JOC Article

Psammaplins from the Sponge *Pseudoceratina purpurea*: Inhibition of Both Histone Deacetylase and DNA Methyltransferase

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Four novel bisulfide bromotyrosine derivatives, psammaplins E (**9**), F (**10**), G (**11**), and H (**12**), and two new bromotyrosine derivatives, psammaplins I (**13**) and J (**14**), were isolated from the sponge *Pseudoceratina purpurea*, along with known psammaplins A (**4**), B (**6**), C (**7**), and D (**8**) and bisaprasin (**5**). The structures of psammaplins E (**9**) and F (**10**), which each contain an oxalyl group rarely found in marine organisms, were determined by spectroscopic analysis. Compounds **4**, **5**, and **10** are potent histone deacetylase inhibitors and also show mild cytotoxicity. Furthermore, compounds **4**, **5**, and **11** are potent DNA methyltransferase inhibitors. The biogenetic pathway previously proposed for the psammaplins class is also revisited.

Introduction

Natural products are finally being explored for their potential as tools to investigate the actions of histone deacetylase (HDAC)[1] and DNA methyltransferase (DNMT).[2] Microbially derived compounds, such as trichostatin A (**1**)[3] and trapoxin A (**2**),[4] were recently described as potent inhibitors of HDAC. These natural products are emerging as important tools because they cause cell cycle arrest,[5] induce differentiation or apoptosis of tumor cells,[6] and exert a p53-independent induction of the cyclin dependent kinase inhibitor, p21.[7] Similarly, the natural product derived compound 5-aza-2′-deoxycytidine (**3**) ranks as the most important among the few reported inhibitors of DNMT. Recent findings with **3** provide a link between DNMT inhibition and epigenetic gene regulation,[8] tumor cell apoptosis,[9] and p21 induction.[10] Adding to this interesting circumstance are the results of a synergistic study with **1** and low doses of **3** to reactivate silenced tumor suppressor genes in colon cancer cells.[11] Thus, while the discovery of novel natural product inhibitors of HDAC or DNMT is a priority, uncovering a single compound to inhibit both enzymes is also quite appealing.

Several years ago we began to screen marine natural products in a mechanism-based HDAC assay relevant to anticancer drug lead compound discovery. As this program developed, a second assay was added that employed

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(1) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Dean, K.; Diamantidis, G.; Green, M. A.; Howell, K. L.; Kashi, R.; Kwon, P.; Lassota, P.; Mou, Y.; Nemzek, R.; Perez, L. B.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Weltchek-Engler, S.; Wood, A. W.; Wu, A. *Abstracts of the 224th National Meeting of the American Chemical Society*; American Chemical Society: Washington, DC, 2002; p 227.

(2) (a) Milutinovic, S.; Knox, J. D.; Szyf, M. *J. Biol. Chem.* **2000**, *275*, 6353−6359. (b) Fournel, M.; Sapieha, P.; Beaulieu, N.; Besterman, J. M.; MacLeod, A. R. *J. Biol. Chem.* **1999**, *274*, 24250−24256.

(3) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174−17179.

(4) Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. *J. Biol. Chem.* **1993**, *268*, 22429−22435.

(5) (a) Yoshida, M.; Horinouchi, S.; Beppu, T. *BioEssays* **1995**, *17*, 423−428. (b) Taunton, J.; Collins, J. L.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 10412−10422. (c) Jung, M.; Hoffmann, K.; Brosch, G.; Loidl, P. *Bioorg., Med. Chem. J. Lett.* **1997**, *7*, 1655−1658. (d) Nakajima, H.; Kim, Y. B.; Terano, H.; Yoshida, M.; Horinouchi, S. *Exp. Cell Res.* **1998**, *241*, 126−133.

(6) (a) Sambucetti, L. C.; Fischer, D. D.; Zabludoff, S.; Kwon, P. O.; Chamberlin, H.; Trogani, N.; Xu, H.; Cohen, D. *J. Biol. Chem.* **1999**, *274*, 34940-34947. (b) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cornell, W. D.; Green, M. A.; Howell, K. L.; Jung, M.; Kwon, P.; Trogani, N.; Walker, H. *J. Med. Chem.* **2002**, *45*, 753−757.

(7) (a) Huang, Y.; Tan, M.; Gosink, M.; Wang, K. W.; Sun, Y. *Cancer Res.* **2002**, *62*, 2913−2922. (b) Medina, V.; Edmonds, B.; Young, G. P.; James, R.; Appleton, S.; Zalewski, P. D. *Cancer Res.* **1997**, *57*, 3697−3707.

(8) (a) Rountree, M. R.; Bachman, K. E.; Herman, J. G.; Baylin, S. B. *Oncogene* **2001**, *20*, 3156−3165. (b) Hendrich, B.; Bird, A. *Mol. Cell. Biol.* **1998**, *18*, 6538−6547.

(9) Zhu, W.; Lakshmanan, R. R.; Beal, M. D.; Otterson, G. A. *Cancer Res.* **2001**, *61*, 1327−1333.

(10) Ramchandani, S.; MacLeod, A. R.; Pinard, M.; von Hofe, E.; Szyf, M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 684−692.

(11) Cameron, E. E.; Bachman, K. E.; Moyhanen, S.; Herman, J. G.; Baylin, S. B. *Nat. Genet.* **1999**, *21*, 103−107.

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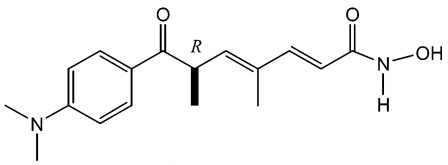
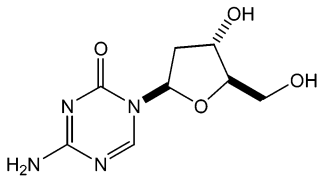
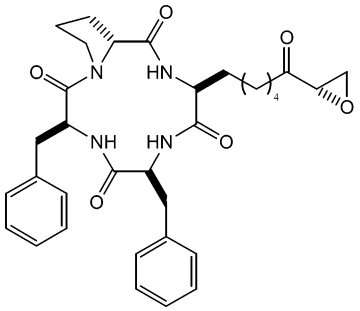


trichostatin (**1**)



5-aza-2'-deoxycytidine (**3**)



trapoxin (**2**)

one isoform of DNMT. Some results from this initial HDAC screening, reported in a meeting abstract,[12] announced that psammaplin A (**4**)[13] was an extremely active hit, and suggested the psammaplins constituted a new class for further structure−activity development. To date there have been few examples described of small molecule inhibitors of DNMT.[14] At this time we report the formal data to substantiate that psammaplins are potent inhibitors of both HDAC and DNMT while also providing a complete outline of the chemistry and biology considerations leading to this discovery.

