Bioactive Bromopyrrole Metabolites from the Caribbean Sponge *Agelas conifera*[1a-c]

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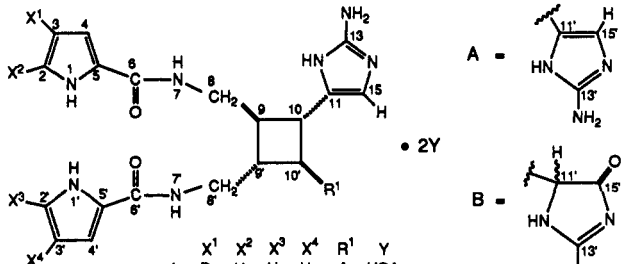
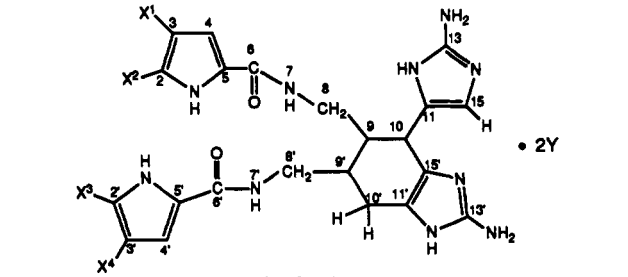
Biologically active extracts of the Caribbean sponge *Agelas conifera* have yielded, in exhaustive studies, the diacetate salts of seven new bromopyrroles (**1**, **3-8**), as well as that of the known debromooroidin dimer sceptrin (**2**). These compounds were found to be antiviral and antibacterial and were active in barnacle settlement and biochemical prophage induction assays. The structures assigned were based on spectroscopic comparisons to sceptrin and two-dimensional NMR data. Synthetic bromopyrroles were used to verify bromine substitution patterns. The oxysceptrins (**4**, **5**) are characterized by their aminoimidazolinone group, the ageliferins (**6-8**) by a unique cyclohexene-based skeleton.

During studies of bioactive marine natural products we found that extracts of some Caribbean *Agelas* sponges possessed activity in antiviral and antibacterial assays.[2] Subsequent exhaustive attempts to isolate the active components resulted in the isolation of numerous biologically active compounds. Reported here are the structures and biological activities of seven new bromopyrrole metabolites isolated as acetate salts **1** and **3-8**, along with sceptrin diacetate (**2**),[3] from the Caribbean sponges *Agelas conifera* and *Agelas* n. sp.[4] (family Agelasidae, order Axinellida[5]).



	X^1	X^2	X^3	X^4	R^1	Y
1:	Br	H	H	H	A	HOAc
2:	Br	H	H	Br	A	HOAc
2a:	Br	H	H	Br	A	HCl
3:	Br	Br	Br	Br	A	HOAc
4:	H	H	H	Br	B	HOAc
5:	Br	H	H	Br	B	HOAc
5a:	Br	H	H	Br	B	HCl



	X^1	X^2	X^3	X^4	Y
6:	Br	H	H	Br	HOAc
6a:	Br	H	H	Br	HCl
7:	Br	Br	H	Br	HOAc
8:	Br	Br	Br	Br	HOAc

Bromopyrroles are characteristic metabolites of sponges of the genus *Agelas*. Forenza et al.[6] isolated 4,5-dibromopyrrole-2-carboxylic acid, its corresponding nitrile and amide, and the antimicrobial oroidin (**9**)[7,8] from *Agelas oroides*; the free acid has also been isolated from *Agelas flabelliformis* and found to possess in vitro immunosuppressive activity.[9] Oroidin (**9**) has also been isolated from *Axinella damicornis*,[10] *Axinella verrucosa*,[11] *Acanthella aurantiaca*,[11] a *Goreauiella* sp.,[12] and *Pseudaxinyssa cantharella*,[13] while debromooroidin (**10**, hymenidin) was isolated from a *Hymeniacidon* sp.[14] and *Agelas clathrodes*.[15] The KB carcinoma-altering *N*-amidino-4-

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(1) (a) Presented in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, 1986, paper no. 1281; at the IUPAC 16th International Symposium on the Chemistry of Natural Products, Kyoto, Japan, May 29-June 3, 1988 (Rinehart, K. L. *Pure Appl. Chem.* **1989**, *61*, 525-528); at the Thirtieth Annual Meeting of the American Society of Pharmacognosy, San Juan, P.R., August 6-10, 1989 (Rinehart, K. L.; et al. *J. Nat. Prod.* **1990**, *53*, 771-792); and at the 49th International Congress of Pharmaceutical Sciences of F.I.P., Munich, F.R.G., September 4-9, 1989 (Rinehart, K. L.; Shield, L. S. In *Topics in Pharmaceutical Sciences* **1989**; Breimer, D. D., Crommelin, D. J. A., Midha, K. K., Eds.; Amsterdam Medical Press BV: Noordwijk, The Netherlands, 1989; pp 613-626). (b) Abstracted in part from Keifer, P. A. Ph.D. Dissertation, 1986 (*Chem. Abstr.* **1987**, *106*, 193150n), and Koker, M. E. S. Ph.D. Dissertation, 1985 (*Chem. Abstr.* **1987**, *106*, 193151p), both University of Illinois. (c) Rinehart, K. L., Jr. U.S. Patent 4 737 510, April 12, 1988 (*Chem. Abstr.* **1988**, *109*, 216002u). (d) Present address: Merck, Sharp & Dohme Research Laboratory, Rahway, NJ 07065. (e) Present address: University of Kent at Canterbury, CT2 7NH, U.K.

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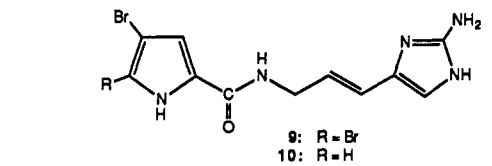
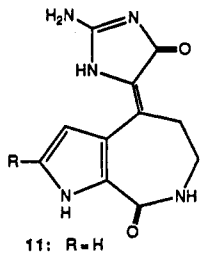
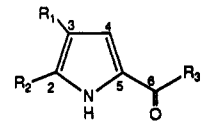
bromopyrrole-2-carboxamide was also found in an *Agelas* sponge,[16] while midpacamide, 5-debromomidpacamide, and methyl *N*-methyl-4,5-dibromopyrrole-2-carboxylate were obtained from *Agelas* cf. *mauritiana*.[17-19] Keramidine, a serotonergic receptor antagonist, was isolated from an Okinawan *Agelas* sp. sponge,[20] while dibromoagelaspongin hydrochloride was found in *Agelas* sp. (Tanzania).[21] Sceptrin was found to be the ichthyotoxic principle from *A. conifera*,[15] and, isolated as the dihydrochloride **2a**, was reported as the major antimicrobial constituent of *Agelas sceptrum*.[3,22]



9: R = Br
10: R = H



11: R = H
12: R = Br



13: $R_1 = R_2 = H$, $R_3 = NHCH_3$
14: $R_1 = Br$, $R_2 = H$, $R_3 = NHCH_3$
15: $R_1 = R_2 = Br$, $R_3 = NHCH_3$
16: $R_1 = R_2 = H$, $R_3 = CCl_3$
17: $R_1 = Br$, $R_2 = H$, $R_3 = CCl_3$
18: $R_1 = R_2 = Br$, $R_3 = CCl_3$

While not isolated from *Agelas* samples, secondary metabolites that appear to be biogenetically related to oroidin include the antibacterial phakellins (from *Phakellia flabellata*[23-27]), dibromocantharelline and its enantiomer dibromoisophakellin (from *Pseudoaxinyssa cantharella*[13] and *Acanthella carteri*,[28] respectively), compounds **11** and **12** (from *Phakellia flabellata*,[29] *Axinella verrucosa* and *Acanthella aurantiaca*,[11,30] and *Hymeniacidon aldis*[31,32]), and odiline (stevensine, found in *P. cantharella*,[13] *Teichaxinella morchella*,[33] a *Goreauiella* sp.,[12] and an unidentified, non-*Agelas*, Micronesian sponge[34]).

Isolation and Characterization. Initial attempts to isolate the bioactive components indicated that the methanol–toluene (3:1) extracts of both *A. conifera* and *Agelas* n. sp. sponges contained toluene- and chloroform-insoluble antibacterial and antiviral components that could be extracted from water into ethyl acetate and 1-butanol. Countercurrent distribution (CCD) of combined ethyl acetate and 1-butanol extracts from both sponges, using water–1-butanol–acetic acid (10:8:1), produced antiviral fractions that showed field desorption mass spectrometry (FDMS) $[M + H]^+$ ions with bromine isotope patterns at m/z 619 (Br_2), 635 (Br_2), and 697 (Br_3); ^1H and ^{13}C NMR spectroscopy suggested the presence of compounds related to sceptrin.[3] *A. conifera* was targeted for further study because it contained more of the compounds of interest than *Agelas* cf. *mauritiana*, although later studies showed that compounds 1–8 were also present in *A. mauritiana* in different ratios.

The isolation procedure started with a solvent extraction scheme similar to that used to isolate sceptrin (Scheme I).[3] The acetone-insoluble portion of a methanolic extract of *A. conifera* (1040 g) was dried to give 35.7 g of brown solids. Fast atom bombardment (FAB) MS and antibacterial and antiviral assays indicated that these solids contained the brominated, bioactive metabolites of interest. The solids were then partitioned by CCD (water–1-butanol–acetic acid–ethanol, 10:8:1:1) to separate partially the brominated compounds of interest. The CCD fractions were characterized by FABMS, high-performance liquid chromatography (HPLC), and the dried residue weights; based on these analyses, they were pooled to give samples A–Z containing partially purified *Agelas* metabolites that gave FABMS ions at m/z 541 (Br), 557 (Br), 619 (Br_2), 635 (Br_2), 697 (Br_3), and 775 (Br_4).

