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Callipeltins B and C; Bioactive Peptides from a Marine Lithistida Sponge *Callipelta* sp.

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Abstract: Following the characterization of callipeltin A (**1**), two new cytotoxic peptides, callipeltin B (**2**) and C (**3**),were isolated from the New Caledonian sponge *Callipelta* sp.. Callipeltin B (**2**) possess the same cyclic depsipeptidal structure as in callipeltin A (**1**) and differs from **1** by having the N-terminal 2,3-dimethylpyroglutamic acid unit instead of the tripeptide chain with the N-terminus blocked by an hydroxyacid. Callipeltin C (**3**) is simply the acyclic callipeltin A. The structures **2-3** have been determined by NMR experiments, FAB mass spectrometry, evaluation of the aminoacids obtained by acid hydrolysis and by comparison of the data with those of callipeltin A (**1**).

Cyclic peptides and depsipeptides have emerged as very important classes of bioactive compounds in marine natural products.[1] Among the marine phyla, sponges of the order Lithistida, are proving to be prominent sources of diverse bioactive peptides containing unusual aminoacid residues,[2] *e.g.* theonellapeptolides,[3] theonellamides,[4] mutoporins,[5] cyclotheonellamides,[6] polytheonamides,[7] theonegramides,[8] from the *Theonella* genus, discodermins[9] and calyculins,[10] from the *Discodermia* genus. The structural relationships between some of lithistid sponge peptides and those isolated from blue-green algae raised the question of the true producer of the metabolites.[11] In the course of our search for bioactive substances from New Caledonian marine invertebrates, we encountered the marine sponge *Callipelta* sp.,[12] which showed marked activity in cytotoxic assays against KB and P388 cells and in the anti-HIV and antifungal tests. From the sponge, we isolated the major active constituent that we designated callipeltin A and assigned the structure **1** from interpretation of spectral data and chemical degradation.[12] We now report the isolation and the structure elucidation of two minor peptides, callipeltins B-C (**2-3**), which are related to callipeltin A.

The sponge *Callipelta* sp. (0.7 Kg freeze-dried) collected off the East Coast of New Caledonia at the depth of 5-10 m was sequentially extracted with *n*-hexane, dichloromethane and 8:2 dichloromethane-methanol. The dichloromethane-methanol extract was separated by DCCC, followed by HPLC, yielding callipeltins A (**1**, 500 mg), B (**2**, 25 mg), and C (**3**, 70 mg). Callipeltin B (**2**) showed a $[M+H]^+$ peak at *m/z* 1031 in the FAB mass spectrum; IR (ν_{max} 1660 and 1740 cm^{-1}), and UV (λ_{max} 232, 275 nm) spectra were similar to those of callipeltin A. The ^1H and ^{13}C NMR (HETCOR) as well as homonuclear COSY, TOCSY (HOHAHA)[13] and NOESY spectra revealed the presence of one residue each of N-methylalanine (MeAla), β-methoxytyrosine (βMeOTyr),

N-methylglutamine (MeGln), Leu, Arg, and two residues of Thr, which were also found in callipeltin A (**1**). One additional spin system corresponding to a 3,4-dimethylpyroglutamic acid residue was also evident in the COSY spectrum; starting at the NH resonance at δ 8.03 ppm leads to the subsequent cross peaks at δ 4.27 ppm (α-H), the CβH at δ 2.99 ppm and CγH at δ 3.54 ppm; the cross peaks CβH-CH_3 at δ 0.89 ppm and CγH-CH_3 at δ 1.26 ppm were also observed in the same spectrum.

