Palau'amine and Its Congeners: A Family of Bioactive Bisguanidines from the Marine Sponge *Stylotella aurantium*[1]

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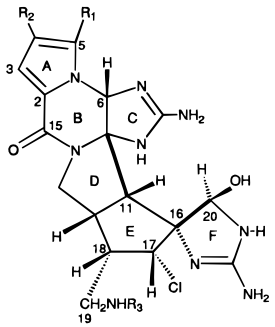
The strongly cytotoxic, antibiotic, and immunosuppressive palau'amine was isolated from the Belau sponge *Stylotella aurantium*, along with less active 4-bromo and 4,5-dibromo derivatives. Three corresponding isomeric compounds, all less active, were also isolated, and full details of the isolation and all structure proofs are provided.

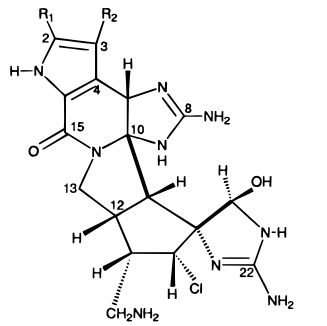
Introduction

The rapidly expanding literature of marine alkaloids contains few bisguanidines.[2] Recent examples include the batzelladines,[3] the phloeodictines,[4] the variolins,[5,6] and mauritiamine.[7] None of these approach in complexity the sponge-derived palau'amine (**1**), which was the subject of a preliminary paper.[1] Palau'amine is a $C_{17}N_9$ bisguanidine alkaloid constructed of six contiguous rings that encompass an unbroken chain of eight chiral centers. A partial synthesis of palau'amine has recently been completed.[8] Its antifungal, antitumor, and immunosuppressive activities are undergoing preclinical studies.[9] In addition to palau'amine and its 4-bromo (**2**) and 4,5-dibromo (**3**) derivatives, we isolated its three ring A regioisomers, which in the meantime have been reported under the name styloguanidine.[10] A full account of the isolation and structures of these six compounds is the subject of this paper.



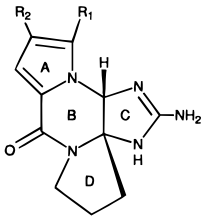
Palau'amines

1, $R_1 = R_2 = R_3 = H$
1a, $R_1 = R_2 = H$; $R_3 = Ac$
2, $R_1 = H$; $R_2 = Br$; $R_3 = H$
3, $R_1 = R_2 = Br$; $R_3 = H$



Styloguanidines (Isopalau'amines)

4, $R_1 = R_2 = H$
5, $R_1 = H$; $R_2 = Br$
6, $R_1 = R_2 = Br$



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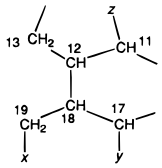

A

Results

Extraction of the freeze-dried sponge with aqueous methanol, followed by weak ion-exchange chromatography of the water-soluble portion of the extract, gave several fractions that were active against *Staphylococcus aureus*. Purification of the active fractions on Sephadex LH-20 and G-15, followed by HPLC, yielded three palau'amine and three styloguanidine[10] derivatives, with palau'amine as the major constituent.

Palau'amine (**1**) was isolated as an off-white amorphous solid of composition $C_{17}H_{23}ClN_9O_2$ based on the HRFABMS of the monoprotonated species. The characteristic chlorine isotope peaks and the ^{13}C NMR data corroborated the molecular formula. Analysis of the spectral data established the structure. Infrared absorption bands at 3600−2500, 1700, and 1660 cm^{-1} pointed to NH and OH functions, a guanidinium ion,[11] and an amide. The UV spectrum, with maxima at 224 and 272

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(1) A preliminary account of part of this work was presented at the Seventh International Symposium on Marine Natural Products, Capri, Italy, July 5−10, 1992; Abstract C7. When it was originally collected and a communication was published, the sponge was identified as *S. agminata*. (Kinnel, R. B.; Gehrken, H.-P.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 3376−3377). The correct species name is *S. aurantium* (Kelly-Borges, M.; Bergquist, P. *Indo-Malayan Zool.* **1988**, *5*, 121−159).

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(10) Kato, T.; Shizuri, Y.; Isumida, H.; Yokoyama, A.; Endo, M. *Tetrahedron Lett.* **1995**, *36*, 2133-2136.

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nm, was particularly informative as it was reminiscent of phakellin (**7**, $R_1 = R_2 = H$),[12] a metabolite of the axinellid sponge *Phakellia flabellata*, whose structure was confirmed by X-ray diffraction.[13] Full NMR data confirmed its distinctive guanidino pyrrolopyrazinone moiety. Comparison of the molecular formulas showed that palau'amine possessed the additional element of $C_6H_9ClN_4O$.

