Investigation of Brominated Tryptophan Alkaloids from Two Thorectidae Sponges: *Thorectandra* and *Smenospongia*

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Chemical investigation of an NCI-DTP collection of *Thorectandra* sp. and a UCSC collection of *Smenospongia* sp. yielded six new brominated tryptophan derivatives: 6-bromo-1′-hydroxy-1′,8-dihydroaplysinopsin (**4**), 6-bromo-1′-methoxy-1′,8-dihydroaplysinopsin (**5**), 6-bromo-1′-ethoxy-1′,8-dihydroaplysinopsin (**6**), (−)-5-bromo-*N*,*N*-dimethyltryptophan (**7**), (+)-5-bromohypaphorine (**8**), and 6-bromo-1*H*-indole-3-carboxylic acid methyl ester (**11**). Additionally, the known compounds aplysinopsin (**1**), 1′,8-dihydroaplysinopsin (**2**), 6-bromo-1′,8-dihydroaplysinopsin (**3**), (1*H*-indole-3-yl)acetic acid (**9**), and (6-bromo-1*H*-indol-3-yl)acetic acid methyl ester (**10**) were also encountered. The structures of **4**−**8** and **11** were confirmed on the basis of analysis of ^{1}H and ^{13}C (1D and 2D) NMR data as well as comparison to known compounds. Compounds **1**, **3**−**8**, **10**, and **11** were found to inhibit the growth of *Staphylococcus epidermidis* with either weak or moderate MICs.

Sponges of the family Thorectidae (order Dictyoceratida) are consistently encountered on many Indo-Pacific reefs. Likewise, its members are of continued interest to us because they have provided a wealth of bioactive compounds: the latrunculins[1] and fijianolides[2] from *Cacospongia mycofijiensis*, the puupehenones[3] from *Hyrtios* sp., and the fascaplysins[4,5] and reticulatines[4,5] from *Fascaplysinopsis reticulata*. During a recent investigation of fascaplysin-containing sponges,[5] we identified the organic extract of an NCI-DTP collection of *Thorectandra* sp. that contained analogues of aplysinopsin (**1**)[6] with molecular weights that differed versus all known derivatives. Parallel to this, a UCSC collection of *Smenospongia* sp. was also found to contain aplysinopsin-related compounds along with numerous other indoles. A project was begun to isolate new and known aplysinopsins along with other tryptophan derivatives using mass spectrometry to guide the isolation. The next goal was to screen all compounds for cytotoxicity and antimicrobial activity. Reported here are the results of a comprehensive investigation of these two sponges. The chemical and biological activity properties are described for six new compounds, **4**−**8** and **11**, in addition to the known compounds **1**−**3**, **9**, and **10**.

Results and Discussion

The constituents of *Thorectandra* sp. (NCI coll. no. 0CDN5714) were pursued first because the crude extracts exhibited numerous unknown molecular ion peaks. A Kupchan-like extraction method was performed on the organic extract to yield four separate extracts (Figure S17). The aqueous MeOH extract labeled "FDFM" and the *sec*-butanol extract labeled "WB" were purified further using a series of preparative and semipreparative HPLC fractionations. This yielded 1′,8-dihydroaplysinopsin (**2**),[7] 6-bromo-1′,8-dihydroaplysinopsin (**3**),[7] and (1*H*-indole-3-yl)acetic acid (**9**)[8] along with four new compounds, 6-bromo-1′-hydroxy-1′,8-dihydroaplysinopsin (**4**), 6-bromo-1′-methoxy-1′,8-dihydroaplysinopsin (**5**), (−)-5-bromo-*N*,*N*-dimethyltryptophan (**7**), and (+)-5-bromohypaphorine (**8**). Similar chromatographic treatment of the dichloromethane extract labeled "FD" from the *Smenospongia* sp. specimen (UCSC coll. no. 91111) provided known compounds aplysinopsin (**1**) and (6-bromo-1*H*-indol-3-yl)acetic acid methyl ester (**10**)[9] as well as two new compounds, 6-bromo-1′-ethoxy-1′,8-dihydroaplysinopsin (**6**) and 6-bromo-1*H*-indole-3-carboxylic acid methyl ester (**11**).

