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## A Chemical Study of Three Novel Cyclic Depsipeptides Produced by a Sponge-Derived Fungus

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### Abstract

Two novel cyclic depsipeptides, guangomides A (**1**) and B (**2**), together with a new destruxin derivative (**3**) were isolated from the cytotoxic extract obtained from the saltwater culture of an unidentified sponge-derived fungus. The new structures were elucidated based on analysis of extensive 1D and 2D NMR data sets, and the absolute configurations of 2*S*, 9*S*, 13*S*, 19*S*, 24*R*, 28*R* of **1** was determined based on the combined X-ray and Marfey's method structure analysis. Identical absolute configurations were assumed for **2**. The cytotoxicity of the extract was found to be due to brefeldin A, while **1** and **2** showed weak antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus durans*.

A few marine invertebrates are known to be a source of structurally fascinating and biologically active peptides and depsipeptides. Some most noteworthy examples include the peptide anticancer drug candidates dolastatin 10,<sup>i</sup> from the sea hare *Dolabella auricularia* (but also the cyanophyte *Symploca* sp VP642ii) and kahalalide F<sup>iii</sup> from the herbivorous marine mollusk *Elysia rufescens* (and also its dietary alga *Bryopsis* sp.). Significantly, both of these chemotypes are continuing subjects of clinical evaluation.<sup>iv</sup> Two closely related PKS/NRPS cyclidepsipeptides — jasplakinolide from sponges<sup>v</sup> and chondramide C from myxobacteria<sup>vi</sup> — are of much interest because they are both potent in causing the hperassembly of G-actin into F-actin.<sup>vii</sup> Our attempts to date to discover marine-derived fungi as a source of unusual peptides have been somewhat successful. These include the isolation of the cytotoxic bicyclic peptide malformin C from *Aspergillus niger*<sup>viii</sup> and highly *N*-methylated linear peptides of the RHM family from an unidentified fungal strain.<sup>ix</sup> Recently we began a project stimulated by the observation that extracts of an unidentified fungal strain separated from an *Inathella* sponge possessed potent cytotoxicity and selectivity in our disk diffusion assay system.<sup>x</sup> Most importantly its <sup>13</sup>C NMR spectra displayed clusters of peaks centered at  $\delta$ 175. The initial bioassay-guided dereplication efforts showed that brefeldin A<sup>xi</sup> was a non-peptide major component responsible for the cytotoxicity. Subsequently, deeper evaluation of crude extract fractions employing bioassay

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Supporting Information Available: <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** – **3**, bioassay data and X-ray data of **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

and LCMS data led to discovery of three novel cyclic depsipeptides named guangomides A (**1**), B (**2**) and homodestcardin (**3**). We now describe the structure elucidation and the biological activity of these depsipeptides.

The molecular formula of  $C_{31}H_{46}N_4O_9$  ( $m/z = 641.3152 [M+Na]^+$ ) was established for guangomide A (**1**) by the HRESIMS data. The low field  $^{13}C$  NMR resonances noted above were confirmed to be associated with peptidic functionality that was further validated by  $^1H$  NMR data showing the presence of two amide protons, six  $\alpha$ -protons, and two *N*-methyl groups. There were a total of six carbonyl carbons in the  $^{13}C$  NMR spectrum (Table 1). Four of those carbonyl carbons were assigned as amide carbons and consistent with the four nitrogen atoms in the molecular formula. Therefore, the assumption that two ester carbons were present was verified by the HMBC correlations from two  $\alpha$ -oxy methines ( $\delta_H$  5.00,  $\delta_C$  70.5 and  $\delta_H$  5.22,  $\delta_C$  76.5) to these carbonyl carbons ( $\delta_C$  172.6, 169.0).

There were six carbonyl containing substructures envisioned based on the 2D NMR data and they are shown in Figure 1. Further, as expected, the gCOSY spectrum of **1** revealed six distinct spin systems. The first included the subset associated with the 2-hydroxyisocaproic acid (**A**). Another cluster included the three sets of overlapping resonances for the alanine residues (**B**, **D** and **E**). The protons associated with the phenylalanine residue (**F**) were identified by HMBC correlations (H28/C30 and H29/C31, C35) shown in Figure 1. Finally, the remaining subunit, 2,3-dihydroxyisovaleric acid (**C**), was established by the HMBC correlations (H13/C14, C15, C16, H15, H16/C13) also shown in Figure 1. Next, the locations of the *N*-methyl groups ( $\delta_H$  3.21,  $\delta_C$  37.1 and  $\delta_H$  2.94,  $\delta_C$  30.3) were affirmed as connected to the alanine (**D**) and the phenylalanine (**F**) based on the HMBC correlations (H22/C19, H37/C28). The final task of sequencing the six subunits was accomplished based on the HMBC correlations from the  $\alpha$ -protons, the amide protons and the *N*-methyl groups to the carbonyl carbons (H2/C8, H9, NH11/C12, H13/C18, H19, H22/C23, H24, NH26/C27, H28, H37/C1) as detailed in Figure 2.

