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Psammaplysin Derivatives from the Balinese Marine Sponge *Aplysinella strongylata*

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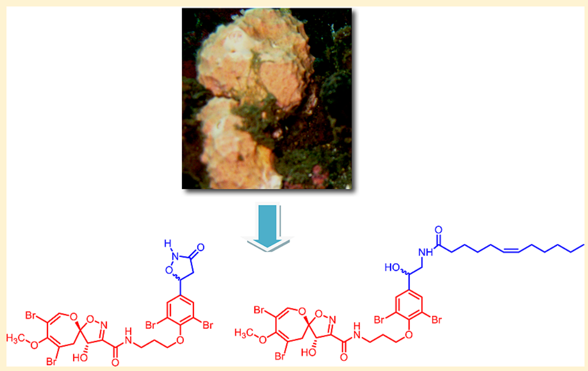
Supporting Information

ABSTRACT: Twenty-one new psammaplysin derivatives (**4**–**24**) exhibiting a variety of side chains, as well as six previously known psammaplysins, were identified from the Indonesian marine sponge *Aplysinella strongylata*. The double bond on the side chain of the fatty acid-containing psammaplysins was located by GC-MS analysis of the fatty acid methyl esters and their pyrrolidide derivatives. HPLC and Mosher ester studies confirmed that the isolated metabolites possessing a 19-OH substituent were mixtures of diastereomers. Selected compounds (**4**, **5**, **7**, **8**, **12**, **18**, and **22**) were screened for in vitro activity against chloroquine-sensitive (3D7) *P. falciparum* malaria parasites. Of the new psammaplysins, 19-hydroxypsammaplysin E (**4**) showed the best antimalarial activity, with an IC_{50} value of 6.4 μM.



Marine sponges of the order Verongida are well known to contain alkaloids derived from bromotyrosine.[1] One of the most interesting and bioactive categories of bromotyrosine-derived metabolites in these sponges is the small group of alkaloids possessing a spirooxepinisoxazoline moiety. To date, there have been 12 such bromotyrosine derivatives, named psammaplysins A–J and ceratinamides A and B, isolated from eight different sponge species. Some of these derivatives contain fatty acyl side chains.[2−9] Psammaplysin C was found to have moderate in vitro cytotoxicity against the human colon tumor cell line HCT116,[3] while psammaplysin D showed activity against a Haitian strain of HIV-1.[4] Psammaplysins G and H have shown promising activity toward *Plasmodium falciparum* malaria parasites.[6,7] We report here the isolation of additional members of the psammaplysin family, along with the known psammaplysins A (**1**), B (**2**), D, and E (**3**) and ceratinamides A and B, from an extract of the sponge *Aplysinella strongylata* (order Verongida, family Aplysinellidae), collected at Tulamben, Bali, Indonesia. Five of the new psammaplysins showed variation in the structural motif attached to C-16 of the aromatic ring, while the remaining compounds contained fatty acid side chains.

RESULTS AND DISCUSSION

The combined MeOH–CH_2Cl_2 extract of the frozen sponge was partitioned between H_2O and various organic solvents including hexanes, EtOAc, and BuOH (see Experimental Section). The EtOAc fraction was resolved by normal-phase Si gel chromatography and was subsequently purified by C_{18} HPLC to afford the known psammaplysins A (**1**), B (**2**), D, and E (**3**), ceratinamides A and B, and 21 new psammaplysin derivatives (**4**–**24**).

Psammaplysin Derivatives with Modified Aromatic Ring Substituents. 19-Hydroxypsammaplysin E (**4**) was obtained as a yellow oil. The presence of four bromine atoms in the molecule was defined from an ion cluster at m/z 874/876/878/880/882 $[M + Na]^+$ in the (+)-LRESIMS. This mass is 16 Da higher than that of psammaplysin E (**3**) and suggested an additional hydroxy group. A molecular formula of $C_{27}H_{25}Br_4N_3O_9$ was derived from (+)-HRESIMS, consistent with 15 double-bond equivalents (DBE).

The presence of the psammaplysin carbon framework in **4** could be deduced from ^{1}H and ^{13}C NMR chemical shifts (MeOH-d_4) compared to those of psammaplysin E (**3**) (Tables 1 and 2). A signal at δ_H 7.15 (1H, s) assigned to H-1 had HMBC correlations to signals at δ_C 103.5 (C-2), 148.9 (C-3), and 122.1 (C-6). Two distinctive geminal protons at δ_H 3.06 and 3.38 could be attributed to H-5a and H-5b, respectively. This AB system correlated to signals for C-4 (δ_C 105.7) and C-

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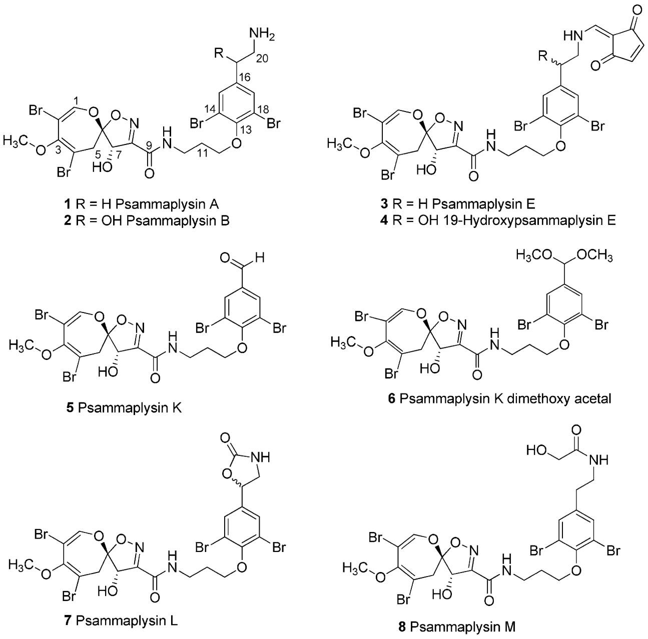

6 as well as to C-7 at δ_C 79.3, diagnostic for a hydroxymethine carbon. The H-7 signal appeared at δ_H 4.99 as an isolated singlet and correlated to an amide carbon atom at δ_C 159.1 (C-9). These assignments secured the presence of the spirooxepinisoxazoline system in **4**.[4] Furthermore, three mutually coupled methylenes at δ_H 3.62, 2.13, and 4.07 suggested the presence of a $-CH_2CH_2CH_2O-$ moiety attached to the amide bond of the spiro ring system. A singlet at δ_H 7.60 was designated to the symmetric aromatic ring protons H-15 and H-17. HMBC correlation between H-12 and C-13 (δ_C 153.2) secured the connection between the propyloxy side chain and the substituted aromatic ring.

The ^{1}H NMR spectra of **3** and **4** were consistent except for the signals corresponding to H-19 and H-20. In psammaplysin E, H-19 appeared at δ_H 2.83 (2H) and H-20 at δ_H 3.55 (2H); in contrast for **4**, H-19 was shifted to δ_H 4.77 (1H, dd, J = 3.7, 7.2 Hz), while H-20 appeared as an AB system [δ_H 3.42 (1H, dd, J = 7.2, 13.4 Hz) and δ_H 3.59 (1H, dd, J = 3.7, 13.4 Hz)]. Since the H-20 signals were partially obscured by those of H-5b and H-10, they were further resolved by a 1D TOCSY experiment involving irradiation of H-19. The chemical shift of C-19 was also shifted downfield from δ_C 36.0 in **3** to δ_C 71.8 in **4**, consistent with a C-19 hydroxy substituent, as found previously in both psammaplysins B and D.[2,4]

