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Isolation and Structure of Hemibastadinols 1−3 from the Papua New Guinea Marine Sponge *Ianthella basta*[1]

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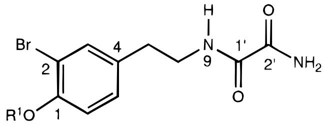
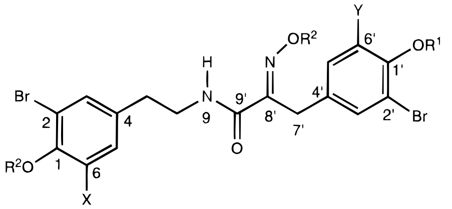
Further investigation of the Bismarck Archipelago (Papua New Guinea) marine sponge *Ianthella basta* for biologically active constituents has led to the isolation of hemibastadins 1 (**2**), 2 (**3**), and 3 (**4**) and the new brominated tyrosine derivatives hemibastadinols 1−3 (**9**, **13**, and **14**). Isolation and structure elucidation of the monomethyl ether derivatives (**7** and **8**) of hemibastadins 1 and 2 and the 3-bromotyramine amide of oxalic acid amide (**1a**) concluded our chemical investigation of *I. basta*. The hemibastadins and hemibastadinols represent important biosynthetic links to a series of bromotyrosine tetramers collectively known as the bastadins. The antimicrobial activity of the bastadins, hemibastadins, and hemibastadinols is summarized.

While ocean water is universally known for its chloride ion content ($\sim$0.5 M), it is also an abundant source of bromide ($\sim$1 mM) and to a lesser extent iodide ($\sim$1 μM).[2] An important consequence of halogen ion availability has been the effective utilization of halogenation reactions by various marine organisms in their evolutionary biosyntheses of defensive and other necessary constituents.[3,4] Illustrative are the tyrosine bromination products characteristic of marine Porifera in the Order Verongida[5−18] and certain marine tunicates.[19,20]

In 1980−83 we began evaluating for antineoplastic constituents specimens of the Verongida species *Ianthella basta* (Pallas, 1776) (also known as *Ianthella ardis*, *Aiolochioia crossa*, and *Pseudoceratina crossia*)[5] collected in Papua New Guinea. Subsequently, we isolated the acyclic and cyclic series of bastadins 1−8, 10, and 12 derived from four units of a brominated tyrosine.[21] Presently, 18[6] bastadins have been isolated from *I. basta* and related sponges. The present investigation of *I. basta* was focused on uncovering new biologically active constituents of this Western Pacific (Bismarck Archipelago) sponge employing minor fractions from a 1983 scaleup (160 kg wet weight) collection. The current study resulted in the isolation of eight new brominated tyrosine dimers and the 3-bromotyramine amide of oxalic acid amide (**1a**).



1a R = H
1b R = COCH$_3$



	X	Y	R^1	R^2
2	H	H	H	H
3	H	Br	H	H
4	Br	H	H	H
5	H	Br	Me	Me
6a	H	H	Me	Me
6b	Br	H	Me	Me
7	H	H	Me	H
8	H	Br	Me	H

Results and Discussion

Amide **1a** was isolated as an amorphous solid. The molecular formula $C_{10}H_{11}BrN_2O_3$, consistent with six unsaturation units, was determined by accurate mass measurement of the electron impact (EI) molecular ion peak cluster at *m/z* 286/288. Analysis of the ^{1}H- and ^{13}C-NMR spectra (pyridine-d_5 and again in CD_3OD) indicated the presence of a 1,2,4-trisubstituted aromatic ring and a 3-bromotyramine partial structure. The presence of phenol and bromo groups in the aromatic ring was supported by characteristic mass spectral fragmentation patterns. The remaining $C_2H_2NO_2$ unit was in turn shown to be composed of an NH_2 unit (^{1}H NMR, δ 9.07, 2H, br s) and two amide carbonyls (^{13}C NMR, 164.0 and 161.8 ppm), which also accounted for the two remaining units of unsaturation. Further support for the amide carbonyls was provided by strong absorption at ν_{max} 1651 cm^{-1} in the IR spectrum. Therefore, the $C_2H_2NO_2$ portion was assigned as CO-CONH$_2$. The carbon at C-1′ was assigned to the 161.8 ppm signal on the basis of an HMBC correlation from H-8. Additional evidence for the structure of the amide **1a** resulted from the preparation of a monoacetate derivative **1b** upon treatment with acetic anhydride−pyridine.

Hemibastadin 1 (**2**) was found to correspond to the molecular formula $C_{17}H_{16}Br_2N_2O_4$ consistent with 10 unsaturation units and was established by accurate mass measurement of the molecular ion peak cluster

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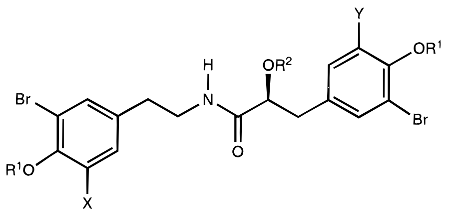
(*m*/*z* 470/472/474) in the EI mass spectrum. The molecular formula was approximately half that of bastadin 1 ($C_{34}H_{30}Br_4N_4O_8$), which suggested a "hemibastadin"-type structure that was deduced as follows. Examination of the ^{1}H and ^{13}C NMR spectra of amide **2** followed by 2D NMR studies employing COSY, HMQC, and HMBC techniques led to the 2-bromo-4-alkylphenol system of amide **1a**. The subunits formed from C-1 to NH-9 and from C-1′ to C-7′ accounted for eight of the 10 unsaturation values and were consistent with the hypothesis that amide **2** represented a bastadin subunit. The two degrees of unsaturation remaining were assigned as amide (165.8 ppm; IR, ν_{max} 1659 cm^{-1}) and oxime groups (153.3 ppm). An HMBC correlation from the C-8 methylene proton resonance (δ 3.38) to the amide carbon allowed the placement of the amide at C-9′. Correlations from the C-7′ methylene proton resonance (δ 3.77) to both the amide and oxime carbons allowed positioning of the oxime at C-8′. The oxime was assigned an *E* geometry on the basis of the ^{13}C NMR chemical shift of C-7′ (28.7 ppm).[18] Methylation of hemibastadin 1 (**2**) with CH_3I/K_2CO_3 in DMF gave trimethyl ether derivative **6a** identical by ^{1}H NMR with that previously reported[12] for the methylation product of the otherwise uncharacterized hemibastadin 1. Thus, hemibastadin 1 was assigned structure **2**.

Hemibastadins 2 (**3**) and 3 (**4**) were isolated as a mixture (3:1) that resisted separation by Sephadex LH-20 partition chromatography or normal-phase HPLC using a variety of solvent systems. The mixture was separated by reversed-phase HPLC using 2:3 CH_3CN-H_2O as eluent. After purification, the quantities of the isolates proved too small for decisive structural studies, and consequently, the mixture was employed for this purpose. Examination of the ^{1}H NMR spectrum of the mixture in CD_3OD indicated that both contained one 1,2,4,6-tetrasubstituted aromatic ring (major: δ 7.38, 2H, s, and minor: δ 7.31, 2H, s), one 1,2,4-trisubstituted aromatic ring (major: δ 7.30, 1H, d, $J = 2.0$ Hz; 6.96, 1H, dd, $J = 2.0, 8.3$ Hz; 6.78, 1H, d, $J = 8.3$ Hz, and minor: δ 7.36, 1 H, d, $J = 2.0$ Hz; 7.04, 1H, dd, $J = 2.0$, 8.3 Hz; 6.76, 1H, d, $J = 8.3$ Hz), a methylene group (major and minor: δ 3.77, s), and a CH_2CH_2 group (major and minor: δ 3.39, t, $J = 7.3$ Hz; 2.68, t, $J = 7.3$ Hz). From the preceeding evidence it seemed likely that amides **3** and **4** were isomers with one more aromatic bromine atom than hemibastadin 1 (**2**). This was confirmed by the single mass spectral ion peak cluster at *m*/*z* 548/550/552/554. The latter was shown by high-resolution EI mass measurement to correspond to $C_{17}H_{16}Br_3N_2O_4$. Comparison of the ^{1}H- and ^{13}C-NMR data exhibited by amides **3** and **4** with those found for hemibastadin 1 (**2**) indicated that the 1,2,4,6-tetrasubstituted aromatic ring was attached to the methylene group in the major isomer and to the CH_2CH_2 group in the minor isomer. The ^{13}C NMR chemical shifts for C-7′ of amides **3** (28.3 ppm) and **4** (28.6 ppm) were consistent with *E* oxime geometries.[18] Methylation of amides **3** and **4** with CH_3I/K_2CO_3 in DMF gave a mixture of the trimethyl ethers **5** and **6b**, which also resisted separation. Comparison of the ^{1}H NMR data arising from the tetramethyl ether derivative of the major isomer **5** compared with those previously reported for permethylhemibastadin 2 (**5**)[12] further supported the MS and NMR conclusions and allowed hemibastadins 2 and 3 to be assigned the structures **3** and **4**, respectively.