Structure elucidation of the new aplysinopsins began with establishing their molecular formulas. Dereplication of the known compound aplysinopsin (**1**) provided important mass spectral and NMR data for characterizing the rest of the compounds isolated. Additionally, ^{1}H and ^{13}C NMR data of known compounds 1′,8-dihydroaplysinopsin (**2**)[7] and 6-bromo-1′,8-dihydroaplysinopsin (**3**),[7] included in Table 1, served as a benchmark to categorize structural variations within the new analogues. Characterization of the first new compound obtained, 6-bromo-1′-hydroxy-1′,8-dihydroaplysinopsin (**4**), began with establishing a molecular formula, $C_{14}H_{15}BrN_4O_2$ (351.0448 $[MH]^+$, calcd for $C_{14}H_{16}BrN_4O_2$, 351.0451), which differed from that of **3** by the addition of an oxygen atom. Side-by-side comparison of the ^{1}H and ^{13}C NMR data of **3** with **4** revealed the H-1′ proton in **3** was absent in **4** and the C-1′ signal was shifted downfield in **4** (δ 89.0) as compared with **3** (δ 64.0). This confirmed that C-1′ in **4** was quaternary with an attached hydroxyl group.

The two other aplysinopsins proved to be derivatives of **4** and consisted of 6-bromo-1′-methoxy-1′,8-dihydroaplysinopsin (**5**) and 6-bromo-1′-ethoxy-1′,8-dihydroaplysinopsin (**6**). The molecular formulas, $C_{15}H_{17}BrN_4O_2$ and $C_{16}H_{19}BrN_4O_2$, were established for **5** and **6** via HRESIMS (*m/z* 365.0641 $[MH]^+$ and 379.0773 $[MH]^+$, respectively). Analysis of their ^{1}H and ^{13}C NMR data verified that **5** and **6** corresponded to the methoxy and ethoxy analogues of **4**, respectively.

After the structural work on **4**−**6** was completed the possibility of these compounds being artifacts of isolation was considered, especially since **4** and **5** gave miniscule $[\alpha]_D$ values ($\leq +3°$). Although it is likely that **5** and **6** are artifacts formed from **4** since large amounts of methanol and ethanol are used during extraction of the sponges, it is unlikely that **4** is an artifact for the following specific reasons. Only the hydroxylated derivative of 6-bromoaplysinopsin was observed by LCMS even though aplysinopsin

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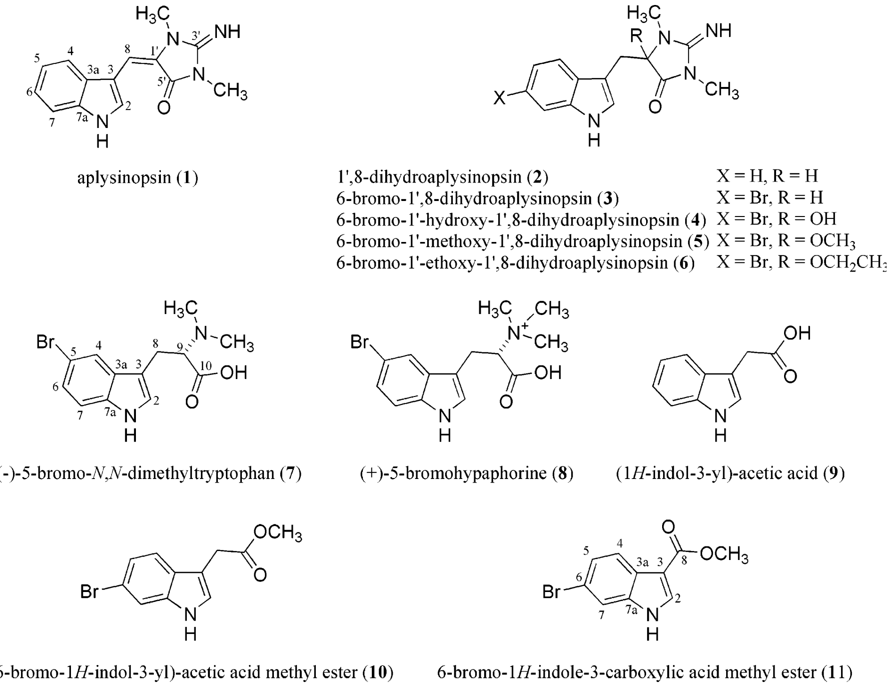
Chart 1



