The Structure of Palau'amide, a Potent Cytotoxin from a Species of the Marine Cyanobacterium *Lyngbya*

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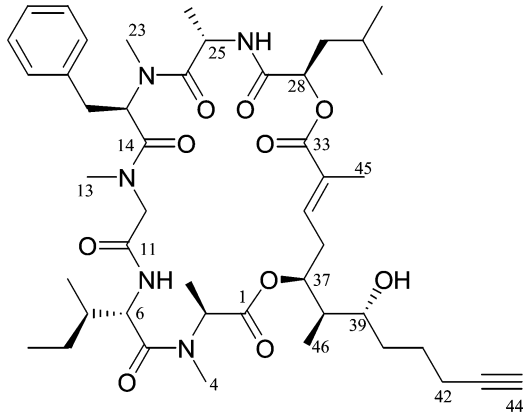
Bioassay-guided fractionation of the extract of a species of *Lyngbya* from Palau has yielded Palau'amide (**1**), which had an IC_{50} value of 13 nM against KB cells. The structure of **1** was elucidated by NMR analysis in a variety of solvents. In particular, a gradient-enhanced band-selective HMBC experiment allowed unambiguous assignment of HMBC correlations to carbon signals that were separated by 0.1 ppm. The relative stereochemistry and the absolute configurations of all but one (C-37) of the nine chiral centers were determined by NMR analysis of the α-methoxyphenylacetic acid (MPA) derivatives and chiral HPLC of the degradation products of **1**. The assignment of *S* rather than *R* stereochemistry at C-37 is discussed in detail.

Since the discovery of penicillin from *Penicillium notatum* in the 1940s, terrestrial microorganisms have been a key source of many of the important products of the drug industry. These encouraging results with terrestrial microorganisms suggest that their marine counterparts might also have the potential to be useful sources of new drug leads. Indeed, investigations of marine cyanobacteria, for example, have shown that these organisms are prolific producers of secondary metabolites, many of which possess a wide range of biological activities.[1]

In the spring of 2000, we collected a strain of cyanobacterium from Ulong Channel, Palau, belonging to the genus *Lyngbya* (Oscillatoriaceae). Since the lipophilic extract of these dark reddish-brown clumps was slightly solid tumor selective in the Corbett cytotoxicity assay,[2] the cyanobacterium was re-collected for further study. Bioassay-guided fractionation of the lipophilic extract provided palau'amide (**1**) in 0.2% yield (2.8 mg).[3]



Palau'amide (**1**)

Results and Discussion

High-resolution mass spectrometry produced a $[M + Na]^+$ ion at *m/z* 874.5003 that afforded a molecular formula of $C_{46}H_{69}O_{10}N_5$ (0.1 mDa error). The proton and carbon NMR data, recorded in $CDCl_3$, of the apparently chromatographically homogeneous material revealed a complex mixture of conformers (1:1:0.3:0.3). By changing the solvent to MeOH-d_3 (Table 1) the conformational ratio improved to an acceptable level (2:1:0.1) and provided reasonably well-dispersed signals. Examination of the ^{1}H NMR (500 MHz) of **1** recorded in MeOH-d_3 indicated the sample was a peptide containing both aliphatic and aromatic residues. The ^{13}C NMR spectrum contained seven carbonyls, five of which were amides based on the presence of two secondary amide (δ_H 8.17, 8.57) and three *N*-methylamide proton signals (δ_H 2.87, 3.01, 3.36). Two degrees of unsaturation could be assigned to a terminal alkyne on the basis of the characteristic carbon chemical shifts (δ_C 85.0, 70.0) and a diagnostic 250 Hz coupling ($^1J_{CH}$) from the δ_C 70.0 (C-44) signal to a proton signal at δ_H 2.20 (H-44).[4] The six remaining double-bond equivalents were assigned to four carbon−carbon double bonds and two rings.