A molecular formula of $C_{18}H_{18}Br_2N_2O_4$ (determined by EI mass measurement of the molecular ion peak cluster at *m*/*z* 485/487/489) was found for 1′-methoxyhemibastadin 1 (**7**). Comparison of the ^{1}H- and ^{13}C-NMR data of amide **7** with those for hemibastadin 1 (**2**) suggested that the methoxyl group resided at C-1′, which was confirmed by an HMBC correlation from the methoxyl proton resonance to C-1′ (155.9 ppm). The ^{13}C-NMR chemical shift value for C-7′ of 28.7 ppm was consistent with an *E* oxime,[18] and thus, amide **7** was shown to be the 1′-methoxy derivative of hemibastadin 1.

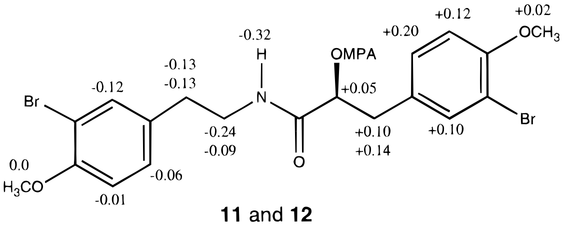
Structural determination of 1′-methoxyhemibastadin 2 (**8**) began with the assignment of molecular formula $C_{18}H_{17}Br_3N_2O_4$ using the ion peak cluster at *m*/*z* 568/570/572/574, as noted for amide **7**. The elemental composition suggested that phenol **8** was closely related to 1′-methoxyhemibastadin 1 (**7**). Examination of the ^{1}H- and COSY-NMR spectra of phenol **8** combined with the NOE and ^{13}C-NMR results confirmed the placement of the tetrasubstituted ring. Comparison of the ^{1}H- and ^{13}C-NMR results associated with the 1,2,4-trisubstituted aromatic ring with those for hemibastadin 1 (**2**) suggested that the methoxyl group was attached to C-1′ and not C-1. An *E* geometry was assigned[18] to the oxime (C-7′: 28.8 ppm), and phenol **8** was thereby shown to be a 1′-methoxy derivative of hemibastadin 2.

Hemibastadinol 1 (**9**) was isolated as an optically active solid. Mass spectral evidence supported a molecular formula $C_{17}H_{16}Br_2NO_4$ consistent with nine unsaturation units. Analyses of the ^{1}H- and COSY-NMR spectra of amide **9** (in CD_3OD) indicated the presence of two 1-hydroxy-2-bromo-4-alkyl aromatic rings. One of the phenol rings was found to be attached to a CH_2CH_2NH group. The other 1-hydroxy-2-bromo-4-alkyl aromatic ring was attached to a methylene that was further coupled to an oxymethine group. Thus, units C-1 to NH-9 and C-1′ to C-8′ accounted for eight of the nine units of unsaturation present.

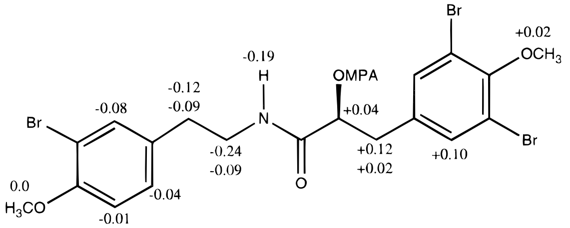


	X	Y	R^1	R^2
9	H	H	H	H
10	H	H	Me	H
11	H	H	Me	(S)-MPA
12	H	H	Me	(R)-MPA
13	H	Br	H	H
14	Br	H	H	H
15	H	Br	Me	H
16	Br	H	Me	H
17	H	Br	Me	(S)-MPA
18	Br	H	Me	(S)-MPA
19	H	Br	Me	(R)-MPA
20	Br	H	Me	(R)-MPA

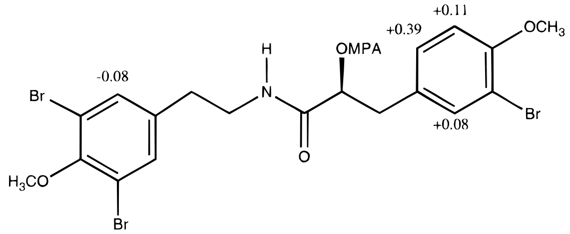
The remaining unit of unsaturation was assigned to



11 and 12



17 and 19



18 and 20

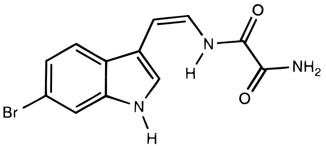
Figure 1. NMR Δδ values for the (*S*)- and (*R*)-MPA derivatives of 1,1′-dimethoxyhemibastadinols 1 (**10**), 2 (**15**), and 3 (**16**).

an amide. The position of the amide carbonyl at C-9′ was supported by HMBC correlations from H-7a′, H-7b′, H-8′, H-7a, and H-7b. Therefore, hemibastadinol 1 (**9**) was shown to be a C-8′ hydroxy derivative of hemibastadin 1 (**2**).

The absolute configuration of the secondary alcohol at C-8′ in hemibastadinol 1 (**9**) was investigated using Trost's modification of the Mosher method[22,23] with the methyl ether derivative **10** formed by reaction of diphenol **9** with ethereal diazomethane. The (*S*)- and (*R*)-α-methoxyphenylacetyl (MPA) derivatives **11** and **12** were prepared by reaction of alcohol **10** with the appropriate epimer of MPA and DCCI/DMAP in dichloromethane. Analysis of the COSY spectra of esters **11** and **12** allowed all the ^{1}H-NMR resonances to be assigned. Negative Δδ values ($\Delta\delta = \delta_S - \delta_R$) for H-3 through NH-9 and positive Δδ values for 1′-OCH_3 through H-7a/H-7b (Figure 1) pointed to a C-8′ (*S*) configuration for the secondary alcohol.

Hemibastadinols 2 (**13**) and 3 (**14**) were isolated as an optically active oily mixture (19:1) that effectively resisted separation. Analysis of the ^{1}H-NMR spectrum (CD_3OD) of the mixture suggested that phenols **13** and **14** were the C-8′ hydroxy derivatives of hemibastadins 2 (**3**) and 3 (**4**). A single molecular ion peak cluster (by EI) at *m*/*z* 535/537/539/541, which corresponded to $C_{17}H_{16}Br_3NO_4$, supported this hypothesis. Comparison of the ^{1}H- and ^{13}C-NMR data exhibited by phenols **13** and **14** with those for hemibastadins 1−3 (**2**−**4**) and hemibastadinol 1 (**9**) suggested that the major isomer **13** contained the 2-bromo-4-alkyl aromatic ring attached to the CH_2CH_2NH group, whereas in the minor isomer the 2,6-dibromo-4-alkyl aromatic ring was attached to the CH_2CH_2NH group. These results allowed structures **13** and **14** to be assigned to hemibastadinols 2 and 3, respectively, and those conclusions were further confirmed by the results of permethylation. When phenols **13** and **14** were methylated using ethereal diazomethane the result was a quantitative yield of methyl ethers **15** and **16**. Application of the Mosher−Trost method to the (*S*)-(**17** and **18**) and (*R*)-(**19** and **20**) MPA derivatives prepared from the mixture of hemibastadinols 2 and 3 methyl ethers gave evidence that both corresponded to the C-8′ (*S*) absolute configuration (Figure 1).