Unique aspects of the structure of the psammaplins are the presence of dense N functionalization accompanied by S and Br heteroatoms. We have an abiding interest in sponge-derived, bioactive nitrogen-containing compounds,[15,16] and note that bisulfide bromotyrosine derivatives from marine sources are distinct in their paucity. The few additional examples of such rare marine-derived bromotyrosine bisulfides all come from Verongid sponges and currently include four members of the psammaplin family isolated from *Pseudoceratina purpurea*,[17] four other psammaplins and two aplysinellins reportedly from *Aplysinella rhax*[18] (thought to be synonomous with *Pseudoceratina purpurea*), and bisaprasin (**5**) isolated from a sponge tentatively identified as *Thorectopsamma xana*.[13c]

To broaden the understanding of sponge-derived bromotyrosine bisulfides and their congeners as HDAC and DNMT inhibitors, the UCSC group began an investigation of two Indo-Pacific *Pseudoceratina purpurea* collections. We now report on the reisolation of known compounds **4**−**8**, the characterization of four novel bisulfide bromotyrosines **9**−**12**, plus two novel bromotyrosine derivatives, psammaplins I (**13**) and J (**14**), which possess sulfoxide and sulfone moieties, respectively. The interesting range of bioactivities observed for these compounds is presented to disclose the first examples of marine natural products acting as dual inhibitors of HDAC and DNMT.

Results and Discussion

The UCSC Kupchan-like solvent partition scheme reported elsewhere[19] was employed to investigate two Papua New Guinea collections of *P. purpurea*. Both yielded crude extract oils which possessed NMR resonances characteristic of the psammaplins.[13,17] The CH_2-Cl_2 partition of the methanol extract of the first organism (coll. no. 96188) was subjected to Sephadex chromatography and further purified by reversed-phase HPLC to yield known psammaplins A (**4**)[13] and D (**8**)[17] and bisaprasin (**5**),[13c] 3-bromo-4-hydroxybenzaldehyde (**15**),[17] together with 3-bromo-4-hydroxyphenylacetonitrile (**16**),[17] and the novel compound psammaplin E (**9**). Purification of the 1:1 MeOH:H_2O partition of this sponge, also proceeded by Sephadex and reversed-phase HPLC, afforded psammaplin F (**10**). Additional compounds were obtained from the second sponge (coll. no. 98171). A *sec*-butanol partition of the methanol extract was subjected to Sephadex and reversed-phase HPLC to yield known psammaplins A (**4**),[13] B (**6**),[17] C (**7**),[17] D (**8**),[17] and bisaprasin (**5**),[13c] and the new psammaplins G (**11**) and H (**12**). Finally, novel psammaplins I (**13**) and J (**14**) were isolated by reversed-phase HPLC of the CH_2Cl_2 extract from this *P. purpurea* sponge.

The major component of the polar partition fractions, psammaplin A (**4**), was easily dereplicated by comparison

(12) Perez, L. B.; Bair, K. W.; Karl, D.; Green, M. A.; Lamberson, C.; Remiszewski, S. W.; Sambucetti, L. C. *Proc. 92nd AACR* **2001**, *42*, 927.

(13) (a) Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 3229−3232. (b) Arabshahi, L.; Schmitz, F. J. *J. Org. Chem.* **1987**, *52*, 3584−3586. (c) Rodriguez, A. D.; Akee, R. K.; Scheuer, P. J. *Tetrahedron Lett.* **1987**, *28*, 4989−4992.

(14) Bigey, P.; Knox, J. D.; Croteau, S.; Bhattacharya, S. K.; Théberge, J.; Szyf, M. *J. Biol. Chem.* **1999**, *274*, 4594−4606.

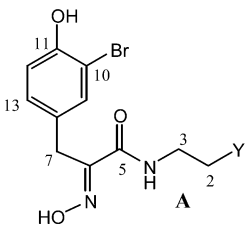
(15) (a) Thale, Z.; Johnson, T.; Tenney, K.; Wenzel, P. J.; Lobkovsky, E.; Clardy, J.; Media, J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews P. *J. Org. Chem.* **2002**, 67, 9384−9391. (b) Thale, Z.; Kinder, F.; Bair, K. W.; Bontempo, J.; Czuchta, A. M.; Versace, R. W.; Phillips, P. E.; Sanders, M. L.; Wattanasin, S.; Crews, P. *J. Org. Chem.* **2001**, *66*, 1733−1741. (c) Odaka, C.; Sanders, M. L.; Crews, P. *Clin. Diag. Lab. Immun.* **2000**, *7*, 947−951. (d) Clark, D. P.; Carroll, J.; Naylor S.; Crews, P. *J. Org. Chem.* **1998**, *63*, 8757−8764. (e) Clark, W. D.; Corbett, T.; Valeriote, F.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285−9286.

(16) (a) Jiménez, C.; Quiñoà, E.; Adamczeski, M.; Hunter, L. M.; Crews, P. *J. Org. Chem.* **1991**, *56*, 3403−3410. (b) Jiménez, C.; Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1991**, *32*, 1843−1846. (c) Jaspars, M.; Rali, T.; Laney, M.; Schatzman, R. C.; Diaz, M. C.; Pordesimo, E. O.; Schmitz, F. J.; Crews, P. *Tetrahedron* **1994**, *50*, 7367−7374.

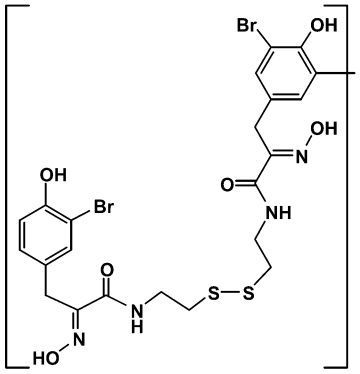
(17) Jiménez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097−2102.