Assignment of the absolute configuration for the amino acid residues was accomplished using complimentary approaches. A *D*-*N*-methyl-phenylalanine was assigned based on results derived from HPLC-analysis of the products obtained from the Marfey's acid hydrolysate.<sup>xii</sup> Though our attempts to determine the relative configurations of chiral centers using NOESY data were unsuccessful, positive results were obtained through X-ray analysis structure of **1** shown in Figure 3.<sup>xiii</sup> Combining the relative configurations deduced from the X-ray data and using the *D*-*N*-methyl-phenylalanine as an anchor point supported the final absolute stereostructure as 2*S*, 9*S*, 13*S*, 19*S*, 24*R*, 28*R*.

The next compound to be analyzed was guangomide B (**2**), whose molecular formula of  $C_{31}H_{46}N_4O_8$  differed from that of **1** by just a single oxygen atom. Not surprisingly, its  $^{13}C$  and  $^1H$  NMR spectra (Table 1) were almost identical with those of **1**. The differences included shifted  $^{13}C$  resonances for the C13/C14/C15/C16 of **2** versus **1** consistent with the proposal that an H group replaced the OH group at C14 of substructure **C** (Figure 1). Likewise the  $^1H$  NMR spectrum of **1** showed diastereotopic H<sub>3</sub>15 and H<sub>3</sub>16 as doublets. Thus, these data were in firm support of substructure **D** as 2-hydroxyisovaleric acid, which was further confirmed by the COSY and the HMBC measurements. The stereochemistry assignments shown for **2** are based on a biogenetic analogy to **1** plus the observations that both compounds have the same sign of the optical rotation and both have parallel NMR shifts at each of the chiral centers.

The last new compound, homodestcardin (**3**) had the molecular formula  $C_{32}H_{55}N_5O_7$  established by the HRESIMS data. This compound was concluded to be a member of the destruxin family based on the similar profile of  $^1H$  and  $^{13}C$  (Table 2) to that of

homodestruxin B (**4**)<sup>xiv</sup> and roseocardin (**5**).<sup>xv</sup> Detail analysis of the 2D NMR data of **3** pinpointed the  $\beta$ -methyl proline and *N*-methyl leucine residues versus the proline residue on **4** and *N*-methyl valine residue on **5**, respectively. Our proposed planar structure derived by analogy to **4** and **5** was confirmed from the 2D NMR data (Figure 4). Extending the comparisons of the NMR data between this trio further revealed that **3** and **4** possessed identical shifts at the western chiral centers (C6, C11, C12, C19 and C20). Alternatively **3** and **5** possessed parallel shifts at the eastern chiral centers (C26, C27 and C33) were almost identical with those of **5**. The significant NOE observed between H26 and H28 was also consistent with these stereochemical conjectures. The final relative stereoassignments proposed for **3** are shown in Figure 4.

The literature, aside from that noted above of cyclic depsipeptides, including those with significant bioactivity properties isolated from marine-derived fungi is rather sparse. One set of compounds includes 15G256 $\gamma$ , 156G256 $\delta$  and 15G256 $\epsilon$  which are lipodepsipeptides isolated from a *Hypoxylon oceanium* separated from mangrove wood.<sup>xvi</sup> Exumolides A and B isolated from a marine plant-derived *Scytaladium* sp. are cytostatic cyclic hexadepsipeptides.<sup>xvii</sup> Sansalvamidexviii and *N*-methylsansalvamide<sup>xix</sup> isolated from two different marine alga-derived *Fusarium* spp. are cytotoxic cyclic pentadepsipeptides. Those previous examples contain one ester group in the molecule. By contrast, the guangomides (**1** and **2**) isolated in this study are different from known cyclic depsipeptides from marine-derived fungi because of the presence of two ester groups in the molecule. In addition, homodestcardin (**3**) is the first example of destruxin analog from marine-derived fungi. The biological properties of **1** and **2** were investigated and deserve brief comment. Guangomide A (**1**) was inactive against murine and human tumor cell lines in a disk diffusion assay. However, **1** and **2** showed weak antibacterial activity against *Staphylococcus epidermidis* (MIC=100 $\mu$ g/mL, each) and *Enterococcus durans* (MIC=100 $\mu$ g/mL, each).