Examination of the NMR data revealed that the C_3H_6 chain of ring D in phakellin (**7**) was replaced by C_6H_8 of partial structure **A**. This hydrocarbon skeleton was secured by COSY, HMBC data, and decoupling experiments. The C13 methylene protons show HMBC correlations to both C10 and C15, which proves its linkage to the amide nitrogen (N14). H11 correlates to both C10 and C6, which indicates that terminus *z* is C10. The chemical shifts of C17 (73.2 ppm) and H17 (4.42 ppm) suggest that *y* is oxygen or chlorine. Oxygen may be excluded since the H17 proton signal remained unchanged in trifluoroacetic acid and since the C17 chemical shift remained a clean singlet in DMSO-d_6 with an added trace of D_2O.[14] Since H17 is a clean doublet, C16 (72.1 ppm) must be quaternary. Both H11 and H17 can be correlated by HMBC to C16.

Acetylation of **1** with acetic anhydride in pyridine, which was accomplished by sonicating the mixture because of the poor solubility of **1** in pyridine, furnished a complex mixture of acetylated products. No pure monoacetylated product was obtained, but mass spectral analyses of several of the fractions revealed that the mixture contained polyacetylated materials with up to five acetyl groups. This suggests that some decomposition had occurred and also confirms that a number of reactive groups are present. However, treatment of **1** with aqueous Ac_2O/NaOAc cleanly afforded a monoacetylated product **1a** in good yield. The ^1H NMR spectrum of **1a** in D_2O more clearly showed the two doublets of doublets for the two C19 protons, which were shifted downfield by about 0.2 ppm. In the ^{13}C spectrum the only significant changes were the downfield shift of C18 and the upfield shift of C19 by 2 ppm. These observations demonstrate that acetylation yielded an acetamide (*x* in A) at the C19 methylene. Interestingly, in DMSO-d_6 H20 in the acetamide no longer shows the coupling to NH21 that was observed for **1**. It is likely that the rate of exchange of the NH is accelerated by an intrinsic pH shift of the solution.

The presence of a hydroxyl group in **1** was inferred by loss of water from the molecular ion in the MS−MS spectrum. A proton signal at 9.55 ppm in the spectrum of **1** in DMSO-d_6 shows COSY cross-peaks with H20 and NH21 and an HMBC correlation to C16; thus, it must be derived from a hydroxyl attached to C20. The signal is broadened slightly and the proton is exchanging too rapidly for observation of any coupling to H20. The hydroxyl signal is affected strongly by the presence of traces of water and shifted to 9.28 ppm as the water peak grew during storage of the sample. In DMSO-d_6 containing a trace of D_2O the ^{13}C NMR signal for C20 is comprised of three peaks, which demonstrates its proximity to the two exchanging protons.[14]

The remaining structural elements were elucidated with the aid of HMBC data. Both H11, a clean doublet, and H17 showed correlations to the quaternary carbon at 72.1 ppm (C16); thus, C16 must be attached to C11 and C17. H11 also correlates to the methine at 83.7 ppm (C20) and to five other carbons. In D_2O, the H20 signal is a singlet, while it is a doublet in DMSO-d_6; hence, it must be vicinal to an exchangeable proton. The NH peak at 7.8 ppm is clearly coupled to H20 by COSY and decoupling experiments. Furthermore, H20 shows an HMBC to C16, C11, and C22, which, together with its ^{13}C chemical shift, confirms that C20 is a carbinolamine and is part of a ring containing the guanidine. These data establish **1** as the overall chemical structure for palau'amine.

Several features of the NMR spectra are noteworthy. The chemical shifts for H20 and C20 are comparable to those found for the carbinolamine (C4) in tetrodotoxin,[15] while the carbon shift for C16 is consistent with a quaternary carbon in a strained bicyclo[3.3.0] system, as in modhephene.[16] In DMSO-d_6, the broad peak at 8.18 ppm for two protons shows ROESY correlations to H17 and weakly to H12, which identifies the NH2 protons on C19. The carbon shifts of the carbonyl and guanidine carbons (C8, C15, and C22) were unambiguously assigned by using HMBC data. Curiously, C15 was at lower field in D_2O (158−160 ppm) in all six compounds, while in DMSO-d_6 it was shifted to near 155 ppm. The guanidine carbon shifts were relatively unaffected by change of solvent. The H12−H18 coupling constant could not be measured by inspection of the spectra, but simulation of the upfield portion of the spectrum of **1** establishes that $J_{12,18}$ is 11.0 Hz. Full NMR data are recorded in Table 1.

