



## New cytotoxic callipeltins from the Solomon Island marine sponge *Asteropus* sp.



Marc Stierhof<sup>a</sup>, Kine Østnes Hansen<sup>b</sup>, Mukesh Sharma<sup>c</sup>, Klaus Feussner<sup>c</sup>, Karolina Subko<sup>a</sup>, Fernando Fernández Díaz-Rullo<sup>d</sup>, Johan Isaksson<sup>e</sup>, Ignacio Pérez-Victoria<sup>f</sup>, David Clarke<sup>g</sup>, Espen Hansen<sup>b</sup>, Marcel Jaspars<sup>a</sup>, Jioji N. Tabudravu<sup>a,\*</sup>

<sup>a</sup> Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, AB24 3UE, Scotland, UK

<sup>b</sup> Marbio, UiT the Arctic University of Norway, N-9037, Tromsø, Norway

<sup>c</sup> Institute of Applied Sciences, Faculty of Science, Technology and Environment, The University of the South Pacific, P.O. Box 1168, Suva, Fiji

<sup>d</sup> Institute of Medical Sciences, University of Aberdeen, AB24 3UE, Scotland, UK

<sup>e</sup> Department of Chemistry, UiT the Arctic University of Norway, Realfagsbygget, Breivika, 9037, Tromsø, Norway

<sup>f</sup> Fundación MEDINA, Parque Tecnológico de la Salud, Av. Conocimiento 34, 18016, Granada, Spain

<sup>g</sup> School of Chemistry, Joseph Black Building, University of Edinburgh, David Brewster Road, Edinburgh, EH9 3FJ, UK

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### ABSTRACT

Four new callipeltin A derivatives (N–Q) have been isolated from the Solomon Island marine sponge *Asteropus* sp. Their structures were established by spectroscopic techniques followed by acid hydrolysis and derivatisation of the free amino acids, and subsequent LCMS analysis of the derivatives. The compounds were evaluated for their activity against cancer cell lines A2058 (melanoma), HT-29 (colorectal adenocarcinoma) and MCF-7 (breast adenocarcinoma) and non-malignant MRC-5 fibroblast cells. While the acyclic callipeltins P and Q were inactive the cyclic callipeltins N and O showed significant cytotoxicity against all exposed cell lines with IC<sub>50</sub> values as low as 0.16 μM confirming the role of cyclic configuration in biological activity.

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## 1. Introduction

Marine invertebrate derived cyclic peptides continue to attract considerable attention due to their strong anticancer and antiviral activities.<sup>1,2</sup> For example, aplidine (dehydrodidemnim B) derived from the ascidian *Aplidium albicans*, is currently in clinical trials phase III for multiple myeloma and in phase II trials for T-cell lymphomas.<sup>3</sup> A group of related sponge derived cyclic peptides such as the callipeltins<sup>4</sup> from *Latrunculia* sp. the neamphamides<sup>5</sup> from *Neamphius huxleyi*, homophymines<sup>6</sup> from *Homophymia* sp., papuamides and theopapuamides<sup>7,8</sup> from *Theonella mirabilis* and *T. swinhoei*, respectively, contain unusual non-proteinogenic units such as (2S,3S,4R)-3,4-dimethylglutamine (3,4-diMeGln), (2S,3R)-β-methoxytyrosine (β-OMeTyr), (2R,3R,4S)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHA) and (2R,3R,4R)-3-hydroxy-

2,4,6-trimethylheptanoic acid (HTMHA) suggesting a common biosynthetic origin.

A sponge collected from a reef in the central province of the Solomon Islands in 2006 was identified as *Asteropus* sp. A methanolic extract of this sponge showed strong inhibition against amphotericin B resistant (ATCC90873) and wild type *Candida albicans* (ATCC32354). A bioassay-guided isolation of this extract resulted in the isolation of cyclic callipeltins N and O as the main components. Also isolated in smaller quantities were their linear forms named callipeltin P and Q, respectively.

The effects of callipeltin N–Q on in vitro survival of human cancer cell lines A2058 (melanoma), HT-29 (colorectal adenocarcinoma) and MCF-7 (breast adenocarcinoma), as well as the non-malignant human fibroblast cell line MRC-5 were evaluated.

## 2. Results and discussion

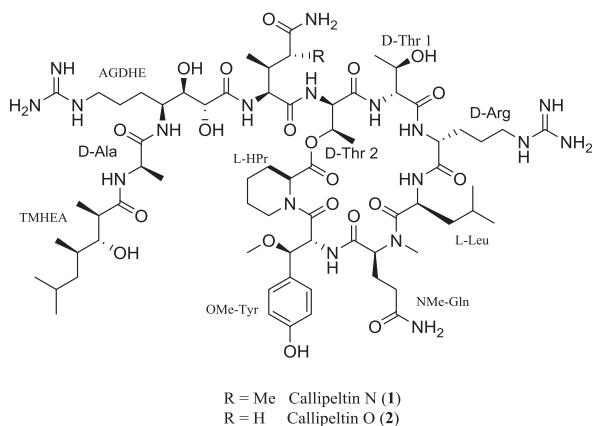
The frozen sponge sample was thawed and extracted three times with MeOH followed by dichloromethane. The combined