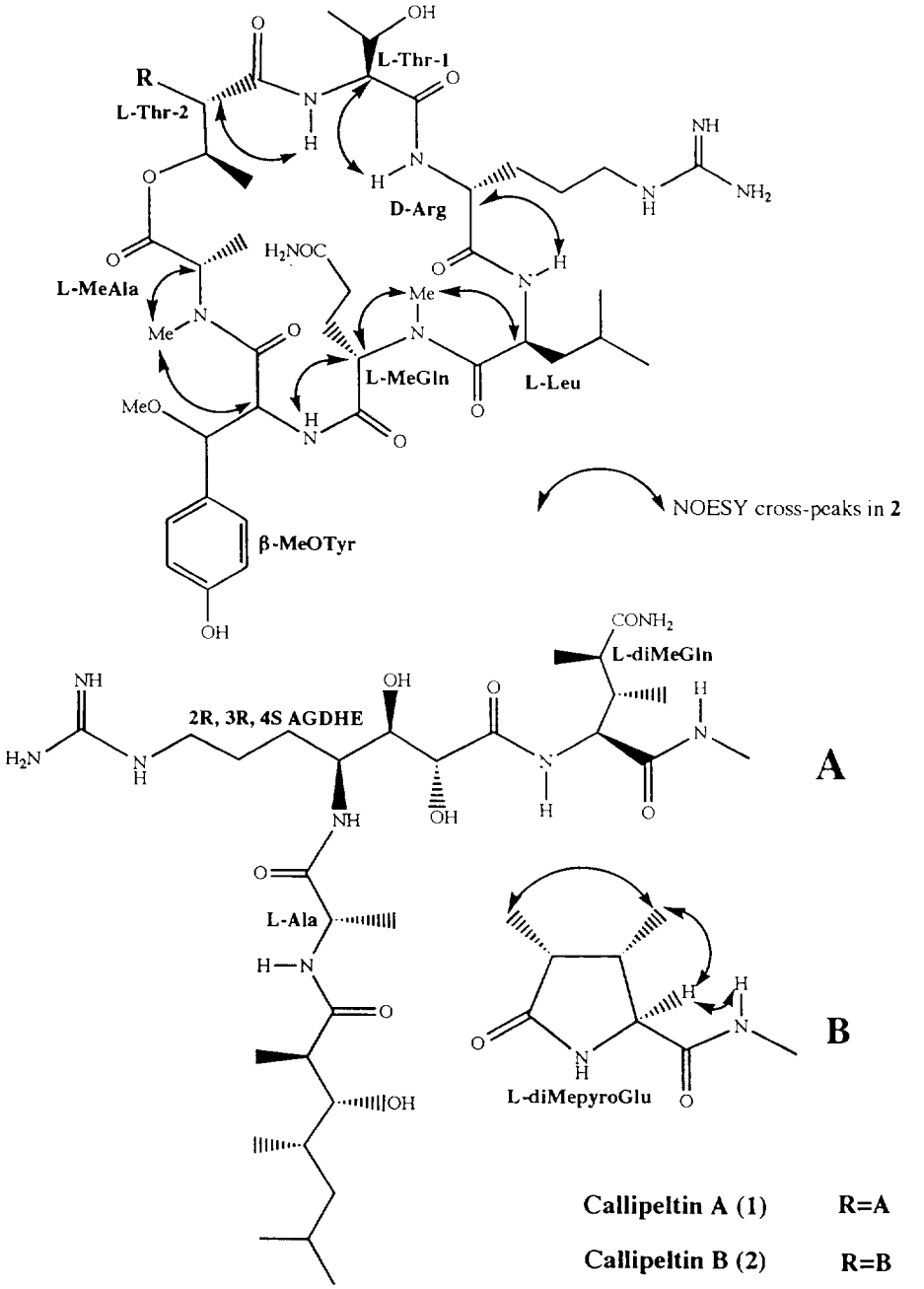


Table 1. ^{1}H and ^{13}C NMR data of callipeltin B (**1**) in pyr-d_5 and methanol-d_4 at 500 MHz.

