JOURNAL OF NATURAL PRODUCTS

Volume 72, Number 9 September 2009

Full Papers

Isolation and Structural Elucidation of Euryjanicins B−D, Proline-Containing Cycloheptapeptides from the Caribbean Marine Sponge *Prosuberites laughlini*†

Brunilda Vera, Jan Vicente, and Abimael D. Rodríguez*

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U. P. R. Station, San Juan, Puerto Rico 00931-3346

Received July 9, 2009

Three new cyclic peptides, euryjanicins B (**2**), C (**3**), and D (**4**), have been isolated from the Puerto Rican marine sponge *Prosuberites laughlini*, and the structures were elucidated by chemical degradation, ESIMS/MS, and extensive 2D NMR methods. When tested against the National Cancer Institute 60 tumor cell line panel, all of the purified isolates displayed weak cytotoxicity.

Cyclic peptides are a frequently encountered class of natural products that often exhibits a wide variety of essential biological functions. A large number of cyclic peptides with unique structures and diverse pharmacological activities have been reported from invertebrate animals and microorganisms typically associated with marine habitats.[1] Additionally, a smaller number of cyclic peptides have been isolated from higher plants.[2] Because of greater resistance to *in vivo* enzymatic degradation and higher bioavailability than their noncyclic counterparts, cyclic peptides often possess superior therapeutic potential.[3] A noteworthy class of marine cyclopeptides is represented by proline-rich compounds, usually containing seven or eight amino acid residues. The role of proline in these molecules, which often occur as complex mixtures of structurally related derivatives, has been associated with control of conformation of the molecule in solution because of the restricted dihedral angle of proline.[4] Examples of this kind of bioactive natural products are the axinellins,[5] axinastatins,[6] phakellistatins,[7] hymenamides,[8] stylopeptides,[9] stylostatin 1,[10] wainunuamide,[11] dominicin,[12] and others.[13]

Within the context of a long-term effort to discover new metabolites with antitumor and anti-infective properties from marine invertebrates from the Caribbean region, we recently had the opportunity to chemically scrutinize the bioactive extract of the marine sponge *Prosuberites laughlini* (order Hadromerida, family Suberitidae) collected in the waters off Puerto Rico.[14] As a result, we recently reported the structure of euryjanicin A (**1**), a crystalline cyclopeptide whose basic structural motif consists of a 21-membered ring constructed from seven amino acid residues, two of which are prolines.[15] In this paper we report the isolation, structure characterization, and bioactivity of three additional members of the family, named euryjanicins B−D (**2**−**4**), from the above marine sponge. All three compounds are proline-containing cycloheptapeptides, which, unlike euryjanicin A (**1**), generate well-resolved NMR spectra in $CDCl_3$. From these spectra it was obvious that for each cyclopeptide one conformation strongly predominated in this solvent.

Results and Discussion

The lyophilized sponge was extracted with MeOH, and the residue obtained was partitioned according to a modified Kupchan procedure, affording two extracts of increasing polarity: EtOAc and *n*BuOH.[16] Our initial biological screenings showed that the EtOAc fraction retained most of the original antitumor activity ascribed to the extract but showed no antituberculosis activity. However, the *n*BuOH extract was strongly antitubercular but weakly cytotoxic. Preliminary chromatographic and ^1H and ^{13}C NMR analyses revealed that the cytotoxic EtOAc fraction contained substances of a peptidic nature, whereas the strongly anti-infective *n*BuOH extract was mainly comprised of a mixture of the known pyrrole alkaloids monobromoisophakellin[17] and hymenidin.[18] Therefore, the EtOAc-soluble material was selected for further isolation work. This extract was subjected to normal-phase Si gel column chro-

† Dedicated to the memory of Dr. Clifford W. J. Chang (1938−2007), who led the discovery of the marine isocyano series of natural products.

* To whom correspondence should be addressed. Tel.: +787-764-0000, ext. 4799. Fax: +787-756-8242. E-mail: abrodriguez@uprrp.edu.

10.1021/np9004135 CCC: $40.75
Published on Web 09/10/2009

Table 1. ^{1}H (500 MHz), ^{13}C NMR (125 MHz), TOCSY, HMBC, and NOESY Correlations for Euryjanicin B (**2**)[a]

amino acid	position	δ_C (mult)[b]	δ_H, mult (J in Hz)	TOCSY[c]	HMBC[d]	NOESY[e]
L-alanine[1]	CO	173.3, qC			α, *β*, αThr, NHThr[2]	
	α	52.4, CH	4.28, dd (7.0, 7.5)	*β*, NH	*β*, NH	*β*
	β	18.7, CH_3	1.54, d (7.5)	α, NH	α, NH	NH
	NH		7.72, d (7.0)	α, *β*		α, *β*, δPro[7], NHThr[2], αPro[6]
L-threonine[2]	CO	169.8, qC			α	
	α	55.3, CH	4.75, dd (3.0, 8.0)	*β*, NH	*γ*, NH	*β*, *γ*, δPro[3]
	β	69.0, CH	4.02, br s	*γ*, NH	α, *γ*	*γ*, δPro[3]
	γ	20.0, CH_3	1.13, d (6.0)	α, *β*, NH		α, *β*
	NH		7.54, d (8.0)	α, *β*, *γ*		α, αPro[3]
L-proline[3]	CO	171.3, qC			α, NHPhe[4]	
trans	α	62.2, CH	4.15, dd (8.5, 8.0)	*β*	*δ*	*γ*, *β*Thr[2]
	β	29.5, CH_2	2.35, m	α, *γ*, *δ*	α, *δ*	α
	γ	25.7, CH_2	1.55, m	*δ*		
			1.95, m	α, *β*, *δ*	*δ*	*δ*
	δ	47.7, CH_2	3.68, m	*γ*, α	*γ*	*γ*
			3.42, m	*γ*		*γ*
L-phenylalanine[4]	CO	171.0, qC			α, *β*, NHVal[5]	
	α	53.6, CH	4.80, dd (9.5, 15.5)	*β*, NH	*β*, NH	*β*
	β	37.7, CH_2	3.13, dd (5.5, 14.0)	NH	α, *ortho*, NH	*ortho*, NH
			3.07, dd (7.5, 14.0)			
	γ	136.9, qC			α, *β*, *meta*	
	ortho	129.7, CH	7.16, d (7.0)		*β*, *meta, para*	α, *β*, NH
	meta	128.6, CH	7.34, m	*ortho*	*para*	
	para	127.0, CH	7.29, m	*ortho*	*ortho*	*ortho*
	NH		6.14, d (9.5)	α, *β*		α, *β*, *ortho*, NH
L-valine[5]	CO	169.5, qC		α	α	
	α	56.5, CH	4.36, dd (8.5, 9.0)	*β*, NH	*β*, *γ*, NH	*γ*, δPro[6]
	β	30.3, CH	2.06, m	*γ*	α, *γ*	α
	γ	19.8, CH_3	0.97, d (6.5)	α, *β*	α	
		17.9, CH_3	0.94, d (6.5)	α, NH		α, NH
	NH		6.60, d (8.5)	α, *β*, *γ*		α, *β*, *γ*, NHPhe[4]
L-proline[6]	CO	170.7, qC			α, *β*	
trans	α	59.3, CH	4.59, dd (5.0, 8.5)	*β*, *γ*, *δ*	*δ*	αPro[7]
	β	28.6, CH_2	2.14, m	*δ*	α, *δ*	α
			1.75, m	*δ*		
	γ	24.8, CH_2	2.11, m		α	
			2.06, m			
	δ	47.6, CH_2	3.63, m	α, *γ*	*β*, *γ*	*γ*
			3.42, m	*γ*		
L-proline[7]	CO	171.4, qC			α, *β*, *γ*, NHAla[1]	
cis	α	60.7, CH	4.35, d (7.0)	*β*, *δ*	*γ*, *δ*	*β*Pro[6], *β*, *γ*
	β	31.8, CH_2	2.44, dd (7.0, 12.3)	α, *γ*, *δ*	α, *δ*	
	γ	22.0, CH_2	1.95, m	α	α, *δ*	
			1.75, m	*δ*		α, *δ*
	δ	46.6, CH_2	3.58, m	α, *γ*	α	*γ*