Selected CCD fractions were subjected to reversed-phase (RP) HPLC using methanol–water–acetic acid–triethylamine mixtures (650–400:350–600:7–14:7–14). Chromatographic peaks containing the brominated compounds of interest (by FABMS) were collected and dried, and the residues were lyophilized and subjected to repeated chromatography on LH-20 (chloroform–methanol–acetic acid, 65:35:1) to produce the diacetate salts **1–8** as amorphous solids. Compounds **1–5** were stable white solids, while **6–8** were prone to discoloration and decomposition.

Compounds **1–8** could be separated into three classes, based on the complexity of their ^1H and ^{13}C NMR spectra (supplementary material) and molecular formulas (from HRFABMS): (1) three compounds, **1–3** (sceptrins), related to sceptrin diacetate (**2**),[3] (2) two oxygenated compounds, **4** and **5** (subsequently named oxysceptrins), having characteristic NMR properties, and (3) three compounds, **6–8** (ageliferins), having sceptrin-like molecular formulas but having more complex NMR spectra. We shall direct our attention first to the compounds similar to sceptrin.

Compounds 1–3 (Sceptrins). The molecular formulas of compounds **1** ($C_{22}H_{25}BrN_{10}O_2$), **2** ($C_{22}H_{24}Br_2N_{10}O_2$), and **3** ($C_{22}H_{22}Br_4N_{10}O_2$), established by HRFABMS, suggested mono-, di-, and tetrabrominated analogues of the same compound. Compound **2**, the most abundant brominated metabolite in *A. conifera*, was shown on the basis of

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Table I. ^{13}C NMR Data for Compounds 13–15, 1–8, and 2a[a]

		13[b]	14[b]	15[b]	1[b]	2[b]	2a[c]	2a[d]	3[b]	4[b]	5[b]	6[b]	7[b]	8[b]
C-2 or C-2′	a	122.6, d[e]			123.1, d					123.1				
	b		122.3, d		122.9, d	122.9, d (2×)	124.0	121.6, d		122.9	123.0, d (2×)	123.0, 123.1, d*[f]	123.0, d	
	c			105.7, s					106.3, s (2×)				106.3, s	106.25, 106.30, s
C-3 or C-3′	a	110.1, d			110.3, d					110.2				
	b		97.2, s		97.5, s	97.5, s (2×)	97.4	95.2, s		97.5	97.5, s (2×)	97.5, 97.6, s*	97.5, s	
	c			99.7, s					100.1, s (2×)				100.1, s	100.0, 100.1, s
C-4 or C-4′	a	111.4, d			112.2, d					112.3				
	b		112.8, d		113.6, d	113.5, d (2×)	113.8	111.6, d		113.8	113.7, 113.8, d	113.7, 114.3, d	113.7, d	
	c			113.8, d					114.7, d (2×)				115.2, d	114.8, 115.2, d
C-5 or C-5′	a	126.8, s			126.6, s					126.6				
	b		127.3, s		127.4, s	127.4, s (2×)	126.2	123.9, s*		127.4	127.26, 127.35, s	128.2*, 128.3*, s	128.5, s*	
	c			128.5, s					128.5, s* (2×)				129.5, s*	128.55*, 128.63*, s
C-6 or C-6′	a	162.4, s			164.1, s					164.1				
	b		163.0, s		162.9, s	163.0, s (2×)	163.2	160.8, s		162.7	162.8, 163.0, s	162.8, 163.0, s	162.8, s	
	c			162.1, s					162.0, s (2×)				162.2, s	162.0, 162.2, s
C-8					42.34, t*	42.4, t			42.5, t	42.5[#], 43.0[#]		42.9, t	42.9, t	42.9, t
C-8′					42.28, t*	42.4, t	43.1	40.9	42.5, t	42.7[#], 43.3[#]	42.2, 42.3, 42.4, 42.9, t	40.5, t	40.6, t	40.6, t
C-9					39.14, d*	39.4, d			39.1, d	33.1*, 35.4*		37.2, d[$]	37.3, d[$]	37.1, d
C-9′					39.10, d*	39.4, d	39.4	36.9	39.1, d	37.7*, 40.4*	32.9, 35.3, 37.9, 40.3, d	33.1, d[$]	33.5, d[$]	33.2, d
C-10					44.4, d	44.3, d			44.2, d	44.4[$], 44.7[$]		43.5, d	43.5, d	43.4, d
C-10′					44.3, d	44.3, d	43.9	41.6	44.2, d	44.8[$], 45.4[$]	43.3, 44.1, 44.7, 45.4, d	23.4, t	23.5, t	23.4, t
C-11					129.0, s	129.4, s (2×)	127.8	*[g]	128.7, s* (2×)	129.4, 129.6	129.2, 129.5, s	122.3, s[#]	122.3, s[#]	122.2, s[#]
C-11′					128.8, s					63.2, exch[h]	63.3, 62.9, exch[h]	127.3, s*	127.3, s*	128.49, s*
C-13					149.2, s (2×)	149.4, s (2×)	148.0	145.7, s	149.2, s (2×)	148.9, 149	148.8, 149.0, s	149.5, 149.7, s	149.5, 149.9, s	149.5, 149.8, s
C-13′										172.7	172.4, s			
C-15					110.0, d (2×)	110.1, d (2×)	110.6	108.3, d	110.1, d (2×)	109.7, 110.2	109.6, 110.0, d	112.4, d	112.4, d	112.4, d
C-15′										190.3, 191.1	190.6, 191.1, s	119.2, s[#]	119.9, s[#]	119.5, s[#]
HOAc					180.1, s	179.8, s			180.2, s	180.5	179.0, s	179.5, s	179.8, s	179.6, s
					23.9, q	23.7, q			24.0, q	24.2	23.2, q	23.5, q	23.7, q	23.6, q
NCH_3		26.2, q	26.1, q	26.1, q										

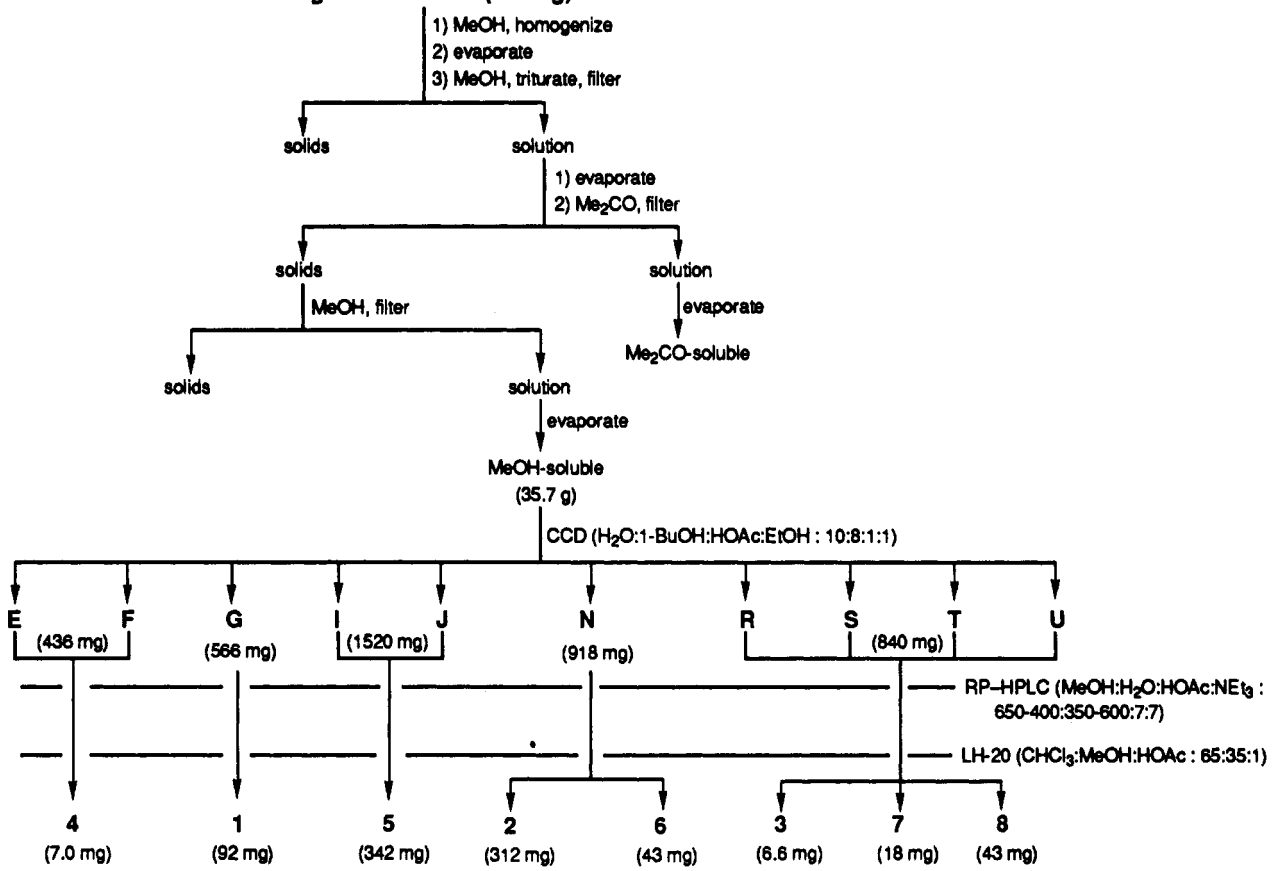
[a] Carbon type: a = in groups containing a nonbrominated pyrrole, b = in groups containing a monobrominated pyrrole, c = in groups containing a dibrominated pyrrole. [b] 90 MHz, methanol-d_4. [c] 90 MHz, DMSO-d_6. [d] D_2O, ref 3. [e] Multiplicities by DEPT or SFORD: s = singlet, d = doublet, t = triplet, q = quartet. [f] Signals with identical superscripts within a column (*, #, $) may be interchanged. [g] Not reported. [h] Signal intensity and multiplicity changed as the directly bound proton exchanged with deuterium from the solvent.