\* Corresponding author. Fax: +44 1224 272921; e-mail address: [j.tabudravu@abdn.ac.uk](mailto:j.tabudravu@abdn.ac.uk) (J.N. Tabudravu).

crude extract (4.2 g) was partitioned using a modified Kupchan method<sup>9,10</sup> followed by reversed phase HPLC to yield callipeltins N–Q. Callipeltin N (**1**) was obtained as a white amorphous solid and showed the doubly charged molecular ion at  $m/z$  766.4563  $[M+2H]^{2+}$  in the HRESIMS spectrum corresponding to the molecular formula  $C_{70}H_{120}N_{18}O_{20}$  ( $\Delta+2.5$  ppm). Extensive analysis of 2D NMR data of COSY, Edited-HSQC, HSQCTOCSY, HMBC and by comparison of chemical shifts with those of callipeltin A<sup>1</sup> revealed one residue each of alanine (Ala),  $\beta$ -methoxytyrosine ( $\beta$ -MeOTyr), 3,4-dimethylglutamine (3,4-diMeGln), *N*-methylglutamine (NMeGln), leucine (Leu), arginine (Arg), threonine (Thr), 4-amino-7-guanidino-2,3-dihydroxy heptanoic acid (AGDHE), and 3-hydroxy-2,4,6-trimethylheptanoic acid (TMHEA). There was however one amino acid that was different from components of known callipeltins. This was the amino acid homoproline (Hpr). Identification of homoproline was carried out using HSQCTOCSY correlations between the  $\alpha$ -carbon at 51.8 ppm and the methylene protons at 2.13/1.58, 1.66/1.50, 1.64/1.47 and 3.78/2.82 ppm, respectively, indicating that the carbons and associated protons were part of the same spin system. The deshielded methylene group at  $\delta_H$  3.78/2.82 ppm and  $\delta_C$  43.2 ppm suggest that this carbon is attached to a nitrogen atom to form a ring thus accounting for the correct number of double bond equivalents in the molecule. Even though homoproline has been reported here for the callipeltins for the first time it has been known to be part of the structurally related neamphamide,<sup>5</sup> homophymine<sup>6</sup> and papuamide.<sup>7</sup>

The sequence of amino acids in callipeltin N (**1**) was determined by HMBC correlations (Fig. 1). It was consistent with those found in callipeltin A<sup>1</sup> with the exception of *N*-methylalanine which has been replaced by homoproline in the structure of callipeltin N (**1**).

The absolute stereochemistry of *D*-alanine, *D*-arginine, *D*-threonine, *L*-homoproline and *L*-leucine in callipeltin N (**1**) was determined by complete acid hydrolysis of **1** and Marfey's derivatisation of the resulting amino acids using 1-fluoro-2,4-dinitrophenyl-5-*L*-leucinamide (FDLA)<sup>11</sup> and the comparison of the LCMS data of the derivatives of **1** with the FDLA derivatives of corresponding standards. The configurations of the remaining stereocenters have not been determined and are assumed to be the same as in callipeltin A based on similarities of <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts.<sup>1</sup>



Callipeltin O (**2**) was obtained as a white amorphous solid and showed the doubly charged molecular ion at  $m/z$  759.4392  $[M+2H]^{2+}$  ( $\Delta+1.7$  ppm) in the HRESIMS spectrum corresponding to the molecular formula  $C_{69}H_{118}N_{18}O_{20}$ . The primary structure of callipeltin O (**2**) was determined by extensive analysis of 2D NMR data and was consistent with that of callipeltin N except for the presence of 3-methylglutamine replacing 3,4-dimethylglutamine in callipeltin N (**1**) (Fig. 2).

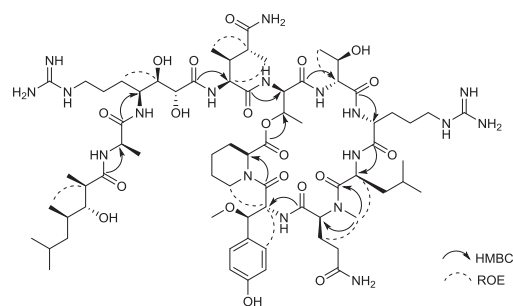


Fig. 1. Selected HMBC and some observed ROE correlations in CD<sub>3</sub>OD for callipeltin N (**1**).

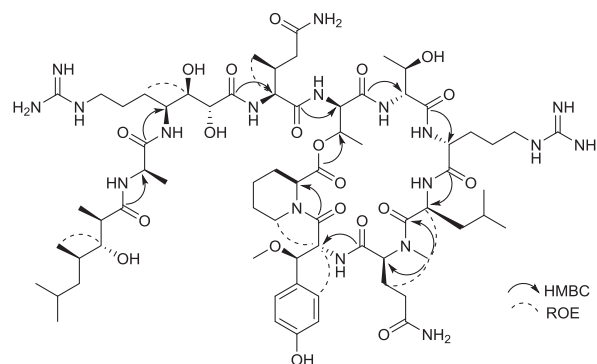
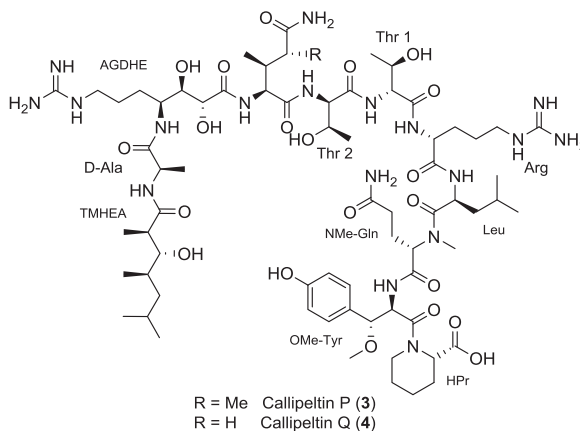


Fig. 2. Selected HMBC and some observed ROE correlations in CD<sub>3</sub>OD for callipeltin O (**2**).