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual $CHCl_3$ (7.25 ppm) or $CDCl_3$ (77.0 ppm) signals. [b] ^{13}C NMR multiplicities were obtained from APT experiments. [c] NMR data recorded in a Bruker (400 Mz) spectrometer. [d] HMBC mixing time = 50 ms. [e] NOESY mixing time = 200 ms.

matography (CC) using a mixture of $CHCl_3$ and MeOH that had been previously saturated with NH_3 (80/20). Similar fractions were pooled together on the basis of their TLC, NMR, and biological activity profiles. Subsequent evaluation of the first fraction by ^{1}H and ^{13}C NMR indicated that this fraction contained a mixture of small peptides. Further fractionation was performed by normal-phase Si gel (100 g) CC with a mixture of hexane and EtOAc (95/5). Close inspection by NMR indicated that subfractions 10 and 11 appeared to contain most of the peptides observed in the initial NMR analysis. Purification of subfraction 10 was achieved by C18 Si gel reversed-phase CC; this yielded pure euryjanicin A (**1**),[15] euryjanicin B (**2**), and the known cyclic octapeptide dominicin (**5**).[12] Subsequent purification of fraction 11 by reversed-phase HPLC afforded pure euryjanicin C (**3**) and euryjanicin D (**4**).

Structure Elucidation of Euryjanicin B. Compound **2** was obtained as an optically active, colorless oil that showed a pseudomolecular ion peak at *m*/*z* 710 in the positive ion ESIMS spectrum. The molecular formula of **2** was determined to be $C_{36}H_{51}N_7O_8$ by the HRESIMS (*m*/*z* 710.3871 $[M+H]^+$), requiring 15 sites of unsaturation. The ^{13}C NMR spectrum revealed resonances consistent with seven amide carbonyls (*δ* 173.3, 171.4, 171.3, 171.0, 170.7, 169.8, 169.5), seven α-methine carbons (*δ* 62.2, 60.7, 59.3, 56.5, 55.3, 53.6, 52.4), a secondary carbinol (*δ* 69.0), and a monosubstituted phenyl [*δ* 136.9 (C), 129.7 (CH), 128.6 (CH), 127.0 (CH)] ring system, suggesting a heptapeptide with phenylalanine and threonine units. The seven amino acids were unambiguously identified by 2D NMR techniques. Three independent spin systems of the type X−CH−CH_2−CH_2−CH_2−X′ were defined using TOCSY, COSY-GPQF, and HSQC (Table 1), indicating the presence of three proline units. This contention was further substantiated by the observation of only four peptide-bond NH proton signals in the ^{1}H NMR spectrum. The spin systems

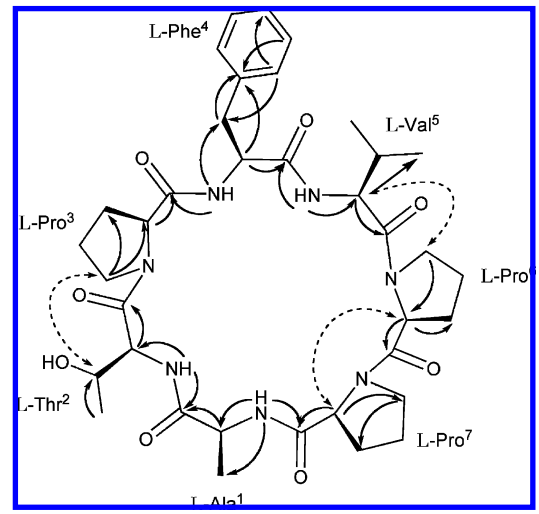


Figure 1. HMBC (H→C) and NOE correlations (dashed arrows) for euryjanicin B (**2**) in $CDCl_3$.

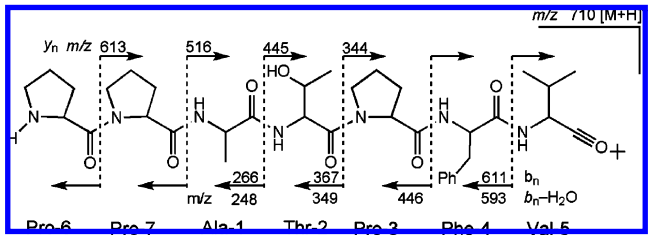


Figure 2. Major fragmentation pathways of **2** following opening of the protonated cyclic structure during ESIMS/MS^n.

X−CH−CH(CH$_3$)$_2$, X−CHCH$_3$, and X−CH(OH)CH$_3$ were identified, suggesting the existence of valine, alanine, and threonine residues. The remaining independent spin system of the type X−CH−CH$_2$−X′ was attributed to phenylalanine by the HSQC correlations: δ 3.13 and 3.07 [βH$_2$(Phe^4)]/δ 37.7 [βC(Phe^4)]. The amino acid composition was confirmed by HPLC analyses of the acid hydrolysate of **2** after derivatization with the Marfey reagent (FDAA), allowing the absolute configurations at the α-carbons to be assigned as the L configuration for all residues.[19] Because only 14 of the calculated 15 degrees of unsaturation could be accounted for by the functionalities in the seven individual amino acids, it became obvious that euryjanicin B was a cyclic peptide. Indeed, the cyclic nature of **2** was evident also by the high degree of chemical shift dispersion observed for the peptide-bond amide proton signals resonating between δ 7.72 and 6.14, its solubility in organic solvents, and the fact that it was negative to a ninhydrin test. The sequence of amino acids was assigned on the basis of a combined approach of 2D-NMR and electrospray tandem mass spectrometry techniques (ESIMS/MS).

