Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1973–1978

BIOORGANIC & MEDICINAL CHEMISTRY

New Apratoxins of Marine Cyanobacterial Origin from Guam and Palau

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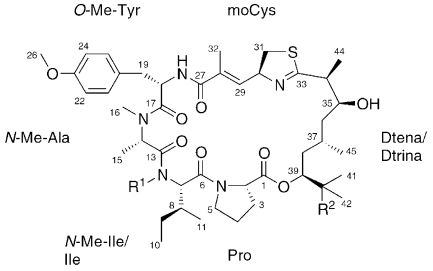
Received 4 September 2001; accepted 13 December 2001

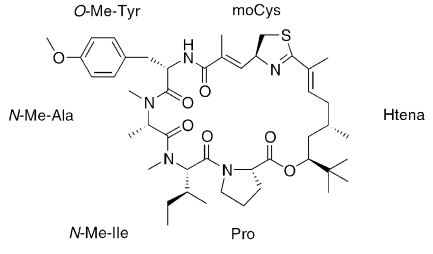
Abstract—Two collections of the marine cyanobacterium *Lyngbya* sp. from Guam and Palau that both afforded the potent cytotoxin apratoxin A (**1**) each yielded different structural analogues with lower degrees of methylation. The new apratoxins, termed apratoxins B (**2**) and C (**3**), were evaluated for their in vitro cytotoxicity along with semisynthetic *E*-dehydroapratoxin A (**4**) to identify key structural elements responsible for the cytotoxicity and to initiate SAR studies on this novel family of depsipeptides. All analogues **2**–**4** displayed weaker cytotoxicity than **1**, but to different extents. While compound **3** closely approached the cytotoxicity of **1**, compounds **2** and **4** exhibited significantly reduced activity, possibly also related to a conformational change. The 16S rRNA genes of the different apratoxin producers have partially been sequenced and compared, and other genetic differences are currently being revealed.

Introduction

Marine cyanobacteria are known for their great biosynthetic potential and ability to produce a wide range of cytotoxic compounds, some with possible therapeutic applications such as curacins[1] and dolastatins.[2] As a result of our ongoing search for new antitumor compounds from marine cyanobacteria, we recently described apratoxin A (**1**) as the most potent cytotoxin produced by a variety of the cyanobacterium *Lyngbya* sp. from Finger's Reef, Apra Harbor, GU, USA.[3] Its mode of action is still unknown at this time. To initiate SAR studies we searched for natural analogues of **1** and discovered apratoxin B (**2**) in minor amounts in the largest recollection of this organism. Populations of the same cyanobacterium, recognized by their dark reddish color, strong attachment to the reef substrate, and predictable coexistence with the red alpheid shrimp *Alpheus frontalis*, were also chemically investigated at other sites around the island of Guam and in Palauan waters. These organisms displayed similar natural products chemistry, including the production of apratoxin A (**1**). A collection from Short Dropoff, Palau, yielded the analogue apratoxin C (**3**) in addition to **1**. A semisynthetic compound, *E*-dehydroapratoxin A (**4**), and the new natural apratoxins **2** and **3** were evaluated for their cytotoxicity compared to apratoxin A (**1**). Comparison of the different apratoxin producers from Guam and Palau on the genetic level has been initiated.



1 $R^1 = CH_3$ $R^2 = CH_3$
2 $R^1 = H$ $R^2 = CH_3$
3 $R^1 = CH_3$ $R^2 = H$



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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00014-7

Results and Discussion

Structure determination

The structures of compounds **2**–**4** were elucidated by interpretation of spectroscopic data and analysis of degradation products. HRFABMS analysis and NMR spectra indicated a molecular formula of $C_{44}H_{67}N_5O_8S$ for compound **2**, one methylene unit less than for **1**. The ^{1}H NMR spectrum of **2** displayed only one *N*-methyl singlet (δ 2.64 in $CDCl_3$) instead of two as in the corresponding spectrum of **1**, accounting for the mass difference. Further 1D NMR (Table 1) and routine 2D NMR analysis in $CDCl_3$ revealed an isoleucine residue instead of an *N*-methylisoleucine moiety and confirmed the presence and connectivity of all other units including the *E* configuration of the double bond as in **1**. The *S*

