Cyclic Peptides from the Ascidian *Lissoclinum patella*: Conformational Analysis of Patellamide D by X-ray Analysis and Molecular Modeling

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Four new cytotoxic, cyclic peptides, patellamide D (**10**) and lissoclinamides 4, 5, and 6 (**11–13**), have been isolated from the ascidian *Lissoclinum patella* collected in Australia and their structures have been determined by ^1H and ^{13}C NMR analyses. Absolute configurations were determined by hydrolysis and analysis of the derivatized constituent amino acids on chiral GC and HPLC columns. Definitive ^{13}C NMR assignments have been made. The structure and absolute configuration of patellamide D were also determined by X-ray crystallography. The solid-state conformation of patellamide D was found to be different from that of the closely related peptide ascidiacyclamide. Computer modeling indicates that the energy-minimized conformation of patellamide D closely resembles that found in the crystalline form and this is ~10 kcal/mol lower in energy than that of an energy minimized "model" of patellamide D based on the reported crystal structure of ascidiacyclamide.

A variety of cyclic peptides[1-6] and depsipeptides[7] exhibiting mild to potent cytotoxicity and in vivo antitumor activity[8,9] have been isolated from ascidians and a sea hare. Structure elucidation of these peptides and depsipeptides has been fraught with difficulty and minor or major revisions have been made for a number of the originally proposed structures.[4,6,8,10-13] The majority of these peptides have been isolated from one species of tunicate, *Lissoclinum patella*, with some metabolite variation being observed with variation in collection site.[1-4,6] Tropical didemnid tunicates like *L. patella* contain symbiotic unicellular algae,[14-16] but it is not known whether these contribute to production of the peptides.

The known metabolites from *L. patella* can be grouped into two sets, **1–5** and **6–9** (Chart I), which differ in the nature of their macrocyclic ring framework. Both sets display unusual amino acids containing thiazole and oxazoline moieties. All of the common amino acids have the L configuration, whereas the thiazole and dihydrothiazole amino acids occur in both L and D forms, with the latter predominating.[6] Structure–activity studies[17] of the set **1–5** and related synthetic or semisynthetic compounds have revealed that the oxazoline ring is vital for the cytotoxic activity of these compounds and that the bridging disulfide unit in **2** (or the corresponding reduced dithiol moiety) greatly enhances cytotoxicity. In connection with these structure–activity studies, the conformation of ascidiacyclamide, **1**, has been investigated by NMR spectroscopy and X-ray diffraction.[18] The results indicate that the solid-state and solution conformations are essentially the same and that **1** adopts a saddle-shape with all of the NH bonds directed to the interior of the macrocyclic ring.

In our continuing search for potential anticancer agents from marine sources[19] we have isolated four new cytotoxic, cyclic peptides related to **2–5** and **6–9** from *L. patella* collected at two different sites on the Great Barrier Reef, Queensland, Australia. We report here their structure determination by spectral analysis and X-ray crystallography. Complete, definitive ^{13}C NMR chemical shift assignments for various members of this group of peptides were made, and this reveals a need to revise some previous tentative assignments for **1–9**. The solid-state conformation of one of the new peptides, patellamide D, **10** (Chart II), was determined by X-ray diffraction and analyzed further by molecular modeling calculations. The conformation observed is entirely different from that reported[18] for the related peptide ascidiacyclamide, **1**.

The new peptides, patellamide D (**10**) and lissoclinamides 4, 5, and 6 (**11–13**) (Chart II), were obtained along with the two known peptides **1** and **2** from extracts of freeze-dried specimens by routine application of adsorption and reverse-phase chromatography. The molecular formulas of all compounds were determined by high-resolution mass spectral analysis. The known compounds were

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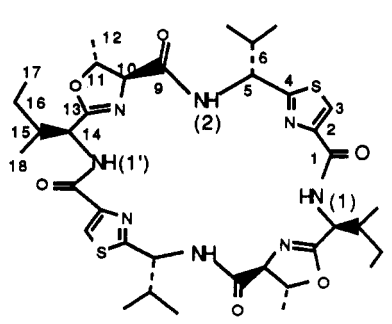
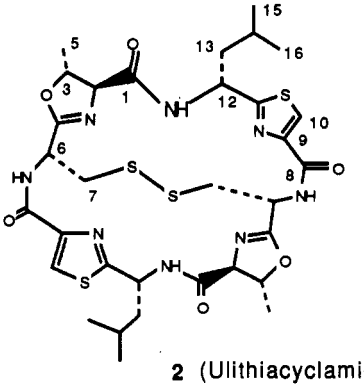
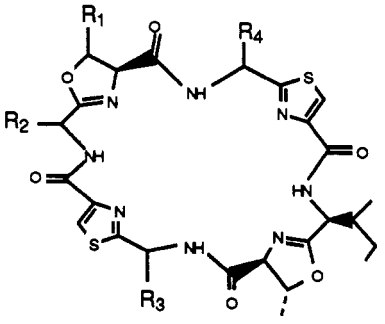
Chart I

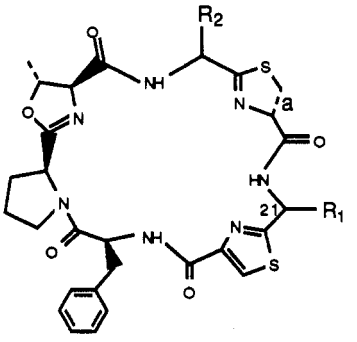


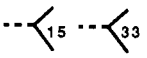
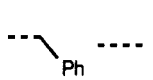
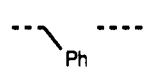
1 (Ascidiacyclamide)

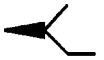
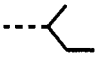
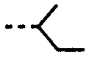
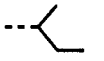


2 (Ulithiacyclamide)





	R_1	R_2	R_3	R_4
3 (Patellamide A)	H			
4 (Patellamide B)	----			
5 (Patellamide C)	---			

	a	R_1	R_2
6 (Ulicyclamide)	=	----	
7 (Lissoclinamide 1)	=		
8 (Lissoclinamide 2)	—	----	
9 (Lissoclinamide 3)	—		

identified by comparison of their spectral data with literature values. However, the ^{13}C NMR chemical shifts for the fully substituted carbons in these compounds have been reassigned based on INAPT[20] experiments (see Table II).

The structure of patellamide D, **10**, $C_{38}H_{48}N_8O_6S_2$, was initially determined from in-depth NMR spectral analyses following a strategy like that described below for lissoclinamides 4, 5, and 6. This provided all of the ^1H and ^{13}C NMR chemical shift and coupling data for **10** in Tables I and II. INAPT experiments confirmed the chemical shift assignments of the non-protonated carbons, see Table II and Figure 1. Fortunately, patellamide D also eventually crystallized and provided the opportunity for an X-ray diffraction analysis to confirm the structure and determine the absolute configuration and solid-state conformation, see below.