Table II. ^{1}H NMR Data for Compounds 13–15, 1–8, and 2a

	δ (ppm), multiplicity[a] (number, J in Hz)												
	13[b]	14[b]	15[b]	1[c]	2[c]	2a[d]	2a[e]	3[c]	4[c]	5[c]	6[c]	7[c]	8[c]
H-2 or H-2′	6.77, dd (2.7, 1.4)	6.77, d (1.2)		6.90, m (2 H)	6.91, m (2 H, 1.5)	6.97, s (2 H)	6.99, s		6.90, br s (2 H)	6.91, d (1.5)[f] 6.9 (1.5)[#]	6.92, d (1.5) 6.95, d (1.5)	6.91, d (1.6)	
H-3 or H-3′	6.03, dd (3.6, 2.7)			6.14, dd (1 H, 3.7, 2.6)					6.14, t (2.7)				
H-4 or H-4′	6.60, dd	6.60, d	6.62, s	6.79, m	6.77, d	6.96, s	6.97, s	6.81, s	6.80, m	6.86, d (1.5);[f] 6.84, d (1.5);[f]	6.80, d (1.4)	6.81, d (2.1)	6.84, s
	(3.6, 1.5)	(1.2)		(2 H)	(2 H, 1.5)	(2 H)		(2 H)	6.87, m	6.77, d (1.2);[f] 6.76 (1.3)[f]	6.90, d (1.4)	6.95, s	6.94, s
H-8 or H-8′				3.49, v br s (4 H)	3.48, v br s (4 H)	3.40, br s (4 H)	3.42, br s (2 H)	3.48, v br s (4 H)	3.3–3.7, m (4 H)	3.35–3.55, m (4 H)	3.36–3.70, m (4 H)	3.45–3.70, m (4 H)	3.3–3.74, m
H-9 or H-9′				2.34, m (2 H)	2.33, m (2 H)	2.24, br s (2 H)	2.29, br s	2.35, v br s (2 H)	2.17, v br s (2 H)	2.15–2.3, m (2 H)	2.22, v br s (2 H)	2.23, v br s[h] (2 H)	2.22, v br s[h] (2 H)
H-10				3.01, m	2.98, m	3.05, m[i]	3.10, d (8)	3.00, m	2.84, t (9.1);[f] 2.94, t (9.0)[f]	2.88, t (9.4);[*f] 2.92, t (8.9)[#f]	3.71, d (4.7)	3.76, d (5.5)	3.3–3.74, m
H-10′				3.01, m	2.98, m	3.05 (9)	3.10, d (8)	3.00, m	2.37, td (9.1);[f,j]	2.45, td (9.0, 4.4);[*+f,j]	2.43, br dd	2.33, br dd	2.43, br dd
									2.51, td (9.1)[f,j]	2.57, td (8.9, 4.4)[*$f,j]	(16.2, 4.6)	(16.2, 4.6)	
											2.72, br dd (16.5, 3.1)	2.71, br dd (15.9, 3.5)	2.71, br dd
H-11′									4.08, d (exch)	4.15, d (4.4, exch)			
H-15				6.52, s (1 H)	6.50, s (2 H)	6.63, s	6.66, s	6.52, s (2 H)	6.41, s;[f] 6.48, s[f]	6.41, s;[+f] 6.49, s[$f]	6.57, s	6.61, s	6.60, s
H-15′				6.51, s (1 H)									
HOAc				1.95, s (6 H)	1.93, s (6 H)			1.93, s (6 H)	1.91, s (6 H)	1.97, s (6 H)	1.91, s (6 H)	1.92, s (6 H)	1.93, s (6 H)
NCH_3	2.74, s (3 H)	2.73, s (3 H)	2.72, s (3 H)										
H-1						7.33, br s (2 H)	7.33, br s (2 H)						
H-7						8.52, br t (2 H, 5)	8.59, br t (5)						

[a] s = singlet, d = doublet, v br = very broad, m = multiplet, br = broad, exch = exchangeable proton; resonances with identical superscripts (*, #, $, +) within a column are coupled. [b] 360 MHz, $CDCl_3$. [c] 360 MHz, methanol-d_4. [d] 360 MHz, DMSO-d_6. [e] DMSO-d_6, ref 3. [f] All 0.5 H. [g] Unresolved overlapping multiplets. [h] $\omega_{1/2}$ = 15 Hz. [i] Appears as an unusual doublet with J = 9 Hz. [j] Smaller (ca. 4 Hz) coupling constant lost when H-11′ proton exchanges with deuterium; smaller coupling constant due to exchangeable proton not measured.

Scheme I



spectroscopic properties (Tables I and II) to be the diacetate of the symmetrical dimer sceptrin, whose structure was previously assigned by X-ray crystallography of the hydrochloride salt **2a**.[3] The ^1H and ^{13}C NMR spectra of **1** and **3** were nearly identical with those of sceptrin (**2**, Tables I and II) except in the pyrrole region. In particular, the cyclobutane signals for H-9, H-9′, H-10, H-10′, and the corresponding carbons were the same, allowing assignment of the same all-trans stereochemistry.

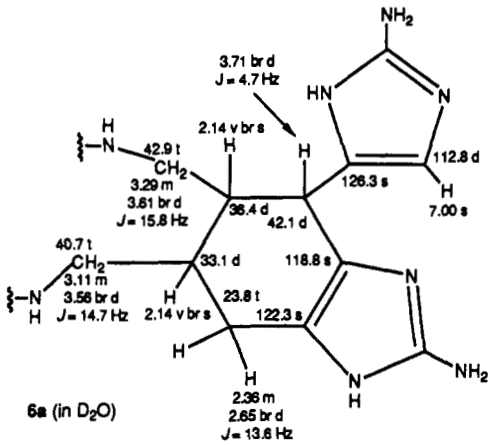
The ^1H NMR spectrum of **3** differed from that of sceptrin only by the loss of both the δ 6.91 resonance and the 1.5-Hz coupling in the δ 6.8 resonance. The ^{13}C NMR spectrum of **3** also had resonances at δ 106.3 (s) and 100.1 (s) instead of the resonances at δ 122.9 (d) and 97.5 (s) found in sceptrin. These data indicated that dibromosceptrin (**3**) had the additional bromines symmetrically located in the two pyrrole rings. ^1H and ^{13}C NMR spectral data for **1** were consistent with a nonsymmetric debromosceptrin structure, as suggested by the molecular formula. The structure of debromosceptrin (**1**) was straightforward since only one enantiomeric compound can be produced by the dehalogenation of either bromine in **2**. The two additional bromines in dibromosceptrin (**3**) were assigned to the pyrroles' α-carbons on the basis of both the biogenetic analogy of oroidin (**9**) and the ^{13}C NMR spectral assignments for midpacamide,[17] keramidine,[18] and synthetic pyrrole-2-*N*-methylcarboxamide (**13**) and its 4-bromo and 4,5-dibromo derivatives (**14** and **15**), which we prepared. NMR spectral assignments of **13–15** (Tables I and II) were based on chemical shift and multiplicity considerations and heteronuclear decoupling studies for the series of three compounds; bromination of 2-carbonylpyrroles also has been shown previously to produce mainly 4-bromo- and 4,5-dibromo-2-carbonylpyrroles.[35-37]

Compounds 6–8 (Ageliferins). The second class of compounds is represented by **6** ($C_{22}H_{24}Br_2N_{10}O_2$), **7** ($C_{22}H_{23}Br_3N_{10}O_2$), and **8** ($C_{22}H_{22}Br_4N_{10}O_2$) (formulas by HRFABMS). Comparisons of the ^1H and ^{13}C NMR spectra of **6–8** demonstrated that all bromines in the three compounds were located on the α- and β-carbons of two nonequivalent pyrrole rings (see Tables I and II). Compound **6** (with two bromines) had two pairs of coupled ^1H doublets at δ 6.92 and 6.80 (J = 1.4 Hz; H-2′ and H-4′) and at δ 6.95 and 6.90 (J = 1.4 Hz; H-2 and H-4), as well as closely related carbon pairs resonating at δ 123.0 and 123.1 (C-2, C-2′), 97.5 and 97.6 (C-3, C-3′), 113.7 and 114.3 (C-4, C-4′), 128.2 and 128.3 (C-5, C-5′), and 163.0 and 162.8 (C-6, C-6′), indicating two slightly nonequivalent 4-bromo-2-pyrrolecarboxamide groups. Similarly, compound **8** (with four bromines) had ^1H singlets at δ 6.94 and 6.84, as well as closely related ^{13}C pairs resonating at δ 106.25 and 106.3 (C-2, C-2′), 100.0 and 100.1 (C-3, C-3′), 114.8 and 115.2 (C-4, C-4′), 128.55 and 128.63 (C-5, C-5′), and 162.0 and 162.2 (C-6, C-6′), indicating again two slightly nonequivalent, 4,5-dibromo-2-carbonylpyrrole groups. The ^1H NMR spectrum of the tribrominated compound **7** [δ 6.91 (d, J = 1.6 Hz), 6.81 (d, J = 2.1 Hz), and 6.95 (s)] and its ^{13}C NMR spectrum [δ 123.0 and 106.3 (C-2, C-2′), 97.5 and 100.1 (C-3, C-3′), 113.7 and 115.2 (C-4, C-4′), 128.5 and 129.5 (C-5, C-5′), and 162.2 and 162.8 (C-6, C-6′)] indicated the presence of both 4-bromo- and 4,5-dibromocarbonylpyrroles. Only one resonance was seen for each carbon, however, arguing the presence of a single regioisomer.

