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Structure and total synthesis of cyclodidemnamide B, a cycloheptapeptide from the ascidian *Didemnum molle*

Axelle Arrault, Anne Witczak-Legrand, Philippe Gonzalez, Nataly Bontemps-Subielos and Bernard Banaigs*

Centre de Phytopharmacie, UMR5054 CNRS, Université de Perpignan, 66860 Perpignan, France

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Abstract—A new cycloheptapeptide, cyclodidemnamide B (**1**), has been isolated from the marine ascidian *Didemnum molle*, collected at Ibo island, Mozambique. The structure of the bisthiazole-containing macrocyclic peptide was initially assigned using 2D NMR analysis and data comparisons with cyclodidemnamide. The absolute configuration was deduced after hydrolysis and characterization of degradation residues by chiral HPLC analysis. The total synthesis served as an unambiguous structural and stereochemical proof. © 2002 Elsevier Science Ltd. All rights reserved.

Lissoclinum and *Didemnum* ascidians are prolific producers of cyclic peptides. A particularly intriguing family of marine cyclopeptides produced by *Lissoclinum patella* and *Didemnum molle* is the 18-, 21- and 24-membered cyclopeptides characterized by an alternating sequence of thiazole, thiazoline or oxazoline heterocycles and hydrophobic amino acids.[1]

Didemnum molle is a common Indo Pacific ascidian associated with endosymbiotic Prochloron algae. Different cyclic peptides were isolated from *D. molle* collected in different locations; the hexapeptides (18-membered) comoramides A and B and the heptapeptides (21-membered) mayotamides A (**2**) and B from two collections in Comoro islands,[2] the heptapeptides cyclodidemnamide (**3**) from Philippine islands[3] and mollamide (**4**) from Australia.[4] As for the *Lissoclinum* azole-containing cycloheptapeptides trunkamide A[5] and lissoclinamides 4, 5[6] and 7,[7] the configuration of the chiral centers adjacent to the thiazole/thiazoline rings in cyclodidemnamide has been revised after total synthesis.[8] The synthesis of mollamide[9] confirmed the published structure.

In this paper, we report the isolation, characterization and total synthesis of a new cycloheptapeptide, we named cyclodidemnamide B, from *D. molle* collected at Ibo island within the Quirimba archipelago, north of Mozambique.

Exhaustive extraction of the alcohol-preserved ascidians with EtOH and CH_2Cl_2 followed by solvent partition of the resulting oil gave a diethylether fraction. The Et_2O sample was subjected successively to silica gel chromatography (heptane/ethyl acetate/methanol) and C_{18} RP HPLC. Cyclodidemnamide B was obtained as colorless amorphous solid[10] and was negative to ninhydrin test suggesting a blocked N-terminus.

The NMR spectra (Table 1) of cyclodidemnamide B were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$ which gave the best dispersion for ^{1}H NMR analysis; one set of resonances was observed for each residue, indicating that one conformation is strongly dominant in this solvent. Spectral data, including IR, UV, ^{1}H and ^{13}C NMR and FABMS, confirmed that the isolated peptide was related to cyclodidemnamide (**3**) which as a model, allowed to elucidate the structure and to describe the complete NMR spectral assignments. The molecular formula $C_{32}H_{47}N_7O_6S_2$ was consistent with the molecular ion $(M+H)^+$ at m/z 689.30 (FABMS) and supported by NMR data. The ^{13}C and DEPT spectra of cyclodidemnamide B, showed 32 unique resonances.

The peptidic nature of the compound was established by the presence of five ^{13}C NMR carbonyl (or carbonyl equivalent) resonances between 169.8 and 171.3 ppm

Keywords: cycloheptapeptide; thiazole-containing peptide; ascidian; *Didemnum molle*.

* Corresponding author. Fax: (33) 0468662223; e-mail: banaigs@univ-perp.fr

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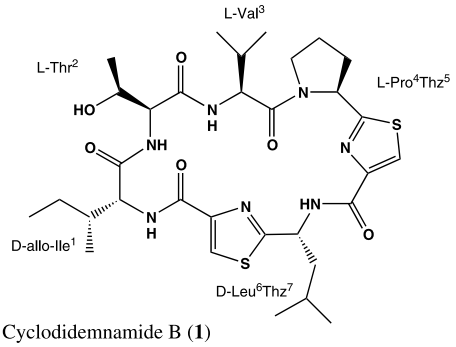
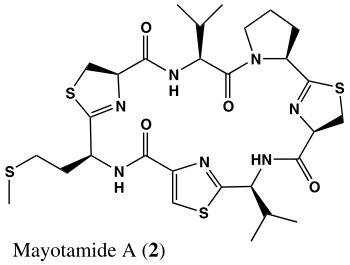
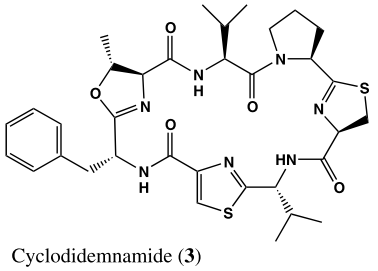