The stereochemistry of the amino acids was determined to be *D*-alanine, *D*-arginine, *L*-homoproline, *D*-threonine, and *L*-leucine by Marfey's method.<sup>11</sup> The configurations of the remaining stereocenters of callipeltin O have not been determined and all except 3-MeGln are assumed to be the same as in callipeltin A based on similarities of <sup>13</sup>C NMR chemical shifts.<sup>1</sup>



Callipeltin P (**3**) was obtained as a white amorphous solid showing the doubly charged molecular ion at  $m/z$  775.4505  $[M+2H]^{2+}$  ( $\Delta-0.72$  ppm) in the HRESIMS spectrum corresponding to the molecular formula  $C_{70}H_{122}O_{21}N_{18}$ . The molecular formula indicated an addition of 18 units to the mass of callipeltin N (**1**). It also indicated an unsaturation number one less than that of callipeltin N suggesting that callipeltin P (**3**) was the linear form of callipeltin N (**1**). The structure was fully supported by both 1D and

**Table 1**  
<sup>1</sup>H and <sup>13</sup>C NMR data (CD<sub>3</sub>OD) for compounds **1**, **2**, **3** and **4**

C-pos	<b>1</b>		C-pos/position	<b>2</b>		C-pos	<b>3</b>		C-pos	<b>4</b>	
	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$		$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$		$\delta_{\text{H}}^{\text{c}}$	$\delta_{\text{C}}^{\text{d}}$		$\delta_{\text{H}}^{\text{c}}$	$\delta_{\text{C}}^{\text{d}}$
<b>HPr</b>			<b>HPr</b>			<b>HPr</b>			<b>HPr</b>		
$\alpha$	5.25 d (4.5)	51.8	$\alpha$	5.24 d (5.7)	51.9	$\alpha$	5.27 ovl.	52.2	$\alpha$	5.29 ovl.	52.2
$\beta$	2.13 bm, 1.58 ovl.	25.1	$\beta$	2.13 d (13.4), 1.58 ovl.	25.1	$\beta$	2.34 d (13.8), 1.67 ovl.	26.2	$\beta$	2.33 d (13.8), 1.67 ovl.	26.4
$\gamma$	1.66 ovl., 1.50 ovl.	19.9	$\gamma$	1.67 ovl., 1.46 ovl.	19.9	$\gamma$	1.77 ovl., 1.43 d (4.0)	20.5	$\gamma$	1.76 ovl., 1.44 d (4.0)	20.6
$\delta$	1.64 ovl., 1.47 d (8.1)	24.9	$\delta$	1.65 ovl., 1.46 ovl.	24.9	$\delta$	1.74 ovl., 1.53 d (12.8)	24.8	$\delta$	1.72 ovl., 1.53 d (12.8)	24.8
$\epsilon$	3.78 d (12.9), 2.82 m	43.2	$\epsilon$	3.81 d (12.2), 2.87 m	43.3	$\epsilon$	4.12 ovl., 3.27 ovl.	43.7	$\epsilon$	4.11 ovl., 3.26 ovl.	43.8
CO		169.7	CO		169.7	COOH		172.3	COOH		172.2
<b><math>\beta</math>-MeOTyr</b>			<b><math>\beta</math>-MeOTyr</b>			<b><math>\beta</math>-MeOTyr</b>			<b><math>\beta</math>-MeOTyr</b>		
$\alpha$	4.95 d (9.3)	51.8	$\alpha$	4.99 d (9.5)	51.8	$\alpha$	5.27 ovl.	52.4	$\alpha$	5.29 ovl.	52.5
$\beta$	4.48 d (9.4)	82.7	$\beta$	4.48 d (9.4)	82.7	$\beta$	4.33 ovl.	83.7	$\beta$	4.33 ovl.	83.7
