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Parguerene and Precarriewamide, Two Classes of Lipopeptides from the Marine Cyanobacterium *Moorea producens*

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Abstract

Two new marine cyanobacterial natural products, parguerene (**1**) and precarriewamide (**2**), were isolated from a collection of *Moorea producens* obtained from La Parguera, Puerto Rico. The planar structures of both were deduced by 2D NMR spectroscopy and mass spectrometry. Parguerene is an modified acyl amide with some structural similarity to the bacterial metabolite, stipiamide (**3**), whereas precarriewamide is a lipopeptide and represents a minor modification compared to two other known metabolites, carriewamide (**4**) and carriewamide sulfone (**5**). The identification of **2** led to an investigation into whether carriewamide and carriewamide sulfone were true secondary metabolites or isolation artifacts.

Marine cyanobacteria are exceptionally prolific producers of structurally diverse secondary metabolites, many of which have intriguing biological properties.¹ The cyanobacterial genus *Moorea*, formally known as *Lyngbya*,² is one of the most prolific producers of these secondary metabolites which include the jamaicamides,³ malyncamides⁴ and apratoxins.⁵ These compounds represent a number of different structure classes (alkaloid, polyketide, peptide or mixed NRPS/PKS metabolites) and have a number of different biological properties (anticancer, antimicrobial, neurotoxic and anti-inflammatory). Such diversity within a single genus makes this an ideal organism for continued study for structurally unique and biologically active secondary metabolites.

In the current effort, several filamentous tuft-forming species of marine cyanobacteria were collected from La Parguera, Puerto Rico in March 2011, and their extracts and reduced complexity chromatography fractions were evaluated in a number of biological assays. One such fraction from a collected *Moorea producens* was found to be moderately cytotoxic to H-460 human lung cancer cells in vitro (41% survival at 30 µg/mL), and thus was chosen for further investigation. As a result of a NMR-guided fractionation process, two major secondary metabolites were isolated and structurally defined, one a linear acyl amide and the other a lipopeptide. The planar structure, double bond geometry and one of two stereocenters were determined for the first metabolite, parguerene (**1**); unfortunately, this

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Supporting Information **Available:** ¹H NMR, ¹³C NMR, COSY, HSQC and HMBC spectra in CDCl₃ for parguerene (**1**). ¹H NMR, ¹³C NMR, COSY, TOCSY, ROESY, HSQC and HMBC spectra in CD₃OD for precarriewamide (**2**). Detailed method descriptions for 16S rRNA strain identification of the producing organism and H460 cytotoxicity assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

metabolite decomposed before the second stereocenter could be defined. The second compound, precarriebowmide (**2**), was a more stable metabolite and was thus fully characterized including the absolute configurations of all stereogenic centers.

The cyanobacterium *M. producens* was collected by hand from shallow water (1 m) where it was found growing on mangrove roots near La Parguera, Puerto Rico, in March 2011. The cyanobacterium was identified by 16S rRNA analysis, where it shares a 100% maximum identity with what is annotated as *Lyngbya majuscula* 3L (Accession # EU315909.1) (currently *M. producens*) (see supporting information). The isopropanol-preserved collection was repetitively extracted (CH₂Cl₂-MeOH, 2:1) and fractionated using normal-phase vacuum liquid chromatography (VLC). Further fractionation using reversed-phase HPLC yielded 4.5 mg of parguerene (**1**), a pale yellow oil, and 2.1 mg of precarriebowmide (**2**), an amorphous solid.

HRESIMS of **1** gave a [M+H]⁺ at *m/z* 398.3056, indicating a molecular formula of C₂₆H₃₉NO₂ and requiring eight degrees of unsaturation. IR spectroscopy suggested the presence of an amide or ester bond and an NH or OH functionality with strong absorption bands at 1646 cm⁻¹ and 3283 cm⁻¹. The ¹³C NMR spectrum also suggested the presence of a mono-substituted phenyl ring (δ_C 141.1, 128.3 × 2, 128.4 × 2 and 125.8), three olefins (δ_C 120.0, 130.0, 130.3, 130.5, 135.3 and 137.4) and one amide or ester carbonyl (δ_C 170.3). The ¹H NMR spectrum corroborated this with protons resonating at δ_H 7.28, 7.19 and 7.18 for the mono-substituted phenyl ring and four olefinic protons at δ_H 6.29, 5.54, 5.49 and 5.10 plus two deshielded olefinic methyl groups at δ_H 1.87 and 1.61.