The remaining structural units of **6–8** were identified by using two-dimensional ^1H–^1H shift correlation spec-

(35) Hodge, P.; Rickards, R. W. *J. Chem. Soc. (London)* **1965**, 459–470.
(36) Anderson, H. J.; Lee, S.-F. *Can. J. Chem.* **1965**, *43*, 409–414.
(37) Bélanger, P. *Tetrahedron Lett.* **1979**, 2505–2508.

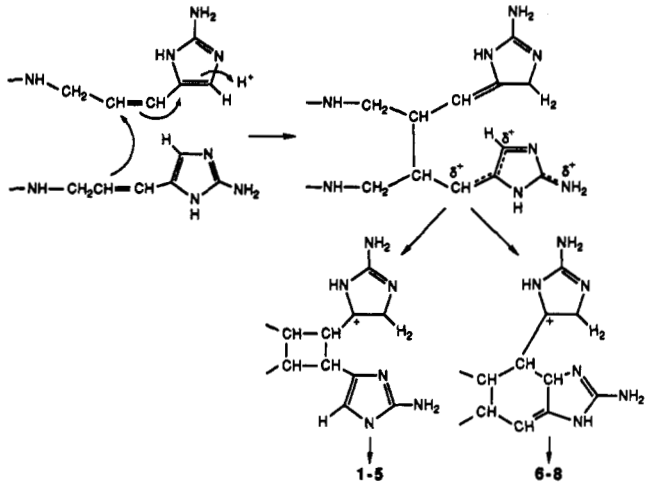
Scheme II



troscopy (COSY) and comparisons of spectral data for **6** to those found for sceptrin. Sceptrin (**2**) contains two guanidinium (C-13) carbons (both at δ 149.4) and two olefinic aminoimidazole (H-15) protons (δ 6.50, s, 2 H); in contrast, **6** had two guanidinium carbons (at δ 149.5 and 149.7) but only one olefinic aminoimidazole proton (at δ 6.57, s, 1 H). Moreover, while sceptrin has only three aliphatic resonances for the six aliphatic carbons (methine carbons at δ 39.4 and 44.3 and a methylene carbon at 42.4), compound **6** contains six separate aliphatic resonances: three methine carbons at δ 33.2, 37.2, and 43.5 and three methylene carbons at δ 23.4, 40.5, and 42.9. Since ^{13}C DEPT (or SFORD) spectra of compounds **2** and **6** account for the same number of carbon-bound protons in each compound (adjusting for the symmetry of sceptrin), it appears that a proton found as an aminoimidazole olefinic proton in sceptrin is found as an aliphatic methine or methylene proton in **6**. The nearly identical UV and IR spectral data for **2** and **6** suggest that both pyrrole and both aminoimidazole units of **2** and **6** are still present, in agreement with the like number of pyrrole- and imidazole-type carbons.

Placement of the nine aliphatic protons of **6** was accomplished by ^1H–^1H COSY studies and verified by selected homonuclear decoupling experiments on both **6** and its corresponding hydrochloride **6a**. The appearance of ^1H NMR spectra of **6** and **6a** depended upon solvent, concentration, and counterion; one sample of **6a** in deuterium oxide (Scheme II) gave a nearly first-order spectrum and was used for both homonuclear (COSY) and heteronuclear (CSCM) shift correlation experiments. COSY indicated that the δ 3.71 (br d, J = 4.7 Hz, 1 H) resonance was coupled to the δ 2.14 (v br s, 2 H) resonance; the latter in turn had coupling to one-proton resonances at δ 2.36 (m, 1 H), 2.65 (br d, J = 13.6 Hz, 1 H), 3.11 (m, 1 H), and 3.29 (m, 1 H) (see Scheme II). The resonances at δ 2.36 and 2.65 showed geminal coupling (13.6 Hz) to one another, while the two-proton resonance appearing as a triplet at δ 3.51–3.66 actually consisted of two uncoupled broad doublets at δ 3.61 (J = 15.8 Hz, 1 H) and 3.56 (J = 14.7 Hz, 1 H), with geminal coupling to the δ 3.29 and 3.11 resonances, respectively. With three methylene and three methine groups in this unit and the three methylenes identified by the large geminal coupling constants, the two protons at δ 2.14 must be coincident methine protons. These data are consistent with the structural unit shown in Scheme II and incorporated into structure **6a** (ageliferin hydrochloride). These chemical shift assignments were verified with ^1H–^{13}C shift correlation spectroscopy (CSCM). The stereochemistry of the cyclohexene ring's substitution could not be assigned; attempts to determine

Scheme III



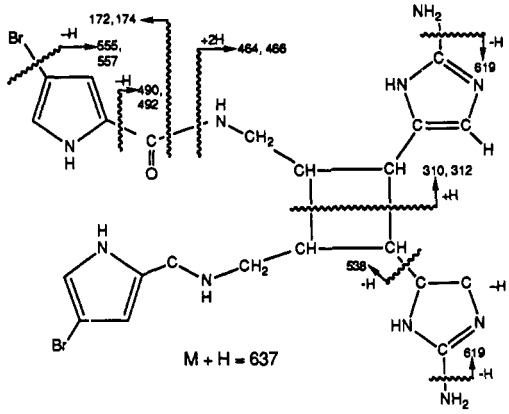
stereochemistry by coupling constants, NOE data, or ROESY on **6** and **7**, all at 500 MHz (see supplementary material, Table IV) were inconclusive.[38] Comparisons of the NMR spectral data of bromoageliferin (**7**) and dibromoageliferin (**8**) with data for ageliferin (**6**) did, however, verify that all three compounds contain the same cyclohexene structure.

Structures **6–8** suggest a [4π + 2π] cycloaddition; however, their optical activity belies their formation via a Diels–Alder cycloaddition. A similar conclusion, noted before,[3] is applicable to sceptrin, which might otherwise be considered the product of a photochemical [2π + 2π] addition. A possible unified mechanism for the formation of both the ageliferins and the sceptrins (as well as the oxysceptrins, cf. below) is shown in Scheme III.

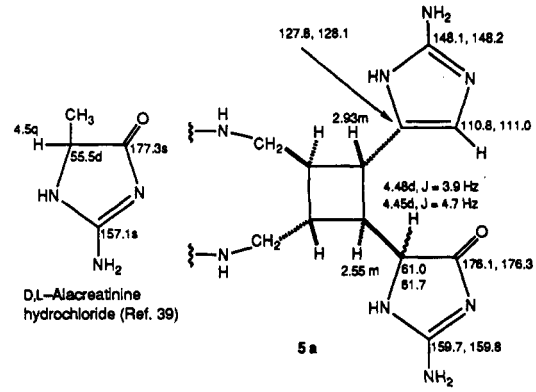
The regiochemistry of mono- vs dibromopyrrole substitution onto the cyclohexene ring of **7** was eventually assigned by the combined use of ROESY with both TOCSY and double-quantum filtered COSY (DQFCOSY) on a sample of **7** in DMSO-d_6 (supplementary material, Table IV). The first step was to assign the regiochemistry of the N-7 amide protons at δ 7.97 and 7.95. The δ 7.97 resonance was shown by DQFCOSY and TOCSY to be scalar coupled to methylene protons δ 3.10 and 3.29 (assigned as H-8′), which in turn were coupled to the proton at δ 2.06 (H-9′), which was coupled to both of the H-10′ methylene protons at δ 2.20 and 2.48. This assigned the δ 7.97 resonance as H-7′. In like manner, the δ 7.95 resonance was shown to be H-7 by its scalar couplings to protons at δ 3.36 and 3.29 (H-8), which were coupled to the H-9 proton at δ 2.24, which was coupled to the H-10 methine proton at δ 3.54. The H-10 proton also had small coupling to the H-15 proton at δ 6.20. This completed the regiochemistry of all aliphatic protons in the molecule, based on the asymmetry of substitution of the cyclohexene ring. (These assignments were substantiated by a similar analysis of the 2D NMR spectra of **6** in DMSO-d_6.) The next step was to link the mono- and dibromopyrrole substituents to their respective amide protons. This was done with ROESY which indicated strong dipolar couplings between the amide H-7 proton at δ 7.95 and the H-4 dibromopyrrole proton at δ 6.88, and between the H-7′ proton at δ 7.97 and the H-4′ monobromopyrrole proton at δ 6.81 (which itself exhibited scalar coupling to H-2′ at δ 6.93). This unequivocably located the dibrominated pyrrole on the same side of the cyclohexene ring as the

(38) Kobayashi et al. (*Tetrahedron* 1990, *46*, 5579–5586) have recently employed extensive NOE studies to assign the stereochemistry of the 6-membered ring on samples of **6–8** isolated from an *Agelas* species.