	pyr-d_5		methanol-d_4	
	δ_H	δ_C	δ_H	δ_C
MeAla				
α	5.73 q (6.8)	51.7	5.34 q (7.1)	52.3
β	1.13 d	13.8	1.30 d (7.1)	14.5
NCH_3	2.86 s	29.8	2.74 s	30.1
CO				171.3
βMeOTyr				
α	5.44 t (9.5)	53.4	4.90 t (9.5)	54.0
β	4.99 d (9.5)	84.1	4.52 d (9.5)	84.2
C4		129.2		129.2
C5\C9	7.80 d (8.0)	131.7	7.26 d (8.1)	131.7
C6\C8	7.14 d (8.0)	116.0	6.78 d (8.1)	115.7
C7		158.5		156.6
OH	7.46 br s[a]			
OCH_3	3.26 s	57.1	3.18 s	56.7
CONH	8.89 d (9.5)		8.44 d (9.5)	170.0
MeGln				
α	5.45 br t (7.2)	56.8	4.94 overlapped	54.0
β	1.80, 1.61 m	26.3	1.59, 1.50 m	25.6
γ	1.95, 1.80 m	32.4	1.94, 1.78 m	32.4
NCH_3	3.03 s	31.0	3.01 s	30.6
CO				172.9*
$CONH_2$	8.56 overlapped			182.0*
Leu				
α	5.03 m	49.3	4.77 m	49.7
β	1.70, 1.34 m	41.0	1.65, 1.38 m	40.6
γ	1.70 m	25.5	1.70 m	26.4
δ	0.78 d (6.4)	21.6	0.96 d (6.7)	23.6
δ_1	0.82 d (6.4)	23.6	0.93 d (6.7)	21.6
CONH	7.80 overlapped		7.44 d (8.1)	175.2
Arg				
α	4.30 m	52.8	4.16 ddd (9.5, 8.1, 5.1)	53.6
β	2.18 m	27.9	1.94, 1.78 m	27.5
γ	1.95, 1.80 m	26.1	1.65, 1.59 m	26.3
δ	3.20 m	42.2	3.19 m	41.9
δNH	9.08 br s			
CONH	9.68 br s		8.26 d (8.1)	172.9*
guan	8.56 overlapped			156.6
Thr-1				
α	4.50 dd (6.8, 4.0)	63.1	4.12 overlapped	62.0
β	4.55 m	66.6	4.12 overlapped	67.0
γ	1.28 d (6.2)	21.5	1.28 d (7.1)	20.4
CONH	8.75 d (4.0)		7.79 d (6.4)	169.9
Thr-2				
α	5.89 t (9.0)	57.1	5.49 overlapped	56.8
β	6.01 m	72.5	5.50 overlapped	72.8
γ	1.47 (6.4)	11.0	1.28 d (6.8)	10.3
CONH	9.88 d (9.0)		8.70 d (9.1)	169.8
DiMepyroGlu				
α	4.27 br s ($W_{1/2}$ 5.1)	62.7	3.97 d (3.0)	63.3
β	2.99 m	39.3	2.77 m	39.9
βMe	0.89 d (6.2)	15.6	1.24 d (7.1)	15.2
γ	3.54 m	39.3	3.19 m	40.0
γMe	1.26 d (6.8)	15.3	1.21 (6.5)	13.6
CONH	8.03 s			168.8
$CONH_2$[a]	7.56 br			182.0

[a] Assignment based on NOESY experiment

Table 2. ^{1}H and ^{13}C NMR data[a] of callipeltin C (**3**) in methanol- d_4 at 500 MHz.

