Callipeltin A, an Anti-HIV Cyclic Depsipeptide from the New Caledonian Lithistida Sponge *Callipelta* sp.

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Abstract: Callipeltin A (**1**) is a cyclic depsidecapeptide from a shallow water sponge of the genus *Callipelta* (order Lithistida), collected in the waters off New Caledonia. The structure of callipeltin A (**1**), which possesses the N-terminus blocked with a β-hydroxy acid, and the C-terminus lactonized with a threonine residue, was determined by interpretation of spectral data, chemical degradation, and evaluation of the amino acids obtained by acid hydrolysis. Along with four common L-, one D-, and two *N*-methyl amino acids, it contains three new amino acid residues: β-methoxytyrosine (βOMeTyr), (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), and (3*S*,4*R*)-3,4-dimethyl-L-glutamine. Callipeltin A (**1**) has been found to protect cells infected by human immunodeficiency (HIV) virus.

Introduction

Sponges of the order Lithistida continue to be exceptionally rich sources of structurally unique and biologically active natural products.[1] Examples include potent cytotoxic macrolides such as swinholides,[2] misakinolide A,[3] theonezolides[4] from the *Theonella* genus, sphinxolides[5] and superstolides[6] from *Neosiphonia superstes*, and discodermolide[7] from *Discodermia dissoluta*, and other strongly bioactive peptides such as theonellapeptolides,[8] theonellamides,[9] mutoporin,[10] cyclotheonamides,[11] polytheonamides,[12] keramamides,[13] theonegramide[14] from the *Theonella* genus, discodermins,[15] polydiscamide A,[16] and calyculins[17] from the *Discodermia* genus, and microscleroderms[18] from *Microscleroderma* sp. In this paper we report the isolation and structural elucidation of a new cyclic depsipeptide, callipeltin A (**1**), from a shallow water lithistid sponge, *Callipelta* sp. Callipeltin A (**1**) showed anti-HIV and antifungal activity.

Results and Discussion

A specimen of the lithistid sponge *Callipelta* sp. was collected in the waters off the East coast of New Caledonia. Preliminary tests of bioactivity of the aqueous and ethanolic crude 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), and anti-HIV activity. The sponge was freeze-dried

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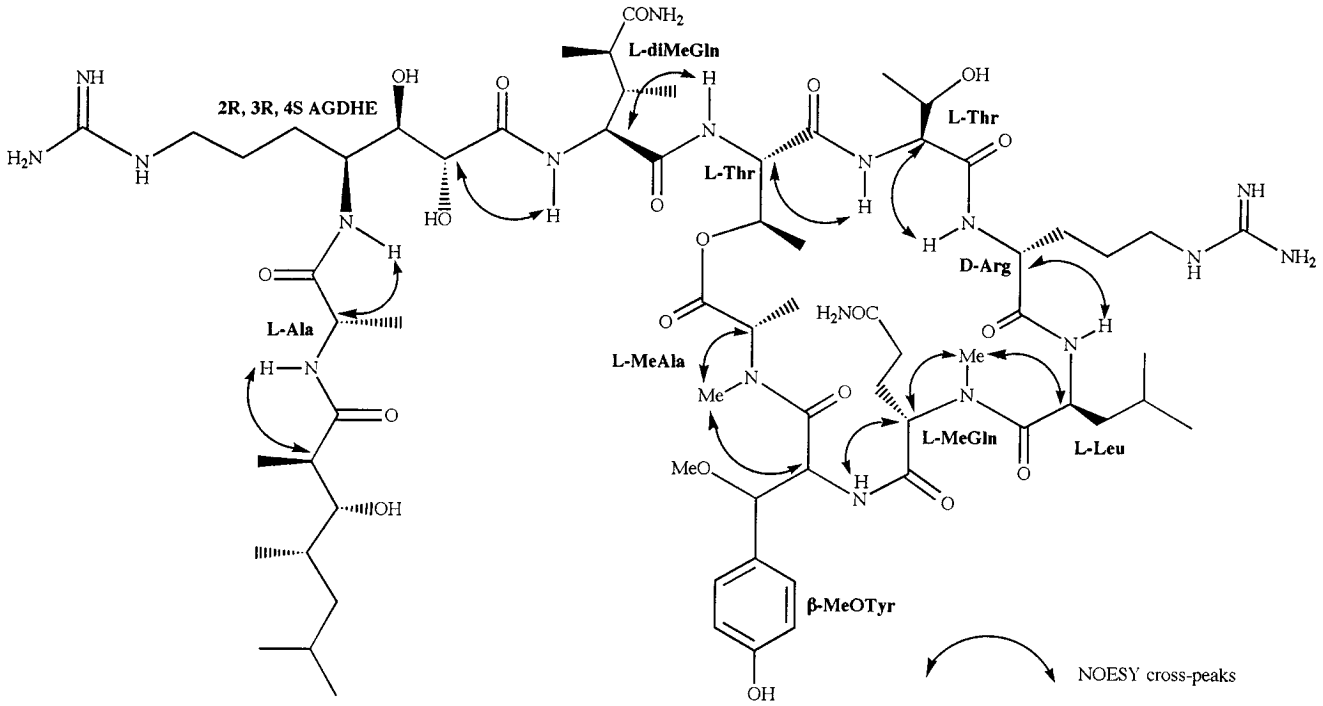
Chart 1



Callipeltin A (1)

(700 g) and extracted sequentially with *n*-hexane, dichloromethane, and a mixture of 8:2 dichloromethane−methanol. Fractionation of the 8:2 dichloromethane−methanol extract by droplet counter current chromatography (DCCC) followed by reversed-phase HPLC yielded callipeltin A (**1**) as colorless solid (500 mg) ($[\alpha]_D$ in methanol = +3.56).