Analysis of the 2D NMR spectra of **1** (COSY, HSQC and HMBC) led to the identification of four partial structures (**a-d**) (Figure 1). The first (**a**) was comprised of one hydroxy group (δ_H 2.93) and one amide NH proton (δ_H 5.82) along with a methylene (δ_H 3.56/3.70), a methine (δ_H 4.12) and a methyl group (δ_H 1.21). Assembly of these atoms by 2D NMR yielded a fragment that resembled alanine but with the carboxy group reduced to a primary alcohol. The second fragment (**b**) consisted of an α,β-unsaturated ketone in which the olefin was α-substituted with a methyl group and possessed a methylene group at the β-position. This was followed by a second carbon-carbon double bond that was substituted at the distal location with one more vinyl methyl group. The third partial structure (**c**) contained three consecutive methylene groups adjacent to a methine bearing a methyl and a fourth methylene group; the latter was downfield shifted due to an adjacent di-substituted olefin. An even further downfield shifted methylene was at the distal side of this olefinic bond. The final fragment (**d**) contained the final four degrees of unsaturation present as a mono-substituted phenyl ring.

Assembly of partial structures **a-d** was accomplished by HMBC (Figure 1). Partial structures **a** and **b** were linked by correlations from the NH proton of **a** as well as the α-methyl protons and β-olefinic proton of **b** to the amide carbonyl. Partial structures **b** and **c** were connected through HMBC correlations from the *bis*-allylic methylene and distal olefinic proton of **b** as well as the allylic methylene of **c** to the quaternary olefinic carbon; this connection was reinforced by an HMBC correlation from the vinyl methyl protons to the nearby methylene carbon atom. Converging HMBC correlations from the C-14 allylic-benzylic methylene of **c** and the C-16 aromatic proton of **d** to the quaternary C-15 carbon served to connect these last two partial structures, thus completing the planar structure of parguerene (**1**).

The configurations of the three olefins were determined by a mixture of ¹³C NMR analysis and by ³J coupling. The olefin between C-12 and C-13 exhibited a coupling constant of 15.5 Hz, indicative of *E* configuration. The other two olefins (C-2/C-3 and C-5/C-6) were each tri-substituted; thus, the distinctive carbon shifts of the two vinyl methyl groups were used to

infer their geometry.⁶ Both olefinic methyl groups showed upfield-shifted carbon resonances (C-23, δ_{H} 12.7; C-22, δ_{H} 16.8), and thus their corresponding olefins were deduced to be of *E* configuration.

The absolute configuration of the reduced alanine residue in **1** was determined by LC-MS analysis of the oxidized acid hydrolysate appropriately derivatized with Marfey's reagent (*D*-FDAA). The two standards, *L*- and *D*-Ala, were also reacted with *D*-FDAA and compared to the derivatized hydrolysate by LC-MS. From the retention times it was clear that the alanine residue produced from compound **1** was of the *L*-configuration. Unfortunately, once purified, parguerene (**1**) proved to be unstable and decomposed shortly after acquiring NMR spectra. As a result, the absolute configuration of C-10 was not determined, and thus either a total synthesis or re-collection of the producing organism are required to determine the full absolute configuration of **1**.

Parguerene is structurally reminiscent of stipiamide (**3**), a highly bioactive natural product isolated from the Gram-negative soil bacterium *Myxococcus stipitatus*.⁷ Because an SAR study of stipiamide revealed that reducing the number of conjugated double bonds significantly reduced its overall toxicity [ED₅₀ 0.01 nM to 14 μ M against adriamycin resistant breast cancer cells (MDR-7adrR)] while maintaining MDR reversing activity,^{8,9} and because parguerene is a more saturated analog of stipiamide, we speculate that it would have less cellular toxicity than stipiamide but perhaps retain its MDR reversing properties. Unfortunately, parguerene decomposed before it could be evaluated for these biological properties. Thus, to confirm structure, clarify the one unresolved stereocenter, and to explore its biological properties, re-isolation or synthetic production of parguerene is needed.