Psammaplysin K (**5**) was obtained as a colorless glass. The (+)-LRESIMS displayed a 1:4:6:4:1 ion cluster at m/z 737/739/741/743/745 $[M + Na]^+$, which indicated the presence of four bromine atoms. The HRESIMS analysis of **5** gave a quasimolecular ion ($M + H^+$) consistent with a molecular formula of $C_{20}H_{18}Br_4N_2O_7$ requiring 10 DBEs. The presence of the psammaplysin scaffold in **5** was evident from the ^{1}H NMR spectrum ($CDCl_3$), in addition to the associated HSQC and HMBC data. There were isolated protons at δ_H 7.02 and 8.03 (2H), two geminal protons at δ_H 3.12 and 3.37, an isolated methine at δ_H 5.14, three mutually coupled methylenes at δ_H 3.75, 2.22, and 4.23, and a methoxy group at δ_H 3.65. The most notable difference between **5** and **3** was the absence of two methylene signals attributed to H-19 and H-20 as well as the disappearance of the signals associated with the cyclopentenedione moiety. Instead, a signal at δ_H 9.86 linked to a carbon signal at δ_C 188.3 indicated the presence of a formyl moiety. HMBC correlations of the formyl proton to both C-15 and C-17, and from H-15/H-17 to C-19, confirmed the

Table 1. ^{1}H NMR Assignments for 3−8

position	δ_H (J in Hz) **3**[a]	δ_H (J in Hz) **4**[b]	δ_H (J in Hz) **5**[a]	δ_H (J in Hz) **6**[a]	δ_H (J in Hz) **7**[c]	δ_H (J in Hz) **8**[c]
1	7.02, s	7.15, s	7.02, s	7.02, s	7.18, s	7.18, s
5	3.12, d (16.0)	3.06, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.14, d (16.2)
	3.37, d (16.0)	3.38, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.45, d (16.2)
7	5.13, s	4.99, s	5.14, s	5.13, s	5.08, s	5.08, d (7.0)
10	3.72, dt (6.4, 6.5)	3.62, t (6.9)	3.75, q (6.5)	3.74, q (6.3)	3.66, m	3.64, m
11	2.12, m	2.13, m	2.22, m	2.12, m	2.12, m	2.15, m
12	4.09, t (5.6)	4.07, t (5.9)	4.23, t (5.6)	4.11, t (5.6)	4.15, t (6.0)	4.09, t (6.1)
15	7.31, s	7.60, s	8.03, s	7.61, s	7.72, s	7.50, s
17	7.31, s	7.60, s	8.03, s	7.61, s	7.72, s	7.50, s
19	2.83, t (6.9)	4.77, dd (3.7, 7.2)		5.31, s	5.65, t (8.3)	2.80, t (6.8)
20	3.55, q (6.9)	3.42, dd (7.2, 13.4)[d]			3.53, m	3.37, q (6.8)
		3.59, dd (3.7, 13.4)[d]			4.05, t (8.3)	
21	7.17, s	7.35, s				
22						3.55, s
24	6.71, d (6.2)	6.69, d (6.2)				
25	6.77, d (6.2)	6.76, d (6.2)				
7-OH	3.89, brs				6.01, d (7.1)	6.02, d (7.0)
3-OCH_3	3.69, s	3.65, s	3.65, s	3.69, s	3.65, s	3.65, s
19-OCH_3				3.32, s		
C-9NH	7.20, t (6.5)		7.10, t (6.5)	7.19, t (6.3)	7.85, brs	7.87, brs
C-20NH	8.38, brt (6.9)					
C-21NH					8.01, s	8.01, s
CHO			9.86, s			

[a]Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.26) at 500 MHz. [b]Chemical shifts (ppm) referenced to CH_3OH (δ_H 3.31) at 500 MHz. [c]Chemical shifts (ppm) referenced to acetone (δ_H 2.05) at 500 MHz. [d]Chemical shifts and multiplicity were resolved by 1D TOCSY experiment.

Table 2. ^{13}C NMR Assignments for 3−8

position	3[a]	4[b]	5[a]	6[d]	7[c]	8[c]
1	145.4	145.2	145.2	145.6	146.4	146.4
2	103.4	103.5	105.8	105.8	103.6	103.6
3	148.6	148.9	149.0	148.9	149.4	149.4
4	105.4	105.7	103.5	103.3	104.2	104.2
5	37.1	37.1	37.2	37.1	37.9	37.6
6	122.0	122.1	122.7	122.3	120.0	120.0
7	79.5	79.3	79.5	79.3	80.2	80.2
8	155.8	156.2	156.1	155.9	158.5	158.6
9	159.0	159.1	159.2	159.1	159.1	159.1
10	37.1	37.1	37.2	37.1	37.5	37.6
11	29.2	29.1	29.2	29.3	30.0	30.1
12	71.1	70.9	71.3	70.8	72.2	72.1
13	151.9	153.2	157.6	152.5	154.0	151.9
14	118.6	118.5	119.6	118.0	119.1	118.5
15	133.1	130.2	133.9	131.4	131.3	134.1
16	135.9	142.3[e]	135.8	134.3	140.0	139.9
17	133.1	130.2	133.9	131.4	131.3	134.1
18	118.6	118.5	119.6	118.0	119.1	118.5
19	36.0	71.8	188.3	101.1	75.9	35.1
20	50.6	56.3			48.5	42.2
21	148.7	149.3			159.1	157.6
22	99.5	99.8				51.5
23	197.8	197.9				
24	142.3	142.1				
25	142.1	142.2				
26	194.1	194.4				
3-OMe	59.1	59.0	58.8	58.9	59.1	59.1
19-OMe				52.6		

[a]Chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.16) at 400 MHz. [b]Chemical shifts (ppm) referenced to CD_3OD (δ_H 49.0) at 400 MHz. [c]Chemical shifts (ppm) referenced to acetone-d_6 (δ_C 29.84) at 400 MHz. [d]Chemical shifts (ppm) taken from HSQC and HMBC spectra referenced to $CDCl_3$ (δ_C 77.16).

substitution of the formyl group at C-16; thus the structure **5** was assigned to psammaplysin K.

Psammaplysin K dimethoxy acetal (**6**), obtained as a colorless glass, had a molecular formula of $C_{22}H_{24}Br_4N_2O_8$ determined by (+)-HRESIMS. The ^{1}H NMR spectrum (MeOH-d_4) of **6** again displayed signals for a spirooxepinisoxazoline moiety. Comparison of the ^{1}H and ^{13}C NMR data of **6** with those of **5** revealed a high degree of structural similarity, but the key difference was that the diagnostic formyl signal in **5** was replaced by a signal at δ_H 3.32 (6H) that was attributed to two methoxy groups. Moreover, acetal functionality could be inferred from signals for H-19 at δ_H 5.31 and C-19 at δ_C 101.1. However, **6** was suspected to be an artifact produced during the chromatographic separation since the diagnostic signal corresponding to the dimethoxy group was absent in the ^{1}H NMR spectrum of the fraction from which **6** was isolated. This was also confirmed by the absence of an ion cluster at m/z 783/785/787/789/791 [M + Na]$^+$ corresponding to **6** in the LRESIMS data of the fraction prior to RP HPLC separation.

Psammaplysin L (**7**) was isolated as a colorless oil by RP HPLC. The (+)-LRESIMS analysis revealed a cluster of ions at m/z 794/796/798/800/802 [M + Na]$^+$ consistent with a molecular formula of $C_{22}H_{21}N_3Br_4O_8$ by HRESIMS measurement. Analysis of the ^{1}H NMR, HSQC, and HMBC data (acetone-d_6) indicated that the molecule also contained the signals anticipated for a spirooxepinisoxazoline carbon framework. The H-10 signal overlapped with the signal of the methoxy group at δ_H 3.66; therefore its chemical shift and multiplicity were resolved by 1D TOCSY irradiation of the H-11 signal at δ_H 2.12. The remaining structural fragment in **7** was determined to be a 2-oxazolidinone group from ^{13}C NMR and HMBC data. A triplet signal resonating at δ_H 5.65 assigned to H-19 had HMBC correlations to the aromatic carbons C-15/C-17 and to a carbonyl at δ_C 159.1 assigned to C-21 (Figure 1).

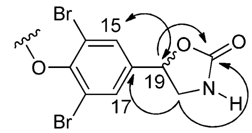

Figure 1. Selected HMBC correlations of the 2-oxazolidinone fragment of **7**.