The relative stereochemistry can be deduced from interproton coupling and NOE's. The bicyclo[3.3.0]-azaoctane ring system (D and E) must be cis-fused, which is supported by the coupling constant (14.1 Hz) between H11 and H12. Although this seems large for a cis-fused bicyclo[3.3.0] system, comparable values are observed in similarly rigid, spiro-annulated five-membered rings.[17] The dihedral angle between these hydrogens is 0.1° according to molecular modeling.[18] In DMSO-d_6, H6 and H11 show a strong ROESY correlation, which places them on the same face of the molecule. This is confirmed by a weak correlation between H6 and 21NH, also observed in the difference NOE. In the ROESY spectrum, H17 is correlated with H12 and with H19a and H19b, but only weakly to H11. Molecular modeling suggests a H17−H12 distance of 4.0 Å, while H17−H11 is 4.2 Å and H6−21NH is 4.3 Å. Also correlated in the ROESY spectrum are H18 and H11; modeling provides a distance of 3.3 Å between them. These data confirm that H11, H12, H17, and H18 are all on the same face of the molecule.

Establishing the configuration at C20 was more challenging. The ROESY spectrum does not allow for un-

(12) Sharma, G. M.; Burkholder, P. R. *J. Chem. Soc., Chem. Commun.* **1971**, 151−152.
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(16) Zalkow, L. H.; Harris, R. N.; Van Derveer, D. *J. Chem. Soc., Chem. Commun.* **1978**, 420−421.
(17) For example, in leucodrin tetraacetate, the comparable hydrogens have a coupling constant of 12.4 Hz (Lowry, J. B.; Riggs, N. V. *Tetrahedron Lett.* **1964**, 2911−2914).
(18) Molecular modeling was carried out with the Discover (BIOSYM) program, using InsightII to construct the models; energy minimization was carried out using the CV force field.

Table 1. ^{1}H and ^{13}C[a] NMR Data for the Palau'amines in D_2O

	1		**2**		**3**		**4**	
carbon	^{13}C, ppm	^{1}H, ppm[b]	^{13}C, ppm	^{1}H, ppm	^{13}C, ppm	^{1}H, ppm	^{13}C, ppm	^{1}H, ppm
2	122.5		123.1		124.4		127.9	7.18, d(J = 2.8)
3	115.6	6.85, dd (J = 3.9, 1.5)	116.8	7.03, d (J = 1.7)	117.7	7.07, s	107.9	6.31, d (J = 2.8)
4	113.8	6.35, dd (J = 3.9, 2.8)	100.6		104.0		120.5	
5	125.2	6.99, dd (J = 2.8, 1.5)	124.5	7.21, d (J = 1.7)	109.2		126.2	
6	69.0	6.33, s	69.1	6.49, s	69.7	6.67, s	56.7	5.81, s
8	157.8		157.8		157.5		158.2	
10	80.8		80.6		80.7		82.7	
11	56.3	3.08, dd (J = 14.1)	56.4	3.23, d (J = 14.1)	56.9	3.27, d (J = 13.9)	56.9	3.13, d (J = 14.1)
12	41.8	2.52, dddd	41.7	2.66, m	41.7	2.70, m	42.13	2.64, m
13	46.1	3.96, dd (J = 10.4, 7.3)	46.1	4.11, dd (J = 10.3, 7.1)	46.0	4.18, dd (J = 10.3, 7.0)	45.7	4.07, dd (J = 10.2, 7.0)
		3.28, dd (J = 10.3, 10.4)		3.44, t (J = 10.3)		3.47, t (J = 10.3)		3.35, t (J = 10.2)
15	159.5		158.5		157.7		160.0	
16	72.1		72.0		72.2		72.2	
17	74.0	4.35, d (J = 7.9)	74.0	4.50, d (J = 7.7)	73.8	4.57, d (J = 7.6)	74.1	4.52, d (J = 7.7)
18	48.6	2.47, dddd	48.5	2.61, m	48.5	2.70, m	48.8	2.60, m
19	41.9	3.32, dd (J = 13.2, 7.0)	41.8	3.46, dd (J = 13.5, 7.1)	41.9	3.50, dd (J = 13.0, 6.9)	42.08	3.47, dd (J = 13.4, 6.7)
		3.24, dd (J = 13.2, 7.0)		3.41, dd (J = 13.5, 6.4)		3.45, dd (J = 13.0, 5.3)		3.41, dd (J = 13.4, 6.4)
20	83.7	5.96, s	83.7	6.11, s	83.8	6.17, s	84.0	6.14, s
22	157.9		157.7		157.9		157.9	

[a] External dioxane reference. [b] Reference was set at 4.63 ppm.

ambiguous assignment, as it demonstrates a correlation between both H17 and H20 and H11 and H20. Difference NOE experiments reveal a 4.9% enhancement for H20 when H11 is irradiated, and only 1.3% enhancement upon irradiation of H17. In **1**, the H11−H20 distance is 2.1 Å, while the distance between H17 and H20 is 3.6 Å as estimated by molecular modeling. Furthermore, H6 also shows a small NOE (0.4% enhancement) to H20, which confirms the configuration at C20 as shown.[19]

The absolute stereochemistry of **1** is not known. However, the CD spectrum displays the same characteristics as that of monobromophakellin hydrochloride,[14] which suggests that the two compounds have the same absolute configuration, as shown in **1**.

