New Cyclic Peptides from the Ascidian *Lissoclinum patella*

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Four new cyclic peptides, patellamide G (**2**) and ulithiacyclamides E−G (**3**−**5**), along with the known patellamides A−C (**6**−**8**) and ulithiacyclamide B (**9**), were isolated from the ascidian *Lissoclinum patella* collected in Pohnpei, Federated States of Micronesia. The planar structures of these peptides were determined from 1D and 2D ^{1}H and ^{13}C NMR spectra. The absolute stereochemistries of the amino acid units, except for cysteine, were assigned by chiral GC analysis of *N*(*O*)-trifluoroacetyl isopropyl ester derivatives of amino acids obtained by acid hydrolysis of the intact and ozonized peptides. The structures of ulithiacyclamides E−G (**3**−**5**) were confirmed by chemical conversion. Patellamides B (**7**) and C (**8**) exhibited in vitro modulation of multidrug resistance in CEM/VBL$_{100}$ cells.

Ascidians are sessile, filter-feeding marine invertebrates belonging to the phylum Chordata. They are a rich source of biologically active cyclic peptides.[1] Of these, didemnin B is probably the most noted because it was the first marine natural product evaluated in clinical trials as an anticancer agent.[2] A variety of cyclic peptides have been isolated from the ascidian *Lissoclinum patella* Gottshaldt (Didemnidae), and some of these exhibit strong in vitro cytotoxicity.[3−15] Our group initially isolated several cyclic peptides from *L. patella* collected in Australia[16] and one of these, patellamide D (**1**), reverses multidrug resistance (MDR) in vitro.[17] In a continuation of our search for anti-MDR agents from marine organisms, we have investigated extracts of *L. patella* collected in Pohnpei, Federated States of Micronesia, and isolated four new cyclic peptides, patellamide G (**2**), and ulithiacyclamides E−G (**3**−**5**), along with four known ones, patellamides A (**6**), B (**7**), and C (**8**),[12,13] and ulithiacyclamide B (**9**).[5] We report here the isolation and structure determination of these compounds.

Results and Discussion

Compounds **2**−**9** were isolated from the CH_2Cl_2 solubles of MeOH and MeOH−CH_2Cl_2 extracts of *Lissoclinum patella* by open column chromatography over Si gel, followed by reversed-phase HPLC on a C_{18} column. The known patellamides A (**6**), B (**7**), and C (**8**),[12,13] and ulithiacyclamide B (**9**)[5] were identified by comparison of their FABMS and ^{1}H and ^{13}C NMR data with literature values. The ^{1}H and ^{13}C NMR data of **9** taken in DMSO-d_6 are reported herein to compare these data with those of new peptides **3**−**5**.

A feature common to the four new peptides (**2**−**5**) was that one of the threonine units or more was not cyclized to an oxazoline ring. All these compounds possessed ^{13}C NMR signals at δ ca. 60 (α-C) and ca. 65 (β-C), which were in agreement with those in prelissoclinamide-2 and preulicyclamide,[13] which also have a noncyclized threonine. Hence, these signals were considered to be characteristic for the presence of a "free" threonine unit. In contrast, the α- and β-carbons of the oxazoline rings absorb at δ ca. 73 and ca. 82, respectively. The ^{1}H NMR signals for the β-H of the "free" threonine units resonated much more upfield (δ 3.6−4.0 ppm in DMSO-d_6) than the β-Hs in the oxazolines (δ ca. 5.0 ppm in $CDCl_3$; 4.75 ppm in DMSO-d_6).