COSY, HMBC, and TOCSY experiments determined the individual units that comprised **1**. TOCSY experiments, which produced spectra with signals that were directly coupled or relay-coupled to the secondary amide protons, established the presence of Ala and Ile units. HMBC correlations to the tertiary *N*-methylamide signals provided the starting point for the elucidation of the *N*-Me-Ala, *N*-Me-Phe, and *N*-Me-Gly units. A TOCSY experiment on the doublet of doublets at δ_H 4.91 (H-28) showed relay-transfer to three multiplets (δ_H 1.79, 1.51, 1.37) and two doublet methyl proton signals (δ_H 0.95, 0.92) that suggested a leucine-derived unit. The downfield chemical shift of the α-carbon C-28 (δ_C 74.2) clearly indicated that this was the acyloxy carbon of an ester linkage; that is, the final amino acid-derived unit was 2-hydroxyisocaproic acid.

The structure of the remaining $C_{14}H_{21}O_3$ unit was assembled as follows. Chemical shift considerations indicated that the last unassigned sp^2 proton signal (δ_H 6.84, H-35) was the *β*-proton of an α,*β*-unsaturated ester bearing an α-methyl group (δ_H 1.93, H-45). The methylene group (H-36) adjacent to H-35 was easily identified by a geminal proton−proton coupling constant of −15.2 Hz. These methylene proton signals showed COSY cross-peaks to an oxygenated methine proton at δ_H 4.93 (H-37), whose proton chemical shift indicated an ester linkage to this oxygen.

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Table 1. Spectral Data for the Major Conformer in MeOH-d_3 (M-R1) of Palau'amide (**1**)

unit	C/H no.	δ_H[a] (J in Hz)	δ_C[b,c]	^{1}H−^{1}H COSY	HMBC[d,e]
N-Me-Ala	1		171.7, s		2, 3
	2	3.84, q (6.7)	61.3, d	3	4
	3	1.44, d (6.7)	13.8, q	2	
	4	3.36, s	39.0, q		2
Ile	5		172.6, s		4, 6
	6	4.89, t (9.6)	53.9, d	6-NH, 7	7, 10
	6-NH	8.17, d (9.6)		6	
	7	1.87, m	38.8, d		
	8_d	1.84, m	25.5, t		9, 10
	8_u	1.32, m		9	
	9	0.94, t (7.4)	10.9, q		7
	10	0.88, d (6.6)	11.5, q	7	7
N-Me-Gly	11		170.6, s		6-NH, 6, 12
	12_d	4.18, d (−18.7)	52.7, t	12_u	13
	12_u	3.13, d (−18.7)		12_d	
	13	2.87, s	36.8, q		12
N-Me-Phe	14		172.4, s		12, 13, 15, 16
	15	5.43, dd (9.9, 5.5)	55.2, d	16	16, 23
	16_d	3.01, dd (−14.5, 9.9)	35.9, t	15	18/22
	16_u	2.95, dd (−14.5, 5.5)		15	
	17		138.3, s		16
	18/22	7.14, d (6.9)	130.7, d		16, 20
	19/21	7.18, t (6.9)	129.1, d		
	20	7.16, t (6.9)	127.5, d		18/22
	23	3.01, s	30.7, q		15
Ala	24		174.9, s		15, 23, 25, 26
	25	4.47, p (6.8)	46.4, d	25-NH, 26	26
	25-NH	8.57, d (6.8)		25	
	26	0.82, d (6.8)	15.3, q	25	25, 25-NH
Hica	27		173.8, s		25-NH, 28
	28	4.91, dd (12.0, 9.8)	74.2, d	29	
	29_d	1.79, m	41.9, t	28, 29_u, 30	
	29_u	1.51, m		28, 29_d, 30	
	30	1.37, m	30.6, t	31, 32	
	31	0.95, d (6.6)	23.8, q	30	32
	32	0.92, d (6.0)	21.7, q	30	31
Dddd	33		169.9, s		35, 45
	34		146.5, s		45
	35	6.84, t (6.7)	141.7, d	36, 45	45
	36_d	2.83, ddd (−15.2, 6.7, 3.7)	29.3, t	35, 36_u, 37	
	36_u	2.46, ddd (−15.2, 6.7, 5.9)		35, 36_d, 37	
	37	4.93, ddd (6.7, 5.9, 3.7)[f]	76.2, d	36, 38	46
	38	1.79, qdd (7.0, 6.7, 5.6)[f]	43.3, d	37, 39, 46	46
	39	3.49, ddd (8.8, 5.6, 2.0)[f]	73.0, d	40	46
	40_d	1.58, m	33.7, t	39, 41_u	39
	40_u	1.41, m			
	41_d	1.65, m	26.0, t	42	42
	41_u	1.48, m		40_u	
	42	2.19, td (7.2, 3.6)	18.8, t	41, 44	
	43		85.0, s		42
	44	2.20, t (3.6)	70.0, d	42	42
	45	1.93, br s	12.9, q	35	35
	46	0.85, d (7.0)	15.9, q	38	

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations were observed for $^nJ_{CH} = 7$ Hz. [f] These coupling constants were determined by selective decoupled 1D TOCSY experiments.