Residue	δ_H	δ_C	Residue	δ_H	δ_C
MeAla			**Thr-2**		
α	5.17 q (7.5)[b]	54.0	α	4.22 d (5.0)	61.3
β	1.43 d (7.5)	14.4	β	4.15 overlapped	68.3
NCH_3	2.86 s	33.4	γ	1.33 d (6.8)	20.2
CO		170.7	CONH		172.6
βMeOTyr			**DiMeGln**		
α	5.26 d (9.5)	54.0	α	4.45 d (9.5)	57.7
β	4.37 overlapped	85.1	β	2.23 m	39.6
C4		129.2	βMe	1.05 d (6.8)	13.7
C5\C9	7.20 d (8.5)	130.3	γ	2.66 m	41.8
C6\C8	6.82 d (8.5)	116.1	γMe	1.21 (6.5)	20.4
C7		158.8	CONH		175.8
OCH_3	3.15 s	56.8	$CONH_2$		179.9
CONH		172.7	**AGDHE**		
MeGln			α	4.00 d (9.1)	72.8
α	4.97 m	56.9	β	3.72 br d (9.1, 2.2)	75.3
β	1.68 m	26.9	γ	4.15 overlapped	50.7
γ	2.00 m	32.4	δ	1.70, 1.60 m	26.2
NCH_3	3.03 s	30.6	ε	1.58 m	25.6
CO		173.0	ζ	3.19 m	42.0
$CONH_2$		179.9	CO		177.5*
Leu			guan		158.4
α	4.74 dd (10.5, 3.0)	49.9	**Ala**		
β	1.70, 1.30 m	39.3	α	4.33 q (7.1)	51.0
γ	1.67 m	25.7	β	1.43 d (7.1)	17.7
δ	0.97 d (6.4)	23.6	CONH		173.5
δ_1	0.95 d (6.4)	21.4	**TMHEA**		
CONH		175.3	1		178.7
Arg			2	2.64 m	44.6
α	4.37 overlapped	53.8	3	3.52 dd (8.8, 3.0)	79.5
β	2.00, 1.67 m	26.0	4	1.79 m	33.4
γ	1.67, 1.53 m	26.2	5	1.26 m	40.4
δ	3.16 m	41.8	6	1.75 m	24.7
CONH		175.7	7	0.96 d (6.4)	24.7
guan		158.4	8	1.11 d (6.4)	14.4
Thr-1			9	1.01 d (6.8)	17.3
α	4.37 overlapped	60.3	10	0.90 d (6.8)	21.5
β	4.15 overlapped	68.3			
γ	1.28 d (7.4)	20.1			
CONH		172.6			

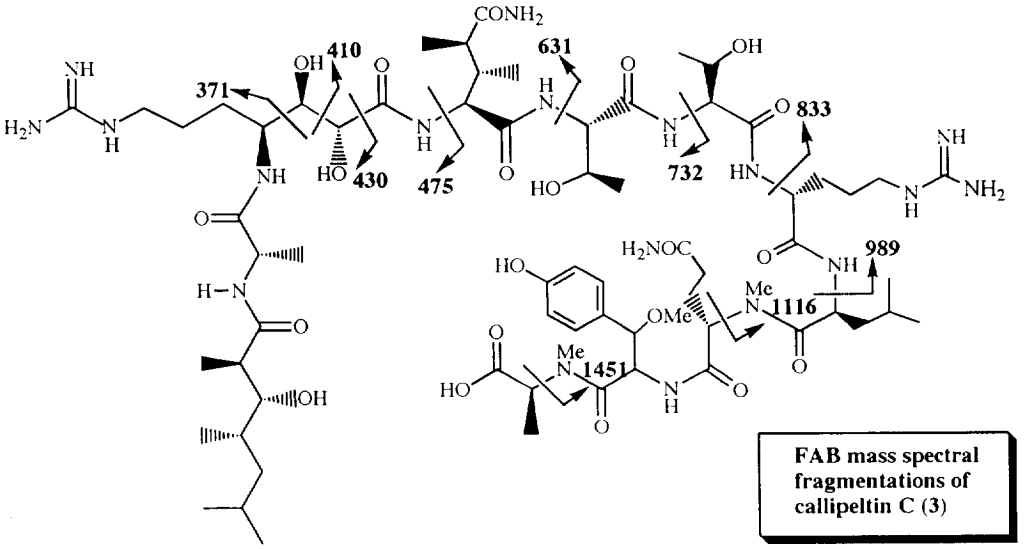
AGDHE: 4-amino-7guanidino-2,3-dihydroxyheptanoyl residue.