HRESIMS of compound **2** gave a $[\text{M}+\text{Na}]^+$ at m/z 887.4706, indicating a molecular formula of C₄₆H₆₈N₆O₈S and requiring 16 degrees of unsaturation (inactive in H-460 cancer cell assay as the IC₅₀ > 10 μ M). IR spectroscopy suggested that **2** was peptidic in nature with a strong absorption band at 1646 cm⁻¹, and was supported by the observation of seven amide or ester type carbonyls by ¹³C NMR analysis (δ_{C} 175.5, 174.1, 174.0, 172.9, 172.0, 170.6 and 170.0). The ¹H NMR spectrum also suggested a peptide with four amide (NH) protons resonating at δ_{H} 7.23, 7.38, 8.60, 8.92 and two *N*-methyl groups at δ_{H} 2.62 and 3.09. The ¹³C and ¹H NMR spectrum indicated the presence of two mono-substituted benzene rings with six peaks, two of which were composed of four carbons each, as indicated by relative peak height (δ_{C} 138.1, 137.5, 130.6 $\times$ 4, 129.9 $\times$ 4, 128.4 and 128.3) and numerous protons resonating between δ_{H} 7.20 and 7.40.

Analysis of 1D and 2D NMR spectra (COSY, TOCSY, ROESY, HSQC and HMBC) led to the identification of five amino acids [alanine (Ala), phenylalanine (Phe), methionine (Met), *N*-methyl-phenylalanine (*N*-MePhe) and *N*-methyl-leucine (*N*-MeLeu)], one hydroxy acid [2-hydroxy-3-methylbutanic acid (Hmba)] and one extended chain polyketide [3-amino-2-methylhexanoic acid (Amha)]. These residues accounted for 15 of the 16 degrees of unsaturation, indicating that the final degree of unsaturation must arise from **2** having an overall cyclic constitution. This conclusion was also apparent from the residue connectivities observed by HMBC and ROESY, along with the comparison to a known compound, carriebowmide (**4**), as described below. ROESY correlations from NH protons to adjacent residue α -protons were used to sequentially connect the Ala, Amha and Hmba residues. HMBC correlations from the *N*-methyl of the *N*-MePhe residue (C-45) to the carbonyl of the Hmba residue (C-46) extended this sequence to Ala – Amha – Hmba – *N*-MePhe. Another fragment was constructed by an HMBC cross-peak between the *N*-methyl of the *N*-MeLeu residue (C-19) and the carbonyl of Phe (C-20). A third fragment was comprised of a Met residue that showed no long-range correlations to any of the other residues. These partial structures were deployed in a search for related compounds in

MarinLit, which revealed that compound **2** was very similar to the known cyanobacterial metabolite, carriebowmide (**4**).¹⁰ A comparison of their ¹³C NMR spectra revealed that the planar structures were identical except for the oxidation state of the sulfur atom in the Met residue. Thus, precarriebowmide was deduced to have a cyclo-[Amha – Ala – *N*-MeLeu – Phe – Met – *N*-MePhe – Hmba] structure.¹¹

The absolute configuration of the *L*-Ala and *L*-Met residues in precarriebowmide (**2**) were determined by LC-MS analysis of the acid hydrolysate appropriately derivatized with Marfey's reagent (*D*-FDAA). As both **2** and **4** had the same *L* configured Ala and Met or Met(SO₂) residues, and the same relative configurations as indicated by their nearly superimposable ¹³C NMR spectra, it was deduced that the two compounds should have the same absolute configurations in the remaining residues [*L*-Phe, *D*-*N*-MePhe, *L*-*N*-MeLeu, (*2S*, *3R*)-Amha and *R*-Hmba].

The similarities between compound **2**, **4** and carriebowmide sulfone (**5**), suggested that **2** might be the actual natural product with the others perhaps representing artifacts as a result of exposure to atmospheric oxygen. Precarriebowmide was extracted and purified from the collected cyanobacterial mass within a few days of collection and preservation, thus potentially preventing **2** from oxidation to carriebowmide. Close inspection of the original LC-MS chromatogram of the semi-crude fraction containing **2** revealed a trace amount of carriebowmide (**4**); however, the major metabolites were compounds **1** and **2**. Upon purification of **2**, there was no indication of carriebowmide; however, after two weeks in CD₃OD the sample was found to be comprised of a mixture of precarriebowmide, carriebowmide and carriebowmide sulfone, in an approximate 60:40:<1 ratio, respectively. These observations taken together with the facile oxidation of the sulfide in methionine, it is conceivable that **2** represents the true natural metabolite and that **4** and **5** are artifacts of the isolation process. Moreover, this conclusion is consistent with the finding that the sulfoxide in carriebowmide (**4**) is racemic, and in fact, represents a mixture of two diastereomeric compounds.