HMBC correlations from a methyl doublet (H-46) to C-37, C-38, and C-39 established this 1,3-dihydroxy-2-methylpropyl subunit. The remainder of this fragment was elucidated by a long-range COSY correlation from the terminal alkyne proton (H-44) to H-42 and HMBC cross-peaks from H-39 and H-42 to C-40 and C-41, respectively. The polyketide unit was therefore 5,7-dihydroxy-2,6-dimethyldodec-2-en-11-ynoic acid (Dddd).

The sequence of **1** was established by correlations observed in a HMBC experiment. Cross-peaks from the tertiary *N*-methylamide proton signals (H-4, H-13, H-23) to the adjacent carbonyls (C-5, C-14, C-24) established two fragments: (*N*-Me-Ala)−(Ile) and (*N*-Me-Gly)−(*N*-Me-Phe)−(Ala). Correlations from the secondary amide proton signals (6-NH and 25-NH) to C-11 and C-27 linked these two units in a linear chain with the 2-hydroxyisocaproic acid appended to the amino terminus of Ala. The carboxy terminus of the Dddd unit (C-33) was linked to the hydroxyisocaproic acid, and the carboxy terminus of *N*-methylalanine (C-1) was linked to C-37. The latter (C-37) was chosen over the other secondary alcohol in the Dddd unit (C-39) on the basis of the proton chemical shifts of the two centers (δ_H 4.93 vs 3.49). Although no HMBC or ROESY correlations, in MeOH-d_3, supported these linkages, the molecular formula necessitated the cyclic structure depicted. Later, both of these connections (C-1/C-37 and C-28/C-33) would be confirmed by HMBC correlations observed in $CDCl_3$ (vide infra).

Several techniques were used to determine the stereochemistry of **1**. The amino acid-derived units were determined by chiral HPLC of the acid hydrolyzate. Comparison with authentic samples established the presence of L-Ala,

Table 2. Selected ^{1}H NMR and ROESY Data for $CDCl_3$ Conformer 2 (C-R2) of Palau'amide (**1**)[a]

unit	C/H no.	δ_H[b] (*J* Hz)	ROESY
Dddd	33		
	34		
	35	6.52, br d (11.8)	37
	36_d	3.03, dt (−15.1, 11.8)	
	36_u	2.20, dd (−15.1, 3.3)	
	37	5.27, dt (11.8, 3.3)	35, 36_u, 38, 46
	38	1.93, m (8.3, 7.0, 3.3)	37
	39	3.52, m (8.3)	36_u, 40, 41_d, 46
	40_d	1.61, m	37
	40_u	1.46, m	
	41_d	1.75, m	
	41_u	1.62, m	
	42	2.23, td (6.0, 2.6)	
	43		
	44	1.954, t (2.6)	
	45	1.75, br s	
	46	0.971, d (7.0)	36_d, 37, 39, 40

[a] See Table S1 in Supporting Information for full NMR data. [b] Recorded at 500 MHz

L-Ile, *N*-Me-L-Ala, *N*-Me-D-Phe, and D-hydroxyisocaproic acid.

The relative configuration of the Dddd unit was established by NOE experiments in a variety of solvents. First, the geometry of the α,*β*-unsaturated ester was determined by an NOE experiment. Irradiation of H-36 revealed the close spatial proximity of H-45 and established an *E* geometry for the double bond.