Patellamide G (**2**) had the molecular formula $C_{38}H_{50}N_8O_7S_2$ based on HRFABMS analysis, *m*/*z* 795.2234 $[M + H]^+$ (Δ −0.2 mmu), and NMR data (Table 1). This formula differed from that of patellamide B (**7**) by 18 mass units. Acid hydrolysis of patellamide G (**2**) yielded L-threonine (two equivalents), L-isoleucine, L-leucine, D-alanine, and D-phenylalanine, just as was found for patellamide B (**7**). Hence, the difference in molecular formulas suggested that one of the threonine units in patellamide G was not cyclized to an oxazoline. The partial structures corresponding to the "free" threonine, the substituted oxazoline, isoleucine, leucine, alanine, phenylalanine, and two thiazole rings were identified from COSY, RELAY−COSY, HMQC, and HMBC experiments, and by comparison of the NMR data of **2** with appropriate patellamide models.[12−15] These partial structures were assembled using HMBC data (Figure 1). Though the amino acid compositions of patellamides B (**7**) and G (**2**) were identical, the amino acid sequence within the macrocycle was different. Interestingly, in peptide **2**, neither the ^{1}H NMR signal of H-2 nor the ^{13}C NMR signal of C-2 was detected in C_6D_6. Though both these signals were observed in $CDCl_3$ (H-2, δ 4.40; C-2, δ 59.7), they were very broad, and no correlation was observed between H-2 and H-3 in the COSY spectrum of **2**. Also, no coupling between H-2 and C-2 was detected in the HMQC spectrum of **2**. The broad signal at δ 4.40 ppm was assigned to H-2 based on result of a 1D decoupling experiment in which irradiation of the broad peak at δ 4.40 simplified a multiplet at δ 4.71 ppm to a pentet. In the spectra obtained in DMSO-d_6 the signals for H-2 and C-2 were clearly observed (Table 1).

Ulithiacyclamides E (**3**), F (**4**), and G (**5**) had the molecular formulas $C_{35}H_{44}N_8O_8S_4$ (HRFABMS *m*/*z* 833.2237 $[M + H]^+$, Δ +0.6 mmu), $C_{35}H_{42}N_8O_7S_4$, and $C_{35}H_{42}N_8O_7S_4$, respectively, based on their FABMS and NMR data (Tables 2 and 3). They all dissolved in $CDCl_3$, but their ^{1}H NMR spectra in $CDCl_3$ (at room temperature and at 50 °C) were not well resolved, and many signals were broad. This was also true for the spectra in DMSO-d_6 at room temperature; however, spectra taken in DMSO-d_6 at 50 °C were well resolved. All three compounds were quite stable under these conditions.

Inspection of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) of ulithiacyclamide E (**3**) revealed striking similarities to

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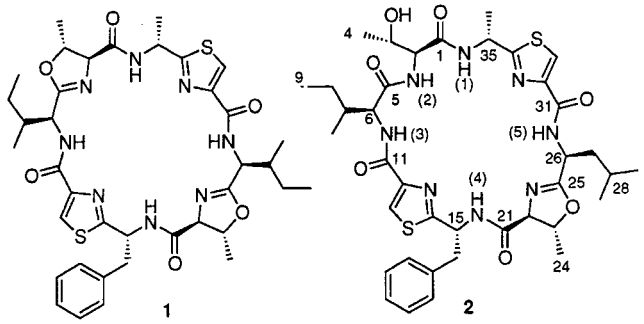

