Tetrahedron Letters 49 (2008) 5297–5299

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Hexamollamide, a hexapeptide from an Okinawan ascidian *Didemnum molle*

Toshiaki Teruya, Hiroaki Sasaki, Kiyotake Suenaga *

Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522, Japan

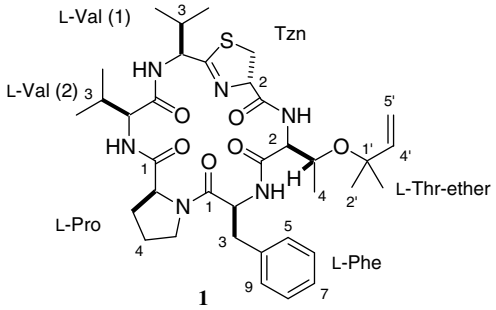
ARTICLE INFO

Article history:
Received 16 May 2008
Revised 16 June 2008
Accepted 19 June 2008
Available online 22 June 2008

ABSTRACT

The bioassay-guided fractionation of the cytotoxic constituents of the Okinawan ascidian *Didemnum molle* led to the isolation of hexamollamide (**1**), a hexapeptide. The gross structure and relative stereostructure of **1** were established by spectroscopic analysis including 2D NMR techniques and single-crystal X-ray diffraction analysis. The absolute stereostructure was determined by chiral HPLC analysis of acid hydrolysates of **1**. Hexamollamide (**1**) exhibits moderate cytotoxicity against HeLa S_3 cells.

Cyclic peptides have been isolated from a number of marine organisms, and many show remarkably high levels of cytotoxicity. Notable examples from ascidians are the thiazole-containing cyclic peptides isolated from *Lissoclinum patella*[1,2] and *L. bistratum*,[3] and the didemnins, which are depsipeptides from the Caribbean ascidians *Tridemnum solidum*[4] and *T. cyanophorum*[5] and from the Mediterranean ascidian *Aplidium albicans*.[6] In our continuing search for new substances from marine ascidians, we investigated the constituents of an Okinawan ascidian *Didemnum molle* collected at Okinawa Prefecture, and isolated a new hexapeptide, hexamollamide (**1**). In this report, we describe the isolation, structural elucidation, and biological activity of hexamollamide (**1**).[7]



1

The Okinawan ascidian *Didemnum molle* (280 g) was extracted with methanol (1 L) for 7 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation using ODS silica gel (40% aqueous MeOH to MeOH) and reverse-phase HPLC (Develosil ODS-HG-5, 80% aqueous MeOH) to give hexamollamide (**1**) (14.3 mg). Hexamollamide (**1**) exhibited moderate cytotoxicity against HeLa S_3 cells, with an IC_{50} value of 17 μg/mL.

The NMR data (Table 1) coupled with $[M+Na]^+$ peaks at *m/z* 719.3550 (Δ −1.7 mmu) in the ESIMS of hexamollamide (**1**) suggested a molecular formula of $C_{36}H_{52}N_6O_6S$. The signals due to the minor rotamers were not observed. The ^1H NMR data showed the presence of four amide NH groups (δ 7.83, 7.56, 7.21, and 6.56). In addition, the ^{13}C NMR spectrum in combination with DEPT experiments confirmed the presence of six carbonyl (or carbonyl equivalent) carbons between 169.5 and 177.3 ppm. A COSY experiment (Fig. 1) was used to characterize the major features of the constituent amino acid, and the resulting assignments were confirmed using HMBC and HMQC spectra. From these data, a phenylalanine (Phe), two valines (Val), proline (Pro), and a threonine (Thr) were identified. In addition, a detailed analysis of the COSY spectra of **1** allowed two partial structures, C2–C3 (Tzn = thiazoline) and C4′–C5′, to be constructed, as shown in Figure 1. Furthermore, HMBC crosspeaks, H-2′/C1′, H-3′/C1′, and H-4′/C1′, suggested the connectivity C1′–C2′, C1′–C3′, and C1′–C4′. Thus, the presence of a dimethylallyl ether group was confirmed. HMBC crosspeaks H-3 (Thr)/C1′ suggested that this dimethylallyl ether group was attached to the threonine oxygen. Furthermore, the sulfur-bearing methylene carbon was considered to be C3 (Tzn) based on its chemical shift (δ_c 38.1). In addition, HMBC crosspeaks H-3 (Tzn)/C1 (Val (1)) and H-2 (Tzn)/C1 (Val (1)) indicated a thiazoline ring. Considering the molecular formula and degree of unsaturation, **1** is a cyclic hexapeptide. Finally, detailed HMBC experiments (Fig. 1) were used to determine the connectivity between all six amino acids, thus constructing the cyclic hexapeptide structure of hexamollamide (**1**) as shown in formula **1**.