Since palau'amine was eluted from the weak ion-exchange column at pH 4.5, it is likely that all of the basic sites are protonated in aqueous solution. The guanidine unit in the phakellin part of the molecule is known to have a pK_a of approximately 8.5, while the primary amine is assuredly more basic. The basicity of second guanidine unit in ring F is not known, but the acetylation data suggests that the amine at C19 is the most basic site.

The structures of the brominated palau'amines (**2** and **3**) can be deduced by comparison of their spectral data with those of **1**. The upfield portions of their ^{1}H and ^{13}C NMR spectra are nearly identical. Different are the resonances for the pyrrole hydrogens and carbons. In 4-bromopalau'amine (**2**), the carbon resonance for C4 is shifted upfield, while in the proton spectrum the resonance at 6.35 ppm in **1** is absent. The coupling constant for the pyrrole hydrogens (1.7 Hz) is characteristic for the H2,H4 protons of pyrrole, while carbon and proton shifts and the HMBC clearly demonstrate that the bromine is on C4. In 4,5-dibromopalau'amine (**3**), only a single pyrrole hydrogen resonance remains at 7.07 ppm (H3); C3 can be identified by its ^{13}C shift, and the hydrogen shows an HMBC to C15, as well as to all the other pyrrole carbons. Thus, the two bromines must be at C4 and C5.

The structures of the bromostyloguanidines were deduced similarly, once the structure of styloguanidine (**4**) was established. Its molecular formula is also $C_{17}H_{23}ClN_9O_2$ by HRFABMS. All of the coupling patterns and chemical shifts for styloguanidine (**4**) were nearly identical to those for palau'amine (**1**), except that the shifts for H6 and C6 were shifted upfield by 0.7 and 10 ppm, respectively, and the pyrrole proton and carbon shifts differed to reflect the regioisomerism in ring A (see Table 1). In **4**, the shifts (7.18, 6.31 ppm) and coupling constant (2.8 Hz) for the two pyrrole hydrogens allow only for 2,3 hydrogen substitution. The ^{1}H NMR spectrum of dibromoisophakellin exhibits the same differences from dibromophakellin.[20] Thus, the structure of **4** is as shown, and the structures for the two bromo derivatives follow.

It is unfortunate that Kato et al.[10] did not record rotations for the styloguanidines. It is conceivable that their styloguanidines may be antipodal to ours, as dibromophakellin has been isolated in two enantiomeric forms, one from *Phakellia flabellata*[12,13] and one from *Pseudoaxinyssa cantharella*.[21]

Palau'amine displayed a remarkable range of biological activities. Its acute toxicity is low (LD_{50} 13 mg/kg ip in mice), and it is more powerfully antibiotic than any of its congeners. Aqueous solutions upon prolonged storage were resistant to fungal growth, and **1** gave a 24 mm zone of inhibition at 50 μg per disk against *Penicillium notatum*. More significant are its immunosuppressive and antitumor activities. In the mixed lymphocyte reaction, **1** showed an IC_{50} < 18 ng/mL, and the cytotoxicity assay against murine lymphocytes showed an activity of 1.5 μg/mL. Against P-388 and A-549 cell lines **1** demonstrated IC_{50}'s of 0.1 and 0.2 μg/mL, respectively. Compounds **2**−**6** showed no comparable activity, although **3** was selective against a human melanoma cell line with an IC_{50} 0.25 μg/mL.

Although the biogenesis of palau'amine is obscure, it appears to be derived from 1 equiv of pyrrole-2-carboxylic acid and 2 equiv of 3-amino-1-(2-aminoimidazolyl)prop-1-ene (AAPE), both present in sponges.[22] One can envision an 11,12-dehydrophakellin, itself derived from pyrrole-2-carboxylic acid and AAPE, plus a second equiva-

(19) This is a correction of that suggested in our earlier communication.[1]