An AB system at δ_H 4.05 and 3.53 attributed to a methylene group (H-20a and H-20b) showed HMBC correlations to the quaternary carbon C-16 as well as to C-21. These data for the oxazolidinone ring matched well with those of the derivative prepared from xestoaminol A isolated from *Xestospongia* sp.[10] and of oxazolidinone derivatives from halaminols from *Haliclona* n. sp.[11] The configuration at C-19 was not determined.

Psammaplysin M (**8**) was isolated as a colorless oil by RP HPLC. Accurate mass determination of **8** confirmed a formula of $C_{23}H_{25}N_3Br_4O_8Na$ at m/z 813.8226 [M + Na]$^+$. The ^{1}H NMR, HSQC, and HMBC data clearly suggested the presence of the same spirooxepinisoxazoline carbon framework as in **7**. Again, the chemical shift and the multiplicity of the H-10 signal were resolved by a 1D TOCSY experiment due to overlap with the methoxy signal at δ_H 3.64. The most prominent differences in the ^{1}H NMR spectra of **7** and **8** were the signals associated with H-19 and H-20. In **7**, the signal corresponding to H-19 appeared at δ_H 5.65 (1H, t), while in **8** it was drastically shifted upfield to δ_H 2.80 (2H, t), where it overlapped with the residual water signal.[12] The chemical shift and its multiplicity were resolved by 1D TOCSY irradiation of H-20, a quartet at δ_H 3.37 in contrast to the two signals observed for H-20 in **7**. An important structural hint was also seen from the ^{13}C NMR data. The signal corresponding to C-19 in **7** was shifted from δ_C 75.9 to δ_C 35.1 in **8**. This inferred that C-19 in **8** no longer had an adjacent oxygen substituent, and as a consequence the AB system was absent from the spectrum.

The side chain in **8** was proposed to be a glycolamide[12] based on the above NMR data and additional HMBC correlations (Figure 2). The H-20 signal was found to correlate to the quaternary aromatic carbon at δ_C 139.9 (C-16) and to the amide carbonyl at δ_C 157.6. Furthermore, H-15 showed HMBC correlations to C-19, and vice versa. A signal corresponding to H-22 correlated only to the carbonyl at C-21.

Psammaplysin Derivatives Containing Saturated Fatty Acid Side Chains. Psammaplysin N (**9**) displayed a

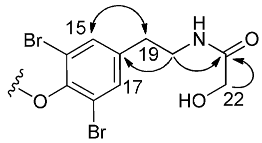

Figure 2. Selected HMBC correlations of the glycolamide fragment of psammaplysin M (**8**).

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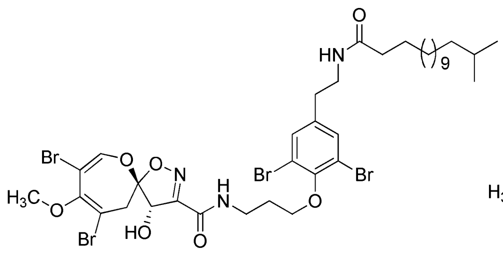
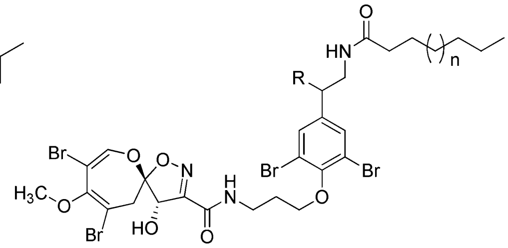
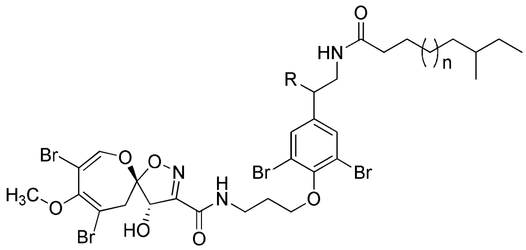
Chart 1



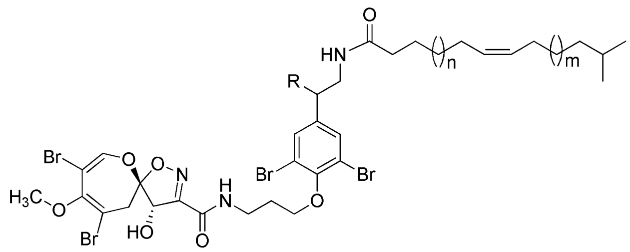
9 Psammaplysin N



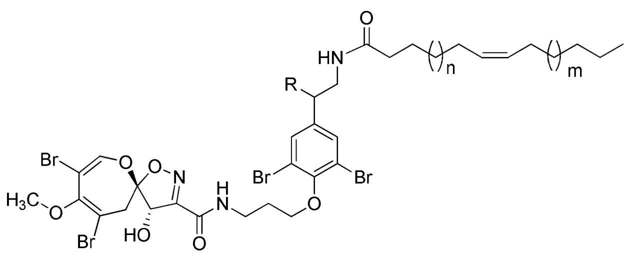
10 R = H, n = 10 Psammaplysin O
11 R = H, n = 12 Psammaplysin P
12 R = OH, n = 12 19-Hydroxypsammaplysin P
13 R = H, n = 8 Psammaplysin Q
14 R = OH, n = 8 19-Hydroxypsammaplysin Q



15 R = OH, n = 8 Psammaplysin R
16 R = H, n = 9 Psammaplysin S
17 R = OH, n = 9 19-Hydroxypsammaplysin S
18 R = H, n = 11 Psammaplysin T
19 R = OH, n = 11 19-Hydroxypsammaplysin T



20 R =H, n = 5, m = 1 Psammaplysin U
21 R = OH, n = 5, m = 1 19-Hydroxypsammaplysin U

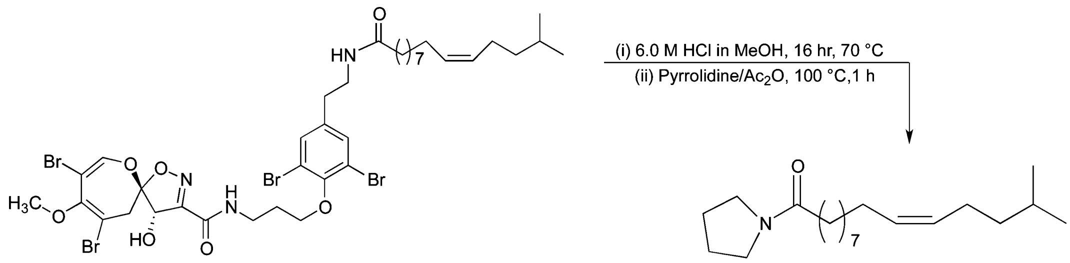


22 R = H, n = 5, m = 1 Psammaplysin V
23 R = H, n = 8, m = 1 Psammaplysin W
24 R = OH, n = 8, m = 1 19-Hydroxysammaplysin W

1:4:6:4:1 ion cluster at m/z 990/992/994/996/998 $[M + Na]^+$, and the molecular formula was defined to be $C_{37}H_{53}N_3Br_4O_7$ from the (+)-HRESIMS. This is 14 Da higher (equivalent to one methylene) than those of 19-deoxypsammaplysin D and ceratinamide B.[9] The ^1H /^{13}C NMR data ($CDCl_3$) of **9** matched those of the known ceratinamide B including the data corresponding to the spirooxepinisoxazoline carbon framework as well as to an *iso* fatty acid chain. The presence of a doublet signal at δ_H 0.86 (6H) further confirmed the presence of an *iso*-branched fatty acyl substituent. The GC-MS chromatogram of the corresponding fatty acid methyl ester (FAME) derivative of **9** showed a single peak with m/z 256. The diagnostic fragment ions at m/z 241 $[M^+ - 15]$, 225 $[M^+ - 31]$, 213 $[M^+ - 43]$, and 74 (McLafferty) indicated a 13-methyltetradecanoate fatty acid ester (*iso* 15:0).