was found to be the major metabolite present. If **4** was formed through a nonspecific reaction, then 1′-hydroxy-1′,8-dihydroaplysinopsin would also be expected to be present; however, this is not the case.

Attention next turned to the two *N*-methyl tryptophan analogues (−)-5-bromo-*N*,*N*-dimethyltryptophan (**7**) and (+)-5-bromohypaphorine (**8**). The identification of **7** began with determination of the molecular formula, $C_{13}H_{15}BrN_2O_2$, based on a HRESIMS *m/z* 311.0369 [MH]$^+$. The ^{1}H and ^{13}C NMR data (1D and 2D), shown in Table 2, revealed four substructures, consisting of a disubstituted indole, a two-carbon saturated side chain off C-3, a dimethylated nitrogen, and a heterosubstituted carbonyl carbon. These moieties were combined to give **7** with a carboxylic acid and not an amide terminus (both *N*-Me's isochronous). The location of the Br was assigned to C-5 on the basis of comparison of the NMR shifts, especially at CH-4 (δ 120.3/7.77 dd, J = 1.8, 0.4), C-5 (δ 112.0), and CH-7 (δ 112.8/7.28 dd, J = 8.6, 0.5), to those of **3**−**6** as well as those of others.[10]

During the course of comparing the levorotatory value of **7** ($[\alpha]_D^{20}$ = −19.3°, MeOH) to several 9*S* and 9*R* tryptophan analogues, a trend was identified between the sign of the rotation and the solvent used. These data are compiled in Table 3 and contain the rotation values for a wide variety of 9*S* tryptophan analogues[11] including *N*-methyl, *N*,*N*,*N*-trimethyl, 5-bromo, 5-bromo-*N*-methyl, 5,6-dibromo-*N*- methyl, and 6-bromo- *N*,*N*,*N*-trimethyl; however, no rotation value has been reported to date for the *N*,*N*-dimethyl analogue or for abrine in organic solvents. Although, the rotations of tryptophans and derivatives are quite variable, the diagnostic patterns of the rotation data for tryptophan congenors (nonesters) are as follows: (a) 9*S* neutral compounds are levorotatory in organic solvents, (b) 9*S* compounds are dextrorotatory in acidic or basic solutions, (c) 9*S* compounds possessing a quaternary nitrogen are dextrorotatory in organic solvents or water. From the above trends (−)-**7** was identified as having the 9*S* configuration.

With the structure of **7** established, a parallel approach was used to characterize **8**. The molecular formula $C_{14}H_{18}BrN_4O_2$ of this cation was provided by the HRESIMS *m/z* of 325.0534 [M]$^+$. This formula differed from **7** by an additional methyl group, and a trimethylated nitrogen cation was envisioned. Comparison of the indole portion of **8** with **7** revealed the same bromination pattern. In view of the above optical rotation analysis the dextrorotatory behavior of **8** ($[\alpha]_D$ = +46.3°, MeOH) was consistent with a 9*S* configuration. Additional support for this conclusion is derived from the comparison to closely related compounds headed by enantiomeric sponge-derived tryptophan alkaloids: 9*S*-6-bromohypaphorine ($[\alpha]_D$ = +58°, MeOH−TFA),[12] 9*R*-6-bromohypaphorine ($[\alpha]_D$ = −27°, MeOH−TFA),[13] and the series including 9*S*-tryptophan ($[\alpha]_D$ = −31.5°, MeOH), synthetic 9*S*-hypaphorine ($[\alpha]_D$ = +117.5°, H_2O),[13d] and synthetic 9*R*-hypaphorine ($[\alpha]_D$ = −87°, MeOH).[15] Elucidation of the last compound, 6-bromo-1*H*-indole-3-carboxylic acid methyl ester (**11**), began with establishing a molecular formula, $C_{10}H_8BrNO_2$, via HRESIMS (*m/z* 253.9808 [M + H]$^+$). Comparison of the ^{1}H and ^{13}C NMR data of **11** to that of the known sponge-derived compound 6-bromo-1*H*-indole-3-carboxylic acid ethyl ester[14] confirmed its structure.