Scheme IV



Scheme V



intact (nonfused) aminoimidazole ring.

Compounds 5 and 4 (Oxysceptrins). The third class of compounds is represented by **5** ($C_{22}H_{24}Br_2N_{10}O_3$) and **4** ($C_{22}H_{25}BrN_{10}O_3$). The molecular formula of **5** contains one oxygen more than that of sceptrin. UV and IR spectra of **5** were very similar to those for sceptrin (**2**); however, **5** showed a very strong hyperchromic effect (8× increase in ϵ) in its 203-nm absorption in the presence of base, while the UV spectrum of sceptrin was relatively insensitive to base. Moreover, a comparison of the IR spectra of **5a** and **2a** showed that **5a** had an additional strong peak at 1707 cm^{-1}.

^1H and ^{13}C NMR spectra (Tables I and II) indicated that **5** exists in solution as two interconverting diastereomers. HPLC of **5** produced two poorly resolved peaks in a ratio of ca. 1:1, which, when individually collected and rechromatographed, again produced a 1:1 peak ratio. On-line polarimetry of the HPLC effluent indicated that both peaks were dextrorotatory, with the faster-eluting peak being more so. ^1H and ^{13}C NMR spectra also indicated that the compound(s) bore a strong resemblance to sceptrin; the chemical shift differences between the two equilibrating isomers suggested that the location of isomerism was distant from the two bromopyrrole rings.

Low-resolution linked-scan (B/E) FABMS indicated that the major fragmentation of **5** ($[M + H]^+ = m/z$ 635:637:639, 1:2:1) was amide bond cleavage, producing a doublet at m/z 464:466 (1:1) from the m/z 637 component corresponding to loss of C_5H_3BrNO - 2 H (bromopyrrolecarbonyl with hydrogen transfer), the same loss seen for sceptrin. This indicated that the extra oxygen was not in the pyrrole groups. Tandem FABMS carried out on a four-sector mass spectrometer (FABMS/MS) proved that the extra oxygen was on the imidazole ring when it located a relatively weak peak at m/z 536 derived from m/z 635 and a corresponding fragment ion at m/z 538 from m/z 637 (Figure 1), both due to the loss of an oxygenated aminoimidazole group ($M - C_3H_5N_3O$).

It is of some interest to note the appearance of the tandem mass spectrum in Figure 1. Since the tandem scan showed is of m/z 637 (containing both ^{79}Br and ^{81}Br) as parent ion, any daughter ion that has lost a bromine, or a unit containing bromine, appears as a doublet, while any daughter ion which retains both bromines appears as a singlet. The extra oxygen is therefore located on an imidazole ring. Other fragments observed by FABMS/MS for both **2** and **5** were consistent with this assignment (see Scheme IV).

The ^{13}C NMR spectrum of **5a** (a pair of diastereomers) in deuterium oxide contained six key resonances (3 pairs) not seen in sceptrin, located at δ 176.1 and 176.3 (amide carbonyls), 159.7 and 159.8 (quaternary carbons), and 61.0 and 61.7 (methine carbons). The δ 61.0 and 61.7 resonances became shorter and broader with time due to hydrogen exchange with the deuterated solvent. The ^1H NMR spectrum of **5** in methanol-d_4 (Table II) indicated a similar exchange of the δ 4.15 (d, J = 4.4 Hz, 1 H) resonance; this exchange resulted in the loss of 4.4-Hz couplings to the resonances at δ 2.45 (td, J = 9.0, 4.4 Hz, 1 H), and 2.57 (td, J = 8.9, 4.4 Hz, 1 H). These data (Scheme V) are similar to data for D,L-alacreatinine hydrochloride (C-2 = δ 157, C-4 = δ 177, and C-5 = δ 155.5)[39,40] and are consistent with a 5-substituted 2-amino-4-imidazolinone group located on the C-10 carbon of sceptrin. The H-11′ proton of **5** exchanges in protic solvents, forming two interconvertible diastereomers.[41] This proton (at δ 4.15 in the two isomers) is coupled to the two H-10′ protons, located at δ 2.45 and 2.57 in the two diastereomers. (Certain dilutions of **5** in methanol-d_4 produced NMR spectra having a pair of doublets for the δ 4.15 resonance.)

The aminoimidazolinone group is substantiated by the IR spectrum of **5a** whose 1707-cm^{-1} peak is consistent with a γ-butyrolactam.[42] The pH-dependent molar absorptivity of the UV amide peak at 203 nm also agrees with this structure.[43] It is significant that a similar 5-substituted 2-amino-4-imidazolinone unit is present in compounds 11 and 12.[11,29-32]

The overall stereochemistry of **5**, which we call oxysceptrin, is the same as that found for sceptrin. 1D NOE and 2D NOESY ^1H NMR spectra of **5** [as well as 2D ROESY of compound **4** (below)] verify that all cyclobutane ring protons possess trans relationships with their neighbors, as in sceptrin (**2**) itself. It is of interest to note that the H-11′ proton of both diastereomers enjoys dipolar relaxation with the H-10 proton; however, the H-15 proton is alternately relaxed by either H-9 or H-10′ in the two diastereomeric forms.

It was noted that the 360-MHz ^1H NMR spectrum of **5** freshly dissolved in methanol-d_4 also contained unexplained minor resonances at δ 4.41 (d, J = 7.8 Hz) and 4.50 (d, J = 5.8 Hz) with peak heights of ca. 2% and 1%, respectively, of the height of the major doublet at δ 4.15 (d, J = 4.4 Hz). These resonances, like the major δ 4.15 resonance, exchanged with methanol-d_4.

The general structure of **4** was straightforward from **5**; NMR data demonstrated that **4** contained one less pyrrolic bromine than **5**. One would predict the existence of two regioisomers of debromooxysceptrin, depending on which

(39) Dietrich, R. F.; Marletta, M. A.; Kenyon, G. L. *Org. Magn. Reson.* **1980**, *13*, 79–88.
(40) Wegner, M. M.; Rapoport, H. *J. Org. Chem.* **1978**, *43*, 3840–3844.
(41) Lee, C. R.; Pollitt, R. J. *Biochem. J.* **1972**, *126*, 79–87.
(42) Bellamy, L. J. *The Infra-Red Spectra of Complex Molecules*, 3rd ed.; Wiley: New York, 1975; p 242.
(43) Matsumoto, K.; Rapoport, H. *J. Org. Chem.* **1968**, *33*, 552–558.

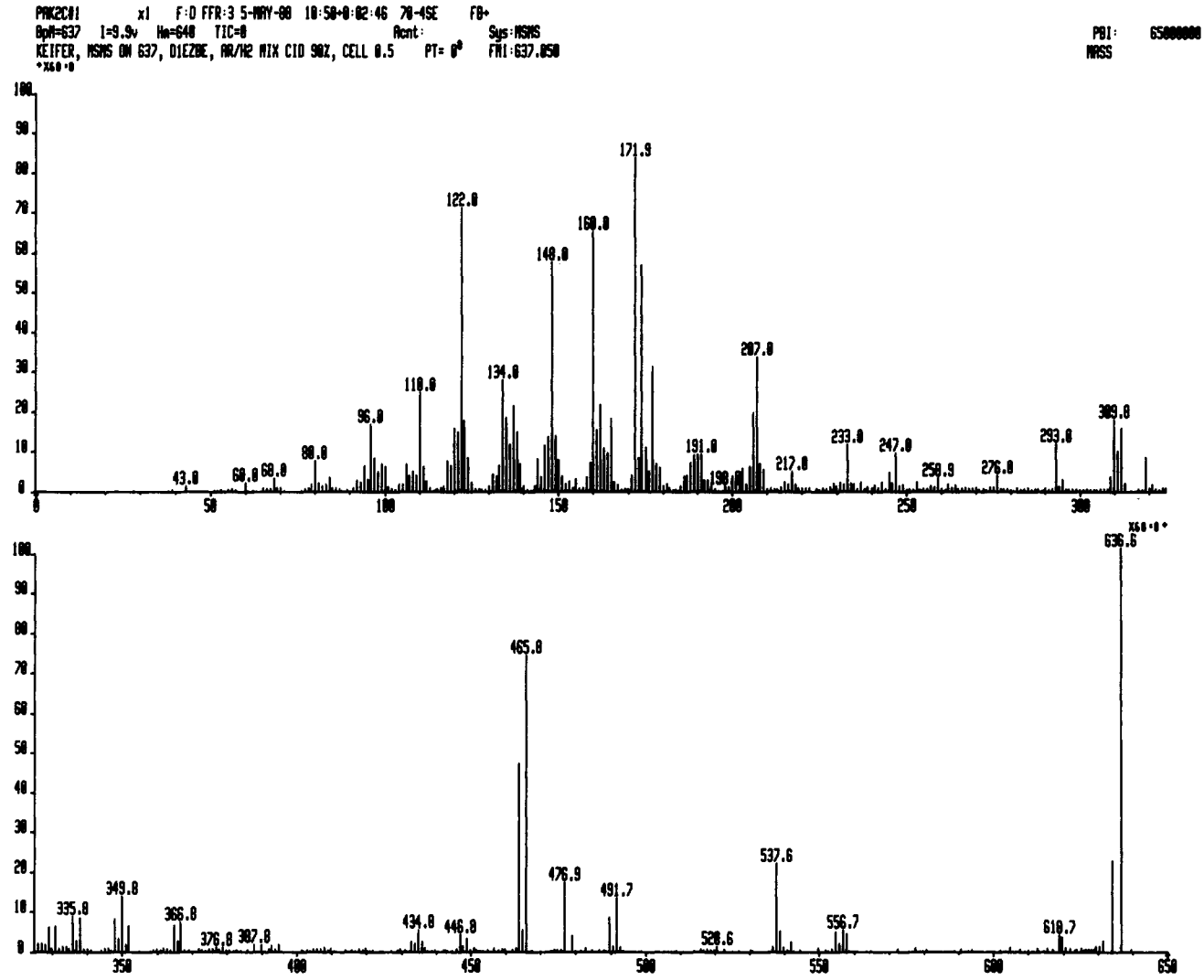


Figure 1. Tandem FAB mass spectrum of m/z 637 of oxysceptrin (5).