The isolation of hemibastadins 1−3 and hemibastadinols 1−3 from *I. basta* provides some further insight into biosynthetic pathways of the cyclic bastadins. For example, the C-8′ alcohol group of hemibastadinols 1−3 suggests that hydroxylation at C-8′ may be a direct precursor (or biosynthetic byproduct) of the bastadin oxime group and that formation of amide **1a** may represent an early event in the overall biosynthetic process. Interestingly, the oxalyl diamide **1a** seems related to the marine sponge constituent igzamide (**21**).[24]



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While the hemibastadins did not display significant activity against the P388 lymphocytic leukemia cell line, they did exhibit promising antimicrobial properties in keeping with the known ability of certain brominated tyrosine derivatives to have antiinflammatory and antimicrobial activities. Bastadins 4, 8, and 9 (Guam *I. basta*) proved to be antiinflammatory in the mouse ear assay.[14] Bastadin 13 (Australian *I. basta*) inhibited growth of the Gram-positive bacterium *Bacillus subtilis*.[9] Bastadins 1−7 (Australian *I. basta*) are apparently inhibitory for Gram-positive bacteria, although data were not provided.[25] We evaluated the ability of amide **1a**, bastadins 1−6, the hemibastadins, and hemibastadinols to inhibit growth of Gram-negative bacteria, Gram-positive bacteria, and two fungi (Table 1). Except for bastadin 6, all of these compounds inhibited growth of the Gram-negative pathogen *Neisseria gonorrhoeae*. Most of the compounds also inhibited growth of the Gram-positive opportunists *Enterococcus faecalis* and *Staphylococcus aureus*. At up to 100 μg/disk, these compounds exhibited no antimicrobial activity against the Gram-negative bacterium *Escherichia coli* or the fungi *Candida albicans* and *Cryptococcus neoformans*.

Experimental Section

General Experimental Procedures. Analytical reversed-phase HPLC was performed using a Merck LiChrospher 100 RP-18 column (250 × 4.6 mm, 5 μm), controlled by an analytical Gilson HPLC system (802B, 811, 2 × 302) fitted with a Rheodyne injector valve (7125 with 20-μL loop), Apple IIe gradient manager (VI.2 Gilson), UV detector, and data system (Hewlett-Packard 1040A) at 276 nm. All other general experimental techniques and instruments were as previously described.[21]

Table 1. Minimum Inhibitory Concentrations (μg/Disk) of Bromotyrosine Derivatives for Bacteria

compd	*E. coli*	*N. gonorrhoeae*	*E. faecalis*	*S. aureus*
bastadin 1	*a*	50–100	12.5–25	6.25–12.5
bastadin 2		50–100	50–100	6.25–12.5
bastadin 3		0.78–1.56	3.12–6.25	1.56–3.12
bastadin 4		12.5–25	50–100	12.5–25
bastadin 5		50–100	50–100	12.5–25
bastadin 6			12.5–25	6.25–12.5
amide **1a**		50–10		
hemibastadin 1		12.5–25.0	50–100	1.56–3.12
1′-(methyloxy)hemibastadin 7		6.25–12.5		6.25–12.5
hemibastadin 2		3.12–6.25		3.12–6.25
hemibastadins 2 and 3		6.25–12.5	50–100	6.25–12.5
hemibastadinol 1		50–100		
hemibastadinols 2 and 3		50–100		

[a] No inhibition at 100 μg/disk.

Animal Collection, Extraction, and Solvent Partitioning. The marine sponge *I. basta* (Pallas, 1776) in the Class Demospongiae (Order Verongia, Family Ianthellida) was collected (160-kg wet weight) in the Bismarck Archipelago (Northern Papua New Guinea). Those details including the extraction and solvent partitioning have been summarized as part of an initial study.[21]

Isolation Sequence. The murine P388 lymphocytic leukemia cell line active fraction (314 g) obtained from the solvent-partitioning procedure[21] was chromatographed on a column of Sephadex LH-20 with CH_3OH as eluent to afford the P388 active fraction (41 g). An aliquot (29 g) of the P388 active fraction was further separated on Sephadex LH-20 using methanol as eluent to give three major fractions, A, B, and a third containing 13.3 g of bastadins as a mixture.

Fraction A (1.16 g) was partitioned with Sephadex LH-20 using 2:1:1 hexane:toluene:methanol as eluent and afforded two P388-active fractions. The first (153 mg) was chromatographed initially over silica gel using 97:3 CH_2Cl_2:CH_3OH and then with normal-phase HPLC (98.5:1.5 CH_2Cl_2:CH_3OH, 1.5 mL/min) to give 1′-methoxyhemibastadin 1 (**7**, 12.9 mg). The second active fraction (144 mg) was separated over silica gel using 3:7 hexane:ethyl acetate to give two additional active fractions. These were further separated using normal-phase HPLC (96:4 CH_2Cl_2:2-propanol, 2.0 mL/min). The first fraction gave amide **1a** (38.3 mg) and a mixture of hemibastadinols 2 and 3 (**13** and **14**, 12.8 mg). The latter afforded 19.9 mg of hemibastadinol 1 (**9**).

Fraction B (5.85 g) was separated in a similar manner using Sephadex LH-20 eluted with 2:1:1 hexane:toluene:methanol to give three initial P388-active fractions. The first (95.2 mg) was separated on a column of silica gel using 96:4 CH_2Cl_2:CH_3OH and then again by column chromatography using silica gel with 13:7 hexane:ethyl acetate as eluent and finally using normal-phase HPLC (7:3 hexane:ethyl acetate, 2.0 mL/min) to give 7.1 mg of 1′-methoxyhemibastadin 2 (**8**) and an additional 8.7 mg of 1′-methoxyhemibastadin 1 (**7**). The second active fraction (57.6 mg) furnished an additional 8.0 mg of amide **1a** using column chromatography on silica gel with 19:1 CH_2Cl_2:2-propanol followed by normal-phase HPLC (7:3 hexane:ethyl acetate, 2.0 mL/min). The third active fraction (262.2 mg) was also separated by silica gel column chromatography using 13:7 to 1:1 hexane:ethyl acetate to give two new active fractions. The first of these (35.5 mg) was subjected to normal-phase HPLC (13:7 hexane:ethyl acetate, 2.0 mL/min) to give a mixture of hemibastadins 2 and 3 (**3** and **4**, 29.5 mg). The second (121.7 mg) furnished 77.4 mg of hemibastadin 1 (**2**) following normal-phase HPLC using 6:4 hexane:ethyl acetate (1.8 mL/min). The remaining sample (12 g) of the original P388-active fraction was separated in an analogous way to give in overall yields hemibastadin 1 (**2**) (106 mg, 5.2×10^{-5}%), hemibastadins 2 (**3**) and 3 (**4**) (mixture, 46.5 mg, 2.2×10^{-5}%), hemibastadinol 1 (**9**) (26.6 mg, 1.3×10^{-5}%), hemibastadinols 2 and 3 (**13** and **14**) (mixture, 15.0 mg, 7.4×10^{-6}%), 1′-methoxyhemibastadin 1 (**7**) (35.4 mg, 1.7×10^{-5}%), 1′-methoxyhemibastadin 2 (**8**) (7.1 mg, 3.5×10^{-6}%) and the 3-bromotyramine amide of oxalic acid amide (**1a**) (65.3 mg, 3.2×10^{-5}%).