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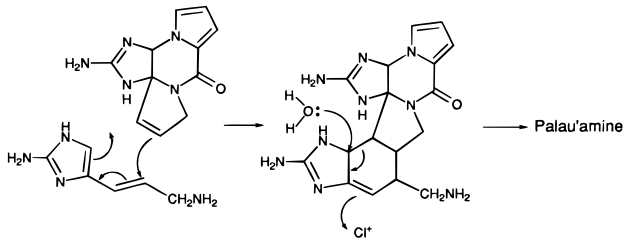
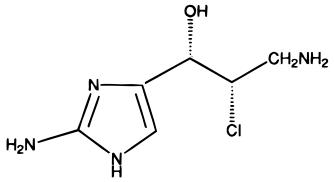
(21) De Nanteuil, G.; Ahond, A.; Guilhem, J.; Poupat, C.; Tran Huu Dau, E.; Potier, P.; Pusset, M.; Pusset, J.; Laboute, P. *Tetrahedron* **1985**, *41*, 6019−6033.

Scheme 1



lent of AAPE undergoing a Diels−Alder reaction, followed by a chloroperoxidase-initiated chlorination, bond migration, and reaction with water, as shown in Scheme 1.[23] AAPE likely undergoes a similar reaction itself to produce the chlorohydrin girolline (**8**), isolated from the axinellid sponge *Pseudoaxinyssa cantharella*,[24] and confirmed by synthesis.[25]



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Our observation that palau'amine has greater activity than the bromo derivatives raises a question in regard to Sharma's phakellin research.[13] In that earlier work, the unbrominated phakellin was never isolated from the sponge. It may well be present in *P. flabellata*, which would explain the as yet unidentified powerful antibiotic activity they report. This activity may also derive from metabolites similar to those reported in very low concentrations in other *Phakellia* species,[26] although the active dibromophakellistatin[27] may be an artifact derived from hydrolysis of dibromophakellin.

Numerous other compounds reported from a wide variety of sponges belonging to the class Demospongia were also identified in our study of this sponge. They include sceptrin,[28] oroidin,[29] dibromophakellin,[12,13] hymenin,[30] hymenidin,[18] hymenialdisine,[25,31] and debromohymenialdisine,[32] also known as "the yellow compound." Members of this class of sponges have wide distribution in tropical and subtropical waters. Several orders are represented, and there seems to be relatively little taxonomic connection among them, even though the chemistry might suggest otherwise.

We have also found that a sample of *S. aurantium* from Guam contains a majority of the compounds that we isolated from the Belau sponge, although the yield of **1** appears to be smaller, and there seems to be greater antibiotic activity in the less polar fractions of the ion-exchange chromatography. Work is continuing on a highly cytotoxic principle present in fractions of medium polarity.

In view of the rich alkaloid chemistry of *S. aurantium*, it is astonishing that in a recent investigation of *S. aurantium* by Pettit et al.[33] only a cyclic peptide was reported from 500 kg of the animal.

Experimental Section

General Aspects. Ion-exchange material was obtained from Bio-Rad (2000 Alfred Nobel Dr., Hercules, CA) and prepared according to the manufacturer's instructions. ^{1}H and ^{13}C NMR spectra were recorded in D_2O unless otherwise specified. Coupling constants (*J*) are reported in Hz. Carbon multiplicities were determined by HMQC, and HMBC spectra were optimized for 7 Hz coupling. Optical rotations and circular dichroic spectra were taken in methanol at 25 °C. FABMS and HRFABMS were obtained by using magic bullet matrix[34] or 3-nitrobenzyl alcohol (3-NBA). HPLC was performed on a YMC A323-AQ column with a variable-wavelength detector set at 272 nm using the solvent specified. Methanol was employed for chromatography over Sephadex LH-20 (LH-20). All solvents were distilled in glass prior to use.

Collection and Isolation. The sponge was first collected in 1977 by Dr. Mark Yunker from this group, then again in November 1991 at −5 to −50 m near Wonder Channel and Rock Islands, Republic of Belau, by personnel from the University of Guam Marine Laboratory. The large orange *S. aurantium* is easy to identify since it is free of colonization by algae and other symbionts.

Freeze-dried sponge (618 g) was broken into small pieces[35] and steeped in 4 L of methanol/water (80:20) at 4 °C for 2−3 days; the extract was decanted through a loose glass wool filter. The sponge was extracted twice more, and the combined extracts were evaporated under reduced pressure to afford 2.4 L of aqueous extract, which consisted of a dark orange supernatant solution, active against *S. aureus*, with suspended yellow solids. This bioassay was used throughout the isolation.

A portion (300 mL) of the solution was filtered through Celite and passed through a column of Cellex CM (85 g, 32 × 4 cm) prepared in 0.05 M NaCl, pH 4.4. The column was washed successively with 1.2 L portions of 0.05, 0.1, 0.3, 0.5, and 1.0 M NaCl solution. Only the last two eluates were active in the bioassay. The 0.5 M NaCl eluate was lyophilized, and the residue was extracted with three 50-mL portions of 95% EtOH. Evaporation of the filtered solution yielded 288 mg of crude palau'amines, contaminated primarily with NaCl and sceptrin.