The FABMS of callipeltin A (**1**) displayed a protonated molecolar ion at *m/z* 1505 $[M + H]^+$, whereas ESIMS showed a major peak (100%) at *m/z* 753.5 for $[M + 2H]^{2+}$ accompained by a minor one (10%) at *m/z* 1506, corresponding to a molecular weight of 1504 daltons. An intense $[M + 2H]^{2+}$ peak in the ESIMS spectrum indicated the presence of two strong basic groups in the molecule, such as two guanidinium units. The peptidal nature of the compound was indicated by NMR (Table 1) and FTIR spectra (ν_{max} 1660 and 1740 cm^{-1}, with the latter absorption supporting also the presence of an ester or lactone function). The ^1H NMR spectrum in pyridine-d_5 contained eight amide NH signals between δ 10.1 and 7.27 as implied by COSY and TOCSY (HOHAHA) experiments revealing NH-CH(α) connectivities for eight amino acid residues and two *N*-methyl signals at δ 2.90 and 3.07, totally accounting for the presence of ten amino acids. The signal at δ 7.40 could be assigned to a phenolic OH on the basis of intraresidual NOESY cross-peak between that signal and the aromatic proton signal at δ 7.25. Other exchangeable signals were observed spread out between δ 7.50 and δ 8.48. ^{13}C spectrum experiments revealed the presence of 13 carbonyls between 180.4 and 171.5 ppm and four quaternary signals at δ 158.3, 158.4, and 158.5, assigned to two guanidino and one phenolic carbons, and at δ 129.2 (aromatic carbon). Further DEPT NMR experiments revealed the presence of 15 methyls, 10 methylenes, and 26 methines.

Standard amino acid analysis revealed the presence of Ala, Leu, Arg, and Thr (2 residues) as common amino acids contained in **1**. Two *N*-methyl groups were assigned to *N*-MeAla and *N*-MeGln (or Glu) residues on the basis of intraresidual cross peaks in the NOESY spectrum in pyridine-d_5 and COLOC spectrum in methanol-d_4 (Table 1).

The aromatic region of the ^1H NMR spectrum (methanol-d_4) contained signals for a *para*-substituted benzene ring at δ 6.81 (d, 2H, J = 8.5 Hz) and 7.24 (d, 2H, J = 8.5 Hz) and a -CHOMe-CH(α) spin system at δ 3.15 (s, 3H), 4.55 (d, 1H, J = 9.5) and 5.01 (d, 1H, J = 9.5 Hz). On the basis of ^1H and ^{13}C NMR chemical shifts and COLOC data, the para-substituted benzene ring was part of a β-methoxytyrosine residue, which appears to be undescribed. It should be noted that the related β-methoxyphenylalanine was detected in discokialides, peptides isolated from *Discodermia kiiensis*[19] and more recently in cyclomarins, potent antiinflammatory cyclopeptides from marine bacteria.[20]

The 3,4-dimethylglutamine (or glutamic acid) was difficult to establish by ^1H NMR spectroscopy in methanol-d_4, because the signals at δ 2.39 (Hβ) and 2.84 (Hγ) did not give any correlation peak in the COSY spectrum, implying that the dihedral angle between the two hydrogens is close to 90 degrees. However, the COLOC spectrum, which showed correlations between the Me carbon signal at δ 14.6 (Me-Cβ) and both Hβ and Hγ *via* $^2J_{CH}$ and $^3J_{CH}$, respectively, indicated the proximity of two methines substituted by methyl groups. A correlation peak between the Hα at δ 4.28 and Hβ at δ 2.39 was observed in the COSY spectrum, whereas correlations between Hα and the carbonyl at 173.5 ppm and between the protons of the methyl on Cγ at δ 1.33 and the carbonyl at 180.4 ppm were observed in the COLOC spectrum. These data indicated the presence of a 3,4-dimethylglutamine (or glutamic acid) residue, which also appears to be undescribed. The isolation on acid hydrolysis of the 3,4-dimethylpyroglutamic acid (see below) confirmed these assignments.

Further analysis of the NMR spectra of **1** revealed the spin system for another unknown amino acid residue **2**. NMR data of **1** showed three contiguous methylenes, which were identical

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Table 1. ^{1}H and ^{13}C NMR Data[a] of Callipeltin A (**1**) in pyr-d_5 and Methanol-d_4 at 500 MHz