We had initially hoped to determine the relative configuration of the diol unit via *J*-based analysis techniques,[5] but HETLOC and HSQMBC spectra, with an adequate signal−noise ratio needed to determine the carbon−hydrogen coupling constants, could not be achieved due to the limited amount of material. Subsequent analysis of the proton−proton couplings observed in MeOH-d_4 indicated that the important $^3J_{HH}$ values in the Dddd unit[6] were of intermediate magnitude ($^3J_{H-37/H-38}$ = 6.7 Hz, $^3J_{H-38/H-39}$ = 5.6 Hz), which was perhaps the result of an interconverting mixture of rotamers around these stereocenters.[5] We sought to change this ratio and increase the magnitude of these coupling constants, which would allow us to determine the configuration through NOE experiments, but cooling the sample from 15 to −50 °C resulted in only a minimal increase in these values.

Analysis of the ^{1}H NMR data recorded in other solvents revealed that in $CDCl_3$ the major conformers of **1** had a large proton−proton coupling ($^3J_{H-38/H-39}$ ca. 8.3 Hz) between H-38 and H-39 and prompted us to characterize the two major conformers of palau'amide in this solvent (see Table 2 for ^{1}H NMR and ROESY data of the Dddd unit in $CDCl_3$ ring conformer 2 (C-R2) and see the Supporting Information for complete tabulated NMR data for both ring conformers).[7] In fact, despite the unfavorable conformational ratio the signals in the ^{1}H NMR spectrum recorded in $CDCl_3$ were actually more dispersed than in MeOH-d_3. Unfortunately, this was not the case with the ^{13}C NMR spectrum in this solvent, as it showed significant overlap in the carbonyl region. This spectral overlap became an issue because the cross-peaks for the linkages (C-28/C-33 and C-37/C-1) that we had been unable to observe in MeOH-d_3 were potentially present in the HMBC spectrum that was recorded in $CDCl_3$. We could not be certain of these assignments though due to insufficient digital resolution in the HMBC spectrum between correlations to three carbonyl signals at approximately δ_C 169.6. However, by selectively exciting the carbonyl region using a gradient-enhanced band-selective HMBC experiment[8] we could assign the correlations to the carbon signals that were separated from each by 0.1 ppm and unambiguously prove the linkage between C-37 and C-1.

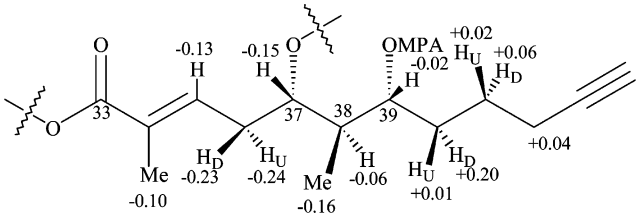

Figure 1. $\Delta\delta^{RS}$ for the MPA derivatives of **1** in MeOH-d_4.

Not surprisingly, the differences in the conformational ratios seen in MeOH-$d_{3/4}$ and $CDCl_3$ are due to slow *cis*/*trans* isomerization of the tertiary amides in **1** within the macrocycle. Analysis of the ROESY correlations observed in $CDCl_3$ suggested chloroform ring conformer 2 (C-R2) had a *cis*-amide bond between *N*-Me-Ala and Ile and a *trans*-amide bond between *N*-Me-Gly and *N*-Me-Phe, since NOE cross-peaks were observed between H-2/H-6 and H-13/H-15, respectively. Conversely, chloroform ring conformer 1 (C-R1) had a *trans*-amide bond between *N*-Me-Ala and Ile and a *cis*-amide bond between *N*-Me-Gly and *N*-Me-Phe, since NOE correlations could be seen between H-4/H-6 and H-12/H-15, respectively. This latter conformation of 4-*trans*-13-*cis*-amide bonds is the major conformer of **1** in MeOH-d_3 (M-R1). This conclusion was supported by a ROESY correlation observed in MeOH-d_3 from H-4 to H-6 and from H-12 to H-15. Thus M-R1 and C-R1 appear to be the same ring conformer.