Although both precarriebowmide and parguerene are ostensibly of mixed biosynthetic origin, they clearly represent two very different structural classes, cyclic lipopeptide versus modified linear acyl amide, respectively.¹² Furthermore, each metabolite has undergone modifications to the core structure, such as the incorporation of a hydroxy acid residue and *N*-methylation events (*N*-MePhe and *N*-MeLeu) in precarriebowmide versus integrated aromatic and aliphatic moieties as well as a presumed reductive offloading of alanine in parguerene.¹³ The location of the methyl groups on the acyl chain of parguerene suggests the possibility of their arising from one of two different biosynthetic pathways, both of which likely begin with phenyl acetic acid. In one scenario, the phenyl acetic acid is condensed with a sesquiterpene moiety whereas in the second phenyl acetic acid is the starter unit for six iterative polyketide synthase (PKS) additions of acetate. In the latter case, *C*-methylation must occur on the C-2 position of every other acetate unit. Differentiation between these two intriguing alternatives may be possible through genome sequencing of DNA preserved at the time of collection.

Experimental Section

General Experimental Procedures

Optical rotations were measured on a JASCO P-2000 polarimeter, UV spectra on a Beckman Coulter DU-800 spectrophotometer, and IR spectra were obtained using a Nicolet IR-100 FT-IR spectrophotometer using KBr plates. NMR spectra were recorded with solvent peaks as internal standards (δ_C 77.0, δ_H 7.26 for CHCl₃, and δ_C 49.0, δ_H 3.31 for CH₃OH) on a Varian Unity 500 MHz spectrometer (500 and 125 MHz for ¹H and ¹³C

NMR, respectively) and Varian Unity 300 MHz spectrometer (300 and 75 MHz for ^1H and ^{13}C NMR, respectively). LR- and HR-ESIMS were obtained on ThermoFinnigan LCQ Advantage Max and Thermo Scientific LTQ Orbitrap-XL mass spectrometers, respectively. All solvents were either distilled or of HPLC quality. Acid hydrolysis was performed using a Biotage (Initiator) microwave reactor equipped with high pressure vessels.

Cyanobacterial Collections and Morphological Identification

The producing cyanobacterium PRM 25-Mar-11-2 was collected by hand using snorkel gear in shallow water off La Parguera on the southwest coast of Puerto Rico. Morphological characterization was performed using an Olympus IX51 epifluorescent microscope (1000 $\times$) equipped with an Olympus U-CMAD3 camera. Morphological comparison and putative taxonomic identification of the cyanobacterial specimen was performed in accordance with modern classification systems.^{14,15}

Extraction and Isolation

The cyanobacterial biomass (10.4 g, dry wt) was extracted with 2:1 CH_2Cl_2 -MeOH to afford 3.9 g of dried extract. A portion of the extract was fractionated by silica gel VLC using a stepwise gradient solvent system of increasing polarity starting from 100% hexanes to 100% EtOAc to 100% MeOH (nine fractions). The fraction eluting with 100% EtOAc was separated further using RP HPLC [4 μ Synergi Fusion, 65% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 50 min to produce six fractions (1-6)] to yield pure parguerene (**1**, 4.5 mg) and precarriebowmide (**2**, 2.1 mg).

Parguerene (1)—pale yellow oil; $[\alpha]_{\text{D}}^{23} +17.3$ (c 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.50), 206 (2.66) nm; IR (neat) ν_{max} 3283, 3050, 2958, 1646, 1541, 1449, 1239, 1185, 1134, 746; ^1H NMR (500 MHz, CDCl_3) and ^{13}C NMR (75 MHz, CDCl_3), see Table 1; HRESIMS $[\text{M}+\text{H}]^+$ m/z 398.3056 (calcd for $\text{C}_{26}\text{H}_{40}\text{NO}_2$, 398.3054).

Precarriebowmide (2)—amorphous solid; $[\alpha]_{\text{D}}^{23} -52.6$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.67), 256.0 (2.91) nm; IR (neat) ν_{max} 3061, 3030, 2958, 1646, 1541, 1239, 1134, 746; ^1H NMR (500 MHz, CDCl_3) and ^{13}C NMR (75 MHz, CDCl_3), see Table 2; HRESIMS $[\text{M}+\text{Na}]^+$ m/z 887.4706 (calcd for $\text{C}_{46}\text{H}_{69}\text{N}_6\text{O}_8\text{S}$, 887.4712).