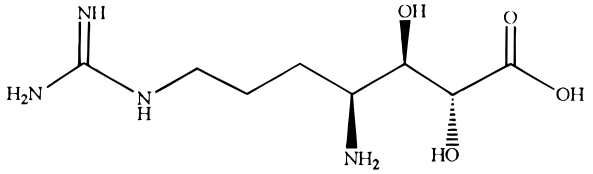
	pyr-d_5[b]		methanol-d_4		
	δ_H	δ_c	δ_H	δ_c	COLOC
			MeAla		
α	5.68 q (6.8)[c]	50.1	5.37 q (7.5)	52.3	NCH_3, Cβ, CO
β	1.13 s	13.7	1.35 d (7.5)	16.3	CO
NCH_3	2.90 s	30.1	2.86 s	30.1	Cα, CO, CO-βMeOTyr
CO				171.5	
			βMeOTyr		
α	5.42 t (9.6)	53.4	5.01 d (9.5)	53.5	CONH
β	4.96 d (9.6)	84.1	4.55 d (9.5)	84.0	CO, Cα, C5\C9, OCH_3
C4		129.2		129.2	
C5\C9	7.64 d (8.0)	131.1	7.24 d (8.5)	130.8	C7
C6\C8	7.25 d (8.0)	116.2	6.81 d (8.5)	115.9	C4
C7		158.5		158.5	
OH	7.40 br s				
OCH_3	3.15 s	56.9	3.15 s	56.7	Cβ
CONH	8.72 d (9.6)			171.6	
			MeGln		
α	5.27 br t (7.2)	56.7	4.82 m	56.7	
β	1.82, 1.78 m	26.4	1.65, 1.50 m	26.5	
γ	2.08, 1.93 m	32.4	1.67 m	32.4	
NCH_3	3.07 s	30.6	3.03 s	30.6	Cα, COLeu
CO				172.9[e]	
$CONH_2$[d]	7.30 overlapped			180.4[e]	
			Leu		
α	4.92 m	49.7	4.67 dd (10.5, 3.0)	50.1	CONH
β	1.74, 1.50 m	39.6	1.73, 1.33 m	39.8	
γ	1.67 m	25.4	1.70 m	26.3	
δ	0.77 d (6.4)	21.6	0.98 d (6.4)	23.4	Cβ, Cγ
ϵ	0.83 d (6.4)	23.5	0.93 d (6.4)	21.5	Cβ, Cγ, Cδ
CONH	7.27 d (8.4)			174.7	
			Arg		
α	4.73 m	52.8	4.36	53.1	
β	2.15, 1.90 m	27.9	2.00, 1.67 m	27.9	
γ	1.90, 1.70 m	26.1	1.67, 1.53 m	26.1	
δ	3.32 m	42.2	3.19 m	41.7	guan
δNH	8.20 br s				
CONH	8.29 br s			176.1[e]	
guan	7.92 overlapped			158.3[e]	
			Thr-1		
α	4.40 d overlapped	63.4	3.96 d (3.7)	63.4	CONH
β	4.56 m	66.8	4.36 overlapped	66.8	
γ	1.45 d (6.0)	21.0	1.33 d (7.4)	20.1	Cα, Cβ
OH	8.65 br d				
CONH	8.54 br s			172.0	
			Thr-2		
α	5.88 (9.2)	56.5	5.48 d (2.7)	56.3	CONH, Cβ
β	6.07 m	72.2	5.60 m	72.4	
γ	1.52 (6.4)	15.1	1.26 d (6.8)	14.6	Cα
CONH	10.1 br s			172.9[e]	
			DiMeGln		
α	5.03 d overlapped	59.3	4.28 d (9.5)	58.7	CONH, Cβ
β	2.81 m	38.5	2.39 m	37.7	βMe
βMe	1.60 d (7.2)	13.3	1.26 d (6.8)	14.6	
γ	3.35 m	40.8	2.84 m	42.8	βMe
γMe	1.40 d (6.8)	16.2	1.33 (6.5)	14.7	$CONH_2$
CONH	9.78 br s			173.5	
$CONH_2$[d]	7.50			180.4[e]	
			AGDHE		
α	4.72 d (8.4)	72.0	3.99 d (9.1)	72.0	
β	4.10 br d (8.4)	75.2	3.65 br d (9.1, 2.2)	75.2	
γ	4.60 overlapped	50.6	4.21 m	50.8	
δ	1.96, 1.78 m	29.6	1.70, 1.60 m	26.1	
ϵ	1.78 m	26.1	1.58 m	25.4	
ζ	3.39 m	42.2	3.19 m	42.1	guan
γNH	8.30 br s				
ζNH	8.50 br s				
CO				177.2[e]	
guan	7.92 overlapped			158.4	

Table 1 (Continued)

	pyr-d_5[b]		methanol-d_4		
	δ_H	δ_c	δ_H	δ_c	COLOC
			Ala		
α	4.80 m	50.4	4.40 overlapped	51.0	CONH, Cβ
β	1.51 d (7.1)	18.3	1.48 d (7.1)	17.8	CONH, Cα, CO-TMHEA
CONH	8.90 d (6.8)			176.2	
			TMHEA		
1				178.7	
2	2.90	44.5	2.68 m	44.7	
3	3.71 dd (8.8, 1.6)	79.5	3.52 dd (8.8, 3.0)	79.5	
4	1.71 m	33.0	1.78 m	33.5	
5	1.31, 1.21 m	38.8	1.26 m	39.3	C9
6	1.50 m	25.7	1.75 m	25.8	
7	0.81 d (6.4)	24.7	0.98 d (6.4)	24.7	C9
8	1.11 d (6.4)	14.6	1.11 d (6.8)	14.4	C2, C4, C1
9	1.01 d (6.8)	17.7	1.01 d (6.8)	17.0	
10	0.74 d (6.4)	21.6	0.91 d (6.8)	21.5	C5

[a] Assignments based on 2D-COSY, 2D-HOHAHA, HETCOR, and COLOC experiments. [b] Quaternary carbons in pyr-d_5 were not assigned due to the failure of obtaining $^1H-^{13}C$ long range connectivities under various experimental conditions in this solvent. [c] Coupling constants are enclosed in parentheses and given in Hz. [d] Tentative assignments based on NOESY experiments: MeGln, cross peak δ 7.30/1.93; DiMeGln cross peak δ 7.50/3.55. [e] These assignments are interchangeable. [f] AGDHE: 4-amino-7-guanidino-2,3-dihydroxyheptanoyl residue. [g] TMHEA: 3-hydroxy-2,4,6-trimethylhepantoyl residue.

to those of Arg, confirmed by the cross peak between the low field resonating methylene protons at δ 3.19 and the guanidino



(2R,3R,4S)-AGDHE (2)

carbon at δ 158.4 ppm in the COLOC spectrum, whereas COSY and HOHAHA[21] spectra revealed three contiguous methines substituted by two oxygen and one nitrogen functionalities. The location of an amide function at C-4 and two hydroxyl groups at C-2 and C-3 was based on the ^1H and ^{13}C NMR chemical shifts. The isolation of the intact C-3 to C-7 unit as Arg after periodate oxidation, oxidative workup, and hydrolysis (see below) confirmed the presence of 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (**2**).

After the ^1H and ^{13}C NMR assignments by COSY, HOHAHA,[21] HMQC,[22] and COLOC[23] of all signals of each amino acid residue (Table 1), there were remaining signals in the spectra for four secondary methyls, four methines, one of which at δ 3.52 (79.5 ppm) assignable to an hydroxymethine, and one carbonyl. The COSY spectrum revealed the presence of two spin systems, C(2)H(Me)-C(3)H(OH)- and $(Me)_2CH-CH_2$-C(4)H(Me)-, which were connected together into the gross structure **3** from key COLOC spectral data *via* $^4J_{CH}$ correlations from the Me proton signal at δ 1.11 (Me at C-2) to the CH carbon at δ 33.5 ppm (C-4). A carbonyl signal at δ 178.7 ppm also correlated with the methyl proton signals at δ 1.11 (Me at C-2). These data indicated the presence of the 3-hydroxy-2,4,6-trimethylheptanoic acid residue (**3**), which was then isolated after acid hydrolysis. The presence of two primary amides was confirmed by methylation of **1** with CH_2N_2, resulting in an increase of only 14 mass units in the FAB mass spectrum, as implied by the presence of the phenolic group of the β-methoxytyrosine residue. All the chemical and spectroscopic data so far collected indicate a $C_{68}H_{116}N_{18}O_{20}$ molecular formula in agreement with the mass spectral data.

