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Stellettapeptins A and B, HIV-inhibitory cyclic depsipeptides from the marine sponge *Stelletta* sp.

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ABSTRACT

Two new HIV-inhibitory depsipeptides, stellettapeptins A (**1**) and B (**2**), were isolated from an extract of the marine sponge *Stelletta* sp., collected from northwestern Australia. Structures of these cyclic nonribosomal peptides were elucidated on the basis of extensive NMR data analysis, and chemical degradation and derivatization studies. Stellettapeptins contain numerous nonproteinogenic amino acid residues and they are the first peptides reported to contain a 3-hydroxy-6,8-dimethylnon-4-(*Z*)-enoic acid moiety. Compounds **1** and **2** potently inhibit infection of human T-lymphoblastoid cells by HIV-1_{RF} with EC_{50} values of 23 and 27 nM, respectively.

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Marine sponges in the genus *Stelletta* have proven to be an extremely rich source of structurally diverse and biologically active natural products.[1–3] Marine sponges frequently contain nonribosomal peptides that have unusual amino acid residues and aliphatic moieties reminiscent of the mixed nonribosomal peptide synthetase (NRPS)–polyketide synthase (PKS) pathways of microorganisms, which suggests the involvement of symbiotic microbes in their production.[4] In the course of our search for bioactive metabolites from marine organisms, we obtained two new cyclic depsipeptides from a sponge of the genus *Stelletta*. We report herein the isolation, structure determination, and HIV-inhibitory properties of stellettapeptins A (**1**) and B (**2**).

Stellettapeptin A (**1**) was isolated as an amorphous solid, which had a molecular formula of $C_{67}H_{108}N_{16}O_{23}$ from analysis of HRESIMS data coupled with ^{1}H and ^{13}C NMR spectral data (Table 1). The presence of a large number of exchangeable amide NH protons (δ_H 6.80–9.98 ppm) and carbonyl resonances (δ_C 170.0–182.4 ppm) in the ^{1}H and ^{13}C NMR spectra of **1** was characteristic of a peptide derivative. Detailed analysis of 2D NMR data enabled us to assign 11 amino acid residues: *N*-methylalanine (*N*MeAla), β-methoxytyrosine (β-*O*MeTyr), *N*-methylglutamine (*N*MeGln), leucine (Leu), glycine (Gly), 3-methoxyalanine (3-*O*MeAla), threonine (Thr), 3,4-dimethylglutamine (3,4-DiMeGln), 2,3-diaminobutanoic acid (Dab), 3-hydroxyglutamine (3-OHGln), and 3-hydroxyasparagine (3-OHAsn) (Table 1). Additionally, a 3-hydroxy-6,8-dimethylnon-4-enoic acid (Hdna) moiety was identified (Fig. 1).

The sequence of amino acid residues in stellettapeptin A (**1**) was deduced from inter-residue NH/CHα ROE interactions, acquired in CD_3OH, and HMBC correlations as shown in Figure 2. The Hdna unit was defined by ^{1}H–^{1}H COSY and heteronuclear correlation NMR data, and its double bond assigned *Z* geometry based on the 10.5 Hz vicinal coupling between the H-4 (δ_H 5.37) and H-5 (δ_H 5.21) olefin protons. The Hdna moiety was linked to the N-terminus of the 3-OHAsn residue by an HMBC correlation from the 3-OHAsn NH (δ_H 8.25) to the carbonyl (δ_C 175.2) of Hdna. This linkage was also supported by ROESY correlations between the 3-OHAsn amide proton and the Hdna H_2-2 (δ_H 2.36, 2.61) methylene protons. The secondary amide NH signal (δ_H 7.88) of 3-OHGln correlated with the C-1 carbonyl (δ_C 171.8) of 3-OHAsn in the HMBC spectrum, which connected these residues, while the 3-OHGln α-methine (δ_H 4.52) showed a ROESY correlation with the amide NH (δ_H 8.75) of Dab. The amino acid sequence was

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Table 1
NMR spectral data for **1** (600 MHz, CD_3OH)