C4		128.1	C4		128.1	C4		128	C4		127.9
C5/C9	7.21 d (8.4)	129.5	C5/C9	7.22 d (8.3)	129.5	C5/C9	7.21 d (8.4)	129.1	C5/C9	7.20 d (8.4)	129.1
C6/C8	6.76 d (8.3)	114.7	C6/C8	6.78 d (8.5)	114.5	C6/C8	6.80 d (8.2)	114.8	C6/C8	6.79 d (8.2)	114.8
C7		157.2	C7		157.2	C7		157.5	C7		158.0
OH	n.a.		OH	n.a.		OH	n.a.		OH	n.a.	
OMe	3.13 s	55.57	OMe	3.13 s	55.6	OMe	3.17 s	55.7	OMe	3.17 s	55.7
CONH	8.37 d (10.0)	169.7	CONH	8.33 d (10.1)	170.0	CONH	7.63 d (9.8)	170.6	CONH	7.63 d (9.8)	170.6
<b>N-MeGln</b>			<b>N-MeGln</b>			<b>N-MeGln</b>			<b>N-MeGln</b>		
$\alpha$	4.81 dd (9.4, 6.1)	55.1	$\alpha$	4.80 dd (8.9, 6.5)	55.3	$\alpha$	4.94 m,	55.9	$\alpha$	4.93 m	55.9
$\beta$	1.58 m, 1.46 d (7.3)	23.9	$\beta$	1.59 ovl., 1.51 ovl.	24.0	$\beta$	1.94 ovl., 1.69 ovl.	23.3	$\beta$	1.92 ovl., 1.70 ovl.	23.3
$\gamma$	1.81 ovl., 1.63 ovl.	30.9	$\gamma$	1.80 ovl., 1.63 ovl.	31.0	$\gamma$	1.98 m	31	$\gamma$	1.97 m	31.1
NMe <sub>3</sub>	2.97 s	29.2	NMe <sub>3</sub>	2.98 s	29.3	NMe <sub>3</sub>	2.82 s	29.7	NMe <sub>3</sub>	2.81 s	29.9
CONH	n.a.	169.8	CONH	n.a.	169.9	CONH	n.a.	169.2	CONH	n.a.	169.2
CONH <sub>2</sub>	n.a.	175.9	CONH <sub>2</sub>	n.a.	175.9	CONH <sub>2</sub>	n.a.	176.1	CONH <sub>2</sub>	n.a.	176.2
<b>Leu</b>			<b>Leu</b>			<b>Leu</b>			<b>Leu</b>		
$\alpha$	4.59 m	49.5	$\alpha$	4.60 m	49.4	$\alpha$	4.73 d (9.6)	48.5	$\alpha$	4.72 d (9.6)	48.7
$\beta$	1.72 ovl., 1.29 d (6.7)	38.5	$\beta$	1.75 ovl., 1.29 d (6.6)	38.5	$\beta$	1.70 ovl., 1.44 d (7.4)	39.2	$\beta$	1.69 ovl., 1.43 d (7.4)	39.2
$\gamma$	1.72 ovl.	24.7	$\gamma$	1.73 ovl.	24.7	$\gamma$	1.69 ovl.	24.5	$\gamma$	1.69 ovl.	24.6
Me- $\delta$	0.94 d (6.7)	22.2	Me- $\delta$	0.95 d (6.5)	22.2	Me- $\delta$	0.96 d (6.6)	22.4	Me- $\delta$	0.96 d (6.7)	22.3
Me- $\epsilon$	0.90 d (6.4)	19.9	Me- $\epsilon$	0.91 d (6.4)	19.9	Me- $\epsilon$	0.94 d (6.4)	20.1	Me- $\epsilon$	0.93 d (6.5)	20.2
CONH	7.28 d (7.6)	173.3	CONH	7.28 d (7.8)	173.3	CONH	8.00 d (8.4)	174.1	CONH	8.00 d (7.8)	174.2
<b>Arg</b>			<b>Arg</b>			<b>Arg</b>			<b>Arg</b>		
$\alpha$	4.36 ovl.	51.9	$\alpha$	4.35 ovl.	52.0	$\alpha$	4.37 d (4.8)	52.7	$\alpha$	4.37 d (4.8)	52.7
$\beta$	1.91 m, 1.63 ovl.	26.8	$\beta$	1.94 m, 1.65 ovl.	26.8	$\beta$	1.93 ovl., 1.71 ovl.	28	$\beta$	1.93 ovl., 1.65 ovl.	28.1
$\gamma$	1.63 ovl.	24.9	$\gamma$	1.58 ovl.	24.8	$\gamma$	1.72 ovl.	25	$\gamma$	1.69 ovl.	25.0
$\delta$	3.14 ovl.	40.6	$\delta$	3.15 ovl.	40.6	$\delta$	3.20 ovl.	40.4	$\delta$	3.2 ovl.	40.4