(22) The carboxamide of pyrrole-2-carboxylic acid was reported in: König, G. M.; Wright, A. M. *Nat. Prod. Lett.* **1994**, *5*, 141−146. AAPE has been reported in two different sponges: Wright, A. E.; Chiles, S. A.; Cross, S. S. *J. Nat. Prod.* **1991**, *54*, 1684−1686.

(23) We are grateful to a reviewer for suggesting this better alternative to our original proposed biogenetic scheme.

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(35) The dust from the dried sponge caused an allergic reaction consisting of severe shortness of breath for about 4 h, which disappeared within 1 day. In addition, some workers also experienced skin rashes from contact with the sponge.

The crude material was mostly dissolved in about 2 mL of MeOH (some salt remains), and the solution was chromatographed over Sephadex LH-20. The first eluate, 42 mg, appeared to be mostly palau'amine by NMR; it was about 70% pure as judged by HPLC. Styloguanidine and the bromopalau'amines were the principal contaminants. This was followed by two fractions, one (168 mg) consisting of a mixture of palau'amines and sceptrin, and a third (22 mg) that was nearly pure sceptrin. Palau'amine was further purified by chromatography over Sephadex G-15. With 0.2 M NaCl (pH 4.5−5.2) as the eluent, palau'amine emerged first, followed by styloguanidine and then the monobromo and finally the dibromo palau'amine/styloguanidine derivatives. Lyophilization, desalting twice with ethanol, and finally another LH-20 column afforded 20 mg (0.2%) of pure palau'amine, which is stable indefinitely in weakly acidic water and in DMSO at 4 °C. In some preparations, the 0.3 M NaCl eluate from the Cellex column also contained palau'amines, and it could be processed in the same manner.

Palau'amine (1): off-white amorphous solid upon lyophilization, dec upon heating; $[\alpha]_D$ −45.2° (c =3.0); IR (KBr) 3600−2500 (br, max 3340, sh 3150), 1700, 1660, 1560, 1430, 1385, 1100 (br) cm^{-1}; UV (MeOH) 224 (7800), 272 nm (7900); CD ([θ] in parentheses) 267 (+9600), 228 (sh, −14 800), 208 nm (−30 000); HRFABMS m/z 420.1669 (MH^+, Δ 0.6 mDa), M + 2, +38; MS/MS (420) m/z 420, 402, 361, 337, 278, 229, 94, 60; ^{1}H NMR (see Table 1); ^{1}H NMR (DMSO-d_6) δ 10.05 (br s, NH), 9.74 (br s, NH), 9.55 (s, OH on C20), 9.07 (br s, NH), 8.32 (br, 2 NH), 8.15 (br, 2 NH), 7.82 (d, J = 5.2, 21NH), 6.73 (dd, J = 3.8, 1.6, H3), 6.34 (dd, J = 2.7, 3.8, H4), 7.16 (dd, J = 2.7, 1.6, H5), 6.20 (s, H6), 3.05 (d, J = 14.4, H11), 2.53 (dddd, H12), 4.04 (dd, J = 10.1, 7.4, H13a), 3.13 (apparent t, J = 10.3, 2H, H13b, H19a), 4.41 (d, J = 9.0, H17), 2.24 (dddd, H18), 2.93 (dd, J = 12.8, 9.0, H19b), 5.75 (d, J = 5.2, H20); ^{13}C NMR (DMSO-d_6) δ 122.5 (C2), 111.7 (C3), 112.3 (C4), 122.7 (C5), 67.1 (C6), 157.8 (C8), 79.6 (C10), 55.6 (C11), 40.8 (C12), 44.8 (C13), 155.7 (C15), 70.1 (C16), 73.2 (C17), 46.2 (C18), 39.8 (C19), 81.8 (C20), 157.2 (C22).

Isolation of the Bromopalau'amines and the Styloguanidines. Dibromo Compounds. The 1.0 M NaCl Cellex column eluate from the extract corresponding to 100 g of dry weight of sponge was lyophilized, and the residual salt was extracted with ethanol to give 171 mg of a mixture that included traces of NaCl. Chromatography over Sephadex LH-20 (MeOH) afforded a single peak that was split according to the bioassay against *S. aureus*. The early part of the peak, which was very active, contained 65 mg of nearly pure **1**, while a center cut consisted of 20 mg of a mixture of **3** and **6**. Purification by HPLC (H_2O/MeCN 80:20, 0.2% TFA) gave 3.2 mg (0.0032%) of **6** and 4.2 mg (0.0042%) of **3**.