Hexamollamide (**1**) was crystallized from toluene at room temperature. The relative stereostructure of **1** was confirmed by X-ray crystallographic analysis (Fig. 2).[8]

The absolute stereostructure of **1** was elucidated as follows. Acid hydrolysis of **1** (9 M HCl, 110 °C, 76 h) followed by reversed-phase HPLC separation afforded Phe.[9] The absolute configuration

* Corresponding author. Tel.: +81 45 566 1820; fax: +81 45 566 1819.
E-mail address: suenaga@chem.keio.ac.jp (K. Suenaga).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.06.086

Table 1
NMR data for hexamollamide in CD_3COCD_3

Position	^{1}H (ppm)[a]	^{13}C (ppm)[b]
L-Thr-ether		
1		169.5
2	4.06 dd (4.9, 4.5)	56.8
3	4.11 m	67.2
4	0.87 d (6.8)	15.9
1′		78.0
2′	1.38 s	27.9
3′	1.44 s	25.2
4′	6.00 dd (17.1, 11.2)	144.5
5′	5.31 d (17.1)	114.4
	5.15 d (11.2)	
NH	7.21 d (4.9)	
Tzn		
1		170.6
2	5.05 dd (9.3, 1.5)	77.7
3	3.77 dd (11.6, 1.5)	38.1
	3.59 dd (11.6, 9.3)	
L-Val (1)		
1		177.3
2	4.77 dd (9.8, 3.9)	57.4
3	2.27 m	32.6
4	0.92 d (6.8)	16.4
5	1.03 d (6.8)	20.2
NH	7.56 d (9.8)	
L-Val (2)		
1		171.3
2	4.61 dd (5.9, 3.0)	57.6
3	2.25 m	34.3
4	0.84 d (6.8)	17.5
5	0.85 d (6.8)	20.0
NH	6.56 d (5.9)	
L-Pro		
1		172.1
2	3.80 d (8.3)	62.6
3	1.42 m, 1.77 m	32.2
4	1.76 m, 1.84 m	22.7
5	3.36 m, 3.55 m	46.8
L-Phe		
1		171.9
2	4.70 dt (8.3, 8.3)	53.0
3	2.94–2.98 t m	39.0
4		137.3
5, 9	7.27 m	130.1
6, 8	7.33 m	129.4
7	7.25 m	127.8
NH	7.83 d (8.3)	

[a] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses.
[b] Recorded at 150 MHz.

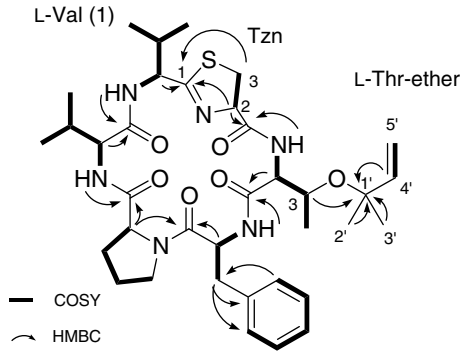


Figure 1. Structure of hexamollamide (**1**), based on 2D NMR correlations.

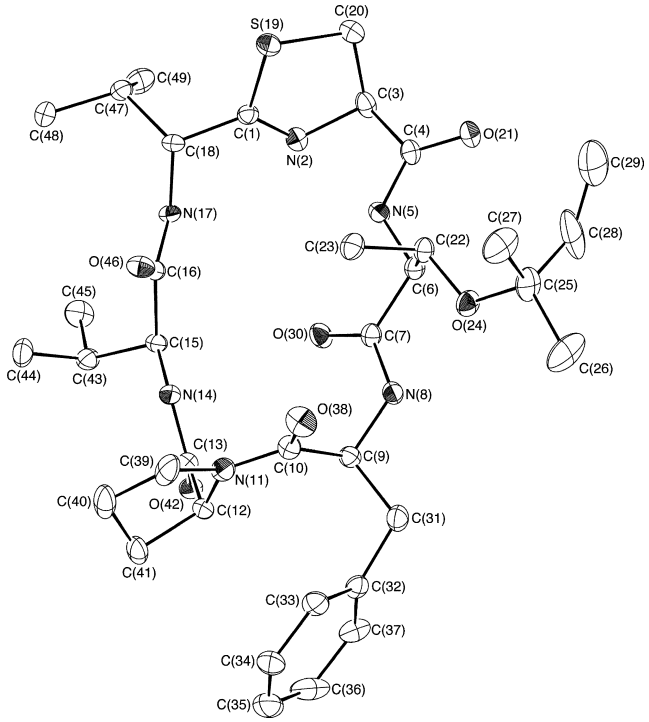


Figure 2. Crystallographically independent two hexamollamide molecules exist in the unit cell, and one of them is shown (because there are essentially no structural differences). Thermal ellipsoids are 30% probability. Hydrogen atoms and solvent molecules are omitted for clarity. Thermal ellipsoids are 30% probability. Hydrogen atoms and solvent molecules are omitted for clarity.

of Phe was determined to be L by chiral HPLC analysis.[10] Thus, the absolute stereostructure of hexamollamide was determined as shown in formula **1**.