Aplysinopsins possess a variety of biological activities including cytotoxic,[15] antiviral,[16] and antidepressant.[17] In addition, members have shown to be inhibitors of monoamine oxidase[20] as well as serotonin 5-HT2 receptor[18] and neuronal nitric oxide synthase.[19] The crude extracts containing **1**−**10** were tested for cytotoxic activity against both leukemia and solid tumor cancer cells and were found to be equally toxic but not potent, which is considered an unfavorable profile.[20] Also compounds **3**, **4**, and **7** were similarly screened in the same panel, and all possessed either minimal or no cytotoxicity and no selectivity. Subsequently, compounds **1**, **3**−**8**, **10**, and **11** were assayed against *Staphylococcus epidermidis*, and the results are shown in Table 4. All of the compounds were found to have either weak or moderate minimum inhibitory concentrations (MIC) ranging from 6.25 to 100 μg/mL as compared to the standard vancomycin (0.625 μg/mL). However, no distinct structure−activity trends could be identified for either the weak or the moderate inhibitors.

Table 1. NMR Data[a] for 1′,8-Dihydroaplysinopsin (**2**), 6-Bromo-1′,8-dihydroaplysinopsin (**3**), 6-Bromo-1′-hydroxy-1′,8-dihydroaplysinopsin (**4**), 6-Bromo-1′-methoxy-1′,8-dihydroaplysinopsin (**5**), and 6-Bromo-1′-ethoxy-1′,8-dihydroaplysinopsin (**6**) in MeOH-d_4

	2		**3**		**4**			**5**			**6**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	gHMBC	δ_C	δ_H (J in Hz)	gHMBC	δ_C	δ_H (J in Hz)
2	123.8	7.07 s	124.8	7.09 s	125.1	7.09 s	3, 3a, 7a	125.4	7.11 d (0.6)	3, 3a, 7a	125.4	7.11 s
3	106.1		106.5		105.3			104.6			104.7	
3a	126.8		125.7		125.6			125.6			125.6	
4	117.7	7.49 ddd (8.0, 1.0, 1.0)	119.3	7.42 dd (8.6, 0.4)	119.3	7.39 d (8.3)	6, 7a	119.4	7.38 dd (8.6, 0.5)	3, 3a, 6, 7a	119.0	7.38 dd (8.6, 0.4)
5	118.7	7.01 ddd (8.0, 7.0, 1.0)	121.9	7.12 dd (8.6, 1.8)	122.0	7.12 dd (8.6, 1.8)	3a, 7	122.1	7.13 dd (8.6, 1.8)	3a, 7	122.0	7.12 dd (8.6, 1.8)
6	121.3	7.08 ddd (8.2, 7.1, 1.1)	114.7		114.8			114.9			114.9	
7	111.1	7.32 ddd (8.0, 0.9, 0.9)	113.9	7.49 dd (1.8, 0.5)	114.0	7.49 d (1.8)	3a, 5, 6	114.0	7.50 dd (1.8, 0.4)	3a, 5, 6	114.0	7.49 dd (1.7, 0.4)
7a	136.4		137.2		137.1			137.2			137.2	
8	29.4	3.50 dd (4.8, 15.4)	29.4	3.47 ddd (15.5, 4.8, 0.5)	30.1	3.44 d (15.4)	2, 3, 1′, 5′	30.1	3.45 dd (15.1, 0.6)	2, 3, 1′, 5′	30.4	3.45 d (14.9)
		3.41 dd (3.9, 15.6)		3.41 ddd (15.5, 4.1, 0.5)		3.38 d (15.4)			3.41 dd (15.1, 0.6)			3.40 d (14.9)
1′	64.1	4.50 t (4.5)	64.0	4.50 dd (4.7, 4.3)	89.0			94.3			93.8	
3′	158.1		158.1		156.6			157.2			157.0	
5′	172.0		171.8		171.9			169.7			170.0	
2′-NCH_3	24.7	3.13 s	24.7	3.14 s	25.3	3.13 s	1′, 3′	25.6	3.13 s	1′, 3′	25.6	3.12 s
4′-NCH_3	24.5	2.87 s	24.1	2.88 s	24.6	2.82 s	3′, 5′	24.7	2.83 s	3′, 5′	24.6	2.81 s
OCH_3								52.3	3.23 s	1′		
OCH_2											61.5	3.38 dq (12.6, 6.1, 6.1, 6.1) 3.36 dq (12.6, 6.1, 6.1, 6.1)
CH_3											13.7	1.25 t (6.1)

