frozen at −20 °C. The extraction procedure was the same as described above.

Chromatography. The crude extract was dissolved in methanol/water (77:23) and applied to a Whatman Partisil ODS-3 9 mm × 500 mm preparative HPLC column. Elution was effected by means of a concave gradient from 77 to 100% methanol over 120 min and the absorbance of the eluate was monitored with a Waters 990 Photodiode Array detector. Fractions corresponding to the various peaks were pooled, evaporated to dryness, and subjected to further purification, either by rechromatography on the same column under isocratic conditions or by chromatography on Sephadex LH-20 using the same solvent. Complete separation of peaks 2 and 3 (Figure 1) required chromatography on silica gel 60 using dichloromethane/ethyl acetate (3:2).

NMR Spectroscopy. Spectra were obtained with a C-5 dual ^{1}H, ^{13}C probe in a JEOL GX400 spectrometer. Compounds were dissolved in deuterated chloroform (Merck) and chemical shifts were derived relative to tetramethylsilane (TMS). For homonuclear correlated experiments (COSY 45), the following parameters were used: Patellamide D: spectral width, 3189 Hz, data matrix, 512 × 2048, scans 32, recycle time 4 s, zero filling in ν_1 domain. Lissoclinamide 4: spectral width 3360 Hz, data matrix 512 × 1024, scans 48, recycle time 3.15 s, zero filling in both domains. Lissoclinamide 5: spectral width 3931 Hz, data matrix 256 × 1024, scans 128, recycle time 4 s, zero filling in both dimensions. The overall time of accumulation was 16–24 h. Sine-bell apodization functions were used. For the heteronuclear correlated experiments the following parameters were used. Patellamide D: data matrix 128 × 4096, scans 192, recycle time 4 s. Lissoclinamide 4: data matrix 128 × 4096, scans 680, recycle time 1.8 s. Lissoclinamide 5: data matrix 64 × 4096, scans 2030, recycle time 1.7 s. Fixed delays of $\Delta1(^{1/2}J)$ and $\Delta2(^{1/4}J)$ were set for J = 138 Hz. Both dimensions were zero filled and multiplied by a sine-bell function before transformation. The COLOC[18] experiments on lissoclinamides 4 and 5 were performed with delay times $\Delta1$ = 25 ms and $\Delta2$ = 30 ms, data matrix 128 × 4096, recycle time 1.722 s, and zero filling in the ν_1 domain. A sine-bell apodization function was applied before transformation.

Mass Spectroscopy. A Kratos MS25RFA instrument was used. For the FAB-MS an Iontech saddle-field FAB source was used with argon gas. Samples were dissolved in methanol at a concentration of 1 mg/mL and diluted 5 times with glycerol for FAB analysis. High-resolution electron-impact mass spectra (HREIMS) were recorded at 70 keV and a resolving power of 3000.

Acid Hydrolysis and Chiral Gas Chromatography. Peptides (0.3 μmol) were hydrolyzed in 6 M HCl (1 mL) and mercaptoethane (100 μL) in vacuo for 3–24 h at 110 °C. Mercaptoethane was added to prevent oxidation of cysteine during hydrolysis. After evaporation to dryness the amino acids were converted to their *N*-pentafluoropropionyl isopropyl esters as described by Frank and co-workers[27] and applied to a Chirasil-Val GC column for separation of D and L isomers.[28]

Cytotoxicity Testing. Two cell lines were used: MRC5CV1 (SV40-transformed human fibroblasts) as control and T24 (transitional cell carcinoma of the bladder) as a tumor line. Cells were maintained in RPMI-1640 medium (Commonwealth Serum Laboratories) containing 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were plated at a density of 5×10^3 cells/16-mm well, 24 h prior to cytotoxicity testing. Initial screening for cytotoxicity was performed by microscopic assessment of cultures 5–7 days after exposure to various concentrations of the compounds for 1 h. Compounds that showed cytotoxicity at this level were studied in more detail by examining their effects on the incorporation of [*methyl*-^{3}H]thymidine into DNA. This method is more rapid and considerably less tedious than the clonogenic assay and has been shown to reflect reliably cell survival when compared to data obtained from the clonogenic assay.[29] Cells were exposed to various concentrations of the compounds for 1 h and after 5 days were labeled with [*methyl*-^{3}H]thymidine (10 μCi/mL, 40 Ci/mmol, Amersham) for 4 h, after which they were extracted with cold 10% trichloroacetic acid (TCA). The TCA-insoluble material was collected onto glass fiber filters and washed with cold 5% TCA followed by 100% ethanol prior to liquid scintillation counting. Each experiment was performed three times with duplicate assays at each point. The statistical significance of differences between mean values was determined by using the student's t test.