The carbonyl carbons within each residue were assigned from HMBC correlations between the C=O and their respective α-protons (Table 1), whereas each peptide-bond amide proton was

Table 2. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), TOCSY, HMBC, and NOESY Correlations for Euryjanicin C (**3**)[a]

amino acid	position	δ_C (mult)[b]	δ_H, mult (*J* in Hz)	TOCSY	HMBC[c]	NOESY[d]
L-serine[1]	CO	169.5, qC			α, NHIle[2]	
	α	54.4, CH	4.50, m	*β*		*β*, *γ*Ile[2], *δ*Ile[2]
	β	63.5, CH$_2$	3.80, d (2.0)			
	NH		6.54, d (8.5)	α, *β*		NHIle[7], α
L-isoleucine[2]	CO	172.9, qC			α	
	α	55.3, CH	4.31, dd (9.3, 10.5)	*β*, *ϵ*	*β*	*β*, *δ*, *ϵ*
	β	37.2, CH	1.79, m	*γ*, *ϵ*	α	
	γ	24.6, CH$_2$	1.63, m	*δ*, *ϵ*	*γ*	
			1.20, m			
	δ	10.6, CH$_3$	0.94, t (7.5)			
	ϵ	14.9, CH$_3$	0.86, d (7.0)		α	
	NH		6.87, d (9.0)	α	COSer[1]	αSer[1], *β*Ser[1], *β*Ile[2]
L-proline[3]	CO	171.8, qC			NHLeu[4], α	
cis	α	61.4, CH	4.46, d (7.0)	*β*, *γ*, *δ*	*β*, *γ*, *δ*	αIle[2], *ϵ*Ile[2], *β*
	β	31.7, CH$_2$	2.40, dd (7.0, 12.0)	*γ*	α, *δ*	
			2.05, m			
	γ	22.1, CH$_2$	1.97, m	*δ*	α, *δ*	*δ*
			1.71, m	*δ*		
	δ	46.4, CH$_2$	3.62, m	*β*, *γ*	α, *β*	*β*, *γ*
			3.49, m	*β*, *γ*		
L-leucine[4]	CO	171.5, qC			α, *β*, NHPhe[5]	
	α	50.5, CH	4.51, m	*β*, *δ*	*β*, *γ*	*β*, *δ*
	β	36.6, CH$_2$	1.61, m	*γ*, *δ*	α	*γ*
			1.42, m	*γ*, *δ*		
	γ	24.8, CH	1.46, m	*δ*	*β*, *δ*	
	δ	16.0, CH$_3$	0.89, d (3.5)			
		21.8, CH$_3$	0.81, d (7.0)		*β*, *δ*	
	NH		7.60, d (6.0)	α	COPro[3], *δ*	α, *β*
L-phenylalanine[5]	CO	171.0, qC			*β*	
	α	54.1, CH	4.62, dt (3.5, 8.3)	*β*	*β*, NHPhe[5]	*β*, αPro[6]
	β	38.0, CH$_2$	3.05, dd (5.0, 8.3)	*γ*	*ortho*	
	γ	134.9, qC			*β*, *meta*	
	ortho	129.7, CH	7.24, d (7.5)		*β*, *para*	α, *β*
	meta	128.9, CH	7.31, m		*ortho*	
	para	127.5, CH	7.28, m		*ortho*	
	NH		7.55, d (3.5)	α		α, *β*, αLeu[4], NHLeu[4]
L-proline[6]	CO	171.0, qC			α, NHIle[7]	
cis	α	61.1, CH	3.89, d (7.0)	*β*, *γ*	*β*, *γ*	*β*, αPhe[5], *β*Phe[5]
	β	30.9, CH$_2$	2.18, dd (7.0, 12.0)	*γ*, *δ*	α, *δ*	
			1.16, m			
	γ	21.7, CH$_2$	1.77, m	*β*	α, *β*, *δ*	
			1.57, m	*δ*	*β*	
	δ	46.5, CH$_2$	3.59, m	*β*, *γ*	α, *β*	*γ*
			3.45, m			*γ*
L-isoleucine[7]	CO	170.8, qC			α, NHSer[1]	
	α	59.6, CH	4.28, dd (5.5, 9.0)	*β*, *δ*, *ϵ*	NHIle[7], *β*, *γ*	*β*, *ϵ*
	β	36.1, CH	2.09, m	*ϵ*	α	
	γ	24.8, CH$_2$	1.42, m	*δ*	α	
			1.21, m	*δ*	α, *γ*	
	δ	11.4, CH$_3$	0.86, t (7.5)			
	ϵ	22.9, CH$_3$	0.90, d (4.0)			
	NH		7.50, d (9.0)	*δ*		α, *β*

[a] Spectra were recorded in CDCl$_3$ at 25 °C. Chemical shift values are in ppm relative to the residual CHCl$_3$ (7.25 ppm) or CDCl$_3$ (77.0 ppm) signals. [b] ^{13}C NMR multiplicities were obtained from APT experiments. [c] HMBC mixing time = 50 ms. [d] NOESY mixing time = 200 ms.

identified from $^1H-^1H$ COSY correlation to its adjacent α-proton and by reference to known values for these residues. The two fragments Pro^7-Ala^1-Thr^2 and Pro^3-Phe^4-Val^5 were assigned by two-bond $^1H-^{13}C$ correlation as follows: NH (Ala^1)/CO (Pro^7), NH (Thr^2)/CO (Ala^1) and NH(Phe^4)/CO (Pro^3), NH(Val^5)/CO (Phe^4). The complete amino acid sequence of euryjanicin B (**2**) was established as *cyclo*-(−Ala-Thr-Pro-Phe-Val-Pro-Pro−) by the following pivotal NOE correlations: αH(Pro^6)/αH(Pro^7); αH(Val^5)/δH_2(Pro^6); βH(Thr^2)/δH_2(Pro^3) (Figure 1). Although the complete sequence was not unequivocally solved using the available NMR data, it was confirmed on the basis of the results of ESIMS/MS experiments. The mass spectrum of euryjanicin B displayed $[M + H]^+$ at *m/z* 710, the fragmentation of which was followed by MS^n. The ESIMS/MS spectrum of euryjanicin B showed preferential opening of the macrocycle at the Val^5-Pro^6 amide bond, followed by a series of major fragmentation pathways. One ion series started with loss of 99 amu due to Val^5, leaving *m/z* 611 (Pro^6-Pro^7-Ala^1-Thr^2-Pro^3-Phe^4 plus H), which then lost 244 amu (Pro^3-Phe^4), affording *m/z* 367 (Pro^6-Pro^7-Ala^1-Thr^2 plus H). The latter fragment lost 101 amu (Thr^2), yielding *m/z* 266 (Pro^6-Pro^7-Ala^1 plus H) (Figure 2). Another pathway left the major fragment *m/z* 593 $[M+H-H_2O-Val^5]^+$ after loss of 99 amu (Val^5) from *m/z* 664 $[M+H-H_2O]^+$, which then lost sequentially 147 amu (Phe^4) and 97 amu (Pro^3), leaving *m/z* 446 $[M+H-H_2O-Val^5\text{-}Phe^4]^+$ and 349 $[M+H-H_2O-Val^5\text{-}Phe^4\text{-}Pro^3]^+$, respectively. The daughter ion spectrum also contained additional fragment ions of lower abundance ascribable to the *y* ion series at *m/z* 613 $[M+H-Pro^6]^+$, 516 $[M+H-Pro^6\text{-}Pro^7]^+$, and 445 $[M+H-Pro^6\text{-}Pro^7\text{-}Ala^1)]^+$.