## Experimental Section

### General Experimental Procedures

UV/vis measurements were recorded on HP 8453 diode array spectrometer. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. The NMR spectra were recorded on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively. High resolution mass measurements were obtained on a bench-top Mariner ESI-TOF mass spectrometer. HPLC was performed with a column of 4 $\mu$ m ODS.

### Biological Materials

The fungus (strain no. 001314c) was isolated from an *Inathella* sp. (coll. no. 00314) collected using SCUBA off the coast of Guango, Papua New Guinea on December 2000 by the same techniques described previously.<sup>xx</sup> Attempts to identify this fungal strain by the alignment of the D2 region of the 25S ribosomal DNA sequence and its fruiting body were unsuccessful (the closest fungus: *Fusarium graminearum* with a genetic distance of 9.75%). This fungus is maintained in a cryopreserved state at UCSC.

### Culture conditions

The fungal strain was grown in a liquid medium (20 L) containing 1.5 % malt extract broth in filtered Monterey Bay seawater adjusted to pH 7.4 at 180 rpm for 28 days at room temperature (25°C).

## Disk Diffusion Soft Agar Colony Formation Assay

An *in vitro* cell-based assay was employed to identify solid tumor selectivity for original extracts, extract partition fractions, and pure compounds. The differential cytotoxicity is expressed by observing a zone differential between any solid tumor cell (Colon 38, Colon H116, Lung H125) and either leukemia cells (L1210 or CEM) or normal cells (CFU-GM). The sample is designated as “solid tumor selective” if the zone units of solid tumor – normal cell or leukemia cells is greater than 250 units.

## Extraction and Isolation

The culture was filtered under suction and the broth was extracted with equal volumes of EtOAc three times. The EtOAc extract was partitioned by Kupchan type extraction reported previously.<sup>xxi</sup> The CH<sub>2</sub>Cl<sub>2</sub> extract (EFD; 2.6 g) contained crystals, which were purified by washing away with MeOH and were identified brefeldin A (560 mg) by comparison of its spectral data to the published data.<sup>8</sup> The CH<sub>2</sub>Cl<sub>2</sub> soluble portion was separated with a flash silica gel chromatography using CH<sub>2</sub>Cl<sub>2</sub>-MeOH stepwise gradient as the eluent. B3 (170 mg) obtained from MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:99) was purified by a reverse phase HPLC with MeOH-H<sub>2</sub>O (3:7 up to 1:0) to furnish **2** (1.5 mg). B4 (190 mg) obtained from MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:49) was purified by a reverse phase HPLC with MeOH-H<sub>2</sub>O (2:3 up to 9:1) to furnish **1** (11.0 mg) and **3** (1.2 mg).

## Guangomide A (1)

Colorless crystals from Hexane/EtOAc/MeOH (1/1/1). Mp. 255-257°C.  $[\alpha]_D^{28} -44.6$  (*c* 0.8, CHCl<sub>3</sub>);  $\lambda_{\max}$  (MeOH)/nm 203 (log  $\epsilon$  4.38); <sup>1</sup>H and <sup>13</sup>C NMR data are listed in Table 1; HRESIMS: *m/z* 641.3152 [M+Na]<sup>+</sup> (calcd for C<sub>31</sub>H<sub>46</sub>N<sub>4</sub>O<sub>9</sub>Na, 641.3157).

## Guangomide B (2)

White amorphous powder.  $[\alpha]_D^{28} -18.1$  (*c* 0.9, CHCl<sub>3</sub>);  $\lambda_{\max}$  (MeOH)/nm 204 (log  $\epsilon$  4.38); <sup>1</sup>H and <sup>13</sup>C NMR data are listed in Table 1; HRESIMS: *m/z* 625.3208 [M+Na]<sup>+</sup> (calcd for C<sub>31</sub>H<sub>46</sub>N<sub>4</sub>O<sub>8</sub>Na, 625.3208).