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Novel Cytotoxic Compounds from the Ascidian *Lissoclinum bistratum*

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The isolation and structures of two new cyclic hexapeptides and two new macrocyclic ethers from the aplousobranch ascidian *Lissoclinum bistratum* are described. Their structures were determined by two-dimensional NMR techniques. The hexapeptides, named bistratamide A and bistratamide B, differ only by the presence or absence of one double bond. They were tested for cytotoxicity toward human fibroblast and tumor cell lines and displayed similar toxicities to the octapeptides called patellamides from *Lissoclinum patella*. The peptides are found within the obligate algal symbiont *Prochloron* but clearly differ from peptides isolated from the same *Prochloron* of *L. patella*. The macrocyclic ethers isolated from *L. bistratum* are exceedingly potent in cytotoxicity. They have been named bistratenes A and B, and structures for these compounds are proposed.

Recent research has confirmed that marine organisms are a valuable source of new organic compounds with potential use as antineoplastic agents.[1] The didemnid ascidians in particular have been a rich source of cytotoxic amino acid-derived metabolites.[2,3] In a related paper[4] we

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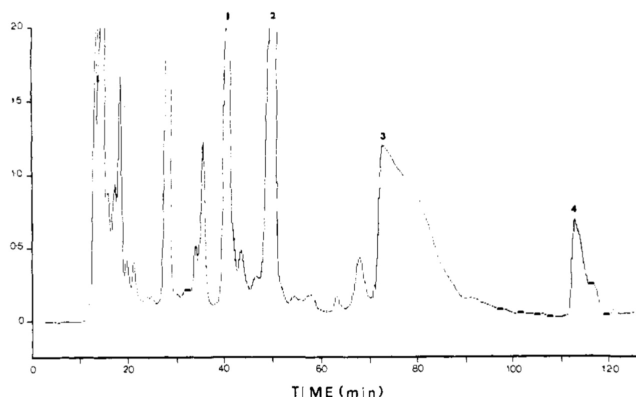

Figure 1. HPLC of *L. bistratum* extract. The extract was applied to a Whatman Partisil 10 ODS-3 column (C-18) in methanol/water (77:23) and eluted as described in the Experimental Section. The major cytotoxic fractions numbered 1–4 are in order of elution bistratamide B, bistratamide A, bistratene A, and bistratene B.

report on the structures of three new cytotoxic cyclic peptides from *Lissoclinum patella*. In this paper we describe the isolation, structure determination, and cytotoxic properties of four new cytotoxic compounds from the related species *L. bistratum*. A recent paper has described a partial structure for one of these compounds.[5] *L. bistratum* contains the same prokaryotic algal symbiont as *L. patella*, namely *Prochloron*. The *Prochloron* was separated from the host, and the types of peptides contained within alga and ascidian were investigated.

Results and Discussion

Isolation and Structure Determination. A crude oil was isolated from *L. bistratum* after homogenization in methanol/toluene (3:1), extraction with 1 M sodium nitrate solution, extraction with chloroform, and evaporation of the dried chloroform layer. The elution profile obtained from preparative reverse-phase high-performance liquid chromatography (HPLC) on Partisil ODS-3 is shown in Figure 1. The major cytotoxic fractions numbered 1–4 are in order of elution: bistratamide B, **1**, bistratamide A, **2**, bistratene A, **4**, and bistratene B, **5**. The name "bistratamide" has been chosen for the family of cyclic hexapeptides from *L. bistratum* because the structures of these compounds[6] are related to the patellamides, a family of cyclic octapeptides from *L. patella*.[2] The name "bistratene" reflects the alkene groups in the other family of compounds from *L. bistratum*. When this work was completed, a partial structure of bistratene A appeared in the literature with the name bistramide A ascribed to it.[5] The structures of the compounds have been determined unambiguously from detailed analyses of ^{1}H and ^{13}C NMR spectra and from 2D COSY 45 and ^{1}H–^{13}C shift correlation experiments.

High-resolution electron-impact mass spectrometry (HREIMS) of bistratamide A, **2** ($C_{27}H_{34}N_6O_4S_2$), gave a molecular weight of 570.2071 (calcd 570.2083). Acid hydrolysis of bistratamide A followed by chiral gas chroma-