The structure of euryjanicin B (**2**) was thus confirmed as *cyclo*-(−Ala-Thr-Pro-Phe-Val-Pro-Pro−). The geometry of the peptidic linkages was assigned on the basis of the differences in ^{13}C chemical shifts of the Cβ and Cγ of the proline residues.[20] The ^{13}C NMR data of euryjanicin B indicated that two proline peptide bonds were *trans*, as shown by the small ^{13}C NMR chemical shift difference of Pro$^3\Delta\delta C\beta-C\gamma = 3.8$ and Pro$^6\Delta\delta C\beta-C\gamma = 3.8$, and one *cis*, as indicated by the large ^{13}C chemical shift difference of Pro$^7\Delta\delta C\beta-C\gamma = 9.8$ (Table 1).[21,22] Adjacent *cis* and *trans* proline residues, which have been previously found in cyclic peptides such as wainunuamide[11] and phakellistatin 8,[23] are known to be powerful β-turn inducers.[4a]

Structure Elucidation of Euryjanicin C. Euryjanicin C (**3**), a white semisolid, showed a high-resolution ESIMS spectral quasimolecular ion peak at *m/z* 768.4662 ($[M+H]^+$) consistent with the molecular formula $C_{40}H_{62}N_7O_8$, requiring 14 degrees of unsaturation. The IR absorption bands at 3324 and 1651 cm^{-1} were attributed to amino and amide carbonyl groups, respectively. The heptapeptide nature of **3** was evident from the molecular formula and ^{13}C NMR spectra, which showed seven carbonyl signals (δ 172.9, 171.8, 171.5, 171.0, 171.0, 170.8, 169.5) and seven α-methine carbons (δ 61.4, 61.1, 59.6, 55.3, 54.4, 54.1, 50.5) (Table 2). Only five amide NH signals were detected in the ^1H NMR spectrum, suggesting a heptapeptide with two proline units. As in **2**, the amino acid residues were identified by extensive NMR analyses—COSY-GPQF, TOCSY, HSQC, HMBC, and NOESY—which allowed us to establish the identity of the seven residues: 2 × Pro, 2 × Ile, Phe, Ser, and Leu. These residues accounted for 13 degrees of unsaturation out of the 14, requiring that **3** is a cyclopeptide, too. The absolute configurations at the α-carbon for these amino acids were based on Marfey's method, and we also found in this case that all residues belong to the L series. The amino acid sequence analysis was conducted in a similar manner to that for **2**, by 2D NMR analysis, e.g., HMBC and NOESY spectra (Table 2). From the results of the useful HMBC correlations, as shown in Figure 3, the structure was established to be *cyclo*-(−Ser-Ile-Pro-Leu-Phe-Pro-Ile−), which was also confirmed by the correlations observed during NOESY NMR experiments (Figure 3).

Analogously to the preceding cyclopeptide, the protonated molecular ion of euryjanicin C at *m/z* 768 was fragmented by ESIMS/MS. A complex spectrum was recorded due to simultaneous fragmentation of two isomeric linear peptides, following the opening of the cyclic structure through preferential fragmentation at the Ser^1-Ile^7 and Pro^6-Ile^7 amide bonds. After loss of H_2O the major linear peptide arising upon fragmentation at Ser^1-Ile^7 left a major fragment at *m/z* 750 $[M+H-H_2O]^+$, which in turn underwent *N*-terminal fragmentation, leaving *m/z* 637 $[M+H-H_2O-Ile^7]^+$, 540 $[M+H-H_2O-Ile^7\text{-}Pro^6]^+$, 393 $[M+H-H_2O-Ile^7\text{-}Pro^6\text{-}Phe^5]^+$, and 280 $[M+H-H_2O-Ile^7\text{-}Pro^6\text{-}Phe^5\text{-}Leu^4]^+$. On the other hand, fragmentation of the lesser abundant linear peptide generated after cleavage of the Pro^6-Ile^7 peptide bond left a major fragment at *m/z* 655, corresponding to $[M+H-Ile^7]^+$, which underwent sequential losses of 87 amu (Ser^1), 113 amu (Ile^2), and 97 amu (Pro^3), leaving *m/z* 568 $[M+H-Ile^7\text{-}Ser^1]^+$, 455 $[M+H-Ile^7\text{-}Ser^1\text{-}Ile^2]^+$, and 358 $[M+H-Ile^7\text{-}Ser^1\text{-}Ile^2\text{-}Pro^3]^+$, respectively. These related patterns of *b* fragments could be clearly distinguished in the daughter ion mass spectrum, leading to the reconstruction of the sequences inferred previously from 2D-NMR experiments.

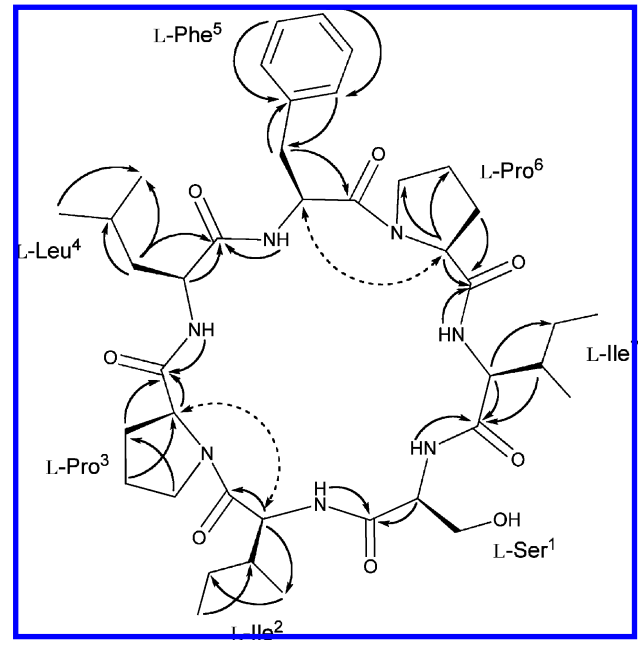


Figure 3. HMBC (H→C) and NOE correlations (dashed arrows) for euryjanicin C (**3**) in $CDCl_3$.

The solution ($CDCl_3$) conformation about the proline peptide linkages of **3** could be assigned from the chemical shift differences between the proline β- and γ-carbons. Thus, Pro^3 and Pro^6 showed $\Delta\delta C\beta-C\gamma = 9.6$ and 9.2, respectively, indicating that these proline peptide bonds are both *cis*. Confirmation for these assignments came from the NOESY spectrum. Cross-peaks between αH(Pro^3)/αH(Ile^2) established the *cis* geometry for the Pro^3 peptide bond, while cross-peaks between αH(Pro^6)/αH(Phe^5) confirmed the *cis*-peptide link for the Pro^6 peptide bond. In point of fact, stylopeptide 1, isolated by the Pettit group from the South and Western Pacific Ocean sponges *Stylotella* sp. and *Phakellia costata* and whose structure was established as *cyclo*-(−Pro-Leu-Ile-Phe-Ser-Pro-Ile−), is a structural isomer of euryjanicin C (**3**).[9a] However, while both cyclopeptides are comprised of the same amino acid residues, there is no sequential homology among them.