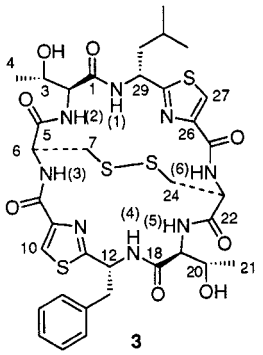

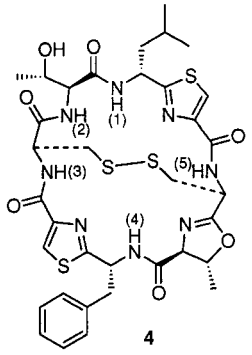

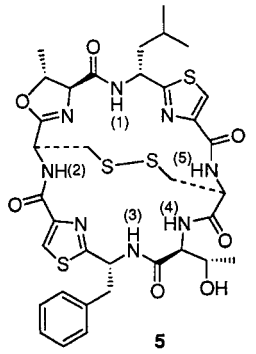

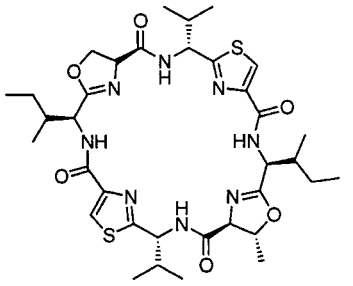

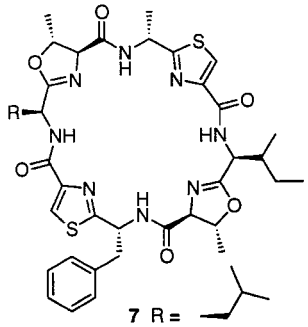

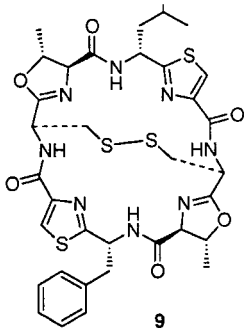

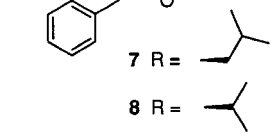

ulithiacyclamide B (**9**),[5] but the former lacked signals for the two substituted oxazolines and instead had resonances corresponding to "free" threonine residues. Thus, **3** exhibited ^{1}H NMR signals at δ 4.31 (t, 8.0, H-2), 3.85 (br, H-3), 4.39 (br t, 7.5, H-19), and 3.99 (br, H-20) and ^{13}C NMR signals at δ 58.8 (d, C-2), 65.2 (d, C-3), 58.6 (d, C-19), and 65.3 (d, C-20). To confirm the structure of ulithiacyclamide E, ulithiacyclamide B (**9**) was treated with 5% H_2SO_4–MeOH, and then 10% aqueous NaOH.[13] After workup, the residue was isolated by reversed-phase HPLC to yield a compound identical to ulithiacyclamide E (**3**) as judged from comparison of its HPLC retention time and ^{1}H NMR spectrum with those of naturally occurring **3**.

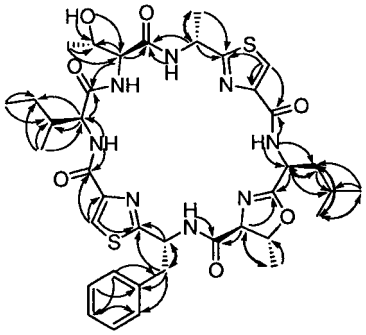

Figure 1. HMBC correlations of patellamide G (**2**).

Ulithiacyclamides F (**4**) and G (**5**), which were isomers and contained one oxazoline and one "free" threonine according to the NMR data (Tables 2 and 3), were anhydro forms of ulithiacyclamide E (**3**). The oxazoline in ulithiacyclamide F (**4**) was located between C-18 and C-23 based on ^{1}H–^{1}H long-range couplings between H-19/H-23 and H-19/H-12 observed in the long-range COSY-45 spectrum of ulithiacyclamide F. Therefore, the structure of ulithiacyclamide F was determined as **4**, and its isomer ulithiacyclamide G was assigned structure **5**. In agreement with this, a long-range coupling between H-2 (δ 4.35) and H-6 (δ 4.71) was observed in the COSY-45 spectrum of ulithiacyclamide G (**5**). The structures of **4** and **5** were verified, and the stereochemistry established to be the same as **3** by chemical conversion of **4** and **5** to **3** by partial hydrolysis.[13] The reaction products were not isolated due to the small amount of material processed; however, reversed-phase HPLC analysis indicated that both reaction mixtures contained primarily **3** based on comparison of retention times with an authentic sample. In addition, ^{1}H NMR spectra of both reaction mixtures showed signals for ulithiacyclamide E (**3**).

To assign the absolute configuration of the amino acid units, each new metabolite was subjected to normal peptide hydrolysis (6N HCl, 110 °C, 22 h) and the resulting acid hydrolysate was analyzed by HPLC to give relative abundances of the amino acids.[18] The absolute configurations of the amino acids were then determined by chiral GC analysis of their *N*(*O*)-trifluoroacetyl isopropyl ester derivatives.[19] These procedures established the presence of L-threonine (2 equivalents), L-leucine, and L-isoleucine for patellamide G (**2**) and L-threonine for ulithiacyclamides E–G (**3**–**5**). At this stage, the stereochemistry of the thiazole amino acids was not established. To determine the absolute configuration of the thiazole amino acids, peptides **2** and **3** were first ozonized to destroy the thiazole ring[14] and then subjected to the same procedures as mentioned above. In addition to the aforementioned amino acids, D-alanine and D-phenylalanine were found in the acid hydrolysate of ozonized **2**; D-leucine and D-phenylalanine were observed in the hydrolysate of ozonized **3**. All amino acids mentioned above were confirmed by GC–MS analysis of the amino acid derivatives. According to these results, and the chemical conversion of **4**, **5**, and **9** to **3**, the stereochemistries of the amino acid units in the new cyclic peptides were determined as shown, though cysteine was not detected in the acid hydrolysate of either intact peptides **3**–**5** or ozonized peptide **3**.