Lissoclinamide 5, **11**, $C_{38}H_{41}O_5N_7S_2$, was ascertained to have the structural units A–G from consideration of $^1H/^1H$ COSY, proton decoupling, and ^{13}C NMR data (Tables I and II). The quaternary carbons and heteroatoms were situated by analogy to structure **10** since the ^1H and ^{13}C NMR chemical shift and multiplicity data for fragments A, C, E and the thiazole units D and F (Chart II) were very similar to data for corresponding features in **10**. The NMR chemical shift assignments of the protonated carbons were confirmed by $^1H/^{13}C$ COSY data. In addition to the data corresponding to these partial structures, there remained four ^{13}C NMR signals compatible with amide carbonyl groups. Two- and three-bond proton–carbon couplings detected by INAPT experiments determined the location of each of these carbonyl groups and also confirmed the chemical shifts of the quaternary carbons of units A, D, and F. The proton signals that were irradiated and the corresponding carbons whose signals were detected in the INAPT experiments are outlined on structure **11'** (Figure 1), and the results are shown in Figure 2. These results established connections between all the fragments A–G except for bonds between C-17 and C-18, between C-27 and C-28, and between C-10 and the proline nitrogen. The first two of these links were confirmed from NOE data, i.e., irradiation of NH(2) enhanced H-19 and irradiation of NH(3) enhanced H-29. The remaining connection between C-10 and the proline nitrogen then necessarily followed to make the macrocyclic ring to satisfy the unsaturation requirement, giving **11** as the final structure. The stereochemistry of the threonine, phenylalanine, and proline portions of **11** were all confirmed to be L (=*S*) by analysis of the amino acids obtained upon hydrolysis of the peptides (Table III). The configuration at C-21 and C-31 are

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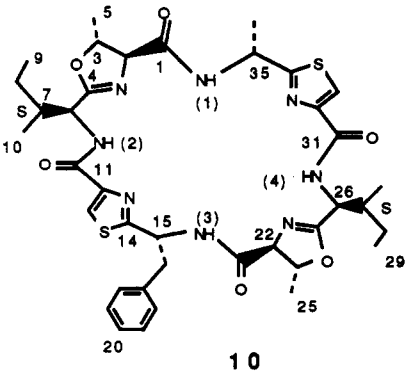
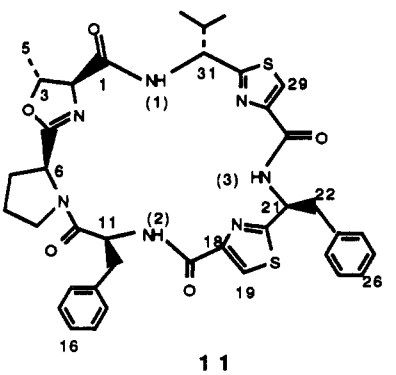
Chart II



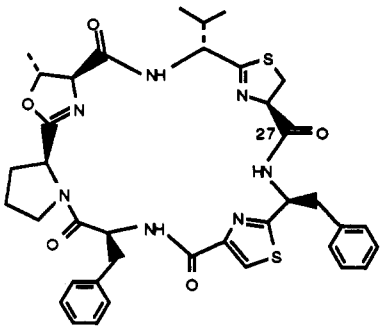
10

(Patellamide D)



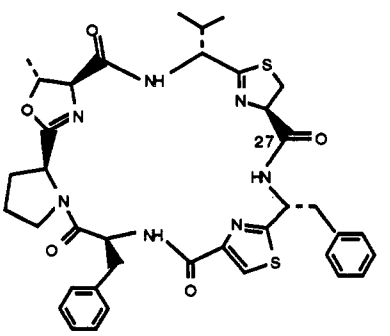
11

(Lissoclinamide 5)



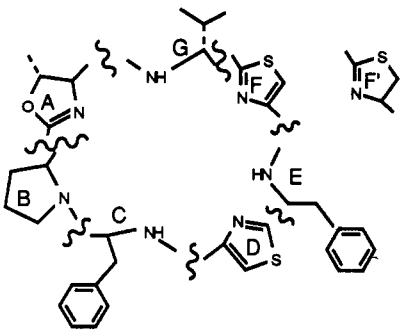
12

(Lissoclinamide 4)



13

(Lissoclinamide 6)



tentatively assigned as L (=*S*) and D (=*R*), respectively, since the ^{13}C NMR chemical shifts for these carbons and those immediately surrounding these centers are essentially the same in peptides **11** and **12** and the C-21 (*S*) and C-31 (*R*) configuration are confirmed for **12**, see below. That changes in configuration should cause significant ^{13}C NMR shifts is evident from the changes in chemical shifts of C-27 in **12** vs **13**, see Table II, of C-27 in ulicyclamide (**6**) vs lissoclinamide 2 (**8**),[6] and C-33 or C-15 in patellamide A (**3**) vs C-31 in lissoclinamide 1 (**7**).[6]

The structure of lissoclinamide 4, **12**, $C_{38}H_{43}N_7O_5S_2$, was deduced by employing the same approach that was used to determine the structure of **11**. Partial structures A–E and G were determined from proton decoupling, $^1H/^1H$ COSY, and ^{13}C NMR data and comparison of these data with that of **10** and **11**. The thiazoline moiety F′ was deduced from coupling between H-28 and H-29, H-29′, long-range H/C coupling (INAPT experiments) between H-28 and C-27, C-30, and comparison of proton and carbon chemical shift data with that reported[6] for the thiazoline group in lissoclinamide 2 and lissoclinamide 3. Connections between these partial structures and the four carbonyl groups remaining from the formula were made on the basis of the two- and three-bond proton–carbon couplings detected from INAPT experiments and selected NOE's indicated on structure **12′**. The INAPT experiments also confirmed the chemical shift assignments of the carbonyl carbons and carbons 4, 18, 20, and 30. In the ^1H NMR spectrum in $CDCl_3$ two protons resonate at 4.66 ppm, H-6 and -31. However, when the spectrum was measured in C_6D_6, these two signals were resolved and the spin system to which each belonged could be definitively assigned. A number of long-range proton–proton couplings useful for defining connections between various fragments of this type of peptide were also detected in a $^1H/^1H$ COSY experiment: H-2 to both NH(1) and H-6; H-6 to H-9; H-28 to both NH(3) and H-31; H-31 to H-29,-29′.

The threonine, proline, phenylalanine, and cysteine amino acid moieties of **12** were all confirmed to have the L configuration, and the valine unit the D configuration by analysis of the amino acids obtained upon hydrolysis, see Table III. The D/L ratios observed for valine and cysteine indicate that some racemization has occurred during the hydrolysis as has been observed by others.[4] C-21 is assigned the *S* configuration for reasons given below in the discussion of the stereochemistry of **13**.

Table I. ^{1}H NMR Data for 10–13[a]