In conclusion, hexamollamide (**1**), a hexapeptide, was isolated from the Okinawan ascidian *Didemnum molle.* The gross structure and relative stereostructure of **1** were established by spectroscopic analysis including 2D NMR techniques and single-crystal X-ray diffraction analysis. The absolute stereostructure was determined by chiral HPLC analysis of acid hydrolysates of **1**. Hexamollamide showed moderate cytotoxicity against HeLa S_3 cells, with an IC_{50} value of 17 μg/mL. Hexamollamide (**1**) contains a thiazoline ring and a threonine amino acid, which is modified by attachment to a dimethylallyl ether. Hexamollamide (**1**) is structurally similar to patellin 2 isolated from *L. patella*.[11,12] The Tzn-Thr dimethylallyl ether is the same in both. Patellins are cyclic hexa-, hepta-, and octa-peptides, where the structure has been modified by inclusion of a thiazoline ring and a dimethylallyl unit associated as a threonine or serine ether residue. Patellin 2 is isolated from *L. patella*. The genus *Lissoclinum* is a rich source of novel biologically active natural products. *L. patella,* for example, has yielded over 20 thiazole-containing macrolides and an antibacterial polyketide lactone and cyclic peptides.[13] Other cyclic peptides that include thiazole and a reverse prenyl unit are mollamide,[14] trunkamide A,[12] comoramide A, B,[15] and mollamide B.[16]

Acknowledgments

We would like to thank Professors Akira Sekiguchi and Masaaki Ichinohe (Univ. of Tsukuba) for the measurement of X-ray crystallographic analysis and helpful discussion. We would like to thank Professor Hideo Kigoshi (Univ. of Tsukuba) for performing the ESIMS analysis. This work was supported by Grant-in-Aid for Young Scientists (B), Keio Leading-edge Laboratory of Science and Technology, Keio Gijuku Academic Development Funds, The Japan Securities Scholarship Foundation.

References and notes

1. Ireland, C. M.; Durso, A. R.; Newman, A. R.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.
2. Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Van den Brenk, A.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1349.
3. Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; Van den Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 1349.
4. Rinehart, K. L.; Kishore, V.; Bible, K. C.; Sakai, R.; Sullins, D. W.; Li, K.-M. *J. Nat. Prod.* **1998**, *51*, 1.
5. Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsac, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
6. Schmitz, F. J.; Yasumoto, T. *J. Nat. Prod.* **1991**, *54*, 1469.
7. hexamollamide (**1**): $[\alpha]_D^{22} + 104.6$ (*c* 1.00, $CHCl_3$). ^1H NMR and ^{13}C NMR see Table 1. HRMS (ESI) Exact mass calcd for $C_{36}H_{52}N_6O_6SNa$ $[M+Na]^+$ requires *m/z* 719.3567, found *m/z* 719.3550.
8. *Crystal data for* **1**·(toluene)·$(H_2O)_{0.5}$. $C_{43}H_{61}N_6O_{6.5}S$, Mw = 798.04, orthorhombic, $P2_12_12_1$ (No. 19), *a* = 12.6110(6) Å, *b* = 25.8190(5) Å, *c* = 27.9540(11) Å, *V* = 9101.9(6) Å^3, *Dc* = 1.165 g/cm^3, *Z* = 8, R_1 = 0.0672 ($I > 2.0\sigma(I)$), wR_2 = 0.1889 (all data), GOF = 1.025 (all data). CCDC-686830.
9. Chiral HPLC analysis was performed for phenylalanine because of its strong UV absorption.
10. Conditions for chiral HPLC analysis: column, CHIRALPAK MA(+) (4.6 × 50 mm); solvent, 2 mM $CuSO_4$/MeCN = 4/1; flow rate 0.5 mL/min; detection at 254 nm. The retention times of authentic samples: L-Phe (13.2 min), D-Phe (9.9 min).
11. Zabriskie, T. M.; Foster, M. P.; Stout, T. J.; Clardy, J.; Ireland, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 8080.
12. Carroll, A. R.; Coll, J. C.; Bourne, D. J.; MacLeod, J. K.; Zabriskie, T. M.; Ireland, C. M.; Bowden, B. F. *Aust. J. Chem.* **1996**, *49*, 659.
13. Faulkner, D. J. *Nat. Prod. Rep.* **1994**, *11*, 355 and earlier reports in the series.
14. Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1994**, *47*, 61.
15. Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *Tetrahedron* **1998**, *54*, 13203.
16. Donia, M. S.; Wang, B.; Dunbar, D. C.; Desai, P. V.; Patny, A.; Avery, M.; Hamann, M. T. *J. Nat. Prod.* **2008**, *71*, 941.