## X-ray crystallography of 1

The single crystal X-ray analysis was conducted as follows. Suitable crystals were obtained from Hexane/EtOAc/MeOH (1/1/1) by the vapor diffusion method. This crystal (0.60 × 0.40 × 0.30 mm<sup>3</sup>) was mounted on a Bruker SMART diffractometer (MoK $\alpha$ ; -100°C). A hemisphere of data was taken using a narrow-scan routine (1406 frames, 0.3° steps  $\omega$ -scan, exposure time was 30 s/frame,  $2\theta_{\max} = 63.62^\circ$ ). Raw data was integrated with the Bruker SAINT+ program<sup>xxii</sup> to yield a total 33908 reflections, of which 10117 were independent ( $R_{\text{int}} = 2.58\%$ , completeness 94.9%) and 8852 with  $I > 2\sigma(I)$ . Data were collected for absorption using SADABS program (min. and max. transmission are 0.9479 and 0.9735, respectively).<sup>xxiii</sup> The structure was solved by direct methods and refined by full matrix least square on  $F^2$  techniques using anisotropic displacement parameters for all non-hydrogen atoms.<sup>xxiv</sup> All hydrogen atoms were found in the difference Fourier map and refined isotropically. At final convergence,  $R_1 = 3.88\%$  and GOF = 1.022 for 581 parameters.

## Amino acid analysis of 1 using Marfey's method

Guangomide A (**1**) (1.4 mg) and 6N HCl (2 mL) were added to MeOH (0.2mL) and heated at 110°C for 14hr in a sealed vial. The cooled reaction mixture was evaporated to dryness. To the residue was added 1M NaHCO<sub>3</sub> (0.1 mL) and 1% Marfey's reagent (FDAA) in acetone (0.1 mL), incubated at 37°C for 0.5 hr. The reaction mixture was quenched with 2N HCl (50  $\mu$ L), analyzed by reverse phase HPLC. The analysis was performed by the

following condition: Alltech altima C18 column (5 $\mu$ , 250mm  $\times$  10mm id.), solvent system acetonitrile/H<sub>2</sub>O (4:1) up to (1:1) over 60 min with 1 mL/min flow rate, UV detection at 340 nm. Separately *N*-methyl-*D*- and *L*-phenylalanine were derivatized with FDAA in the same manner as that of **1**. The HPLC conditions gave the distinguishable retention times for *D*- and *L*-forms (51.0 min and 50.3 min, respectively). The configuration of the *N*-methylphenylalanine was determined to be *D*-form based on co-injection of each form of the standard amino acids and **1**.

### Homodestcardin (3)

White amorphous powder.  $[\alpha]_D^{27} -143.8$  (*c* 0.9, CHCl<sub>3</sub>);  $\lambda_{\max}$  (MeOH)/nm 204 (log  $\epsilon$  3.75); <sup>1</sup>H and <sup>13</sup>C NMR data are listed in Table 2; HRESIMS: *m/z* 622.4170 [M+H]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>56</sub>N<sub>5</sub>O<sub>7</sub>, 622.4174).

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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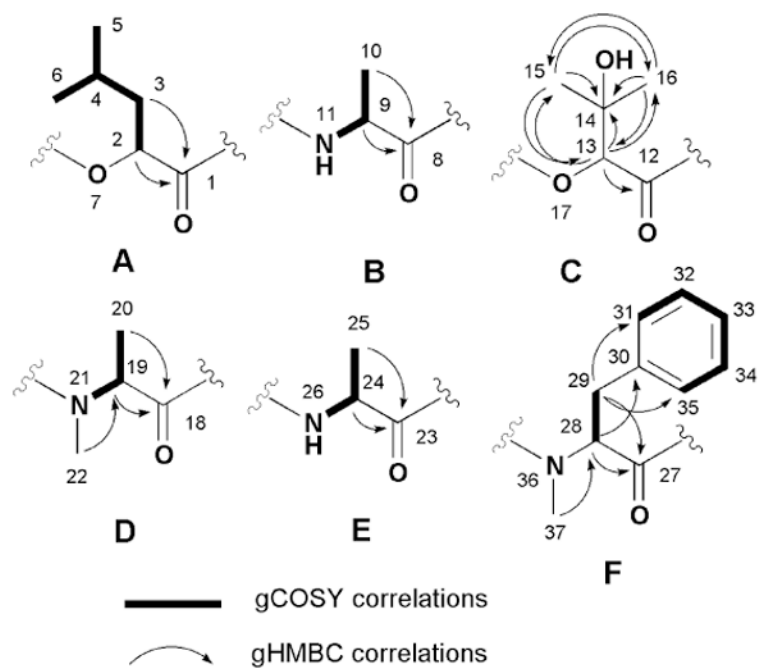