H at C no.	10[b]	10[c]	11[b]	12[b]	13[b]
2	4.25 (d, 2.5)	4.36 (d, 2.5)	4.28 (d, 4.0)	4.29 (d, 5.8)	4.27 (d, 5.5)
3	4.95 (m)	5.12 (m)	4.88 (m)	4.81 (m)	4.79 (dq, 5.5, 7.0)
5	1.43 (d, 7.0)	1.06 (d, 7.0)	1.45 (d, 6.5)	1.46 (d, 7.0)	1.50 (d, 7.0)
6	4.71 (dd, 7.7, 11.6)	5.04 (dd, 7.0, 12.0)	4.56 (t, 7.8)	4.66 (dd, 8.0, 8.5)	4.60 (t, 8.0)
7	2.23 (m)	2.16 (m)	1.88 (m)	1.85 (m)	1.98 (m)
7			2.15 (m)	2.14 (m)	2.32 (m)
8	1.63 (m)	1.15 (m)	1.75 (m)	1.72 (m)	1.98 (m)
8	1.50 (m)	1.34 (m)	1.75 (m)	1.72 (m)	2.14 (m)
9	0.88 (t, 6.7)	0.56 (t, 6.7)	2.07 (m)	2.0 (m)	3.40 (m)
9			3.26 (m)	3.21 (m)	3.78 (br dd, 8.0, 10.0)
10	1.04 (d, 6.7)	0.79 (d, 7)			
11			4.87 (ddd, 4.5, 7.5, 10.0)	4.78 (ddd, 10.0, 6.6, 4.3)	5.15 (m)
12			2.88 (dd, 10, 12.6)	2.79 (dd, 12.8, 10.0)	2.98 (dd, 14.0, 6.2)
12			3.25 (dd, 4.5, 12.6)	3.38 (dd, 12.8, 4.3)	3.14 (dd, 14.0, 6.2)
13	7.38 (s)	7.04 (s)			
14			7.11 (d, 7)	7.09 (d, 7)	7.10 (m)
15	5.49 (dt, 6.1, 10.0)	5.72 (ddd, 12.0, 10.0, 6.0)	7.25 (m)	7.28 (m)	7.14 (m)
16	3.30 (dd, 6.1, 14)	3.12 (dd, 14.0, 12.0)	7.25 (m)	7.28 (m)	7.26 (m)
16	3.44 (dd, 9.9, 14)	3.44 (dd, 14.0, 6.0)			
18	7.30 (m)	7.38 (br d, 8.0)			
19	7.30 (m)	7.15 (m)	7.88 (s)	7.92 (s)	7.99 (s)
20	7.30 (m)	7.07 (m)			
21			5.43 (ddd, 3.9, 5.7, 10.0)	5.39 (ddd, 4.0, 6.0, 9.0)	5.57 (dt, 8.0, 6.2)
22	4.35 (d, 2.6)	4.07 (d, 2.5)	2.74 (dd, 10.0, 13.0)	2.88 (dd, 13.3, 9.0)	3.23 (dd, 14.0, 6.2)
22			3.87 (dd, 3.9, 13.0)	3.63 (dd, 13.3, 4.0)	3.14 (dd, 14.0, 6.2)
23	4.98 (m)	5.04 (m)			
24			7.11 (d, 7)	7.09 (dd, 7)	7.10 (m)
25	1.47 (d, 6.3)	0.94 (d, 7.0)	7.25 (m)	7.28 (m)	7.14 (m)
26	4.75 (dd, 7.7, 11.6)	5.04 (dd, 7.0, 12.0)	7.25 (m)	7.28 (m)	7.26 (m)
27	2.23 (m)	2.16 (m)			
28	1.50 (m), 1.63 (m)	1.15 (m), 1.34 (m)		5.28 (t, 9.5)	5.15 (m)
29	0.90 (t, 6.7)	0.57 (t, 7.0)	8.06 (s)	3.59 (dd, 11.4, 9.5)	3.35 (dd, 2.0, 6)
29				3.75 (dd, 11.4, 9.5)	3.46 (t, 12.0)
30	1.04 (d, 6.7)	0.78 (d, 7.0)			
31			5.18 (t, 10.0)	4.66 (t, 10.0)	4.92 (dt, 10.0, 2.5)
32			2.80 (m)	2.37 (m)	2.53 (m)
33	7.49 (s)	7.09 (s)	0.77 (d, 6.5)	0.88 (d, 7)	0.72 (d, 7.0)
34			1.07 (d, 6.5)	0.94 (d, 7)	0.98 (d, 7.0)
35	5.63 (dq, 7.3, 10.0)	5.44 (dq, 7.0, 10.0)			
36	1.74 (d, 7.3)	1.44 (d, 7.0)			
N-1	7.57 (d, 10.0)	7.88 (d, 10.0)	7.92 (d, 10.0)	7.77 (d, 10.0)	7.54 (d, 10.0)
N-2	7.53 (d, 7.7)	7.77 (d, 7.0)	8.70 (d, 7.5)	8.65 (d, 6.6)	7.89 (d, 8.5)
N-3	7.63 (d, 10.0)	7.94 (d, 10.0)	9.22 (d, 5.7)	8.51 (d, 6.0)	6.82 (d, 8.0)
N-4	7.56 (d, 7.7)	7.75 (d, 7.0)			

[a] 300 MHz; δ (mult, J in hertz). [b] $CDCl_3$. [c] C_6D_6.

Lissoclinamide 6, **13**, $C_{38}H_{43}N_7O_5S_2$, was obtained as a trace component, 2.5 mg. Proton decoupling and COSY spectra provided evidence for the partial structures A–E, F′, and G. NMR chemical shifts of protonated carbons were assigned from $^1H/^{13}C$ COSY data. Because only a small quantity of material was available, the more sensitive ^1H detected heteronuclear multiple quantum correlation (HMQC) experiment[23] (maximized for long-range coupling) was used to make the NMR chemical shift assignments for the nonprotonated carbons. The results that are outlined on structure **13′**, definitively fixed the location of three of the carbonyl groups and the quaternary carbons of the heterocyclic rings, and provided spectral evidence for connections between all of the partial structures except A to B, B to C, and C to D. The A/B connection was established by observation of a long-range coupling between H-2 and H-6 (COSY). Similarly, the B/C connection was supported by coupling observed between H-6 and H-11. By default the remaining carbonyl group to which no direct long-range couplings were observed must be assigned to the C-17 position. Combination of this data yields structure **13** for lissoclinamide 6. The NMR chemical shift of C-17 is consistent with that confirmed by long-range proton–carbon couplings for C-17 in **11** and **12**.

The threonine, proline, phenylalanine, and cysteine amino acid moieties were confirmed to have the L configuration, and the valine unit the D configuration, just as in the case of **12**, see Table III. Hence **12** and **13** differ only in configuration at C-21, the only center at which the configuration was not determined by the hydrolysis results. The stereochemistry at C-21 for these epimers could be assigned by comparison of their ^{13}C NMR data with that of another closely related epimeric pair, **6** and **8**, which also differ in configuration at C-21, but have the same configuration as **12** and **13** at all other chiral centers. Thus **6/8** and **12/13** are sets of closely related epimeric pairs differing in configuration at the same position on the macrocyclic ring. For the former pair the configuration was determined at all centers by chemical degradation,[4] but the ^{13}C NMR shifts of the carbonyl carbons were not assigned experimentally; for the latter pair we have assigned all ^{13}C NMR signals by long-range H/C correlations. One member of each of these epimeric pairs, namely **8** and **13**, displays one carbonyl carbon signal at 180–182 ppm, a shift quite distinct from the 160–172 ppm range noted for all other amide carbonyl signals in the entire set of cyclic peptides **1–13**. For **13** we confirmed by long-range H/C coupling that the 182 ppm signal is due to C-28, the thiazoline amino acid carbonyl carbon that is near the chiral

(23) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.

Table II. ^{13}C NMR Data for 1, 2, 10–13[a]

C	1[b,d]	2[b,d]	10[b,d]	10[c,d]	11[b,d]	12[b,d]	13[b,d]
1	160.5[e]	170.0[e]	173.1[g]	173.0[e]	171.4[e]	171.3[e]	172.2[f]
2	149.7[e]	74.2	73.7	74.3	75.2	75.2	75.9
3	123.1	81.6	82.5	82.3	82.6	82.3	81.0
4	169.1[e]	167.4[e]	168.2[g]	167.9[e]	169.7[e]	169.2[e]	169.6[f]
5	54.9	22.3	21.1	21.1	21.9	21.8	21.9
6	33.5	48.3	53.2	53.4	56.6	56.1	56.9
7	19.3	46.4	32.8	33.4	28.1	28.5	29.0
8	18.0	160.1[e]	25.0	25.2	25.1	25.3	25.6
9	171.5[e]	149.9[e]	8.7	9.0	47.2	46.9	47.5
10	73.6	124.1	15.1	15.3	171.0[e]	170.4[e]	169.3[f]
11	81.7	170.4[e]	161.8[g]	162.1[e]	54.0	54.1	52.2
12	21.8	47.9	147.4	148.2[e]	40.8	40.4	38.2
13	168.6[e]	46.5	123.7	123.9	136.2[e]	136.1[e]	135.0[f]
14	52.2	25.9	171.9[g]	171.6[e]	128.6	128.0	128.4
15	37.1	22.4	52.3	53.0	129.9	129.0	129.9
16	24.8	22.6	40.9	41.5	127.1	127.3	126.9
17	10.8		137.0[g]	137.3[e]	159.8[e]	159.6[e]	159.7
18	15.0		129.5	129.7	148.0[e]	148.4	148.6[f]
19			129.1	129.0	123.0	123.0	124.2
20			127.2	127.3	167.6[e]	167.8[e]	170.1[f]
21			173.1[g]	173.2[e]	54.5	54.3	52.2
22			73.7	74.2	42.8	42.4	41.7
23			82.4	85.5	136.0[e]	135.9[e]	135.7[f]
24			168.2[g]	167.8	128.0	128.0	128.9
25			21.1	21.1	129.6	129.0	129.4
26			53.2	53.5	127.2	127.3	127.6
27			32.8	33.4	160.5[e]	170.5[e]	182.1[f]
28			25.0	25.2	150.5[e]	80.1	79.1
29			8.7	9.0	122.9	33.9	36.4
30			15.1	15.0	168.7[e]	173.3[e]	172.1[f]
31			161.9[g]	161.9[e]	55.2	55.4	56.4
32			147.2[g]	148.3[e]	32.9	33.3	31.9
33			123.6	123.9	20.0	20.0	19.6
34			172.9[g]	173.3[g]	20.3	19.4	15.8
35			46.7	47.1			
36			20.8	21.0			

[a] 75.4 MHz. [b] $CDCl_3$. [c] C_6D_6. [d] Multiplicities by DEPT, protonated carbon assignments by H–C COSY experiments. [e] Assignments by INAPT. [f] Assignments by 500-MHz H-detected HMQC experiment. [g] Assignments by analogy to compound **10** in C_6D_6.