Guan	7.32 t (5.7)	157.2	Guan	7.31 t (5.8)	157.1	Guan	7.43 ovl.	157.2	Guan	7.43 ovl.	157.2
CONH	7.76 ovl.	172.6	CONH	7.84 d (8.4)	172.5	CONH	8.22 d (7.2)	172.4	CONH	8.23 d (7.4)	172.3
<b>Thr-1</b>			<b>Thr-1</b>			<b>Thr-1</b>			<b>Thr-1</b>		
$\alpha$	3.90 ovl.	62.3	$\alpha$	3.94 ovl.	62.2	$\alpha$	4.24 d (6.4)	59.8	$\alpha$	4.23 d (6.5)	59.8
$\beta$	4.31 ovl.	65.5	$\beta$	4.29 ovl.	65.6	$\beta$	4.09 d (7.4)	67.1	$\beta$	4.09 d (7.4)	67.1
$\gamma$	1.27 d (6.2)	18.8	$\gamma$	1.28 d (6.7)	18.9	$\gamma$	1.31 d (5.9)	19.1	$\gamma$	1.31 d (5.1)	19.2
OH	n.a.		OH	n.a.		OH	n.a.		OH	n.a.	
CONH	8.32 bs	170.6	CONH	8.21 d (4.4)	170.7	CONH	8.22 d (7.2)	171.3	CONH	8.23 d (7.4)	171.5
<b>Thr-2</b>			<b>Thr-2</b>			<b>Thr-2</b>			<b>Thr-2</b>		
$\alpha$	5.41 m	55.2	$\alpha$	5.36 d (8.9)	55.3	$\alpha$	4.38 d (6.3)	59	$\alpha$	4.38 d (6.3)	59.1
$\beta$	5.57 m	70.9	$\beta$	5.58 m	70.8	$\beta$	4.13 ovl.	67	$\beta$	4.13 ovl.	67.1
$\gamma$	1.2 ovl.	13.8	$\gamma$	1.22 d (4.3)	13.3	$\gamma$	1.27 d (6.4)	19	$\gamma$	1.27 d (6.4)	19.0
CONH	8.84 d (9.9)	171.8	CONH	8.76 d (9.7)	171.6	CONH	n.a.	171.7	CONH	n.a.	
<b>3,4-diMeGln</b>			<b>3-MeGln</b>			<b>3,4-diMeGln</b>			<b>3-MeGln</b>		
$\alpha$	4.18 d (9.7)	57.6	$\alpha$	4.22 m	59.3	$\alpha$	4.44 d (4.3)	55.6	$\alpha$	4.44 d (4.3)	57.3
$\beta$	2.39 m	36.4	$\beta$	2.59 ovl.	31.9	$\beta$	2.23 m	38.2	$\beta$	2.55 m	32.9
$\beta$ -Me	1.21 ovl.	14.1	$\beta$ -Me	1.21 d (4.5)	16.2	$\beta$ -Me	1.03 d (7.2)	12.4	$\beta$ -Me	1.06 d (6.9)	15.9
$\gamma$	2.75 m	41.9	$\gamma$	2.52 dd (14.4, 5.4) 2.31 dd (14.4, 7.4)	38.8	$\gamma$	2.67 ovl.	40.6	$\gamma$	2.40 dd (14.4, 5.8) 2.20 m	38.1
$\gamma$ -Me	1.27 d (6.7)	13.2	$\gamma$ -Me	n.a.		$\gamma$ -Me	1.20 d (7.3)	14.4	$\gamma$ -Me	n.a.	
CONH <sub>2</sub>	n.a.	179.4	CONH <sub>2</sub>	n.a.	176.2	CONH <sub>2</sub>		178.6	CONH <sub>2</sub>	8.36 s	176.1
CONH	9.36 d (3.0)	172.3	CONH	8.81 d (5.7)	172.1	CONH	8.66 d (8.2)	172.1	CONH	n.a.	171.9
<b>AGDHE</b>			<b>AGDHE</b>			<b>AGDHE</b>			<b>AGDHE</b>		
$\alpha$	3.91 d ovl.	71.2	$\alpha$	3.93 ovl.	71.3	$\alpha$	4.00 d (7.7)	71.5	$\alpha$	4.00 d (7.4)	71.5
$\beta$	3.61 dd (9.0, 1.8)	73.9	$\beta$	3.62 dd (8.6, 2.0)	73.7	$\beta$	3.72 dd (7.5, 2.9)	74	$\beta$	3.71 d (7.5)	74.0
$\gamma$	4.17 ovl.	49.5	$\gamma$	4.18 m	49.5	$\gamma$	4.16 ovl.	49.5	$\gamma$	4.15 ovl.	49.6
$\delta$	1.71 ovl., 1.57 ovl.	28.5	$\delta$	1.70 ovl., 1.58 ovl.	28.5	$\delta$	1.66 ovl.	28.3	$\delta$	1.65 ovl.	28.3
$\epsilon$	1.58 ovl.	25.2	$\epsilon$	1.59 ovl.	25.1	$\epsilon$	1.63 ovl.	25.1	$\epsilon$	1.63 ovl.	25.0

