (3.2 mg). Tripeptide **6**: isolated as a clear oil; $[\alpha]^{25}_D = -73.2°$ (*c* 0.21, MeOH); UV [CH_3CN-H_2O] λ_{max} 240 (ϵ 675), 276 (ϵ 654) nm; HRFABMS provided *m*/*z* 438.2235 $(M + H)^+$, calculated 438.2240 for $C_{21}H_{32}N_3O_7$ and *m*/*z* 460.2068 $(M + Na)^+$, calculated 460.2060 for $C_{21}H_{31}N_3O_7Na$; ^{1}H NMR (500 MHZ, DMSO-d_6) δ **Hpr** H-2, 5.14 (1H, br d, $J = 5.0$ Hz); H-3, 1.55 (1H, m) and 2.15 (1H, br d, $J = 13.0$ Hz); H-4, 1.26 (1H, m) and 1.67 (1H, m); H-5, 1.42 (1H, m) and 1.67 (1H, m); H-6, 3.12 (1H, t, $J = 12.3$ Hz) and 4.21 (1H, br d, $J = 12.3$ Hz); ***β*-OMeTyr** H-α, 5.19 (1H, t, $J = 9.7$ Hz); H-*β*, 4.31 (1H, d, $J = 9.7$ Hz); H-2 and H-6, 7.14 (2H, d, $J = 8.0$ Hz); H-3 and H-5, 6.70 (2H, d, $J = 8.0$ Hz); OMe on C-*β*, 3.00 (3H, s); NH, 9.17 (1H, d, $J = 9.7$ Hz); **NMeThr** H-2, 3.27 (1H, m); H-3, 3.42 (1H, m); H-4, 0.32 (3H, d, $J = 6.0$ Hz); *N*-Me, 2.28 (3H, m); NH, 8.55 (1H, br s); NH′, 8.95 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ **Hpr** CO, 172.0; C-2, 51.5; C-3, 26.2; C-4, 20.4; C-5, 24.7; C-6, 43.3; ***β*-OMeTyr** CO, 169.6; C-α, 51.7; C-*β*, 82.6: OMe on C-*β*, 56.1; C-1, 127.4; C-2 and C-6, 129.2; C-3 and C-5, 114.9; C-4, 157.5; **NMeThr** C-1, 164.5; C-2, 66.7; C-3, 65.0; C-4, 18.8; *N*-Me, 31.2. Many protons and carbons appeared as two resonances, in which case only the signal for the major one (>90% excess) is reported above. Assignments based on HMQC, HMBC, COSY, ROESY, and NOE data.

Sodium Borohydride Reduction of Papuamide A (1). A solution of papuamide A (**1**) (5.4 mg) in 2 mL of 70% aqueous 2-propanol and excess $NaBH_4$ (19.8 mg) was stirred for 16 h at room temperature. The solution was evaporated to dryness in vacuo and the reaction mixture chromatographed in a manner identical to that described above for the purification of **6**, to give both the tripeptide **6** (0.5 mg) (retention time 40.3 min) and the tetraol **7** (0.1 mg) (retention time 48.7 min). Tetraol **7**: isolated as a clear oil; UV [CH_3CN-H_2O] λ_{max} 236 (ϵ 9030) nm; HRFABMS provided *m*/*z* 409.2324 $(M + Na)^+$, calculated 409.2315 for $C_{19}H_{34}N_2O_6Na$, *m*/*z* 391.2215 $(M + Na - H_2O)^+$, calculated 391.2209 for $C_{19}H_{32}N_2O_5Na$, and *m*/*z* 369.2403 $(M + H - 2H_2O)^+$, calculated 369.2389 for $C_{19}H_{31}N_2O_4$; ^{1}H NMR (500 MHZ, DMSO-d_6) δ **Dhtda** Me on C-2, 1.08 (3H, s); H-3, 4.60 (1H, d, $J = 10.3$ Hz); H-4, 5.39 (1H, m); H-5, 5.98 (1H, d, $J = 12.3$ Hz); Me on C-6, 1.73 (3H, dd, $J = 4.1$ and 1.1 Hz); H-7, 5.22 (1H, m); H-8, 2.32 (1H, m); Me on C-8, 0.91 (3H, d, $J = 6.7$ Hz); H-9, 1.21 (1H, m) and 1.33 (1H, m); H-10, 0.82 (3H, m); **Gly** H-2, 3.65−3.75 (2H, m); NH, 7.98 (1H, d, $J = 6.1$ Hz); **Dhab** H-1, 3.30 (1H, dd, $J = 10.7$ and 6.7 Hz) and 3.42 (1H, dd, $J = 10.7$ and 6.7 Hz); H-2, 3.62 (1H, m); H-3, 3.82 (1H, m); H-4, 0.96 (3H, d, $J = 6.3$ Hz); NH, 7.29 (1H, d, $J = 9.3$ Hz); ^{13}C NMR (125 MHZ, DMSO-d_6) δ **Dhtda** C-1, 175.4; C-2, 76.7; C-3, 70.8; C-4, 126.9; C-5, 135.7; C-6, 130.6; C-7, 137.5; C-8, 33.5; C-9, 29.8; C-10, 11.9; **Gly** C-1, 168.9; C-2, 42.2; **Dhab** C-1, 60.4; C-2, 55.8; C-3, 64.4; C-4, 20.1; Many protons and carbons appeared as two resonances, in which case only the signal for the major one (>90% excess) is reported above. Assignments based on HMQC, HMBC, COSY, ROESY, and NOE data.