Structure Elucidation of Euryjanicin D. Euryjanicin D (**4**) was obtained as an optically white semisolid that gave a $[M + H]^+$ ion in the HRESIMS at *m/z* 802.4514, appropriate for a molecular formula of $C_{43}H_{59}N_7O_8$, requiring 18 sites of unsaturation. General features of both the ^1H and ^{13}C NMR spectra (Table 3) suggested **4** was a peptide. Seven ^{13}C NMR resonances had chemical shifts appropriate for amide carbonyls, and seven resonances had chemical shifts appropriate for amino acid α-carbons,

Table 3. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), TOCSY, HMBC, and NOESY Correlations for Euryjanicin D (**4**)[a]

amino acid	position	δ_C (mult)[b]	δ_H, mult (*J* in Hz)	TOCSY	HMBC[c]	NOESY[d]
L-phenylalanine[1]	CO	170.8, qC			α, *β*, NHSer[2]	
	α	53.8, CH	4.51, m	*β*	*β*	*β*, *ortho*
	β	38.6, CH_2	3.05, m		*ortho*	*ortho*
	γ	135.0, qC			*β*, *meta*	
	ortho	129.2, CH	7.22, d (7.0)		*β*, *para*	
	meta	128.9, CH	7.33, m			
	para	127.5, CH	7.27, m		*ortho*	
	NH		7.26, d (5.0)	α, *β*	*β*	α, *β*Ile[7]
L-serine[2]	CO	170.0, qC			*β*	
	α	54.9, CH	4.51, m	*β*	*β*	*β*, αPro[7], NH
	β	63.2, CH_2	3.89, br d (14.5)		α	
			3.71, dd (5.0, 11.0)			*β*Phe[1]
	NH		7.47, d (6.0)	α, *β*	α, COPhe[1]	α
L-proline[3]	CO	171.7, qC			α, NHIle[4]	
cis	α	61.4, CH	3.59, d (7.5)	*β*, *γ*, *δ*	*β*, *γ*, *δ*	*β*
	β	30.9, CH_2	1.96, m	*γ*	*γ*, *δ*	
			0.95, m	*γ*		
	γ	21.6, CH_2	1.68, m	*β*	α, *β*, *δ*	
			1.44, m	*β*		
	δ	46.1, CH_2	3.38, d (9.5)	*β*, *γ*	α, *β*	
L-isoleucine[4]	CO	171.4, qC			α, NHPhe[5]	
	α	60.4, CH	4.15, d (8.8)	*β*, *ϵ*	*ϵ*, NH	*δ*, *ϵ*
	β	32.3, CH	1.91, m	α, *δ*, *ϵ*	α, *δ*, *ϵ*	*γ*, *δ*, *ϵ*
	γ	24.4, CH_2	1.40, m	*δ*	α, *δ*, *ϵ*	*δ*
			0.95, m			
	δ	9.89, CH_3	0.77, d (7.0)			
	ϵ	15.8, CH_3	0.84, d (6.5)		α, *β*, *γ*	
	NH		7.11, d (8.8)	α, *ϵ*	*β*, *γ*, *δ*, *ϵ*	
L-phenylalanine[5]	CO	171.6, qC			*β*	
	α	53.2, CH	4.51, m	*β*	*β*, *ortho*, NH	*β*, αPro[6], NH
	β	37.8, CH_2	3.08, m		*ortho*, *meta*, NH	*ortho*, NH
	γ	135.4, qC			α, *meta*	
	ortho	129.6, CH	7.26, d (5.0)		*para*	
	meta	128.9, CH	7.33, m			
	para	127.5, CH	7.27, m		*ortho*	
	NH		7.71, d (7.0)	α, *β*		*β*, *γ*, *ϵ*
L-proline[6]	CO	170.6, qC			α, NHIle[7]	
cis	α	61.1, CH	3.92, d (7.5)	*β*, *γ*, *δ*	*β*, *δ*	*β*, *ortho*Phe[5]
	β	30.7, CH_2	2.18, dd (6.0, 12.0)	*γ*	α, *γ*	
			1.04, m	*γ*		*γ*
	γ	21.7, CH_2	1.45, m	*β*	α, *β*	*β*
			1.74, m	*β*		
	δ	46.4, CH_2	3.54, dt (8.0, 11.8)	*β*, *γ*	α, *β*	
			3.41, m			*γ*
L-isoleucine[7]	CO	171.6, qC			α, NHPhe[1]	
	α	56.4, CH	4.12, d (9.3)	*β*	*γ*, *δ*	NHPhe[1]
	β	36.2, CH	2.09, m	*ϵ*	α, *δ*, *ϵ*	*γ*, *δ*, *ϵ*
	γ	25.0, CH_2	1.44, m	*δ*	*ϵ*, α, *δ*	
			1.12, m			*δ*
	δ	11.1, CH_3	0.85, d (7.0)	α, *γ*	*γ*	
	ϵ	15.8, CH_3	0.90, d (7.0)		α, *γ*	
	NH		7.67, d (9.3)	α, *β*, *δ*		*β*, *γ*, *ϵ*

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual $CHCl_3$ (7.25 ppm) or $CDCl_3$ (77.0 ppm) signals. [b] ^{13}C NMR multiplicities were obtained from APT experiments. [c] HMBC mixing time = 50 ms. [d] NOESY mixing time = 200 ms.

consistent with a heptapeptide. Detailed analyses of the COSY-GPQF, TOCSY, HSQC, and HMBC data (Table 3) revealed that euryjanicin D (**4**) contained one serine and two each of proline, isoleucine, and phenylalanine residues. Hydrolysis of peptide **4** with 6 N HCl followed by Marfey's derivatization and HPLC analyses of the amino acids in the hydrolysate confirmed the presence of these four amino acids and established that all seven amino acid residues had the L configuration. The seven amino acid residues accounted for all of the atoms in the molecular formula of euryjanicin D (**4**) and 17 of the sites of unsaturation. Furthermore, there was no evidence for terminal amino or carboxylic acid functionalities, and therefore, euryjanicin D (**4**) was presumed to be a cyclic peptide.

The sequence analysis was conducted in a similar fashion to those in **2** and **3** by 2D-NMR analysis, e.g., HMBC and NOESY spectra. From the HMBC correlations as shown in Figure 4, the structure was established to be *cyclo*-(−Phe-Ser-Pro-Ile-Phe-Pro-Ile−), which was also confirmed by NOE correlations observed in the NOESY spectrum. Finally, this sequence was confirmed by tandem MS fragmentation analysis of a linear acylium ion at *m/z* 784 generated after loss of H_2O following preferential opening of the macrocycle at the Phe[1]-Ile[7] amide bond (Figure 5). Once the amino acid sequence of euryjanicin D (**4**) was determined, the final structural feature to be elucidated was the geometry of the peptidic linkages at the Pro residues. In this instance, the NMR data of **4** pointed to

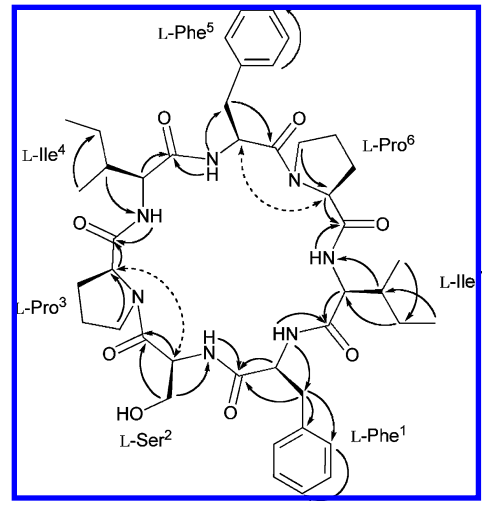


Figure 4. HMBC (H→C) and NOE correlations (dashed arrows) for euryjanicin D (**4**) in $CDCl_3$.