(continued on next page)

Table 1 (continued)

C-pos	1		C-pos/position	2		C-pos	3		C-pos	4	
	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$		$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$		$\delta_{\text{H}}^{\text{c}}$	$\delta_{\text{C}}^{\text{d}}$		$\delta_{\text{H}}^{\text{c}}$	$\delta_{\text{C}}^{\text{d}}$
$\zeta$	3.20 ovl.	40.8	$\zeta$	3.19 ovl.	40.8	$\zeta$	3.20 ovl.	40.6	$\zeta$	3.20 ovl.	40.8
$\gamma$ -NH	7.74 ovl.		$\gamma$ -NH	7.68 d (9.4)		$\gamma$ -NH	7.77 d (9.8)		$\gamma$ -NH	7.74 d (9.6)	
Guan	7.43 t (5.6)	157.2	Guan	7.38 t (5.7)	157.2	Guan	7.43 ovl.	157.2	Guan	7.43 ovl.	157.2
CO	n.a.	174.9	CO		175.1	CO	n.a.	174.5	CO	n.a.	174.4
<b>Ala</b>			<b>Ala</b>			<b>Ala</b>			<b>Ala</b>		
$\alpha$	4.31 ovl.	49.8	$\alpha$	4.30 ovl.	49.8	$\alpha$	4.33 ovl.	49.6	$\alpha$	4.32 ovl.	49.8
$\beta$	1.42 d (7.3)	16.6	$\beta$	1.41 d (7.2)	16.5	$\beta$	1.43 d (7.4)	16.3	$\beta$	1.42 d (7.4)	16.4
CONH	8.25 d (6.3)	174.9	CONH	8.23 d (6.8)	174.8	CONH	8.27 d (7.0)	174.6	CONH	8.27 d (7.0)	174.6
<b>TMHEA</b>			<b>TMHEA</b>			<b>TMHEA</b>			<b>TMHEA</b>		
1	n.a.	177.4	1	n.a.	177.5	1	n.a.	177.6	1	n.a.	177.5
2	2.60 m	43.5	2	2.60 ovl.	43.5	2	2.64 m	43.4	2	2.63 m	43.4
3	3.47 dd (8.8, 3.1)	78.2	3	3.47 dd (8.7, 3.2)	78.3	3	3.51 dd (8.5, 2.2)	78.3	3	3.51 d (8.8)	78.4
4	1.73 ovl.	32.3	4	1.73 ovl.	32.4	4	1.77 ovl.	32.3	4	1.76 ovl.	32.4
5	1.16 m	38.2	5	1.17 m	38.2	5	1.21 m	38.1	5	1.20 m	38.2
6	1.61 ovl.	24.9	6	1.58 ovl.	25.0	6	1.63 ovl.	25	6	1.66 ovl.	25.0
7	0.92 d (6.6)	23.4	7	0.93 d (6.5)	23.4	7	0.96 d (6.6)	23.4	7	0.96 d (6.7)	23.4
8	1.07 d (6.9)	13.3	8	1.07 d (6.9)	13.3	8	1.09 d (7.1)	13.1	8	1.09 d (7.1)	13.3
9	0.96 d (7.0)	16.1	9	0.97 d (6.9)	16.1	9	1.01 d (7.0)	16	9	1.00 d (7.0)	16.1
10	0.85 d (6.5)	20.2	10	0.86 d (6.5)	20.3	10	0.90 d (6.6)	20.2	10	0.89 d (6.6)	20.3

<sup>1</sup>H and <sup>13</sup>C assignments aided by HSQC, COSY, HMBC and HSQCTOCSY experiments.

Coupling constants are in brackets and given in Hz. Overlap (ovl.); Not available (n.a.).

<sup>a</sup> 600 MHz.

<sup>b</sup> 150 MHz.

<sup>c</sup> 500 MHz.

<sup>d</sup> 125 MHz.

2D NMR data (Table 1, Fig. S25) and MS/MS fragmentation data (Fig. S18). The stereochemistry of each of the amino acid has not been determined and is assumed to be the same as in callipeltin N (1). Callipeltin C the acyclic version of callipeltin A was found to have the same amino acid configurations as callipeltin A.<sup>2</sup>

Callipeltin Q (4) was obtained as a white amorphous solid showing the doubly charged molecular ion at  $m/z$  768.4437 [M+2H]<sup>2+</sup> ( $\Delta$  0.66 ppm) in the HRESIMS spectrum corresponding to the molecular formula C<sub>69</sub>H<sub>120</sub>O<sub>21</sub>N<sub>18</sub>. The molecular formula indicated an addition of 18 units to the mass of callipeltin O (2), with an unsaturation number one less suggesting that callipeltin Q (4) was the linear form of callipeltin O (2). The structure was supported by both 1D and 2D NMR data (Table 1, Fig. S36) and MS/MS fragmentation data (Fig. S29). The stereochemistry of each of the amino acids has not been determined and is assumed to be the same as in callipeltin O (2).

The compounds were evaluated for their antiproliferative activity against A2058 (melanoma), HT-29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma) and human lung fibroblast MRC-5 cell lines. The cyclic compounds, 1 and 2, showed antiproliferative activity against all cell lines, while their linear forms, 3 and 4, had no apparent effect on the cells up to a concentration of 20  $\mu$ M (Table 2). This clearly suggests that peptide cyclisation is essential for the observed activity. With IC<sub>50</sub> values of 0.17, 0.19, 0.21 and 0.16  $\mu$ M against A2058, HT-29, MCF-7 and MRC-5,

Table 2

Cytotoxicity data of callipeltin N–Q (1–4) towards normal cell line MRC-5 and cancer cell lines A2058, HT-29 and MCF-7. Values are means  $\pm$ SD based on 3 independent determinations

Compound	IC50 ( $\mu$ M)			
	A2058	HT-29	MCF-7	MRC-5
1	0.17 $\pm$ 0.04	0.19 $\pm$ 0.05	0.21 $\pm$ 0.21	0.16 $\pm$ 0.14
2	0.67 $\pm$ 0.03	2.08 $\pm$ 0.08	0.48 $\pm$ 0.08	1.37 $\pm$ 0.23
3	n.a.	n.a.	n.a.	n.a.
4	n.a.	n.a.	n.a.	n.a.

n.a.=not active at 20  $\mu$ M.

respectively, compound 1 showed a 2 to 11-fold higher cytotoxic potency than 2, which displayed IC<sub>50</sub> values of 0.67, 2.08, 0.48 and 1.37  $\mu$ M against the same cell lines. No distinction could be seen for the cytotoxic potency of compound 1 and 2 between the non-malignant and the malignant cell lines indicating high cellular non-specificity.