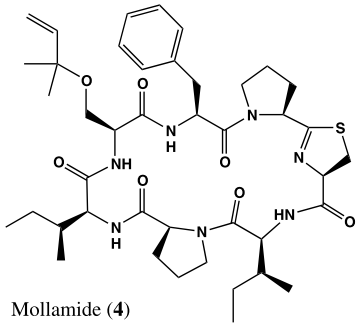
Cyclodidemnamide B (**1**)



Mayotamide A (**2**)



Cyclodidemnamide (**3**)



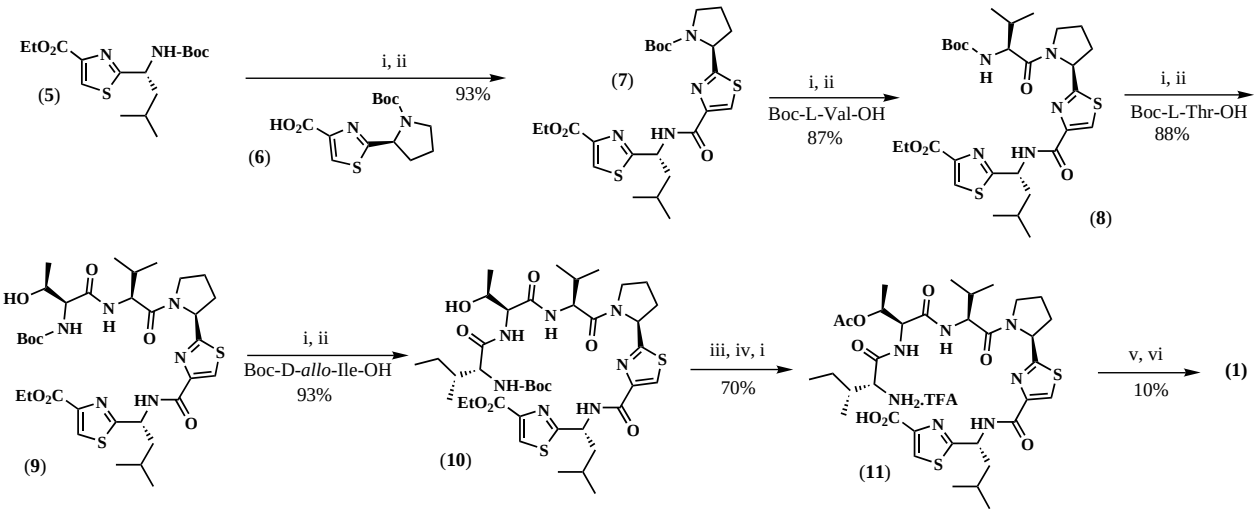
Mollamide (**4**)

and by the presence of four amide doublets in the ^{1}H NMR spectrum. A combination of DQF-COSY and HOHAHA experiments allowed a straightforward identification of spin systems for one isoleucine, one threonine, one proline, one valine and one leucine. Two additional 1H singlets at δ 7.92 and 7.94 in the ^{1}H NMR spectrum were observed as a key signature of thiazole-containing peptides. A HSQC experiment was used to correlate all the protonated carbons with their respective protons. Further evidence for the two thiazole rings was provided by long range heteronuclear correlations from the isolated protons singlets at δ 7.92 and 7.94 with the corresponding quaternary Cα-Thz (δ 150.8 and 150.2) and carbon atoms (δ 170.1 and 169.2) adjacent both to the nitrogen and sulfur atoms. HMBC experiments, optimized for 8 and 4 Hz couplings, supported the assignment of the leucylthiazole and prolylthiazole units and were used to establish the Ile-Thr-Val-ProThz-LeuThz sequence. Break in the assignments of the peptide occurred for the connection LeuThz-Ile. However, the cyclic nature of the peptide could be confirmed by phase-sensitive NOESY and ROESY experiments with a correlation observed between the H-CβThz^7 and the H-CβIle.[1]

Hydrolysis of (**1**) followed by Marfey's derivatization[11] and HPLC analysis of the formed diastereomers, assigned the chirality of Thr^2 and Val^3 as L and Ile^1 as D. The configuration of the β Ile carbon (D-Ile or D-*allo*-Ile) could not be determined in our conditions. It is known that the Cα next to the thiazole and thiazoline racemizes readily during hydrolysis, making the stereochemical assignments for the thiazole-amino acid units unreliable.[6] So the configuration of Pro^4 and Leu^6

Table 1. NMR assignments of cyclodidemnamide B (**1**) in $CDCl_3$ (ppm)