With the NMR assignments for the two major conformers of **1** in $CDCl_3$ (C-R1/-R2) secure, we returned our attention to the relative stereochemistry of C-38/C-39. Subsequent NOE experiments recorded in $CDCl_3$ revealed a strong correlation between H-40 and H-46 that indicated the *erythro* configuration of C-38 and C-39. Unfortunately, no solvent system could be found where the magnitude of the proton coupling constant between H-37 and H-38 was large enough to determine the relative configuration of the other two stereogenic carbons in the Dddd unit by NOE correlations. Owing to the limited amount of material, the absolute configuration of C-39 was determined prior to attempting to degrade **1**. To this end, the α-methoxyphenylacetic acid (MPA) derivatives were prepared and purified by silica chromatography. The chemical shifts of the protons in the Dddd units were determined by 1D TOCSY experiments in MeOH-d_4 at 50 °C.[6,9] Comparison of the $\Delta\delta^{RS}$ values for these derivatives of **1** established the *R* absolute configuration of C-39 (Figure 1).[10]

Unfortunately, attempts to degrade **1** in such a manner as to be able to determine the configuration of C-37 via the acetonide derivative failed, as no trace of a product containing this stereogenic center could be found after cleavage of the ester linkages with 6 N HCl, NaOMe, or DIBAL-H.

While the configuration of C-37 could not be rigorously established by chemical means, analysis of molecular models in conjunction with NOE data suggested an *S*-configuration for this chiral center.[11] The reasoning is as follows: Owing to free rotation around the C-37/C-38 bond, the side chain (C-38 to C-44) should adopt a low-energy conformation that is essentially independent from that of the ring system (C-1 through C-37). So if the configuration of C-37 is *R*, then the Dddd unit will adopt a *single* conformation that is *s-trans* from the methine carbon (C-35) to the alkyne carbon (C-42) because for the *R*-configuration this is the only conformation of the side chain

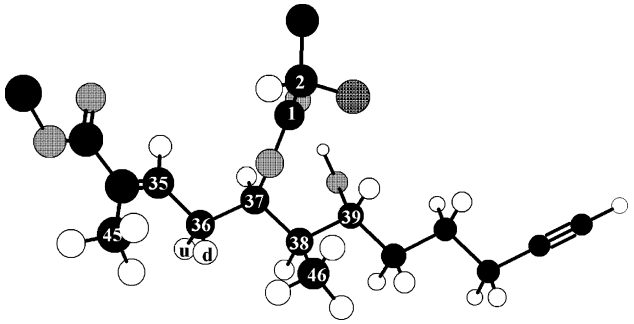

Figure 2. (37*R*,38*R*,39*R*)-Dddd unit with an *s-trans* conformation. The NOE correlations observed between H-37/H-46 and H-36$_u$/H-39 are not consistent with this stereochemistry.

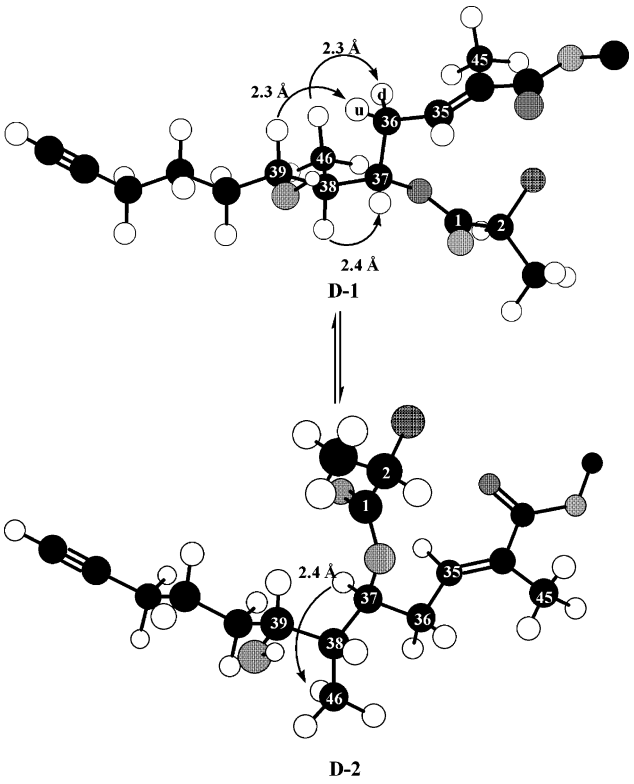

Figure 3. The two conformers of the (37*S*,38*R*,39*R*)-Dddd unit that explain the NOE data. The D-2 conformer is also supported by an NOE between H-37/H-40$_d$ (2.1 Å in model).