In summary, we have discovered four new depsipeptides callipeltins N–Q that add to the class first exemplified by callipeltin A. Callipeltins N and O showed cytotoxic activities similar to those observed for other related macrocyclic depsipeptides such as callipeltins A and B<sup>2</sup>, homophyamine,<sup>6</sup> theopapuamide,<sup>8a</sup> neamphamide A<sup>5</sup>, pipecolidepsin A<sup>12</sup> underlying the importance of macrocyclisation as well amino acid composition in biological activity. Of the three cell lines that were tested the results showed that callipeltin N containing the 3,4-dimethylGln was on average six times more cytotoxic than callipeltin O containing the 3-methylGln suggesting the significance of that position to bioactivity. The strong antifungal activities observed in the crude extracts may have been attributed to the presence of macrocyclic callipeltins (N and O) and linear callipeltins (P and Q) as both macrocyclic and linear callipeltins are known for their antifungal activities.<sup>14</sup> The compound map<sup>13</sup> (Fig. S38) shows the close distribution of the depsipeptides that share a high degree of structural homology to the callipeltins where differences are due to different amino acids introduced at selected positions. The variability could be explained by the presence of various isoforms of the bacterial NRPS enzymes or alternatively enzymes that have broad substrate specificity<sup>14</sup> allowing the introduction of new amino acids into the peptidic frame work in response to selection pressure.

### 3. Experimental section

#### 3.1. General experimental procedures

NMR data, both 1D and 2D were recorded on a Varian Unity Inova spectrometer at 600 and 150 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively, using an ID probe. High resolution mass spectrometry data were recorded using a Waters LCT Premier coupled to a Waters

Acquity UPLC system and a ThermoScientific LTQXL-Discovery Orbitrap coupled to an Accela HPLC system. Optical rotation measurements were recorded using a Perkin Elmer, Model 343 Polarimeter at 589 nm. Semi-preparative HPLC purifications were performed on an Agilent 1100 HPLC system consisting of a binary pump, degasser, DAD and a preparative fraction collector. All solvents used were of HPLC grade.

### 3.2. Collection

The sponge *Asteropus* sp. was collected by hand using SCUBA at a depth of 7–15 m from Mary Island, Russell group, Solomon Islands (9.02°S, 158.74°E) in June 2006 during a sample collection expedition in the central province of the Solomon Islands. The sponge (greenish exterior and light brown inside) was spongy and soft to touch and had a porous interior. Oscules were very small (0.5 mm). It was identified by Dr J. Hooper, Queensland Museum, Australia. A voucher specimen (SI06-5s) has been preserved at the Marine Reference Collection, The University of the South Pacific, Suva, Fiji Islands.

### 3.3. Extraction and isolation

150 g frozen sample was thawed and extracted three times with MeOH followed by dichloromethane. The combined crude extract (4.2 g) was partitioned using a modified Kupchan method.<sup>9,10</sup> The antifungal activities were found to be in the dichloromethane fraction. Further purification of this fraction was performed using a semi-preparatory C18 HPLC column (Ace 5 C18-HL, 50×10 mm) using water–acetonitrile (with 0.05% TFA) as solvent and a solvent gradient system from 0 to 100% acetonitrile in 30 min to afford **1** (38 mg), **2** (7.7 mg), **3** (2.9 mg) and **4** (2.8 mg).

**3.3.1. Callipeltin N (1).** White amorphous solid, 38.5 mg;  $[\alpha]_D^{20} -17.2^\circ$  (c 0.0015 M, methanol) IR (MeOH,  $\text{cm}^{-1}$ ) 3338, 3213, 2959, 2936, 2871, 1657, 1532, 1520, 1452, 1384, 1201, 1186, 1137, 837, 803, 719. <sup>1</sup>H and <sup>13</sup>C NMR data in CD<sub>3</sub>OD are given in Table 1. HRESIMS  $m/z$  766.4563  $[M+2H]^{2+}$   $\Delta +2.5$  ppm from calculated for C<sub>70</sub>H<sub>120</sub>O<sub>20</sub>N<sub>18</sub>.

**3.3.2. Callipeltin O (2).** White amorphous solid, 7.7 mg;  $[\alpha]_D^{20} -11.2^\circ$  (c 0.00023 M, methanol); IR (MeOH,  $\text{cm}^{-1}$ ) 3338, 3213, 2959, 2936, 2871, 1657, 1532, 1520, 1452, 1384, 1201, 1186, 1137, 837, 803, 719. <sup>1</sup>H and <sup>13</sup>C NMR data in CD<sub>3</sub>OD are given in Table 1. HRESIMS  $m/z$  759.4385  $[M+2H]^{2+}$   $\Delta 1.7$  ppm from calculated for C<sub>69</sub>H<sub>118</sub>O<sub>20</sub>N<sub>18</sub>.

**3.3.3. Callipeltin P (3).** White amorphous solid, 2.9 mg;  $[\alpha]_D^{20.0} -14.8^\circ$  (c 0.000048 M, methanol); IR (MeOH,  $\text{cm}^{-1}$ ) 3338, 3213, 2959, 2936, 2871, 1657, 1532, 1520, 1452, 1384, 1201, 1186, 1137, 837, 803, 719. <sup>1</sup>H NMR (MeOD- $\delta_4$ ) are given in Table 1.