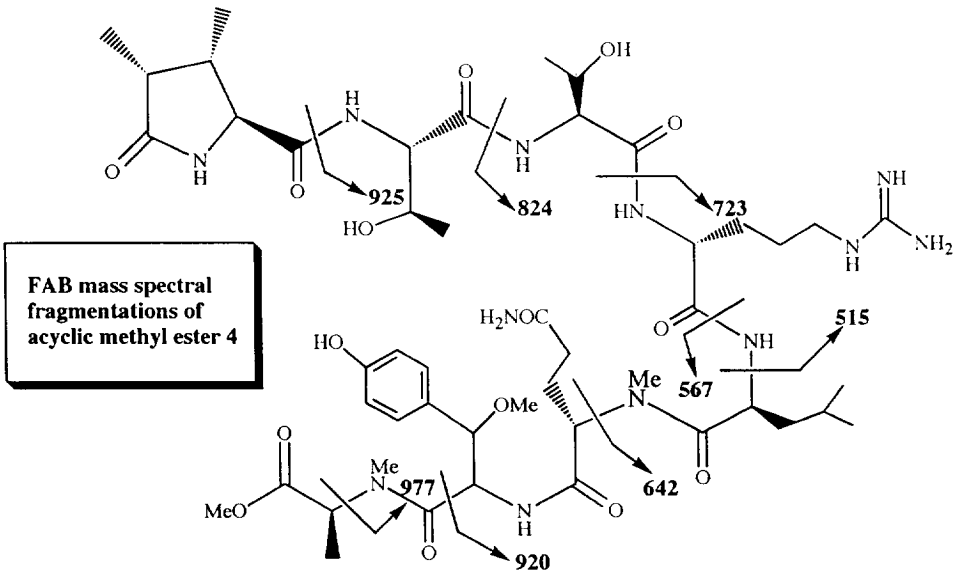
TMHEA: 3-hydroxy-2,4,6-trimethylheptanoyl residue.

[a]Assigments based on 2D-COSY, 2D-HOHAHA, NOESY and HETCOR experiments.[b]Coupling constants are enclosed in parenthesis and given in Hertz. Quaternary carbons are assigned by comparison with callipeltin A (**1**).

Amino acids analysis together with HPLC analysis of the acid hydrolyzate derivatized with Marfey's reagent[14] indicated the same absolute stereochemistry for the common amino acids (L-Ala, L-NMeAla, L-Leu, L-Thr and D-Arg). The uncommon β-Methoxytyrosine residue was not released intact from **2** by acid hydrolysis, just as in the case of the hydrolysis of callipeltin A (**1**), and was identified only by analysis of ^{1}H and ^{13}C NMR data (Table 1). The relative stereochemistry of the 3, 4-diMepyroGlu residue in **2** was suggested by NOE data which indicated that Hα and both the methyls were located on the same side of the molecule. The L (2S) configuration of both 3, 4-diMepyroGlu and NMeGln residues was suggested by analogy with callipeltin A.[12] The sequence of cyclic peptide was found to be identical to callipeltin A (**1**) by NOESY experiment in pyridine-d_5, which gave

sequential informations from correlation between amide protons and α-protons of the adjacent residue via amide bond, as shown in **2**. The β-proton of Thr-2 resonating at low field, δ 6.01 in pyridine-d_5 and 5.50 in methanol-d_4, very close to the same signal in callipeltin A, confirmed the participation of this residue in the lactone ring formation with the C-terminal MeAla, whose α-proton did not give rise to cross peaks with any amide protons in the NOESY spectrum. Further, a NOE cross-peak between NH-Thr-2 and Hα 3, 4-diMepyroGlu confirmed the 3, 4-diMepyroGlu N-terminal to be linked to the Thr-2 residue. Next, callipeltin B (**2**) was treated with sodium methoxide in methanol to furnish the acyclic methyl ester **4**, which was generated by methanolysis of the lactone ring of **2**. The FAB MS of **4** provided, in addition to the quasi-molecular ion MH^+ at *m/z* 1063, several fragment ion peaks, which were consistent with the amino acid sequence of **2** as shown in **4**.