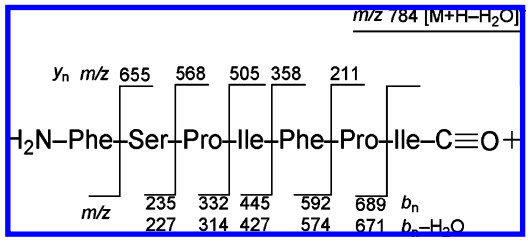


Figure 5. *N*-Terminal and *C*-terminal MS^n fragmentations of the acyclic acylium ion at *m/z* 784 generated from cyclopeptide **4** after in-source protonation, ring-opening, and loss of H_2O.

a *cis* geometry for both the Ser^2-Pro^3 and Phe^5-Pro^6 peptide bonds ($\Delta\delta C\beta-C\gamma = 9.3$ and 9.0, respectively). Subsequent confirmation for these assignments followed from the NOESY spectrum. Strong cross-peaks between $\alpha H(Ser^2)/\alpha H(Pro^3)$ and $\alpha H(Phe^5)/\alpha H(Pro^6)$ confirmed that indeed the proline peptide bonds were *cis*.

Our overall chemical investigation of the cell growth inhibitory sponge extract yielded four new cyclic heptapeptides, euryjanicin A–D (**1**–**4**), and the previously known cyclic octapeptide dominicin (**5**). These molecules are structurally related to several other bioactive proline-rich cyclopeptides reported from marine sponges.[5–13] Yet, the isolated peptides **1**–**5** were found to be only marginally active to inactive against the National Cancer Institute (NCI) 60 tumor cell line panel (Table 4). The problem of lost activity associated with proline-rich cyclic peptides has been observed by others.[9a,13b] This phenomenon may be due to changes in the conformation of the cyclic peptide during isolation or to its ability to bind to a potent antineoplastic substance present in very low concentration and therefore detectable only during biological screenings.[7,24] Peptides **1**–**5** have been tested for their antimalarial (*Plasmodium falciparum* W2 clone), anti-Mtb, antiviral [HBV, HSV-1, HSV-2, FluA (H5N1), FluB, RSV A, WNV, Rift Valley fever], and anti-inflammatory activity in rat neonatal microglia and were inactive in all of the assays.

Cycloheptapeptides **1**–**4** and cyclooctapeptide **5** are characterized by the presence of two or three prolines, an array of apolar residues such as Leu, Ile, and Val, and (in the case of **1**–**4**) one or two aromatic residues such as Phe or Trp. Euryjanicins A (**1**), C (**3**), and D (**4**) each contains a serine residue, whereas euryjanicin B (**2**) and dominicin (**5**) possess one threonine unit. Except for metabolites **1** and **5**, one set of resonances was observed for each of the amino acid residues in compounds **2**–**4**, indicating that in each instance one rigid solution conformation dominates in $CDCl_3$. On the other hand, the ^1H and ^{13}C NMR spectra of **1** and **5** in $CDCl_3$ gave broad signals resulting from the equilibria of slow exchanging conformers about the peptidic ring.[12,15] The remarkable analogy in structure and in amino acid content between cyclic peptides isolated from marine sponges and those stemming from marine-derived cultured bacteria or fungi suggests a possible microbial or symbiotic origin for sponge-derived cyclic peptides such as **1**–**5**.[25]

Table 4. Percent Growth of Selected Human Cancer Cell Lines Treated with Euryjanicins A–D (**1**–**4**) and Dominicin (**5**)[a]

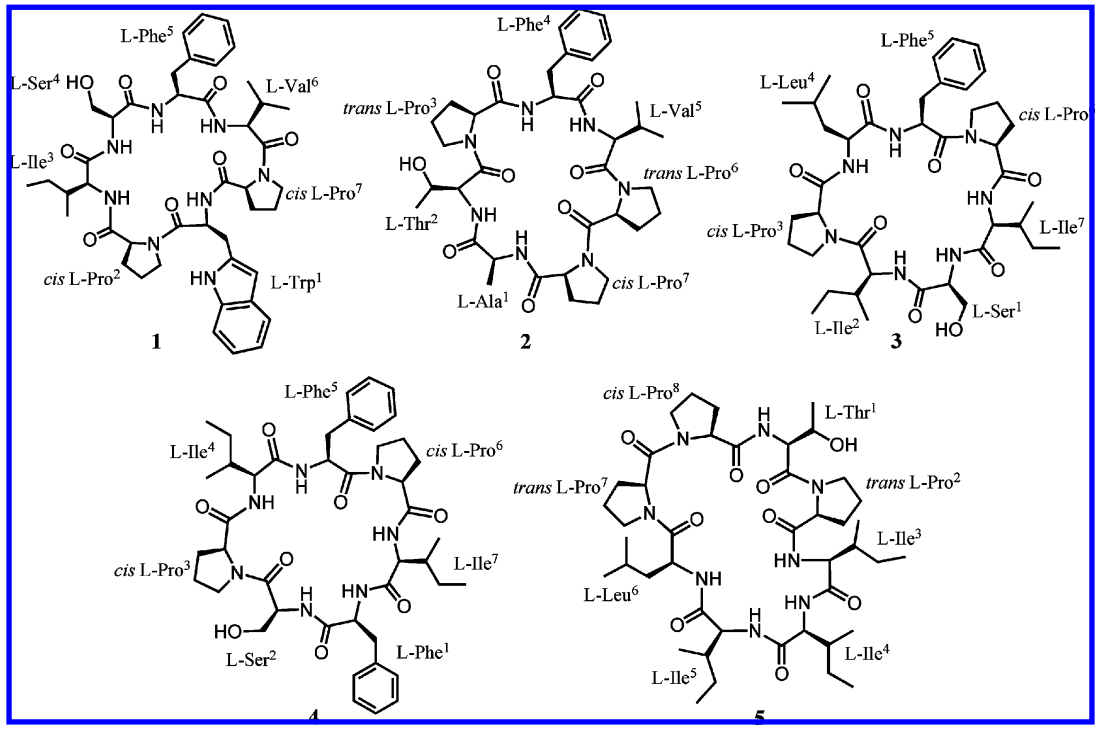
cell line	1	2	3	4	5
NCI-H522[b]	72.2	83.9	95.1	94.6	100
LOX IMVI[c]	89.0	88.6	88.0	87.6	100
IGROV1[d]	82.6	91.3	100	100	100
UO-31[e]	73.6	76.3	83.4	82.7	32.8

[a] Inhibitory activity tested at a single high dose (10^{-5} M) against the full NCI 60 cell line panel. [b] Non-small cell lung cancer. [c] Melanoma. [d] Ovarian cancer. [e] Renal cancer.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Rudolph Autopol IV polarimeter. The UV data were recorded with a Shimadzu UV-2401PC spectrometer, and the IR analyses were performed with a Nicolet Magna IR 750 spectrometer. 1D and 2D NMR data were recorded on a Bruker DRX-500 or Bruker AV-500 FT-NMR spectrometer. ^1H NMR chemical shifts were recorded with respect to the residual $CHCl_3$ signal (7.26 ppm), and ^{13}C NMR chemical shifts were reported in ppm relative to the $CDCl_3$ signal (77.0 ppm). Mass spectrometric measurements were generated at the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. Column chromatography was performed on Analtech Si gel (35–75 mesh) and monitored with TLC analyses carried out on Analtech glass precoated Si gel plates and visualized using UV light and I_2 vapors. All solvents were distilled from glass prior to use.