(18) (a) Tabudravu, J. N.; Eijsink, V. G. H.; Gooday, G. W.; Jaspars, M.; Komander, D.; Legg, M.; Synstad, B.; van Aalten, D. M. F. *Bioorg. Med. Chem.* **2002**, *10*, 1123−1128. (b) Shin, J.; Lee, H.; Seo, Y.; Rho, J.; Cho, K.; Paul, V. *Tetrahedron* **2000**, *56*, 9071− 9077. (c) Pham, N.; Butler, M.; Quinn, R. *J. Nat. Prod.* **2000**, *63*, 393−395.

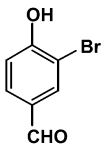
(19) (a) Jaspars, M.; Crews, P. *Tetrahedron Lett.* **1994**, *35*, 7501−7504. (b) Rodríguez, J.; Nieto, R.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034−2040.



A



bisaprasin (**5**)



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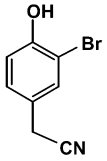
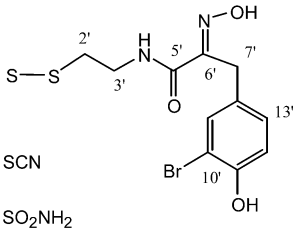


16

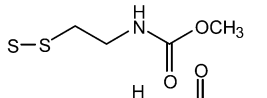
psammaplin A (**4**) Y =



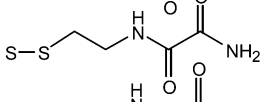
psammaplin B (**6**) Y = SCN

psammaplin C (**7**) Y = SO_2NH_2

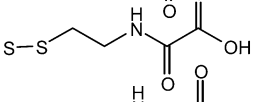
psammaplin D (**8**) Y =



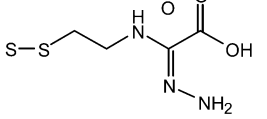
psammaplin E (**9**) Y =



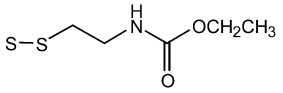
psammaplin F (**10**) Y =



psammaplin G (**11**) Y =

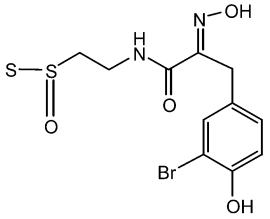


psammaplin H (**12**) Y =



psammaplin I (**13**) Y = SO_2CH_3

psammaplin J (**14**) Y =



of NMR and MS data with those in the literature.[13] The *E*-oxime geometry in this and other psammaplins was further defined by the diagnostic resonances of the benzylic position (Table 1).[13b,15] Another major component also quickly dereplicated by ESIMS (molecular ion *m*/*z* 1322, tetrabrominated cluster) and comparison to the NMR data reported by Scheuer was bisaprasin (**5**).[17c] Finally, previously reported compounds **6**,[17] **7**,[17] and **8**[17] were characterized in a similar fashion.

The structure determination of the first new compound isolated, psammaplin E (**9**), began by establishing the molecular formula as $C_{15}H_{19}N_4O_5S_2{}^{79}Br$ (HRFABMS [M + H]$^+$ *m*/*z* 479.0094, Δ −3.5 mmu of calcd). The molecular ion doublet in an ESIMS ([M + Na]$^+$ *m*/*z* 501.16 and 503.08) spectrum confirmed the presence of a single bromine atom. The APT and HMQC NMR data verified the presence of 5 sp^3 CH_2, 3 sp^2 CH, and 7 sp^2 C. Two *ortho* benzene ring protons, H-12 and H-13, were recognized by their mutual coupling of 8 Hz. The 2 Hz coupling of H-9 to H-13 secured their *meta* relationship. Other connectivities supporting the C-5 to C-13 substructure were derived from HMBC correlations, including those from H-13 to C-9 and the oxygenated C-11, and those from H-7 to C-5, C-6, C-8, C-9, and C-13. The initial partial structure could be expanded to include all the atoms of substructure **A**, and additionally the disulfide to C-3′ (totaling $C_{13}H_{16}N_2O_3S_2{}^{79}Br$) by comparison of NMR data (see Table 1) to that of psammaplin A (**4**). The remaining atoms, $C_2H_3N_2O_2$ (2 unsaturation equivalents), comprised the second partial structure and all of its hydrogens were determined to be exchangeable by ^{1}H NMR analysis in MeOH-d_4. The δ 162.1 signal was assigned to C-5′ based on the HMBC correlation to H-3′, and additionally, the ^{13}C shifts at C-5′ and C-3′ were consistent with these two atoms being interconnected by a nitrogen. The constraints of the preceding formula and relatively low-field shifts of both C-5′ (δ 162) and C-6′ (δ 164) supported the proposal of an *N*-substituted oxalamide residue to complete the overall structure of **9**.

The second novel compound, psammaplin F (**10**), possessed the molecular formula $C_{15}H_{18}N_3O_6S_2{}^{79}Br$ (HR-FABMS *m*/*z* 501.9724 [M + Na]$^+$, Δ 0.6 mmu of calcd). Again, the ESIMS ion cluster at *m*/*z* 478 and 480 ([M − H]$^-$) suggested a monobrominated species. A comparison of its NMR data to that of **4** and **9** (see Table 1) established the presence of the same partial structure from C-13 to C-3′ discussed above. The terminal group now consisted of $C_2H_2NO_3$. The broad IR absorption at 3600−2600 cm^{-1} suggested the existence of a carboxylic acid moiety and, by default, a secondary amide functional group was also indicated. The HMBC correlation from H-3′ (δ 40.1) to C-5′ (δ 161.9), along with the shift at C-6′ (δ 159.2), indicated the presence of an *N*-substituted oxalamic acid group to complete the structure.