The stereochemistry of standard amino acids was determined by HPLC analysis of the acid hydrolysate derivatized with Marfey's reagent,[24] which allowed us to assign the L configuration for the Ala, *N*-MeAla, Leu, and Thr residues and the D configuration for the Arg residue. In our hands the Marfey's derivatives of D-Arg and L-Thr had identical retention times. The configuration of both residues were solved by applying the Marfey's method on the amino acids isolated after acid hydrolysis (see below).

The *S* configuration at C-4 of the new 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid residue (AGDHE, **2**) was determined by cleavage of the C-2, C-3 diol in **1** with sodium periodate, followed by oxidative workup and hydrolysis with 6 N hydrochloric acid, giving L-Arg. Coupling constants in the ^1H NMR spectrum of **1** relative to the AGDHE residue were consistent with the β-hydroxycarbonyl residue existing in a hydrogen bonded half chair conformation with H-2 and H-3 quasi-axial, *i.e.*, 2*R*,3*R* or 2*S*,3*S* ($J_{2,3}$ = 9.1 Hz in methanol-d_4). The value of the coupling constant H-3/H-4 (2.2 Hz), in good agreement with that calculated for the minimum energy conformation for the 2*R*,3*R*,4*S* configuration ($J_{3,4}$ = 2.0 Hz) and not for 2*S*,3*S*,4*S* configuration ($J_{3,4}$ = 4.7 Hz), allowed the 2*R*,3*R*,4*S* configuration of the AGDHE unit to be proposed (Figure 1).

In order to determine the configuration of the amino acid residues NMeGln and 3,4-diMeGln and of the hydroxy acid **3** as well as to confirm the structures of the new amino acids, the acid hydrolysate, after extraction with dichloromethane to remove the lipidic 3-hydroxy-2,4,6-trimethylheptanoic acid (**3**) from the mixture, was subjected to droplet counter current chromatography (DCCC) in 10:10:1:6 chloroform−methanol−butanol−water (1% TFA), with the organic phase as the mobil phase, which achieved successful separation of the constituents. The 3-hydroxy-2,4,6-trimethylheptanoic acid (**3**), *m/z* 189 $[M + H]^+$ in FABMS, was characterized by ^1H NMR spectroscopy and sequential decoupling experiments (see Experimental Section). The relative stereochemistry as 2*R**,3*R**,4*S** is tentatively proposed on the same coupling constants arguments used for

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	ϕ (H_β-C_β-C_γ-H_γ)	J (H_β-H_γ)	
		calculated	experimental
4S, 3R, 2R	68.2	2.0 Hz	2.2 Hz
4S, 3S, 2S	48.1	4.7 Hz	

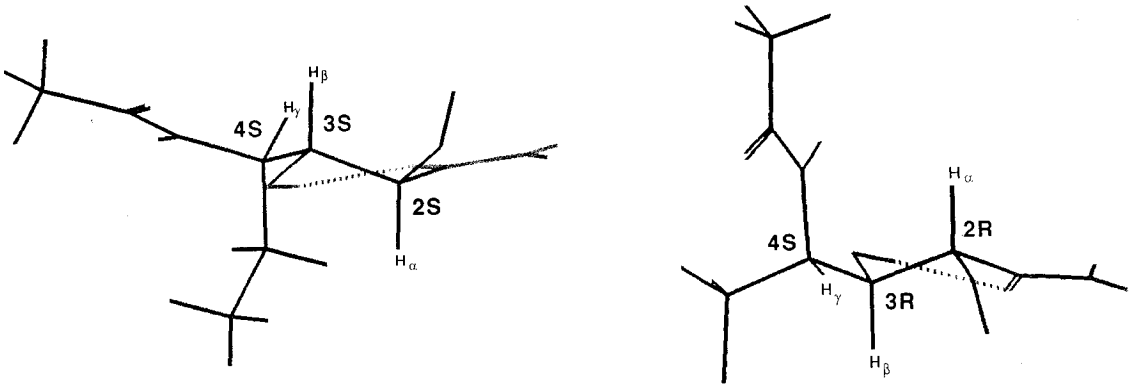
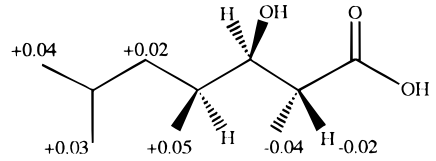


Figure 1. CHARm calculated lowest energy conformers of (4*S*,3*R*,2*R*)- and (4*S*,3*S*,2*S*)-4-amido-7-guanidino-2,3-dihydroxyheptanoyl residue (AGDHE)



3-hydroxy-2,4,6-trimethylheptanoic acid (3)

Figure 2. $\Delta\delta$ (δ_S-δ_R) values in ppm obtained at 500 MHz for the MTPA esters of **3**.

the AGDHE residue. Coupling constants in the ^{1}H NMR spectrum of **3** were consistent with the β-hydroxycarbonyl residue existing in a hydrogen bonded half chair conformation with H-2 and H-3 quasi-axial ($J_{2,3}$ = 7.7 Hz), *i.e.*, 2*R**,3*R**. The value of the coupling constant H-3/H-4 (4.1 Hz) is in good agreement with that calculated for the minimum energy conformation for the 2*R**,3*R**,4*S** configuration ($J_{3,4}$ = 3.85 Hz) and not for 2*R**,3*R**,4*R** configuration ($J_{3,4}$ = 1.27 Hz). The determination of the absolute stereochemistry at C-3 of **3** was achieved by the modified Mosher method.[25] The O-(*R*)- and -(*S*)-MTPA (2-methoxy-2-(trifluoromethyl)-2-phenylacetic) derivatives of **3** were prepared, and $\Delta\delta$ values ($\delta_S-\delta_R$) were determined at 500 MHz. Negative $\Delta\delta$ were found for the protons on C2(CH_3) side of the MTPA plane, whereas positive values were found for protons on C4-C7 side (Figure 2). This meant that C3 had to have the *R* configuration and that the absolute configuration of **3** can be proposed as 2*R*,3*R*,4*S* as shown.

