Citronamides A and B, Tetrapeptides from the Australian Sponge *Citronia astra*

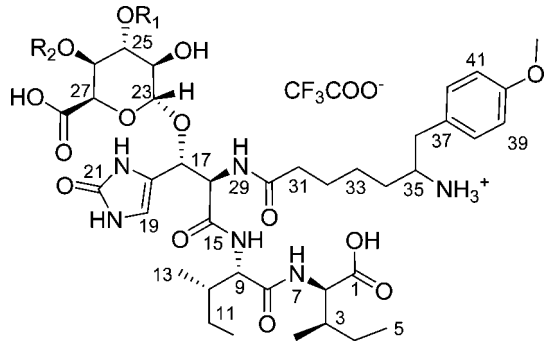
Anthony R. Carroll,*[,†,‡] Sandra Duffy,[‡] and Vicky M. Avery[‡]

Australian Rivers Institute, Griffith University, Gold Coast, Queensland, Australia 4222, and Eskitis Institute, Griffith University, Brisbane, Queensland, Australia 4111

Received December 31, 2008

Two new linear tetrapeptides, citronamides A (**1**) and B (**2**), were isolated from the Australian sponge *Citronia astra* and their structures determined by 1D and 2D NMR spectroscopy. The peptides contain two previously unreported amino acids, 3-(2-oxo-2,3-dihydro-1*H*-imidazol-4-yl)serine and 6-amino-7-(4-methoxyphenyl)heptanoic acid, and α-iduronic acid modified at either C-3 or C-4 with a carbamate. Citronamide A showed moderate antifungal activity against *Saccharomyces cerevisiae*.

Citronia is a newly described sponge genus belonging to the family Dysideidae.[1] Related sponges, such as *Dysidea* and *Lamellodysidea,* have proven to be rich sources of novel compounds, many of which show remarkable similarity to compounds that have been isolated from cyanobacteria and actinomycetes.[2,3] This is not all that surprising when one considers that for some Dysideidae species over 50% of their biomass can be attributed to symbiotic microorganisms, especially cyanobacteria.[4] The structure and biological activity of a potent serine protease (thrombin and FVIIa) inhibitor, dysinosin A, isolated from the Northern Australian sponge *Citronia astra* was reported in a previous communication.[5] Dysinosin A is structurally related to the cyanobacterial compounds the aeurginosins,[6] and its biological activity warranted further evaluation as a potential lead for the development of therapeutic drugs to treat blood clotting disorders such as thrombosis and stroke. In the process of reisolating larger quantities of dysinosin A from a large re-collection of *C. astra* for *in vivo* evaluation two unrelated compounds, citronamides A (**1**) and B (**2**), were isolated from fractions eluting close to dysinosin A. The citronamides are unique. They contain two previously unreported amino acids, 3-(2-oxo-2,3-dihydro-1*H*-imidazol-4-yl)serine (ODIS) and 6-amino-7-(4-methoxyphenyl)heptanoic acid (AMPH), either 3- or 4-*O*-(aminocarbonyl)-α-iduronic acid, and both D- and L-Ile.



1 $R_1 = CONH_2$, $R_2 = H$
2 $R_1 = H$, $R_2 = CONH_2$

MeOH and H_2O extracts of the sponge were combined and partitioned between CH_2Cl_2 and H_2O. The H_2O layer was fractionated on C_{18} silica with a step gradient from H_2O to MeOH. The fractions eluting between 15% and 45% MeOH were combined and purified by gradient C_{18} silica HPLC. Dysinosin A eluted in 30% MeOH, while a mixture of **1** and **2** eluted slightly later in 43% MeOH. Final purification of **1** and **2** was achieved by C_{18} silica HPLC elution with 40% CH_3CN, 1% TFA, and 59% H_2O. Both **1** and **2** were isolated in very low yield.