Interconversion of Papuamide A (1) to Papuamide C (3) by Hofmann Elimination. To 6.8 mg of papuamide A (**1**) was added 3 mL of freshly distilled DMF and 0.5 mg of K_2CO_3. The solution was stirred for 2 h, and then 100 μL of MeI (passed over dry basic alumina) was added and the reaction stirred for a further 15 h at room temperature. The volume of the solution was reduced in vacuo to 0.25 mL. The resulting solution was fractionated by reversed-phase C_{18} HPLC with 39:61 $CH_3CN-0.05\%$ aqueous TFA as eluent to yield one major product (5.7 mg) that was identical to papuamide C (**3**) by co-injection on HPLC (a single sharp peak was observed by analytical HPLC), ^{1}H NMR, and positive ion HRFABMS $(M + Na)^+$ *m*/*z* 1421.7183 ($C_{66}H_{102}N_{12}O_{21}Na$, calculated 1421.7181).

Synthesis of *N*-Methylthreonine Standards. The synthetic method described by Beulshausen et al.[22] was modified as described below. $SOCl_2$ (0.26 mL) was added dropwise to 1 mL of dry MeOH, and the solution was cooled to −10 °C. To this solution was added 100 mg of L-threonine (2*S*,3*S*-threonine), and the mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated to dryness, and the resulting threonine methyl ester (Thr-OMe) was crystallized from petroleum ether. To a solution of Thr-OMe in CH_2Cl_2 (10 mL) was added 0.1 N aqueous trifluoroacetic acid (TFA) (10 mL), and the mixture was cooled to 0 °C. With vigorous stirring, a 37% aqueous solution of formaldehyde (62 μL) was added dropwise, and stirring was continued at room temperature for 8 h. The reaction mixture was neutralized with $NaHCO_3$ and the product isolated by extraction with CH_2Cl_2 (4 × 10 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated in vacuo to give the oxazolidine derivative, which was used without further purification. A solution of the oxazolidine in CH_2Cl_2 (6 mL) was cooled to 0 °C, and TFA (6 mL) and triethylsilane (0.6 mL) were added. The mixture was stirred at room temperature for 24 h, then the solvent was removed in vacuo, and the residue was dissolved in 1 N HCl and washed with petroleum ether. Hydrolysis in 6 N HCl (400 μL) at 110 °C for 16 h provided (2*S*,3*S*)-*N*-MeThr. The identical procedure using L-*allo*-threonine, D-threonine, or D-*allo*-threonine as starting material provided (2*S*,3*R*)-, (2*R*,3*S*)-, or (2*R*,3*R*)-*N*-MeThr, respectively.