Position	δ_C	δ_H (J in Hz)	HMBC
*N*MeAla			
1	171.4		
2	51.9	5.42 q (7.0)	1, 3, N-Me, $1_{3\text{-OMeTyr}}$
3	13.3	1.28 d (7.0)	1, 2
N-Me	29.5	2.68 s	2, $1_{3\text{-OMeTyr}}$
3-*O*MeTyr			
1	170.0		
2	53.8	4.92[b] dd (10.5, 9.8)	1, 3
3	84.4	4.53[a] d (9.8)	1, 2, 4, 5, 9
3-OMe	56.9	3.10 s	3
4	129.5		
5, 9	131.5	7.17 d (8.3)	3, 6, 7, 8
6, 8	116.2	6.80 d (8.3)	4, 7
7	158.8		
NH		8.23 d (10.5)	2, 1_{NMeGln}
*N*MeGln			
1	170.8		
2	55.8	4.76[a] m	1, 4, N-Me, 1_{Leu}
3	25.1	1.29 m	5
		1.54 m	2, 4, 5
4	32.1	1.61 m	2, 3, 5
		1.69 m	2, 3, 5
5	177.9		
5-NH_2		6.80 br s	4, 5
		7.05 br s	5
N-Me	30.4	2.93 s	2, 1_{Leu}
Leu			
1	174.0		
2	49.4	4.72 m	3, 1_{Gly}
3	40.7	1.23 m	
		1.61 m	5
4	26.2	1.66 m	3
5	21.5	0.90[a] d (6.5)	3, 4, 6
6	23.6	0.95 d (6.5)	3, 4, 5
NH		7.20 d (9.2)	1_{Gly}
Gly			
1	172.3		
2	44.2	3.51 dd (17.0, 5.2)	1, $1_{3\text{-OMeAla}}$
		3.95 dd (17.0, 6.1)	1, $1_{3\text{-OMeAla}}$
NH		9.08 dd (6.1, 5.2)	2, $1_{3\text{-OMeAla}}$
3-OMeAla			
1	172.8[a]		
2	55.4	4.48 q (7.2)	1, 3, 1_{Thr}
3	71.6	3.74 m	1, 2, 3-OMe
		3.79 m	1, 2, 3-OMe
3-OMe	59.5	3.39 s	3
NH		8.47 d (7.2)	2, 3, 1_{Thr}
Thr			
1	172.9[a]		
2	57.4	5.20 dd (10.2, 2.8)	1, 3, 4
3	71.6	5.60 dq (6.6, 2.6)	4, 1_{NMeAla}
4	14.8	1.18 d (6.3)	2, 3
NH		8.93 d (10.2)	2, $1_{3,4\text{-DiMeGln}}$
3,4-DiMeGln			
1	174.1		
2	59.3	4.09 dd (9.3, 2.9)	1, 3, 3-Me, 4
3	36.8	2.47 m	2, 3-Me, 4-Me, 5
3-Me	17.3	1.28 d (6.9)	2, 3, 4
4	44.9	2.72 m	2, 3, 3-Me, 4-Me, 5
4-Me	14.0	1.32 d (7.1)	3, 4, 5
5	182.4		
5-NH_2		7.14 br s	4, 5
		7.87[a] br s	5
NH		9.98 br s	1_{Dab}
Dab			
1	171.6		
2	56.4	4.56 t (6.1)	1, 3, 4, $1_{3\text{-OHGln}}$
3	49.5	3.95 m	
3-NH_2		7.73 2H, br s	
4	16.8	1.43 d (6.8)	2, 3
NH		8.75 d (6.9)	2, 3, $1_{3\text{-OHGln}}$

Table 1 (*continued*)

Position	δ_C	δ_H (*J* in Hz)	HMBC
3-OHGln			
1	172.9[a]		
2	58.7	4.52[a] dd (8.5, 1.3)	1, 3
3	68.7	4.75[a] m	
4	40.6	2.37 m	5
		2.45 m	2, 3, 5
5	176.0		
5-NH_2		6.88 br s	4, 5
		7.54 br s	5
NH		7.88[a] d (8.5)	2, 3, $1_{3\text{-OHAsn}}$
3-OHAsn			
1	171.8		
2	59.5	4.73 dd (6.9, 3.9)	1, 3, 4, 1_{Hdna}
3	72.7	4.61 br d (3.9)	4
4	176.6		
4-NH_2		7.36 br s	3, 4
		7.80 br s	4
NH		8.25 d (6.9)	1_{Hdna}
Hdna			
1	175.2		
2	45.4	2.36 m	1, 3, 4
		2.61 m	1, 3, 4
3	66.6	4.90[b] m	
4	131.0	5.37 dd (10.5, 9.1)	2, 6
5	139.3	5.21 dd (10.5, 5.0)	3, 4, 6, 6-Me, 7
6	31.3	2.63 m	4, 5, 6-Me, 7
6-Me	22.1	0.92[a] d (6.7)	5, 6, 7
7	48.2	1.13 2H, m	5, 6, 6-Me, 8
8	26.9	1.57 m	6, 7, 8-Me
8-Me	23.7	0.87[a] d (6.7)	7, 8
9	23.0	0.88[a] d (6.7)	7, 8