bromine was removed from oxysceptrin. NMR spectra of 4, however, do not indicate the presence of significant amounts of more than one diastereomeric regioisomer. HPLC and NMR do demonstrate that 4 exists in solution as a pair of interconverting diastereomers like those for oxysceptrin. ^{1}H NMR spectra also indicate that the H-15 proton of 4 exchanges with deuterium during extended exposure to deuterated solvents. This slow exchange was also occasionally observed for the H-15 protons of 1–3 and 5–8.

The regiochemistry of pyrrole vs bromopyrrole substitution in 4 (with respect to the aminoimidazolinone substituent on the cyclobutane ring) was assigned in a manner similar to that used for compound 7. The complexity of NMR spectra of 4 in DMSO-d_6 precluded a direct assignment without first assigning the NMR spectra of 5 in DMSO-d_6 (see supplementary material, Table IV). That being done, the following analysis of TOCSY, DQFCOSY, and ROESY spectra of 4 in DMSO-d_6 was possible. (Lowercase "a" and "b" designations refer to the two different diastereomers.) The H-11′ proton resonated at both δ 3.76 (J = 10 Hz) and 3.82 (J = 5 Hz). The δ 3.76 resonance, arbitrarily designated diastereomer a, was coupled to H-10′a at δ 1.74, which was coupled to H-9′a at δ 2.12, which was coupled to both H-8′a protons at δ 3.09 and 3.52, which in turn were coupled to H-7′a(NH) at δ 8.72. ROESY and 1D NOE indicated that H-7′a enjoyed dipolar relaxation to both H-10′a (δ 1.74) and a resonance at δ 2.09 (H-9a). This H-9a resonance showed scalar coupling to H-10a at δ 2.70 and to both H-8a protons at δ 3.3–3.4, which were in turn coupled to H-7a(NH) at δ 8.28 The H-7a(NH) and H-9′a protons enjoyed mutual dipolar relaxation, as did H-10a with both H-9′a and H-11′a. This traced out the regio- and stereochemistry of all aliphatic protons within the all-trans cyclobutane ring; the dipolar relaxation found between the H-7′a amide proton and the bromopyrrole H-4′a proton at δ 6.97 (which was scalar coupled to H-2′a at δ 6.8 and had dipolar relaxation to H-1′a at δ 11.8) located the bromopyrrole substituent on the same "oroidin subunit" as the aminoimidazolinone group. This was confirmed by the dipolar relaxation between the nonbrominated pyrrolic resonance H-4a (δ 6.8) and H-7a (δ 8.28); dipolar and scalar couplings were shared among H-4a and H-3a (δ 6.04), H-2a (δ 6.8), and H-1a (δ 11.4).

The b diastereomer was assigned in a similar manner. The H-11′b resonance (δ 3.82) was coupled to H-10′b (δ 2.34), which was coupled to H-9′b (δ 1.98), which was coupled to both H-8′b protons at δ 3.04, which were coupled to H-7′b (δ 7.85). This amide proton enjoyed dipolar relaxation with H-4′b (δ 6.96) which was further relaxed by H-1′b at δ 11.8 and was coupled to H-2′b at δ 6.8, hence this amide proton (δ 7.85) was adjacent to the brominated pyrrole. The unbrominated pyrrole (H-1b at δ 11.4; H-2b, H-3b, and H-4b at δ 6.8, 6.04, and 6.8, respectively) enjoyed dipolar relaxation with H-7b (δ 8.32) which was in a scalar

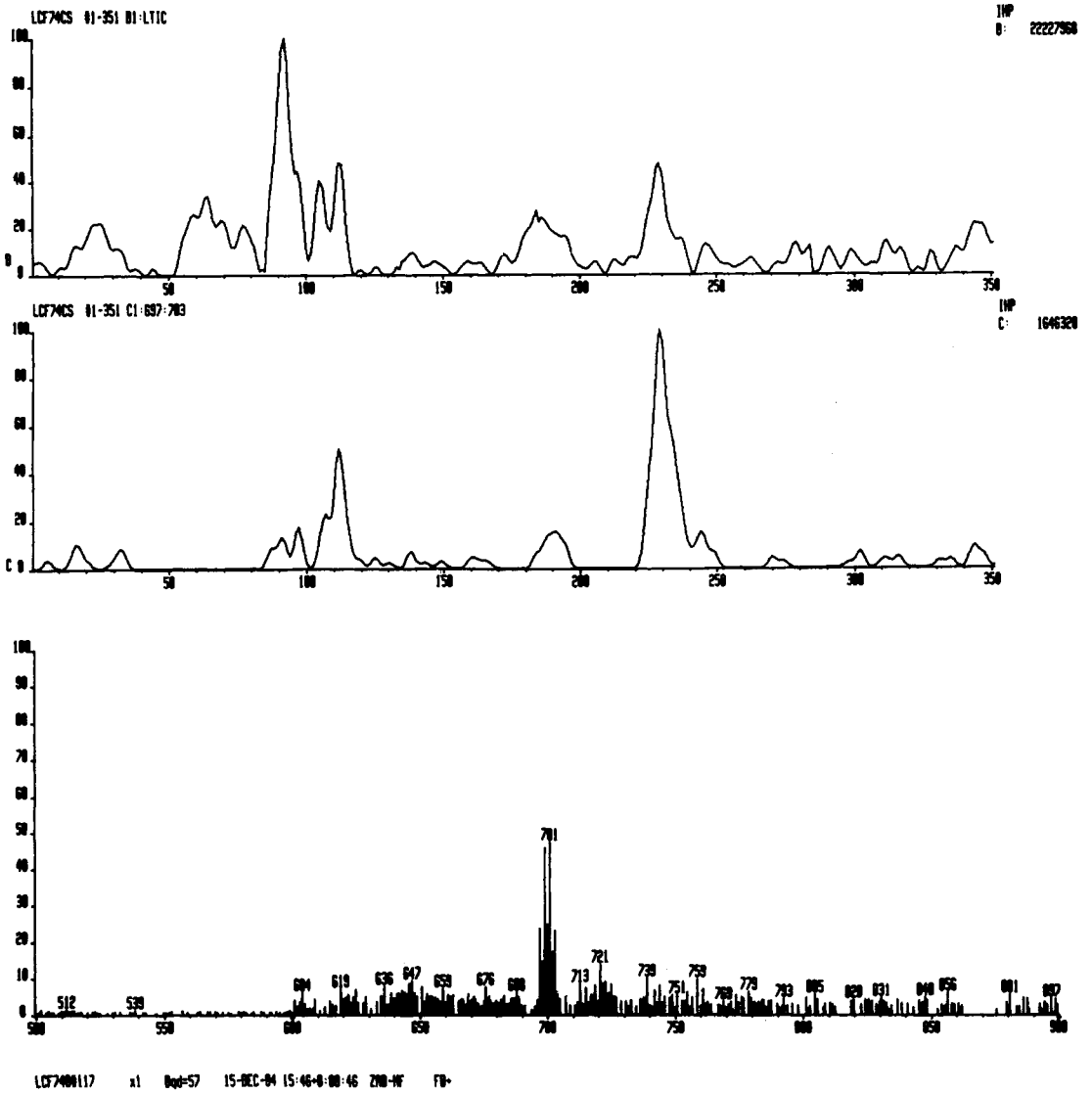


Figure 2. Top: limited total ion current. Middle: selected ion plot (697–703). Bottom: FAB mass spectrum of scan no. 230 (note the near absence of a dibromo ion at m/z 619–621–623).

coupling network that included H-8b (δ 3.2), H-9b (δ 2.00), and H-10b (δ 2.64). This verified that the regiochemistry was the same for both diastereomers; ROESY and 1D NOE of these resonances verified that the stereochemistry was also the same (H-7b and H-8b both relaxed H-9′b; H-7′b and H-8′b relaxed both H-10′b and H-9b).

It was noted that the FAB mass spectra of 1–8 characteristically showed $[M + H - Br + H]^+$ ion peaks at 1–10% of the intensity of the $[M + H]^+$ ion peaks. ^1H NMR and HPLC analyses of the same samples, however, indicated the presence of <0.5% of the debrominated impurities. This FABMS dehalogenation reaction has been reported by McCloskey et al.[44] and by Nakamura et al.[45] who found that reductive dehalogenation of halogenated aromatic rings occurs as a major process during FABMS. This process was not seen in moving-belt liquid chromatography (LC)/FABMS spectra of 3, 7, and 8, where no matrix was used (Figure 2).[46]

A number of additional peaks were observed during the HPLC purifications of 1–8. Two of these peaks were individually collected and analyzed by FABMS to give compounds with ions of m/z 713 (Br_3) and 791 (Br_4); these might correspond to oxygenated forms of bromosceptrin and dibromosceptrin, respectively. Also, an LC/FABMS[45] study of one CCD fraction of an *Agelas* n. sp. extract located an unidentified but fairly abundant compound with m/z 742 (Br_2) along with compounds 3, 7, and 8.