In summary, the four new cyclic peptides, patellamide G (**2**) and ulithiacyclamides E–G (**3**–**5**) have been characterized. These new metabolites have one or two noncyclized threonine units. Similar peptides having the "free" threonine unit have been reported.[13]

Table 1. NMR Data for Patellamide G (**2**)[a]

	^{1}H (*J*, Hz, DMSO-d_6)[b]	^{13}C (DMSO-d_6)[b]	^{1}H (*J*, Hz, $CDCl_3$)[c]	^{13}C ($CDCl_3$)[c]	HMBC (carbon)[d]
1		167.9 (s)		167.7 (s)	
2	3.86 (dd, 4.5, 7.4)	61.0 (d)	4.40 (br)	59.7 (d)	1, 3, 5
3	4.33 (m)	64.8 (d)	4.71 (dp, 3.5, 6.5)	66.2 (d)	
4	1.11 (d, 6.5)	21.2 (q)	1.19 (d, 6.5)	19.1 (q)	2, 3
5		174.0 (s)		169.5 (s)	
6	4.51 (m)	53.1 (d)	4.60 (dd, 6.2, 11.5)	54.7 (d)	5, 7, 8, 11*
7	2.27 (m)	33.2 (d)	2.17 (m)	33.7 (d)	
8	1.60 (m); 1.17 (m)	24.6 (t)	1.61 (m); 1.17 (m)	25.9 (t)	
9	0.83 (t, 7.4)	9.7 (q)	0.87 (t, 7.5)	10.1 (q)	7, 8
10	0.92 (d, 6.5)	14.6 (q)	0.98 (d, 6.5)	15.0 (q)	6, 7, 8
11		161.3 (s)		162.1 (s)	
12		147.1 (s)		148.0 (s)	
13	7.82 (s)	124.2 (d)	7.35 (s)	123.5 (d)	11, 12, 14
14		171.1 (s)		171.3 (s)	
15	5.38 (td, 9.9, 5.4)	52.0 (d)	5.48 (td, 9.5, 6.0)	52.1 (d)	14, 16
16	3.30 (m)	39.9 (t)	3.43 (dd, 9.5, 14.0)	40.5 (t)	14, 15, 17, 18
			3.30 (dd, 6.0, 14.0)		
17		137.2 (s)		136.2 (s)	
18	7.41 (d, 7.1)	129.0 (d, 2C)		129.2 (d, 2C)	16, 20
19	7.34 (t, 7.6)	128.1 (d, 2C)	7.25−7.39 (5H)	128.8 (d, 2C)	17
20	7.25 (t, 7.1)	126.4 (d)		127.2 (d)	18
21		172.0 (s)		173.3 (s)	
22	4.18 (d, 3.4)	73.2 (d)	4.25 (d, 3.0)	73.5 (d)	21, 24, 25
23	4.77 (dq, 3.4, 6.5)	80.5 (d)	4.96 (dq, 3.0, 6.0)	82.4 (d)	21*, 25*
24	1.29 (d, 6.5)	20.3 (q)	1.42 (d, 6.0)	21.0 (q)	22, 23
25		167.2 (s)		168.7 (s)	
26	4.51 (m)	52.7 (d)	4.32 (m)	54.4 (d)	25, 27, 28
27	1.95 (m); 1.60 (m)	39.2 (t)	2.04 (m); 1.61 (m)	40.3 (t)	26, 28, 29
28	1.64 (m)	24.3 (d)	1.75 (m)	24.9 (d)	
29	0.92 (d, 6.5)	22.5 (q)	0.95 (d, 6.5)	22.6 (q)	27, 30
30	0.99 (d, 6.3)	21.8 (q)	1.02 (d, 6.5)	22.3 (q)	27, 28, 29
31		161.3 (s)		162.8 (s)	
32		146.6 (s)		146.9 (s)	
33	7.82 (s)	125.0 (d)	7.51 (s)	123.9 (d)	31, 32, 34
34		172.3 (s)		172.9 (s)	
35	5.24 (dq, 9.1, 6.8)	46.0 (d)	5.42 (dq, 9.5, 7.0)	48.0 (d)	1, 34, 36
36	1.57 (d, 6.8)	21.1 (q)	1.69 (d, 7.0)	21.0 (q)	34, 35
N-1	7.82 (d, 9.1)		7.38 (br)		1
N-2	9.19 (br)		7.20 (br)		
N-3	8.70 (d, 7.3)		8.15 (br)		6, 11
N-4	7.68 (d, 9.9)		7.82 (d, 9.5)		21
N-5	7.94 (d, 7.1)		7.70 (br)		26, 27, 31
OH	4.98 (d, 7.9)				2, 3

[a] Measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C; δ in ppm (*J* in Hz); ^{13}C multiplicities assigned by DEPT experiment. [b] Spectra taken at 50 °C. [c] Spectra taken at 25 °C. [d] Obtained from HMBC spectra in DMSO-d_6 (50 °C) and $CDCl_3$ (25 °C); the long-range correlations with an asterisk were only observed in the HMBC spectrum taken in $CDCl_3$.