[a] Signals overlapped.
[b] Buried under CD_3OH signal.

further extended by a ROESY between the Dab α-methine (δ_H 4.56) and the secondary amide NH (δ_H 9.98) of 3,4-DiMeGln. An HMBC correlation from the Thr NH (δ_H 8.93) to C-1 (δ_C 174.1) of 3,4-DiMeGln linked these two residues, while an HMBC from 3-OMeAla NH (δ_H 8.47) to the Thr carbonyl (δ_C 172.9) joined these subunits. ROESY interactions observed between the α-methine (δ_H 4.48) of 3-OMeAla and the Gly NH (δ_H 9.08), and between the Gly methylene resonances (δ_H 3.51, 3.95) and the Leu NH (δ_H 7.20) helped define neighboring residues. HMBC correlations between the methyl group (δ_H 2.93) of *N*MeGln and the Leu carbonyl (δ_C 174.0), and between the 3-*O*MeTyr NH (δ_H 8.23) and C-1 (δ_C 170.8) of *N*MeGln linked these residues. An HMBC correlation from the methyl group (δ_H 2.68) of *N*MeAla to the 3-*O*MeTyr carbonyl (δ_C 170.0) completed the amino acid sequence. An ester bond linking *N*MeAla and the Thr residue was evident from the deshielded chemical shift of the Thr oxymethine (δ_H 5.60) and an HMBC correlation from this proton to the *N*MeAla carbonyl (δ_C 171.4). Thus the planar structure of **1** was elucidated.

The absolute configurations of L-*N*MeAla, L-*N*MeGln, L-Leu, D-*allo*-Thr, D-3-*O*MeAla, (2*R*,3*R*)-3-OHGln, and (2*R*,3*S*)-3-OHAsn

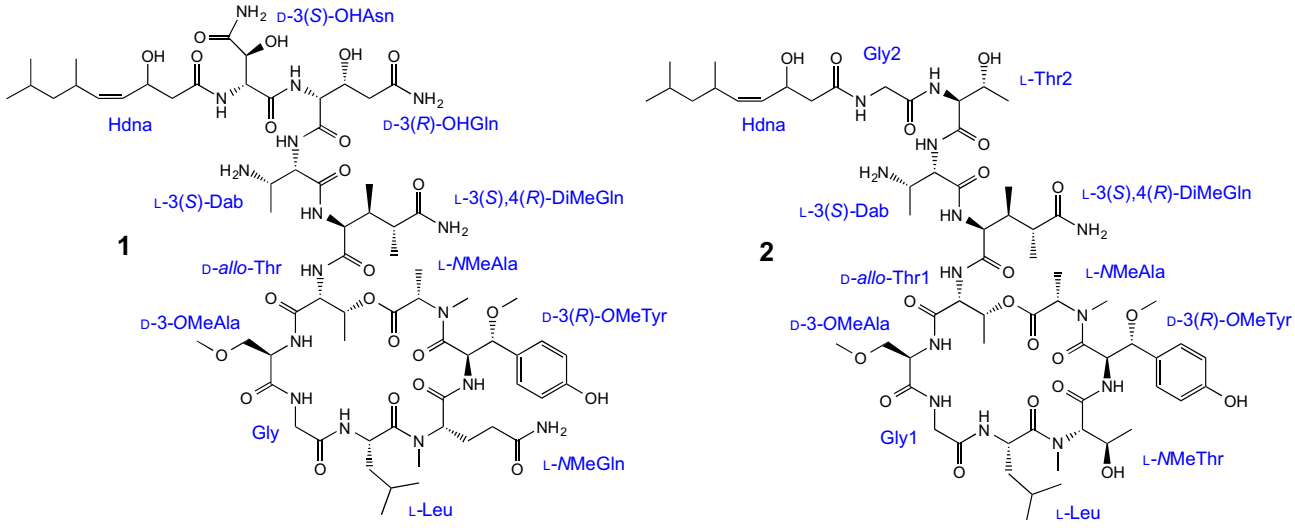

Figure 1. Structures and composition of stellettapeptins A (**1**) and B (**2**).