Determination of Absolute Stereochemistry. (a) Peptide Hydrolysis. Peptide samples (200 μg) were dissolved in degassed 6 N HCl (0.5 mL) in an evacuated glass tube and heated at 108 °C for 16 h. The solvent was removed in vacuo and the residue placed under high vacuum.

(b) LC−MS Analysis of Marfey's (FDAA) Derivatives.[23] An aqueous solution of the hydrolysate (20 μL) was treated with 6% triethylamine (10 μL) and 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (20 μL) at 40 °C for 1 h. The reaction mixture was diluted with 50 μL of H_2O and an aliquot applied to a C_{18} HPLC column eluted with $CH_3CN-0.05\%$ aqueous TFA using a linear gradient from 10% CH_3CN to 50% CH_3CN over 20 min. Derivatized amino acids were detected by absorption at 340 nm and by MS (mass range 300−800 Da). Retention times (min) are given in parentheses: L-Thr (9.94), L-*allo*-Thr (10.46), *N*-Me-L-Thr (11.07), D-*allo*-Thr (11.78), *N*-Me-D-Thr (12.07), OMe-L-Ala (12.50), L-Ala (12.86), d-Thr (13.47), *N*-Me-L-*allo*-Thr (13.89), *N*-Me-D-*allo*-Thr (14.60), OMe-D-Ala (15.76), D-Ala (15.82), D-Hpr (18.46), L-Hpr (19.74).

(c) LC−MS Analysis of 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl Isothiocyanate (GITC) Derivatives. An aqueous solution of the hydrolysate (20 μL) was treated with 6% triethylamine (10 μL) and 1% 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate in acetone (20 μL) at room temperature for 5 min. The reaction mixture was applied directly to a C_{18} HPLC column eluted as described above for the FDAA derivatives. Derivatized amino acids were detected by

absorption at 254 nm and by MS (mass range 400−950). Retention times (min) are given in parentheses: L-*allo*-Thr (12.08), L-Thr (12.40), *N*-Me-L-*allo*-Thr (12.45), D-*allo*-Thr (12.53), L-Ala (12.60), *N*-Me-L-Thr (12.76), *N*-Me-D-*allo*-Thr (13.17), D-Thr (13.52), OMe-L-Ala (13.54), D-Ala (13.67), *N*-Me-D-Thr (14.01), OMe-D-Ala (14.44), L-Hpr (15.79), D-Hpr (16.83).

(d) GC−MS Analysis of Trifluoroacetyl Isopropyl Ester Derivatives. A premixed solution of acetyl chloride−isopropyl alcohol (1:4) was added to the hydrolysate and heated to 100 °C for 1 h. The solution was dried under a stream of N_2, and trifluoroacetic anhydride (50 μL) in CH_2Cl_2 (200 μL) was added to the residue. The reaction mixture was heated to 100 °C for 20 min, then cooled to 0 °C, and evaporated under a stream of N_2. The product was taken up in ethyl acetate and immediately analyzed by GC−MS using a Chirasil-L-Val capillary column ramped from 40 to 240 °C at 10 °C/min, and a mass range of 40−650 Da was recorded every 1 s. Retention times (min) are given in parentheses: (2*R*,3*S*)-OHLeu (8.97), (2*S*,3*R*)-OHLeu (9.17), OMe-d-Ala (9.26), NMe-L-Thr (9.37), D-Ser (9.93), (2*R*,3*R*)-OHLeu (10.87), (2*S*,3*S*)-OHLeu (11.18), L-Hpr (11.42), (2*S*,3*S*,4*R*)-diMePyroGlu (12.77), (2*R*,3*S*)-Dab (14.48), (2*S*,3*S*,4*R*)-diMeGlu (14.74), (2*S*,3*R*)-Dab (14.80), (2*R*,3*R*)-Dab (15.60), (2*S*, 3*S*)-Dab (16.18).

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Supporting Information Available: NMR spectral data for papuamide A (**1**), papuamide B (**2**), and the diacetate derivative of papuamide A (**5**), including ^{1}H NMR, ^{13}C NMR, HSCQ, HMBC, TOCSY, and ROESY spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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