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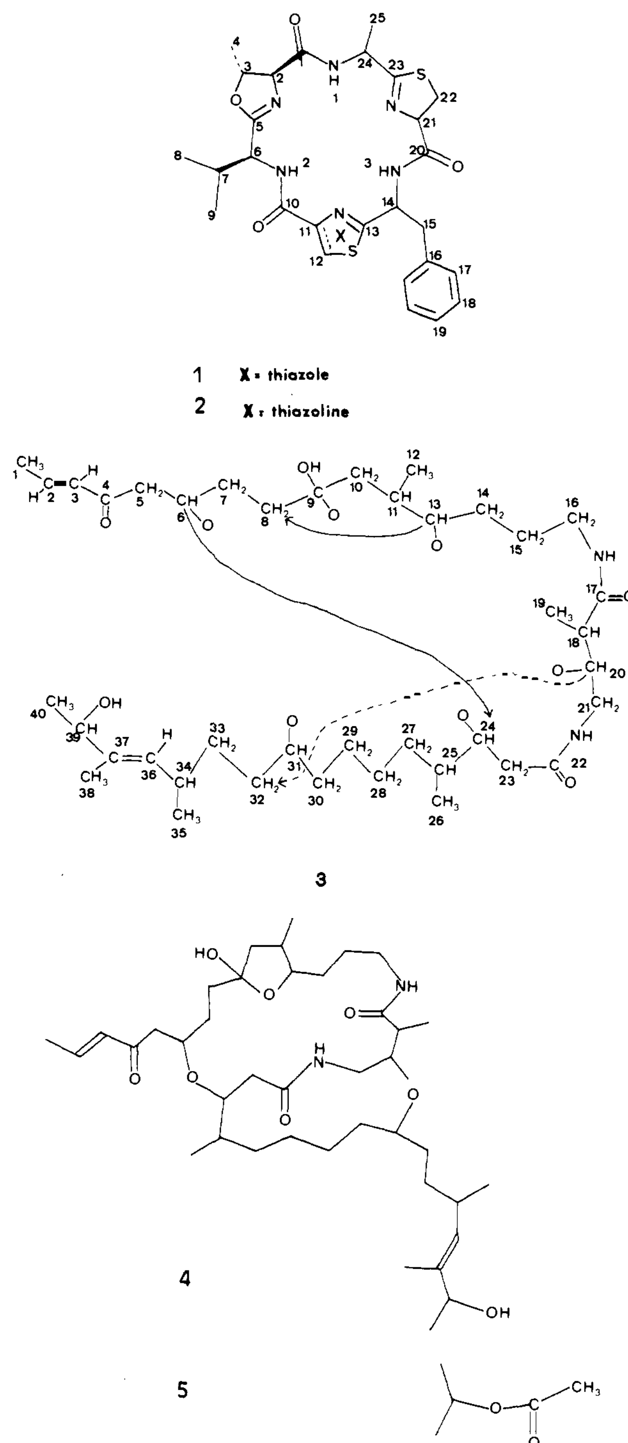

tography yielded L-threonine, L-valine, L-cysteine, DL-alanine, and DL-phenylalanine. The proportions of L-alanine and L-phenylalanine were always greater than the proportions of the D isomers (by a factor of 3:2) so it may be assumed that the configuration of the amino acids is L in all cases. We are presently unable to resolve the problem of racemization of amino acids adjacent to the thiazoline rings.

The NMR data for **2** are given in Table I. Long-range ^{1}H–^{1}H coupling is important in determining the order of amino acids around the ring. This long-range coupling is observed between H-2 and H-6, H-2 and H-24, and H-21 and H-24, unambiguously placing the alanine and not the phenylalanine residue at position 24. Long-range coupling is also observed between one of the thiazoline protons H-11 and the phenylalanine α-C proton (H-14). The long-range coupling constants in bistratamide A (ca. 2 Hz) are much

Table I. NMR Assignments for Bistratamide A (2)

carbon no.	δ(^{13}C)	δ(^{1}H)	multiplicity (^{1}H)	coupling constant (^{1}H–^{1}H): *J*, Hz
1	172.21[a]			
2	73.90	4.16	dd	9.6, 2.2
3	81.95	4.80	dq	9.6, 6.2
4	21.71	1.61	d (3 H)	6.2
5	168.33[a]			
6	52.44	4.67	ddd	8.2, 3.1, 2.2
7	31.46	2.27	m	
8	17.26	0.83	d (3 H)	6.9
9	18.92	0.92	d (3 H)	6.9
10	170.12[a]			
11	77.63	4.90	ddd	12.2, 9.3, 2.5
12	36.14	3.75, 3.48	dd, dd	11.3, 9.3, 11.3, 12.2
13	169.60[a]			
14	52.63	5.00	m	
15	39.22	3.34, 3.17	dd, dd	13.9, 4.9; 13.9, 4.1
16	135.29			
17	129.56 (2 C)	7.05–7.13	m (5 H)	
18	128.10 (2 C)	7.05–7.13	m	
19	127.04	7.05–7.13	m	
20	175.49[a]			
21	78.01	4.98	dd	10.0, 2.2
22	36.90	3.78, 3.54	dd, dd	11.3, 10.0; 11.3, 10.0
23	168.94[a]			
24	48.06	4.76	m	
25	21.83	1.32	d (3 H)	7.0
NH(1)		7.59	d	7.5
NH(2)		7.48	d	8.2
NH(3)		7.89	d	6.5

[a] Interchangeable assignments.