3-Bromotyramine amide of oxalic acid amide (1a): colorless, amorphous solid; UV (CH_3OH) λ_{max} (log ϵ) 212 (3.89), 281 (3.14) nm; IR (NaCl, film) ν_{max} 3387, 3310, 1651 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 2.72 (2H, t, $J = 7.4$ Hz, H_2-7), 3.41 (2H, t, $J = 7.4$ Hz, H_2-8), 6.81 (1H, d, $J = 8.2$ Hz, H-6), 7.01 (1H, dd, $J = 2.0$, 8.2 Hz, H-5), 7.33 (1H, d, $J = 2.0$ Hz, H-3); ^{13}C NMR (100 MHz, CD_3OD) 35.0 (C-7), 42.2 (C-8), 110.8 (C-2), 117.3 (C-6), 130.0 (C-5), 132.8 (C-4), 134.3 (C-3), 154.0 (C-1), 161.8 (C-1′), 164.0 ppm (C-2′); EI mass spectrum *m/z* (rel int) [M$^+$] 288 (6), 286 (6), 200 (99), 199 (13), 198 (100), 187 (32), 185 (32), 120 (20), 101 (10), 77 (11); HREIMS [M$^+$] 287.9935 ($C_{10}H_{11}{}^{81}BrN_2O_3$, calcd 287.9933), 285.9948 ($C_{10}H_{11}{}^{79}BrN_2O_3$, calcd 285.9953), mass ions 199.9666 ($C_8H_7{}^{81}BrO$ calcd 199.9660), 197.9683 ($C_8H_7{}^{79}BrO$, calcd 197.9680), 186.9593 ($C_7H_6{}^{81}BrO$, calcd 186.9582), 184.9615 ($C_7H_6{}^{79}BrO$, calcd 184.9602), 120.0573 (C_8H_8O, calcd 120.0575), 101.0353 ($C_3H_5N_2O_2$, calcd 101.0351).

The phenol (**1a**, 12.7 mg) was dissolved in pyridine (1 mL) and Ac_2O (0.5 mL), and the solution was stirred at room temperature for 24 h. The reaction mixture was poured into water (50 mL) and extracted with Et_2O (2 × 50 mL). The combined ethereal extract was washed with water (2 × 50 mL) and dried (sodium sulfate) and solvent removed in vacuo to yield the acetate **1b** (14.5 mg, 99%) as an amorphous powder: UV (CH_2Cl_2) λ_{max} (log ϵ) 230 (3.58), 276 (3.14), 286 (3.06) nm; IR (NaCl, film) ν_{max} 3391, 3312, 1767, 1651, 1599, 1547, 1416, 1229, 1213, 1192 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 2.26 (3H, s, 1-$OCOCH_3$), 2.88 (2H, t, $J = 6.0$ Hz, H_2-7), 3.66 (2H, dt, $J = 6.0$, 6.0 Hz, H_2-8), H-3, H-5, H-6 obscured by solvent, 9.02 (2H, br s, 2′-NH_2), 9.63 (1H, br t, $J = 6.0$ Hz, NH-9); ^{13}C NMR (125 MHz, pyridine-d_5) 20.5 (1-$OCOCH_3$), 34.9 (C-9), 41.1 (C-8), 116.6 (C-2), 124.3 (C-6), 129.5 (C-5), 133.7 (C-3), 139.5 (C-4), 147.4 (C-1), 161.7 (C-1′), 163.5 (C-2′), 168.7 ppm (1-$OCOCH_3$); EI mass spectrum *m/z* (rel int) [M^+] 330 (1), 328 (1), 288 (22), 286 (23), 201 (13), 200 (96), 199 (15), 198 (100), 187 (22), 186 (23), 120 (11), 101 (9), 91 (7), 77 (6); HREIMS [M^+] 328.0067 ($C_{12}H_{13}{}^{79}BrN_2O_4$ calcd 328.0059), 330.0041 ($C_{12}H_{13}{}^{81}BrN_2O_4$ calcd 330.0039).

Hemibastadin 1 (2): colorless oil; UV (CH_3OH) λ_{max} (log ϵ) 210 (4.53), 281 (3.88) nm; IR (NaCl, film) ν_{max} 3383, 3000, 1659, 1537, 1495, 1422, 1283, 1256 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 2.67 (2H, t, *J* = 7.3 Hz, H_2-7), 3.38 (2H, t, *J* = 7.3 Hz, H_2-8), 3.77 (2H, s, H_2-7′), 6.76 (1H, d, *J* = 8.3 Hz, H-6′), 6.77 (1H, d, *J* = 8.3 Hz, H-6), 6.95 (1H, dd, *J* = 2.0, 8.3 Hz, H-5), 7.03 (1H, dd, *J* = 2.0, 8.3 Hz, H-5′), 7.29 (1H, d, *J* = 2.0 Hz, H-3), 7.35 (1H, d, *J* = 2.0 Hz, H-3′); ^1H NMR (300 MHz, pyridine-d_5) δ 2.87 (2H, t, *J* = 6.5 Hz, H_2-7), 3.67 (2H, dt, *J* = 6.5, 6.5 Hz, H_2-8), 4.32 (2H, s, H_2-7′), 7.05 (2H, br s, H-5, H-6), 7.12 (1H, d, *J* = 8.3 Hz, H-6′), 7.53 (1H, s, H-3), 7.55 (1H, dd, *J* = 2.1, 8.3 Hz, H-5′), 8.06 (1H, d, *J* = 2.1 Hz, H-3′), 8.60 (1H, t, *J* = 6.5 Hz, NH-9); ^{13}C NMR (100 MHz, CD_3OD) 28.7 (C-7′), 35.3 (C-7), 42.0 (C-8), 110.7, 110.5 (C-2, C-2′), 117.3, 117.1 (C-6, C-6′), 130.1, 130.4 (C-5, C-5′), 130.7 (C-4′), 133.1 (C-4), 134.3, 134.5 (C-3, C-3′), 153.3 (C-8′), 153.9, 153.8 (C-1, C-1′), 165.8 ppm (C-9′); ^{13}C NMR (125 MHz, pyridine-d_5) 28.9 (C-7′), 35.0 (C-7), 41.5 (C-8), 111.0, 111.1 (C-2, C-2′), 117.2, 117.2 (C-6, C-6′), 129.6, 130.3 (C-5, C-5′), 130.4 (C-4′), 132.2 (C-4), 133.8, 134.5 (C-3, C-3′), 153.1 (C-8′), 154.1, 154.2 (C-1, C-1′), 164.6 ppm (C-9′); EI mass spectrum *m/z* (rel int) [M^+] 474 (10), 472 (19), 470 (10), 458 (7), 456 (16), 454 (7), 256 (9), 214 (23), 213 (91), 212 (33), 211 (84), 201 (26), 200 (97), 199 (27), 198 (98), 188 (13), 187 (95), 186 (15), 185 (100), 132 (43), 120 (31), 77 (42); HREIMS [M^+] 469.9437 ($C_{17}H_{16}{}^{81}Br_2N_2O_4$, calcd 473.9430, 471.9445 ($C_{17}H_{16}{}^{79}Br^{81}BrN_2O_4$, calcd 471.9457), 469.9467 ($C_{17}H_{16}{}^{79}Br_2N_2O_4$, calcd 473.9477).