in which there is no steric hindrance (1,3-eclipsing) between the oxygen substituents on C-37 and C-39 (Figure 2). A comparison of the theoretical and observed NMR data for this scenario revealed the following inconsistencies that ruled out the possibility of a 37*R*-configuration: (1) In this conformation of the side chain H-37/H-38 and H-38/H-39 are *gauche* (3.3 Hz) and *anti* (8.3 Hz), respectively, as seen in C-R2 (Table 2), but the calculated magnitudes of these coupling constants[12] using the dihedral angles from a MM2 model are not in full agreement with the experimental data (calcd 1.2, 9.8 Hz, respectively),[13,14] and (2) the NOE correlations observed between H-37/H-46 and H-39/H-36$_u$ cannot be reconciled by this conformation since the protons of each of these pairs are *anti* to one another. If on the other hand, the Dddd unit has a 37*S*-configuration, then *two* conformers, designated D-1 and D-2, exist in which no 1,3-eclipsing interactions are present (Figure 3).[15] Individually neither conformer satisfies all of the NOE constraints, but interestingly the cross-peaks that are not in accord with the D-1 conformer of the side chain are compatible with the D-2 conformer of the side chain. In other words, rapid oscillation between these two conformers of the side chain, D-1 and D-2, explains the observed NOE data, in both $CDCl_3$ and MeOH-$d_{3/4}$. The intermediate magnitudes (6.7, 5.6 Hz) of the $^3J_{H-37/H-38}$ and $^3J_{H-38/H-39}$ coupling constants recorded in MeOH-d_3 are evidence that this unit exists as a rapidly interconverting mixture of conformers in the side chain.[5] The conformational ratio of the side chain does appear to be solvent dependent. For example, in $CDCl_3$ the observed proton−proton coupling constants for H-37/H-38 (3.3 Hz) and H-38/H-39 (8.3 Hz) are consistent with approximately a 8:2 ratio of conformers of the side chain (D-1 vs D-2),[16] while in MeOH the ratio appears closer to 4:6.[17] On the basis of this analysis we therefore propose an *S*-configuration for C-37.

Palau'amide (**1**) displayed strong cytotoxicity against KB cells with an IC_{50} value of 13 nM, but due to the paucity of material, no in vivo biological evaluation of **1** was undertaken.[18] Compounds that display a similar level of activity against KB cells are lyngbyabellin A[19] and lyngbyastatin 1,[20] both of which have been the focus of recent synthetic endeavors.

Experimental Section

General Experimental Procedures. The optical rotation was measured on a Jasco-DIP-700 polarimeter at the sodium D line (589 nm). The UV spectrum was taken on a Hewlett-Packard 8453 spectrophotometer. The IR spectrum was recorded on a Perkin-Elmer 1600 FTIR instrument as a film on a NaCl disk. The NMR spectra of **1** were collected at 500 and 125 MHz at 15 °C on a Varian using the residual solvent signals as the internal reference. The FABMS was recorded in the positive mode on a VG ZAB2SE spectrometer and the MALDI spectrum on a DE-STR. HPLC separations were performed on a Beckman 110B apparatus coupled to an Applied Biosystems 759A absorbance detector.

Biological Material. The dark reddish-black clumps of cyanobacterium, designated VP755, were collected at Ulong Channel in Palau. The sample was identified by V. J. Paul, and a voucher is maintained at the Smithsonian Marine Station, Fort Pierce, FL.

Extraction and Isolation of VP755. VP755 was extracted with 1:1 EtOAc/MeOH to yield 1.11 g of lipophilic extract that was subsequently partitioned between hexane and 80% aqueous MeOH. After drying, the aqueous methanol residue was partitioned between water and *n*-butanol. Normal-phase flash chromatography of the organic layer with increasing amounts of methanol in dichloromethane resulted in the cytotoxicity concentrated primarily in the 5% methanol fraction. Subsequent separation on a C_{18} column with increasing amounts of MeCN in H_2O resulted in the activity concentrated primarily in the 60% MeCN in H_2O fraction. This sample was purified twice by RP-HPLC [Ultracarb ODS 30, 250 × 10 mm, flow rate 3 mL/min, detection at 220 nm], first with 70% MeCN in H_2O (t_R 21 min) and then with 80% MeOH in H_2O to yield 2.8 mg of palau'amide (**1**, t_R 28 min).