The first eluted fractions from DCCC contained a mixture of *N*-methylpyroglutamic acid and 3,4-dimethylpyroglutamic acid, which were separated by HPLC using a Vydac C_{18} column equipped with an UV detector operating at 205 nm and gradient elution from 100% H_2O to 80% of aqueous methanol (0.1% TFA). The first HPLC peak contained the *N*-methylpyroglutamic acid, which was identified by ^{1}H NMR and ^{13}C NMR and comparison with a reference compound, obtained by heating in water commercial *N*-methyl-L-glutamic acid.[26] The CD spectra of both the reference *N*-methyl-L-pyroglutamic acid and the *N*-methylpyroglutamic acid obtained from **1** showed positive maxima at 217 nm, thus establishing the L-configuration of NMeGln residue in **1**.[27] The ^{1}H NMR spectrum of the second HPLC eluted peak revealed signals for two contiguous methines substituted by methyls and one CH(α). These data indicated that the less polar component of this mixture was related to 3,4-dimethylglutamic residue. Since mass spectroscopy with various ionization sources, EI, CI, and FAB, did not give useful information, we resorted to the derivatization procedure with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) as donor of the *tert*-butyldimethysilyl groups[28] followed by EI mass spectrum, which was then taken to a GC-MS apparatus. The mass spectrum was dominated by diagnostic ions at *m/z* 370 $(M-15)^+$ (weak), *m/z* 328 $(M-57)^+$ (intense), and *m/z* 229 $(M-159)^+$ (intense), originating from the molecular ion by loss of a methyl and a *tert*-butyl group, and by cleavage at the α-carbon (loss of CO_2TBDMS). Other diagnostic ions were represented by *m/z* 385 $(M-43)^+$ and *m/z* 300 $(M-85)^+$. These fragments indicated the presence of an amino acid with molecular weight of 157 daltons, containing two TBDMS groups, which corresponded to the structure of 3,4-dimethylpyroglutamic acid. The stereochemistry of C-2 was assigned to be L (2S) on the basis of a positive Cotton effect at 217 nm.[27] The stereochemistry at C-3 and C-4 was suggested from the NMR NOE data, which indicated that the H-2 and both the methyls were located on the same side of the molecule. From the remaining DCCC fractions of the acid hydrolysate of **1**, we isolated *N*-MeAla, Ala, and Thr, in that order, followed by a mixture of Arg and 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE, **2**) which was characterized by ^{1}H NMR (see Experimental Section) of the mixture and FABMS (+), which gave a major peak at *m/z* 252, corresponding to its ammonium salt. The fractions containing Thr and Arg were subjected to Marfey's analysis and confirmed the

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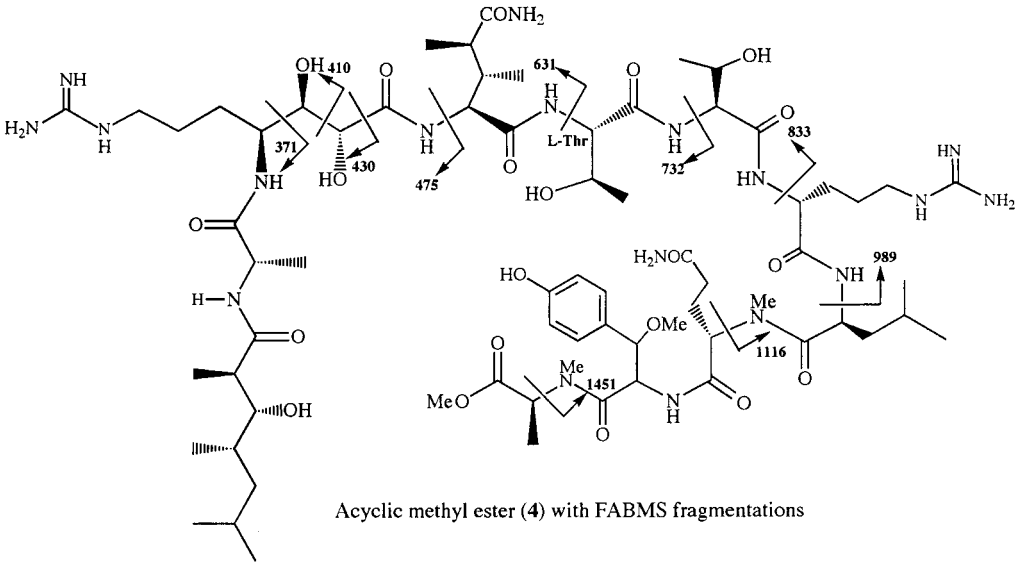
Chart 2



Acyclic methyl ester (**4**) with FABMS fragmentations

L-configuration of Thr and the D-configuration of Arg. The β-methoxytyrosine, labile under acid conditions, was the only piece lost during the acid hydrolysis. The ^{1}H NMR spectrum of the total hydrolysate mixture revealed only dispersed aromatic signals confused in the background of the spectrum.

Amino acid sequence of callipeltin A (**1**) was deduced by interpretation of the NOESY spectrum in pyridine-d_5, which gave sequential information from correlations between amide protons and α-protons of the adjacent residue *via* amide bond, as shown in **1**. The attachment of the 3-hydroxy-2,4,6-trimethylheptanoyl residue to the α-amino group of the N-terminus Ala, as first indicated by the COLOC correlation between the Ala α-proton and the carbonyl of the heptanoyl residue at 178.7 ppm and supported by a NOESY cross peak between the Ala amide proton and the proton attached to C-2 of the heptanoyl residue, was definitively confirmed by isolation, after mild acid hydrolysis in 6 N HCl at 140 °C for 1 h, of the *N*-(3-hydroxy-2,4,6-trimethylheptanoyl)-L-alanine. The amino acid was characterized by EIMS, *m/z* 259, M^+, and ^{1}H NMR spectroscopy. The remaining connection to be clarified was that in the macrocyclic lactone between the carbonyl group of the C-terminal MeAla, whose α-proton did not give rise to cross peaks with any amide protons in the NOESY spectrum, and one of the hydroxyl groups of the two Thr residues. The β-proton of Thr-2 resonated at low-field, δ 5.60 in methanol-d_4 and 6.07 in pyridine-d_5, thereby revealing partecipation of the hydroxyl group of this residue in the lactone formation. Furthermore, treatment of callipeltin A (**1**) with sodium methoxide in methanol dry furnished the acyclic methyl ester **4**. In addition to the pseudomolecular ion at *m/z* 1537 $[M + H]^+$, corresponding to the introduction of 32 mass units in the molecule, the FAB mass spectrum provided several fragment ion peaks, which were consistent with the amino acids sequence of callipeltin A (**1**) as shown in **4**. The major peaks correspond to N-terminal fragments due to the cleveage of the linkage adjacent to the amide bond and 1H transfer, referred to as C1-fragments in the Roepstorff and Folhlman peptide fragmentation nomenclature.[29]