Table 1. NMR spectral data for apratoxin B (**2**) and apratoxin C (**3**) in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C)

Unit	C/H no.	Apratoxin B (**2**) (major conformer)		Apratoxin C (**3**)	
		δ_H (*J* in Hz)	δ_C[a]	δ_H (*J* in Hz)	δ_C[a]
Pro	1		172.9, s		172.8, s
	2	4.46, dd (8.9, 4.8)	59.0, d	4.17, t (7.6)	59.7, d
	3a	1.96, m	29.1, t	1.88, m	29.2, t
	3b	2.24, m		2.23, m	
	4a	1.96, m	24.8, t	1.89, m	25.6, t
	4b	1.98, m		2.06, m	
	5a	3.68, m	47.4, t	3.66, m	47.6, t
	5b	4.19, m		4.23, m	
Ile[b]/*N*-Me-Ile[c]	6		170.2, s		170.5, s
	7	4.17, dd (11.0, 8.4)	54.9, d	5.19, d (11.5)	56.6, d
	8	1.90, m	35.0, d	2.22, m	31.7, d
	9a	0.82, m	24.7, t	0.95, m	24.6, t
	9b	1.08, m		1.31, m	
	10	0.71, t (7.4)	9.4, q	0.91, t (7)	9.1, q
	11	0.95, d (6.7)	14.2, q	0.92, d (6.5)	14.0, q
	NH[b]/12[c]	7.89, d (8.4)		2.69, s	30.4, q
N-Me-Ala	13		168.9, s		170.0, s
	14	4.91, q (6.7)	55.0, d	3.29, br q (6.7)	60.6, d
	15	0.64, d (6.7)	14.1, q	1.21, d (6.7)	13.9, q
	16	2.64, s	28.4, q	2.80, s	36.2, q
O-Me-Tyr	17		171.3, s		170.4, s
	18	5.28, ddd (11.3, 8.9, 5.0)	50.3, q	5.05, ddd (11.2, 9.2, 4.8)	50.5, d
	19a	2.87, dd (−12.4, 5.0)	37.6, t	2.86, dd (−12.5, 4.8)	37.1, t
	19b	3.23, dd (−12.4, 11.3)		3.11, dd (−12.5, 11.2)	
	20		128.2, s		128.1, s
	21/25	7.08, d (8.5)	130.1, d	7.15, d (8.6)	130.6, d
	22/24	6.81, d (8.5)	114.0, d	6.80, d (8.6)	113.8, d
	23		158.6, s		158.6, s
	26	3.77, s	55.3, q	3.78, s	55.3, q
	NH	6.51, d (8.9)		6.07, d (9.2)	
MoCys	27		167.3, s		169.3, s
	28		126.6, s		130.4, s
	29	6.93, br d (9.1)	144.7, d	6.36, dd (9.8, −1.3)	136.2, d
	30	5.25, br dd (9.1, 8.2)	72.3, d	5.26, ddd (9.8, 8.9, 4.0)	72.3, d
	31a	3.11, br d (−10.6)	38.0, t	3.14, dd (−11.0, 4.0)	37.6, t
	31b	3.38, dd (−10.6, 8.2)		3.46, dd (−11.0, 8.9)	
	32	1.93, br s	12.2, q	1.96, d (−1.3)	13.3, q
Dtena[b]/Dtrina[c]	33		178.2, s		177.5, s
	34	2.68, dq (9.9, 7.0)	48.5, d	2.63, dq (10.0, 7.0)	49.1, d
	35	3.54, dddd (11.5, 10.6, 9.9, 3)	72.2, d	3.55, dddd (11.5, 10.9, 10.0, 2.9)	71.6, d
	36a	1.10, ddd (−13, 12.0, 3)	39.0, t	1.12, ddd (−13.5, 12.0, 2.9)	38.2, t
	36b	1.56, ddd (−13, 11.5, 4)		1.54, ddd (−13.5, 11.5, 4)	
	37	2.05, ddqdd (12.0, 11.7, 6.5, 4, 3)	24.2, d	2.18, ddqdd (12.0, 11.8, 6.5, 4, 2.9)	24.2, d
	38a	1.25, ddd (−13.7, 11.7, 2)	37.4, t	1.29, ddd (−13.9, 11.8, 2.3)	40.4, t
	38b	1.72, ddd (−13.7, 12.5, 3)		1.77, ddd (−13.9, 12.6, 2.9)	
	39	4.94, dd (12.5, 2)	77.6, d	4.98, ddd (12.6, 8.5, 2.3)	75.2, d
	40		34.7, s	1.71, dqq (8.5, 7.0, 6.9)	33.1, d
	41		3×	0.85, d (6.9)	18.0, q
	42	3×0.88, s	26.0, q	0.89, d (7.0)	18.8, q
	43			—	—
	44	1.11, d (7.0)	16.4, q	1.06, d (7.0)	16.7, q
	45	0.97, d (6.5)	20.2, q	0.98, d (6.5)	19.6, q
	OH	3.67, d (10.6)		4.69, d (10.9)	

