ORGANIC LETTERS

2008
Vol. 10, No. 20
4569−4572

Isolation, Structure Determination, and Synthesis of Galaxamide, A Rare Cytotoxic Cyclic Pentapeptide from a Marine Algae *Galaxaura filamentosa*

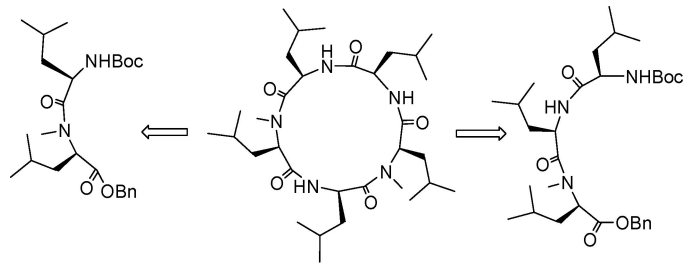
Wen-Jie Xu,[†] Xiao-Jian Liao,[‡] Shi-Hai Xu,[*,‡] Jian-Zhong Diao,[‡] Bin Du,[§] Xu-Long Zhou,[§] and Shan-Shan Pan[‡]

Institute of Hydrobiology, Department of Chemistry, and Medical College, Jinan University, Huangpu Road West 601, Guangzhou, Guangdong 510632, People's Republic of China

txush@jnu.edu.cn

Received August 4, 2008

ABSTRACT



Galaxamide (**1**)

Galaxamide (1), a rare cyclic pentapeptide, was isolated from the marine algae *Galaxaura filamentosa*. A preliminary bioassay of Galaxamide showed remarkable in vitro antiproliferative activities against GRC-1 and $HepG_2$ cell lines. The first total synthesis of the cyclic peptide was achieved for further biological evaluation.

Cyclopeptides have many physiological functions and curatorial worthiness; for example, aplidine (dehydrodidemnin B) from the *ascidian Aplidium albicans*.[1,2] As part of our continuing search for structurally and pharmacologically interesting secondary metabolites from marine algae, *Galaxaura filamentosa*, we found a rare cyclopeptide (**1**) composed of a kind of amino acid and its derivates. Herein, we report the isolation, structure elucidation, biological activity, and synthesis of the peptide that we have named Galaxamide (**1**).

Specimens of *Galaxaura filamentosa* were collected from the Xisha Island of South China Sea in May of 2001. The EtOH extract from *Galaxaura filamentosa* was concentrated under vacuum to yield an orange−brown gum. The crude residue was partitioned between water and petroleum ether. The organic layer was concentrated under vacuum to yield a greenish gum (20.5 g), and this residue was then subjected to silica gel column chromatography using petroleum ether (60−90) containing an increasing amount of EtOAc and $CHCl_3$ containing an increasing amount of MeOH as an eluent, respectively. The fraction eluted with petroleum−EtOAc (5:5) was subjected to Sephadax LH-20 (MeOH:CH_2Cl_2 1:1), yielding colorless needles, compound **1** (15 mg).

Galaxamide (**1**), $[\alpha]^{20}_D$ −130° (C 0.1, MeOH), mp 208−210 °C, was obtained as colorless needles and showed

[†] Institute of Hydrobiology
[‡] Department of Chemistry
[§] Medical college

(1) Rinehart, K. L. *Med. Res. Rev.* **2000**, *20*, 1–27.
(2) Schmitz, F. J.; Yasumoto, T. *J. Nat. Prod.* **1991**, *54*, 1469–1490.