Compounds **2**−**8** were evaluated for anti-MDR activity against vinblastine-resistant CCRF−CEM human leukemic lymphoblasts, according to a procedure described by Williams and Jacobs.[17] The IC_{50} for vinblastine against the drug resistant cells (CEM/VBL_{100}) was 90 nM. The IC_{50}s (nM) for vinblastine in the presence of 2.5 μg/mL of the cyclic peptides were: **2**, 60; **3**, 112; **4**, 44; **5**, 90; **6**, 90; **7**, 12; **8**, 12. Patellamides B (**7**) and C (**8**), which reduced drug resistance about ten-fold, are the only ones considered significantly active.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector (235 nm). IR spectra were taken on a Bio-Rad 3240-SPC FT instrument and optical rotations on a Rudolph Autopol III automatic polarimeter. NMR experiments were conducted with Varian XL-300 and VXR-500 instruments; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB E mass spectrometer. Columns used for reversed-phase HPLC were from Phenomenex (Torrance, CA).

Animal Material. The ascidian *Lissoclinum patella* was collected in 1986, in Pohnpei, Federated States of Micronesia, and frozen shortly after collection. A voucher specimen (ID 2-PO-86) has been deposited in the University of Oklahoma.

Extraction and Isolation. Freshly thawed specimens (147 g dry wt after extraction) were cut into small pieces and extracted with MeOH (3 × 1.5 L) and then MeOH−CH_2Cl_2 (1:1). The extracts were concentrated in vacuo and combined to give a residue that was subjected to solvent partitioning as described previously.[20] Three organic fractions were obtained after removal of solvents: hexane (3.09 g); CH_2Cl_2 (4.77 g); and *n*-BuOH (2.16 g). The CH_2Cl_2 solubles showed cytotoxicity against P-388 cell lines and were therefore fractionated on an open column of Si gel using increasing amounts of EtOAc as eluent. In all, 22 fractions were collected. The 13th fraction, which contained predominantly patellamide A (**6**) and a trace amount of ulithiacyclamide B (**9**), was subjected to a C_{18} open column (30% H_2O−MeOH) to give the more polar patellamide A and a mixture of patellamide A and ulithiacyclamide B. This mixture was resolved by reversed-phase HPLC (10 μM, ODS-2 column) using 20% H_2O−MeOH as eluent. Fraction 14 was passed through a C_{18} open column (30% H_2O−MeOH) to remove the dark green pigment and yielded patellamide B (**7**).

Reversed-phase HPLC (5 μM, ODS-3 column) of fraction 16 using 30% H_2O in MeOH as eluent, furnished patellamide C (**8**) as the major component, in addition to small amounts of patellamide B(**7**) and a mixture of ulithiacyclamide F (**4**) and G (**5**), which was further separated by reversed-phase HPLC

Table 2. ^{1}H NMR Data for Ulithiacyclamides E−G (**3**−**5**) and B (**9**)[a]