Citronamide A (**1**) was obtained as a colorless gum. An $[M + H]^+$ ion in the (+)-HRESIMS at *m/z* 866.4171 allowed the molecular formula $C_{39}H_{59}N_7O_{15}$ to be assigned to **1**. Absorption bands at 3365, 1724, 1708, and 1661 cm^{-1} in the IR spectrum suggested that the molecule contained hydroxyl, amine, amide, and carboxylic acid functionalities. The ^1H NMR spectrum of **1** (Table 1) contained signals for two downfield exchangeable proton singlets at δ_H 9.60 and 9.51, a broad three-proton singlet at δ_H 7.68, three amide doublets, a *p*-methoxyphenyl group, a broad exchangeable two-proton singlet at δ_H 6.50, an olefinic singlet, several hydroxymethines, two methyl doublets, two methyl triplets, and a large number of aliphatic multiplets. The HSQC spectrum allowed 27 protonated carbon signals to be identified including four aliphatic methyls, one methoxyl, seven aliphatic methylenes, two aliphatic methines, four aminomethines, six hydroxymethines, an olefinic methine, and two aromatic methines (each representing two carbons). Analysis of the COSY spectrum indicated that **1** had eight discrete proton spin systems: two Ile, a *p*-methoxylphenyl, $-CH_2CH_2CH_2CH_2CH(NH_3^+)CH_2-$, $-OCH(O)CH(OH)-CH(OR)-CH(OH)CH(O)-$, $NHCHCH(OR)-$, and $-NHCH{=}CNH-$. A midfield two-proton exchangeable signal at δ_H 4.05 was assigned to two hydroxyl protons since a correlation was observed from this signal to oxygenated methine signals at δ_H 3.38 and 3.75 in the COSY spectrum. Acid hydrolysis, derivatization using Marfey's reagent, and analysis of the products by HPLC indicated that **1** contained both D- and L-Ile.[7] Correlations obtained from a HMBC spectrum provide evidence to assemble the identified partial structures. Correlations between the Ile α proton, H-9, and the Ile amide proton H-7, and the amide carboxyl carbon C-8 indicated an amine bond linked the two Ile. A urea carbonyl carbon, C-21, could be assigned from correlations between the olefinic proton H-19 and the downfield exchangeable protons H-22 and H-20 and a carbon at δ_C 154.7. Both H-20 and H-22 also correlated to the olefinic methine carbon, C-19, and a quaternary olefinic carbon at δ_C 118.8, C-18, suggesting that they were part of a 2-oxo-2,3-dihydro-1*H*-imidazole. Correlations were also observed between the oxygenated methine H-17 and amino methine H-16 and the quaternary olefinic carbon C-18, indicating that the molecule contained a 3-(2-oxo-2,3-dihydro-1*H*-imidazol-4-yl)serine (ODIS). Correlations from the Ile α proton H-9, the Ile amide proton H-14, and the ODIS α proton H-16 to the ODIS carbonyl carbon C-15 indicated an amide linkage between the second Ile and the ODIS. The presence of a 6-amino-7-(4-methoxyphenyl)heptanoyl group (AMPH) was deduced from HMBC correlations between the

* To whom correspondence should be addressed. Tel: 61 7 5552 9187. Fax: 61 7 5552 7785. E-mail: A.Carroll@griffith.edu.au.
† Australian Rivers Institute.
‡ Eskitis Institute.

10.1021/np800832w CCC: $40.75
Published on Web 03/04/2009

Table 1. NMR Data for Citronamides A (**1**) and B (**2**) in d_6-DMSO[a]