Chart 1



Animal Material. The Caribbean sponge *Prosuberites laughlini* (Diaz, Alvarez & van Soest, 1987)[26] (phylum: Porifera; class: Demospongiae; order: Hadromerida; family: Suberitidae) was collected at a depth of 50 feet by scuba off Aguadilla, Puerto Rico, in April 2006. A voucher specimen (No. PLAG-01) is stored at the Chemistry Department of the University of Puerto Rico. The encrusting (0.3−4.0 cm thick) sponge was dull orange to yellow externally, lighter internally with surface visually smooth in thin specimens, rugose on thicker ones. The specimen collected possessed oscules with transparent membranes (1−6 mm in diameter) with thin (1−2 mm) canals departing radially and was soft and compressible, easy to tear.

Extraction and Isolation. The freshly collected sponge was freeze-dried for 5 days, and the dried organism (622.7 g) was repeatedly extracted with MeOH (16 L). The combined MeOH extracts were evaporated to dryness, and the resulting brown oil (83.1 g) was partitioned between EtOAc (3 × 1 L), *n*BuOH (3 × 1 L), and H_2O (1 × 1.5 L). The combined EtOAc extracts were concentrated *in vacuo* to give 6.1 g of a dark brown oil that was chromatographed over Si gel (201 g) with 20% MeOH·NH_3 in $CHCl_3$ as eluent. The first fraction (2.79 g) was subsequently chromatographed over Si gel (100 g) with 5% EtOAc in hexane as eluent. Subfractions 10 (182.4 mg) and 11 (155.3 mg) contained all of the peptides. Fraction 10 was purified by reversed-phase C18 column chromatography (5 g) using 15% H_2O in MeOH, yielding pure euryjanicin A (**1**) (43.6 mg; 0.007%), euryjanicin B (**2**) (21 mg; 0.003%), and dominicin (**5**) (32.3 mg; 0.005%). Purification of fraction 11 via C18 reversed-phase HPLC using a 10 mm × 25 cm Ultrasphere ODS column, 5 μm, with 30% H_2O in MeOH yielded pure euryjanicin C (**3**) (15.3 mg; 0.002%) and euryjanicin D (**4**) (39.9 mg; 0.006%).

Euryjanicin B (2): colorless oil; $[\alpha]^{20}_D$ −55 (*c* 0.8, $CHCl_3$); IR (film) ν_{max} 3345, 3087, 3064, 2973, 1667, 1518, 1454, 753 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 204 (4.2) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRESIMS *m*/*z* $[M + H]^+$ 710.3871 (calcd for $C_{36}H_{52}N_7O_8$, 710.3877).

Euryjanicin C (3): white semisolid; $[\alpha]_D^{20}$ −60 (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3324, 3063, 3028, 2961, 1651, 1530, 1449, 754 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 204 (3.9) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRESIMS *m*/*z* $[M + H]^+$ 768.4662 (calcd for $C_{40}H_{62}N_7O_8$, 768.4660).

Euryjanicin D (4): white semisolid; $[\alpha]_D^{20}$ −123 (*c* 0.85, $CHCl_3$); IR (film) ν_{max} 3307, 3063, 3029, 2963, 1667, 1516, 1454, 1347, 752, 702 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 205 (4.0); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3); HRESIMS *m*/*z* $[M + H]^+$ 802.4514 (calcd for $C_{43}H_{60}N_7O_8$, 802.4503).

Acid Hydrolysis of Euryjanicins B−D (2−4). Pure euryjanicins B−D (0.5 mg) were hydrolyzed in 0.5 mL of 6 N HCl at 110 °C for 12 h in a 1.0 mL reaction vial. The cooled reaction mixture was evaporated to dryness, and traces of HCl were removed from the residual hydrolysate by repeated evaporation from H_2O (3 × 0.5 mL) using N_2 gas.

Absolute Configuration of Amino Acids. To a 4 mL vial containing 1 μmol of pure amino acid standards in 200 μL of H_2O was added 1 μmol of *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alanine amide (L-FDAA) in 400 μL of acetone followed by 100 μL of 1 N $NaHCO_3$. The mixture was heated for 1 h at 40 °C. After cooling to rt, 100 μL of 2 N HCl was added and the resulting solution was filtered through a small 4.5 mm filter and stored in the freezer until HPLC analysis. Half of each peptide hydrolysate mixture was dissolved in 200 μL of H_2O, and to this was added 1.5 μmol of L-FDAA in 400 μL of acetone followed by 100 μL of 1 N $NaHCO_3$. The derivatization reaction was carried out and worked up as described above. An 8 μL aliquot of the resulting mixture of L-FDAA derivatives was analyzed by reversed-phase HPLC. A 5 mm × 250 mm Spheri-5 C18 column, 5 μm, with a linear gradient of (A) 9:1 triethylammonium phosphate (50 mM, pH 3.0)/CH_3CN and (B) CH_3CN with 0% B at start to 40% B over 55 min (flow rate = 1 mL/min) was used to separate the L-FDAA derivatives with UV detection at 340 nm. Each chromatographic peak was identified by comparing its retention time with the L-FDAA derivative of the pure L-amino acid standard and by co-injection. In all cases a peak at 39.2 min was observed, which was attributed to excess L-FDAA. Retention times (min) are given in parentheses: L-Pro (32.11), L-Ile (46.85), L-Leu (47.40), L-Thr (26.15), L-Ala (31.74), L-Val (40.70), L-Phe (39.23), L-Ser (23.2), L-Trp (46.0).

Electrospray Ionization Mass Spectroscopic (ESIMS/MS) Analyses. ESIMS/MS analyses were performed with a LTQ ion trap mass spectrometer (Thermo Fisher). Aliquots of the peptide (100 μL) in solution (50% CH_3CN(aq)/0.1% formic acid) were injected in the ion source at a flow rate of 1 μL/min, the capillary temperature was 200 °C, and the applied voltage was 1 kV. Fragmentation experiments were carried out using a collision energy of dissociation of 20%. All ESIMS are reported as averaged mass.

Biological Assays. Additional experimental details for our primary *in vitro* antimicrobial assays against *Mycobacterium tuberculosis* and *Plasmodium falciparum* have been previously described.[27,28] All of the *in vitro* antiviral, anti-inflammatory, and cancer cell cytotoxicity assays for cyclopeptides **1**−**5** were used as indicated.[29−31]

Acknowledgment. We thank Dr. I. I. Rodríguez for helping us during the collection of *P. laughlini* and the UPR-RISE and UPR-MARC Fellowship Programs for financial support to B.V. and J.V., respectively. Sponge extracts were screened for antitumor activity by Dr. S. Nam at the City of Hope Beckman Research Institute and for anti-TB activity by Dr. Y. Wang at the Institute for Tuberculosis Research of the University of Illinois. Antitumor, antimycobacterial, antiviral, antiplasmodial, and anti-inflammatory bioassays of the pure cyclopeptides were conducted at the National Cancer Institute (NCI), the Institute for Tuberculosis Research of the UIC, the NIAID's Antimicrobial Acquisition and Coordinating Facility (AACF), the Instituto de Investigaciones Avanzadas y Servicios de Alta Tecnología (Panama), and the Midwestern University (by Dr. A. M. S. Mayer), respectively. ESIMS/MS data were kindly provided by Dr. I. E. Vega (Department of Biology, University of Puerto Rico). Major financial support was provided by the NIH-MBRS SCORE program (Grant S06GM08102) of the University of Puerto Rico.