[a]Multiplicity deduced from the HSQC spectrum.
[b]Refers to compound **2**.
[c]Refers to compound **3**.

stereochemistry of the amino acid derived units was established as for **1** by chiral HPLC analysis of degradation products. The configuration of the 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid portion C33–C45 (Dtena) was the same as in **1** based on similar chemical shifts (δ_H, δ_C) and proton–proton coupling constants (Table 1). However, the lack of the *N*-methyl group affected the conformation of **2**. The optical rotation taken in MeOH was significantly lower. Two conformers were observed in the NMR solvent $CDCl_3$ (ratio 3:1), and amino acid signals were strongly shifted compared to **1**. Differences in the NMR data were especially pronounced near the *N*-methyl group, pointing to a *trans–cis* isomerization around the *O*-Me-Tyr–*N*-Me-Ala amide bond. *CIS* configuration for the major conformer was confirmed by a cross-peak in the ROESY spectrum between the α-protons of both residues[4] which had not been observed for compound **1** because of a *trans* configuration there. ROESY correlations across the ring, for example, between H_3-11 and H-34, suggested that the folding of **2** was otherwise close to the one for **1** in $CDCl_3$. The lowest-energy conformation of **1** in $CDCl_3$ which had been generated using distance geometry followed by restrained molecular dynamics (NOE restraints, dihedral restraints)[3] served thus as a reasonable starting structure for simple modeling studies of **2**. Energy minimization, after formal *N*-demethylation of **1** and isomerization of the discussed amide bond from *trans* to *cis*, generated the structure shown in Figure 1. The model is able to explain electronic differences in the modified cysteine (moCys) unit between **1** and **2**. ^{13}C chemical shifts for the double bond of this unit were affected by the lack of the *N*-methyl group (**2** vs **1**: δ_{C28} 126.6 vs 130.5 and δ_{C29} 144.7 versus 136.3). This shifting can be explained best by intramolecular hydrogen bonding between the NH of the isoleucine residue and the carbonyl oxygen of the moCys unit, increasing the double bond character of the C27–C28 bond and deshielding C29 (Fig. 1). As expected, the signal for H-29 is downfield shifted as well (**2** vs **1**: $\delta_{H\text{-}29}$ 6.93 vs 6.35).

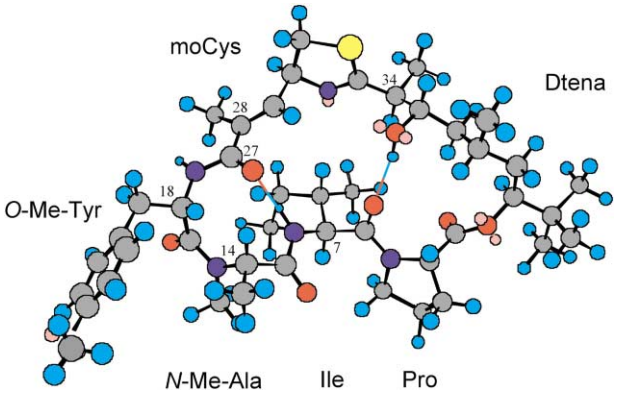

Figure 1. Model of the major conformer of compound **2** (*cis* configuration around the *O*-Me-Tyr—*N*-Me-Ala-bond) which is in agreement with NOE data and with *J* couplings in the Dtena unit. Spatial proximity between NH of Ile and the moCys carbonyl oxygen is suggested and potentially allows hydrogen bonding (indicated), leading to more double bond character between C27 and C28 and thus to carbon chemical shifts observed.