Table III

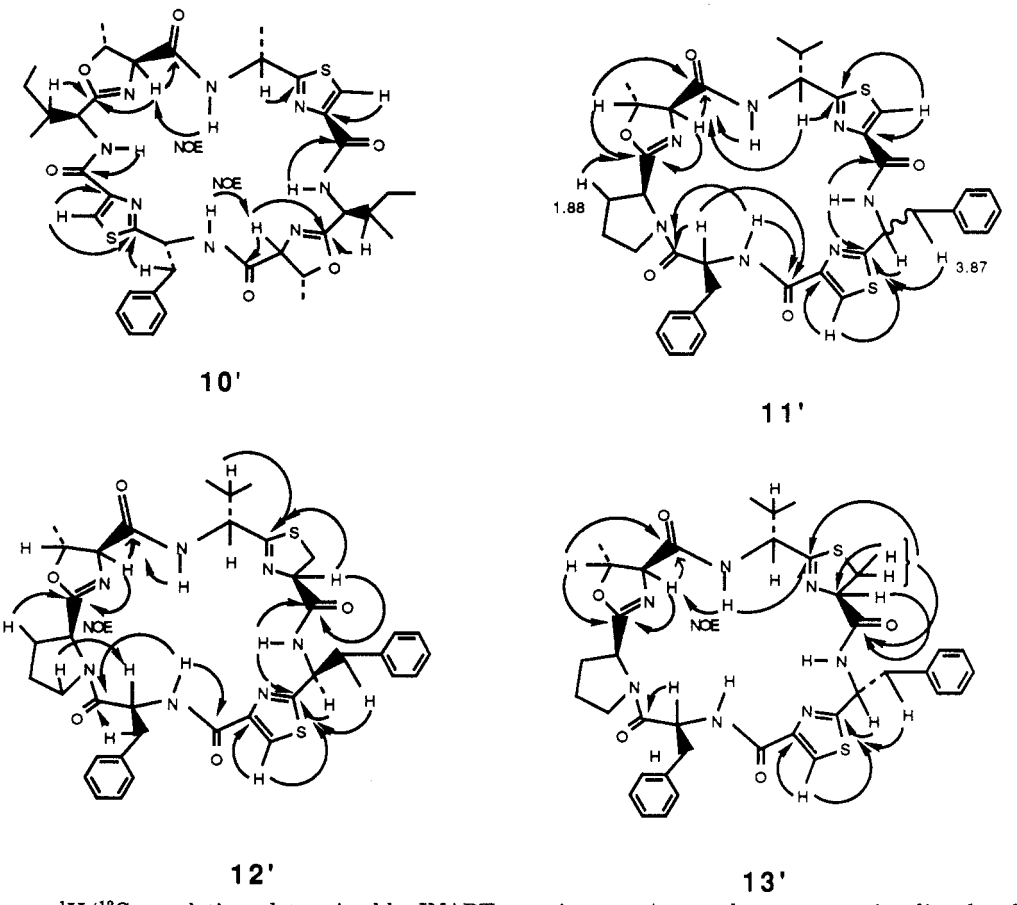
compd	Thr	Val	Phe	ILE	Pro	Cys[d]
			Amino Acid Abundances[a]			
10	1.00	na[b]	na	1.01	na	na
11	1.00	na	0.92	0.11[c]	~1.0	na
12	1.00	1.05	1.02	0.02[c]	~1.0	~0.5
13	1.00	1.11	1.06	na	~1.0	~0.5
			Amino Acid D/L Values[e]			
10	0.00	na	na	0.01	na	na
11	0.00	na	0.03	0.00	0.00	na
12	0.00	1.51	0.03	0.00	0.00	0.12
13	0.00	2.08	0.04	na	0.04	0.12

[a] Amino acid abundances were determined by HPLC[21] and are reported relative to Thr. Whereas the relative abundances of proline could not be determined by the HPLC procedure that was employed, the gas chromatographic anlyses indicate that proline is present in approximately equal abundance to threonine in compounds **11**–**13**. Proline was not detected in the hydrolyzate of compound **10**. [b] na, not applicable; amino acid not detected in the HCl hydrolyzate of this compound. [c] Although ILE was not expected based on the structure, small peaks corresponding to ILE were definitely observed. Trace impurity peaks were also evident in the proton NMR spectrum. [d] Cysteine appears to be partially destroyed in the hydrolysis procedure since no precautions were taken to prevent decomposition or oxidation. Some cysteic acid was detected. [e] Amino acid D/L values were determined by gas chromatographic analyses of the N(O)-TFA-isopropyl ester derivatives of the amino acids isolated from the acid hydrolyzates of the respective compounds. Details of this anlytical procedure are reported elsewhere.[22] The experimental error for all D/L values is ±0.01.

center C-21, and hence we propose that the 180 ppm signal of **8** is due to the corresponding thiazoline amino acid carbonyl carbon. The distinctive downfield shift of the thiazoline amino acid carbonyl carbon thus correlates with the confirmed C-21 (*R*) chirality in **8**. Hence we assign the *R* configuration to C-21 in **13** and, by difference, the *S* chirality to C-21 in **12**.

Subsequent to completion of the structure elucidation work we noted that the ^1H NMR spectrum of a sample of **12** that had been standing in $CDCl_3$ solution for over a month exhibited a signal corresponding to a mixture of **12** and **13**. Since **13** was not isolated from our more recent collection of *L. patella*, it is possible that **13** is an artifact.

The ^{13}C NMR chemical shift assignments for the nonprotonated carbons of ascidiacyclamide and ulithiacylamide were established by INAPT and long-range ^1H–^{13}C COSY experiments, see Table II. These values parallel those for related carbons in the other peptides reported in this paper but differ from earlier assignments which were made without the aid of long-range proton–carbon coupling information. Our results require that the values previously assigned to the thiazolecarboxamide moieties



10' 11' 12' 13'

Figure 1. Long-range $^1H/^{13}C$ correlations determined by INAPT experiments. Arrows denote proton irradiated and carbon signal detected.

in **1–9**, prelissoclinamide-2,[6] prepatellamide-B-formate,[6] and preulicyclamide[6] be reassigned to parallel those reported here. One universal change required is reversal of the assignments of the carboxamide carbonyl carbon and C-2 in the thiazole ring.

All of the new cyclic peptides showed marginal cytotoxicity against lymphocytic leukemia cells (PS); ED_{50} values (μg/mL) for **10–13** are 11, 12, 10, and 6.9, respectively.[24] This level of activity is essentially the same as that reported for **1** and **4–6**.[2,3,14]

X-ray Crystallographic Analysis and Conformation of Patellamide D (10). The final atomic parameters of the non-hydrogen atoms are listed in Table IV. Figure 3 shows the numbering scheme followed in the description of crystallographic results. A stereoview of a single molecule of patellamide D is shown in Figure 4. The conformation of the peptide ring is further illustrated in Figure 5 in which the side-chain atoms are excluded for clarity.