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).

Table 2. NMR Data[a] for (−)-5-Bromo-*N*,*N*-dimethyltryptophan (**7**), (+)-5-Bromohypaphorine (**8**), and 6-Bromo-1*H*-indole-3-carboxylic Acid Methyl Ester (**11**) in MeOH-d_4

	7			**8**			**11**	
position	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)
1′							165.8	
2	125.3	7.28 d (0.6)	3, 3a, 7a, 8	125.5	7.21 s	3, 3a, 7a, 8	132.4	7.93 s
3	106.6			106.1			107.1	
3a	128.6			128.5			124.8	
4	120.3	7.77 dd (1.8, 0.4)	3, 3a, 5, 6, 7a	120.2	7.75 dd (1.9, 0.5)	3, 3a, 5, 6, 7a	121.9	7.93 dd (8.5, 0.5)
5	112.0			112.0			124.2	7.27 dd (8.6, 1.8)
6	124.2	7.21 dd (8.6, 1.8)	4, 5, 7a	124.1	7.20 dd (8.8, 1.8)	4, 5, 7a	115.6	
7	112.8	7.28 dd (8.6, 0.5)	3a, 5	112.7	7.27 dd (8.7, 0.5)	3a, 5	114.5	7.59 dd (1.8, 0.4)
7a	135.2			135.2			137.4	
8	23.5	3.49 ddd (15.2, 6.2, 0.6) 3.44 ddd (15.2, 7.4, 0.6)	2, 3, 3a, 9, 10	22.5	3.54 dd (13.8, 4.2) 3.43 dd (13.5, 11.5)	2, 3, 3a, 9, 10		
9	68.4	4.27 dd (7.3, 6.2)	3, 8, 10	76.2	4.30 dd (11.6, 4.0)	3, 10		
10	169.7			168.8				
$N(CH_3)_2$	40.9	2.95 s	9					
$N(CH_3)_3$				51.4	3.35 s	9		
OCH_3							50.0	3.85 s

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).

Although aplysinopsins have been isolated from numerous sponge genera, scleractinian corals, and one sea anemone, this constitutes the first report of aplysinopsins and their brominated analogues from a collection of *Thorectandra*. Likewise, hypaphorine has an even larger prevalence with sponges, tunicates, plants, insects, and fungi. Similarly, aplysinopsins, widely distributed in the Pacific, Caribbean, and Mediterranean, have not previously