	citronamide A (**1**)		citronamide B (**2**)	
position	δ_C[b]	δ_H (J in Hz)	δ_C[b]	δ_H (J in Hz)
1	173.5		173.5	
2	54.5	4.32, dd (4.8, 9.0)	54.5	4.33, dd (4.2, 8.4)
3	36.1	1.84, m	36.2	1.84, m
4a	25.5	1.22, m	25.4	1.24, m
4b		1.10, dqd (7.2, 6.6, 13.8)		1.10, dqd (7.2, 6.6, 13.8)
5	11.3	0.80, t (6.6)	11.3	0.80, t (6.6)
6	14.9	0.80, d (6.6)	14.9	0.80, d (6.6)
7		8.00, d (9.0)		8.02, d (9.0)
8	170.4		170.2	
9	56.5	4.35, dd (5.6, 9.0)	56.3	4.38, dd (5.4, 9.0)
10	37.6	1.62, m	37.8	1.65, m
11a	23.4	0.91, m	23.8	0.93, m
11b		1.22, m		1.23, m
12	11.2	0.72, t (6.6)	11.2	0.73, t (7.2)
13	15.2	0.67, d (6.6)	15.1	0.68, d (7.2)
14		7.69, d (9.0)		7.68, d (9.0)
15	168.6		168.5	
16	55.6	4.76, dd (8.4, 9.0)	55.7	4.76, dd (7.3, 9.0)
17	73.0	4.54, d (8.4)	72.9	4.59, d (7.3)
18	118.8		118.7	
19	107.3	6.11, bs	107.4	6.11, bs
20		9.60, bs		9.65, s
21	154.7		154.5	
22		9.51, bs		9.52, s
23	101.2	4.92, d (3.3)	101.3	4.90, d (3.2)
24	67.5	3.38, dd (3.3, 4.7)	69.3	3.30, dd (3.2, 4.9)
24-OH		4.05, bs		4.45, bs
25	70.9	4.56, dd (4.7, 4.7)	68.5	3.63, dd (4.8, 4.9)
25-OH				5.00, bs
26	67.2	3.75, dd (2.8, 4.7)	70.2	4.67, dd (3.3, 4.8)
26-OH		4.05, bs		
27	69.0	4.25, d (2.8)	67.2	4.43, d (3.3)
28	170.2		169.9	
29		7.80, d (9.0)		7.86, d (8.4)
30	172.1		171.9	
31	34.7	2.11, m	34.8	2.13, m
32	24.6	1.42, m	24.7	1.42, m
33	24.3	1.36, m	24.2	1.36, m
34	31.3	1.42, m	31.7	1.42, m
35	51.9	3.25, m	52.0	3.25, m
35-NH_3^+		7.68, bs		7.67, bs
36a	37.1	2.75, dd (6.0, 13.8)	37.3	2.75, dd (6.0, 13.8)
36b		2.70, dd (7.8, 13.8)		2.70, dd (7.8, 13.8)
37	127.8		128.1	
38	130.2	7.13, d (8.4)	130.2	7.13, d (8.4)
39	113.8	6.87, d (8.4)	113.9	6.87, d (8.4)
40	158.1		158.2	
40-OCH_3	55.7	3.71, s	55.5	3.71, s
41	113.8	6.87, d (8.4)	113.9	6.87, d (8.4)
42	130.2	7.13, d (8.4)	130.2	7.13, d (8.4)
$CONH_2$	155.7	6.50, bs	155.7	6.39, bs
				6.57, bs

[a] ^{1}H NMR at 600 MHz referenced to residual DMSO solvent (δ_H 2.49) and ^{13}C NMR at 150 MHz referenced to DMSO (δ_C 39.5). [b] ^{13}C chemical shifts obtained from correlations observed in gHSQC and gHMBC spectra.

methylene protons 36-CH_2 and the aromatic carbons C-37, C-38, and C-42 and correlations from the methylene protons 31-CH_2 to the amide carbonyl carbon C-30. A correlation from the ODIS α proton H-16 to C-30 indicated that an amide bond linked the amide nitrogen of the ODIS to the carbonyl carbon of the AMPH. A pyranuronic acid was present in the molecule since HMBC correlations were observed between the anomeric proton H-23 and C-27, while H-27 showed correlations back to C-23 and also to a carboxylic acid carbon, C-28. The most downfield of the oxygenated methine protons, H-25, correlated to a downfield quaternary carbon at δ_C 155.7, suggesting that there was an ester substituent at C-25. The chemical shift of the quaternary carbon was not typical for an ester carbonyl but more appropriate for a carbamate,[8] and the broad two-proton singlet at δ_H 6.50 was assigned to the NH_2 of this system. This assignment was corroborated by the appearance of a peak at m/z 823 $[MH^+ - CONH]^+$ in the (+)-LRESIMS when the cone voltage was increased from 35 eV to 100 eV. A HMBC correlation between the anomeric proton H-23 and the ODIS oxygenated methine carbon C-17 and from H-17 to C-23 indicated that the pyranuronic acid was ether linked to the ODIS at C-17. The above data defined the planar structure of **1**.