Hemibastadin 2 (3) and Hemibastadin 3 (4). Mixture: IR (NaCl, film) ν_{max} 3393, 1657, 1476 cm^{-1}; EI mass spectrum *m/z* (rel int) [M^+] 554 (4), 552 (14), 550 (14), 548 (5), 293 (33), 291 (67), 289 (34), 267 (24), 265 (50), 263 (24), 213 (41), 212 (51), 211 (24), 198 (76), 187 (91), 185 (100), 132 (28), 120 (25), 77 (49); HREIMS [M^+] 553.8549 ($C_{17}H_{15}{}^{81}Br_3N_2O_4$ calcd 553.8522), 551.8536 ($C_{17}H_{15}{}^{79}Br^{81}Br_2N_2O_4$, calcd 551.8542), 549.8562 ($C_{17}H_{15}{}^{79}Br_2{}^{81}BrN_2O_4$, calcd 549.8562), 547.8570 ($C_{17}H_{15}{}^{79}Br_3N_2O_4$, calcd 547.8582). Hemibastadin 2 (**3**): ^1H NMR (500 MHz, CD_3OD) δ 2.68 (2H, t, *J* = 7.3 Hz, H_2-7), 3.39 (2H, t, *J* = 7.3 Hz, H_2-8), 3.77 (2H, s, H_2-7′), 6.78 (1H, d, *J* = 8.3 Hz, H-6), 6.96 (1H, dd, *J* = 2.0, 8.3 Hz, H-5), 7.30 (1H, d, *J* = 2.0 Hz, H-3), 7.38 (2H, s, H-3′, H-5′); ^{13}C NMR (75 MHz, CD_3OD) 28.3 (C-7′), 35.1 (C-7), 42.0 (C-8), 110.9, 112.2 (C-2, C-2′), 117.5, 112.2 (C-6, C-6′), 130.2, 134.2 (C-5, C-5′), 132.6 (C-4′), 133.3 (C-4), 134.5, 134.2 (C-3, C-3′), 152.9 (C-8′), 154.1, 151.0 (C-1, C-1′), 165.9 ppm (C-9′); hemibastadin 3 (**4**): ^1H NMR (500 MHz, CD_3OD) δ 2.68 (2H, t, *J* = 7.3 Hz, H_2-7), 3.39 (2H, t, *J* = 7.3 Hz, H_2-8), 3.77 (2H, s, H_2-7′), 6.76 (1H, d, *J* = 8.3 Hz, H-6′), 7.04 (1H, dd, *J* = 2.0, 8.3 Hz, H-5′), 7.31 (2H, s, H-3, H-5), 7.36 (1H, d, *J* = 2.0 Hz, H-3′); ^{13}C NMR (75 MHz, CD_3OD) 28.6 (C-7′), 34.8 (C-7), 41.7 (C-8), 112.3, 110.7 (C-2, C-2′), 112.3, 117.3 (C-6, C-6′), 133.9, 130.5 (C-5, C-5′), 130.9 (C-4′), 135.0 (C-4), 133.9, 134.8 (C-3, C-3′), 153.5 (C-8′), 154.0, 151.0 (C-1, C-1′), 166.2 ppm (C-9′).

1′-Methoxyhemibastadin 1 (7): colorless oil; UV (CH_3OH) λ_{max} (log ϵ) 213 (4.53), 280 (3.87) nm; IR (NaCl, film) ν_{max} 3385, 3283, 1657, 1537, 1497, 1283, 1256 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.67 (2H, t, *J* = 7.2 Hz, H_2-7), 3.40 (2H, t, *J* = 7.2 Hz, H_2-8), 3.80 (2H, s, H_2-7′), 3.82 (3H, s, 1′-OCH_3), 6.77 (1H, d, *J* = 8.3 Hz, H-6), 6.89 (1H, d, *J* = 8.4 Hz, H-6′), 6.95 (1H, dd, *J* = 2.0, 8.3 Hz, H-5), 7.14 (1H, dd, *J* = 2.3, 8.4 Hz, H-5′), 7.29 (1H, d, *J* = 2.0 Hz, H-3), 7.43 (1H, d, *J* = 2.3 Hz, H-3′); ^{13}C NMR (100 MHz, CD_3OD) 28.7 (C-7′), 35.3 (C-7), 41.9 (C-8), 56.7 (1′-OCH_3), 110.7 (C-2), 112.2 (C-2′), 113.2 (C-6′), 117.2 (C-6), 130.0 (C-5), 130.4 (C-5′), 131.6 (C-4′), 133.0 (C-4), 134.2 (C-3), 134.8 (C-3′), 153.1 (C-8′), 153.8 (C-1), 155.9 (C-1′), 165.7 ppm (C-9′); EI mass spectrum *m/z* (rel int) [M^+] 488 (6), 486 (13), 484 (6), 472 (6), 470 (11), 468 (6), 288 (12), 286 (12), 228 (21), 227 (97), 226 (20), 225 (100), 201 (38), 200 (30), 199 (38), 198 (27), 187 (50), 185 (53), 146 (52), 120 (18), 103 (33), 77 (38); HREIMS [M^+] 487.9615 ($C_{18}H_{18}{}^{81}Br_2N_2O_4$, calcd 487.9593), 485.9609 ($C_{18}H_{18}{}^{79}Br^{81}BrN_2O_4$, calcd 485.9614), 483.9633 ($C_{18}H_{18}{}^{79}Br_2N_2O_4$, calcd 485.9634).

1′-Methoxyhemibastadin 2 (8): colorless solid; UV (CH_3OH) λ_{max} (log ϵ) 213 (4.50), 281 (3.87) nm; IR (NaCl, film) ν_{max} 3300, 1661, 1537, 1497, 1472, 1422, 1260, 993 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 2.68 (2H, t, *J* = 7.3 Hz, H_2-7), 3.40 (2H, t, *J* = 7.3 Hz, H_2-8), 3.80 (3H, s, 1′-OCH_3), 3.82 (2H, s, H_2-7′), 6.79 (1H, d, *J* = 8.3 Hz, H-6), 6.97 (1H, dd, *J* = 2.0, 8.3 Hz, H-5), 7.30 (1H, d, *J* = 2.0 Hz, H-3), 7.47 (2H, s, H-3′, H-5′); ^{13}C NMR (125 MHz, CD_3OD) 28.8 (C-7′), 35.2 (C-7), 42.0 (C-8), 61.0 (1′-OCH_3), 110.7 (C-2), 118.6 (C-2′, C-6′), 117.2 (C-6), 130.0 (C-5), 133.0 (C-4), 134.2 (C-3), 134.5 (C-3′, C-5′), 137.4 (C-4′), 152.1 (C-8′), 153.8 (C-1′), 153.9 (C-1), 165.3 ppm (C-9′); EI mass spectrum *m/z* (rel int) [M^+] 568 (2), 566 (6), 564 (7), 562 (2), 552 (2), 550 (7), 548 (8), 546 (2), 307 (30), 305 (61), 303 (30), 292 (14), 290 (25), 288 (13), 281 (10), 279 (17), 277 (9), 271 (7), 269 (23), 267 (10), 201 (15), 200 (96), 199 (18), 198 (100), 187 (44), 185 (48), 183 (17), 181 (15), 143 (18), 120 (18), 77 (17); HREIMS [M^+] 567.8672 ($C_{18}H_{18}{}^{81}Br_3N_2O_4$, calcd 567.8679), 565.8701 ($C_{18}H_{18}{}^{79}Br^{81}Br_2N_2O_4$, calcd 565.8699), 563.8715 ($C_{18}H_{18}{}^{79}Br_2{}^{81}BrN_2O_4$, calcd 563.8719), 561.8716 ($C_{18}H_{18}{}^{79}Br_3N_2O_4$, calcd 561.8739).

Hemibastadinol 1 (9): colorless solid; $[\alpha]^{23}{}_D$ −31° (*c* =1.83, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 208 (4.43), 281 (3.69) nm; IR (NaCl, film) ν_{max} 3381, 1643, 1541, 1495, 1420, 1289 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.57 (2H, m, H_2-7), 2.72 (1H, dd, *J* = 7.2, 14.0 Hz, H-7a′), 2.90 (1H, dd, *J* = 4.0, 14.0 Hz, H-7b′), 3.26 (1H, m, H-8a), 3.38 (1H, m, H-8b), 4.13 (1H, dd, *J* = 4.0, 7.2, H-8′), 6.79 (2H, d, *J* = 8.3 Hz, H-6, H-6′), 6.92 (1H, dd, *J* = 1.9, 8.3 Hz, H-5), 7.02 (1H, dd, *J* = 1.9, 8.3 Hz, H-5′), 7.28 (1H, d, *J* = 1.9 Hz, H-3), 7.34 (1H, d, *J* = 1.9 Hz, H-3′); ^1H NMR (300 MHz, pyridine-d_5) δ 2.82 (2H, m, H_2-7), 3.18 (1H, dd, *J* = 7.8, 13.5 Hz, H-7a′), 3.47 (1H, dd, *J* = 3.5, 13.5 Hz, H-7b′), 3.69 (2H, m, H_2-8), 4.73 (1H, dd, *J* = 3.5, 7.8 Hz, H-8′), 7.08 (2H, br s, H-5, H-6), 7.12 (1H, d, *J* = 8.3 Hz, H-6′), 7.32 (1H, dd, *J* = 1.9, 8.3 Hz, H-5′), 7.56 (1H, obscured, H-3), 7.82 (1H, d, *J* = 1.9 Hz, H-3′), 8.39 (1H, t, *J* = 5.6 Hz, NH-9); ^{13}C NMR (100 MHz, CD_3OD) 35.4 (C-7), 40.6 (C-7′), 41.5 (C-8), 73.7 (C-8′), 110.4 (C-2′), 110.7 (C-2), 116.9 (C-6′), 117.3 (C-6), 130.0 (C-5), 131.0 (C-5′), 131.6 (C-4′), 133.0 (C-4), 134.2 (C-3), 135.2 (C-3′), 153.9 (C-1), 154.0 (C-1′), 176.2 ppm (C-9′); ^{13}C NMR (125 MHz, pyridine-d_5) 35.2 (C-7), 40.6 (C-7′), 40.9 (C-8), 73.4 (C-8′), 110.7 (C-2′), 111.0