Figure 1. Structural units for 1

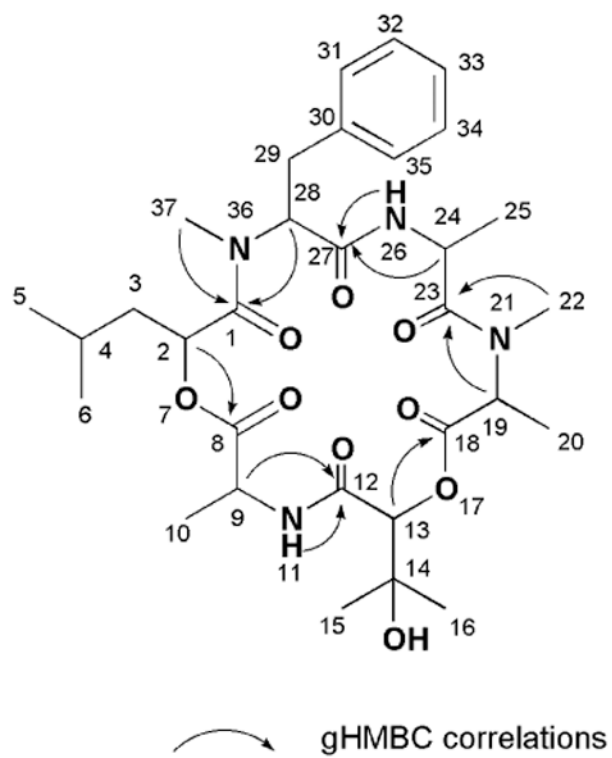


Figure 2. Selected gHMBC correlations for 1



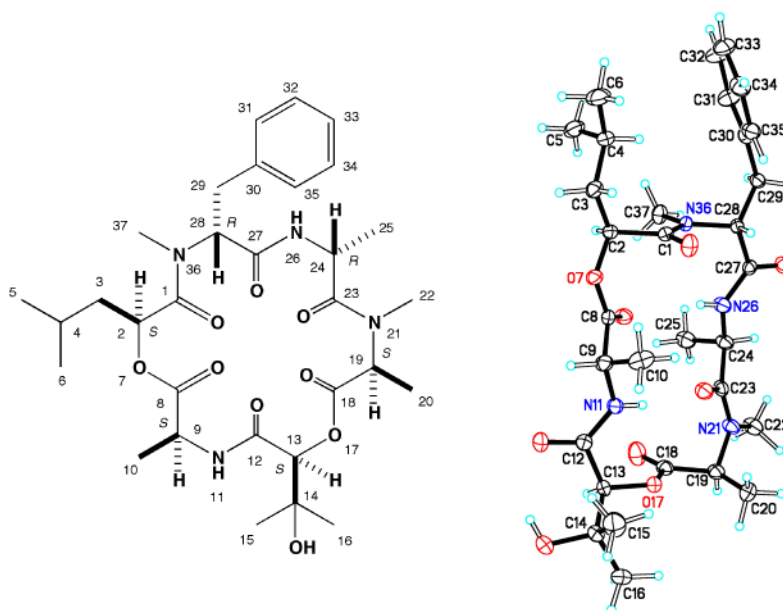