In addition to some N-terminal fragments due to the cleavage of the linkage adjacent to the amide bond and 1H trasfer, the spectrum showed a series of C-terminal fragments, *i.e.* with the charge retained on the C-terminus, originating by protonation of the amide followed by the cleavage of the amide peptide bond. Minor satellite peaks, $[MH-15]^+$ confirmed these ions as C-terminus fragments; 925-910, 824-809; 723-708; 567-552[15]

Callipeltin C (**3**) is closely related to Callipeltin A (**1**). The MH^+ at *m/z* 1523 in the FAB mass spectrum, 18 mass units higher than callipeltin A, together with ^1H and ^{13}C NMR (Table 2) spectra suggested callipeltin C (**3**) to be the acyclic callipeltin A (**1**). The ^1H and ^{13}C NMR spectra were very similar with those of callipeltin A except for the signals assigned to Hβ and Cβ of the Thr-2 residue. In **3** these signals were found shifted upfield to δ 4.50 and 68.3 ppm (CD_3OD) respectively, relative to the same signal in the spectra of **1** (δ_H 5.60, δ_C 72.4). FAB mass spectrum showed identical fragmentation ion peaks observed in the spectrum of the acyclic methyl ester derived from callipeltin A by treatment with sodium methoxide. The N-terminus fragmentation due to the cleavage of the linkage adjacent to the amide bond and 1H transfer confirmed the aminoacid sequence as shown in **3**.

Callipeltins A-C were cytotoxic against various human carcinoma cells *in vitro* (Table 3) and the activities of **1** and **2** exceed significantly those of **3**, thus suggesting the importance of the macrocycle for the bioactivity. Compounds **1** and **3** also exhibited antifungal activity against *Candida albicans* showing 30 mm and 9 mm inhibition at 100 μg/disc, respectively. Whereas callipeltin A (**1**) showed anti-HIV activity,[12] callipeltins B and C proved to be inactive as antiviral compounds.

Table 3. *In vitro* cytotoxic activity (IC_{50} in μg/ml) of callipeltins A-C[a]

Tumor cells	1	2	3
NSCLC-N6	<1.1	1.3	53.5
NSCLC-N6 C15	>30	22.5	-
NSCLC-N6 C92	<3.3	>30	-
NSCLC-N6 C98	<3.3	<3.3	-
E39	<1.1	>10	36.1
P388	<3.3	<3.3	-
M96	<3.3	<3.3	-

a) NSCLC-N6: human bronchopulmonary non-small-cell-lung-carcinoma; [C 15, C92, C98 are clones of NSCLC-N6 cell line; they differ in chemiosensibility, having different doubling times, 91, 108 and 74 hours, respectively. One might consider C15 and C 92 as being well differentiated clones, whereas C98 is a less differentiated clone of NSCLC-N6 cell line]. E 39: human renal carcinoma; P 388: murine leukemia; M 96: human melanoma. Assays made at three diluitions 3.3 μg/ml; 10 μg/ml and 30 μg/ml

Experimental Section

General Information. For general details see: D'Auria M.V. et. al[16]

Isolation. *Callipelta* sp. (Demospongiae, Lithistida, Corallistidae), was collected in 1992 and '93 in the shallow waters of East coast of New Caledonia. Taxonomic identification was performed by Professor Claude Lévi, Muséum National d'Histoire Naturelle, Paris, France, and reference specimens are on file (reference 1572) at the ORSTOM Centre of Noumea. Preliminary tests of bioactivity on polar extracts showed antifungal activity against *Fusarium oxysporum, Helminthosporium sativum* and *Phytophtora hevea*, cytotoxic activity against KB and P388 cells (10 μg/ml, *ca.* 80% inhibition, in both cases) and anti-HIV activity.