Palau'amide (1): colorless oil: $[\alpha]^{23}_D$ −22° (*c* 0.4 MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.54), 225 (sh) nm; IR (film) ν_{max} 3444, 1737, 1713, 1644, 1455, 1414, 1247, 1094 cm^{-1}; ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMBC, TOCSY, and ROESY data, see Table 1, Table S1, Table S2; FABMS *m*/*z* $[M + Na]^+$ 874; HR-MALDI *m*/*z* 874.5003 (calcd for $C_{46}H_{69}O_{10}N_5Na$ 874.5004).

Absolute Stereochemistry of the Amino Acid-Derived Units: Chiral HPLC. The acid hydrolyzate of **1** [0.3 mg, 6 N HCl, 18 h, 118 °C] was analyzed by chiral HPLC, and the retention times were compared with authentic standards [Column Chirex Phase 3126 (D), 250 × 4.6 mm, Phenomenex, solvent flow rate 0.8 mL/min, detection at 254 nm, except 2-hydroxy-3-methylvaleric acid, which was determined on a CHIRALPAK MA(+), 50 × 4.6 mm, Diacel Chemical Industries, Ltd., flow rate 0.7 mL/min, detection at 254 nm]. The retention times of the amino acids in the hydrolyzate were

L-Ala (14.8), *N*-Me-L-Ala (18.2), D-2-hydroxyisocaproic acid (28.4), L-Ile (54.5), and *N*-Me-D-Phe (57.3). The retention times of the standards not present in the hydrolyzate were D-Ala (19.2), *N*-Me-D-Ala (21.2), L-2-hydroxyisocaproic acid (35.8), L-allo-Ile (38.2), D-allo-Ile (58.0), D-Ile (76.2), and *N*-Me-D-Phe (49.5). The identities of the peaks were confirmed by co-injection. The solvent systems were as follows: 1 mM aqueous $CuSO_4$ for *N*-Me-Ala and Ala; 95:5 2 mM aqueous $CuSO_4$/CH_3CN for Ile; 85:15 2 mM aqueous $CuSO_4$/CH_3CN for the remaining standards.

Calculation Methods. All calculations were initially performed with CS Chem3D Pro V4.0 (CambridgeSoft Corporation) using semiempirical quantum chemical calculations AM1 with a closed shell and subsequently repeated using HyperChem 7.0 (Hypercube, Inc) using molecular mechanics force field program MM+. All computations were carried out in the gas phase. To search for stable conformations, the configuration of C-37 was fixed as either *S* or *R* and then specific dihedral angles (H-39/H-38, H-38/H-37, H-37/H-36) were rotated between 0° and 360° by increments. At each stage the geometry was optimized until a minimum RMS gradient equaling 0.01.[21] This led to conformers D-1 and D-2. The latter was consistently lower in energy by approximately 0.9 kcal/mol.

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Supporting Information Available: NMR spectra for **1** in MeOH-$d_{3/4}$ and in $CDCl_3$, tabulated NMR data of the two major conformers of **1** in $CDCl_3$, and drawings of the models of the Dddd unit used in the conformational analysis. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(15) An *s-trans* conformation from C-36 to C-42 is unfavorable if C-37 has an *S*-configuration because now 1,3-eclipsing is present between the oxygen substituents on C-37 and C-39 (Figure S1). To relieve the steric hindrance, a 60° rotation can occur around either the C-37/C-38 bond or the C-38/C-39 bond, resulting in the two conformers shown in Figure 3.
(16) According to ref 5 the relative configuration of C-38/C-39 can still be assigned by NOE correlations despite this conformational ratio.
(17) These ratios are based on comparison with the calculated $^3J_{HH}$ values from the modified Karplus curve using ref 12.
(18) It should be noted that palau'amide was accompanied by trace amounts of a related compound. While insufficient material was available for adequate NMR characterization of this sample, MS provided pseudo-molecular ion peaks at *m/z* 874 MNa^+ and 852 MH^+, which suggest that a 2° amide had replaced one of the ester linkages in **1**. This compound had an IC_{50} against KB cells of 1 nM.
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