The absolute configuration of patellamide D was determined by the Bijvoet method[25] using the anomalous dispersion of Cu radiation by the sulfur atoms. Of the 20 Friedel's pairs with most significant Bijvoet differences, 19 pairs agreed with the configuration shown in the schematic of **10**.

The patellamide D molecule assumes a severely folded conformation in which the 24-membered modified peptide backbone is shaped like a twisted "figure eight" and deviates significantly from its potential 2-fold symmetry. This conformation of the peptide molecule is stabilized by four transannular N–H···O hydrogen bonds. The two thiazole rings are nearly parallel to each other (dihedral angle of 8°) with a separation distance of 4.1 Å. The phenyl ring of the phenylalanyl residue protrudes out of the peptide backbone, whereas the aliphatic side chains of both the isoleucine residues and the threonine methyl groups cluster on the same surface of the peptide backbone.

The folding of the peptide chain results in a pair of β-turns with isoleucine and cyclized threonine residues at the corners of the turns. The (ϕ,ψ) torsion angles of these two β-turns, [(–80,113), (102,–1)] and [(–77,121), (102,–14)], are close to the theoretically expected values of [(–60,120), (80,0)] for a β(II)-turn.[26] The presence of a β(II) turn with two L residues at the corners is unusul. Normally a β(II) turn has L–D residues at the corners.[27] In the present structure, the cyclization of the threonine may be responsible for the uncommon conformation. Both the β-turns are stabilized by 4→1 type N–H···O hydrogen bonds (10-atom loop). The two hydrogen bonds involving the isoleucine amide group with the oxygen atom in the oxazoline ring can be described as a 5→1 type hydrogen bond (13-atom loop) observed in other cyclopeptide antibiotic structures.[27] The presence of both 4→1 type and 5→1 type hydrogen bonds in the same molecule has been reported in the case of the toxic octapeptide β-amanitin,[28] in iso-

(24) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, Part 3, 3(2), 1–103. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in μg/mL of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an $ED_{50} < 20$ μg/mL. PS refers to in vitro lymphocytic leukemia.

(25) Bijvoet, J. M.; Peerdeman, A. F.; van Bommel, A. J. *Nature (London)* **1951**, *168*, 271.

(26) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425.

(27) Karle, I. L. *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 4, pp 1–53.

(28) Kostansek, E. C.; Lipscomb, W. N.; Yocum, R. R.; Thiessen, W. E. *Biochemistry* **1978**, *17*, 3790.

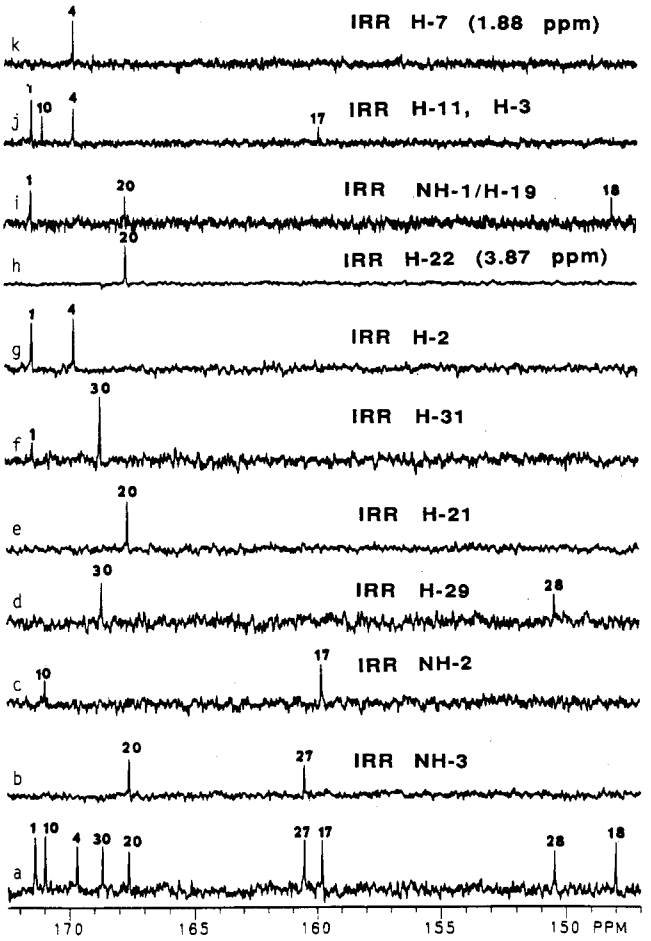


Figure 2. (a) Downfield region of broadband decoupled ^{13}C spectrum of 11 in $CDCl_3$ at 75 MHz. (b–k) INAPT spectra obtained by transfer from the proton indicated on each spectrum. All INAPT spectra are the results of 1024 scans.

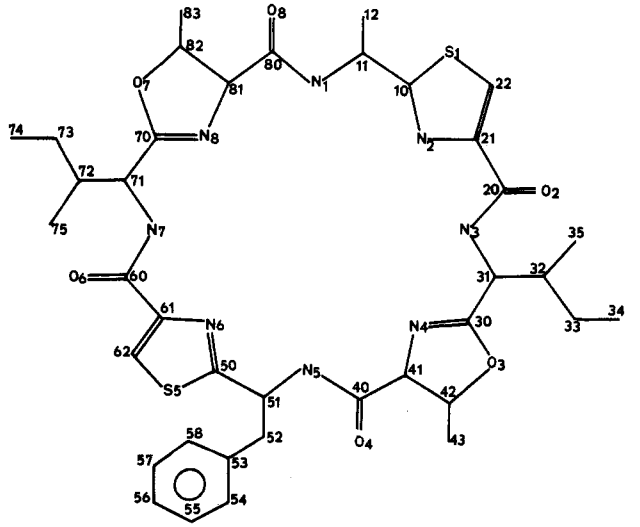


Figure 3. Atom numbering scheme used in the description of crystal structure results of **10**.

leucinomycin,[29] and in some of the valinomycin conformers.[27] All the intramolecular hydrogen bond parameters are listed in Table V.

The bond distances and angles observed in the molecule are normal. The dimensions of the thiazole ring and its neighboring amide bonds compare well with those observed

Table IV. Atomic Parameters of Non-Hydrogen Atoms of 10 (Estimated standard deviations are in parentheses)