Citronamide B (**2**) was obtained as a colorless gum. The (+)-HRESIMS of **2** also had a pseudomolecular ion at m/z 866.4165, which indicated that the molecular formula $C_{39}H_{59}N_7O_{15}$ was the same as that assigned to **1**. The ^{1}H NMR spectrum of **2** (Table 1) was very similar to that of **1**. The upfield and downfield regions of the spectra were almost superimposable. The differences were observed in the midfield region (3−5 ppm) of the spectrum, and this suggested that the pyranuronic acid was modified. The proton−proton couplings observed for the pyranuronic acid protons in **1** and **2** were also very similar, and this suggested that the same pyranuronic acid was present in both compounds. Two-dimensional NMR analysis (COSY, HSQC, and HMBC) confirmed that **1** and **2** were identical apart from the position of the carbamate on the pyranuronic acid. The chemical shift of H-25 was shifted upfield 0.93 ppm while H-26 was shifted downfield 0.92 ppm in **2** compared with **1**. This suggested that the carbamate was attached at C-26 in **2**. Correlations from two exchangeable proton signals at δ_H 4.45 and 5.00 to oxygenated methine protons at δ_H 3.30 (H-24) and 3.63 (H-25), respectively, in the COSY spectrum were also in agreement with the placement of the carbamate at C-26. A HMBC correlation from H-26 to the carbamate carbon δ_C 155.7 confirmed this assignment. The larger vicinal coupling between H-26 and H-27 in **2** compared to **1** was also in keeping with a less electronegative substituent attached to C-26 in **2**.

Analysis of $^3J_{HH}$ and $^1J_{CH}$ coupling constants and correlations observed in HMBC, COSY, and ROESY spectra were used to establish the identity of the sugar residues in **1** and **2**. A small vicinal coupling between H-23 and H-24 (3.3 Hz in **1** and 3.2 Hz in **2**) and a strong ROESY correlation observed between H-23 and H-24 in **1** suggested that H-24 was equatorial. Small vicinal couplings between H-24 and H-25 (4.7 Hz in **1** and 4.9 Hz in **2**) and between H-25 and H-26 (4.7 Hz in **1** and 4.8 Hz in **2**) together with strong ROESY correlations between H-24 and H-25 and between H-25 and H-26 in **1** suggested that H-25 and H-26 were also equatorial. Furthermore, small COSY correlations observed between H-23 and H-25 and between H-24 and H-26 in both **1** and **2** could be assigned to *W* couplings that would only be present if these four protons adopted equatorial positions.[12,15] H-27 was axial since a weaker ROESY correlation and a small coupling (J = 2.8 Hz in **1** and 3.3 Hz in **2**) was observed between H-26 and H-27. The relative configurations assigned to C-25, C-26, and C-27 in **1** were corroborated by the observation of a small ROESY correlation between H-27 and the axial carbamate protons attached to C-25 (δ 6.50, $CONH_2$). These data suggested that the pyranuronic acid residue in both **1** and **2** was α-iduronic acid.

Iduronate residues are known to exist as a mixture of three interconverting low-energy conformers in solution: two chair isomers, $^1C_4$, $^4C_1$, and a skew boat isomer, $^2S_0$ (Figure 1). The proportion of each isomer is dependent upon substituents attached to the sugar, and as a consequence, the ^{1}H−^{1}H coupling constants and chemical shifts of the protons are an average of the contributions of each isomer.[12−14] In oligosaccharides the $^1C_4$ conformer predominates, but a small contribution of the $^4C_1$ and $^2S_0$ conformers generally also occurs.[12−14] A small ROESY correlation between H-27 and H-24 in **1** was consistent with a small contribution of the $^2S_0$ isomer, while a small ROESY correlation between H-23 and H-25 was consistent with a small contribution of the $^4C_1$ isomer. $^1J_{CH}$ coupling constants have been measured for pyranoses,[9] and in general the $^1J_{CH}$ coupling for C-1 is ∼10 Hz greater in α anomers compared to β anomers. Although the $^1J_{CH}$ coupling constants in iduronates are affected by the contribution of the various conformations, the $^1J_{CH}$ coupling constants for the anomeric position in

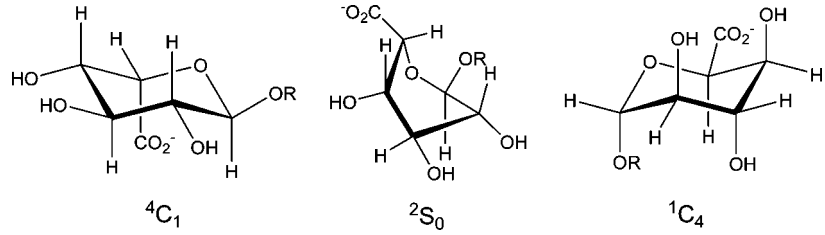


Figure 1. The three major conformers ($^4C_1$, $^2S_0$, and $^1C_4$) found in iduronates.