Oxidation, Acid Hydrolysis and Marfey's Analysis of Parguerene (1)

Parguerene (**1**, 0.5 mg) was dissolved in 200 μL of acetone and cooled to 0 $^\circ\text{C}$, then treated with 10 μL of 0.25 M Jones reagent (CrO_3 , H_2SO_4). After 20 min the reaction was quenched with 200 μL of isopropyl alcohol and the reaction mixture was extracted with EtOAc 5 $\times$ to yield the desired product. The reaction product was then treated with 300 μL of 6 N HCl in a microwave reactor at 160 $^\circ\text{C}$ for 5 min. The reaction product was dissolved in 300 μL of 1 M sodium bicarbonate, and then 56 μL of 0.5% D-FDAA (1-fluoro-2,4-dinitrophenyl-5-D-alanine amide) was added in acetone. The solution was maintained at 40 $^\circ\text{C}$ for 80 min at which time the reaction was quenched by the addition of 300 μL of 1 N HCl. The reaction mixture was diluted with 300 μL of CH_3CN and 10 μL of the solution was analyzed by LC-ESIMS.

The Marfey's derivatives of the hydrolysate and standards were analyzed by RP HPLC using a Phenomenex Luna 5 μm C_{18} column (4.6 $\times$ 250 mm). The HPLC conditions began with 10% $\text{CH}_3\text{CN}/90\%$ H_2O + 0.1% formic acid (FA) followed by a gradient profile to 50% $\text{CH}_3\text{CN}/50\%$ H_2O + 0.1% FA over 85 min at a flow of 0.4 mL/min, monitoring from 200 to 600 nm. The retention times of the D-FDAA derivatives of the authentic amino acids were D-Ala (50.62 min), and L-Ala (56.44 min); the derivative of the hydrolysate product gave a peak with a retention time of 56.68 min, corresponding to L-Ala .

Acid Hydrolysis and Marfey's Analysis of Precarriebowmide (2)

Precarriebowmide (**2**, 0.3 mg) was treated with 300 μ L of 6 N HCl in a microwave reactor at 160 °C for 5 min. The reaction product was dissolved in 200 μ L of 1 M sodium bicarbonate, and then 32 μ L of 0.5% D-FDAA was added in acetone. The solution was maintained at 40 °C for 70 min at which time the reaction was quenched by the addition of 100 μ L of 2 N HCl. The reaction mixture was diluted with 200 μ L of CH₃CN and 10 μ L of the solution was analyzed by LC-ESIMS.

The Marfey's derivatives of the hydrolysate and standards were analyzed by RP HPLC using a Phenomenex Luna 5 μ m C₁₈ column (4.6 $\times$ 250 mm). The HPLC conditions were identical to the method described above. The retention times of the D-FDAA derivatives of the authentic amino acids were D-Ala (50.17), L-Ala (56.33), D-Met (62.35) and L-Met (70.49); the hydrolysate product gave peaks with retention times of 56.51 and 70.56 min, according to L-Ala and L-Met, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Parguerene (1)

Precarriebowmide (2)

— COSY/TOCSY
— HMBC
— ROESY

Figure 1. Partial structures for parguerene (1) and select 2D NMR data for both parguerene (1) and precarriebowmide (2).

Table 1¹H and ¹³C NMR Assignments for Parguerene (**1**) in CDCl₃.

Position	δ_{C}^b , type	δ_{H}^a (J in Hz) ^a	HMBC ^a
1	170.3, C		
2	130.3, C		
3	135.3, CH	6.29, t (7.3)	1, 4, 5, 23
4	27.3, CH ₂	2.85, t (7.2)	1, 3, 5, 6
5	120.0, CH	5.10, t (7.0)	3, 4, 7, 22
6	137.4, C		
7	39.8, CH ₂	1.95, t (7.4)	8, 9, 22, 6
8	25.3, CH ₂	1.40, m	7, 9, 10
9a	36.2, CH ₂	1.29, m	7, 10, 11, 21
9b		1.09, m	7, 10, 11, 21
10	33.0, CH	1.47, m	8, 9, 11, 21
11a	39.9, CH ₂	2.02, dt (14.0, 6.3)	9, 10, 12, 21
11b		1.86, dt (14.0, 6.9)	9, 10, 12, 21
12	130.5, CH	5.49, dt (15.5, 6.3)	10, 11, 13, 14
13	130.0, CH	5.54, dt (15.5, 6.5)	14, 15, 16, 17
14	39.1, CH ₂	3.34, d (6.5)	13, 15, 16
15	141.1, C		
16/20 ^c	128.3, CH	7.28, m	15, 17
17/19 ^c	128.4, CH	7.18, m	16, 18
18	125.8, CH	7.19, m	17
21	19.5, CH ₃	0.88, d (6.8)	9, 10, 11
22	16.1, CH ₃	1.61, s	5, 6, 7
23	12.8, CH ₃	1.87, s	3
1'	48.1, CH	4.12, m	2', 3'
2a'	67.7, CH ₂	3.70, dd (10.9, 3.2)	1', 3'
2b'		3.56, dd (10.9, 6.5)	1', 3'
3'	17.1, CH ₃	1.21, d (6.7)	1', 2'
OH		2.93, bs	
NH		5.82, d (5.5)	