Table II. Assignments for Bistratamide B (1)

carbon no.	δ(^{13}C)	δ(^{1}H)	multiplicity (^{1}H)	coupling constant (^{1}H–^{1}H): *J*, Hz
1	170.23[a]			
2	73.87	4.23	dd	7.2, 2.1
3	80.83	4.91	m	
4	21.40	1.50	d (3 H)	6.2
5	168.38[a]			
6	51.74	4.76	m	
7	31.32	2.11	m	
8	15.76	0.42	d (3 H)	7.0
9	18.79	0.74	d (3 H)	6.7
10	159.36			
11	148.90			
12	123.73	7.93	s	
13	170.12[a]			
14	53.00	5.36	ddd	9.3, 7.3, 4.9
15	43.56	3.46, 2.90	dd, dd	13.2, 4.9; 13.2 9.3
16	135.85			
17	129.63 (2 C)	7.17–7.27	m (3 H)	
18	128.62 (2 C)	7.02	dd (2 H)	
19	127.22	7.17–7.27	m	
20	175.69[a]			
21	77.93	5.17	dd	9.8, 8.7
22	36.50	3.70, 3.77	dd, dd	11.4, 9.8; 11.4, 8.7
23	168.08[a]			
24	48.14	5.05	m	
25	21.80	1.56	d (3 H)	6.8
NH(1)		8.40	d	7.3
NH(2)		7.40	d	9.6
NH(3)		8.14	d	7.3

[a] Interchangeable assignments.

larger than for the corresponding couplings in the patellamides and lissoclinamides, and splitting due to the long-range coupling can be observed in the 1-D ^{1}H NMR spectrum. For example, the signal from H-2 is a doublet of doublets instead of a doublet as would be expected (see Table I) and the signal from H-11 is split into a doublet of doublet of doublets.

HREIMS of bistratamide B, 1, indicated a molecular weight of 568.1922 (calcd for $C_{27}H_{32}N_6O_4S_2$ 568.1926). 1 differs from bistratamide A only in the conversion of one thiazoline to a thiazole ring. NMR data for this compound are presented in Table II. Long-range ^{1}H–^{1}H couplings are observed between protons H-2 and H-6, H-21 and H-24, and H-3 and H-24. This establishes the same sequence of amino acids as in bistratamide A. Acid hydrolysis followed by chiral gas chromatography yielded L-threonine, L-valine, L-cysteine, and DL-alanine. Attempts to determine the stereochemistry of the phenylalanine thiazole were unsuccessful.[7]

Bistratamides A and B were obtained in approximately equal amounts comprising 0.3% of dried extract each (equivalent to 5 mg/kg wet weight).

Conformational Information. The ^3J(NHCH) vicinal coupling constants were used to obtain information about the dihedral angle (θ) between NH and α-CH.[8] For bistratamide A the coupling constants are NH(1)–CH(24) 7.5 Hz, NH(2)–CH(6) 8.2 Hz, and NH(3)–CH(14) 6.5 Hz. The corresponding dihedral angles are 150° to 130° or 30° to 15°, 155° to 140° or 20° to 0°, and 145° to 135° or 45° to 25°, respectively.

For bistratamide B the respective coupling constants are 7.3, 9.6, and 7.3 Hz with corresponding angles of $150 \geq \theta \geq 130°$ or $30° \geq \theta \geq 15°$, $170° \geq \theta \geq 155°$, and $150° \geq \theta \geq 130°$ or $30° \geq \theta \geq 15°$. Using Dreiding models and the larger set of angles, it was observed that all nitrogen atoms pointed toward the center of the ring. If the smaller set of angles was used, only the heterocyclic N atoms pointed toward the center.

The α-CH–β-CH_2 vicinal coupling constants for the phenylalanine residues can be analyzed by the method of Feeney[9] to yield populations of rotamers about C-α–C-β, where rotamer I has the phenyl group between CO and α-CH, rotamer II between α-CH and NH, and rotamer III between CO and NH. The coupling constants for bistratamide A are 4.1 and 4.9 Hz and for bistratamide B 9.3 and 4.9 Hz. In each case two solutions are possible. For bistratamide A they are pI 0.07, pII 0.14, pIII 0.80 or pI 0.16, pII 0.21, pIII 0.63, where pI, pII, pIII represent the mole fractions of rotamers I, II, and III. Thus the preferred orientation of the phenylalanine ring is between the amide NH and the thiazoline ring (rotamer III) with the aromatic ring positioned above the macrocyclic ring. For bistratamide B the solutions are pI 0.68, pII 0.14, and pIII 0.18 or pI 0.28, pII 0.69, and pIII 0.03. Thus the preferred orientation of the phenylalanine is either between the thiazole ring and the α-CH (rotamer I) with the phenyl ring positioned near the thiazole ring or between the amide NH and the α-CH (rotamer II) with the phenyl ring pointing away from the macrocyclic ring.