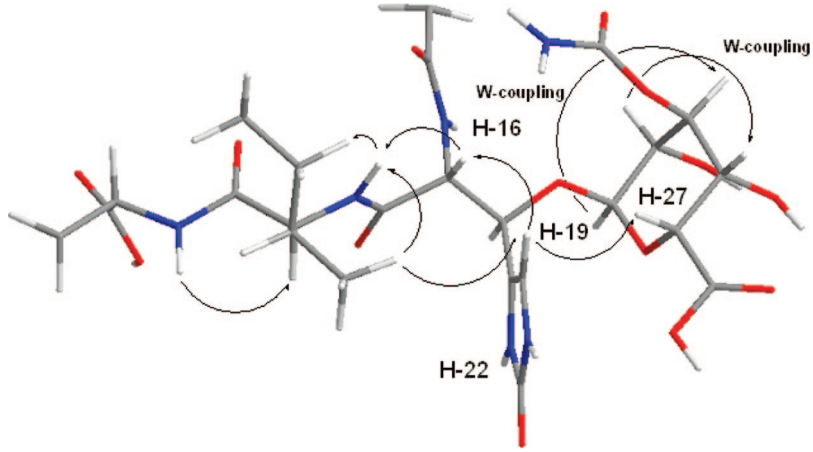


Figure 2. ROESY correlations and *W* couplings associated with the ODIS, isoleucines, and α-iduronate in citronamide A (**1**).

substituted α-iduronates have consistently been measured to be >170 Hz.[10,11] A $^1J_{CH}$ of 170 Hz between H-23 and C-23 observed in the HMBC spectrum of **1** and 171 Hz observed in a C coupled HSQC spectrum of **2** suggested that the anomeric proton, H-23, was equatorial in the major $^1C_4$ conformer in both compounds, and this assignment was consistent with the observed *W* couplings observed between H-23 and H-25.[10,11]

Energy-minimized structures constrained by observed coupling constants and ROESY correlations were generated for **1**. This proved invaluable for assigning the relative configuration of the stereogenic centers in the flexible open-chain portion of the molecule (Figure 2). A dihedral angle of ~180° between H-16 and H-17 in **1** was supported by a 8.4 Hz coupling between these protons and the observation of ROESY correlations between H-16 and both H-19 and H-22. A ROESY correlation was also observed between H-27 and the imidazole proton H-19. The ODIS α proton, H-16, was *syn* to the amide proton H-14 since a strong ROESY correlation was observed between them. A strong ROESY correlation was also observed between H-7 and H-9, indicating that they too were *syn* to each other. A correlation from the imidazole proton, H-19, to the Ile methyl 13-CH_3 indicated that the Ile side chain was on the same face as the imidazole, and this provided evidence to assign the relative configuration of all of the stereogenic centers, except C-35, in **1** (and **2**). Since amino acid analysis using Marfey's procedure showed that citronamide A (**1**) contained both a D- and a L-Ile residue, the structures HO-(D-Ile)-(L-Ile)-(16*R*,17*R*-ODIS-L-α-iduronate)-AMPH and HO-(L-Ile)-(D-Ile)-(16*S*,17*S*-ODIS-D-α-iduronate)-AMPH are equally plausible for **1** from interpretation of the ROESY correlations and molecular modeling analysis. Assignment of the absolute configuration of the iduronate residue would distinguish between these two alternative structures, but unfortunately, the very low isolated yield of both **1** (0.000018%) and **2** (0.000013%) meant that insufficient material was available to hydrolyze the sugar residues and undertake comparative HPLC analysis with sugar standards to determine the absolute configuration of the iduronate residues. The absolute configurations of **1** and **2** therefore remain unassigned, and the structures depicted in the text imply only the relative configuration of the stereogenic centers in **1** and **2**.

Citronamide A (**1**) was tested for *in vitro* antimalarial activity against both chloroquine-resistant (Dd2) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum*, antitrypanosomal activity against *Trypanosoma brucei* (BS427), cytotoxicity against both HeLa and HEK 293 cells, antibacterial activity against *Escherichia coli* and *Staphylococcus albus*, and antifungal activity against *Saccharomyces cerevisiae*. It showed only weak activity (24% inhibition at 120 μM) against the Dd2 malarial strain, was inactive in the cytotoxicity and trypanosomal assays up to 120 μM, was inactive against *S. albus* at 800 μg/mL, was weakly active against *E. coli* (MIC 80 μg/mL), and showed moderate antifungal activity against *S. cerevisiae* (MIC 8 μg/mL).