Identifying yet another exotic functional group was the challenge accompanying the characterization of psammaplin G (**11**), isolated from the second *P. purpurea* collection. Its ESIMS ion cluster at *m*/*z* 494/496 [M + H]$^+$ required a single Br, and the entire formula of $C_{15}H_{20}N_5O_5S_2{}^{79}Br$ (*m*/*z* 494.0055 [M + H]$^+$, Δ 3.4 mmu of calcd)

TABLE 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Psammaplins A (4), E (9), and F (10) in MeOH-d_4

	4[a]		**9**		**10**	
atom no.	C (δ)	H (δ, mult, J in Hz)	C (δ)	H (δ, mult, J in Hz)	C (δ)	H (δ, mult, J in Hz)
2	38.7	2.79 (t, 6.0)	38.8	2.84 (t, 6.0)	38.6	2.84 (t, 6.0)
2′	38.7	2.79 (t, 6.0)	38.2	2.84 (t, 6.0)	37.9	2.84 (t, 6.0)
3	39.7	3.51 (t, 6.0)	39.9	3.54 (t, 6.0)	39.8	3.54 (t, 6.0)
3′	39.7	3.51 (t, 6.0)	39.8	3.53 (t, 6.0)	40.1	3.54 (t, 6.0)
5	166.0		166.0		166.0	
5′	166.0		162.1		161.9	
6	153.3		153.4		153.3	
6′	153.3		164.0		159.2	
7	28.8	3.78 (s)	28.8	3.78 (s)	28.8	3.78 (s)
7′	28.8	3.78 (s)				
8	130.7		130.2		130.7	
8′	130.7					
9	134.6	7.35 (d, 2.0)	134.6	7.35 (d, 2.0)	134.6	7.35 (d, 2.0)
9′	134.6	7.35 (d, 2.0)				
10	110.6		111.0		110.6	
10′	110.6					
11	153.8		154.5		153.9	
11′	153.8					
12	117.1	6.75 (d, 8.0)	117.4	6.75 (d, 8.0)	117.2	6.75 (d, 8.0)
12′	117.1	6.75 (d, 8.0)				
13	130.5	7.06 (dd, 8.0, 2.0)	130.5	7.06 (dd, 8.0, 2.0)	130.5	7.05 (dd, 8.0, 2.0)
13′	130.5	7.06 (dd, 8.0, 2.0)				

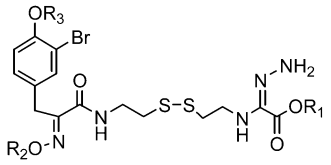
[a] Also see ref 13.

TABLE 2. ^{13}C (125 MHz) NMR Spectral Data for Psammaplins A (4), D (8), and F−J (10 − 14)

no.	**4**[a]	**8**[c]	**10**[a]	**11**[a]	**11**[b]	**12**[a]	**13**[a]	**14**[a]
2	38.7	39.0	38.6	38.6	36.9	39.4	55.8	39.3
2′	38.7	38.2	37.9	37.9	36.3	38.5	54.1	55.0
3	39.7	39.1	39.8	39.7	38.2	39.8	31.9	33.5
3′	39.7	40.6	40.1	40.1	38.3	41.0		32.2
5	166.0	164.1	166.0	166.0	163.2	166.0	164.5	164.6
5′	166.0	158.2	161.9	161.9	160.9	159.2		164.6
6	153.3	153.4	153.3	153.2	151.8	153.2	151.6	151.6
6′	153.3	52.4	159.2	159.2	156.8	61.9		151.6
7	28.8	28.5	28.8	28.8	27.7	28.8	27.2	27.2
7′	28.8					15.1		27.2
8	130.7	130.9	130.7	130.7	128.8	130.6	129.1	129.2
8′	130.7							129.2
9	134.6	134.1	134.6	134.6	132.8	134.6	133.0	133.0
9′	134.6							133.0
10	110.6	109.9	110.6	110.6	108.9	110.6	109.0	109.0
10′	110.6							109.0
11	153.8	152.8	153.9	153.8	152.3	153.9	152.4	152.3
11′	153.8							152.3
12	117.1	117.1	117.2	117.2	116.1	117.2	115.6	115.6
12′	117.1							115.6
13	130.5	130.4	130.5	130.5	129.1	130.5	129.0	129.0
13′	130.5							129.0

[a] In MeOH-d_4. [b] In DMSO-d_6. [c] Data from ref 13 in CH_3CN-d_3.

was established by HRFABMS. Again, the NMR data (^{1}H, ^{13}C, HMQC, and HMBC) easily revealed that substructure **A** was attached to the four-atom chain S−S−C-2′−C-3′, as in the psammaplins A (**4**), D (**8**), E (**9**), and F (**10**). Completing the structure of **11** rested on rationalizing the 14-amu difference between **11** and **10**. That the greater mass of **11** was not associated with an extra methyl or methylene was confirmed by comparison of its NMR spectrum to that of **10** (see Table 2), and thus chemical transformation was explored. Esterification of **11** with diazomethane in Et_2O at 0 °C afforded three products (see Figure 1). The major component was determined to be the methyl ester, **11a** (HRFABMS [M + Na]$^+$ m/z 530.0132, Δ 1.1 mmu of calcd, $C_{16}H_{22}N_5O_5S_2{}^{79}$BrNa), supported by the ^{1}H NMR shift at δ 3.82. This

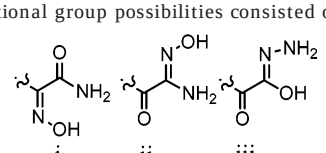


11a R1 = Me, R2 = R3 = H
11b R1 = R2 = Me, R3 = H
11c R1 = R2 = R3 = Me
11d R1 = R2 = H, R3=Ac

FIGURE 1. Synthetic derivatives of psammaplin G (**11**).