(C-2), 116.8 (C-6′), 117.2 (C-6), 129.4 (C-5), 130.6 (C-5′), 131.7 (C-4′), 132.2 (C-4), 133.8, (C-3), 134.8 (C-3′), 154.1 (C-1, C-1′), 174.3 ppm (C-9′); EI mass spectrum *m/z* (rel int) [M^+] 461 (<1), 459 (0.3), 457 (<1), 443 (6), 441 (13), 439 (6), 244 (30), 242 (35), 241 (18), 227 (14), 225 (15), 201 (19), 200 (98), 199 (21), 198 (100), 187 (28), 185 (28), 120 (20), 107 (23), 77 (18); HREIMS [M^+] 460.9477 ($C_{17}H_{17}{}^{81}Br_2NO_4$ calcd 460.9494), 458.9483 ($C_{17}H_{17}{}^{79}Br^{81}BrNO_4$, calcd 458.9514), 456.9507 ($C_{17}H_{17}{}^{79}Br_2NO_4$, calcd 456.9534).

Hemibastadinol 2 (13) and Hemibastadinol 3 (14). Mixture: $[\alpha]^{23}_{D}$ −24° (c = 0.10, CH_3OH); IR (NaCl, film) ν_{max} 3381, 1643, 1539, 1478, 1279 cm^{-1}; EI mass spectrum *m/z* (rel int) [M^+] 541 (<1), 539 (<1), 537 (<1), 535 (<1), [$M^+ - H_2O$] 523 (2), 521 (7), 519 (6), 517 (2), 244 (9), 242 (10), 201 (15), 200 (100), 199 (16), 198 (100), 187 (16), 185 (17), 120 (20), 77 (13); HREIMS [M^+] 540.8558 ($C_{17}H_{16}{}^{81}Br_3NO_4$ calcd 540.8572), 538.8586 ($C_{17}H_{16}{}^{79}Br^{81}Br_2NO_4$, calcd 538.8593), 536.8631 ($C_{17}H_{16}{}^{79}Br_2{}^{81}BrNO_4$, calcd 536.8612), 534.8605 ($C_{17}H_{16}{}^{79}Br_3NO_4$, calcd 534.8633), [$M^+ - H_2O$] 522.8446 ($C_{17}H_{14}{}^{81}Br_3NO_3$, calcd 522.8464), 520.8471 ($C_{17}H_{14}{}^{79}Br^{81}Br_2NO_3$, calcd 520.8484), 518.8497 ($C_{17}H_{14}{}^{79}Br_2{}^{81}BrNO_3$, calcd 518.8504), 516.8509 ($C_{17}H_{14}{}^{79}Br_3NO_3$, calcd 516.8524). (8′*S*)-Hemibastadinol 2 (**13**): ^1H NMR (500 MHz, CD_3OD) δ 2.53 (1H, ddd, J = 7.0, 8.0, 14.0 Hz, H-7a), 2.60 (1H, ddd, J = 6.5, 8.5, 14.0 Hz, H-7b), 2.75 (1H, dd, J = 4.0, 14.0 Hz, H-7a′), 2.88 (1H, dd, J = 7.0, 14.0 Hz, H-7b′), 3.23 (1H, ddd, J = 6.5, 8.0, 15.0 Hz, H-8a), 3.40 (1H, ddd, J = 7.0, 8.5, 15.0 Hz, H-8b), 4.14 (1H, dd, J = 4.0, 7.0, H-8′), 6.80 (1H, d, J = 8.0 Hz, H-6), 6.92 (1H, dd, J = 2.0, 8.0 Hz, H-5), 7.28 (1H, d, J = 2.0 Hz, H-3), 7.34 (2H, s, H-3′, H-5′); ^{13}C NMR (125 MHz, CD_3OD) 35.5 (C-7), 41.5 (C-7′, C-8), 73.3 (C-8′), 110.7 (C-2), 111.8 (C-2′, C-6′), 117.3 (C-6), 129.9 (C-5), 132.9 (C-4′), 133.1 (C-4), 134.2 (C-3), 134.6 (C-3′, C-5′), 150.9 (C-1′), 153.8 (C-1), 175.8 ppm (C-9′). (8′*S*)-Hemibastadinol 3 (**14**): ^1H NMR (500 MHz, CD_3OD) δ 6.80 (1H, d, J = 8.0 Hz, H-6′), 7.02 (1H, dd, J = 2.0, 8.0 Hz, H-5′), 7.31 (2H, s, H-3, H-5), 7.34 (1H, d, J = 2.0 Hz, H-3′); ^{13}C NMR (125 MHz, CD_3OD) 35.1 (C-7), 40.6 (C-7′), 41.2 (C-8), 73.7 (C-8′), 110.4 (C-2′), 112.2 (C-2, C-6), 116.9 (C-6′), 130.9 (C-5′), 131.6 (C-4′), 131.9 (C-4), 133.6 (C-3, C-5), 135.2 (C-3′), 150.8 (C-1), 153.9 (C-1′), 176.2 ppm (C-9′).

1,1′,8′(*S*)-Trimethoxyhemibastadin 1 (6a). A mixture of hemibastadin 1 (**2**, 2.6 mg), K_2CO_3 (15 mg), and methyl iodide (0.25 mL) in dry DMF (1 mL) was stirred at room temperature for 40 h. The reaction mixture was poured into water (20 mL) and extracted with Et_2O (2 × 25 mL). The combined ethereal extract was washed with water (2 × 25 mL), and dried (sodium sulfate) and solvent removed in vacuo to give methyl ether **6a** as a colorless solid (1.8 mg, 64%): ^1H NMR (300 MHz, $CDCl_3$) δ 2.75 (2H, t, J = 6.9 Hz, H_2-7), 3.49 (2H, dt, J = 6.9, 6.9 Hz, H_2-8), 3.81 (2H, s, H_2-7′), 3.85, 3.87, 3.98 (3 × 3H, 3s, 1-OCH_3, 1′-OCH_3, 8′-$NOCH_3$), 6.79 (1H, br t, J = 6.9 Hz, NH-9), 6.79 (1H, d, J = 8.5 Hz, H-6′), 6.82 (1H, d, J = 8.5 Hz, H-6), 7.06 (1H, dd, J = 2.1, 8.5 Hz, H-5), 7.20 (1H, dd, J = 2.1, 8.5 Hz, H-5′), 7.37 (1H, d, J = 2.1 Hz, H-3), 7.46 (1H, d, J = 2.1 Hz, H-3′), identical (by ^1H-NMR) to that previously reported.[12]