HRESIMS  $m/z$  775.4505  $[M+2H]^{2+}$   $\Delta -0.72$  ppm from calculated for C<sub>70</sub>H<sub>122</sub>O<sub>21</sub>N<sub>18</sub>.

**3.3.4. Callipeltin Q (4).** White amorphous solid, 2.8 mg;  $[\alpha]_D^{20.4} -20.0$  (c 0.000016 M, methanol) IR (MeOH,  $\text{cm}^{-1}$ ) 3338, 3213, 2959, 2936, 2871, 1657, 1532, 1520, 1452, 1384, 1201, 1186, 1137, 837, 803, 719. <sup>1</sup>H NMR (CD<sub>3</sub>OD) are given in Table 1. HRESIMS  $m/z$  768.4437  $[M+2H]^{2+}$   $\Delta 0.66$  ppm from calculated for C<sub>69</sub>H<sub>120</sub>O<sub>21</sub>N<sub>18</sub>.

### 3.4. Determination of absolute stereochemistry

**3.4.1. General procedure for peptide hydrolysis.** To determine the absolute stereochemistry of callipeltins N and O, 0.2 mg of the compounds were hydrolysed by 6N HCl aq at a temperature of 110 °C for 24 h. The reaction mixture was subsequently cooled and dried under nitrogen. To remove the residual HCl, water (0.4 mL) was added and subsequently removed three times and the hydrolysate was dried under N<sub>2</sub> for 24 h. To 50  $\mu\text{L}$  of each of 50 mM aqueous solution of standard amino acids and the hydrolysate, 1N NaHCO<sub>3</sub> (20  $\mu\text{L}$ ) and 1% L-FDLA (1-fluoro-2,4-dinitrophenyl-5-L-leucinamide) (100  $\mu\text{L}$ ) in acetone were added. The reaction mixtures were incubated at 40 °C for 1 h, with frequent mixing. After cooling at room temperature, the reactions were quenched by the addition of 10  $\mu\text{L}$  of 2 N HCl. The samples were diluted by making the volume up to 1 mL with MeOH. The derivatised amino acids were analysed by LCMS (Table 3).

**3.4.2. General procedure for LCMS analysis of Marfey's FDLA derivatives.** 2  $\mu\text{L}$  of each of the derivatised mixture of hydrolysed **1** and **2** and standards were injected into a Waters Acquity Xylene bridged hybrid (BEH) C18 column (2.1×150 mm, 1.7  $\mu\text{m}$ ) using a water–acetonitrile solvent system, starting from 10 to 100% acetonitrile in 4.5 min and flushing the column using 100% acetonitrile with a further 1.5 min before returning to the starting gradient. The eluted compounds were detected by a Waters LCT Premier MS system using the following conditions: Capillary voltage 2600 V, cone voltage 50 V, desolvation temperature 350 °C, desolvation gas 650 L/h, cone gas 5 L/h, mass range 150–1500 amu. The instrument was tuned to a resolution of 10,000 (FWHM) and leucine enkephalin were used as lock mass for internal calibration.

### 3.5. Biological activities

**3.5.1. Antifungal.** The crude extract showed inhibition zones at 20 and 21 mm for amphotericin resistant (ATCC90873) and wild type *Candida albicans* (ATCC32354), respectively, using susceptibility discs (6 mm in diameter) and at a concentration of 2.5 mg crude extract per disc.

**3.5.2. Cell growth inhibitory assay.** The cytotoxicity of compounds **1–4** were evaluated at ranging concentrations against the

**Table 3**  
HPLC retention time in minutes of derivatised callipeltins N (**1**), O (**2**) amino acids and standards

Amino acid		D-HPr	L-HPr	D-Ala	L-Ala	D-Leu	L-Leu	D-Arg	L-Arg	D-Thr	L-Thr
Standard <sup>a</sup>	tR <sup>b</sup>	2.73	2.85	2.63	2.37	3.26	2.77	1.79	1.88	2.39	2.10
	$m/z$	424.18	424.18	384.15	384.15	426.20	426.20	469.21	469.21	414.16	414.16
Callipeltin N	tR	—	2.85	2.63	—	—	2.75	1.82	—	2.28	—
	$m/z$	—	424.18	384.15	—	—	426.20	469.21	—	414.16	—
Callipeltin O	tR	—	2.85	2.63	—	—	2.76	1.82	—	2.28	—
	$m/z$	—	424.18	384.15	—	—	426.20	469.21	—	414.16	—

<sup>a</sup> Amino acid standards.

<sup>b</sup> LCMS retention time.

malignant cell lines A2058 (ATCC CRL-11147TM), HT-29 (ATCC HTB-38) and MCF-7 (ATCC HTB-22) and the non-malignant cell line MRC-5 (ATCC CCL-171) using a previously reported MTS assay. RPMI containing the maximum DMSO concentration used in each assay (0.4%) was used as negative control and Triton<sup>®</sup> X-100 (Sigma Aldrich) as positive control.

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### Supplementary data

Supplementary data (HRESIMS, LCMS/MS, and NMR data of **1**, **2**, **3** and **4** including <sup>1</sup>H, <sup>13</sup>C, COSY, HSQC, HMBC, HSQCTOCSY and ROESY) associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.tet.2016.09.016>.

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