Figure 3. Absolute structure of 1 including X-ray crystal structure

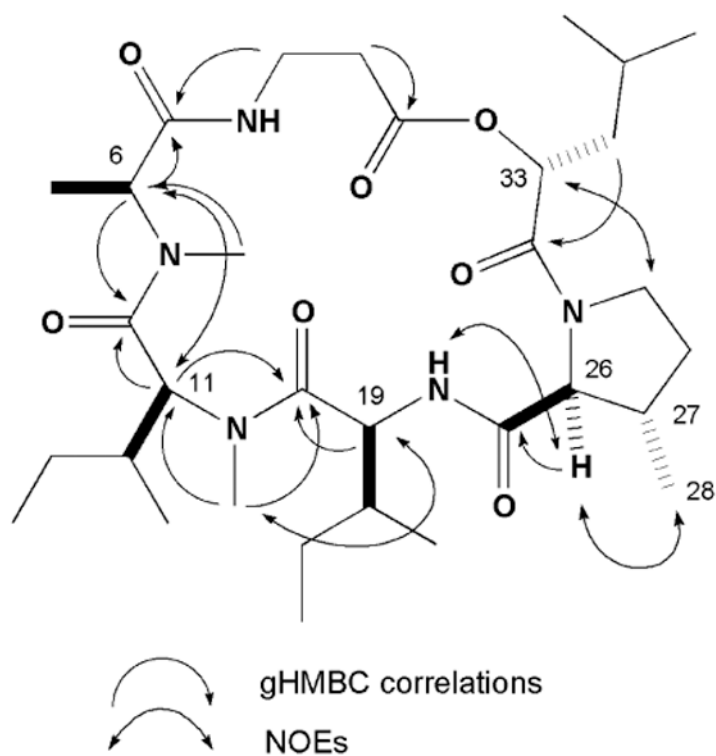
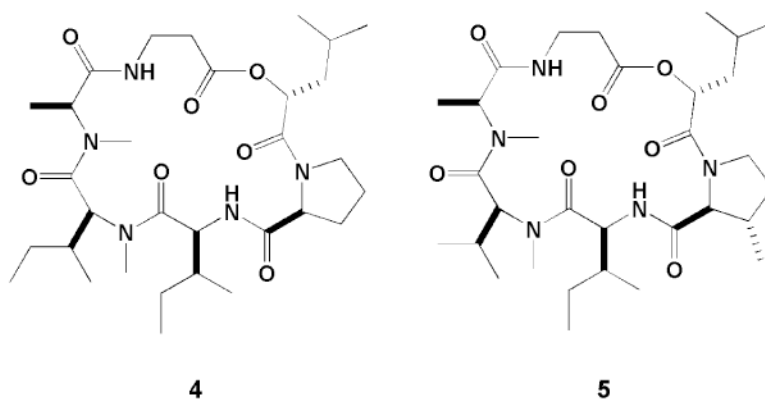
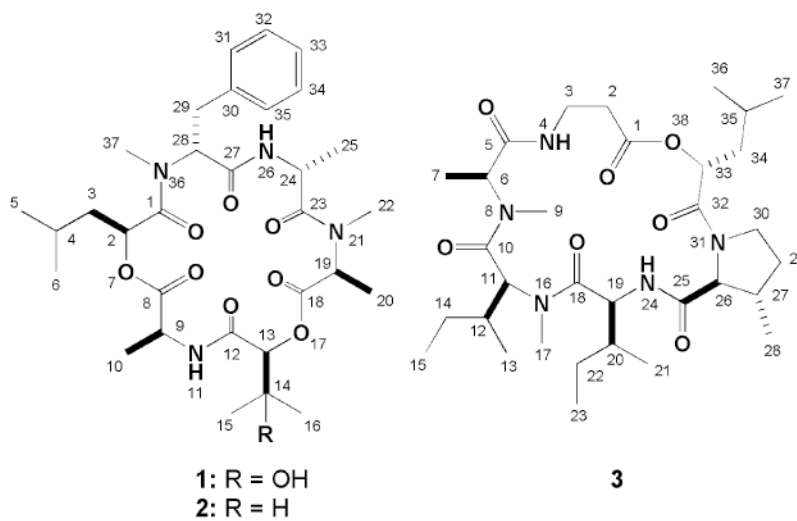