1,1′,8′(*S*)-Trimethoxyhemibastadin 2 (5) and 1,1′,8′(*S*)-Trimethoxyhemibastadin 3 (6b). Methylation of hemibastadins 2 (**3**) and 3 (**4**) (total 5.0 mg) in dry DMF (1 mL) was conducted with methyl iodide (0.5 mL) and K_2CO_3 (25 mg) as noted above (cf.) to give a mixture of ethers of **5** and **6b** as a colorless solid (total 3.6 mg, 71%). 1,1′,8′(*S*)-Trimethoxyhemibastadin 2 (**5**): ^1H NMR (300 MHz, $CDCl_3$) δ 2.76 (2H, t, J = 7.0 Hz, H_2-7), 3.49 (2H, dt, J = 7.0, 7.0 Hz, H_2-8), 3.82 (2H, s, H_2-7′), 3.85, 3.86, 3.99 (3 × 3H, 3s, 1-OCH_3, 1′-OCH_3, 8′-$NOCH_3$), 6.76 (1H, br t, J = 7.0 Hz, NH-9), 6.79 (1H, d, J = 8.4 Hz, H-6), 7.20 (1H, dd, J = 2.0, 8.4 Hz, H-5), 7.33 (2H, s, H-3′, H-5′), 7.47 (1H, d, J = 2.0 Hz, H-3), identical (by ^1H-NMR) to that previously reported.[12] 1,1′,8′(*S*)-Trimethoxyhemibastadin 3 (**6b**): ^1H NMR (300 MHz, $CDCl_3$) δ 2.76 (2H, t, J = 7.0 Hz, H_2-7), 3.51 (2H, dt, J = 7.0, 7.0 Hz, H_2-8), 3.81 (2H, s, H_2-7′), 3.84, 3.87, 3.99 (3 × 3H, 3s, 1-OCH_3, 1′-OCH_3, 8′-$NOCH_3$), 6.76 (1H, br t, J = 7.0 Hz, NH-9), 6.83 (1H, d, J = 8.4 Hz, H-6′), 7.08 (1H, dd, J = 2.2, 8.4 Hz, H-5′), 7.38 (1H, d, J = 2.2 Hz, H-3′), 7.42 (2H, s, H-3, H-5).

1,1′-Dimethoxy-8′(*S*)-hemibastadinol 1 (10). Freshly distilled ethereal diazomethane (1 mL) was added to a solution of hemibastadinol 1 (**9**) in methanol−ethyl ether (1:3, 2 mL). After being stirred for 30 min at 0−5 °C, the solution was warmed to room temperature in a gentle stream of argon to remove the excess diazomethane. Solvent was removed in vacuo to give ether **10** as a colorless solid (6.8 mg, 100%): $[\alpha]^{27}_{D}$ −23° (c = 0.66, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 208 (4.45), 281 (3.60) nm; IR (NaCl, film) ν_{max} 3387, 1651, 1497, 1279, 1256, 1057 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 2.60 (2H, m, H_2-7), 2.77 (1H, dd, J = 6.9, 14.0 Hz, H-7a′), 2.93 (1H, dd, J = 4.1, 14.0 Hz, H-7b′), 3.25 (1H, ddd, J = 7.4, 7.4, 15.0 Hz, H-8a), 3.41 (1H, ddd, J = 6.5, 7.9, 13.3 Hz, H-8b), 3.82 (3H, s, 1-OCH_3), 3.83 (3H, s, 1′-OCH_3), 4.15 (1H, dd, J = 4.1, 6.9 Hz, H-8′), 6.92 (2H, d, J = 8.4 Hz, H-6, H-6′), 7.04 (1H, dd, J = 2.1, 8.4 Hz, H-5), 7.15 (1H, dd, J = 2.1, 8.4 Hz, H-5′), 7.35 (1H, d, J = 2.1 Hz, H-3), 7.42 (1H, d, J = 2.1 Hz, H-3′); HRFABMS [M + H], 485.9897 ($C_{19}H_{22}{}^{79}Br_2NO_4$, calcd 485.9915).

1,1′-Dimethoxy-8′(*S*)-[α(*S*)-methoxyphenylacetyl]-hemibastadinol 1 (11). A mixture of 1,1′-dimethoxy-hemibastadinol 1 (**10**) (6.5 mg), 4-(dimethylamino)-pyridine (6 mg), 1,3-dicyclohexylcarbodiimide (8 mg), and α(*S*)-methoxyphenylacetic acid (7.5 mg) in dry CH_2Cl_2 (0.75 mL) was stirred under argon for 4 h. The solution was filtered and concentrated to dryness and the residue dissolved in CH_2Cl_2. The solution was chromatographed on a silica gel column (Pasteur pipette) and eluted with CH_2Cl_2 (5 mL) followed by 97:3 CH_2Cl_2−MeOH (5 mL). Ester **11** contaminated with 1,3-dicyclohexylurea was eluted by the latter solvent. Normal-phase HPLC (1:1 hexane/EtOAc) led to a pure specimen of ester **11** as a colorless solid (5.1 mg; 60%): $[\alpha]^{26}_{D}$ +13° (c = 0.51, CH_3OH); IR (NaCl, film) ν_{max} 3422, 2927, 2853, 1759, 1682, 1499, 1258 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 2.31 (1H, m, H-7a), 2.39 (1H, m, H-7b), 2.87 (1H, m, H-8a), 3.04 (1H, dd, J = 6.0, 14.5 Hz, H-7a′), 3.10 (1H, dd, J = 4.5, 14.5 Hz, H-7b′), 3.29 (1H, m, H-8b), 3.33 (3H, s, 2″-OCH_3), 3.87 (3H, s, 1′-OCH_3), 3.89 (3H, s, 1-OCH_3), 4.72 (1H, s, H-2″), 5.23 (1H, br t, J = 7.0 Hz, NH-9), 5.41 (1H, dd, J = 4.5, 6.0 Hz, H-8′), 6.76 (1H, dd, J = 2.0, 8.5 Hz, H-5), 6.78 (1H, d, J = 8.5 Hz, H-6′), 6.81 (1H, d, J = 8.5 Hz, H-6), 7.02 (1H, dd, J = 2.0, 8.5 Hz, H-5′), 7.10 (1H, d, J = 2.0 Hz, H-3), 7.30

(5H, m, 2″-Ph), 7.36 (1H, d, J = 2.0 Hz, H-3′); HRFABMS [M$^+$], 634.0440 ($C_{28}H_{30}{}^{79}Br_2NO_6$, calcd 634.0439).

1,1′-Dimethoxy-8′(*S*)-[α(*R*)-methoxyphenylacetyl]hemibastadinol 1 (12). A mixture of 1,1′-dimethoxyhemibastadinol 1 (**10**) (3.3 mg), DMAP (8 mg), DCCI (7 mg), and α(*R*)-methoxyphenylacetic acid (5.0 mg) in dry CH$_2$Cl$_2$ (0.75 mL) was allowed to react and the product isolated as outlined above (see the procedure for **11**). Ester **12** was obtained as a colorless solid (3.0 mg, 70%): $[\alpha]^{26}_{D}$ −5.0° (c = 0.3, CH$_3$OH); IR (NaCl, film) ν_{max} 3320, 2926, 2851, 1755, 1667, 1499, 1258 cm^{-1}; ^{1}H NMR (500 MHz, CD$_3$OD) δ 2.44 (1H, m, H-7a), 2.52 (1H, m, H-7b), 3.00 (2H, m, H-7a′, H-7b′), 3.12 (1H, m, H-8a), 3.34 (3H, s, 2″-OCH$_3$), 3.38 (1H, m, H-8b), 3.85 (3H, s, 1′-OCH$_3$), 3.89 (3H, s, 1-OCH$_3$), 4.72 (1H, s, H-2″), 5.36 (1H, dd, J = 5.5, 5.5 Hz, H-8′), 5.55 (1H, br t, J = 7.0 Hz, NH-9), 6.66 (1H, d, J = 8.5 Hz, H-6′), 6.82 (3H, m, H-5, H-6, H-5′), 7.22 (1H, d, J = 2.0 Hz, H-3), 7.26 (1H, d, J = 2.0 Hz, H-3′), 7.34 (5H, m, 2″-Ph); HRFABMS [M$^+$], 634.0436 ($C_{28}H_{30}{}^{79}Br_2NO_6$, calcd 634.0439).