Table 3. Optical Properties of Tryptophan

compound	rotation neutral	rotation acidic	rotation basic
S/L − tryptophan	"−" (−31.5/MeOH)[a]	"+" (+2.4/0.5 N HCl)[a]	"+" (+0.15/0.5 N NaOH)[a]
S/L − tryptophan Me ester	"+" (+37/MeOH)[b]		
S/L − tryptophan *N*-Me (abrine)		"+" (+44/0.5 N HCl)[a]	"+" (+65.1/0.5 N NaOH)[d]
S/L − tryptophan *N*-Me, Me ester HCl	"+"(+47.2/MeOH)[b]		
S/L − tryptophan *N*,*N*-diMe			
S/L − tryptophan *N*,*N* -diMe, Me ester	"+" (+65/EtOH)[b]	"+" (+70/)0.5 dil HCl)[b]	
S/L − tryptophan *N*,*N*,*N*-triMe (hypaphorine)	"+" (+133.6/H_2O)[b]		
R/D − tryptophan *N*,*N*,*N*- triMe (hypaphorine)	"−" (−87/MeOH)[e]		
S/L − 5-Br-tryptophan	"−" (−24/MeOH)[c]	"+" (+32/1 M HCl)[b]	
S/L − 5-Br-tryptophan *N*-Me (5-Br-abrine)	"−" (−34/MeOH)[c]	"+" (+46/1 N HCl)[c]	"+" (+46/1 N NaOH)[c]
S/L − 5,6-di-Br-tryptophan *N*-Me (5,6-di-Br-abrine)	"−" (−31/MeOH)[c]	"+" (+44/1 N HCl)[c]	"+" (+44/1 N NaOH)[c]
S/L − 6-Br-tryptophan *N*,*N*,*N* -triMe (6-Br-hypaphorine)	"+" (+58/MeOH−TFA, 8:1)[b]		
R/D − 6-Br-tryptophan *N*,*N*,*N*-triMe (6-Br-hypaphorine)	"−" (−27/MeOH−TFA, 8:1)[b]		

[a] Merck Index. [b] The Chapman & Hall Dictionary of Natural Products. [c] Reference 13d. [d] Aldrich catalog. [e] Reference 15.

Table 4. MIC of **1**, **3**−**8**, **10**, and **11** against *Staphylococcus epidermidis*

compound	MIC (μg/mL)
vancomycin	0.625
1	25
3	25
4	12.5
5	100
6	12.5
7	25
8	100
10	6.25
11	50

been reported from sponges collected in Papua New Guinea. Of additional note is that the new aplysinopsins **4**−**6** represent the first naturally occurring oxidized 2-aminoimidazolinones to be described. The pattern of indole ring bromination varied as a function of sponge taxa investigated here, and this deserves brief comment. Either unbrominated or 6-brominated tryptophan derivatives were isolated from *Smenospongia*. By contrast a more complex biosynthetic situation is represented in the metabolites of the *Thorectandra* collection, as all three types of metabolites co-occurred, consisting of unbrominated, 5-brominated, and 6-brominated tryptophans. Further study of these two sponges on the mechanism of indole bromination is warranted.

Experimental Section

General Experimental Procedures. Optical rotations were acquired using a digital polarimeter model JASCO DPI-370. The NMR spectra were recorded at 500 MHz (^{1}H, MeOH-d_4) and 125 MHz (^{13}C, MeOH-d_4). Final NMR assignments were based on comparison to previously published data and 2D NMR data derived from gHMQC and gHMBC. LCMS was performed with a ODS reversed-phase analytical column, particle size 5 μm, using photodiode array (PDA) and evaporative light scattering (ELS) detection with an electrospray ionization time of fight (ESITOF) mass spectrometer. Sephadex LH-20 was used for separation of the crude fractions. Preparative HPLC was performed using a ODS reversed-phase column, particle size 6 μm, with a single wavelength ($\lambda = 254$ nm) UV detector and ELS detector were in series. HPLC was performed with a ODS reversed-phase column, particle size 5 μm. A ESITOF mass spectrometer was employed for HRESITOFMS.

Biological Material, Collection, and Identification. The UCSC specimen of *Smenospongia* sp. was collected from the Milne Bay region of Papua New Guinea (coll. no. 91111). It was gathered off the coast Nuakata Island, using scuba, at depths of 30−50 feet: MLN#14 (Nuak#2) (S 10°16′15″, E 151°1′45″). The NCI-DTP specimen of *Thorectandra* sp. was collected from the Milne Bay Province of Papua New Guinea (coll. no. 0CDN5714) in a bay on the southwest tip of Misima Island in the Louisiade Achipelago, using scuba, at depths of 20−100 feet (S 10°37.67′, E 152°31.42′).