The citronamides are structurally unique but are very distantly related to the antibiotic, antitumor glycopeptides the bleomycins and tallysomycins that have been isolated from various actinomycetes, because they contain a β-hydroxylated histidine connected to a sugar containing a carbamate ester group.[16,17] Well over 40 natural products containing β-hydroxylated histidine residues have been isolated exclusively from actinomycetes, but to the best of our knowledge no natural products containing an oxohistidine residue have been isolated previously.[18] The ODIS residue is therefore a novel amino acid. Oxohistidine residues are however known to occur in proteins, and their formation is thought to occur through the oxidation of histidine by metal catalysts.[19,20] These residues do not however contain β-hydroxyl groups. The amino acid AMPH is probably biosynthesized by condensation of the amino acid carboxyl group of tyrosine with two acetate residues. Over 20 natural products isolated from microorganisms and the sponge *Theonella swinhoei* contain tyrosine residues condensed at the amino acid carboxyl group with either an acetate residue or a propionate residue, but there are no previous reports of natural products in which two acetate residues have been condensed with tyrosine.[18] AMPH is therefore also a novel amino acid. The closest

residue that occurs in a natural product is a doubly acetate extended serine residue that occurs in the cyanobacterial compound anachelin H isolated from *Anabaena cylindrical*.[21] *C. astra* produces peptides with structural similarities to microbial products, and this suggests that these compounds may be of microbial origin. Initial studies directed at identifying the origin of dysinosin A and the citronamides by isolating and culturing microorganism symbionts from *C. astra* have proved inconclusive. None of the microbial strains cultured from the sponge produced either dysinosin A or either of the citronamides.

Experimental Section

General Experimental Procedures. All solvents used for HPLC, UV, $[\alpha]_D$, and MS were Merck Omnisolv grade, and the H_2O used was Millipore Milli-Q PF filtered. Optical rotations were measured on a JASCO P-1020 polarimeter (10 cm cell). UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer, and IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded at 30 °C. NMR spectra were recorded on Varian Inova 600 MHz NMR spectrometer. The ^1H and ^{13}C chemical shifts were referenced to the DMSO-d_6 solvent peak at δ_H 2.49 and δ_C 39.5. Standard parameters were used for the 2D NMR spectra obtained, which included gCOSY, gHSQC ($^1J_{CH}$ = 140 Hz), gHMBC ($^{3-2}J_{CH}$ = 8.3 Hz), and ROESY (mixing time 300 ms). HRESIMS were recorded on a Mariner Biospectrometry TOF workstation using positive electrospray ionization. Alltech Davisil 30−40 μm 60 Å C_{18} bonded silica was used for MPLC work. A YMC 5 μm 120 Å aqueous C_{18} silica HPLC column (10 mm × 150 mm) was used for HPLC semipreparative separations.

Animal Material. The sponge *Citronia astra* (Dysideidea) was collected from Day Reef far North Queensland in January 1997. A voucher specimen (G312968) has been lodged at the Queensland Museum.

Extraction and Isolation. The freeze-dried and ground sponge (670 g) was extracted exhaustively with CH_2Cl_2 (3 × 350 mL), MeOH (4 × 400 mL), and H_2O (4 × 400 mL). The MeOH and H_2O extracts were combined, yielding a black gum (55 g), which was partitioned between CH_2Cl_2 (500 mL) and H_2O (3 × 500 mL). The aqueous layer (1500 mL) was filtered through C_{18} silica (200 g) and fractionated with a step gradient from H_2O to MeOH. Ten fractions, 10% MeOH increments (300 mL for each fraction), were collected. Mass spectroscopic analysis indicated that dysinosin A was located in fractions eluting between 15% MeOH and 45% MeOH. These fractions were combined and evaporated to yield a gum (2.8 g), and this was purified by HPLC on C_{18} silica, eluting with a gradient from 10% MeOH/90% H_2O to 50% MeOH/50% H_2O. Dysinosin A (110 mg) eluted in 30% MeOH/70% H_2O. A mixture of citronamides A (**1**) and B (**2**) eluted in 43% MeOH/57% H_2O. The citronamides were further separated by C_{18} silica HPLC eluting with 40% CH_3CN/1% TFA/59% H_2O, yielding citronamide A (**1**, 1.2 mg, 0.000018%) and citronamide B (**2**, 0.9 mg, 0.000013%), respectively.