The next group of psammaplysin derivatives identified from this sponge extract all contained saturated straight-chain fatty acids (**10**−**14**). Psammaplysin O (**10**) displayed a molecular formula of $C_{37}H_{53}O_7N_3Br_4$, and its ^1H NMR data were similar to those of **9** except that the terminal methyl signal in **10** appeared as a triplet at δ_H 0.88 (3H) and so confirmed a linear fatty acid chain. FAME analysis concluded that **10** contained a 16:0 straight-chain fatty acid residue. Psammaplysin P (**11**) was found to return a molecular formula of $C_{39}H_{57}O_7N_3Br_4$, i.e., a molecular mass 28 Da larger than in **10**, suggesting the presence of two additional methylenes in the fatty acid component. GC-MS analysis of the associated FAME derivative indicated the presence of a C18:0 fatty acid chain attached to the psammaplysin structure. Metabolite **12** gave an adduct ion peak at m/z 1034.0771 $[M + Na]^+$, larger than that of **11** by 16 Da, suggesting the presence of an additional oxygen in the molecule. The ^1H and 2D NMR data of **12** were similar to those in **11** except for the signals attributed to H-19 and H-20. The signal for H-19 was a doublet of doublets at δ_H 4.81 (1H, J = 3.0, 6.5 Hz), and H-20 presented as an AB system at δ_H 3.31 (dd) and 3.67 (dd). These data indicated that a hydroxy group was present at C-19; thus **12** was determined to be the 19-hydroxy derivative of **11**. Previous research has identified the *iso* C15:0-containing psammaplysin D, which also has a 19-hydroxy substituent.[4] Similarly, **12**, with a molecular formula of $C_{35}H_{49}O_8N_3Br_4$, was deduced as the 19-hydroxy derivative of **13**. Compounds **13** and **14** displayed molecular formulas of $C_{35}H_{49}O_7N_3Br_4$ and $C_{35}H_{49}O_8N_3Br_4$, respectively, which revealed an additional hydroxy group in **14** compared to **13**. The ^1H and 2D NMR data of **14** matched a 19-hydroxy derivative of **13**. Both compounds yielded a C14:0 straight-chain fatty acid on GC-MS analysis of their FAME derivatives.

The next group of psammaplysin derivatives (**15**−**19**) all contained an *anteiso*-branched saturated fatty acid. Psammaplysin R (**15**) was obtained as a colorless oil by RP HPLC with



(21)

Figure 3. Preparation of FAME and pyrrolidide derivatives of **21**.

a molecular formula of $C_{37}H_{53}O_8N_3Br_4$ obtained from HRESIMS data. The ^{1}H and 2D NMR data of **15** displayed a high degree of similarity with those of **14**, except that the signal attributed to the terminal methyl of the linear fatty acyl chain was absent; instead there were methyl signals at δ_H 0.88 (t) and at δ_H 0.83 (d). HMBC correlations on the fatty acid side chain provided further support for this *anteiso* assignment. $^3J_{CH}$ HMBC correlations were observed between the terminal triplet methyl at δ_H 0.88 and the methylene carbon at δ_C 22.5 (C-34) and the methine carbon at δ_C 31.9 (C-33). FAME analysis confirmed that **15** is a psammaplysin derivative featuring an *anteiso* C16:0 fatty acid chain. A pair of psammaplysins, **16** and **17**, both shared the same *anteiso* C17:0 fatty acid chain, but differed in that **17** had a 19-hydroxy group. These two compounds showed almost superimposable ^{1}H NMR signals, except for those of H-19 and H-20. 19-Hydroxypsammaplysin S (**17**) displayed a 1:4:6:4:1 ion cluster at m/z 1020/1022/1024/1026/1028 $[M + Na]^+$, which is 16 Da larger than that of **16**. The molecular formulas of **16** and **17** were deduced from (+)-HRESIMS as $C_{38}H_{55}O_7N_3Br_4$ and $C_{38}H_{55}O_8N_3Br_4$, respectively. A final pair of compounds, **18** and **19**, possessed similar ^{1}H NMR and 2D NMR data including the signals corresponding to an *anteiso*-branched C19:0 fatty acyl side chain, but differed in the signals of H-19 and H-20. A molecular formula of $C_{40}H_{59}O_8N_3Br_4$ was deduced for **19** by HRESIMS.

Psammaplysin Derivatives Containing Monoenoic Fatty Acid Side Chains. Five new psammaplysin derivatives containing a monoenoic fatty acid side chain were isolated from the sponge extract. Two of them (**20** and **21**) contained an *iso*-branched fatty acid, and the rest (**22**–**24**) featured unbranched chain fatty acids. Psammaplysin U (**20**) showed a molecular formula of $C_{38}H_{53}Br_4N_3O_7$, while **21** was the 19-hydroxy derivative of **20** due to a molecular weight 16 Da larger than that of **20**. The presence of the hydroxy group at C-19 in **21** was again evident from the ^{1}H and ^{13}C NMR chemical shifts of the signals at C-19 and C-20. In addition, an *iso*-branched fatty acid was inferred from a signal at δ_H 0.86 (d) integrating for six protons. In the side chain, an olefinic functionality was apparent from ^{1}H NMR signals at δ_H 5.36; a *Z* geometry was inferred from the carbon chemical shifts of the adjacent vinylic carbon atoms, which were less than 30 ppm.[13] GC-MS analysis of the FAME derivative of **21** revealed a molecular ion at m/z 282 accompanied by ions at m/z 250 [M − 32] and m/z 208 [M − 74; McLafferty] as well as a base peak at m/z 74. The location of the double bond was deduced from the GC-MS pattern of the corresponding pyrrolidide derivative (Figure 3), which showed a molecular ion peak at m/z 321 together with the characteristic base peak of a pyrrolidide derivative [m/z 113] formed by a McLafferty rearrangement.[14] By implementing the rule formulated by Anderson and Holman,[15] the double bond in **21** was located between C-10 and C-11.

The relative configuration of psammaplysin A (**1**) has been determined as 6*R**,7*S** by single-crystal X-ray crystallographic analysis of psammaplysin acetamide acetate.[2] By chemical shift comparison, and also based on ROESY correlation data and modeling studies,[8] other known psammaplysins have been shown to possess the same relative configuration. The close similarity of ^{1}H and ^{13}C NMR shifts with those of known psammaplysins supports a 6*R**,7*S** configuration for the spirooxepinisoxazoline ring in psammaplysins **4**–**24**.

The specific rotations of psammaplysin A (**1**), psammaplysin B (**2**), **5**, and **6** were calculated as follows: **1** $[\alpha]^{24}_D = -57.8$ (*c* 0.32, MeOH); **2** $[\alpha]^{24}_D = -55.6$ (*c* 0.28, MeOH); **5** $[\alpha]^{24}_D = -16$ (*c* 0.09, $CHCl_3$); **6** $[\alpha]^{24}_D = -15$ (*c* 0.03, $CHCl_3$). The specific rotations of previously isolated psammaplysins are all negative;[2−8] thus it is likely that the various psammaplysins all share the same absolute configuration. Both psammaplysins and related spirocyclohexadienyloxazolines may derive biosynthetically from the same bromotyrosine precursor as shown in Figure 4. The spirocyclohexadienyloxazolines may derive from nucleophilic attack of the oxime hydroxy group directly onto the arene oxide (path a) with C−O bond cleavage and protonation, giving a hydroxy group at C-1.[16] In contrast, the psammaplysin skeleton may be derived by a two-step process involving first arene oxide−oxepin rearrangement,[2] then a ring closure that involves protonation of a double bond and nucleophilic attack by the oxime hydroxy group. Alternatively in a concerted mechanism, as shown in path b of Figure 4, the psammaplysin skeleton could be generated by hydroxy attack on the epoxide, with simultaneous C−C bond cleavage, and protonation at C-5. Whereas the stereochemical consequences of path a are well understood, the stereochemical implications of path b (or an equivalent two-step mechanism) are not as predictable. The ECD spectrum of psammaplysin A (**1**) when run in MeOH showed a positive Cotton effect at 240 nm. The relationship between ECD data and absolute configuration in bromotyrosine-derived spirocyclohexadienyloxazolines is well documented.[17−21] Despite the close structural similarities of the

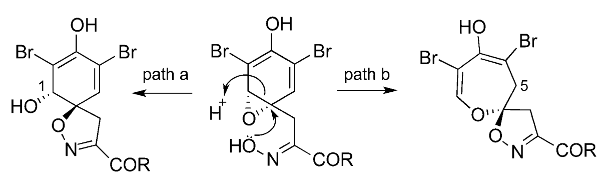


Figure 4. Proposed biosynthesis of spirooxepinisoxazoline and spirocyclohexadienyl-oxazoline ring systems (arrows show proposed mechanistic steps for path b).