The ^1H NMR spectrum of compound **3** was almost superimposable with the one of **1**, but disclosed the lack of the *tert*-butyl group by the absence of the intense singlet around δ 0.87. Replacement by an isopropyl group was consistent with the molecular formula of $C_{44}H_{67}N_5O_8S$ deduced from HRFABMS analysis; the Dtena unit was substituted by a 3,7-dihydroxy-2,5,8-trimethylnonanoic acid (Dtrina) spin system. This structural difference did not affect the conformation of **3** compared with **1**. NMR data (Table 1) and optical rotation matched closely for both compounds.

During our extensive NMR analysis of apratoxin A (**1**)[3] we noticed its slow, presumably acid-catalyzed decomposition in $CDCl_3$ to one major compound. HPLC purification provided us with an additional probe for SAR studies, *E*-dehydroapratoxin A (**4**). 1D NMR data (Table 2) and 2D NMR analysis disclosed that dehydration had occurred in the Dtena unit to a 7-hydroxy-2,5,8,8-tetramethyl-2-nonenoic acid (Htena) residue, in agreement with the molecular formula of $C_{45}H_{67}N_5O_7S$ derived from HRFABMS analysis. The ROESY spectrum revealed the *E* configuration of the new double bond by showing cross-peaks between H_3-44 and H-36b. All amino acid derived units retained their stereochemical integrity which was determined as above. The dehydration had an effect on the conformation. Two conformers (ratio 3:2) were present in the NMR solvent $CDCl_3$. Again, the conformers arose from restricted rotation around the *O*-Me–Tyr–*N*-Me-Ala amide bond. Chemical shift data (Table 2) and ROESY analysis were indicative for isomerization around that bond. The major conformer of **4** was ascribed a *cis* configuration due to observed ROESY cross-peaks between H-14 and H-18. The minor conformer of **4** lacked this correlation between the α-protons of the two amino acid units and thus had to be *trans* configurated. Prominent ROESY correlations across the ring, again involving *N*-Me-Ile residue signals, were observed between H_3-11 and H_3-44, between H_3-12 and H-29, and between H_3-12 and H_3-32, thus indicating similar folding of this depsipeptide as for the other apratoxins. The ^{13}C NMR shifts for the α,β-unsaturated system of the moCys were comparable to the ones for **1** and **3**. This fact supports that the significant change in the chemical shifts observed for **2** is specifically linked to the lack of the *N*-methyl group of the isoleucine residue and thus in agreement with the proposed hydrogen bonding.

Cytotoxicity and SAR implications

The new apratoxins **2**–**4** were evaluated for their cytoxicity against human solid tumor cell lines, that is, KB and LoVo, in comparison with **1** (Table 3).[5] Apratoxin C (**3**) possesses almost the same IC_{50} values for in vitro cytotoxicity, indicating that the *tert*-butyl group is not essential for activity and can be replaced at least by the sterically somewhat less demanding isopropyl group. Apratoxin B (**2**) and especially *E*-dehydroapratoxin A (**4**) were significantly less cytotoxic. This suggested that the *N*-methyl group at the Ile residue might play a role in the cytotoxicity as well as the 1,2-methine system (C34–C35) bearing the hydroxyl

group. NMR data had also revealed a conformational change for compounds **2** and **4** in $CDCl_3$ (see above). Molecular modeling studies for apratoxin A (**1**) in $CDCl_3$ had demonstrated the spatial proximity of the *N*-methyl group of Ile and the hydroxyl group of the Dtena unit.[3] Their lack does not appear to be well tolerated by the (unknown) receptor. Their presence and/or interaction possibly also somehow favors the *trans* configuration of the *O*-Me-Tyr–*N*-Me-Ala amide bond in the NMR solvent. Perhaps this conformational behavior is of some importance under (aqueous) assay conditions and contributes to the observed activity loss as well.