The antiviral activity of callipeltin A (**1**) was measured on CEM4 lymphocytic cell lines infected with HIV-1 (Lai strain). In order to evaluate the antiviral activity of callipeltin A (**1**), we studied the inhibition of cytopathic effects (CPE) induced by HIV-1, using the MTT cell viability to determine the CD_{50} (50% cytotoxic dose) and ED_{50} (50% effective dose) as described previously.[30] At day six post-infection, callipeltin A (**1**) exhibited a CD_{50} of 0.29 μg/mL and a ED_{50} of 0.01 μg/mL giving a selectivity index (SI ratio CD_{50}/ED_{50}) of 29. AZT reference has a CD_{50} of 50 μM and a ED_{50} of 30 nM. The antifungal activity of callipeltin A (**1**) was measured against *Candida albicans*, whose growth was inhibited at 100 μg/disc (6 mm) with 30 mm of inhibition.

Conclusion

Callipeltin A (**1**) is a novel antiviral and antifungal cyclodepsipeptide which contains three unusual amino acid residues: (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), which is apparently derived by a two carbon atoms homologation of arginine; β-methoxytyrosine, whose stereochemistry waits to be elucitated; and (3*S*,4*R*)-3,4-dimethyl-L-glutamine. All three, to the best of our knowledge, have not been isolated from natural sources. The general structure of callipeltin A (**1**) with the N-terminus blocked and the C-terminus lactonized with a Thr residue, giving rise to a macrocyclic lactone, is similar in these aspects to other marine cyclodepsipeptides such as, for example, didemnins, a family of potent antitumoral and antiviral compounds from *Tridemnum* tunicate,[31] discodermins, potent inhibitors against phospholipase A2, from sponges of the genus *Discodermia*,[15] and theonellapeptolides from the sponge *Theonella sp*.[8]

Experimental Section

General Information. NMR measurements were performed on a Bruker AMX-500. The instrument was interfaced with an Bruker X-32 computer. The callipeltin A (**1**) samples were prepared dissolving 20 mg in 0.4 mL of methanol-d_4 or in 0.4 mL of pyridine-d_5.

Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were performed in methanol-d_4 and in pyr-d_5 by

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(31) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsaki, S. A.; Scahill, T.A. *J. Am. Chem. Soc.* **1981**, *103*, 1857. For a review, see: Rinehart, K. L., Jr.; Shield, L. S.; Cohen-Parson, M. *Antiviral Substances in Marine Biotechnology*; Attaway, D. H., Zaborsky, O. Q., Eds.; Plenum Press: New York, London, 1993; Vol. 1, pp 309−342.

employing the conventional pulse sequence. The COSY spectra were obtained using a data set ($t_1 \times t_2$) of 1024 × 512 points for a spectral width of 4201.7 Hz (relaxation delay 1 s) in methanol-d_4 and 5154.6 Hz in pyr-d_5, respectively . The data matrix was processed using a unshifted sine bell window function, following transformation to give a magnitude spectrum with symmetrization (digital resolution in F2 dimension: 4.10 Hz/pt in methanol-d_4 and 5.03 Hz/pt in pyr-d_5).

The 2D-HOHAHA[21] (pyr-d_5) experiment was performed in the phase sensitive mode (TPPI) using a MLEV 17 sequence for mixing. The spectral width (t_2) was 5434.8 Hz; 512 experiments of 48 scans each (relaxation delay 1.5 s, mixing time 100 ms) were aquired in 1K data points. For processing, an unshifted sine bell window function was applied in both dimension before transformation. The resulting digital resolution in F2 was 5.30 Hz/pt.

The NOESY (pyr-d_5) experiment was performed in the phase-sensitive mode (TPPI). The spectral width (F_2) was 6172.8 Hz; 512 experiments of 64 scans each (relaxation delay 1.0 s, mixing time 400 ms) were acquired in 2K data points. For processing, a sine bell window function was applied in both dimension before transformation. The resulting digital resolution in F2 was 3.01 Hz/pt.

^{13}C, ^{1}H shift correlation experiment (HETCOR) was performed in methanol-d_4 (125 MHz). The spectral width in ^{13}C dimension was 17 241.3 (1024 points) and 3703.7 Hz (128 time increments) along the ^{1}H domain; for each FID 256 scans were recorded. The digital resolution in F2 was 37.5 Hz/pt.

The HMQC experiment (pyr-d_5) was performed according to Bax *et al.*[22] The spectral width in ^{1}H dimension was 3703.7 Hz; 512 experiments of 32 scans each were acquired in 1K data points (relaxation delay 1.5 s). A sine square window function was applied in both dimension before transformation (digital resolution in F2 was 3.62 Hz/pt).

^{13}C, ^{1}H longe range shift correlation experiment (COLOC[23]) was performed in methanol-d_4 (125 MHz). Spectral width in F2 was 22 727.23; 256 experiments of 128 scans were aquired in 1K data points (relaxation delay 1.5 s). The digital resolution in F2 was 22.2 Hz/pt.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Fast atom bombardment mass spectra (FAB MS) were recorded in a glycerol-matrix in the positive ion mode on a VG ZAB instrument (Argon atoms of energy of 2−6 KV). IR spectrum was performed on a IFS 48 Bruker instrument. UV spectra were recorded on a Beckman DU70 spectrophotometer, and CD spectra were recorded on a JASCO 710 spectrometer.