For bistratene A, fast atom bombardment mass spectrometry gave a molecular ion peak $(M + H)^+$ of 705 consistent with a chemical formula of $C_{40}H_{68}N_2O_8$ (calcd 704.4975). After this work was completed, another group published a partial structure for bistratene A which they called bistramide A.[5] They reported that they were unable to deduce the complete structure using currently available 2D NMR techniques. The results of our 2D NMR experiments, COSY 90, COSY 45, ^{1}H–^{13}C shift correlation

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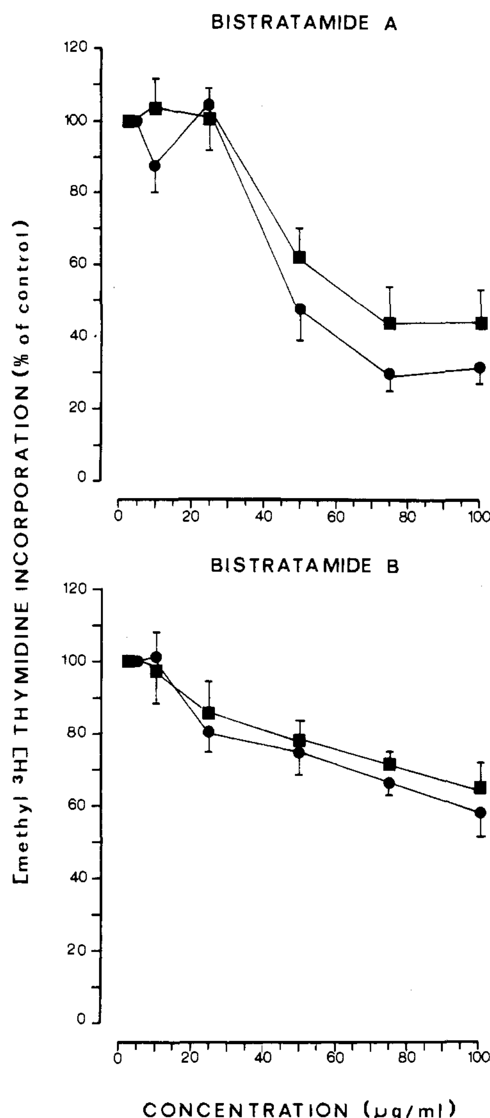

Figure 2. Cytotoxicity of bistratamides A and B. Fibroblast cells (MRC5CV1, ■) and bladder carcinoma cells (T24, ●) were exposed for 1 h to the indicated concentrations of the four compounds examined. Five days later the incorporation of [*methyl*-^{3}H]-thymidine was assessed and expressed as a percentage of the incorporation of untreated cells (100%). Each experiment was performed three times with duplicate assays at each point. The error bars show the standard error of the mean.

(HETCOR), and long-range ^{1}H–^{13}C shift correlations, are entirely consistent with the linear structure they have presented, **3**. The NMR data for bistratene A have been tabulated in Table III. Three key long-range ^{1}H–^{13}C correlations (marked with an asterisk) enable us to propose the structure **4** for bistratene A. These key couplings were observed using, not the COLOC pulse sequence,[10] but the ordinary ^{1}H–^{13}C shift correlation pulse sequence[11] with delay times optimized for long-range couplings. Two experiments were necessary to observe all three couplings: one with Δ1 and Δ2 set at 50 and 33 ms, respectively, and the other with Δ1 and Δ2 set at 30 and 20 ms. Bistratene B, **5**, is an acetylated derivative of bistratene A. FAB mass spectrometry of bistratene B gave a molecular ion peak $(M + H)^+$ of 747 consistent with a molecular formula of $C_{42}H_{70}N_2O_9$ (calcd 746.5081). NMR data for bistratene B are given in Table IV. Evidence from NOESY experiments[5] suggests that the two allylic side chains in bistratene A are in close proximity to each other.

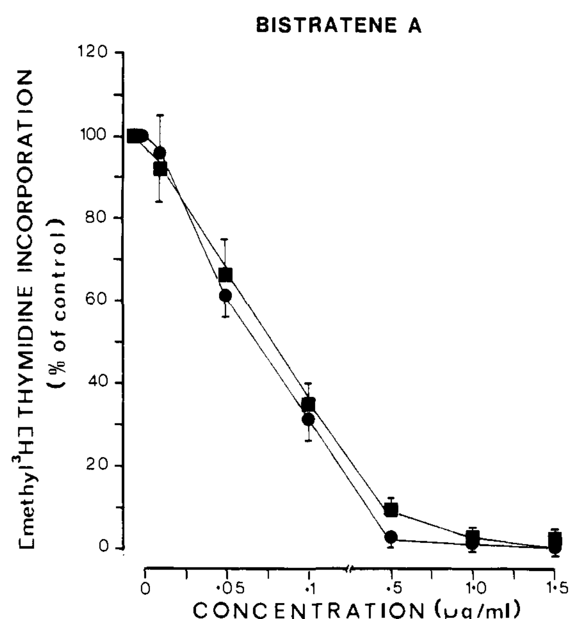

Figure 3. Cytotoxicity of bistratene A. For experimental details, see Figure 2.