Supporting Information Available: Representative copies of the NMR (^1H and ^{13}C), 2D NMR (TOCSY, HSQC, HMBC, and NOESY), and ESIMS/MS spectra for euryjanicins B−D (**2**−**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Piel, J. *Curr. Med. Chem.* **2006**, *13*, 39–50. (b) Aneiros, A.; Garateix, A. *J. Chromatogr. B* **2004**, *803*, 41–53. (c) Tincu, J. A.; Taylor, S. W. *Antimicrob. Agents Chemother.* **2004**, *48*, 3645–3654. (d) Matsunaga, S.; Fusetani, N. *Curr. Org. Chem.* **2003**, *7*, 945–966. (e) Moore, R. E. *J. Ind. Microbiol. Biotechnol.* **1996**, *16*, 134–143. (f) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793–1806.

(2) (a) Ma, X.; Wu, C.; Wang, W.; Li, X. *Asian J. Tradit. Med.* **2006**, *1*, 85–90. (b) Tan, N. H.; Zhou, J. *Chem. Rev.* **2006**, *106*, 840–895.

(3) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115–2134.

(4) (a) Herald, D. L.; Cascarano, G. L.; Pettit, G. R.; Srirangam, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 6962–6973. (b) Mechnich, O.; Hessler, G.; Kessler, H. *Helv. Chim. Acta* **1997**, *80*, 1338–1353.

(5) (a) Randazzo, A.; Piaz, F. D.; Orru, S.; Debitus, C.; Roussakis, C.; Pucci, P.; Paloma, L. G. *Eur. J. Org. Chem.* **1998**, 2659–2665. (b) Kong, F.; Burgoyne, D. L.; Andersen, R. J. *Tetrahedron Lett.* **1992**, *33*, 3269–3272. (c) Tabudravu, J. N.; Morris, L. A.; Van den Bosch, J. J. K.; Jaspars, M. *Tetrahedron* **2002**, *58*, 7863–7868.

(6) (a) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N.; Rutzler, K. C. *J. Med. Chem.* **1991**, *34*, 3339–3340. (b) Pettit, G. R.; Gao, F.; Cerny, R. *Heterocycles* **1993**, *35*, 711–717. (c) Pettit, G. R.; Gao, F.; Schmidt, J. M.; Chapuis, J.-C.; Cerny, R. L. *Biorg. Med. Chem. Lett.* **1994**, *4*, 2935–2940. (d) Pettit, G. R.; Gao, F.; Cerny, R. L.; Doubek, D. L.; Tackett, L. P.; Schmidt, J. M.; Chapuis, J.-C. *J. Med. Chem.* **1994**, *37*, 1165–1168.

(7) Pettit, G. R.; Tan, R. *J. Nat. Prod* **2005**, *68*, 60–63, and references therein.

(8) (a) Kobayashi, J.; Tsuda, M.; Nakamura, T.; Mikami, Y.; Shigemori, H. *Tetrahedron* **1993**, *49*, 2391–2402. (b) Tsuda, M.; Shigemori, H.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1993**, *49*, 6785–6796. (c) Tsuda, M.; Sasaki, T.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 4667–4680. (d) Kobayashi, J.; Nakamura, T.; Tsuda, M. *Tetrahedron* **1996**, *52*, 6355–6360.

(9) (a) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Xu, J.-P.; Boyd, M. R.; Cichacz, Z.; Kamano, Y.; Schmidt, J. M.; Erickson, K. L.

J. Org. Chem. **1995**, *60*, 8257–8261. (b) Brennan, M. R.; Costello, C. E.; Maleknia, S. D.; Pettit, G. R.; Erickson, K. L. *J. Nat. Prod.* **2008**, *71*, 453–456.
(10) (a) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Erickson, K. L.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Bakus, G. J. *J. Org. Chem.* **1992**, *57*, 7217–7220. (b) Forns, P.; Piró, J.; Cuevas, C.; García, M.; Rubiralta, M.; Giralt, E.; Diez, A. *J. Med. Chem.* **2003**, *46*, 5825–5833.
(11) Tabudravu, J.; Morris, L. A.; Van den Bosch, J. J. K.; Jaspars, M. *Tetrahedron Lett.* **2001**, *42*, 9273–9276.
(12) Williams, D. E.; Patrick, B. O.; Behrisch, H. W.; Soest, R. V.; Roberge, M.; Andersen, R. J. *J. Nat. Prod.* **2005**, *68*, 327–330.
(13) (a) Shin, J.; Seo, Y.; Lee, H.-S.; Rho, J.-R.; Mo, S. J. *J. Nat. Prod.* **2003**, *66*, 883–884. (b) Mohammed, R.; Peng, J.; Kelly, M.; Hamann, M. T. *J. Nat. Prod.* **2006**, *69*, 1739–1744.
(14) This sponge was formerly described as *Eurypon laughlini*.
(15) Vicente, J.; Vera, B.; Rodríguez, A. D.; Rodríguez-Escudero, I.; Raptis, R. G. *Tetrahedron Lett.* **2009**, *50*, 4571–4574.
(16) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Siegel, C. W. *J. Org. Chem.* **1994**, *38*, 178–179.
(17) Assmann, M.; Köck, M. *Z. Naturforsch.* **2002**, *57*, 153–156.
(18) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Experientia* **1986**, *42*, 1176–1177.
(19) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(20) Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702–703; *Angew. Chem.* **1975**, *88*, 712−714.
(21) Dorman, D. E.; Borvey, F. A. *J. Org. Chem.* **1973**, *38*, 1719–1722.
(22) Dorman, D. E.; Borvey, F. A. *J. Org. Chem.* **1973**, *38*, 2379–2383.
(23) Pettit, G. R.; Xu, J.-P.; Dorsaz, A.-C.; Williams, M. D.; Boyd, M. R.; Cerny, R. L. *Biorg. Med. Chem. Lett.* **1995**, *5*, 1339–1344.
(24) Tabudravu, J. N.; Jaspars, M.; Morris, L. A.; Van den Bosch, J. J. K.; Smith, N. *J. Org. Chem.* **2002**, *67*, 8593–8601.
(25) (a) Tan, L. T.; Cheng, X.-C.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2003**, *68*, 8767–8773. (b) Renner, M. K.; Shen, Y.-C.; Cheng, X.-C.; Jensen, P. R.; Frankmoelle, W.; Kauffman, C. A.; Fenical, W.; Labbovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1999**, *121*, 11273–11276.
(26) Díaz, M. C. *Caribb. J. Sci* **2005**, *41*, 465–475.
(27) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
(28) Corbett, Y.; Herrera, L.; González, J.; Cubilla, L.; Capson, T.; Colley, P. D.; Kursar, T. A.; Romero, L. I.; Ortega-Barria, E. *J. Trop. Med. Hyg.* **2004**, *70*, 119–124.
(29) Mayer, A. M. S.; Oh, S.; Ramsey, K. H.; Jacobson, P. B.; Glaser, K. B.; Romanic, A. M. *Shock* **1999**, *11*, 180–186.
(30) Rodríguez, I. I.; Shi, Y.-P.; García, O. J.; Rodríguez, A. D.; Mayer, A. M. S.; Sánchez, J. A.; Ortega-Barria, E.; González, J. *J. Nat. Prod.* **2004**, *67*, 1672–1680.
(31) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paul, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

NP9004135