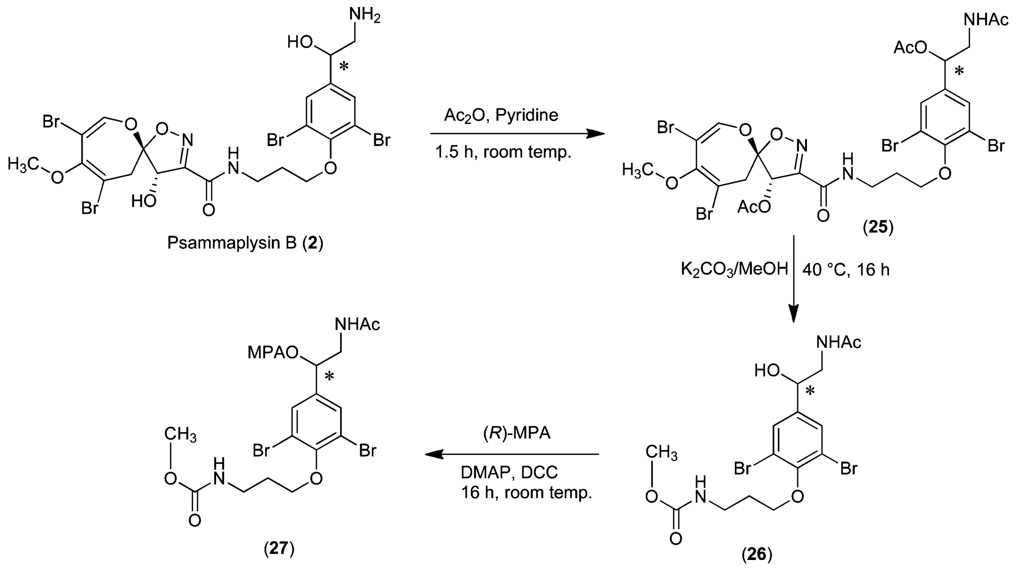
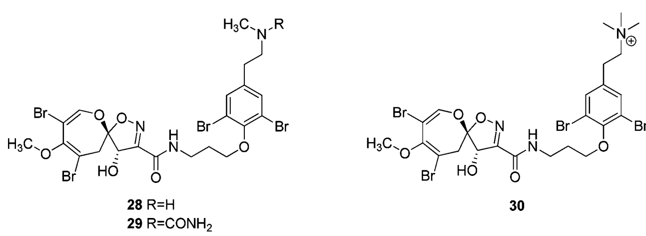


Figure 5. Acetylation, hydrolysis, and MPA ester analysis of psammaplysin B (**2**).

spirocyclohexadienyloxazoline and psammaplysin chromophores, the experimental ECD data do not necessarily secure the absolute configuration of the psammaplysins due to the greater conformational flexibility of the spirocycloheptadiene ring system compared to the spirocyclohexadienes.

The absolute configuration of psammaplysin A was not pursued in the original X-ray crystallographic work due to inferior crystal quality.[2] In principle, this information is accessible if the diffraction data are collected using Cu Kα radiation at low temperature.[22] Acetylation of psammaplysin A (**1**) or B (**2**) gave the known psammaplysin A acetamide acetate and psammaplysin B acetamide diacetate (**25**), respectively,[2] but neither product produced crystals suitable for X-ray crystallography. HPLC of **25** using a DAICEL Chiralpak AD column with UV detection at 254 nm revealed two components, each possessing a negative optical rotation, and therefore suggesting the presence of diastereomers differing in configuration at C-19. Hydrolysis of **25** gave carbamate **26**, the 19-OH analogue of a carbamate product that had been characterized in the original report of Kashman et al., although their proposed structure was incorrect.[23] Carbamate **26** had a specific rotation close to zero, in agreement with it being a racemate; however HPLC as above failed to differentiate the two enantiomers of **26**. Preparation of the (*R*)-MPA ester derivative of **26** was undertaken in an attempt to establish the presence of diastereomers differing in configuration at C-19; however the ^{1}H NMR spectra of the reaction product did not clearly show the presence of diastereomers. Attempts to purify individual (*R*)-MPA ester derivatives were complicated by their ease of hydrolysis.

Owing to recent literature reports detailing the antimalarial activity of psammaplysins F (**28**), G (**29**), and H (**30**),[6,7] we tested the more abundant metabolites (>2 mg) from this study (**4**, **5**, **7**−**9**, **12**, **18**, **22**) in an in vitro growth inhibition assay using the chloroquine-sensitive *P. falciparum* 3D7 malaria parasite line. Psammaplysins F, G, and H had been previously shown to display IC_{50} values of 1.92, 5.22, and 0.41 μM, respectively, against *P. falciparum* 3D7 parasites.[7] In this study, compounds **4**, **5**, **7**−**9**, **12**, **18**, and **22** were initially screened at 10 μM against the same *P. falciparum* line; however only compound **4** showed any inhibition of parasite growth at this concentration. Further biological evaluation of **4** showed an IC_{50} value of 6.4 (±1.4) μM.



The biological data of **4**, in conjunction with the reported antimalarial data for psammaplysins F (**28**), G (**29**), and H (**30**), clearly identify the importance of the *N*-substitution of the ethylamino moiety to antimalarial activity. Furthermore, the data indicate that the replacement of an amine, urea, or enamine derivative with a secondary amide functionality adversely effects antiparasite activity. However, the higher lipophilicity (i.e., log P) and larger molecular weights associated with the new amide psammaplysin analogues (**8**, **9**, **12**, **18**, **22**) tested in this study would also minimize bioavailability,[24] thus reducing the biological activity. Further analogues of this structure class are required in order to elucidate more detailed structure−activity relationships.

■ CONCLUSIONS

In this study, 21 new psammaplysin derivatives were identified from an extract of the marine sponge *A. strongylata* collected from Bali. A group of psammaplysins including the 19-hydroxy derivative of psammaplysin E (**4**) together with four derivatives containing modified side chains (**5**−**8**) were identified. Another group of psammaplysin derivatives containing an unbranched chain, an *iso*- or *anteiso*-branched chain, or monoenoic fatty acid (**9**−**24**) side chains were also isolated. An HPLC study using a chiral column revealed that psammaplysin B acetamide diacetate (**25**) was a mixture of diastereomers differing in configuration at C-19. Hydrolysis of **25** gave a racemic carbamate product (**26**). 19-Hydroxypsammaplysin E (**4**) displayed modest in vitro growth inhibition of chloroquine-sensitive *P. falciparum* parasites with an IC_{50} value of 6.4 μM.