Citronamide A (1): colorless gum (1.2 mg, 0.000018%); $[\alpha]_D$ −15 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 nm (3.8), 275 (3.4); IR (film) ν_{max} 3365, 2963, 2931, 1724, 1708 1661 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, Table 1; (+)-LRESIMS *m/z* (35 eV) (rel int) 866 (100%) $[MH]^+$, *m/z* (100 eV) 866 (100%), 823 (20%) [MH − CONH]; (+)-HRESIMS *m/z* 866.4171 (calcd for $C_{39}H_{60}N_7O_{15}$, 866.4147).

Citronamide B (2): colorless gum (0.9 mg, 0.000013%); $[\alpha]_D$ −17 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 nm (ϵ 3.9), 275 (3.4); IR (film) ν_{max} 3406, 2965, 2932, 1728, 1708, 1664 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, Table 1; (+)-LRESIMS *m/z* (rel int) 866 (100%) $[MH]^+$; (+)-HRESIMS *m/z* 866.4165 (calcd for $C_{39}H_{60}N_7O_{15}$, 866.4147).

Amino Acid Analysis. Citronamide A (**1**) (0.5 mg) was heated in 6 N HCl (5 mL) at 105 °C for 36 h. The resulting hydrolysate was dried, dissolved in distilled water (100 μL), and derivatized with 1-fluoro-2,4-dinitrophen-5-yl-L-alanine amide (FDAA) (0.76 mg) in acetone (250 μL) and 1 N sodium bicarbonate (50 μL) at 50 °C for 3 h. Upon completion of the reaction, the solution was acidified with 2 N HCl (250 μL) and stored in the dark until analyzed. HPLC analysis (Hypersil CPS Supelco column, 4.6 mm × 250 mm, 5 μm; linear gradient elution, triethylammonium phosphate (50 mM, pH 3.0)/CH_3CN, 100:0−40:60 in 60 min; 1.0 mL/min; PDA detection at 340 nm) of the FDAA-derivatized hydrolysate established the configuration of the Ile amino acids. Peaks in the chromatogram were identified by comparing their retention times with that of the FDAA derivatives of the pure amino acid standards. The acid hydrolysis of citronamide A (**1**) showed peaks at 13.49, 14.19, 19.55, 30.37, and 35.53 min. L-Ile gave a retention time of 30.35, and D-Ile gave a retention time of 35.50 min. This confirmed that L-Ile and D-Ile were present in **1**.

Biological Assays. Antimalarial Assays. Compounds were incubated in the presence of 2 or 3% parasite (3D7 or Dd2) and 0.3% hematocrit in a total assay volume of 50 μL, for 72 h at 37 °C and 5% CO_2, in poly-D-lysine-coated CellCarrier imaging plates.

After incubation, plates were stained with DAPI (4′,6-diamidino-2-phenylindole) in the presence of Saponin and Triton X-100 and incubated for a further 5 h at RT in the dark before imaging on the OPERA HTS confocal imaging system. The digital images obtained were then analyzed using the Perkin-Elmer Acapella spot detection software, where spots that fulfill the criteria established for a stained parasite are counted. The % inhibition of parasite replication was calculated using DMSO and artemisinin control data.

Antimicrobial Assays. Citronamide A (**1**) was tested against *Escherichia coli* (K-12), *Staphylococcus albus* (epidermis strain), and the yeast *Saccharomyces cerevisiae*. The two bacterial strains were grown in trypticase soy broth overnight, and then Laboratory-Lemco nutrient agar plates were inoculated with the broth cultures. The yeast was grown in malt nutrient broth overnight, and then malt agar plates were inoculated with the culture.

Bioassays were carried out using the agar hole punch diffusion assay method. Three 4 mm holes, punched using a 4 mm cork punch, were made in 90 mm agar plates inoculated with either *E. coli*, *S. albus*, or *S. cerevisiae*. A 40 μL aliquot of compound dissolved in MeOH was added to each 4 mm hole. Plates were incubated overnight (19−20 h) at 34−37 °C, after which zones of inhibition were measured. Compounds were tested at three concentrations: 800, 80, and 8 μg/mL. MIC values for the isolated compound were obtained by testing three wells per concentration.