10.1021/ol801799d CCC: $40.75
Published on Web 09/25/2008

Table 1. ^{1}H and ^{13}C NMR Data for **1** at 600 MHz in Pyridine-d_5

position		^{13}C	^{1}H (δ_H, J in Hz)	position		^{13}C	^{1}H (δ_H, J in Hz)
N-MeLeu1	N-CH_3	40.2	3.43 (s)	Leu2	NH		8.59 (d, 9.8)
	αCH	65.8	3.90 (dd, 10.7, 4.7)		αCH	48.3	5.28 (m)
	βCH_2	37.5	2.41 (m); 1.86 (m)		βCH_2	40.7	1.65 (m); 1.52 (m)
	γCH	25.4	1.69 (m)		γCH	25.2	1.84 (m)
	δCH_3	22.8	0.87 (m)		δCH_3	21.6	0.97 (d, 6.6)
		23.4	0.76 (d, 6.6)			20.7	0.83 (d, 6.7)
	C=O	172.0			C=O	172.1	
Leu1	NH		8.33 (d, 9.1)	Leu3	NH		8.65 (d, 7.9)
	αCH	49.3	5.30 (m)		αCH	53.5	4.88 (m)
	βCH_2	42.2	2.09 (m); 1.80 (m)		βCH_2	40.3	2.15 (m); 1.97 (m)
	γCH	24.7	1.76 (m)		γCH	25.3	1.92 (m)
	δCH_3	22.9	1.02 (d, 6.5)		δCH_3	21.7	0.89 (m)
		22.7	0.88 (m)			23.2	0.87 (m)
	C=O	173.0			C=O	174.7	
N-MeLeu2	N-CH_3	39.4	3.27 (s)				
	αCH	71.4	3.81 (t, 8.0)				
	βCH_2	39.2	2.64 (m); 2.25 (m)				
	γCH	26.3	1.72 (m)				
	δCH_3	23.0	0.93 (d, 6.6)				
		21.9	0.89 (m)				

negative reaction to ninhydrin reagent but positive reaction after hydrolysis with 6 mol/L of HCl. The moleclular formula of **1** was deduced as $C_{32}H_{59}N_5O_5$ by means of spectral analysis and TOF-MS-ES+ [$(M + H)^+$ 594.4597, calcd *m/z* 594.4594]. The IR absorption bands of **1** at 3351, 3305, and 1638 cm^{-1} were characteristic of amino and amide carbonyl groups. The structure of **1** was assigned using a combination of one- and two-dimensional NMR spectroscopic methods. The ^{1}H and ^{13}C NMR spectral data acquired for **1** illustrated typical resonances for a cyclic peptide (Table 1). The presence of five carbonyl resonances at 172.0, 172.1, 173.0, 173.2, and 174.7 together with the presence of three NH protons and two N-Me functionality suggested Galaxamide was a monocyclic pentapeptide. The ^{13}C NMR spectrum (Table 1) displayed signals for 32 unique carbons, and the DEPT experiment indicated that **1** contained 12 methyl, 5 methylene, and 10 methine carbons. The five signals at δ_H 3.81 (t, $J = 8.0$), 3.90 (dd, $J = 10.7, 4.7$), 4.88 (m), 5.28 (m), and 5.30 (m) were indicative of α-proton resonances for amino acids. Analysis of 1D-TOCSY, HSQC, and HMBC data established the presence of three leucine (Leu) and two *N*-methylleucine (N-Me-Leu) residues (Table 1). The sequence of the cyclic peptide was established by analysis of HMBC data, which generally showed correlations of the carbonyl carbons of each amino acid with the corresponding NH or N-CH_3 proton of the adjacent residue. Further evidence was provided by TOF-MS-MS-ES+ which showed the fragments of I to V as follows:

I m/z 594 [Leu-N-MeLeu-Leu-N-MeLeu-Leu+H]$^+$

II m/z 481 [Leu-N-MeLeu-Leu-N-MeLeu+H]$^+$

III m/z 368 [N-MeLeu-Leu-N-MeLeu+H]$^+$

IV m/z 354 [Leu-N-MeLeu-Leu+H]$^+$

V m/z 241 [Leu-N-MeLeu+H]$^+$

Therefore, the structure of compound **1**, a new pentapetide, was elucidated as cyclo(-N-Me-Leu1-Leu1-N-Me-Leu2-Leu2-Leu3-).

The absolute stereochemistry for the amino acids of **1** was determined by hydrochloric acid hydrolysis of **1** followed by treatment of the hydrolysate with Marfey's reagent.[3] Analysis of the mixture of FDAA derivatives by HPLC, using retention times and coinjections with standards, revealed all leucines of **1** were assigned to L-configuration.