The organism were freeze-dried and the lyophilized matherial (700 g) was extracted with *n*-hexane and CH_2Cl_2 in a Soxhlet apparatus, then with CH_2Cl_2:MeOH 8:2 (3x2 L) at room temperature. The dichloromethane-

methanol extract was filtered and concentrated under reduced pressure to give 10 g of a brown amorphous solid. The crude dichloromethane-methanol extract was injected in a DCCC apparatus in five runs ($CHCl_3$:MeOH:H_2O 7:13:8, ascending mode; fractions of 4 ml were collected). Fractions 10-12, mainly containing callipeltin C (**3**), were further purified by HPLC on a Vydac 218 TP proteine-peptide RP column (flow rate 4 ml/min) with CH_3CN:H_2O (30:70) as eluent to give 70 mg of pure **3** (t_r=6.8 min). Fractions 13-17 contained callipeltin A (**1**) while fractions 18-23, mainly containing callipeltin B(**2**), were further purified by HPLC on a Vydac column (flow rate 4 ml/min) with CH_3CN:H_2O (32:68) as eluent to give 25 mg of pure **2** (t_r=5.2min).

Callipeltin B (**2**): $[\alpha]_D$=+11.3° (*c* 0.0057 M, MeOH); UV (MeOH) λ_{max} 230 (ε=7023), 274 (ε=1300); IR (KBr) 3335, 1740, 1660, 1520 cm^{-1}; ^1H and ^{13}C NMR in the Table 1; FABMS *m/z* 1031, MH^+

Callipeltin C (**3**): $[\alpha]_D$=-15.3° (*c* 0.0053 M, MeOH); UV (MeOH) λ_{max} 230 (ε=6890), 274 (ε=1320); IR (KBr) 3330, 1740, 1660, 1520 cm^{-1}; ^1H and ^{13}C NMR in the Table 2; FABMS *m/z* 1523, MH^+

Determination of Amino Acid Absolute Configuration. (a) Hydrolysis of callipeltins. 100 μg of callipeltin B (**1**) and callipeltin C (**3**)were dissolved respectively in 0.5 ml of 6 N HCl in two evacuated glass tubes and heated at 110 °C for 16 h. The residues were taken to dryness. **(b) HPLC Analysis of the Marfey's Derivatives.**[14] To a 10% acetone solution (50 μl) of 1-fluoro-2,4-dinitrophenyl-5-alanine amide (L-FDAA) and 1N $NaHCO_3$ (40 μl) were added 100 μg of the peptide acid hydrolysate of callipeltin B and the mixture was kept at 50 °C for 1 h. After cooling to room temperature 2N HCl (40 μl) was added and the resulting solution was taken to dryness and then dissolved in 500 μl of DMSO. A 5 μl aliquot of the FDAA derivatives was analyzed by reversed-phase HPLC. A linear gradient from triethylammonium phosphate (50 nM, pH 3.0)/MeCN 90% to triethylammonium phosphate (50 nM, pH 3.0)/MeCN 50% over 45 min (flow rate 2 ml/min) was used to separate the FDAA derivatives which were detected by uv at 340 nm.[14] Amino acids were derivatized as described above. The peaks were identified by co-injection with a DL-mixture of standard amino acids. Retention times (min) are given in parentheses. L-Thr and D-Arg (18.1), L-MeAla (21.6), L-Leu (37.6). The same procedures were apllied to 100 μg of callipeltin C. Retention times (min): L-Thr and D-Arg (18.1), L-Ala (21.6), L-MeAla (28.3), L-Leu (37.6).

Methanolysis of Callipeltin B (1) Giving the Opened Methyl Ester (4). A solution of 1 mg of callipeltin B (**1**) was treated with 1.1 mg of NaOMe in dry methanol (0.2 ml) at room temperature for 2 h. The reaction mixture was neutralized with 0.1N HCl, poured into ice-water and then extracted with *n*-BuOH. The *n*-BuOH phase was evaporated under reduced pressure and the crude product (0.8 mg) directly subjected to FABMS analysis.