The yields of bistratenes A and B were 10% and 2.8% of the dried extract, respectively (equivalent to 0.56 g/kg wet weight and 0.15 g/kg, respectively).

The bistratenes bear gross structural similarity to the bryostatins, macrocyclic lactones isolated by Pettit's group from the bryozoan *Bugula neritina*.[12]

Cytotoxicity Studies. It has been shown that a number of compounds isolated from ascidians have potentially useful biological activities and we have described here two new classes of metabolites. It provides a useful approach to studying structural functional relationships. In this paper we compare the cytotoxicity of these compounds toward human cell lines, MRC5CV1 fibroblasts and T24 bladder carcinoma cells, by incorporation of [*methyl*-^{3}H]thymidine into cells 5 days after a 1-h exposure (Figures 2 and 3). Bistratamide A has an IC_{50} value of about 50 μg/mL and bistratamide B an IC_{50} value greater than 100 μg/mL. Their toxicity is similar to that of the cyclic octa- and heptapeptides from *L. patella*, patellamide D and lissoclinamide 5.[4] The conversion of one thiazoline in bistratamide A to a thiazole in bistratamide B results in a less toxic compound. A similar observation was made with lissoclinamides 4 and 5.[4]

The bistratenes are the most cytotoxic compounds we have yet discovered in ascidians. Bistratene A has an IC_{50} value of 0.07 μg/mL (see Figure 3). The cytotoxicity of bistratene B is similar to that of bistratene A (IC_{50} = 0.09 μg/mL); thus acetylation of the free hydroxyl group of bistratene A does not affect its toxicity. The potency of the bistratenes rivals that of the bryostatins whose IC_{50} values range from 0.89 μg/mL to 10^{-7} μg/mL with the P388 cell line in vitro.[12]

None of the compounds described here showed preferential cytotoxicity to the tumor cell line T24, although further studies with a variety of cell types will be more revealing. However, the high potency of the bistratenes indicates that they may be potential candidates as antitumor agents. Preliminary studies using flow cytometry demonstrate that the bistratenes kill cells at all phases of

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Table III. NMR Assignments and Correlations in Bistratene A (4)

carbon no.	$\delta(^{13}C)$ (DEPT)	$\delta(^{1}H)$ (from HETCOR)	$^{1}H-^{1}H$ couplings (COSY 45 and COSY 90)	$^{1}H-^{13}C$ long-range couplings
1	18.36 (CH_3)	1.92 (3 H) dd (J = 6.8, 1.6 Hz)	H2, H3	
2	144.37 (CH)	6.91 dd (J = 15.8, 6.8 Hz)	H1, H3	H1
3	132.17 (CH)	6.13 dd (J = 15.8, 1.6 Hz)	H2, H1	H1
4	198.87			H3, H5
5	45.30 (CH_2)	2.54, 2.90 dd, dd (J = 16.8, 3.2; 16.8, 8.8 Hz)	H6	
6	64.93 (CH)	4.20 q (J = 6.3 Hz)	H7, H5	H8, H24*
7	30.76 (CH_2)	1.37, 1.69	H6	
8	36.14 (CH_2)	1.47, 1.61		
9	95.47			
10	27.95 (CH_2)	1.45, 1.58		H12
11	34.88 (CH)	1.29	H12	H10, 12
12	17.99 (CH_3)	0.80 (3 H) d (J = 6.6 Hz)	H11	
13	74.28 (CH)	3.15	H16, H14	H8*
14	30.48 (CH_2)	1.35, 1.71	H13	
15	25.85 (CH_2)	1.52, 1.83	H16, H13	H18
16	39.54 (CH_2)	3.29 (2 H)	H15, NH(1)	
17	175.18			
18	43.40 (CH)	2.39		H19
19	15.53 (CH_3)	1.25 (3 H) d (J = 7.2 Hz)	H20	
20	73.85 (CH)	3.71	H19, H21	H19, H30*
21	44.68 (CH_2)	3.24, 3.48	NH(2), H20	
22	173.42			H24, H23
23	32.47 (CH_2)	2.13, 2.75 dd, dd (J = 15.2, 1.65; 15.2, 11.5 Hz)	H24	
24	74.80 (CH)	4.09 dd (J = 10.6, 4.8 Hz)	H25, H23	H23, H26
25	33.33 (CH)	1.92	H26, H27, H24	H26
26	17.07 (CH_3)	0.86 (3 H) d (J = 7 Hz)	H25,	
27	26.56 (CH_2)	1.32, 1.63	H25	H26, H28
28	35.52 (CH_2)	1.57, 1.35	H25, H29	H29
29	19.25 (CH_2)	1.52, 1.81	H28, H30	H27
30	31.36 (CH_2)	1.13, 1.53	H31, H29	
31	69.09 (CH)	3.45	H30	H30
32	34.12 (CH_2)	1.27, 1.41		
33	33.50 (CH_2)	1.28, 1.40	H36	H35
34	31.89 (CH)	2.35	H35, H33, H36	H36, H35
35	20.94 (CH_3)	0.95 (3 H) d (J = 6.8 Hz)	H34, H33	H36
36	131.38 (CH)	5.18 d (J = 9.5 Hz)		H35, H38
37	137.23			H38, H40
38	11.79 (CH_3)	1.62 (3 H) s	H36	H38
39	73.29 (CH)	3.15	H40, H36	H40
40	21.80 (CH_3)	1.24 (3 H) d (J = 6.4 Hz)	H39	
NH(1)		7.00 t (J = 5.5 Hz)		
NH(2)		7.35 t (J = 5.9 Hz)		