Smenospongia sp.[21] (UCSC coll. no. 91111) (Thorectidae, order Dictyoceratida): The sponge is a massive flabellate specimen, yellow in color internally and gray green externally, compressible, and dense in consistency. The skeleton consisted of a regular fiber rectangular reticulation, with few primaries (120−200 μm), and dominated mostly by secondaries (60−100 μm). The fibers are all clear, laminated, and uncored. The specimens fit very well the genus *Smenospongia* Wiedenmayer, 1977, with a clear, uncored regular fiber reticle dominated by secondary fibers.

Thorectandra sp.[22] (NCI coll. no. 0CDN5714) (Thorectidae, order Dictyoceratida): The NCI was unable to provide either a photograph or a taxonomic description of the sponge due to a memorandum of understanding between the source country and the NCI-DTP. However, Michelle Kelly was identified as the taxonomist who assigned that specimen.[22]

Extraction and Isolation. The *Smenospongia* sp. specimen was preserved according to our standard procedure as described previously[23] and then transported to the home laboratory at ambient temperature. The organism was soaked three successive times for 24 h in 100% MeOH. The resulting oil was partitioned as described elsewhere.[23]

The *Thorectandra* sp. specimen was frozen in the field and transported to the NCI-DTP laboratory. The organism was later thawed and soaked in a solution of 50:50 MeOH−CH_2Cl_2 to give an organic extract (no. C18709). A 1 g portion of C18709 was subsequently sent to the UCSC lab. The organic extract, C18709, was partitioned between water (the *sec*-butanol-soluble sample coded "WB") and CH_2Cl_2 (sample coded "F"). The concentrated F was next partitioned between hexanes (sample coded "FH") and 10% aqueous MeOH (sample coded "FDFM").

Pure compounds were obtained as follows (also see Figure S17). The FDFM extract of *Thorecandra* sp. was fractionated using semipreparative reversed-phase HPLC with a gradient of 80:20 up to 0:100 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to yield **3** (18.2 mg), **4** (2.9 mg), and **5** (6.4 mg). A 250 mg portion of the WB extract of *Thorectandra* sp. was fractionated using preparative HPLC with a gradient of 80:20 up to 0:100 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to give 10 fractions. Fraction 4 supplied **9** (28.6 mg), and the sixth fraction provided **2** (30.2 mg). The eighth HPLC fraction was separated using semipreparative reversed-phase HPLC with a gradient of 70:30 up to 30:70 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to yield **8** (6.3 mg) and **7** (7.5 mg).

A 1.5 g portion of the FD extract of *Smenospongia* sp. was fractionated (see Figure S17) using preparative reversed-phase

HPLC with a gradient of 80:20 up to 0:100 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to give seven fractions. The second fraction was purified using semipreparative reversed-phase HPLC with a gradient of 80:20 up to 50:50 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to give **1** (3.7 mg). The fifth preparative HPLC fraction was further fractionated using semipreparative reversed-phase HPLC with a gradient of 60:40 up to 20:80 H_2O−MeOH (0.1% trifluoroacetic acid in both solvents) to yield **6** (8.5 mg), **10** (1.7 mg), and **11** (6.9 mg).

Antibacterial Assay. Compounds **1**, **3**−**8**, **10**, and **11** were tested against *Staphylococcus epidermidis* (ATCC 12228) following the procedure published elsewhere.[24]

Aplysinopsin (1): yellow solid; ^1H and ^{13}C NMR (MeOH-d_4) data were in agreement with literature values;[6] LRESITOFMS *m/z* 255 [M + H]$^+$.

1′, 8-Dihydroaplysinopsin (2): red solid; ^1H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 1; LRESITOFMS *m/z* 257 [M + H]$^+$.