Table 3. ^{1}H NMR Assignments for 9−16^{a}

position	9	10	11	12	13	14	15	16
1	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s
5	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)	3.12, d (16.0)
	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)	3.37, d (16.0)
7	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s
10	3.74, q (6.5)	3.74, q (6.0)	3.74, q (6.2)	3.73, q (6.5)	3.73, q (6.5)	3.73, q (6.2)	3.74, q (6.2)	3.74, q (6.2)
11	2.14, m	2.14, m	2.13, m	2.12, m	2.14, m	2.12, m	2.11, m	2.12, m
12	4.09, t (5.5)	4.09, t (5.5)	4.09, t (5.6)	4.10, t (5.6)	4.09, t (5.5)	4.10, t (5.6)	4.10, t (5.6)	4.09, t (5.6)
15	7.34, s	7.34, s	7.34, s	7.52, s	7.34, s	7.52, s	7.52, s	7.34, s
17	7.34, s	7.34, s	7.34, s	7.52, s	7.34, s	7.52, s	7.52, s	7.34, s
19	2.74, t (6.8)	2.75, t (6.8)	2.74, t (6.8)	4.81, dd (3.0, 6.5)	2.74, t (6.8)	4.81, dd (2.5, 6.5)	4.81, dd (2.5, 7.0)	2.74, t (6.9)
20	3.46, q (6.8)	3.46, q (6.8)	3.46, q (6.8)	3.31, dd (3.0, 13.0)	3.45, q (6.8)	3.31, dd (2.5, 13.0)	3.31, dd (2.5, 13.0)	3.45, q (6.9)
				3.65, dd (6.5, 13.0)		3.65, dd (6.5, 13.0)	3.65, dd (7.0, 13.0)	
22	2.14, m	2.14, m	2.14, m	2.21, t (6.0)	2.14, m	2.21, t (6.0)	2.21, t (5.6)	2.14, m
23	1.60, m^{b,c}	1.61, m^{b,c}	1.60, m^{b,c}	1.63, m^{b,c}	1.61, m^{b,c}	1.64, m^{b,c}	1.62, m^{b,c}	1.60, m^{b,c}
24−31	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
32	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.26, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
33	1.15, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.29, m^{b,c}	1.28, m^{b,c}	1.26, m^{b,c}	1.25−1.28, m^{b,c}
34	1.51, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	0.88, t (6.5)	0.88, t (6.7)	0.86, m	1.26, m^{b,c}
							1.28, m	
35	0.86, d (7.0)	1.29, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}			0.88, t (6.8)	0.85, m
								1.28, m
36	0.86, d (7.0)	0.88, t (6.5)	1.25, m^{b,c}	1.26, m^{b,c}			0.83, d (6.8)	0.88, t (6.7)
37			1.28, m^{b,c}	1.29, m^{b,c}				0.83, d (6.7)
38			0.88, t (6.7)	0.88, t (6.8)				
OCH_3	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s
C-9NH	7.19, t (6.5)	7.19, t (6.0)	7.20, t (6.2)	7.19, t (6.5)	7.19, t (6.5)	7.19, t (6.2)	7.19, t (6.2)	7.20, t (6.2)
C-21NH	5.44, t (6.5)	5.84, t (6.5)	5.43, t (6.4)	5.84, t (6.0)	5.44, t (6.5)	5.83, t (6.0)	5.83, t (5.9)	5.86, t (6.0)

[a]Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.26) at 500 MHz. [b]Unresolved chemical shifts due to overlapping signals. [c]Signal multiplicity unresolved due to overlapping signals.

■ EXPERIMENTAL SECTION

General Experimental Procedures. All NMR spectra were referenced to solvent signals as follows: δ 7.26 and 77.16 ppm for $CDCl_3$; δ 2.05 and 29.84 for acetone-d_6; and δ 3.31 and 49.00 ppm for methanol-d_4. 1D and 2D NMR spectra were acquired using a Bruker Avance 400 or a Bruker Avance 500 spectrometer at 298 K. Optical rotations were obtained using a Jasco P2000 polarimeter. The electronic circular dichroism spectra were run on a Jasco J-710 spectrophotometer in MeOH solution. Positive ion electrospray mass spectra (LRESIMS) were determined using a Bruker Esquire HCT or (HRESIMS) using a MicroTof Q instrument each with a standard ESI source. Reversed-phase HPLC was carried out on an Agilent 1100 Series instrument fitted with a variable-wavelength UV and refractive index detector, an Agilent D1311A quaternary pump, and a semipreparative Phenomenex C_{18} Gemini 5 μm 110 Å column (10 mm × 250 mm). Analytical HPLC was performed on an Agilent 1200 Series liquid chromatograph system equipped with both a UV detector (set at 254 nm) and an ALP detector (Advanced Laser polarimeter, PDR-Chiral Inc.) using a Chiralpak AD column (4.6 × 250 mm, DAICEL Chemical IND, LDT) and with a gradient of ^{i}PrOH−hexanes (5 to 40% ^{i}PrOH) at a flow rate of 0.5 mL per min. Normal-phase flash column chromatography was performed by wet-packing a glass column, containing a glass frit, to give a column bed height of 18 cm. The column packing used was silica gel 60 (40−63 μm; Scharlau). Gas chromatography was carried out on a Shimadzu GCMS-QP2010 Plus. Initial temperature was 100 °C, isothermal for 3 min, then ramped 16 °C/min for 10 min. The final temperature was 270 °C, injection temperature 250 °C, and flow rate 1.5 mL/min.

Biological Material. The sponge *Aplysinella strongylata* was harvested in Tulamben Bay, Bali, at a depth of approximately 20 m by hand using scuba in November 2010. The sponge was spherical in shape, fleshy to the touch, and compressible. The color in life was milky-colored inside and gray outside, compressible with mucus secretion, turning deep brown in 70% EtOH preservative. The sponge identification was undertaken at the Research Center of Oceanography, Indonesian Institute of Science, where a voucher specimen (TL-20) is deposited.

Extraction and Purification. The freshly collected sponge (147 g wet wt) was frozen before being extracted with 1:1 DCM−MeOH (3 × 200 mL) at room temperature. The combined extracts were dried under vacuum to produce 64.5 g (wet wt) crude extract. The crude extract was sequentially partitioned between hexanes (3 × 200 mL) and H_2O, followed by EtOAc and H_2O (3 × 200 mL), and finally BuOH and H_2O (3 × 200 mL). This gave hexanes (1.2 g), EtOAc (3.1 g), and BuOH (0.84 g) fractions, respectively. A portion of the EtOAc fraction (3.1 g) was resolved by normal-phase VLC (3 × 10 cm in diameter) with step gradient elution from 100% hexanes to DCM, EtOAc, and 100% MeOH and gave 11 fractions. Based on TLC analysis, the fourth and the sixth fractions were combined to give a 433 mg fraction coded as TL-20EV4-6, while the seventh until the 11th fractions were grouped to produce a 2.1 mg fraction coded as TL-20EV7-11. Subsequent normal-phase flash column chromatography on the more polar fraction TL-20EV7-11 employing step gradient elution from 100% DCM to 100% MeOH gave nine fractions. These fractions were combined on the basis of their TLC profile to give five fractions, coded as TL-20EV7-11F2-3 (47.0 mg), TL-20EV7-11F4 (902 mg), TL-20EV7-11F5 (636 mg), TL-20EV7-11F6 (433 mg), and TL-