^a 500 MHz for ¹H NMR, HMBC, and COSY^b 75 MHz for ¹³C NMR^c Carbons 16/20 and 17/19 each form a single peak

Table 2¹H and ¹³C NMR Assignments for Precarriebowmide (**2**) in CD₃OD.

Residue	position	δ_C^b , type	δ_H (J in Hz) ^a	HMBC ^a
AMHA	1	175.5, C		
	2	43.2, CH	2.65, m	1, 3, 4
	3	9.1, CH ₃	0.90, d (6.9)	1, 2, 4
	4	52.0, CH	4.28, m	2, 3, 5
	5a	35.0, CH ₂	1.52, m	4, 6, 7
	5b		1.50, m	4, 6, 7
	6a	20.6, CH ₂	1.43, m	5, 7
	6b		1.37, m	5, 7
	7	13.8, CH ₃	0.97, d (7.4)	5, 6
	8-NH		7.38	
Ala	9	174.1, C		
	10	48.8, CH	4.50, m	9, 11, 13
	11	16.4, CH ₃	1.16, d (6.8)	9, 10
	12-NH		8.92, d (9.0)	13
N-MeLeu	13	170.6, C		
	14	59.8, CH	4.69, m	13, 15, 19
	15a	37.8, CH ₂	1.74, m	14, 16, 17, NH
	15b		-0.23, td (10.9, 3.5)	14, NH
	16	25.7, CH	1.44, m	15, 17
	17	21.8, CH ₃	0.78, d (6.5)	15, 16, 18
	18	23.9, CH ₃	0.73, d (6.5)	17
	19-N-Me	29.7, CH ₃	2.62, s	14, 20
Phe	20	174.0, C		
	21	52.8, CH	4.77, dd (9.9, 6.0)	22
	22a	38.8, CH ₂	3.09, m	20, 21, 24
	22b		3.03, m	20, 21, 24
	23	137.5, C		
	24/28 ^c	130.6, CH	7.21, d (7.5)	22, 23, 25, 26
	25/27 ^c	129.9, CH	7.28, d (7.5)	24, 26
	26	128.3, CH	7.24, t (7.5)	24, 25
	29-NH		7.23, m	30
Met	30	172.9, C		
	31	52.0, CH	4.55, m	30, 32
	32a	30.1, CH ₂	2.19, m	34
	32b		1.31, m	31, 33
	33a	33.5, CH ₂	1.88, m	34
	33b		1.67, m	32

Residue	position	δ_C^b , type	δ_H (J in Hz) ^a	HMBC ^a
N-MePhe	34-S-Me	15.2, CH ₃	2.05, s	32, 33
	35-NH		8.60, d (8.8)	
	36	170.0, C		
	37	62.2, CH	4.62, m	36, 38, 45
	38a	37.7, CH ₂	3.41, dd (13.5, 9.9)	36, 37, 39
	38b		2.95, dd (13.5, 5.6)	36, 37, 39
	39	138.1, C		
	40/44 ^c	130.6, CH	7.25, d (7.5)	39, 41, 42
	41/43 ^c	129.9, CH	7.37, t (7.5)	40, 42
	42	128.4, CH	7.27, t (7.5)	40, 41
Hmba	45-N-Me	30.1, CH ₃	3.09, s	37, 46
	46	172.0, C		
	47	76.1, CH	5.16, d (2.81)	48, 49, 50
	48	30.6, CH	1.70, m	47, 49, 50
	49	19.7, CH ₃	1.18, d (6.8)	47, 48, 50
	50	16.9, CH ₃	0.88, d (6.8)	47, 48, 49

^a 500 MHz for ¹H NMR, HMBC, and COSY

^b 75 MHz for ¹³C NMR

^c Carbons 24/28/40/44 and 25/27/41/43 each form a single peak