		^{1}H	^{13}C			^{1}H	^{13}C
Ile^1	NH	8.00		Pro^4	CαH	5.74	56.69
	CαH	4.58	57.64		$CβH_2$	2.32	30.20
	CβH	2.05	37.68		$CγH_2$	2.11	24.92
	$CγH_2$	1.55/1.77	26.03		$CδH_2$	3.43/3.54	46.35
	CH_3-Cβ	0.97	11.40		CS		170.19
	$CδH_3$	1.03	14.96	Thz^5	Cα		150.77
	CO		173.28		CβH	7.97	122.82
Thr^2	NH	7.05			CO		160.40
	CαH	4.43	56.69	Leu^6	NH	7.89	
	CβH	4.47	64.85		CαH	5.53	48.22
	CH_3-Cβ	1.20	18.41		$CβH_2$	1.92/2.09	44.32
	CO		171.47		CγH	1.53	24.95
Val^3	NH	7.94			$CδH_3$	0.94	22.31
	CαH	4.55	55.21		$Cδ'H_3$	0.99	22.58
	CβH	1.75	31.74		CS		169.08
	$CγH_3$	0.15	15.53	Thz^7	Cα		150.27
	$Cγ'H_3$	0.63	19.93		CβH	7.99	123.25
	CO		169.90		CO		160.13



Scheme 1. Synthesis of cyclodidemnamide B (**1**). *Reagents and conditions*: (i) TFA:CH_2Cl_2 (1:9), rt, 1 h; (ii) EDC, HOBt, TEA, THF, 0°C–rt, 18 h; (iii) aq. NaOH, dioxanne:H_2O (1:3), rt, 2 h; (iv) Ac_2O, TEA, cat. DMAP, DMF, rt, 4 h; (v) DPPA, $i Pr_2NEt$, DMF, 0°C–rt, 3 days; (vi) aq. K_2CO_3, CH_3OH, 0°C, 1 h.

residues was assumed to be, respectively L and D as the corresponding prolylthiazoline and valylthiazole units in the revised structure of cyclodidemnamide[12] and lissoclinamides 4, 5 and 7.[8] Furthermore the very similar ^{13}C and ^{1}H chemical shifts of the Val[3] methyl groups were indicative of a similar conformation of the macrocycle with that of cyclodidemnamide;[4] an absolute L-Pro[4]Thz[5]–D-Leu[6]Thz[7] configuration should be the most favorable for the stabilization of these conformationally preorganized 21-membered cyclopeptides. The L chirality of Val[6]Thz[7] in mayotamide A (**2**) is an exception to this rule.[2]

Since no more natural product was available for further investigations, total synthesis of cyclodidemnamide B stereomers was the only way to solve the stereochemical problem. Among the synthetic targets that we considered essential as reference materials for an unambiguous structural correlation were the D-Ile[1] and D-*allo*-Ile[1] stereomers. The total synthesis of the D-*allo*-Ile[1] isomer is summarized in Scheme 1. Compounds **5** and **6** were synthesized employing the modified Hantzsch method.[13] After removing the BOC protecting group in **5**, a coupling reaction between the corresponding free amine and **6** then gave rise to the compound **7**. Upon the same procedure, **7** was fused on Boc-L-Val-OH, Boc-L-Thr-OH and Boc-D-*allo*-Ile-OH, leading to compound **10**. After saponification of **10** to its carboxylic acid and acetylation of the hydroxy group of this compound, cyclization using DPPA–$i Pr_2NEt$–DMF[6] at room temperature for three days gave cyclopeptide **11**. Removal of the hydroxy protecting group in **11** produced finally cyclodidemnamide B. In a similar fashion, the D-Ile isomer was prepared from intermediate **9** of the previous synthesis.

A careful comparison of ^{1}H and ^{13}C NMR resonances for the two synthetic cyclodidemnamide B analogues with the natural product revealed that the D-*allo*-Ile isomer provided an excellent spectroscopic match. Any difference in ^{1}H NMR with $\Delta\delta > 0.05$ ppm between the natural compound and the D-Ile isomer, was observed.

Some minor but significant differences in ^{13}C (4 carbons shifted in the range from 0.2 to 0.8 ppm) NMR resonances occurred between the two peptides. The changes occurred solely for the Ile residue (Cβ, $\Delta\delta$ 0.22; Cγ, $\Delta\delta$ 0.86; Cδ, $\Delta\delta$ 0.57; CH3-Cβ, $\Delta\delta$ 0.41). Most significantly; the ^{1}H and ^{13}C NMR spectra of natural product and synthetic D-*allo*-isomer were superimposable.

In summary, from these results we proposed structure **1**, cyclo-[(2*R*,3*R*)-Ile-(2*S*,3*S*)-Thr-(2*S*)-Val-(2*S*)-ProThz-(2*R*)-LeuThz], for cyclodidemnamide B. The total synthesis has provided an unambiguous structural and stereochemical proof.

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