Table 4. ^{1}H NMR Assignments for 17−24^{a}

position	17	18	19	20	21	22	23	24
1	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s	7.02, s
5	3.11, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)	3.12, d (16.0) 3.37, d (16.0)
7	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s	5.13, s
10	3.74, q (6.0)	3.74, q (6.2)	3.74, q (6.0)	3.75, q (6.2)	3.74, q (6.0)	3.75, q (6.2)	3.74, q (6.2)	3.75, q (6.1)
11	2.12, m	2.13, m	2.13, m	2.13, m	2.13, m	2.14, m	2.12, m	2.13, m
12	4.09, t (5.5)	4.09, t (5.5)	4.09, t (5.5)	4.09, t (5.6)	4.09, t (5.5)	4.09, t (5.5)	4.09, t (5.6)	4.10, t (5.6)
15	7.51, s	7.34, s	7.51, s	7.34, s	7.51, s	7.34, s	7.34, s	7.34, s
17	7.51, s	7.34, s	7.51, s	7.34, s	7.51, s	7.34, s	7.34, s	7.34, s
19	4.80, dd (2.5, 7.0)	2.74, t (7.0)	4.80, dd (3.0, 7.0)	2.74, t (6.9)	4.80, dd (2.5, 7.0)	2.74, t (6.8)	2.74, t (6.8)	4.80, dd (2.5, 7.0)
20	3.31, dd (2.5, 12.5) 3.65, dd (7.0, 12.5)	3.46, q (7.0)	3.30, dd (3.0, 12.5) 3.66, dd (7.0, 12.5)	3.45, q (6.9)	3.30, dd (2.5, 12.5) 3.66, dd (7.0, 12.5)	3.45, q (6.8)	3.46, q (6.8)	3.30, dd (2.5, 12.7) 3.66, dd (7.0, 12.7)
22	2.20, t (7.0)	2.13, m	2.13, m	2.13, m	2.21, m	2.14, m	2.14, m	2.13, m
23	1.62, m^{b,c}	1.60, m^{b,c}	1.60, m^{b,c}	1.60, m	1.62, m	1.62, m	1.60, m	1.60, m
24−28	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
29	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	2.00, m^{b,c}	2.00, m^{b,c}	2.01, m	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
30	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	5.34, m^{b,c}	5.34, m^{b,c}	5.34, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
31	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	5.34, m^{b,c}	5.34, m^{b,c}	5.34, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}
32	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	2.00, m^{b,c}	2.00, m^{b,c}	2.01, m	2.00, m	2.00, m
33	1.06, m,^{b,c} 1.25, m	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.25, m^{b,c}	1.25, m^{b,c}	1.26, m^{b,c}	5.35, m^{b,c}	5.35, m^{b,c}
34	1.25, m^{b,c}	1.25−1.28, m^{b,c}	1.25−1.28, m^{b,c}	1.16, m^{b,c}	1.16, m	1.26, m	5.35, m^{b,c}	5.35, m^{b,c}
35	0.86, m, 1.28, m	1.07, m^{b,c}, 1.26, m	1.07, m^{b,c}, 1.26, m	1.51, m	1.51, m	1.29, m	2.00, m	2.00, m
36	0.87, t (6.5)	1.25, m^{b,c}	1.34, m^{b,c}	0.86, d (6.5)	0.86, d (6.5)	0.88, t (7.0)	1.25, m^{b,c}	1.25, m^{b,c}
37	0.83, d (6.5)	0.85, m 1.28, m	0.85, m 1.28, m	0.86, d (6.5)	0.86, d (6.5)		1.25, m^{b,c}	1.25, m^{b,c}
38		0.88, t (6.7)	0.88, t (6.8)				1.28, m^{b,c}	1.29, m^{b,c}
39		0.83, d (6.7)	0.83, d (6.8)				0.88, t (6.6)	0.88, t (6.6)
OCH_3	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s	3.69, s
C-9NH	7.21, t (6.0)	7.20, t (6.2)	7.20, t (6.0)	7.21, t (6.2)	7.20, t (6.0)	7.20, t (6.2)	7.20, t (6.2)	7.20, t (6.1)
C-21NH	5.89, t (6.0)	5.44, t (6.4)	5.44, t (5.9)	5.86, t (5.5)	5.86, t (5.5)	5.44, t (6.5)	5.44, t (5.6)	5.44, t (6.0)

^{a}Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.26) at 500 MHz. ^{b}Unresolved chemical shifts due to overlapping signals. ^{c}Signal multiplicity unresolved due to overlapping signals.

20EV7−-11F7−9 (4128 mg). Fraction TL-20EV7-11F5 was purified through HPLC (isocratic MeCN−H_2O, 85:15% for 40 min) to give psammaplysins A (**1**) and B (**2**).[2] The other fraction obtained after the vacuum liquid chromatography, TL-20EV4-6, was put through NP flash column chromatography eluting with gradient solvent from 100% hexanes to hexanes−$CHCl_3$ (3:1, 1:1, 1:3) to MeOH 100% to yield 13 fractions. These fractions were then grouped into four fractions based on their TLC profile: TL-20EV4-6F4 (20 mg), TL-20EV4-6F5 (48 mg), TL-20EV4-6F6−7 (60 mg), and TL-20EV4-6F8 (209 mg). The last fraction was then chromatographed using C_{18} bonded silica HPLC (15:85% MeOH−H_2O for 45 min) to yield 19-hydroxypsammaplysin E (**4**) (3.2 mg). The less polar fraction, TL-20EV4-6F6-7, was subjected to RP HPLC (a gradient of 50% to 100% MeOH−H_2O over 20 min) to give psammaplysin D,[4] ceratinamide A,[9] and seven psammaplysin derivatives (**12**, **14**, **15**, **17**, **18**, **21**, and **24**). Fraction TL-20EV4-6F5 gave psammaplysin E (**3**)[4] and four psammaplysins (**9**, **13**, **22**, and **23**) after RP HPLC eluting with 85:15% MeCN−H_2O for 45 min. The least polar fraction in this group, TL-20EV4-6F4, gave ceratinamide B[9] and seven psammaplysins (**5**, **6**, **10**, **11**, **16**, **19**, and **20**) by RP HPLC using 95:5% MeOH−H_2O for 35 min. Besides containing mostly lipid components, the hexanes fraction (TL-20H) also afforded psammaplysin M (3.7 mg) (**7**) and psammaplysin N (1.5 mg) (**8**) after successive NP flash column and RP HPLC (90:10 MeOH−H_2O for 50 min).

19-Hydroxypsammaplysin E (4): yellowish oil; $[\alpha]^{22}_{D}$ = −79.6 (*c* 0.21, $CHCl_3$); ^{1}H and ^{13}C NMR (methanol-d_4, 500 MHz), see Table 1 and Table 2; (+)-LRESIMS *m/z* (rel int) 874 (23), 876 (66), 878 (100), 880 (70), 882 (24) $[M + Na]^+$; (+)-HRESIMS *m/z* 877.8148 $[M + Na]^+$ (calcd for $C_{27}H_{25}Br_4N_3O_9Na$, 877.8176; Δ 3.1 ppm).

Psammaplysin K (5): colorless glass; $[\alpha]^{22}_{D}$ = −16 (*c* 0.09, $CHCl_3$); ^{1}H and ^{13}C NMR (acetone-d_6) see Table 1 and Table 2. (+)-LRESIMS *m/z* (rel int) 737 (10), 739 (50), 741 (100), 743 (43), 745 (14) $[M + Na]^+$; (+)-HRESIMS *m/z* 740.7698 $[M + Na]^+$ (calcd for $C_{20}H_{18}Br_4N_2O_7Na$, 740.7699; Δ 0.1 ppm).

Psammaplysin K dimethoxy acetal (6): colorless glass; $[\alpha]^{24}_{D}$ = −15 (*c* 0.03, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; (+)-LRESIMS *m/z* (rel int) 783 (30), 785 (70), 787 (100), 789 (79), 791 (30) $[M + Na]^+$; (+)-HRESIMS *m/z* 786.8107 $[M + Na]^+$ (calcd for $C_{22}H_{24}Br_4N_2O_8Na$, 786.8117; Δ 1.4 ppm).

Psammaplysin L (7): colorless glass; $[\alpha]^{22}_{D}$ = −65.6 (*c* 0.19, acetone); ^{1}H and ^{13}C NMR (acetone-d_6, 500 MHz), see Table 1 and Table 2; (+)-LRESIMS *m/z* (rel int) 794 (21), 796 (58), 798 (100),

Table 5. ^{13}C NMR Assignments for 9–24[a]