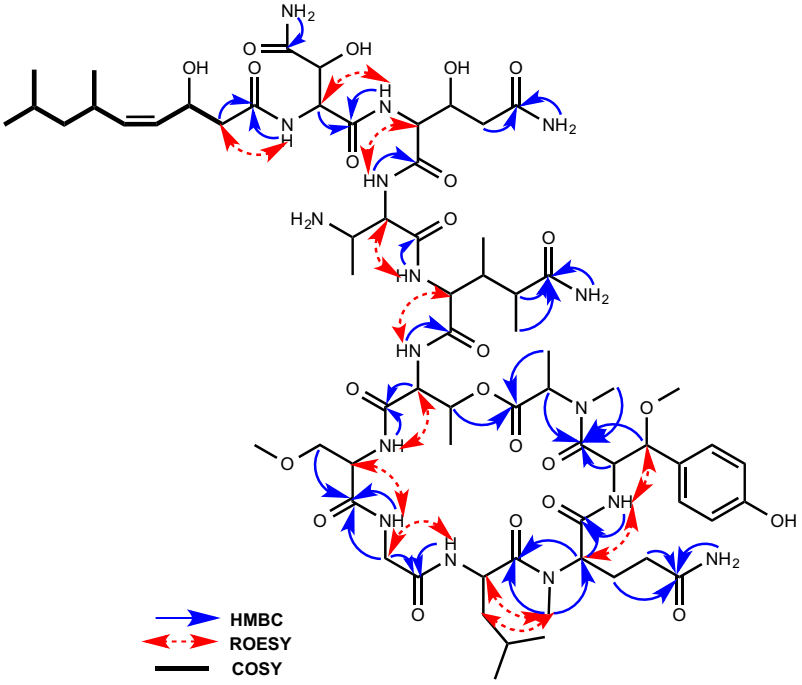

Figure 2. Key HMBC, ROESY, and COSY correlations for **1**.

were determined by LC–MS analysis of the acid hydrolysate derivatized with Marfey's reagent and comparison with appropriate amino acid standards. Both the L- and D-FDLA derivatives were prepared and analyzed for amino acids with multiple chiral centers for which a complete set of standards was not available.[5] Due to decomposition of β-*O*MeTyr during acid hydrolysis of **1**, this residue was converted to β-*O*MeAsp via ozonolysis prior to acid hydrolysis and derivatization, as described by Zampella et al.[6] Samples of **1**, callipeltin A,[7] and papuamide B[8] were ozonized and hydrolyzed, and then subjected to Marfey's analysis. Both the L- and D-FDLA derivatives were prepared and ion selective monitoring for β-*O*MeAsp [*m*/*z* 458, $(M+H)^+$] of the hydrolysates from all three peptides showed the same retention times (Supplementary data) corresponding to (2*R*,3*S*)-3-*O*MeAsp. Thus the configuration of the β-*O*MeTyr residue in **1** was assigned as 2*R*,3*R*.[6] The coupling constant (9.8 Hz) between H-2 (δ_H 4.92) and H-3 (δ_H 4.53) of this residue was in good agreement with those of callipeltin A and papuamide B, which also have a (2*R*,3*R*)-3-*O*MeTyr, while neamphamide[9] contains (2*S*,3*R*)-β-*O*MeTyr and its oxymethine proton (H-3, δ_H 5.03) appears as a broadened singlet. These data supported the (2*R*,3*R*)-configuration of β-*O*MeTyr in **1**. Comparison by LC–MS of both the L- and D-FDLA derivatives of 3,4-DiMeGln and Dab of **1** with similar derivatives from the hydrolysates of authentic samples of callipeltin A and/or papuamide B indicated these residues have the same configuration in all three peptides. Thus, their configurations were assigned as (2*S*,3*S*,4*R*)-DiMeGln and (2*S*,3*S*)-Dab. As (2*S*,3*S*)-Dab is a common fragment of papuamides and callipeltin A, considerable effort has been made toward its synthesis.[10–13] In these synthetic studies, the diastereomers of Dab showed distinct differences in the coupling constants, with the H-2/H-3 coupling constant in the (2*R*,3*S*)-isomer being ~3 Hz, while in the (2*S*,3*S*)-diastereoisomer it is 6.1–7 Hz, supporting the presence of (2*S*,3*S*)-Dab in **1**. Thus the absolute configurations of all 17 stereogenic carbons in the amino acid portion of stellettapeptin A (**1**) were successfully established. The polyketide moiety was unstable and it decomposed during repeated cleavage attempts, so the configuration of this portion of **1** could not be assigned.