the cell cycle (unpublished results). The results suggest that these compounds may be acting at the level of the cell membrane rather than inhibiting protein or nucleic acid synthesis. Studies are in progress to investigate this possibility.

Location of Bistratamides and Bistratenes. When the *Prochloron* algal cells are removed from the host ascidian and extracted, bistratamides A and B are found in large concentration. Workup of the remaining host animal yields the bistratenes. Hence the cyclic peptides are presumed to be synthesized by the algal cells and the complex macrocyclic ethers by the ascidian itself. The cyclic peptides are clearly different from the peptides found in the same *Prochloron* from a related host *L. patella*.[2] Thus it will be interesting to determine just how the compounds are synthesized: whether they are made by the ascidian and transferred to the algal symbiont.

Bistratamide B and the patellamides and lissoclinamides contain thiazole rings, which are commonly present in a number of peptide antibiotics elabatorated by many strains of *Streptomyces*, e.g. thiostrepton[13] and nosiheptide.[14] Preliminary results show that none of the cyclic thiazole containing peptides we have isolated have significant antibacterial activity. The function of these peptides in the ascidian remains to be elucidated.

In summary we have isolated two new classes of cytotoxic compounds from the ascidian *L. bistratum*. One of these, the bistratenes, is extremely potent. The mode of action of these compounds is currently under study.

Experimental Section

Collection of Animals. *L. bistratum* was collected at Heron Island Reef on the Great Barrier Reef, Australia, and identified by Dr. P. Kott of the Queensland Museum. Animals were cleaned of debris and frozen at −20 °C within 4 h of collection. The prokaryotic algal symbiont in association with *L. bistratum* has been classified in the same taxonomic group as the *Prochloron* from *L. patella*, by Dr. G. Cox, (University of Sydney) in a study of the structural, ultrastructural, and biochemical characteristics of *Prochloron* from different ascidian hosts.[15]

Extraction of Cytotoxic Compounds. A typical sample size of 250 g (wet weight) of frozen animals was homogenized in a Waring Blendor with 1 L of a 3:1 methanol/toluene mixture.[16] The homogenate was filtered to remove particulate material and the filtrate extracted with 800 mL of 1 M aqueous sodium nitrate. The aqueous layer was extracted with chloroform (6 × 100 mL) and the chloroform layer dried and evaporated, yielding a

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Table IV. NMR Assignments for Bistratene B (**5**)