Figure 4. Selected 2D NMR correlations and the relative structure of 3



**Table 1**  
 $^1\text{H}$  and  $^{13}\text{C}$  NMR data for guangamide A (1) and B (2) in  $\text{CDCl}_3^d$

Structural unit	1						2					
	$^{13}\text{C}$ ( $\delta$ , mult)	$^1\text{H}$ ( $\delta$ , mult., J, Hz)	gCOSY	gHMBC	NOESY	$^{13}\text{C}$ ( $\delta$ , mult)	$^1\text{H}$ ( $\delta$ , mult., J, Hz)	gHMBC				
S-Hic	1	172.4 (s)				172.4 (s)						
	2	70.5 (d)	3a, 3b	1, 3, 4, 8	3a, 3b, 4, 5, 6, 37	70.3 (d)	5.00 (dd, 8.9, 6.4)	1, 3, 4, 8				
	3	38.8 (t)	a	1, 4, 5, 6	2, 3b, 4, 5, 6, 37	38.8 (t)	0.96 (ddd, 14.2, 6.4, 5.3)	2, 4, 5, 6				
			b	1, 2, 4, 5, 6	2, 3a, 4, 5, 6		1.54 m	4, 5, 6				
	4	23.8 (d)	3a, 5, 6	3, 5, 6	2, 3a, 3b, 5, 6, 31, 32, 34, 35, 37	23.8 (d)	1.19 (hept, 6.7)					
	5	22.8 (q)	4	3, 4, 6	2, 3a, 3b, 4, 31, 32, 34, 35, 37	22.9 (q)	0.78 (d, 6.6)	3, 4, 6				
	6	22.3 (q)	4	4, 5, 6	2, 3a, 3b, 4, 31, 32, 34, 35, 37	22.2 (q)	0.72 (d, 6.6)	4, 5, 6				
L-Ala	8	172.6 (s)				172.9 (s)						
	9	47.1 (d)	11	8, 10, 12	10, 11	47.0 (d)	4.98 (dq, 8.9, 7.4)	8, 10				
	10	19.3 (q)		8, 9	9, 11	19.3 (q)	1.52 (d, 7.4)	8, 9				
	11		9	12	9, 10, 13, 15		7.89 (d, 8.5)					
S-Dhiv	12	169.8 (s)				168.5 (s)						
(S-Hiv)	13	76.5 (d)		12, 14, 15, 16, 18	11, 15, 16	78.0 (d)	5.23 (d, 2.5)	12, 14, 15, 16				
	14	71.8 (s)				30.0 (d)	2.61 (hept, d, 6.7, 2.5)					
	15	26.7 (q)		12, 13, 14, 16	11, 13, 16, OH	19.2 (q)	0.93 (d, 6.7)	13, 14, 16				
	16	24.1 (q)		13, 14, 15	13, 15, OH	15.8 (q)	0.92 (d, 6.7)	13, 14, 15				
OH				15, 16								
L-N-MeAla	18	169.0 (s)				169.7 (s)						
	19	60.5 (d)	20	18, 20, 22, 23	20, 22	60.7 (d)	3.69 (q, 6.8)	18, 20, 22, 23				
	20	13.5 (q)	19	18, 19	19, 22	13.6 (q)	1.54 (d, 6.9)	18, 19				
	22	36.9 (q)		19, 23	19, 20, 24, 25	36.9 (q)	3.19 s	19, 23				
D-Ala	23	171.3 (s)				171.1 (s)						
	24	46.2 (d)	25, 26	23, 25, 27	22, 25, 26	46.3 (d)	4.84 (quint, 7.1)	23				
	25	18.1 (q)	24	23, 24	22, 26, 37	18.1 (q)	1.42 (d, 6.7)	23, 24				

Structural unit	1					2				
	<sup>13</sup> C (δ, mult)	<sup>1</sup> H (δ, mult., J, Hz)	gCOSY	gHMBC	NOESY	<sup>13</sup> C (δ, mult)	<sup>1</sup> H (δ, mult., J, Hz)	gHMBC	gHMBC	
	26	7.09 (d, 7.3)	24	27	24, 25, 28, 29a		7.12 (d, 7.4)			
D-N-Me-Phe	27	168.5 (s)				168.5 (s)				
	28	56.6 (d)	29a, 29b	1, 27, 29, 37	26, 29a, 29b, 31, 35	56.5 (d)	5.76 (dd, 11.8, 5.3)	1, 27, 29, 37	1, 27, 29, 37	
	29	33.2 (t)	28, 29b	27, 28, 30, 31, 35	26, 29b, 28, 31, 35	33.2 (t)	2.92 (dd, 15.1, 11.8)	a	27, 30, 31, 35	
	30	137.1 (s)	28, 29a	30, 30, 31, 35	28, 29a, 31, 35	137.1 (s)	3.47 (dd, 15.1, 5.3)	b	27, 30, 31	
	31,35	128.6 (d)	32, 34	29, 30, 32, 33, 34	5, 6, 28, 29a, 29b	128.6 (d)	7.16 m		29, 30, 32, 33, 34	
	32,34	128.4 (d)	31, 33, 35	30, 31, 33, 35	5, 6	128.4 (d)	7.24 m		30, 31, 33, 35	
	33	126.6 (d)	32, 34	31, 32, 34, 35		126.6 (d)	7.19 m		31, 32, 34, 35	
	37	30.2 (q)		1, 28	2, 3a, 4, 5, 6, 25	30.1 (q)	2.92 s		1, 28	

<sup>a</sup>Measured at 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C).