position	3	4	5	9
2	4.31 (t, 8.0)	4.37 (m)	4.35 (d, 5.0)	4.32 (d, 6.3)
3	3.85 (br)	3.89 (m)	4.75 (dq, 5.0, 6.0)	4.69 (dq, 6.3, 6.3)
4	0.83 (br d, 5.5)[b]	1.13 (d, 6.2)	1.30 (d, 6.0)	1.34 (d, 6.3)
6	4.82 (td, 7.0, 2.5)	4.83 (br s)	4.71 (m)	5.00 (br m)
7	3.32 (m); 3.18 (m)	3.60 (m); 3.26 (m)	3.25 (m); 3.00 (m)	3.23 (m)
10	8.18 (s)	8.19 (s)	8.25 (s)	8.28 (s)
12	5.37 (q, 8.0)	5.45 (dt, 5.1, 8.0)	5.45 (ddd, 5.0, 8.5, 10.0)	5.58 (dt, 6.3, 7.7)
13	3.24 (m); 3.11 (m)	3.28 (m); 2.99 (m)	3.25 (m); 3.10 (m)	3.32 (m), 3.13 (dd, 13.5, 8)
15−17	7.18−7.26 (m, 5H)	7.07−7.26 (m, 5H)	7.18−7.37 (m, 5H)	7.14−7.29 (m, 5H)
19	4.39 (br t, 7.5)	4.32 (d, 4.8)	4.20 (t, 7.5)	4.36 (dd, 5.7, 1.4)
20	3.99 (br)	4.75 (dq, 4.8, 6.5)	3.64 (m)	4.70 (dq, 5.7, 6.4)
21	1.12 (d, 6.0)	1.29 (d, 6.5)	0.60 (br d, 5.0)b	1.32 (d, 6.4)
23	4.87 (td, 7.0, 2.5)	4.70 (m)	4.78 (br s)	4.93 (br m)
24	3.32 (m); 3.18 (m)	3.30 (m); 2.99 (m)	3.64 (m); 3.27 (m)	3.16 (m)
27	8.21 (s)	8.27 (s)	8.29 (s)	8.34 (s)
29	5.18 (m)	5.19 (m)	5.33 (m)	5.42 (dt, 5.7, 8.6)
30	1.86 (m); 1.65 (m)	1.89 (m); 1.63 (m)	1.65 (m)	1.82 (m); 1.67 (m)
31	1.65 (m)	1.79 (m)	1.62 (m)	1.67 (m)
32	0.90 (d, 6.0)	0.93 (d, 6.5)	0.90 (d, 5.0)	0.93 (d, 6.8)
33	0.94 (d, 6.0)	0.95 (d, 6.5)	0.94 (d, 5.5)	0.95 (d, 6.6)
N-1	8.20 (br)	8.00 (br d, 7.3)	8.88 (br)	7.96 (br d, 8.6)
N-2	8.28 (d, 8.5)	7.94 (br)	8.62 (d, 8.0)	9.08 (br)
N-3	8.15 (br)	8.80 (br)	8.88 (br)	8.12 (br d, 6.9)
N-4	8.40 (br)	8.60 (d, 8.0)	7.78 (br d, 6.5)	9.08 (br)
N-5	8.33 (d, 8.5)	8.75 (d, 4.5)	7.94 (br)	
N-6	8.15 (br)			

[a] Spectra recorded at 500 MHz in DMSO-d_6 at 50 °C, referenced to the residual DMSO signal (δ 2.49 ppm). Assignments based on COSY, LR-COSY, and COSY-RELAY. [b] Broadness not due to long-range coupling, but probably to slowly interconverting rotational conformers.

(polymeric reversed-phase C_{18}) using 30% H_2O−MeOH as eluent. The 17th fraction was subjected to reversed-phase HPLC (5 μM, ODS-3) using 30% H_2O in MeOH as eluent, to afford ulithiacyclamide E (**3**) and patellamides C (**8**) and G (**2**).

Patellamide G (**2**) (14 mg): amorphous solid, $[\alpha]_D$ +40.6° (*c* 0.35, MeOH); IR (film) ν_{max} 3400, 3165, 1680, 1650, 1540,-1480, 1380 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m/z* 795 [M + H]$^+$, 817 [M + Na]$^+$; HRFABMS *m/z* 795.3324 [M + H]$^+$, calcd for $C_{38}H_{51}N_8O_7S_2$ 795.3322.