Biological Activities. Since these compounds were all isolated using bioassay-guided isolation schemes, it was important to evaluate fully the biological activities of the pure compounds. To that end, compounds 1–8 were screened for activity in antimicrobial, antiviral, cytotoxicity, and biochemical prophage induction (BIA) assays using 6.5-mm filter disk-agar diffusion methods,[47] while compounds 2, 5, and 6 were also subjected to barnacle settlement assays (Table III). Compounds 1, 2, and 6–8 inhibited growth of *Bacillus subtilis* at 10 μg/disk, with 3, 4, and 5 being somewhat less active. Compounds 2, 6, and 7 were active against *Escherichia coli* at 10 μg/disk; all compounds except 4 were active at 50 μg/disk. Compounds 1–3 and 6–8 were active against *Herpes simplex* virus, type 1 at 20 μg/disk and against *Vesicular stomatitis* virus at 100 μg/disk; 4 and 5 were less active against both.

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Table III. Biological Activities of 1–8[a]

	E.c.[b] 50 μg	B.s.[b] 50 μg	HSV-1[b] 50 μg	VSV[b] 100 μg	BIA[c] 10 μg	I-EC_{50}[d]	F-EC_{50}[d]
1	8	11	12	7	++		
2	10	16	13	11	++	21	none
3	7	10	11	10	+		
4	0	7	0	-	+		
5	8	14	e	8	++	none	0.017
6	9	15	13	10	+gray	44	0.029
7	8	12	11	10	+gray		
8	7	9	11	9	+gray		

[a] E.c. = *Escherichia coli*; B.s. = *Bacillus subtilis*; HSV-1 = *Herpes simplex* virus, type 1; VSV = *Vesicular stomatitis* virus; BIA = biochemical prophage induction assay; I-EC_{50} = barnacle settlement inhibition EC_{50}; F-EC_{50} = barnacle settlement facilitation EC_{50}. [b] Disk diffusion assay on agar, employing listed amount of sample on 6.5-mm disks, zone of inhibition in millimeters. [c] Subjectively rated with a "+/-" system. [d] Effective solution concentrations, μg/mL. [e] Active, but too diffuse to assign a meaningful zone diameter. In all agar-based tests, 4 and 5 gave more diffuse zones, presumably due to increased water solubility.

All compounds were inactive against *Penicillium atrovenetum* and *Saccharomyces cerevisiae* and were noncytotoxic to monkey kidney cells at 200 μg/disk. In the BIA assay,[48] 1, 2, and 5 were moderate inducers at 10 μg/disk, 3 and 4 were less active, and compounds 6–8 were only questionably active at 50 μg/disk. These compounds produced an atypical gray response in the BIA assay; it is also unusual for compounds to be active in the BIA assay but noncytotoxic to mammalian cells.

Barnacle settlement assays[49,50] on 2, 5, and 6, using *Balanus amphitrite amphitrite* (Darwin, 1854), gave settlement-inhibition EC_{50} values of 44 and 21 μg/mL for compounds 2 and 6, respectively, and settlement-facilitation EC_{50} values of 29 and 17 ng/mL for compounds 2 and 5. This indicates moderate settlement inhibition and represents potential antifouling activity.

Experimental Section

IR and UV spectra were obtained on IR/32 FTIR and Lambda 3 UV/vis spectrophotometers, respectively, and optical rotations were obtained on an Autopol III automatic polarimeter. NMR spectra were recorded on Unity 500 and XL-200, NT-360, and QE-300 and GN-500 FT spectrometers using MeOH-d_4 or DMSO-d_6 as solvent and internal standard (δ 3.30 and 49.0 or 2.49, respectively) or D_2O with dioxane as an internal standard (δ 3.70 and 67.8). Mass spectra were run on MAT 311A, MAT 731, VG 7070-EH, ZAB-HF, ZAB-SE, or 70-4SE spectrometers operating in the fast atom bombardment (FAB) mode employing Xe atoms at 8–9 keV, typically using a matrix of either dithiothreitol:dithioerythritol (DT's, "magic bullet")[51] or thioglycerol. A VG ZAB (B-E-E-B) mass spectrometer at the National Institute for Environmental Health Sciences was used for collision-induced decomposition (He or Ar:H_2 = 1:1) tandem FABMS. Liquid chromatography (LC)/FABMS was performed using a moving-belt interface with surfactant-treated belts, no FAB matrix, and a ZAB spectrometer.[52]

Countercurrent distribution (CCD) was carried out on a 400-tube (20 mL/tube) 3-B-X Craig-Post automatic liquid–liquid fractionator. HPLC was performed with 114M and 110 pumps and 165 and 153 variable- and fixed-(254 nm) wavelength UV detectors; columns used were Econosphere ODS (Alltech; 5 μm, 4.6 × 250 mm or 10 μm, 10 × 250 mm), Spherisorb ODS, ODS-2, and Phenyl (Alltech or Chromanetics; all 5 μm, 4.6 × 250 mm), Altex ODS (5 μm, 10 × 250 mm); all were equipped with guard columns. Gravity flow chromatography ($CHCl_3$–MeOH–HOAc, 65:35:1 v/v) was carried out on Sephadex LH-20 (Pharmacia; 25-100 μm) on a 3 × 40-cm column (UV 254 nm detection).

Sample IRCE 2-VII-81-1-1, identified as *A. conifera*,[4] was collected by using SCUBA techniques at St. George's Reef (–55 m) off the coast of Belize, Central America. Sample AHCE 20-III-78-1-4 (no. 631), identified as *Agelas* n. sp.,[4] was collected at Punta Oeste, Isla Roatán, Honduras (86° 38′ W, 16° 15′ N; –3 to –20 m). Preliminary bioactivities were determined in the field and in Urbana. The sponges were stored at –20 °C until needed.

Sample Isolations. Sample IRCE 2-VII-81-1-1 (1040 g) was homogenized and washed with MeOH (4 × 2 L) (see Scheme I). The filtered MeOH solution was flash evaporated and dried in vacuo to a brown oil. This oil was triturated with MeOH (800 mL) to produce a suspension, which was filtered, flash evaporated, and dried in vacuo to 41.5 g of brown solids. These solids were washed and filtered with Ac_2O (2 × 250 mL) at 40 °C, and the retained solids were triturated with MeOH (3 × 175 mL) at 40 °C. The filtered MeOH solution was flash evaporated and dried in vacuo to produce 35.7 g of MeOH-soluble material (3.4% yield from the wet sponge), which by FABMS and antibacterial and antiviral assays contained the brominated bioactive metabolites.

This sample (35.6 g, MeOH-soluble) was partitioned by CCD (1036 steps, 400 tubes) using H_2O–1-BuOH–AcOH–EtOH (10:8:1:1 v/v). The mobile phase was eluted after tube 400 and diverted to a fraction collector. The effluents were analyzed by dried residue weight, FABMS, and HPLC and pooled accordingly.

Compounds 1–3 and 6–8 were isolated from the pooled CCD fractions by using similar procedures. Pooled fractions containing the compounds of interest were subjected to reversed-phase (C18) preparative HPLC using various combinations of H_2O–MeOH–AcOH–Et_3N (solvent system 1; 650–400:350–600:7–14:7–14 v/v; pH 5). Samples were chromatographed 1–3 times until shown to be pure by analytical HPLC. Appropriate HPLC fractions were flash evaporated and dried at 0.2 Torr for several days over H_2SO_4 and KOH. The resulting oils were dissolved in H_2O, filtered (0.4 μm), and lyophilized, and the solids obtained were subjected to repeated LH-20 chromatography ($CHCl_3$–MeOH–AcOH, 65:35:1) to remove all of the faster eluting triethylammonium acetate. Appropriate LH-20 fractions were lyophilized to produce the diacetate salts, compounds 1–3 and 6–8, as amorphous, white-to-tan solids. Colored impurities were removed either by eluting the acetate salts as hydrochlorides from Dowex 1 × 2 [Cl^-] with dilute HCl, or, much less efficiently, by passing the acetate salts over a column of Sephadex G-10 with 5% AcOH in H_2O.

The isolations of 4 and 5 required a modified technique; appropriate CCD fractions were subjected to LH-20 chromatography both before and after HPLC to remove nonoxygenated analogues and triethylammonium acetate, respectively

Debromosceptrin Acetate (1). Sample G (Scheme I; 269 mg) was subjected to HPLC using solvent system 1 (600:400:7:7) to give one major UV-absorbing peak. Workup as before gave 92.2 mg of 1: $[\alpha]^{25}_D$ –30° (*c* 1.03, MeOH); UV (MeOH) λ_{max} 203 nm (ε 25 700), 212 (26 700), 265 (25 600); IR (KBr) 3350 (br), 1682, 1618, 1565, 1528, 1409, 1330, 922 cm^{-1}; MS calcd for $C_{22}H_{26}{}^{79}BrN_{10}O_2$ (M + H) 541.1424, found 541.1434 (HRFABMS).