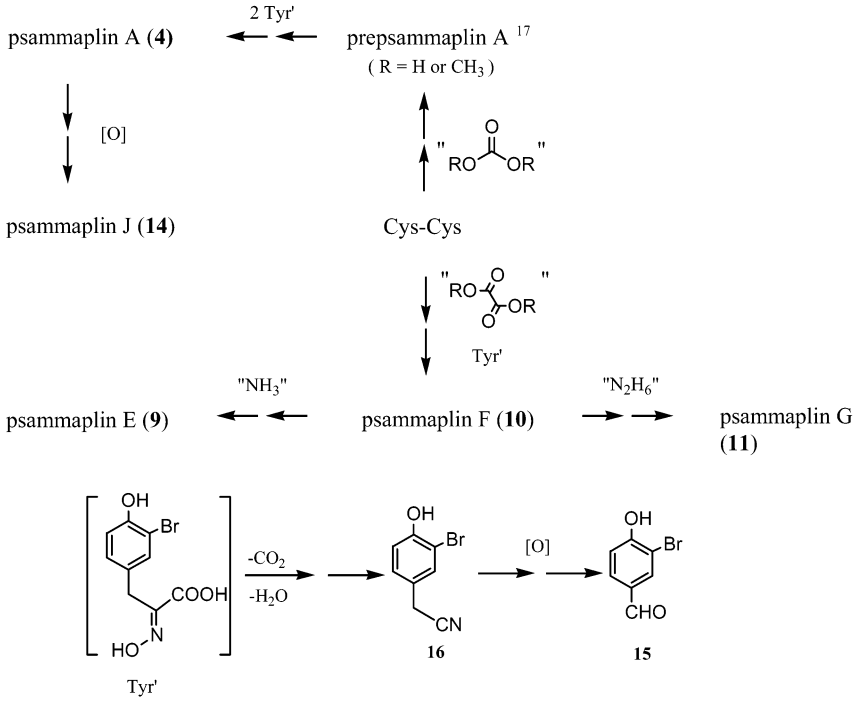
confirmed a carboxylic acid moiety for the starting material **11** and ruled out the three other interesting possibilities for the $C_2H_3N_2O_2$ functional group.[20] Two additional minor components, **11b** and **11c**, the di- and tri-methylated derivatives, respectively, were also isolated as a mixture from the reaction product. Their molecular formulas were determined by HRFABMS to be $C_{17}H_{24}N_5O_5S_2{}^{79}$BrNa ([M + Na]$^+$ m/z 544.0275, Δ 2.5 mmu of calcd) and $C_{18}H_{26}N_5O_5S_2{}^{79}$BrNa ([M + Na]$^+$ m/z 558.0472, Δ 1.6 mmu of calcd), respectively. These data indicated that the very unusual oxalamic acid in psammaplin F (**10**) was replaced by an even more striking functionality, which does not appear to have a precedent. Last, acetylation of **11** yielded **11d** whose structure was established by the molecular formula $C_{17}H_{22}N_5O_6S_2{}^{79}$BrNa (HRFABMS [M + Na]$^+$ m/z 558.0060, Δ 3.2 mmu of calcd), as well as from ^{1}H resonances for the single OAc (δ 2.14, s, 3H) and the singlet at δ 3.96 (H-7, 2H).

(20) The functional group possibilities consisted of **i**−**iii**:



i ii iii

SCHEME 1



Another compound, psammaplin H (**12**), was isolated as a yellow oil. The HRFABMS provided data to set the molecular formula as $C_{16}H_{22}N_3O_5S_2{}^{79}BrNa$ ($[M + Na]^+$ *m*/*z* 502.0102, Δ 2.0 mmu of calcd). Similar comparisons to the previously analyzed psammaplins supplied the substructure C-3′−C-13, and NMR afforded the fragments included in C-3′−C-13, NH, and OC_2H_5. At this point, a close comparison of the ^{13}C NMR data of **12** with that of psammaplin D (**8**) was made (see Table 2) and showed that they differed only in an ester moiety. The presence of an ethoxy carbamate in **12** was clearly supported by the carbonyl shift at δ_C 158.2, the oxygenated methylene at δ_C 61.9, the methyl at δ_C 15.1, and the coupled protons at δ_H 4.05 (2H, q, J = 7.0 Hz) and 1.21 (3H, t, J = 7.0 Hz) (see Table 3).

The structure elucidation of psammaplins I (**13**) and J (**14**) relied heavily on comparative molecular formula data. Such an approach was needed because the NMR spectra of both compounds contained just the signature resonances of substructure **A** present in **4**−**12**. The analysis began by comparing the formula of substructure **A** ($C_{11}H_{12}N_2O_3S^{79}Br$) to that established for psammaplin I (**13**), $C_{12}H_{15}N_2O_5S_2{}^{79}Br$ ($[M + Na]^+$ *m*/*z* 400.9795, Δ 1.2 mmu of calcd), and psammaplin J (**14**) $C_{22}H_{24}N_4O_7S_2{}^{79}Br_2$ ($[M + Na]^+$ *m*/*z* 700.9396, Δ 4.5 mmu of calcd). Subtracting the atom count of substructure **A** from that of **13** gave a residual of SSO_2CH_3 revealing that an interesting functional group capped the terminal end of the molecule. Also consistent with this assignment versus other possibilities such as $-SO_2SCH_3$ were similar C-2 shifts (see Table 2) observed for **13** (δ_C 55.8) and psammaplin C (**7**) (δ_C 54.5). Last, the resonance position of the terminal methyl (δ_C 54.1) along with the infrared absorption at 1284 cm^{-1} supported the presence of the terminal sulfone group. The overall structural features of **14** were also quickly assembled by subtracting the atom count of twice substructure **A** plus the disulfide ($C_{22}H_{24}N_4O_6S_2Br_2$) from that of psammaplin J (**14**). The residual atom consisted of just one oxygen, which explained the nearly identical ^{13}C NMR shifts observed between psammaplin J (**14**) and psammaplin A (**4**). The single exception was at C-2′ (**14** δ_C 55.0 vs **4** δ_C 38.7). In view of these observations, the only way to construct the overall structure of **14** was to replace a sulfur atom of **4** with a sulfoxide group, consistent with the infrared absorption at 1043.5 cm^{-1}. Unfortunately, samples of **14** had decomposed prior to measuring its optical properties.

The biosynthetic pathway we previously proposed for psammaplins A−D (**4**−**8**)[17] can now be extended to cover the new psammaplins E (**9**), F (**10**), G (**11**), and J (**14**), plus compounds **15** and **16**. These ideas are shown in Scheme 1 and utilize a modified tyrosine (Tyr′),[17] which is also the logical precursor to compounds **15** and **16**. The disulfide presammaplin A, previously isolated by the UCSC group,[17] must be derived from cystine. Likewise, cystine can lead to psammaplin F (**10**), which is envisioned to be the precursor of psammaplins E (**9**) and G (**11**). Oxidation of psammaplin A (**4**) (possibly by a cytochrome P450 reaction) provides a connection to psammaplin J (**14**). The two esterified psammaplins D (**8**) and H (**12**) may be artifacts of isolation derived from the acid formed via condensation of cystine and Tyr′.