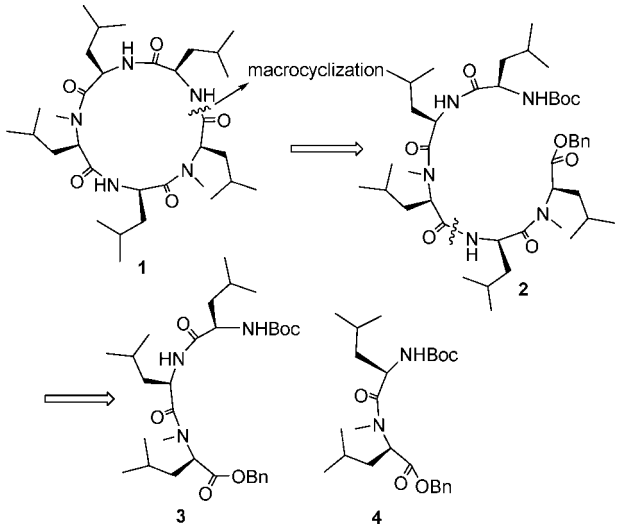
In vitro cytotoxic assay accomplished by the MTT method[4] following exposure of cells to the tested compounds for 72 h showed that **1** was remarkably active against the human renal cell carcinoma GRC-1 and human hepatocellular carcinoma $HepG_2$ cell lines with corresponding IC_{50} values of 4.26 and 4.63 μg/mL, and those of the commercially available Mitomycin C which tested as a positive reference are 6.01 and 0.41 μg/mL, respectively. Following the experiment that indicated Galaxamide possesses potent antitumor properties, we thus carried out the total synthesis of **1** to provide access to additional material for further biological evaluation.

Compoud **1** is a high symmetry cyclopeptide, which is composed of three leucines and two *N*-methyl leucines. In the previous paper, Sakurai[5] described the synthesis of the cyclic peptide c (-Leu_5-) which is a analogue of **1**. Here we designed a solution phase route for the synthesis of **1** and chose to elaborate **3** from the tripeptide **4**, then synthesized **2** from the tripeptide **3**. The target product **1** was to be produced in a final step of cyclization from pentapeptide **2** (Scheme 1).

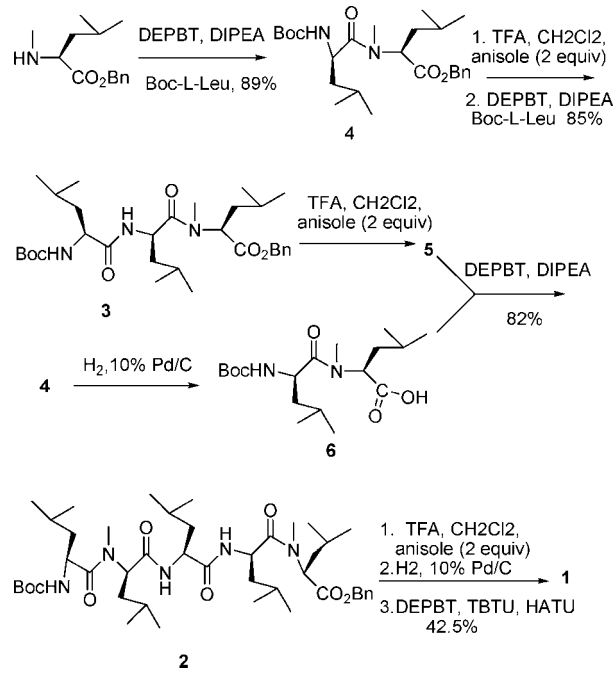
Following the strategy, our synthesis started from dipeptide **4**. Reaction of the commercially available *tert*-butyl-L-leucine with *N*-methyl-L-leucine benzyl ester in the presence of a coupling reagent, 3-(diethoxyphosphoryloxy)-3*H*-benzo[d][1,2,3] triazin-4-one (DEPBT), and

(3) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(4) Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55–63.
(5) Sakurai, A.; Okumura, Y. *Bull. Chem. Soc. Jpn.* **1979**, *52* (2), 540–543.