Sceptrin Acetate (2) and Ageliferin Acetate (6). Sample N (918 mg) was subjected to preparative HPLC using solvent system 1 (500:500:7:7) to give two major UV-absorbing peaks. The earlier peak was worked up as before to give 311.8 mg of 2: $[\alpha]^{25}_D$ –18° [*c* 4.33, MeOH (lit.[3] +8°)]; UV (MeOH) λ_{max} 205 nm (ε

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27 300), 214 (28 300), 267 (25 200); IR (KBr) 3350 (br), 1683, 1624, 1566, 1527, 1408, 1332, 922 cm^{-1}; MS calcd for $C_{22}H_{25}{}^{79}Br^{81}BrN_{10}O_2$ (M + H) 621.0508, found 621.0520 (HRFABMS).

The smaller, later eluting peak was also rechromatographed by HPLC and worked up as before to give 43.3 mg of **6**: $[\alpha]^{25}_D$ +4.1° (*c* 0.146, MeOH); UV (MeOH) λ_{max} 204 nm (ϵ 25 500), 220 (27 100), 267 (25 900); IR (KBr) 3350 (br), 1681, 1629, 1565, 1526, 1407, 1330, 922 cm^{-1}; MS found for $C_{22}H_{25}{}^{79}Br^{81}BrN_{10}O_2$ (M + H) 621.0526 (HRFABMS).

Dibromosceptrin Acetate (3), Bromoageliferin Acetate (7), and Dibromoageliferin Acetate (8). Samples R through U (total, 840.6 mg) were subjected individually to HPLC (solvent system 1, 350–370:650–630:7:7) to give a large number of UV-absorbing peaks. These were collected and analyzed by FABMS and NMR; the three major peaks were shown to contain compounds **3**, **7**, and **8**. The earliest peak was rechromatographed twice by HPLC, subjected to LH-20 chromatography, and then rechromatographed by HPLC (solvent system 1, 400:600:14:14). Workup as before gave 18.5 mg of **7**: $[\alpha]^{25}_D$ +6.9° (*c* 0.058, MeOH); CD (*c* 0.0016, MeOH) $[\theta]_{270}$ +1900, $[\theta]_{250}$ +1200, $[\theta]_{230}$ −3800, $[\theta]_{220}$ +2600; UV (MeOH) λ_{max} 203 nm (ϵ 30 600), 220 (26 300), 270 (24 500); IR (KBr) 3400 (br), 1684, 1652, 1627, 1565, 1525, 1410, 1327, 922 cm^{-1}; MS calcd for $C_{22}H_{24}{}^{79}Br_2{}^{81}BrN_{10}O_2$ (M + H) 698.9613, found 698.9608 (HRFABMS).

The middle peak was rechromatographed by HPLC and worked up to give 6.6 mg of **3**: $[\alpha]^{25}_D$ −44° (*c* 0.108, MeOH); UV (MeOH) λ_{max} 202 nm (ϵ 27 300), 215 (24 800), 273 (26 300); IR (KBr) 3350 (br), 1635, 1568, 1527, 1414, 1327, 924 cm^{-1}; MS calcd for $C_{22}H_{23}{}^{79}Br_2{}^{81}Br_2N_{10}O_2$ (M + H) 778.8698, found 778.8712 (HRFABMS).

The latest eluting peak was rechromatographed by HPLC and worked up to give 42.8 mg of **8**: $[\alpha]^{25}_D$ −11.6° (*c* 0.276, MeOH); CD (*c* 0.00185, MeOH) $[\theta]_{300}$ −500, $[\theta]_{270}$ −1100, $[\theta]_{240}$ 0, $[\theta]_{230}$ +400, $[\theta]_{220}$ +3700; UV (MeOH) λ_{max} 202 nm (ϵ 24 300), 214 (24 500), 274 (25 000); IR (KBr) 3400 (br), 1684, 1631, 1570, 1526, 1415, 1328 cm^{-1}; MS found for $C_{22}H_{23}{}^{79}Br_2{}^{81}Br_2N_{10}O_2$ (M + H) 778.8698 (HRFABMS).

Debromooxysceptrin Acetate (4). Samples E and F (total, 435.8 mg) were chromatographed on LH-20 ($CHCl_3$–MeOH–AcOH, 65:35:1) to remove the faster eluting debromosceptrin (**1**) from **4**. Fractions devoid of **1** were subjected to RP-HPLC using solvent system 1 (600:400:14:14). The major UV-absorbing peak was worked up as described above to give 7.0 mg of **4**: $[\alpha]^{25}_D$ −25° (*c* 0.108, MeOH); UV (MeOH) λ_{max} 203 nm (ϵ 32 400), 220 (24 600), 265 (26 600); IR (KBr) 3350 (br), 1685, 1632, 1568, 1531, 922 cm^{-1}; MS calcd for $C_{22}H_{26}{}^{79}BrN_{10}O_3$ (M + H) 557.1373, found 557.1363 (HRFABMS).

Oxysceptrin Acetate (5). Samples I and J (total, 1518 mg) were chromatographed on LH-20 ($CHCl_3$–MeOH–AcOH, 65:35:1) to remove any of the faster-eluting sceptrin (**2**). Fractions containing oxysceptrin but no sceptrin (701 mg) were subjected to RP-HPLC using solvent system 1 (500:500:14:14). The major UV-absorbing peak was worked up as described above to give 342 mg of **5**: $[\alpha]^{25}_D$ +4.5° (*c* 1.37, MeOH); UV (MeOH) λ_{max} 203 nm (ϵ 35 100), 221 (30 700), 267 (28 100); IR (KBr) 3350 (br), 1681, 1632, 1565, 1523, 921 cm^{-1}; MS calcd for $C_{22}H_{25}{}^{79}Br^{81}BrN_{10}O_3$ (M + H) 637.0457, found 637.0472 (HRFABMS).

Formation of the Dihydrochloride Salts 2a, 5a, and 6a. Compounds **2a**, **5a**, and **6a** were formed quantitatively from their respective diacetate salts (**2**, **5**, and **6**) by treatment with 0.1 N HCl (30 molar equiv), followed by flash evaporation and desiccation in vacuo. Compound **2a**: IR (KBr) 3300 (br), 1677, 1564, 1523, 921 cm^{-1} [lit.[3] $[\alpha]^{25}_D$ −7.4° (*c* 1.2, MeOH); UV (MeOH) λ_{max} 265 nm (ϵ 20 850)]. Compound **6a**: IR (KBr) 3350 (br), 1677, 1622, 1565, 1522, 922 cm^{-1}. Compound **5a**: IR (KBr) 3300 (br), 1776, 1678, 1627, 1565, 1524, 921 cm^{-1}. Colored impurities in **2a** were removed either with activated charcoal (DARCO) or by passing **2a** over Dowex 1 × 2[Cl^-] ion exchange resin with H_2O. Decolorized **2a** tended to crystallize.

Syntheses of Pyrrolecarboxamides 13, 14, and 15. The reaction of pyrrole with $AcCCl_3$ produced 2-(trichloroacetyl)pyrrole (**16**)[53] in 53% yield: mp 73.5–74.5 °C (lit.[50] mp 73–75 °C); ^1H NMR ($CDCl_3$) δ 9.6 (s br, 1 H), 7.4 (s, 1 H), 7.18 (s, 1 H), 6.4 (s, 1 H). $MeNH_2$ was bubbled for 30 min through a stirred solution of **16** (550 mg) in $CHCl_3$ (25 mL), then filtered and flash evaporated to produce pyrrole-2-*N*-methylcarboxamide (**13**) in 90% yield: mp 156–157 °C (lit.[54] 151 °C); UV (MeOH) λ_{max} 261 nm (ϵ 13 000), 207 (4400); IR (KBr) 3285, 1642, 1578 cm^{-1}; MS calcd for $C_6H_8N_2O$ M_r 124.0636 (M^+), found M_r 124.0637 (HREIMS).

4-Bromo- and 4,5-dibromo-2-(trichloroacetyl)pyrroles (**17** and **18**) were obtained by treating **16** (2.2 g, 0.01 mol) in $CHCl_3$ (10 mL, 0 °C) with Br_2 (0.81 mL, 1.5 equiv) in $CHCl_3$ (15 mL) over 4.5 h. The mixture was poured on ice and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo to a mixture which was chromatographed (Si, hexane–EtOAc, 9:1) to produce **17** and **18** in 12% and 10% yields, respectively. These compounds were individually treated with $MeNH_2$, as above, to generate 4-bromopyrrole-2-*N*-methylcarboxamide (**14**) and 4,5-dibromopyrrole-2-*N*-methylcarboxamide (**15**) in 90–100% yields. Compound **14**: mp 178–180 °C; UV (MeOH) λ_{max} 266 nm (ϵ 9900), 231 (4700), 218 (4700); IR (KBr) 3320, 1600, 1490 cm^{-1}; MS calcd for $C_6H_7{}^{79}BrN_2O$ M_r 201.9741 (M^+), found M_r 201.9744 (HREIMS).

Compound **15**: mp 187–189 °C; UV (MeOH) λ_{max} 271 nm (ϵ 13 700), 232 (5400), 214 (6300); IR (KBr) 3243, 1642, 1614, 1570 cm^{-1}; MS calcd for $C_6H_6{}^{79}Br_2N_2O$ M_r 279.8846 (M^+), found M_r 279.8848 (HREIMS).

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Supplementary Material Available: Representative ^1H and ^{13}C NMR spectra and tables of additional NMR spectral data, bioassay data, and countercurrent distribution fractionation information (11 pages). Ordering information is given on any current masthead page.

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