6-Bromo-1′, 8-dihydroaplysinopsin (3): red solid; $[\alpha]_D^{25}$ −8.4° (*c* 2.5, MeOH); ^1H NMR (500 MHz), Figure S1 and Table 1; ^{13}C NMR (125 MHz), Figure S2 and Table 1; HRESITOFMS *m/z* 335.0486 [M + H]$^+$, Δ 1.6 mmu of calcd.

6-Bromo-1′-hydroxy-1′-8-dihydroaplysinopsin (4): red solid; $[\alpha]_D^{25}$ +1.0° (*c* 0.5, MeOH); ^1H NMR (500 MHz), Figure S3 and Table 1; ^{13}C NMR (125 MHz), Figure S4 and Table 1; HRESITOFMS *m/z* 351.0448 (calcd for $C_{14}H_{16}BrN_4O_2$ [M + H]$^+$ 351.0457, Δ 0.3 mmu).

6-Bromo-1′-methoxy-1′-8-dihydroaplysinopsin (5): red solid; $[\alpha]_D^{25}$ +3.0° (*c* 1.4, MeOH); ^1H NMR (500 MHz), Figure S6 and Table 1; ^{13}C NMR (125 MHz), Figure S7 and Table 1; HRESITOFMS *m/z* 365.0641 (calcd for $C_{15}H_{18}BrN_4O_2$ [M + H]$^+$ 365.0608, Δ 3.3 mmu).

6-Bromo-1′-ethoxy-1′-8-dihydroaplysinopsin (6): red solid; $[\alpha]_D^{25}$ 19.3° (*c* 0.05, MeOH); ^1H NMR (500 MHz), Figure S8 and Table 1; ^{13}C NMR (125 MHz), Figure S9 and Table 1; HRESITOFMS *m/z* 379.0773 (calcd for $C_{16}H_{20}BrN_4O_2$ [M + H]$^+$ 379.0764, Δ 0.9 mmu).

(−)-5-Bromo-*N*,*N*-dimethyltryptophan (7): yellow solid; $[\alpha]_D^{25}$ −19.3° (*c* 0.5, MeOH); ^1H NMR (500 MHz), Figure S10 and Table 2; ^{13}C NMR (125 MHz), Figure S11 and Table 2; HRESITOFMS *m/z* 311.0369 (calcd for $C_{13}H_{16}BrN_2O_2$ [M + H]$^+$ 311.0390, Δ 2.1 mmu).

(+)-5-Bromohypaphorine (8): yellow solid; $[\alpha]_D^{25}$ +46.3° (*c* 0.5, MeOH); ^1H NMR (500 MHz), Figure S12 and Table 2; ^{13}C NMR (125 MHz), Figure S13 and Table 2; HRESITOFMS *m/z* 325.0534 (calcd for $C_{14}H_{18}BrN_2O_2$ [M]$^+$ 325.0546, Δ 1.2 mmu).

(1*H*-Indole-3-yl)acetic acid (9): yellow solid; ^1H and ^{13}C NMR (MeOH-d_4) data were in agreement with literature values;[8] LRESITOFMS *m/z* 176 [M + H]$^+$.

(6-Bromo-1*H*-indol-3-yl)acetic acid methyl ester (10): yellow solid; ^1H NMR (MeOH-d_4) data were in agreement with literature values;[9] LRESITOFMS *m/z* 268 [M + H]$^+$.

6-Bromo-1*H*-indole-3-carboxylic acid methyl ester (11): yellow solid; ^1H NMR (500 MHz), Figure S14 and Table 2; ^{13}C NMR (125 MHz), Figure S15 and Table 2; HRESITOFMS *m/z* 253.9808 (calcd for $C_{10}H_9BrNO_2$ [M + H]$^+$ 253.9811, Δ 0.3 mmu).

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Supporting Information Available: ^1H and ^{13}C NMR spectra data of **3**−**8** and **11**, gHMBC spectrum of **4**, and above-water photograph of collection no. 91111. This material is available free of charge via the Internet at http://pubs.acs.org.

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