Experimental procedures for cytotoxicity and antitrypanosomal bioassays are described in the Supporting Information

Acknowledgment. We thank Mr. P. Baron for obtaining accurate mass measurements. We thank Dr. J. N. A. Hooper (Queensland Museum) for collecting and identifying the sponge. We thank Ms. M. Sykes for antitrypanosomal testing and Ms. D. McElroy for antimicrobial testing. We thank Dr. R. Hill formerly from the Australian Institute of Marine Science for isolating and culturing microbes from the sponge.

Supporting Information Available: 1D and 2D NMR spectra for citronamides A and B and experimental procedures for the cytotoxicity and antitrypanosomal bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Cook, S. de C.; Bergquist, P. R. *Systema Porifera*, 1st ed.; Hooper, J. N. A., Van Soest, R. W. M., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002; Vol. 1, p 1066.
(2) König, G. M.; Kehraus, S.; Seibert, S., F.; Abdel-Lateff, A.; Müller, D. *ChemBioChem.* **2006**, *7*, 229–238.
(3) Carroll, A. R.; Buchanan, M. S.; Edser, A.; Hyde, E.; Simpson, M.; Quinn, R. J. *J. Nat. Prod.* **2004**, *67*, 1291–1294.
(4) Thacker, R. W. *Integr. Comp. Biol.* **2005**, *45*, 369–376.
(5) Carroll, A. R.; Pierens, G. K.; Fechner, G., A.; Leone, P. de A.; Ngo, A.; Simpson, M.; Hyde, E., G.; Hooper, J. N. A.; Boström, S.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340–13341.
(6) Ishida, K.; Okita, Y.; Matsuda, H.; Okina, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971–10988.
(7) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
(8) Kenani, A.; Lamblin, G.; Hénichart, J.-P. *Carbohydr. Res.* **1988**, *177*, 81–89.
(9) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307–3330.
(10) Gettins, G.; Horne, A. G. *Carbohydr. Res.* **1992**, *223*, 81–89.
(11) Yates, E. A.; Santini, F.; De Cristofano, B.; Payre, N.; Cosentino, C.; Guerrini, M.; Naggi, A.; Torri, G.; Hricovini, M. *Carbohydr. Res.* **2000**, *329*, 239–247.
(12) Ferro, D., R.; Provasoli, A.; Ragazzi, M.; Torri, G.; Casu, B.; Gatti, G.; Jacquinet, J.-C.; Sinaÿ, P.; Petitou, M.; Choay, J. *J. Am. Chem. Soc.* **1986**, *108*, 6773–6778.

(13) Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Casu, B.; Torri, G.; Bossennec, V.; Perly, B.; Sinaÿ, P.; Petitou, M.; Choay, J. *Carbohydr. Res.* **1990**, *195*, 157–167.
(14) Ernst, S.; Venkatramaran, G.; Sasisekharan, V.; Langer, R.; Cooney, C. L.; Sasisekharan, R. *J. Am. Chem. Soc.* **1998**, *120*, 2099–2107.
(15) Bootsma, J.; Wagenaars, G.; Dreef, E.; Hout, F.; Kellenbach, E. *Magn. Reson. Chem.* **2001**, *39*, 288–293.
(16) Umezawa, H.; Maeda, K.; Takenchi, T.; Okami, Y. *J. Antibiot. Ser. A* **1966**, *19*, 200–209.
(17) Kawaguchi, H.; Tsukiura, H.; Tomita, K.; Konishi, M.; Saito, K.-I.; Kobaru, S.; Numata, K.-I.; Fujisawa, K.-I.; Miyaki, T.; Hatori, M.; Koshiyama, H. *J. Antibiot.* **1977**, *30*, 779–788.
(18) Dictionary of Natural Products on CD-ROM, version 17.2; Chapman and Hall Electronic Publishing Division, 2009.
(19) Schöneich, C. *J. Pharm. Biomed. Anal.* **2000**, *21*, 1093–1097.
(20) Traoré, D. A. K.; Ghazouani, A. E.; Jacquamet, L.; Borel, F.; Ferrer, J.-L.; Lascoux, D.; Ravanat, J.-L.; Jaquinod, M.; Blondin, G.; Caux-Thang, C.; Duarte, V.; Latour, J.-M. *Nat. Chem. Biol.* **2009**, *5*, 53–59.
(21) Gademann, K.; Bethuel, Y.; Locher, H. H.; Hubschwerlen, C. *J. Org. Chem.* **2007**, *72*, 8361–8370.

NP800832W