Atom	x	y	z	U (equiv)
S(1)	0.95266 (6)	0.21214 (3)	−0.13670 (6)	0.0363 (2)
S(5)	0.69955 (6)	0.15866 (3)	0.01621 (6)	0.0344 (2)
N(1)	0.9080 (2)	0.37619 (9)	−0.1630 (2)	0.0306 (7)
N(2)	0.9409 (2)	0.29545 (9)	−0.0105 (2)	0.0285 (7)
N(3)	0.9491 (2)	0.30696 (8)	0.2115 (2)	0.0269 (6)
N(4)	0.8307 (2)	0.27647 (8)	0.4430 (2)	0.0261 (6)
N(5)	0.7453 (2)	0.19846 (8)	0.3316 (2)	0.0276 (7)
N(6)	0.7135 (2)	0.24470 (8)	0.1349 (2)	0.0270 (6)
N(7)	0.7079 (2)	0.35547 (8)	0.0875 (2)	0.0273 (7)
N(8)	0.8285 (2)	0.45778 (8)	−0.0485 (2)	0.0304 (7)
O(2)	0.9489 (2)	0.21451 (8)	0.2344 (1)	0.0366 (6)
O(3)	0.7858 (1)	0.34673 (7)	0.3345 (1)	0.0286 (5)
O(4)	0.5957 (1)	0.22541 (8)	0.3754 (2)	0.0373 (6)
O(6)	0.7034 (2)	0.33750 (8)	−0.0932 (1)	0.0349 (6)
O(7)	0.8793 (1)	0.41796 (7)	0.1083 (1)	0.0278 (5)
O(8)	1.0564 (1)	0.41436 (8)	−0.1581 (2)	0.0413 (7)
C(10)	0.9423 (2)	0.2824 (1)	−0.1138 (2)	0.0280 (8)
C(11)	0.9351 (2)	0.3232 (1)	−0.2064 (2)	0.0308 (8)
C(12)	0.8636 (2)	0.3041 (1)	−0.2921 (2)	0.043 (1)
C(20)	0.9480 (2)	0.2551 (1)	0.1740 (2)	0.0282 (8)
C(21)	0.9475 (2)	0.2497 (1)	0.0542 (2)	0.0277 (8)
C(22)	0.9547 (2)	0.2011 (1)	0.0006 (2)	0.0325 (9)
C(30)	0.8546 (2)	0.3132 (1)	0.3746 (2)	0.0251 (7)
C(31)	0.9533 (2)	0.3191 (1)	0.3277 (2)	0.0262 (8)
C(32)	0.9982 (2)	0.3758 (1)	0.3476 (2)	0.0322 (9)
C(33)	1.0998 (2)	0.3793 (1)	0.3011 (2)	0.0360 (9)
C(34)	1.1711 (2)	0.3379 (1)	0.3471 (3)	0.047 (1)
C(35)	0.9962 (2)	0.3892 (1)	0.4697 (2)	0.039 (1)
C(40)	0.6830 (2)	0.2310 (1)	0.3825 (2)	0.0268 (8)
C(41)	0.7248 (2)	0.2783 (1)	0.4486 (2)	0.0267 (8)
C(42)	0.6989 (2)	0.3340 (1)	0.3987 (2)	0.0304 (8)
C(43)	0.6815 (2)	0.3792 (1)	0.4793 (2)	0.040 (1)
C(50)	0.7107 (2)	0.1913 (1)	0.1410 (2)	0.0274 (8)
C(51)	0.7161 (2)	0.1601 (1)	0.2479 (2)	0.0259 (8)
C(52)	0.7850 (2)	0.1109 (1)	0.2441 (2)	0.0307 (8)
C(53)	0.7902 (2)	0.0824 (1)	0.3530 (2)	0.0320 (8)
C(54)	0.7297 (2)	0.0389 (1)	0.3773 (3)	0.043 (1)
C(55)	0.7336 (3)	0.0138 (1)	0.4788 (3)	0.054 (1)
C(56)	0.7983 (3)	0.0318 (1)	0.5573 (3)	0.052 (1)
C(57)	0.8578 (2)	0.0756 (1)	0.5329 (3)	0.045 (1)
C(58)	0.8545 (2)	0.1003 (1)	0.4315 (3)	0.0369 (9)
C(60)	0.7064 (2)	0.3210 (1)	0.0018 (2)	0.0275 (8)
C(61)	0.7062 (2)	0.2618 (1)	0.0278 (2)	0.0271 (8)
C(62)	0.6989 (2)	0.2212 (1)	−0.0471 (2)	0.0327 (8)
C(70)	0.8070 (2)	0.4326 (1)	0.0392 (2)	0.0267 (8)
C(71)	0.7089 (2)	0.4151 (1)	0.0723 (2)	0.0274 (8)
C(72)	0.6743 (2)	0.4449 (1)	0.1748 (2)	0.0318 (8)
C(73)	0.5714 (2)	0.4285 (1)	0.2041 (2)	0.040 (1)
C(74)	0.4942 (2)	0.4457 (1)	0.1235 (4)	0.059 (1)
C(75)	0.6838 (2)	0.5072 (1)	0.1591 (3)	0.042 (1)
C(80)	0.9713 (2)	0.4142 (1)	−0.1315 (2)	0.0298 (8)
C(81)	0.9345 (2)	0.4577 (1)	−0.0535 (2)	0.0298 (8)
C(82)	0.9662 (2)	0.4438 (1)	0.0632 (2)	0.0328 (9)
C(83)	0.9915 (2)	0.4929 (1)	0.1327 (3)	0.048 (1)
W(1)	0.4707 (2)	0.3022 (1)	0.4590 (3)	0.087 (1)
W(2)[a]	1.0549 (4)	0.1099 (2)	−0.7942 (5)	0.082 (2)
W(2a)[a]	1.1294 (5)	0.1953 (4)	−0.6943 (6)	0.085 (3)
W(2b)[a]	0.130 (1)	0.0080 (7)	0.136 (1)	0.069 (7)

[a] Disordered.

Table V. Hydrogen Bond Parameters

donor, D	acceptor, A	D–A	D–H	H–A	D–H–A angle, deg
N(1)	O (6)	3.131 (3)	0.83 (3)	2.39 (3)	149 (2)
N(3)	O (7)	3.142 (3)	0.79 (3)	2.39 (3)	159 (2)
N(5)	O (2)	3.111 (3)	0.85 (3)	2.39 (3)	143 (2)
N(7)	O (3)	3.231 (3)	0.89 (3)	2.42 (3)	151 (2)

in other thiazole containing peptide structures as in nosiheptide[30] and in cyclo[L-pro-L-Leu-L-val-(Gly)Thz-

(29) Pletnev, V. J.; Galitskii, M. K.; Smith, G. D.; Weeks, C. M.; Duax, W. *Biopolymers* **1980**, *19*, 1517.

(30) Pascard, C.; Decruix, A.; Lunel, J.; Prange, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418.

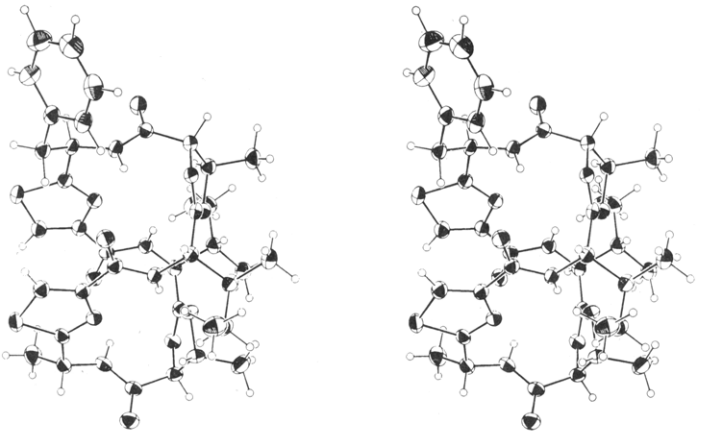


Figure 4. A stereoview of an ORTEP drawing of a patellamide D (50% probability thermal ellipsoids, hydrogen temperature factor is arbitrary).

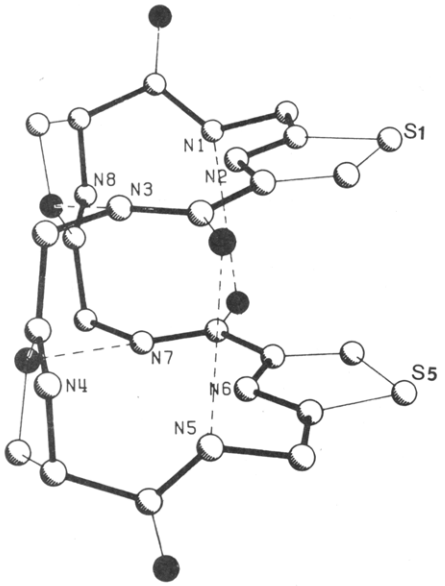


Figure 5. A perspective view of patellamide D, excluding the side-chain atoms. Peptide chain is drawn in bold lines. Hydrogen bonds are indicated by dashed lines.

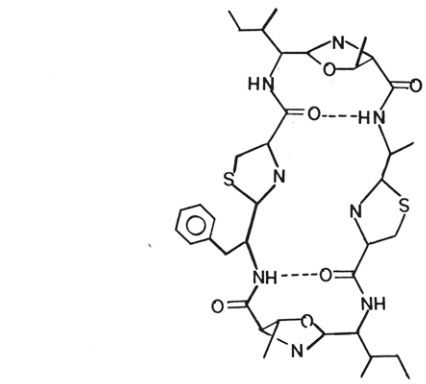
(Gly)Thz], a dolastatin 3 analogue.[31] The root-mean-square deviations of the respective five non-hydrogen atoms from their best planes are 0.003 Å for the thiazole rings and 0.09 Å for the oxazoline rings.