Genetic analysis

Interestingly, apratoxins with an isopropyl group in the starter unit for the polyketide synthase (PKS) that is putatively involved in the biosynthesis[6] were never obtained from Guamanian collections over the last decade, but found in Palauan samples (**3**). Different methylation patterns in the starter unit for a possible

Table 2. NMR spectral data for both conformers of *E*-dehydroapratoxin A (**4**) in $CDCl_3$ (ratio 3:2) at 500 MHz (^1H) and 125 MHz (^{13}C)

Unit	C/H no.	Major conformer		Minor conformer	
		δ_H (*J* in Hz)	δ_C[a]	δ_H (*J* in Hz)	δ_C[a]
Pro	1		171.0, s		173.0, s
	2	4.37, dd (8.3, 5.7)	58.8, d	4.17, t (7.8)	59.6, d
	3a	1.93, m	29.4, t	1.85, m	29.3, t
	3b	2.27, m		2.25, m	
	4a	1.93, m	25.1, t	1.90, m	25.4, t
	4b	1.95, m		2.04, m	
	5a	3.60, m	47.4, t	3.63, m	47.3, t
	5b	4.02, m		4.13, m	
N-Me-Ile	6		170.0, s		170.4, s
	7	4.80, d (11.4)	57.9, d	5.10, d (11.4)	56.9, d
	8	2.00, m	33.89, d	1.93, m	33.7, d
	9a	0.92, m	25.8, t	0.94, m	25.7, t
	9b	1.25, m		1.28, m	
	10	0.82, t (7.4)	9.7, q	0.92, t (7.0)	10.2, q
	11	1.12, d (6.5)	13.9, q	0.96, d (6.3)	14.3, q
	12	2.78, s	30.0, q	2.85, s	30.8, q
N-Me-Ala	13		170.3, s		169.9, s
	14	4.74, q (6.8)	53.8, d	3.37, q (6.8)	60.4, d
	15	0.63, d (6.8)	15.3, q	1.24, d (6.8)	13.8, q
	16	2.59, s	28.8, q	2.90, s	36.7, q
O-Me-Tyr	17		172.0, s		170.2, s
	18	5.34, ddd (10.6, 9.2, 4.6)	50.2, d	5.07, ddd (11.2, 9.7, 5.6)	50.6, d
	19a	2.89, dd (−12.6, 4.6)	39.2, t	2.81, dd (−13.1, 5.6)	36.9, t
	19b	3.21, dd (−12.6, 10.6)		3.15, dd (−13.1, 11.2)	
	20		128.5, s		128.5, s
	21/25	7.15, d (8.7)	130.4, d	7.16, d (8.8)	130.6, d
	22/24	6.81, d (8.7)	113.9, d	6.80, d (8.8)	113.7, d
	23		158.5, s		158.5, s
	26	3.77, s	55.3, q	3.78, s	55.3, q
	NH	6.33, d (9.2)		5.97, d (9.7)	
MoCys	27		167.4, s		169.4, s
	28		128.2, s		131.6, s
	29	6.51, dd (10.2, −1.2)	136.3, d	6.18, dd (9.4, −1.2)	135.5, d
	30	5.46, ddd (10.2, 8.0, 1.6)	72.8, d	5.39, ddd (9.4, 8.2, 3.5)	73.0, d
	31a	3.14, dd (−11.0, 1.6)	37.0, t	3.07, dd (−11.1, 3.5)	38.7, t
	31b	3.42, dd (−11.0, 8.0)		3.48, dd (−11.1, 8.2)	
	32	1.98, d (−1.2)	12.7, q	1.94, d (−1.2)	13.3, q
Htena	33		173.1, s		172.6, s
	34		132.0, s		130.6, s
	35	6.34, dd (7.3, 5.3)	139.3, d	6.35, dd (7.3, 6.5)	139.4, d
	36a	1.54, ddd (−12.1, 12.1, 5.3)	33.94, t	1.53, ddd (−13, 13, 6.5)	34.1, t
	36b	2.64, ddd (−12.1, 7.3, 3)		2.60, ddd (−13, 7.3, 2)	
	37	1.58, ddqdd (12.1, 9.8, 6.1, 3, 2.1)	29.6,[b] d	1.59, ddqdd (12.0, 11.0, 6.5, 2.2, 1.3)	29.5,[b] d
	38a	1.37, ddd (−14.0, 9.8, 1.7)	37.86, t	1.31, ddd (−14.1, 11.0, 1.3)	37.92, t
	38b	1.64, ddd (−14.0, 11.4, 2.1)		1.69, ddd (−14.1, 12.0, 2.2)	
	39	4.94, dd (11.4, 1.7)	77.8, d	5.00, dd (12.0, 1.3)	77.8, d
	40		35.0, s		34.7, s
	41/42/43	3×0.88, s	3×26.1, q	3×0.87, s	3×25.9, q
	44	1.93, br s	14.1, q	1.92, br s	14.6, q
	45	1.01, d (6.1)	21.1, q	0.95, d (6.5)	21.2, q