Once all of the psammaplins were characterized, the process of evaluating their bioactive properties began. A total of eleven compounds isolated here were analyzed simultaneously in the HDAC enzyme assay and the results are shown in Table 4. The compounds were tested at concentrations ranging from 16 nM to 10 μM, and the data obtained were compared to that of two standards, trichostatin A and trapoxin. There were three psammaplin derivatives whose IC_{50} values were in a range of very potent activity (≤10 nM) and these included the following: psammaplin A (**4**) IC_{50} = 4.2 nM, bisaprasin (**5**) IC_{50} = 10.7 nM, and psammaplin F (**10**) IC_{50} = 8.6 nM. By

TABLE 3. ^{1}H (500 MHz) NMR Spectral Data (δ, mult, J in Hz) for Psammaplins A (4) and F−J (10−14)

no.	**4**[a,c]	**10**[a]	**11**[b]	**12**[a]	**13**[a]	**14**[a]
2	2.79 (t, 6.0)	2.84 (t, 6.0)	2.83 (t, 6.5)	2.82 (t, 7.0)	2.96 (m)	3.51 (m)
2′	2.79 (t, 6.0)	2.84 (t, 6.0)	2.83 (t, 6.5)	2.78 (t, 7.0)	3.75 (s)	3.22 and 3.32 (m)
3	3.51 (t, 6.0)	3.54 (t, 6.0)	3.42 (t, 6.5)	3.54 (t, 7.0)	3.62 (dt, 6.5, 2.0)	3.62 (m)
3′	3.51 (t, 6.0)	3.54 (t, 6.0)	3.42 (t, 6.5)	3.36 (t, 7.0)		3.29 (m)
6′				4.05 (q, 7.0)		
7	3.78 (s)	3.78 (s)	3.69 (s)	3.78 (s)	3.78 (s)	3.78 (s)
7′	3.78 (s)			1.21 (t, 7.0)		
9	7.35 (d, 2.0)	7.35 (d, 2.0)	7.28 (d, 2.5)	7.36 (d, 2.5)	7.35 (d, 2.0)	7.35 (d, 2.0)
9′	7.35 (d, 2.0)					
12	6.75 (d, 8.0)	6.75 (d, 8.0)	6.83 (d, 8.0)	6.75 (d, 8.5)	6.75 (d, 8.5)	6.75 (d, 8.5)
12′	6.75 (d, 8.0)					
13	7.06 (dd, 8.0, 2.0)	7.05 (dd, 8.0, 2.0)	7.01 (dd, 8.0, 2.0)	7.06 (dd, 8.0, 2.5)	7.05 (dd, 8.5, 2.0)	7.05 (dd, 8.5, 2.0)
13′	7.06 (dd, 8.0, 2.0)					

[a] In MeOH-d_4. [b] In DMSO-d_6. [c] Also see ref 13.

TABLE 4. Enzyme-Based Histone Deacetylase (HDAC) and DNA Methyltransferase (DNMT) Inhibition Data, and Cell-Based p21 Promoter Activity Data, for the Psammaplins

compd	HDAC enzyme inhibition IC_{50} (nM)[a]	cell-based fold induction activity AC_{50} (μM)[b]	max fold induction (22 MFI is produced at 0.5 μM)	DNMT enzyme inhibition IC_{50} (nM)
psammaplin A (**4**)	4.2 ± 2.4	7.5	15	18.6
psammaplin B (**6**)	48 ± 12	15.0	7	nt
psammaplin C (**7**)	nt	nt	nt	nt
psammaplin D (**8**)	44 ± 10	>15.0	5	> 30.0
psammaplin E (**9**)	327 ± 39	>15.0	2	> 30.0
psammaplin F (**10**)	2.1 ±0.4	0.7	18	> 30.0
psammaplin G (**11**)	18 ± 8	7.5	7	12.8
psammaplin H (**12**)	79 ± 22	3.8	7	> 30.0
psammaplin I (**13**)	299 ± 70	2.6	9	> 30.0
psammaplin J (**14**)	20 ± 17	3.6	8	nt
bisaprasin (**5**)	9 ± 5	0.7	9	3.4
trichostatin A (**1**)	14 ± 1	0.2	7.2	nt
trapoxin A (**2**)	9 ± 3	0.005	28.5	nt

[a] Compounds were titrated in a 1:5 dilution series (10 μM, 2 μM, 400 nM, 80 nM, and 16 nM); averages from duplicate trials are shown, except for known HDAC inhibitors, trichostatin A[3] and trapoxin A.[4] [b] Concentration of compound required to produce 50% p21 promoter activity by psammaplin A (**4**).

contrast, psammaplins B (**6**), C (**7**), D (**8**), G (**11**), H (**12**), and J (**14**) were only considered moderately active (IC_{50} values >40 nM), while the other two psammaplins E (**9**) and I (**13**) were weakly active (IC_{50} values >100 nM). These trends are mirrored in the activation of p21 promoter regions in cell-based assays (see Table 4). The factors responsible for the structure−activity relationship (SAR) variations in Table 4 based on potency are clearly complex. It would appear that the structural features required for the best HDAC inhibition activity include the disulfide spacer linked to a hydroxyimino amide capped on each end by modified tyrosine residues. Deletion of one of the tyrosine end groups was not tolerated, but in one case it could be replaced by an oxalamic acid group (i.e. **10**).

During the final efforts on the structure elucidation of novel psammaplins E−J, the DNA methyltransferase assay became operational. The DNMT assay provided data indicating significant activity in the crude CH_2Cl_2 solvent partition extract of *Pseudoceratina purpurea* collected from Madagascar (coll no. 00254). It was quickly determined by ESITOF mass spectrometry and ^{1}H NMR analysis that this crude extract contained psammaplins A (**4**), D (**8**), and J (**14**). Eight psammaplin analogues from our repository were then assessed in the DNA methyltransferase assay and the results are presented in Table 4. Unfortunately, scarcity of material prevented the testing of three of the analogues, psammaplins B (**6**), C (**7**), and J (**14**). Only one compound, bisaprasin (**5**), was observed as very potent (≤10 nM) and two other compounds, psammaplin A (**4**) and G (**11**), were also potent. There are scant quantitative SAR inhibition data in the literature for small molecule inhibitors of DNMT. The best example pertains to a family of synthetic biopolymers comprised of 25 nucleotide bases that form hairpin loops whose K_i values against DNMT are 30 nM or greater.[14] Interestingly, two of these, psammaplin A (**4**) and bisaprasin (**5**), were also potent HDAC inhibitors. Although our data set is limited, it would appear that the unifying feature for dual activity against both HDAC and DNMT is the presence of the disulfide spacer linked to a hydroxyimino amide capped on each end by aromatic moieties.