carbon no.	$\delta(^{13}C)$ (DEPT)	$\delta(^{1}H)$ (from HETCOR)	$^{1}H-^{1}H$ coupling (COSY 45)	$^{1}H-^{13}C$ couplings (COLOC, L-R HETCOR)
1	18.38 (CH_3)	1.92 (3 H) dd (J = 6.8, 1.7 Hz)	H2, H3	
2	144.39 (CH)	6.90 dd (J = 15.6, 6.8 Hz)	H1, H3	H1
3	132.187 (CH)	6.12 dd (J = 15.8, 1.7 Hz)	H1, H2	H1
4	198.87			H3, H5
5	45.32 (CH_2)	2.50, 2.90 dd, dd (J = 17.0, 3.0; 17.0, 8.8 Hz)	H6	H6
6	64.92 (CH)	4.19	H7, H5	H7, H24*
7	30.79 (CH_2)	1.36, 1.65	H8, H6	
8	36.15 (CH_2)	1.45, 1.52	H7	H7
9	95.46			H8
10	27.93 (CH_2)	1.46, 1.62		H8, H12
11	34.81 (CH)	1.28	H12, H13	H10, H12
12	17.99 (CH_3)	0.81 (3 H) d (J = 6.6 Hz)	H11	
13	74.37 (CH)	3.14	H11, H14	H12
14	30.55 (CH_2)	1.32, 1.68	H13, H15	H15
15	25.83 (CH_2)	1.50, 1.82	H14, H16	H14
16	39.64 (CH_2)	3.29 (2 H)	H15	
17	175.21			H16, NH(1), H18, H20
18	43.43 (CH)	2.36 dd (J = 7.0, 4.8 Hz)	H19, NH(1)	H19
19	15.54 (CH_3)	1.26	H18	H18, H30*
20	73.86 (CH)	3.71	H18	H19, H21, H30*
21	44.67 (CH_2)	3.22, 3.48	NH(1)	
22	173.41			NH(2), H21, H23, H24
23	32.45 (CH_2)	2.12, 2.75 dd, dd (J = 15.2, 1.7; 15.2, 11.6 Hz)	H24	H25
24	74.82 (CH)	4.07	H23, H25	H26, H27
25	33.36 (CH)	1.87	H24, H26, H27	H26
26	17.70 (CH_3)	0.86 (3 H) d (J = 7.1 Hz)	H25	
27	26.57 (CH_2)	1.60, 1.30	H25, H28	H26, H24
28	35.50 (CH_2)	1.32, 1.46	H27, H29	H29
29	19.24 (CH_2)	1.80, 1.52	H28	H28, H30
30	31.39 (CH_2)	1.50, 1.15	H31	H29
31	69.01 (CH)	3.42	H30, H32	H30, H32
32	34.14 (CH_2)	1.32		H33
33	33.38 (CH_2)	1.32		H32, H35
34	31.98 (CH)	2.32	H35	H35
35	20.77 (CH_3)	0.94 (3 H) d (J = 6.6 Hz)	H34	
36	133.77 (CH)	5.20	H38	H35, H38
37	132.98			H38, H40
38	12.13 (CH_3)	1.62	H36	
39	75.59 (CH)	5.22	H40	H36, H40
40	19.29 (CH_3)	1.28 (3 H) d (J = 6.6 Hz)	H39	
41	170.33			H42
42	21.43 (CH_3)	2.03 (3 H) s		H40
		NH(1) 6.92	H16	
		NH(2) 7.28	H21	

brownish oil (0.22 g). *Prochloron* was separated from the host ascidian as described in the accompanying paper and extracted as described above.

High-Performance Liquid Chromatography. The crude oil was dissolved in methanol/water (77:23) and applied to a Whatman Partisil ODS-3, 9 mm × 50 cm preparative HPLC column. Elution was effected by means of a concave gradient from 77 to 100% methanol over 120 min. Cytotoxic fractions were pooled, evaporated to dryness, and subjected to further purification by chromatography on Sephadex LH-20 using methanol/water (77:23) as solvent.

NMR Spectroscopy. The compounds were characterized by detailed analysis of their ^{1}H and ^{13}C NMR spectra and 2D COSY 45 and $^{1}H-^{13}C$ shift correlation experiments. All spectra were obtained with a C-5 dual ^{1}H, ^{13}C probe in a JEOL GX400 NMR spectrometer. Compounds were dissolved in deuterated chloroform (Merck), and chemical shifts are reported relative to tetramethylsilane (TMS). For the COSY 45 experiments the following parameters were used: Bistratamide A, spectral width 3076.9 Hz, data matrix 512 × 1024, scans 32, recycle time 3 s, with zero filling in the ν_1 domain; bistratamide B, spectral width 3450.7 Hz, data matrix 512 × 2048, scans 32, recycle time 3.23 s with zero filling in the ν_1 domain. Sine-bell apodization functions were used. For the heteronuclear correlated experiments the parameters were: bistratamide A, data matrix 128 × 4096, scans 592, recycle time 1.6 s; bistratamide B, data matrix 128 × 4096, scans 2000, recycle time 1.7 s; bistratene A, data matrix, 128 × 4096, scans 320, recycle time 1.9 s. Fixed delays of Δ1 and Δ2 ($^{1/2}J$ and $^{1/4}J$, respectively) were set for J = 138 Hz. Both dimensions were zero filled and multiplied by a sine-bell function before transformation. For long-range $^{1}H-^{13}C$ shift correlations in bistratene A, two experiments were used with Δ1 and Δ2 ($^{1/2}J$ and $^{1/3}J$, respectively) set for J = 10 Hz and J = 16.6 Hz.

Mass spectrometry, acid hydrolysis, chiral gas chromatographic analyses, and determination of cytotoxicity were performed as described in the accompanying paper.

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