Ulithiacyclamide E (**3**) (18 mg): amorphous solid, $[\alpha]_D$ +4.9° (*c* 0.82, MeOH); IR (film) ν_{max} 3450, 3350, 1685, 1650, 1540, 1490, 1370 cm^{-1}; ^{1}H (DMSO-d_6) and ^{13}C NMR (DMSO-d_6; CD_3OD), see Tables 2 and 3, respectively; ^{1}H NMR (500 MHz, CD_3OD) δ 8.16, 8.13 (1H each, s, H-10, H-27), 7.18−7.28 (5H, m, H-15−H-17), 5.53 (1H, dd, J = 8.3, 7.4 Hz, H-12), 5.32 (1H, dd, J = 5.0, 9.9 Hz, H-29), 4.95 (1H, dd, J = 5.0, 5.8 Hz, H-6), 4.92 (1H, dd, J = 5.2, 5.8 Hz, H-23), 4.60 (1H, br d, J = 5.8 Hz, H-2), 4.47 (1H, d, J = 6.6 Hz, H-19), 4.09 (1H, pentet, J = 6.6 Hz, H-3), 3.95 (1H, br m, H-20), 3.30 (4H, m, H-6, H-24), 3.20 (2H, m, H-13), 1.91 (1H, m, H-30), 1.75 (1H, m, H-31), 1.68 (1H, m, H-30), 1.23 (3H, d, J = 6.6 Hz, H-4), 1.00 (3H, d, J = 6.6 Hz, H-33), 0.98 (3H, d, J = 6.6 Hz, H-32), 0.89 (3H, br d, J = 6.6 Hz, H-21); FABMS *m/z* 833 [M + H]$^+$, 855 [M + Na]$^+$; HRFABMS *m/z* 833.2237 [M + H]$^+$, calcd for $C_{35}H_{45}N_8O_8S_4$ 833.2243.

Ulithiacyclamide F (**4**) (8 mg): amorphous solid, $[\alpha]_D$ +29.6° (*c* 0.27, MeOH); IR(film) ν_{max} 3200 (br), 1655 (br), 1540, 1480, 1370 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3, respectively; FABMS *m/z* 815 [M + H]$^+$.

Ulithiacyclamide G (**5**) (5 mg): amorphous solid, $[\alpha]_D$ +25.6° (*c* 0.18, MeOH); ^{1}H and ^{13}C NMR, see Tables 2 and 3, respectively; FABMS *m/z* 815 [M + H]$^+$, 837 [M + Na]$^+$.

Patellamide A (**6**) (500 mg): amorphous powder, $[\alpha]_D$ + 114.0° (*c* 1.0, CH_2Cl_2), [lit.[12] +113.9°]; FABMS *m/z* 743 [M + H]$^+$; NMR data identical to lit.[12]

Patellamide B (**7**) (475 mg): amorphous solid, $[\alpha]_D$ + 43.0° (*c* 0.86, CH_2Cl_2), [lit.[12] +29.4°]; +50.6° (synthetic);[21] FABMS *m/z* 777 [M + H]$^+$; NMR data identical to lit.[12]

Patellamide C (**8**) (234 mg): amorphous solid, $[\alpha]_D$ + 23.7° (*c* 3.04, CH_2Cl_2), [lit.[12] +19°]; +32° (synthetic);[21] FABMS *m/z* 763 [M + H]$^+$, 785 [M + Na]$^+$; NMR data identical to lit.[12]

Table 3. ^{13}C NMR Data for Ulithiacyclamides E−G (**3**−**5**), and B (**9**)[a]