Inspired by its HDAC and DNMT enzyme inhibitory actions and unique structural features, the antiproliferative properties of psammaplin A (**4**) were thoroughly explored. Encouraging *in vitro* results were found as **4** inhibited cells grown in monolayers and in soft agar at the following levels (IC_{50} values in μM): A549 lung tumor at 1.35 and 2.5, and MDA-MB-435 breast tumor at 1.15, and 2.0, respectively. These positive results were also observed *in vivo* as psammaplin A (**4**) inhibited tumor growth in the A549 lung xenograph mouse model while also maintaining low toxicity.[12]

Conclusions

The results described above are significant from both a structural chemical and biological view. To the best of our knowledge, psammaplin F (**10**) is the first marine natural product containing oxalamic functionality. The *N*-substituted oxalamide functionality of psammaplin E (**9**) is also rare among marine natural products. The only other marine natural products that possess an oxalamide functional group are igzamide, isolated from the marine sponge *Plocamissa igzo*,[21] and 3-bromotyramine amide, isolated from the Papua New Guinea marine sponge *Ianthella basta*.[22] Even more amazing is that the functionality reported in psammaplin G (**11**) has no precedent in natural products literature. Recent findings implicate an interesting relationship between DNMT and HDAC as epigenetic modifiers in the silencing of tumor supressor genes, but to date no single compound has been shown to exhibit dual activity for both of these distinct targets. For the first time, single compounds are reported herein which inhibit both of these enzymes. These dual-inhibiting compounds are psammaplin A (**4**) and bisaprasin (**5**). Furthermore, while psammaplin F (**10**) is selective for HDAC, psammaplin G (**8**) is selective for DNMT. The unique structural differences between these various psammaplins make them relevant for further SAR studies. In summary, two of the compounds of this study, psammaplin A (**4**) and bisaprasin (**5**), are μM inhibitors of both HDAC and DNMT and may be useful as biomolecular probes of epigenetic gene regulation pathways. Finally, the logical next step has been taken by the Novartis group to design a synthetic compound, NVP-LAQ824,[6b] based in part on the trichostatin A (**1**), tripoxin B (**2**), and psammaplin A (**4**) pharmacophores, and this HDA inhibitor has been advanced for an anticancer Phase I clinical trial.[23]

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 500 MHz for ^{1}H NMR and 125.7 MHz for ^{13}C NMR. Multiplicities of ^{13}C NMR were determined from APT data and HMQC (500 MHz). High-resolution mass spectra were recorded with quadrupole (FAB and ESI) and ESITOF instrumentation. Chromatography was performed with Sephadex LH20 and 5 μm C_{18} ODS columns. The IR data were recorded with FT instrumentation and the UV/vis data were obtained with photodiode array spectrophotometry.

Collection and Identification. Specimens were collected with SCUBA from two different sites in Papua New Guinea: coll. no. 96188 (300 g dry weight) from Bagabag Island in the Madang Lagoon, and coll. no. 98171 (500 g dry weight) from the Hermit Islands. A third specimen was also collected by SCUBA, coll. no. 00254 (200 g dry weight), from the east coast of Madagascar. All sponges were identified as *Pseudoceratina purpurea*[24] (order Verongida; family Aplisysinellidae) by Dr. M. C. Diaz (UCSC). The fresh sponges were preserved, transported to our lab, and extracted as previously described.[18] They were found growing on ledges as a dark purple conulose encrusting form, 0.5−1 cm in thickness. The color became dull brown above water. The texture was compressible, rubbery, and easily fragmented. Regular, raised (0.5−1 cm in height) membranous oscules were apparent underwater. The fibers are darkly pithed without bark (10−100-μm diameter). An underwater photograph and a voucher sample are available from PC (UCSC).

Extraction and Isolation. The methanol extract of each specimen was initially partitioned between water and methylene chloride. The resulting organic layer was further partitioned between MeOH (90% aq) and hexane, followed by partition between MeOH (50% aq) and CH_2Cl_2 to produce two fractions (FM and FD, respectively). The aqueous layer from the initial partition was extracted with butanol (WB). To complete the process of extraction, the methylene chloride soluble material from the residual sponge was partitioned between MeOH (90% aq) (DMM) and hexane (DMH). Yields obtained from this extraction were as follows: 96188 FD, 2248 mg; 96188 FM, 788 mg; 98171 WB, 12 g; 98171 DMM, 4.44 g.

The 96188 FD partition was subjected to Sephadex chromatography to yield 10 fractions. Fraction 10 was purified by reversed-phase HPLC to produce psammaplin A (**4**) (180.5 mg), psammaplin D (**8**) (6.8 mg), bisaprasin (**5**) (91.6 mg), psammaplin E (**9**) (4.0 mg), (3-bromo-4-hydroxy)benzaldehyde (**15**) (2.0 mg), and (3-bromo-4-hydroxyphenyl)acetonitrile (**16**) (3.5 mg). Following a similar purification procedure, the 96188 FM partition afforded psammaplin F (**10**) (20.3 mg). With use of the same strategy on the second collection, the 98171 WB partition afforded the known psammaplins B (**6**) and C (**7**), together with the new psammaplins G (**11**) (4.9 mg) and H (**12**) (3.7 mg). A similar procedure on 98171 DMM yielded the novel psammaplins I (**13**) (2.6 mg) and J (**14**) (3.1 mg).

Biological Evaluation. The *in vitro* cell proliferation assay was carried out as recently described.[15] The HDAC enzyme inhibition assay was also carried out as previously reported.[6] Semipurified DNMT-1 derived from chromatography at a final total protein concentration of 2 mg/mL was used for the enzyme assay. Preparations of partially purified DNMT-1 were thawed and mixed with test compounds or DMSO vehicle, biotinylated Poly(dI-dC)·Poly(dI-dC) substrate, and 50 mM Tris pH 8.0 buffer in a 96-well Isoplate. The methyl donor, *S*-adenosyl-L-[methyl-^{3}H]methionine, in buffer, was added to initiate the reaction. A typical reaction contained 4 μL of partially purified enzyme, 5 μL of vehicle or inhibitor, 1 μL of substrate, 30 μL of buffer, and 10 μL of ^{3}H-SAM for a final reaction volume of 50 μL. The reaction was run for 3.5 h at 37 °C. The reaction was stopped by adding 50 μL of streptavidin SPA beads diluted in deionized H_2O. The plates were briefly mixed and the beads were allowed a binding time of 30 min at room temperature. The plates were then centrifuged at 1000 g, and read on a *microplate* liquid scintillation and luminescence counter with a color/chemical quench and DPM correction. Control samples produced a signal resulting from the interaction of radiolabeled methylated oligo with the SPA bead. Wells with low signal were scored as inhibitors. An assay to determine DMSO sensitivity was run at a final concentration of 1 nM substrate, 0.25 mg/mL of partially purified enzyme, and 5 μM ^{3}H-SAM for 3.5 h at 37 °C, and stopped with SPA beads at a final concentration of 1.5 mg/mL. DMSO was titrated from a final concentration of 0.5% to 10%. DMSO had no significant effect on assay signal up to a final concentration of 10%. Compounds were screened at various concentrations to generate the IC_{50} values. As a positive control, COP-1, a known DNMT-1 inhibitor,[25] had an IC_{50} value of 160 nM in the assay.