Determination of biological activity. NSCLC-N6-L16, C15, C92, C98, E39, M96, cells were mainteined in a suspension culture of RPMI supplemented with 5% calf serum containing 1% of glutamine solution at 200 mM and 1% of a penicillin-streptomycin mixture (10000 UI/ml). A 50 μl aliquot of each cell culture [P388 cells (5x103)] was mixed with 50 μl aliquot of serial dilution (3.3 μg/ml; 10 μg/ml and 30 μg/ml) of compounds and the mixture was incubated in a microtited well plate (96-well Falcon 3072) for 72 h at 37 °C in a humified incubator comtaining 5% CO_2 in air.Cell proliferation was estimated by a colorimetric test: 10 μl of MTT was added. After 4 h the dark blu cristals, formed in mitochondria of living cells during the reduction of MTT, were solubilized with 100 μl of isopropanoic acid. Microplates were read by ELISA using a multiskar Titertek multiscan MK2 with a 570 nm filter.

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REFERENCES AND NOTES

1. Ireland, C.M.; Molinski, T.F.; Roll, D.M.; Zabriskie, T.M.; McKee, T.C.; Swersey, J.C.; Foster, M.P. Natural Product Peptides from Marine Organisms in *Bioorganic Marine Chemistry*, Paul J. Scheuer (Ed.), Springer-Verlay Berlin Heidelbery, 1989, pp. 1-46
2. Fusetani, N.; Matsunaga, S. *Chem. Rev*, **1993**, 93, 1793-1806.
3. Kitagawa, I.; Lee, N.K.; Kobayashi, M.; Shibuya, H. *Chem. Pharm. Bull.* **1987**, 35, 2129-2132; Kitagawa, I.; Lee, N.K.; Kobayashi, M.; Shibuya, H. *Tetrahedron* **1991**, 47, 2169-2180; Kobayashi, M.; Lee, N.K.; Shibuya, H.; Momose, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1991**, 39, 1177-1184; Kobayashi, M.; Kanzaki, K.; Katayama, S.; Ohashi, K.; Okada, H.; Ikegami, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, 42, 1410-1415.
4. Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Walchli, M. *J. Am. Chem. Soc.* **1989**, 111, 2582-2588; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, 60, 1177-1181.
5. de Silva, E.D.; Williams, D.E.; Andersen, R.J.; Klix, H.; Holmes, C.F.B.; Allen, T.M. *Tetrahedron Lett.* **1992**, 33, 1561-1564.
6. Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, 112, 7053-7054.
7. Hamada, T.; Suyawara, T.; Matsunaga, S.; Fusetani, N.*Tetrahedron Lett.* **1994**, 35, 609-612.
8. Bewley, C.A.; Faulkner, D.J. *J. Org. Chem.* **1994**, 59, 4849-4852.
9. Fusetani, N.; Matsunaga, S.; Konosu, S. *J. Nat. Prod.*, **1985**, 48, 236-241; Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, 25, 5165-5168; Fusetani, N.; Matsunaga, S.; Konosu, S.*Tetrahedron Lett.* **1985**, 26, 855-856; Ryu, G.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1994**, 35, 8251-8254; Ryu, G.; Matsunaga, S.; Fusetani, N.;*Tetrahedron* **1994**, 50, 13409-13416.
10. Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, 108, 2780-2781; Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K.J. *J. Org. Chem.* **1988**, 53, 3930-3932; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.*, **1991**, 32, 5605-5606.
11. Bewley, C.A.; Debitus, C.; Faulkner, D.J. *J. Am. Chem. Soc.* **1994**, 116, 7631-7636. Faulkner, D.J.; He, H.; Unson, M.D.; Bewley, C.A. *Gazz. Chim. Ital.* **1993**, 123, 301-307.
12. Zampella, A.; D'Auria, M.V.; Gomez Paloma, L.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.*, in press.
13. Edwards, M.W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918-932.
14. Marfey, P. *Carlsberg Res. Commun.* **1984**, 49, 591-596.
15. Larsen, B.S. *Tips for the Analysis of Peptides using FAB in Mass Spectrometry of Biological Materials* Mc Even, C.N.; Larsen, B.S. ed's, Pratical Spectroscopy series vol. 8, pp. 197-214, Marcel Dekker, Inc. New York, Basel **1990**.
16. D'Auria, M.V.; Debitus, C.; Gomez Paloma, L.; Minale, L.; Zampella, A.;. *J. Am. Chem. Soc.*, **1994**, *116*, 6658-6663.

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