Molecular Mechanics Calculations. All the calculations were carried out on a SGI Personal Iris 35/G using the force field CHARMm (QUANTA 4.0 software package). The two diastereomeric molecular models for the AGDHE residue (2*R*,3*R*,4*S*) and (2*S*,3*S*,4*S*) were generated by imposing an hydrogen bond between the amide carbonyl and the hydroxyl group attached to C-3. Then the lowest energy conformation for each diastereomer was searched by energy minimizing a number of different initial conformations. Energy minimization was performed using a conjugate gradient algorithm and a distance dependent dielectric (to partially compensate for the absence of the solvent) until the energy gradient was less then 0.001 Kcal/mol. The two conformations (one for each diastereomer) displaying the lowest potential energy were selected and the ϕ dihedral angle (Hβ−Cβ−Cγ−Hγ) measured, so that the coupling constants for the two stereoisomers could be calculated (by means of a modified Karplus equation) and then compared to the corresponding experimental value. The same procedure was applied for searching the lowest energy minimum conformations of the diastereomeric β-hydroxy acid (**3**).

Isolation of Callipeltin A (1). *Callipelta* sp. (Demospongiae, Lithistida, Corallistidae) was collected in 1992 and 1993 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 Nouméa. 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 and then with 8:2 CH_2Cl_2:MeOH (3 × 2 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 applied in five runs to a DCCC apparatus (7:13:8 $CHCl_3$:MeOH:H_2O, ascending mode; fractions of 4 mL were collected). Fractions 13−17, mainly containing callipeltin A (**1**), were further purified by HPLC on a Waters C-18 μ-Bondapak column (7.8 mm i.d. × 30 cm) with MeOH:H_2O (50:50) as eluent to give 500 mg of pure **1** (t_r =10.8 min).

Callipeltin A (**1**): $[\alpha]_D = +3.56°$ (*c* 0.012 M, MeOH); UV (MeOH) λ_{max} 232 (ϵ = 6919), 274 (ϵ = 1272); IR (KBr) 3330, 1740, 1660, 1520 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; FABMS and ESMS in the text.

Amino Acid Analysis of Callipeltin A (1). For standard amino acid analysis, 100 μg of callipeltin A (**1**) was dissolved in 0.5 mL of 6 N HCl in a evacuated glass tube and heated at 110 °C for 16 h. After evaporation, the residue was dissolved in 0.5 mL of HCl and subjected to amino acid analysis on a Beckman 118BL system. Retention times in the amino acid analysis (min) were as follows: Thr (18.9), Ala (33.3), Leu (44.2), Arg (102.4), NH_3 (160.0).

For a large scale hydrolysis, a 50 mg sample of callipeltin A (**1**) dissolved in 5 mL of 6 N HCl and heated at 130 °C for 4 h. The crude hydrolysate was extracted (2 × 1mL) with CH_2Cl_2. The CH_2Cl_2 layer afforded 4 mg of 3-hydroxy-2,4,6-trimethylheptanoic acid (**3**); FABMS *m/z* 189 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, d, J = 6.8 Hz, H-9), 0.92 (3H, d, J = 6.8 Hz, H-10), 0.96 (3H, d, J = 5.8 Hz, H-7), 1.12−1.18 (2H, m, H-5 and H-5′), 1.26 (3H, d, J = 7.7 Hz, H-8), 1.64 (1H, m, H-6), 1.70 (1H, m, H-4), 2.73 (1H, quintet, J = 7.7 Hz, H-2), 3.48 (1H, dd, J = 4.1, 7.7 Hz, H-3). The aqueous layer was fractionated by DCCC [10:10:1:6 $CHCl_3$:MeOH: *n*BuOH:H_2O (0.1% TFA), descending mode], and 4 mL fractions were collected. Fractions 18−43 (A) contained a mixture of 3,4-dimethylpyroglutamic acid and *N*-methylpyroglutamic acid; fractions 59−70 (B) contained Leu, fractions 143−158 (C) contained a mixture of Ala and Me-Ala, fractions 197−217 (D) contained Thr and, fractions 231−241 (E) contained a mixture of Arg and 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (**2**): FABMS *m/z* 252 (4-amino-7-guanidino-2,3-dihydroxyheptanoic acid, $[M + NH_4]^+$);^{1}H NMR (pyr-d_5) δ 1.90 (4H, m, H-6 AGDHE and H-4 Arg), 2.15 (2H, m, H-5 AGDHE and H-3 Arg), 2.25 (2H, m, H-5′ AGDHE and H-3′ Arg), 3.30 (4H, m, H-7 AGDHE and H-5 Arg), 4.13 (2H, m, H-4 AGDHE and H-2 Arg), 4.59 (1H, dd, J = 7.5, 3.0 Hz, H-3 AGDHE), 4.98 (1H, d, J = 7.5 Hz, H-2 AGDHE). Fraction A was further purified by HPLC on a Vydac 218 TP protein-peptide RP column (flow rate 4 mL/min, eluent: H_2O (0.1% TFA) for 10 min, CH_3CN−H_2O (0.1% TFA) 10−80% in 30 min) to give 0.5 mg of pure *N*-methylpyroglutamic acid (t_r = 23.2 min) and 0.4 mg of pure 3,4-dimethylpyroglutamic acid (t_r = 23.4 min).

N-Methylpyroglutamic acid: CD $[\theta]_{216nm} = +3151$ (*c* 0.13 10^{-4} M, H_2O); ^{1}H NMR (pyr-d_5) δ 2.05 (1H, m, H-3), 2.12 (1H, m, H′−3), 2.20 (1H, m, H-4), 2.40 (1H, m, H-4′), 2.88 (3H, s, N-Me), 4.12 (1H, dd, J = 9.3, 3.3 Hz, H-2); ^{13}C NMR: δ 22.6, 28.4, 29.2, 61.7, 174.5 and 175.0. The compound shows identical spectral properties with a synthetic sample obtained by heating in water under reflux overnight the commercial L-*N*-methylglutamic acid.[26]

3,4-Dimethylpyroglutamic acid: CD $[\theta]_{208nm} = +1534$ (*c* 0.001 M, H_2O); ^{1}H NMR (pyr-d_5) δ 1.01 (3H, d, J = 6 Hz, Me on C-3), 1.04 (3H, d, J = 6.8 Hz, Me on C-4), 2.65 (1H, m, H-4) 2.71 (1H, m, H-3), 4.02 (1H, d, J = 3.8 Hz, H-2). A 0.1 mg aliquot of compound was treated with 50 μL of MTBSTFA [*N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide] in 0.5 μL of CH_3CN at 150 °C for 150 min. After cooling to room temperature the solution was directly applied to GC-MS under conditions reported by Des Neves *et* Vasconcelos;[28] *m/z* 370, 328, 229, 385, 300.