Scheme 1. Retrosynthesis Strategy of **1**



Scheme 2. Synthesis of **1**



diisopropylethylamine (DIPEA) gave the monocrystal dipeptide **4**[6] in 89% yield.[7] Removal of the Boc group in **4** using TFA was coupled with Boc-L-leucine using DEPBT as a coupling reagent, leading to the tripeptide **3** in 85% yield (from **4** to **3**). We removed the Boc group of **3** using TFA to **5** and took off the benzyl from **4** using hydrogen reduction with Pd/C[8] to **6**. The synthesis of the pentapeptide **2** was achieved by coupling of **5** and **6** in the presence of DEPBT and DIPEA in 82% yield (from **3** to **2**) (Scheme 2). We found, upon completion, each reaction was concentrated, needed not carry out the postproccessing, and directly subjected to silica gel column chromatography using *n*-hexane/acetone (20:1) isocratic elution when we prepared the three intermediates **2**−**4** using DEPBT as the coupling reagent.

The cyclization is a key step in the synthesis of cyclic peptides. After the Boc group and benzyl in pentapeptide **2** were removed using TFA and hydrogen reduction in Pd/C, respectively, the dried, crude, free amine/free acid linear pentapeptide was dissolved in a 2:2:1 ratio of THF/CH_3CN/CH_2Cl_2 (0.004 M). Then, three coupling agents, DEPBT, [2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (0.7 equiv each), and DIPEA (6 equiv) were added to the reaction.[9,10] The reaction was usually completed within about 4 days. The final product was generated after removing the solvent by distillation under reduced pressure, and the mixture was disssolved in EtOAc and washed with ammonium chloride. The organic layers were combined, dried, filtered, and concentrated. The crude mixture was purified by reverse phase HPLC to the target product **1** in 42.5% yield (from **2** to **1**) (Scheme 2).

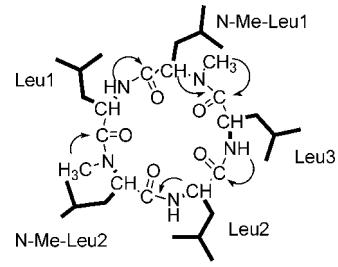


Figure 1. TOCSY and key HMBC (H→C) correlation of **1**.

In summary, we have isolated the rare cytotoxic cyclic pentapeptide **1** from the marine algae *Galaxaura filamentosa*. The structure as well as the absolute configuration were determined by a combination of its spectral data and Marfey's method. The efficient synthesis described herein should allow for the preparation of various analogues of this bioactive cyclic peptide. We believe that the characteristic cyclopeptide composed of a kind of amino acid will be a potential lead compound. Further studies on analogues and bioactivity of **1** are underway.

(6) Liao, X. J.; Xu, W. J.; Xu, S. H.; Dong, F. F. *Acta Crystallogr. E* **2007**, *63*, O3313–U4398.
(7) Li, H. T.; Jiang, X. H.; Ye, Y. H.; Fan, C. X.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1* (1), 91–93.
(8) Zhu, J. D.; Ma, D. W. *Angew. Chem., Int. Ed. Engl.* **2003**, *42* (43), 5348–5351.
(9) Belofsky, G. N.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1999**, *40* (15), 2913–2916.
(10) Rodriguez, R. A.; Pan, P. S.; Pan, C. M.; Ravula, S.; Lapera, S.; Singh, E. K.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; McAlpine, S. R. *J. Org. Chem.* **2007**, *72* (6), 1980–2002.

Acknowledgment. This work was supported by grants from the National High Technology Development Project (863 Project) (Nos. 2006AA09Z408 GDSFC 06025194, 2005A30503001, and 2006Z3-E4041) and National natural Science Fund (Nos. 20772048).

Supporting Information Available: Experimental details, NMR assignments for Galaxamide, NMR spectra of **1** and synthetic intermediates, and Marfey's method. This material is available free of charge via the Internet at http://pubs.acs.org.

OL801799D