The two water molecules form hydrogen bonds with the peptide molecule at O(2), O(4), O(6), and N(4), but the solvent interactions do not seem to have any significant role in the conformation of the peptide backbone.

The only reported crystal structure among the group of cytotoxic cyclopeptides from ascidians is that of ascidiacyclamide[18] in which the 24-membered macrocyclic backbone assumes a nearly square shape with the four heterocyclic rings at the corners. The authors showed that this is also the predominant form in solution. They further suggested that the patellamides, which have the same backbone configuration as ascidiacyclamide and show similar cytotoxic activities, are likely to have the same conformation. Two kinds of basic conformation were proposed for the 24-membered modified peptide ring, a square one (type II) without any intramolecular hydrogen bonds, see structure 1, and a rectangular form (type I) stabilized by two intramolecular –H···O hydrogen bonds

(31) Stezowski, J. J.; Pöhlmann, H. W.; Haslinger, E.; Kalchhauser, H.; Schmidt, U.; Pozolli, B. *Tetrahedron* **1987**, *43*, 3923.

Chart III



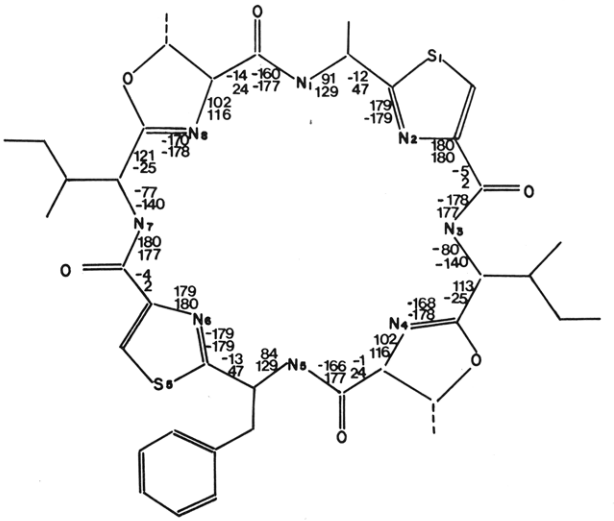


Figure 6. Conformational angles of the macrocyclic peptide chain for patellamide D (upper) and ascidiacyclamide (lower). Angles are rounded off [esd's range 0.3 → 0.5° (patellamide D)].

as shown in Chart III. While ascidiacyclamide has the type II conformation, patellamide D assumes a distinctly different conformation (type III) in which the rectangle of the type I conformation is twisted so that two additional hydrogen bonds are formed between the isoleucine amide groups and the oxygen atoms of the oxazoline rings.

The peptide backbone torsion angles (ω,ϕ,ψ) of patellamide D and asicidiacyclamide are compared in Figure 6. Except for those belonging to the planar segments containing thiazole rings, the conformational angles in the two molecules are significantly different. The largest

differences (60–150°) lie in the ϕ,ψ angles of the two isoleucine residues, although for both molecules these values fall within allowed low-energy regions on a Ramachandran plot[32] (helical region for ascidiacyclamide, small-peptide region for patellamide D). The differences in the ϕ,ψ values for the two D-residues (alanine and phenylalanine in patellamide D, two valines in ascidiacyclamide) are also very large (38–60°). On a Ramachandran plot, these ϕ,ψ angles lie close to the allowed regions. For the cyclized threonine residues, the differences in the ϕ,ψ angles are smaller (14–38°) but still significant. In general, the ϕ,ψ angles for the peptide chain fragments containing the five-membered rings fall outside the allowed regions on a Ramachandran plot for both compounds.

The planarity imposed by the conjugation of the thiazole ring and its attached amide bond seems to be a common feature in all related structures.[18,30,31] In both the molecules, the alkyl side chains protrude outward, which would explain the lipophillic character of these compounds. In ascidiacyclamide, all eight nitrogen atoms are pointed inward while the six oxygen atoms in the structure are directed away from the ring center. In patellamide D, all four NH groups are internally hydrogen bonded.

The distinctly different solid-state conformation of patellamide D compared to that of ascidiacyclamide clearly demonstrates that there is no single preferred conformer for the 24-membered peptide ring modified by the presence of two thiazole and two oxazoline rings as suggested by Ishida et al.[18] In fact, the present results shows that despite the modification of the peptide backbone, the macrocyclic ring retains the flexibility normally seen in regular polypeptide macrocycles.

In order to investigate the energetics of the different conformers of the macrocyclic peptide backbone, energy-minimization calculations were performed on the patellamide D crystal structure and a "model structure" of patellamide D, which was based on the crystal structure of ascidiacyclamide. The program AMBER[33] with all atom force field was used. The results show, interestingly, that the energy minimized structure of patellamide D, which does not differ significantly from its crystal structure, has appreciably lower energy (~10 kcal/mol) than the energy minimized "model structure". This energy difference may be attributed to the four intramolecular N–H···O hydrogen bonds in the patellamide D structure.

The work of Shioiri et al.[17] has shown that the oxazoline ring is crucial to manifestation of good levels of cytotoxicity in the cyclic peptides **1–5**. However, the ~100-fold increase in cytotoxicity of ulithiacyclamide, **2**, relative to other members of the group prompted the suggestion that the fixed conformation imposed by the disulfide link or the disulfide link itself may be necessary for optimum activity. On the basis of a CPK model the Japanese group found that ulithiacyclamide may have a conformation similar to the type II conformation found by X-ray for ascidiacyclamide.

Our X-ray results show that the structurally similar peptides ascidiacyclamide and patellamide D can have quite different preferred conformations and hence care must be taken in making predictions for related peptides. Our own computer molecular modeling indicates that a disulfide bridge is more easily accommodated in the ascidiacyclamide crystal conformation (type II) than in the one found for patellamide D (type III). Yet both compounds show similar cytotoxicity values. We conclude that the observations that have been made to date do not prove that the superior activity of ulithiacyclamide is related solely to conformation, and, hence, we assume that the optimum activity is due to a combination of conformation enforced by the disulfide link and chemical reactivity or nonbonding interactions of the disulfide group. We are continuing to determine the conformation of other peptides in this class and to probe the role that the disulfide group plays in the cytotoxicity of ulithiacyclamide.

Note added in proof: While this article was in press the ACS Publications Division made us aware that an in press issue of *J. Med. Chem.* contained an article that also reported lissoclinamides 4 and 5. (Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1349.) In order to avoid confusion in the literature, we have made the minor adjustment of making our names vs structures coincide with those of Degnan et al. As a consequence lissoclinamide 5 is discussed prior to lissoclinamide 4 since we had originally assigned the names in the reverse order.

Experimental Section

Melting points were taken on an A. H. Thomas Unimelt apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane; 2-D experiments were run using Varian software V. 6.1c. IR spectra were measured on a Perkin-Emer Model 298 spectrometer. Low- and high-resolution mass spectra were recorded on Hewlett-Packard 5985B and VG-ZAB E spectrometers, respectively. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Waters Associates silica gel Sep-Paks were used. Altex 5 μm × 9.6 mm × 25 cm semipreparative silica gel (Li Chrosorb 60) and Adsorbosphere 5 μm × 9.6 × 25 cm reverse-phase C_{18} columns were used for HPLC separation and purification.

Extraction and Isolation Procedures. Specimens of *L. patella* were collected at approximately 10-m depths using SCUBA (a) in August, 1983, at Dalton Reef, and (b) in June, 1987, at an unnamed reef 146° 44′ E: 17° 53′ S on the Great Barrier Reef, Australia, and identified by Dr. Patricia Kott, Queensland Museum, Brisbane, Australia. Freeze-dried material (65.8 g) from the 1983 collection was extracted in a Soxhlet extractor successively with pentane (4 h), hexane (24 h), and CH_2Cl_2 (2 days) to yield 566-, 324-, and 519-mg samples of viscous oil, respectively. The pentane and hexane extracts were combined and chromatographed on 150 g of silica gel with methylene chloride–ethyl acetate (6:4). Slowly eluting fractions of like composition (TLC) were combined and resolved by silica gel HPLC [hexane–acetone (7:3)] into ulithiacyclamide (**2**), 40 mg, and ascidiacyclamide (**1**), ~150 mg. The ^1H and ^{13}C NMR data for both peptides were identical with those reported[1,3] and correct elemental compositions were obtained by positive ion FAB mass spectral analysis.