Psammaplin E (9): UV λ_{max} (CH_3OH) 206 (ϵ 18070), 284 (1858). IR ν_{max} (film) 3500−3100 (br), 3327, 1668, 1539, 1420,

(21) Dumdei, E.; Andersen, R. J. *J. Nat. Prod.* **1993**, *56*, 792−794.
(22) Pettit, G. R.; Butler, M. S.; Williams, M. D.; Filiatrault, M. J.; Pettit, R. K. *J. Nat. Prod.* **1996**, *59*, 927−934.
(23) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cesarz, D.; Chandramouli, N.; Cheung, M.; Cornell, W.; Dean, K.; Green, M. A.; Howell, K. L.; Lassota, P.; Sorensen, E.; Sheng, T.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Wood, A. *Proc.92nd AACR* **2002,** *43***,** 740.
(24) For recent taxonomic revisions that involve previous names of Psammaplysilla to Druinella to Pseudoceratina see: Bergquist P. R. *Mem. Queensl. Mus.* **1995**, *38*, 1−51.
(25) van Putten A. J.; de Lang R.; Veltkamp E.; Nijkamp, H. J.; van Solingen, P.; van den Berg, J. A. *J. Bacteriol.* **1986**, *168*, 728−733.

1358, 1287, 1221, 1010, 985. HRFABMS *m/z* 479.0094 [M + H]$^+$ (Δ −3.5 mmu of calcd). See Table 1 for ^{1}H and ^{13}C NMR.

Psammaplin F (10): UV λ_{max} (CH_3OH) 206 (ϵ 25935), 280 (2057). IR ν_{max} (film) 3600−2600 (br), 1647, 1527, 1424, 1390, 1287, 1219, 1047, 1019, 985. HRFABMS *m/z* 501.9724 [M + Na]$^+$ (Δ 1.1 mmu of calcd). See Table 1 for ^{1}H and ^{13}C NMR.

Psammaplin G (11): IR ν_{max} (film) 3600−2600 (br), 1666, 1631, 1596, 1531, 1437, 1408, 1354, 1290, 1220, 1145, 1043, 1025, 996. HRFABMS *m/z* 494.0055 [M + H]$^+$ (Δ 3.4 mmu of calcd). See Tables 2 and 3 for ^{13}C and ^{1}H NMR.

Esterification of 11 To Form the Methyl Ester of Psammaplin G (11a). A solution of diazomethane was added to a cold ether solution of psammaplin G (**11**) (2 mg) and the mixture was stirred continuously for 8 h. After the removal of the solvent in vacuo, the crude oil was partitioned on a short Si gel column (2.5 g) eluted with methylene chloride. The ester of psammaplin G (**11a**) was obtained pure (1.5 mg) as a colorless oil. The properties of **11a** are as follows: ^{1}H NMR (500 MHz) (CD_3OD) δ 7.39 (1H, d, 2.0 Hz), 7.17 (1H, dd, 8.0, 2.0 Hz), 6.91 (1H, d, J = 8.0 Hz), 4.01 (2H, s), 3.82 (3H, s), 3.55 (4H, t, 7.0 Hz), 2.84 (4H, t, 7.0 Hz). LRESIMS showed the ^{79}Br/^{81}Br clusters *m/z* 508/510 [M + H]$^+$ and 506/508 [M + H]$^-$. HRFABMS *m/z* 530.0132 [M + Na]$^+$ ($C_{16}H_{22}N_5O_5S_2{}^{79}$BrNa, 530.0143 calcd).

Acetylation of 11 To Form Psammaplin G Acetate (11d). Psammaplin G (**11**) (1.5 mg) was treated with 1 mL of Ac_2O−pyridine (1:1) and left overnight at room temperature with magnetic stirring. After removal of the solvents in vacuo the crude oil was analyzed by NMR and MS, confirming the formation of the acetate derivative of psammaplin G (**11d**). The properties of **11d** are the following: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.85 (1H, d, 2.0 Hz), 8.47 (1H, dd, 8.0, 2.0 Hz), 7.91 (1H, d, J = 8.0 Hz), 3.96 (2H, s), 3.64 (2H, t, 7.0 Hz), 3.58 (2H, t, 7.0 Hz), 2.84 (4H, t, 7.0 Hz), 2.14 (3H, s). LRESIMS showed the ^{79}Br/^{81}Br clusters *m/z* 536/538 [M + H]$^+$ and 534/536 [M + H]$^-$. HRFABMS *m/z* 558.0060 [M + Na]$^+$ ($C_{17}H_{22}N_5O_6S_2{}^{79}$BrNa, 558.0092).

Psammaplin H (12): IR ν_{max} (film) 3500−3000 (br), 1660, 1531, 1419, 1384, 1360, 1260, 1231, 1037, 985. HRFABMS *m/z* 502.0102 [M + Na]$^+$ (Δ 2.0 mmu of calcd). See Tables 2 and 3 for ^{13}C and ^{1}H NMR.

Psammaplin I (13): IR ν_{max} (film) 3600−3000 (br), 1654, 1531, 1501, 1420, 1361, 1284, 1214, 1043, 973. HRFABMS *m/z* 400.9795 [M + Na]$^+$ (Δ 1.2 mmu of calcd). See Tables 2 and 3 for ^{13}C and ^{1}H NMR.

Psammaplin J (14): IR ν_{max} (film) 3600−2900 (br), 1660, 1625, 1531, 1502, 1449, 1425, 1361, 1287, 1284, 1213, 1149, 1044. HRFABMS *m/z* 700.9396 [M + Na]$^+$ (Δ 4.5 mmu of calcd). See Tables 2 and 3 for ^{13}C and ^{1}H NMR.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **9**, **10**, **13**, and **14**, ^{13}C NMR spectrum of **12**, HRFAB$^+$ MS and HMBC of **11**, and gHMQC of **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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