[a]Multiplicity deduced from the HSQC spectrum.
[b]No correlation observed in the HSQC spectrum.

PKS involved in apratoxin biosynthesis could suggest relaxed substrate specificity of this enzyme or methylation at a later stage. Subtle distinctness in the metabolite production of both cyanobacterial populations led us to examine differences more closely on the genetic level. Analysis of the 16S ribosomal RNA gene revealed that the collection from Apra Harbor, GU, USA, yielding **1** and **2**, actually consisted of at least two different organisms (VP417a and VP417b, GenBank accession nos AY049750 and AY049751). The sequences differed only at the 3′-end of the PCR-amplified 1.5 kb fragment, representing almost the entire 16S rRNA gene, and showed 97.4% identity. Both organisms most likely belonged to the same species. Organism NIH309 from Short Dropoff, Palau, producing **1** and **3**, seemed to be a single cyanobacterium since PCR amplified only one variant of a 16S rRNA gene (GenBank accession no. AY049752). Its DNA sequence was 99.7% identical to the one amplified from VP417a and 97.4% identical to the one from VP417b. PCR amplification of partial nonribosomal peptide synthetase (NRPS) genes and PKS genes from genomic DNA of both organisms is in progress. Both types of genes are expected to be involved in the biosynthesis of the apratoxins.[6]

Conclusion

In summary, we have described two new natural analogues, **2** and **3**, of the potent cytotoxin apratoxin A (**1**) from Micronesian cyanobacterial collections. SAR studies have been initiated by comparing their in vitro cytotoxicity with the ones of **1** and its dehydration product **4**. This information could be helpful for synthetic and medicinal chemists targeting this compound group. The biosynthesis of the apratoxins is under investigation.

Experimental

General

All NMR experiments were run on a Varian Unity Inova 500 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz, respectively, using residual solvent signals as internal references. The HSQC experiments were optimized for $^{1}J_{CH} = 140$ Hz, and the HMBC experiments for $^{n}J_{CH} = 7$ Hz. HRMS were obtained by FAB in the positive mode using a VG-ZAB spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR. UV spectra were obtained on a Hewlett-Packard 8453 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Table 3. In vitro cytotoxicity data for apratoxins **1**–**4** toward human solid tumor cell lines

	IC_{50} in nM (KB)	IC_{50} in nM (LoVo)
Apratoxin A (**1**)	0.52	0.36
Apratoxin B (**2**)	21.3	10.8
Apratoxin C (**3**)	1.0	0.73
E-Dehydroapratoxin A (**4**)	37.6	85.1

Biological material. *Lyngbya* sp. VP417 was collected on 19 January 1999, at Finger's Reef, Apra Harbor, GU, USA. The isolation of lyngbyabellins A[7] and B,[8] lyngbyapeptin A,[8] and apratoxin A (**1**)[3] from this collection has already been described. A specimen preserved in formalin has been deposited at the University of Guam Marine Laboratory. *Lyngbya* sp. NIH309 was collected on 7 April 1999, at Short Dropoff, Palau.

Isolation of apratoxin B (2). Extraction and fractionation of VP417 collected in January 1999 has previously been discussed.[3,7] Apratoxin B (**2**) eluted from silica gel with 6% *i*-PrOH in CH_2Cl_2, as did apratoxin A (**1**). Semipreparative reversed-phase HPLC (Ultracarb, 5 ODS 30, 250×10 mm, 2.0 mL/min; UV detection at 220 nm) allowed separation and purification to afford **1** (10.0 mg, t_R 43.2 min) and **2** (1.7 mg, t_R 60.0 min).