Table 2

**<sup>1</sup>H and <sup>13</sup>C NMR data for homodestcardin (3) in CDCl<sub>3</sub><sup>a</sup>**

3

Structural unit	<sup>13</sup> C (δ, mult)	<sup>1</sup> H (δ, mult, J, Hz)	gCOSY	gHMBC	NOESY
β-Ala	1	173.8 (s)			
	2	34.5 (t)	a 2.56 (ddd, 18.5, 5.0, 1.8) b 2.68 (ddd, 18.5, 11.5, 2.1)	2b, 3b 2a, 3a	1, 3 2b, 3a 2a, 3a, 4 2a, 2b, 3b, 4 3a 2b, 3a, 6, 20, 22b, 24
N-Me-Ala	3	33.2 (t)	3.08 (m) 4.05 (m)	2b, 3b, 4 2a, 3a, 4	
	4		8.27 (d, 8.5)	3a, 3b	
N-Me-Ile	5	169.7 (s)			
	6	55.5 (d)	5.16 (q, 6.8)	7	5, 8
	7	15.3 (q)	1.30 (d, 6.9)	6	5, 6
	9	28.1 (q)	2.73 (s)		6, 10
	10	171.1 (s) <sup>b</sup>			
Ile	11	56.8 (d)	5.03 (d, 10.9)	12	9, 10, 12, 14, 18
	12	33.5 (d)	2.06 (m)	11, 13	6, 12, 14a, 14b, 15
	13	16.2 (q)	0.85 (m) <sup>c</sup>	11	11, 13, 14b, 15, 17
	14	25.8 (t)	a 0.98 (m) b 1.43 (m)	14b, 15 14a, 15	11, 12, 14 11
	15	11.1 (q)	0.91 (t, 7.2)	14a, 14b	11, 12, 14b, 17
	17	31.0 (q)	3.20 (s)		11, 12, 14a
	18	173.6 (s)			12, 14b, 19, 20
	19	53.5 (d)	4.83 (dd, 9.2, 6.8)	20, 24	17, 20, 21, 22a, 22b, 24
	20	37.5 (d)	1.92 (m)	19, 21	4, 17, 19, 21, 22b, 24
	21	15.3 (q)	0.84 (m) <sup>c</sup>	20	19, 20
	22	24.6 (t)	a 1.29 (m) b 1.44 (m)	22b, 23 22a, 23	19, 23, 24
	23	11.4 (q)	0.85 (m) <sup>c</sup>	22a, 22b	4, 19, 20, 22b, 23, 24
	24		7.07 (d, 8.8)	19	22a, 22b 4, 19, 20, 22a, 22b, 26, 30b

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Structural unit	<sup>13</sup> C (δ, mult)	<sup>1</sup> H (δ, mult., J, Hz)	gCOSY	gHMBC	NOESY
3-Me-Pro	25	171.0 (s) <sup>b</sup>			
	26	67.1 (d)	27	25, 27, 28, 29	24, 27, 28
	27	36.2 (d)	26, 28, 29b		26, 28, 29a, 29b
	28	19.0 (q)	27	26, 27, 29	26, 27, 29a, 30a
	29	30.9 (t)	29b, 30a, 30b		28, 29b, 30a, 27
		a	27, 29a, 30a, 30b		27, 29a, 30b
		b	29a, 29b, 30b		28, 29a, 30b, 33, 34a
	30	44.9 (t)	29a, 29b, 30b		24, 29b, 30a, 33
		a	29a, 29b, 30a		
		b			
Hica	32	169.9 (s)			
	33	71.9 (d)	34a, 34b		30a, 30b, 34a, 34b, 37
	34	39.3 (t)	34b, 35	32, 35, 36	30a, 33, 34b, 35
		a	34a	32, 35, 36	33, 34a, 35, 36
		b			
	35	24.4 (d)	34a, 36, 37		34a, 34b, 36, 37
	36	23.4 (q)	35	35, 37	34b, 35
	37	21.7 (q)	35	35, 36	33, 35

<sup>a</sup> Measured at 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C).

<sup>b</sup> Assignment may be interchangeable.

<sup>c</sup> Coupling constant could not be measured due to signal overlap.