Stellettapeptin B (**2**) analyzed for a molecular formula of $C_{63}H_{103}N_{13}O_{20}$ by HRESIMS coupled with ^1H and ^{13}C NMR spectroscopic data (Supplementary data). Its ^1H and ^{13}C NMR spectra were also characteristic of a peptide and suggested that **2** was similar in structure to **1**. Detailed analysis of 2D NMR data of **2** led to the identification of 12 residues: *N*MeAla, β-*O*MeTyr, *N*MeThr, Leu, Gly (2), 3-*O*MeAla, Thr (2), 3,4-DiMeGln, Dab, and Hdna (Fig. 1). The sequence of these residues was deduced from analysis of ROESY and HMBC correlations, as in **1**. The structure of **2** differed from **1** by the presence of *N*MeThr, Thr, and Gly in place of *N*MeGln, 3-OHGln, and 3-OHAsn residues, respectively. Similar to compound **1**, macrocyclic ring closure in **2** was established by an HMBC correlation from the Thr1 oxymethine (δ_H 5.65) and the carbonyl (δ_C 171.7) of the C-terminal *N*MeAla residue. The absolute configurations of the amino acid residues of **2** were also determined by Marfey's analysis of the acid hydrolysate using a similar strategy employed with **1**. Compound **2** was shown to have the same absolute configuration for each amino acid residue it has in common with **1**. It was also analyzed for L-*N*MeThr and both a D-*allo*-Thr and a L-Thr residue. Based on the close structural similarity between compounds **1** and **2**, and the fact that all cyclic depsipeptides in this structural class that form a macrocycle via esterification of the C-terminus with the hydroxyl group of a threonine utilize a D-*allo*-Thr residue, the L-Thr was assigned to be adjacent to the N-terminal Gly residue in **2**.

The anti-HIV activity of **1** and **2** was evaluated in an XTT based cell viability assay using the human T-cell line CEM-SS infected with HIV-1_{RF}.[14] After a six day incubation period, compounds **1** and **2** effectively inhibited the cytopathic effect of HIV-1 infection with EC_{50} values (concentration at which 50% of the target cells are protected from death by the virus) of 23 and 27 nM, respectively (Fig. 3 and Supplementary data). Direct cytotoxicity of **1** and **2** against the host cells was observed with IC_{50} values (concentration at which 50% of cells are killed by the test sample) of 367 and 373 nM, respectively.

In conclusion, stellettapeptins A (**1**) and B (**2**) are new depsipeptides with structural features characteristic of the family of anti-HIV peptides which includes the callipeltins,[6,7,15–17] papuamides,[8]

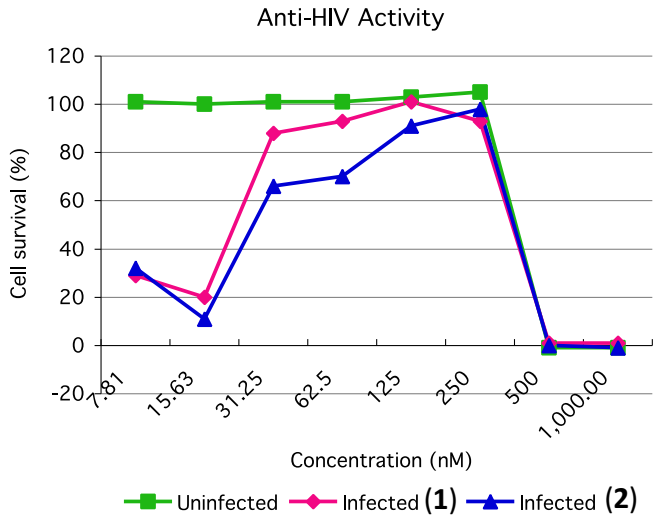

Figure 3. Anti-HIV activity of compounds **1** and **2**. Uninfected cell cultures reveal direct cytotoxicity of the test samples. HIV infected cultures show cytoprotective effects of **1** and **2** against viral infection at lower concentrations, and cytotoxic effects at higher concentrations.

mirabamides,[1,18] and neamphamide.[9] Compounds **1** and **2** contain a previously undescribed polyketide subunit, 3-hydroxy-6,8-dimethylnon-4-enoic acid, and the 3-OHGln and 3-OHAsn residues in **1** are rarely found in peptides. Compounds **1** and **2** potently inhibited the cytopathic effect of HIV-1 infection, providing additional evidence that this class of peptides may hold promise as anti-HIV agents. Isolation of the stellettapeptins from *Stelletta* sp., which is phylogenetically distinct from sponges the callipeltins and papuamides were isolated from, and the fact that **1** and **2** have characteristic structural features of nonribosomal peptides, suggest a possible microbial origin for the stellettapeptins.

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Supplementary data

Supplementary data (1D and 2D NMR spectral data, ozonolysis, amino acid analysis, anti-HIV data, and isolation of **1** and **2**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.05.058. These data include MOL files and InChiKeys of the most important compounds described in this article.

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