Chromatography of the methylene chloride solubles (500 mg) over 50 g of silica gel using methylene chloride–ethyl acetate (6:4) yielded eight fractions. Fractions 4 and 5 contained ascidiacyclamide and ulithiacyclamide, and these were purified by HPLC as above. Fraction 6 was subjected to C_{18} reverse-phase HPLC using methanol–water (9:1) and yielded 9.5 mg of patellamide D (**10**). Fraction 7 (25 mg) was passed through a silica gel Sep-Pak column and eluted with hexane–acetone (95:5→0:100); eight fractions (10 mL each) were collected, a–h. Fractions d and e were combined and chromatographed by C_{18} reverse-phase HPLC [methanol–water (75:25)] to obtain 3 mg of patellamide D, 7 mg of lissoclinamide 5 (**12**), and 2.5 mg of lissoclinamide 6 (**13**).

From a 1987 collection, 900 g of ground, freeze-dried tissue was soaked in hexane (3 L, 12 h) and then chloroform (2 × 3 L, 24 h each). Evaporation of the combined extracts yielded 12 g of oily residue, which was subjected to flash chromatography using a step gradient commencing with hexane and then adding acetone

(32) Ramachandran, G. M.; Ramakrishnan, C.; Sasisekharan, V. J. *Mol. Biol.* **1963**, *7*, 95.

(33) Singh, U. C.; Weiner, P. K.; Caldwell, J.; Kollman, P. A.; Siebel, G. AMBER (USCF) Version 3.0. A set of Computer Programs for Molecular Mechanics; University of California: San Francisco, CA, 1986.

in steps up to a 1:1 ratio (total of 16 500-mL fractions). A portion of fraction 15 was subjected to HPLC using a C_{18} column and methanol–water (3:1) to give three pure compounds: **10**, 40 mg; **11**, 7 mg; and **12**, 70 mg. HPLC of portions of fractions 13 and 14 over C_{18} using methanol–water (7:3) afforded compounds **1**, **2**, and **10** (10, 8, and 4 mg, respectively).

Patellamide D (10): mp 144–145 °C (slow evaporation of methanol solution); IR 3380, 3325, 1660 (brd) cm^{-1}; $[\alpha]_D$ 32° (*c* 0.37, $CHCl_3$); HR EI MS 776.3099 $C_{38}H_{48}N_8O_6S_2$ (−5 ppm).

Lissoclinamide 5 (11): IR ($CHCl_3$) 3380, 3320, 3120, 1655, 1635 cm^{-1}; HR EI MS 739.2638 $C_{38}H_{41}N_7O_5S_2$ (3.7 ppm).

Lissoclinamide 4 (12): mp 152–154 °C (powder precipitated from ether); IR ($CHCl_3$) 3380, 3325, 1655, 1630 cm^{-1}; $[\alpha]_D$ 45° (*c* 0.7, $CHCl_3$); HR EI MS 741.2767 $C_{38}H_{43}N_7O_7S_2$ (15.2).

Lissoclinamide 6 (13): FT IR (film) 3380, 3325, 1677, 1640 cm^{-1}; HR EI MS 741.2818 $C_{38}H_{43}N_7O_5S_2$ (6.9 ppm).

X-ray Analysis of 10. Well-formed prismatic crystals of patellamide D were obtained by slow evaporation from a methanol solution. A crystal of dimensions 0.10 × 0.10 × 0.40 mm was selected for X-ray measurements. The cell parameters were determined from the least-square fit of $\pm 2\theta$ values of 48 reflections measured at 163 K using Cu $K\alpha_1$ radiation. Patellamide D crystallizes in the orthorhombic space group $P2_12_12_1$ with a = 13.976 (2), b = 24.360 (4), and c = 12.289 (3) Å; V = 4183.9 Å^3; Z = 4; D = 1.288 gm/cm^3 at 163 K. Intensities of all unique reflections within $0 \leq 2\theta \leq 150°$ were measured on an Enraf-Nonius CAD-4 diffractometer at 163 ± 2 K using Cu $K\alpha$ radiation. A $\theta - 2\theta$ scan technique was employed using a variable scan width of $(0.90 + 0.15 \tan \theta)°$. Intensities of three standard reflections were monitored every 2 h of X-ray exposure and they showed no significant variation. Out of the total of 4798 reflections, 4274 were considered observed on the basis of $I > 2\sigma(I)$. Data were corrected for Lorentz and polarization effects, but no absorption correction was made (μ = 15.2 cm^{-1}). The structure was solved by direct methods using the program MULTAN[34] and refined by a full-matrix least-squares routine[35] in which the quantity, $\sum\omega(|F_o| - |F_c|)^2$, was minimized. All the hydrogen atoms in the molecule were located from the difference Fourier maps and were refined with isotropic thermal parameters. One of the two water molecules in the structure was found to be disordered. The structure was refined to a final R factor of 0.034, R_w = 0.035 and an S = 1.67. The final difference map was featureless and contained peaks of height +0.2 e/Å^3. The molecular dynamics and energy minimization calculations were performed using the AMBER all atom force field.[33] Charges for the nonstandard peptide fragments of the molecule were calculated with the ab initio GAUSSIAN80 USCF program.

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Supplementary Material Available: Bond lengths, bond angles, anisotropic thermal parameters for non-hydrogen atoms, and hydrogen atom parameters (10 pages). Ordering information is given on any current masthead page.

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Notes

Sulfircin: A New Sesterterpene Sulfate from a Deep-Water Sponge of the Genus *Ircinia*

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Relatively few sulfate-containing compounds have been reported from marine sources. Sponges have been the source of sterol sulfates[1] while saponin sulfates have been reported from echinoderms.[2] Recently, suvanine, a sesterterpene sulfate, has been reported from a sponge of the genus *Coscinoderma*.[3] From our efforts to identify antifungal agents from marine organisms, we report the isolation and structure elucidation of a new sesterterpene sulfate, **1**, for which we have assigned the common name sulfircin. Sulfircin was isolated as its *N,N*-dimethylguanidinium salt from a deep-water collection of the marine sponge *Ircinia* sp.[4] The presence of sulfate functionality in **1** makes it unusual, but even more unusual was its isolation as its *N,N*-dimethylguanidinum salt. To the best of our knowledge, suvanine is the only other example of a marine natural product isolated as its *N,N*-dimethylguanidinium salt.

(1) See, for example: Fusetani, N.; Matsunaga, S.; Konosu, S. *Tetrahedron Lett.* **1981**, *22*, 1985–1988. Nakatsu, T.; Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *Experimentia* **1983**, *39*, 759–761.

(2) See, for example: Kitagawa, I.; Kobayashi, M. *Tetrahedron Lett.* **1977**, 859–862; *Chem. Pharm. Bull.* **1978**, *26*, 1864–1873.

(3) Manes, L. V.; Crews, P.; Kernan, M. K.; Faulkner, D. J.; Fronczek, F. R.; Gandour, R. D. *J. Org. Chem.* **1988**, *53*, 570–575.

(4) Taxonomic classification carried out by Cristina Diaz as per Wiedenmayer and van Soest: Wiedenmayer, F. *A Monograph of the Shallow Water Sponges of the Western Bahamas*; Birkhauser Verlag: Basel, 1977; pp 287 (*Experientia* supplement 28). van Soest, R. W. M., personal communication. A voucher of the sponge is kept for reference at Harbor Branch Oceanographic Institution in Ft. Pierce.

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