carbon	3[b]	3[c]	9[b]	4[b]	5[b]
1	169.4 (s)	172.2 (s)	169.9 (s)	169.8 (s)	169.7 (s)
2	58.8 (d)	60.1 (d)	73.4 (d)	59.8 (d)	73.0 (d)
3	65.2 (d)	67.7 (d)	80.4 (d)	65.6 (d)	79.2 (d)
4	20.0 (q)	20.3 (q)	21.3 (q)	19.95 (q)	21.0 (q)
5	169.3 (s)	171.4 (s)	166.6 (s)	168.0 (s)	168.0 (s)
6	53.6 (d)	55.4 (d)	47.6 (d)	51.8 (d)	52.1 (d)
7	43.6 (t)	44.5 (t)	46.1 (t)	44.3 (t)	43.0 (t)
8	159.4 (s)	162.4 (s)	160.0 (s)	159.0 (s)	159.0 (s)
9	148.6 (s)	149.9 (s)	148.0 (s)	147.6 (s)	147.4 (s)
10	124.2 (d)	125.6 (d)	126.2 (d)	124.4 (d)	124.0 (d)
11	170.6 (s)	171.9 (s)	170.6 (s)	168.5 (s)	170.1 (s)
12	52.2 (d)	54.0 (d)	52.1 (d)	52.0 (d)	52.1 (d)
13	41.4 (t)	43.2 (t)	42.1 (t)	42.5 (t)	41.2 (t)
14	136.9 (s)	138.1 (s)	136.4 (s)	135.9 (s)	137.1 (s)
15	129.2 (d)	130.4 (d)	129.6 (d)	129.2 (d)	129.2 (d)
16	128.1 (d)	129.6 (d)	128.5 (d)	128.1 (d)	127.9 (d)
17	126.5 (d)	128.1 (d)	127.0 (d)	126.6 (d)	126.3 (d)
18	169.5 (s)	172.0 (s)	169.9 (s)	169.6 (s)	169.4 (s)
19	58.6 (d)	60.1 (d)	73.6 (d)	73.1 (d)	60.1 (d)
20	65.3 (d)	67.5 (d)	80.4 (d)	79.2 (d)	65.3 (d)
21	20.2 (q)	20.0 (q)	21.4 (q)	20.97 (q)	19.5 (q)
22	169.6 (s)	171.4 (s)	166.5 (s)	168.0 (s)	167.9 (s)
23	53.6 (d)	55.4 (d)	47.6 (d)	49.6 (d)	49.7 (d)
24	43.6 (t)	44.5 (t)	46.1 (t)	43.0 (t)	44.3 (t)
25	159.4 (s)	162.3 (s)	160.0 (s)	160.0 (s)	160.0 (s)
26	148.8 (s)	149.8 (s)	148.4 (s)	147.7 (s)	148.1 (s)
27	123.8 (d)	125.3 (d)	125.8 (d)	124.7 (d)	125.2(d)
28	171.7 (s)	173.4 (s)	169.0 (s)	172.8 (s)	171.5 (s)
29	48.8 (d)	50.6 (d)	48.5 (d)	48.6 (d)	48.5 (d)
30	44.4 (t)	46.2 (t)	46.3 (t)	44.3 (t)	46.6 (t)
31	24.4 (d)	26.3 (d)	24.6 (d)	24.3 (d)	24.2 (d)
32	21.4 (q)	21.7 (q)	22.2 (q)	20.65 (q)	22.5 (q)
33	22.7 (q)	23.4 (q)	22.6 (q)	22.96 (q)	22.2 (q)

[a] Spectra recorded at 125 MHz referenced to the solvents peaks, δ 39.5 ppm for DMSO-d_6, δ 49.0 ppm for CD_3OD; multiplicities determined by DEPT experiment; protonated carbons assigned by HMQC experiments, some of assignments for the quarternary carbons confirmed by HMBC experiments. [b] Recorded in DMSO-d_6. [c] Recorded in CD_3OD.

Ulithiacyclamide B (**9**) (4 mg): amorphous solid, $[\alpha]_D$ +108.6° (*c* 0.25, MeOH), [lit.[5] +117°]; ^{1}H and ^{13}C NMR($CDCl_3$);[5]

^{1}H NMR (DMSO-d_6), see Table 2; ^{13}C NMR (DMSO-d_6), see Table 3; FABMS m/z 797 $[M + H]^+$.

Conversion of Ulithiacyclamide B (9) to Ulithiacyclamide E (3). Ulithiacyclamide B (**9**) (3.5 mg) was refluxed in 5% H_2SO_4−MeOH (5 mL) for 1 h. The reaction mixture was then added to a solution of 10% aqueous NaOH (5 mL) and allowed to stir overnight. The mixture was then neutralized with 1N HCl and partitioned with $CHCl_3$ (10 mL × 3), and the combined $CHCl_3$−soluble layers were dried over anhydrous Na_2SO_4. After removal of solvent, the residue was subject to reversed-phase HPLC (10 μM, ODS-2, 300 × 10 mm, 30% H_2O−MeOH) to afford ulithiacyclamide E (**3**) (2.1 mg).

Conversion of Ulithiacyclamides F (4) and G (5) to Ulithiacyclamide E (3). Ulithiacyclamides F (**4**) (1 mg) and G (**5**) (0.7 mg) were processed, respectively, in the same manner as ulithiacyclamide B (**9**). After workup, the reaction residues were analyzed by reversed-phase HPLC and ^{1}H NMR. The HPLC (10 μM, ODS-2, 300 × 10 mm, 30% H_2O−MeOH, flow rate = 1.5 mL/min) of the reaction residues revealed a peak at t_R = 30.7 min, which was exactly the same as that of the natural product, ulithiacyclamide E (**3**).

Ozonolysis of Patellamide G (2) and Ulithiacyclamide E (3). A stream of ozone was passed through a solution of each peptide in ca. 8 mL of CH_2Cl_2 or MeOH at −78 °C until the color of the solution turned pale blue (approximately 15 min).[14] After removal of the solvent under a stream of N_2, the residue was subjected to hydrolysis and derivatization as described previously.[19]

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