4-Bromopalau'amine (2). The water-soluble portion of the extract from 600 g of sponge was added to 100 g of Sephadex-CM (swollen in 1.0 M NaCl and stored in 0.05 M NaCl) and then allowed to stand for 1 h. The Sephadex was removed by filtration and then soaked successively in 400 mL each of 0.05, 0.1, 0.5, 1.0, 2.0, and 3.0 M NaCl solution. The last four salt solutions were separately lyophilized, and the residues were extracted with EtOH. The EtOH solubles were chromatographed separately over Sephadex LH-20, and samples with similar NMR spectra were combined, affording 903 mg of a mixture of palau'amines. Two sequential chromatographies on Sephadex LH-20 afforded 107 mg of a mixture of palau'amine and its bromo derivatives. Purification by HPLC (H_2O/MeCN 85:15, 0.1% TFA) gave 3.5 mg (0.0009%) of **2**.

Styloguanidine (4). A 141 mg sample of a mixture of **1** and **4** plus some sceptrin, isolated from the 0.5 M NaCl wash of the Cellex column, was chromatographed over Sephadex LH-20. The first part of the peak that was eluted contained mostly **1**; a middle cut (37 mg) contained both **1** and **4** by NMR, and this was followed by a fraction that was mostly sceptrin. Separation of the middle cut by HPLC (H_2O/MeCN, 85:15, 0.1% TFA) gave 12.6 mg (0.001%) of **4** along with 16.8 mg of **1**.

3-Bromostyloguanidine (5). The 0.5 M eluate of the Cellex column from 618 g of lyophilized sponge (367 mg after desalting with EtOH) was subjected to Sephadex LH-20 chromatography. After elution of 41 mg of almost pure **1**, 168 mg of a mixture of **1**, **4**, sceptrin, and minor bromopalau'amines, estimated by ^{1}H NMR, was eluted. This fraction was rechromatographed on Sephadex LH-20 to give a peak that was divided into three parts. The middle fraction, 44 mg, contained a mixture of **1** and **4** (about 60:40), along with minor materials. Separation by HPLC (H_2O/MeCN 90:10, 0.1% TFA) gave 17 mg of **4**, 24 mg of **1**, and 3.2 mg (0.0005%) of **5**.

4-Bromopalau'amine (2): tan amorphous solid; $[\alpha]_D$ −64.4° (c = 2.6); HRFABMS m/z 498.0769 (MH^+ Δ 0.1 mDa), M 81, M + 2 100, M + 4 27; MS/MS (498) m/z 498, 480, 415, 356, 308, 172, 60; ^{1}H, ^{13}C NMR, see Table 1.

4,5-Dibrompalau'amine (3): white solid upon lyophilization, dec when heated; $[\alpha]_D$ −115.3° (c = 2.7); HRFABMS m/z 575.9877 (MH^+, Δ 0.7 mDa) M 48, M + 2 100, M + 4 74, M + 6, 21; MS/MS (576) m/z 576, 558, 493, 434, 60; ^{1}H, ^{13}C NMR, see Table 1.

Styloguanidine (4): white amorphous, hygroscopic solid upon lyophilization; $[\alpha]_D$ +20.7° (c = 3.5); HRFABMS m/z 420.1666 (MH^+, Δ 0.3 mDa) M + 2 37; ^{1}H, ^{13}C NMR, see Table 1.

3-Bromostyloguanidine (5): tan amorphous solid upon lyophilization; $[\alpha]_D$ +57.5° (c = 0.7); ^{1}H NMR δ 7.27 (s, H2), 6.15 (s, H20), 5.87 (s, H6), 4.51 (d, J = 7.7, H17), 4.07 (dd, J = 7.0, 10.7, H13a), 3.46 (dd, J = 7.0, 13.7, H19a), 3.42 (dd, 6.4, 13.7, H19b), 3.36 (apparent t, J = 10.0, H13b), 3.16 (d, J = 14.4, H11), 2.61 (m, H12, H18); ^{13}C NMR δ 159.2 (C15), 158.0 (C8), 157.9 (C22), 127.3 (C2), 124.1 (C5), 121.2 (C4), 95.1 (C3), 84.0 (C20), 82.8 (C10), 74.1 (C17), 72.3 (C16), 56.9 (C11), 56.0 (C6), 48.8 (C18), 45.7 (C13), 42.0 (C12, C19).