2: colorless, amorphous solid; $[\alpha]_D^{25}$ −73° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.64), 230 (4.36) nm; IR (film) ν_{max} 3442, 2956, 2920, 2849, 1742, 1641, 1540, 1510, 1451, 1374, 1243, 1172, 1113, 1036 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* $[M+H]^+$ 826.4753 (calcd for $C_{44}H_{68}N_5O_8S$, 826.4789).

Isolation of apratoxin C (3). The freeze-dried organism NIH309 (∼40 g) was extracted with CH_2Cl_2–MeOH (2:1) to afford a lipophilic extract (2.80 g) that was partitioned between hexanes and 80% aq MeOH. The latter portion was evaporated to dryness and the residue then partitioned between *n*-BuOH and H_2O. The concentrated *n*-BuOH phase was subjected to silica gel chromatography, eluting first with CH_2Cl_2 followed by CH_2Cl_2 solutions containing progressively increasing amounts of *i*-PrOH, and finally with MeOH. The mixture that eluted with 6% *i*-PrOH in CH_2Cl_2 was subjected to semipreparative reversed-phase HPLC using the Ultracarb column mentioned above (65% aqueous MeCN, 3.0 mL/min) to give apratoxin C (**3**) (0.5 mg, t_R 55.0 min) and apratoxin A (**1**) (0.8 mg, t_R 69.5 min).

3: colorless, amorphous solid; $[\alpha]_D^{25}$ −171° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.64), 230 (4.31) nm; IR (film) ν_{max} 3421, 2956, 2928, 2861, 1736, 1623, 1511, 1451, 1374, 1249, 1178, 1089, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* $[M+H]^+$ 826.4822 (calcd for $C_{44}H_{68}N_5O_8S$, 826.4789).

***E*-Dehydroapratoxin A (4).** Compound **1** converted mainly to **4** by exposure to acidic $CDCl_3$ in the NMR tube. Purification was achieved by reversed-phase HPLC on the Ultracarb column using a step-gradient of 80% aqueous MeCN (30 min) followed by MeCN (30 min). Residual amounts of compound **1** eluted at t_R 26.5 min, and compound **4** at t_R 40.5 min.

4: colorless, amorphous solid; $[\alpha]_D^{25}$ −133° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.64), 230 (4.34), 245 (sh) (4.13) nm; IR (film) ν_{max} 3431, 2956, 2920, 2849, 1742, 1641, 1510, 1457, 1249, 1178 cm^{-1}; ^{1}H and

^{13}C NMR data, see Table 2; HRFABMS m/z $[M+H]^+$ 822.4831 (calcd for $C_{45}H_{68}N_5O_7S$, 822.4839).

Absolute stereochemistry of the amino acid-derived units

The analysis was carried out as described for **1**.[3] Samples of compounds **2**, **3**, and **4** were each either hydrolyzed (6 N HCl) to liberate the regular and methylated amino acids, or subjected to oxidative ozonolysis followed by acid hydrolysis to additionally liberate cysteic acid. The chiral HPLC profiles of **3** and **4** corresponded to the ones for **1**. The profiles of **2** differed by showing a peak for L-Ile instead of for *N*-Me-L-Ile. Therefore, all stereocenters were concluded to be *S*.

Acknowledgements

Funding was provided by NCNPDDG grant CA53001 from the National Cancer Institute. The upgrade of the 500 MHz NMR spectrometer used in this research was funded by grants from the CRIF Program of the National Science Foundation (CHE9974921) and the Elsa U. Pardee Foundation. We thank the Division of Marine Resources of the Republic of Palau and the Koror State Government for marine research permits. Jason Biggs and Dr. Edwin Cruz-Rivera helped collecting the cyanobacterium in Palau. Cytotoxicity assays were performed by Dr. M. Lieberman and G. Tien, Department of Chemistry, University of Hawaii at Manoa. Mass spectral analyses were conducted at the UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Riverside. We thank Prof. D. Hoffmann, Department of Chemistry, University of Hawaii at Manoa, for guidance in obtaining genetic data on the cyanobacteria.

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