1,1′-Dimethoxy-8′(*S*)-hemibastadinol 2 (15) and 1,1′-Dimethoxy-8′(*S*)-hemibastadinol 3 (16). A mixture of hemibastadinol 2 (**13**) and hemibastadinol 3 (**14**) weighing 7.1 mg was methylated with ethereal diazomethane (cf. the procedure for **10**) to give methyl ether derivatives **15** and **16** as a colorless solid (7.3 mg, 100%): IR (NaCl, film) ν_{max} 3393, 2930, 1651, 1537, 1497, 1472, 1258 cm^{-1}; HRFABMS [M + H], 563.9019 ($C_{19}H_{20}{}^{79}Br_3NO_4$, calcd 563.9020). 1,1′-Dimethoxy-8′-(*S*)-hemibastadinol 2 (**15**): ^{1}H NMR (300 MHz, CD$_3$OD) δ 2.59 (2H, m, H$_2$-7), 2.80 (1H, dd, J = 6.8, 14.0 Hz, H-7a′), 2.93 (1H, dd, J = 4.1, 14.0 Hz, H-7b′), 3.24 (1H, ddd, J = 6.9, 8.0, 14.6 Hz, H-8a), 3.42 (1H, ddd, J = 6.3, 8.3, 14.6 Hz, H-8b), 3.80 (3H, s, 1-OCH$_3$), 3.83 (3H, s, 1′-OCH$_3$), 4.17 (1H, dd, J = 4.1, 6.8, H-8′), 6.92 (1H, d, J = 8.3 Hz, H-6′), 7.05 (1H, dd, J = 2.0, 8.3 Hz, H-5′), 7.36 (1H, d, J = 2.0 Hz, H-3′), 7.45 (2H, s, H-3′, H-5′). 1,1′-Dimethoxy-8′(*S*)-hemibastadinol 3 (**16**): ^{1}H NMR (300 MHz, CD$_3$OD) δ 2.59 (2H, m, H$_2$-7), 2.80 (1H, dd, J = 6.8, 14.0 Hz, H-7a′), 2.93 (1H, dd, J = 4.1, 14.0 Hz, H-7b′), 3.24 (1H, ddd, J = 6.9, 8.0, 14.6 Hz, H-8a), 3.42 (1H, ddd, J = 6.3, 8.3, 14.6 Hz, H-8b), 3.82 (3H, s, 1′-OCH$_3$), 3.83 (3H, s, 1-OCH$_3$), 4.14 (1H, dd, J = 4.1, 6.8 Hz, H-8′), 6.92 (1H, d, J = 8.3 Hz, H-6′), 7.16 (1H, dd, J = 2.0, 8.3 Hz, H-5′), 7.40 (2H, s, H-3, H-5), 7.42 (1H, d, J = 2.0 Hz, H-3′).

1,1′-Dimethoxy-8′(*S*)-[α(*S*)-methoxyphenylacetyl]hemibastadinol 2 (17) and 1,1-Dimethoxy-8′(*S*)-[α-(*S*)-methoxyphenylacetyl]hemibastadinol 3 (18). A mixture of 1,1′-dimethoxyhemibastadinols 2 (**15**) and 3 (**16**) (total 4.7 mg), DMAP (8 mg), DCCI (8 mg), and α-(*S*)-MPA (6.0 mg) in dry methylene chloride (0.75 mL) was employed and product isolated as summarized for ester **11** to yield a mixture of esters **17** and **18** as a colorless solid (total 3.0 mg, 60%). Mixture: IR (NaCl, film) ν_{max} 3420, 2928, 2855, 1759, 1682, 1499, 1472, 1258 cm^{-1}; HRFABMS [M + H], 711.9560 ($C_{28}H_{29}{}^{79}Br_3NO_6$, calcd 711.9545). 1,1′-Dimethoxy-8′(*S*)-[α(*S*)-methoxyphenylacetyl]hemibastadinol 2 (**17**): ^{1}H NMR (500 MHz, CD$_3$OD) δ 2.28 (1H, m, H-7a), 2.42 (1H, m, H-7b), 2.85 (1H, m, H-8a), 3.06 (2H, m, H-7a′, H-7b′), 3.31 (1H, m, H-8b), 3.37 (3H, s, 2″-OCH$_3$), 3.86 (3H, s, 1′-OCH$_3$), 3.89 (3H, s, 1-OCH$_3$), 4.73 (1H, s, H-2″), 5.23 (1H, br t, J = 7.0 Hz, NH-9), 5.41 (1H, dd, J = 5.2, 5.2 Hz, H-8′), 6.79 (1H, dd, J = 1.8, 8.3 Hz, H-5), 6.82 (1H, d, J = 8.3 Hz, H-6), 7.14 (1H, d, J = 1.8 Hz, H-3), 7.31 (5H, m, 2″-Ph), 7.32 (2H, s, H-3′, H-5′). 1,1′-Dimethoxy-8′(*S*)-[α(*S*)-methoxyphenylacetyl]hemibastadinol 3 (**18**): ^{1}H NMR (500 MHz, CD$_3$OD) δ 6.79 (1H, d, J = 8.3 Hz, H-6′), 7.09 (2H, s, H-3, H-5), 7.20 (1H, dd, J = 2.1, 8.3 Hz, H-5′), 7.36 (1H, J = 2.1 Hz, H-3′).

1,1′-Dimethoxy-8′(*S*)-[α(*R*)-methoxyphenylacetyl]hemibastadinol 2 (19) and 1,1′-Dimethoxy-8′(*S*)-[α(*R*)-methoxyphenylacetyl]hemibastadinol 3 (20). A mixture of 1,1′-dimethoxyhemibastadinol 2 (**15**) and 3 (**16**) (total 3.6 mg), DMAP (6 mg), DCCI (8 mg), and α(*R*)-MPA (4.3 mg) in dry CH$_2$Cl$_2$ (0.75 mL) provided (refer to ester **12**) a mixture of esters **19** and **20** as a colorless solid (total 3.0 mg, 66%): IR (NaCl, film) ν_{max} 3412, 2928, 2855, 1755, 1667, 1499, 1472, 1258 cm^{-1}; HRFABMS [M + H], 711.9560 ($C_{28}H_{29}{}^{79}Br_3NO_6$, calcd 711.9545). 1,1′-Dimethoxy-8′(*S*)-[α(*R*)-methoxyphenylacetyl]hemibastadinol 2 (**19**): ^{1}H NMR (500 MHz, CD$_3$OD) δ 2.40 (1H, m, H-7a), 2.53 (1H, m, H-7b), 2.94 (1H, dd, J = 4.5, 14.5 Hz, H-7a′), 3.04 (1H, dd, J = 6.3, 14.5 Hz, H-7b′), 3.09 (1H, m, H-8a), 3.35 (3H, s, 2″-OCH$_3$), 3.40 (1H, m, H-8b), 3.84 (3H, s, 1′-OCH$_3$), 3.89 (3H, s, 1-OCH$_3$), 4.74 (1H, s, H-2″), 5.37 (1H, dd, J = 4.5, 6.3 Hz, H-8′), 5.54 (1H, br t, J = 7.0 Hz, NH-9), 6.83 (2H, m, H-5, H-6), 7.22 (2H, s, H-3′, H-5′), 7.22 (1H, d, J = 2.1 Hz, H-3), 7.34 (5H, m, 2″-Ph). 1,1′-Dimethoxy-8′(*S*)-[α(*R*)-methoxyphenylacetyl]hemibastadinol 3 (**20**): ^{1}H NMR (500 MHz, CD$_3$OD) δ 6.68 (1H, d, J = 8.3 Hz, H-6′), 6.81 (1H, dd, J = 2.1, 8.3 Hz, H-5′), 7.17 (2H, s, H-3, H-5), 7.28 (1H, d, J = 2.0 Hz, H-3′).

Antimicrobial Susceptibility Testing. Antimicrobial disk susceptibility tests were performed according to the methods established by the National Committee for Clinical Laboratory Standards.[26] Mueller-Hinton agar was used for susceptibility testing of *S. aureus* (ATCC #29213), *E. faecalis* (ATCC #29212), and *E. coli* (ATCC #25922), Gonococcal Typing agar was used for *N. gonorrhoeae* (ATCC #49226) and YM agar for *C. albicans* (ATCC #90028) and *C. neoformans* (ATCC #90112). Compounds were reconstituted in sterile DMSO, and 2-fold dilutions applied to sterile 6-mm disks. Zones of inhibition were recorded after 16 h for bacterial cultures, and 42 h for fungal cultures. Results are the average of two experiments.

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