2,3-Dibromostyloguanidine (6): off-white microcrystalline solid from 2-PrOH/MeOH, dec before melting; $[\alpha]_D$ −70.8° (c = 0.6); HRFABMS m/z 575.9883 (MH^+, Δ 1.7 mDa) M 49, M + 2 100, M + 4 67, M + 6 19; MS/MS (576) m/z 558, 434, 346, 318, 146, 60; ^{1}H NMR δ 6.13 (s, H20), 5.84 (s, H6), 4.48 (d, J = 7.7, H17), 4.05 (dd, J = 7.2, 10.4, H13a), 3.45 (dd, J = 6.7, 13.6, H19a), 3.39 (dd, J = 6.4, 13.6, H19b), 3.34 (apparent t, J = 9.9, H13b), 3.14 (d, J = 14.4, H11), 2.58 (m, H12, H18); ^{13}C NMR δ 158.2 (C15), 157.8 (C22), 157.7 (C8), 124.8 (C5), 122.0 (C4), 111.0 (C2), 97.9 (C3), 83.8 (C20), 82.5 (C10), 74.0 (C17), 72.1 (C16), 56.8 (C11), 55.7 (C6), 48.6 (C18) 45.6 (C13), 41.9 (C12), 41.7 (C19).

Acetylation of 1. To a solution of 15.3 mg (0.034 mmol as the monhydrochloride) of **1** dissolved in 2.5 mL of H_2O was added 0.1 mL of Ac_2O followed by 30 mg of NaOAc·3H_2O. The solution was stirred for 30 min, and then an additional 50 μL of Ac_2O was added. Stirring was continued for an additional 1 h, and then the solution was lyophilized. The residue was dissolved in 2 mL of H_2O, and 1.1 mL of 0.25 M HCl was added. The solution was lyophilized again. The residue (30.5 mg) was triturated with 1 mL of 95% EtOH, and the solution was filtered through a cotton plug. Evaporation of the EtOH afforded 15.4 mg of crude acetate. Addition of H_2O followed by lyophilization to remove residual EtOH gave 12.6 mg (82%) of the **1a** as an amorphous solid: HRFABMS m/z 462.1765 (MH^+, Δ 0.4 mDa) M + 2 40; IR (KBr) 3600−2400 (br), 1700, 1649, 1639, 1556, 1429, 1050, 744 cm^{-1}; ^{1}H NMR δ 7.15 (dd, J = 2.8, 1.4, H5), 7.00 (dd, J = 3.9, 1.4, H3), 6.51 (dd, J = 3.9, 2.8, H4), 6.46 (s, H6), 6.10 (s, H20), 4.36 (d, J = 8.8, H17), 3.98 (dd, J = 10.2, 7.4, H13a), 3.61 (dd, J = 14.1, 4.9, H19a), 3.48 (dd, J = 14.1, 7.8, H19b), 3.37 (dd, J = 10.2, 10.6, H13b), 3.10 (d, J = 14.8, H11), 2.53 (m, H12), 2.39 (m, H18), 2.09 (s, CH_3CO); ^{1}H NMR (DMSO-d_6) δ 10.18 (br s, NH), 9.79 (t, NH), 9.20 (m, NH), 9.12 (d, NH), 8.30 (d, 2 NH), 8.21 (t, NH of acetamide), 7.81 (br, NH), 7.27 (t, H5), 6.72 (dd, H3), 6.31 (dd, H4), 6.12 (s, H6), 5.65 (s, H20), 4.29 (d, J = 10.2, H17), 3.86 (dd, J = 9.8, 6.8, H13a), 3.44 (dt, J = 13.5, 3.6, 3.5, H19a), 3.12 (d, J = 14.6, H11), 3.06 (ddd, J = 13.5, 7.2, 2.1, H19b), 3.02 (t, J = 10.3, H13b), 2.75 (m, H12), 1.98 (m, H18), 1.90 (s, C19 acetamide CH_3); ^{13}C NMR δ 175.7 (s, C19 amide CO), 159.6 (s, C15), 158.0 (s, C22), 157.7 (s, C8), 125.1 (d, C5), 122.6 (s, C2), 115.5 (d, C3), 113.8 (d, C4), 83.7 (d, C20), 80.8 (s, C10), 73.6 (d, C17), 72.0 (s, C16), 68.9 (d, C6), 56.3 (d, C11), 50.5 (s,

C18), 46.5 (t, C13), 41.8 (d, C12), 40.7 (t, C19), 23.1 (s, C19 amide CH_3).

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Supporting Information Available: ^{13}C NMR and ^{1}H NMR spectra of **1**, **1a**, and **2**−**6** in D_2O; ^{13}C NMR and ^{1}H NMR spectra of **1a**, ROESY, COSY, ^{1}H NMR and HMBC spectra of **1** in DMSO-d_6; HMQC of **1** and HMBC spectra of **1**, **1a**, **2**−**4**, and **6** in D_2O (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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