Determination of Absolute Configuration. (a) HPLC Analysis of the Marfey's Derivatives.[24] To a 10% acetone solution (50 μL) of 1-fluoro-2,4-dinitrophenyl-5-alanine amide (L-FDAA) and 1 N $NaHCO_3$ (40 μL) was added 100 μg of the peptide acid hydrolysate, and the mixture was kept at 50 °C for 1 h. After cooling to room temperature 2 N 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[24]. Amino acids were derivatized as described above. The peaks were identified by co-injection with a DL-mixture of standard amino acids. Due to the overlapping of L-Thr and D-Arg peaks, we used the DCCC enriched fractions D and E for the identification of these amino acids. Retention times (min) are given in parentheses. L-Thr and D-Arg (18.1), L-Ala (21.4), L-MeAla (28.3), L-Leu (37.6).

(b) Periodate Oxidation of Callipeltin A (1). A solution of callipeltin A (**1**) (1.5 mg) and sodium periodate (200 mg) in water (0.5 mL), adjusted to pH 4.0 with acetic acid, was stirred overnight at 25 °C. The solvent was lyophilized, and the residue was dissolved in hydrogen peroxide (2 mL) and formic acid (1 mL). The solution was heated at 70 °C for 20 min and cooled, and the solvent was evaporated under reduced pressure. The reaction product was subjected to acid hydrolysis and Marfey's derivatization as described above. An additional peak, corresponding to L-Arg (retention time: 16.4 min), was observed in the HPLC trace of Marfey's derivatives.

(c) *R* (+) and *S* (−) Mosher Esters of 3. Freshly distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) chloride (6 μL) was added to a solution of **3** (2 mg) with a catalytic amount of 4-(dimethylamino)pyridine and 5 μL of freshly distilled triethylamine, in 1 mL of freshly distilled CH_2Cl_2 and allowed to stand at room temperature for 12 h under argon atmosphere. The residue, obtained after evaporation of the solvent, was subjected to reverse-phase HPLC using a linear gradient from water to CH_3CN (100%), UV detector: λ = 260 nm, to obtained 0.5 mg of (*R*)-(+)-MTPA ester of **3**. The same procedure was used to obtain 0.4 mg of (*S*)-(−)-MTPA ester of **3**. 3-(+)-(*R*)-MTPA ester of **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (3H, d, J = 5.8 Hz, H-7), 0.87 (3H, d, J = 6.8 Hz, H-10), 0.92 (3H, d, J = 6.8 Hz, H-9), 1.10 (2H, m, H-5 and H-5′), 1.36 (3H, d, J = 7.5 Hz, H-8), 1.62 (1H, m, H-6), *ca.* 1.70 (1H, overlapped with H_2O, H-4), 3.16 (1H, quintet, H-2), 3.70 (1H, dd, H-3). 3-(−)-(*S*)-MTPA ester of **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (3H, d, J = 5.8 Hz, H-7), 0.90 (3H, d, J = 6.8 Hz, H-10), 0.97 (3H, d, J = 6.8 Hz, H-9), 1.13 (2H, m, H-5 and H-5'), 1.32 (3H, d, J = 7.5 Hz, H-8), 1.64 (1H, m, H-6), *ca* 1.70 (1H,overlapped with H_2O, H-4), 3.14 (1H, quintet, H-2), 3.72 (1H, dd, H-3).

Methylation of Callipeltin A (1). A solution of 1 mg of callipeltin A (**1**) in MeOH was treated with an excess of CH_2N_2 in ether at room temperature for 1 h. The solution was taken to dryness under a stream of N_2 and analyzed by FABMS, *m/z* 1519 $[M + H]^+$, and ^{1}H NMR: the spectrum contained a new methyl signal at δ 3.80 (3H, s).

Methanolysis of Callipeltin A (1) Giving the Opened Methyl Ester (4). A solution of 1 mg of callipeltin A (**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 0.1 N 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) was directly subjected to FABMS analysis. FAB-MS fragmentations are shown in **4**.

Partial Hydrolysis of Callipeltin A (1) Giving the *N*-(3-Hydroxy-2,4,6-trimethylheptanoyl)-L-Alanine Residue. Callipeltin A (**1**) (30 mg) was treated with 2 N HCl (2 mL), and after degasing the reaction mixture was heated at 145 °C for 1 h in a sealed tube. The reaction mixture was extracted with CH_2Cl_2 (3 × 2 mL) and the dichloromethane layer afforded 1.5 mg of *N*-(3-hydroxy-2,4,6-trimethylheptanoyl)-L-alanine: EIMS *m/z* 259 (M^+); ^{1}H NMR (methanol-d_4) δ 0.87 (3H, d, J = 6.4 Hz, H-13), 0.94 (3H, d, J = 6.5 Hz, H-14), 0.95 (3H, d, J = 6.5 Hz, H-10), 1.11 (1H, ddd, J = 14.0, 9.0 and 4.5 Hz, H-8), 1.18 (3H, d, J = 7.1 Hz, H-12), 1.33 (1H, ddd, J = 14.0, 9.5 and 3.0Hz, H-14), 1.41 (3H, d, J = 7.5 Hz, H-11), 1.67 (2H, m, H-7 and H-9), 2.60 (1H, quintet, J = 7.1 Hz, H-5), 3.67 (1H, t, J = 7.1 Hz, H-6), 4.38 (1H, q, J = 7.5 Hz, H-2).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, 2D-COSY, and 2D-NOESY spectra of callipeltin A (**1**) (12 pages). See any current masthead page for ordering and Internet access instructions.

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