position	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	145.4	145.2	145.2	145.2	145.0	145.0	145.2	145.2	145.2	145.2	145.1	145.3	144.7	145.2	145.3	145.0
2	105.5	105.6	105.8	105.6	105.6	105.6	105.8	105.6	105.4	105.8	105.7	105.2	105.2	105.7	105.6	105.8
3	149.0	148.7	149.2	148.5	149.0	149.0	149.1	149.2	149.8	148.9	148.8	148.9	148.7	148.9	149.1	148.9
4	103.4	103.5	103.5	103.3	103.3	103.3	103.6	103.4	103.6	103.5	103.6	103.5	103.4	103.4	103.6	103.3
5	36.9	37.3	37.0	37.2	37.3	37.3	37.0	37.2	37.2	37.1	37.0	37.1	37.2	37.2	37.1	37.1
6	122.5	122.1	122.2	122.2	122.3	122.3	122.4	122.6	122.3	122.2	122.5	122.4	122.2	122.5	122.7	122.2
7	79.5	79.3	79.4	79.4	79.6	79.6	79.5	79.3	79.5	79.5	79.4	79.6	79.4	79.9	79.5	79.5
8	155.8	155.6	155.5	155.6	155.6	155.6	155.9	156.1	155.9	155.9	155.8	155.7	155.8	155.5	155.8	155.5
9	159.1	159.0	159.3	159.2	159.1	159.1	159.3	159.3	159.2	159.9	159.4	159.0	159.3	159.2	159.2	159.2
10	37.2	37.1	37.1	37.3	37.0	37.0	37.1	36.8	37.3	37.2	37.1	37.2	37.2	37.4	37.2	37.4
11	29.1	29.3	29.1	29.3	29.4	29.4	28.9	29.1	29.3	29.1	29.1	29.1	29.3	29.4	29.2	29.4
12	70.9	71.1	71.0	71.2	71.2	71.2	71.0	70.8	71.0	71.1	71.0	70.1	70.9	70.9	71.1	71.1
13	151.2	151.2	151.6	152.0	152.2	152.2	152.2	151.6	151.9	151.3	151.3	151.4	152.3	151.4	151.5	152.0
14	118.1	118.2	118.5	118.5	118.5	118.5	118.5	118.1	118.3	118.2	118.5	118.5	118.3	118.3	118.2	118.3
15	133.2	133.1	132.8	130.3	130.6	130.6	130.4	132.7	130.2	133.0	130.3	133.2	130.0	132.9	132.3	130.0
16	138.0	138.2	138.4	138.4	141.3	138.6	141.0	138.4	141.3	138.2	141.3	138.2	140.9	138.3	138.0	140.9
17	133.2	133.1	132.8	130.3	130.6	130.6	130.4	132.7	130.2	133.0	130.3	133.2	130.0	132.9	133.3	130.1
18	118.1	118.2	118.5	118.5	118.5	118.5	118.5	118.1	118.3	118.2	118.5	118.5	118.3	118.3	118.2	118.3
19	34.4	34.6	34.5	72.7	34.4	72.7	72.9	34.2	72.7	34.7	72.8	34.5	72.9	34.7	34.4	72.7
20	40.3	40.4	40.2	47.9	41.9	47.9	47.7	39.9	47.9	40.3	47.7	40.2	47.8	40.3	40.3	47.9
21	173.5	173.5	173.6	175.9	176.2	176.2	176.3	173.7	176.1	173.3	176.0	173.0	175.8	173.3	173.3	175.9
22	36.7	36.6	36.9	36.6	36.6	36.6	36.1	36.8	36.7	36.9	36.4	36.7	36.5	36.9	36.8	36.7
23	25.8	25.8	25.7	25.8	25.9	25.9	25.2	25.9	25.6	25.7	25.8	25.9	25.7	25.7	25.7	25.6
24–28	29.3[b]	29.7[b]	29.5[b]	29.7[b]	29.7[b]	29.7[b]	29.4[b]	29.5[b]	29.6[b]	29.5[b]	29.6[b]	29.5[b]	29.6[b]	29.4[b]	29.7[b]	29.5[b]
29	29.3[b]	29.7[b]	29.5[b]	29.7[b]	29.7[b]	29.7[b]	29.4[b]	29.5[b]	29.6[b]	29.5[b]	29.6[b]	27.1	27.2	27.3	29.7[b]	29.5[b]
30	29.3[b]	29.7[b]	29.5[b]	29.7[b]	29.7[b]	29.7[b]	29.4[b]	29.5[b]	29.6[b]	29.5[b]	29.6[b]	130.1	129.7	129.8	29.7[b]	29.5[b]
31	29.3[b]	29.7[b]	29.5[b]	29.7[b]	29.7[b]	29.7[b]	29.4[b]	29.5[b]	29.6[b]	29.5[b]	29.6[b]	130.1	129.7	129.8	29.7[b]	29.5[b]
32	29.3[b]	29.7[b]	29.5[b]	29.7[b]	31.9	31.9	37.0	29.5[b]	29.6[b]	29.5[b]	29.6[b]	27.1	27.2	27.3	27.3	27.3
33	39.2	29.7[b]	29.5[b]	29.7[b]	22.7	22.7	31.9	37.1	37.2	29.5[b]	29.6[b]	29.5[b]	29.6[b]	29.4[b]	130.2	130.2
34	27.9	31.9	29.5[b]	29.7[b]	14.4	14.4	22.5	31.9	32.0	29.5[b]	29.6[b]	39.0	39.2	31.7	130.2	130.2
35	22.6	22.8	29.5[b]	29.7[b]			13.8	22.6	22.8	37.2	36.9	28.1	28.1	22.5	27.3	27.3
36	22.6	14.4	32.1	32.0			19.5	14.8	14.4	32.1	32.6	22.5	22.9	14.2	29.7[b]	29.5[b]
37			22.7	22.8				20.0	19.9	22.7	22.6	22.5	22.9		31.8	32.1
38			14.0	14.3						14.2	14.1				22.6	22.7
39										19.8	19.6				14.2	14.3
3-OMe	59.1	59.2	59.0	59.3	59.2	59.2	59.0	59.1	59.1	59.1	58.9	59.1	59.0	59.0	59.3	59.1

[a]Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.16). [b]Unresolved chemical shifts due to overlapping signals

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800 (83), 802 (38) [M + Na]$^+$; (+)-HRESIMS m/z 797.7930 [M + Na]$^+$ (calcd for $C_{22}H_{21}Br_4N_3O_8Na$, 797.7913; Δ −2.0 ppm).

Psammaplysin M (**8**): colorless glass; $[\alpha]^{22}_D$ = −33 (*c* 0.05, acetone); ^{1}H and ^{13}C NMR (acetone-d_6, 500 MHz), see Table 1 and Table 2; (+)-LRESIMS m/z (rel int) 810 (17), 812 (67), 814 (100), 816 (63), 818 (18) [M + Na]$^+$; (+)-HRESIMS m/z 813.8226 [M + Na]$^+$ (calcd for $C_{23}H_{25}Br_4N_3O_8Na$, 813.8226; Δ 0.1 ppm).

Psammaplysin N (**9**): colorless glass; $[\alpha]^{22}_D$ = −43 (*c* 0.01, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 990 (29), 992 (78), 994 (100), 996 (76), 998 (30) [M + Na]$^+$; (+)-HRESIMS m/z 994.0495 [M + Na]$^+$ (calcd for $C_{37}H_{53}Br_4N_3O_7Na$, 994.0468; Δ −2.7 ppm).

Psammaplysin O (**10**): colorless glass; $[\alpha]^{24}_D$ = −74 (*c* 0.08, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 990 (17), 992 (67), 994 (100), 996 (70), 998 (20) [M + Na]$^+$; (+)-HRESIMS m/z 994.0488 [M + Na]$^+$ (calcd for $C_{37}H_{53}Br_4N_3O_7Na$, 994.0468; Δ −2.0 ppm).

Psammaplysin P (**11**): colorless glass; $[\alpha]^{24}_D$ = −11 (*c* 0.09, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 1018 (18), 1020 (68), 1022 (100), 1024 (66), 1026 (24) [M + Na]$^+$; (+)-HRESIMS m/z 1022.0799 [M + Na]$^+$ (calcd for $C_{39}H_{57}Br_4N_3O_7Na$, 1022.0799; Δ −1.8 ppm).

19-Hydroxypsammaplysin P (**12**): colorless glass; $[\alpha]^{24}_D$ = −74 (*c* 0.08, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 1034 (20), 1036 (52), 1038 (100), 1040 (80), 1042 (22) [M + Na]$^+$; (+)-HRESIMS m/z 1034.0748 [M + Na]$^+$ (calcd for $C_{39}H_{57}Br_4N_3O_8Na$, 1034.0771; Δ 2.3 ppm).

Psammaplysin Q (**13**): colorless glass; $[\alpha]^{24}_D$ = −55 (*c* 0.01, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 962 (38), 964 (70), 966 (100), 968 (78), 970 (40) [M + Na]$^+$; (+)-HRESIMS m/z 966.0137 [M + Na]$^+$ (calcd for $C_{35}H_{49}Br_4N_3O_7Na$, 966.0155; Δ 1.9 ppm).

19-Hydroxypsammaplysin Q (**14**): colorless glass; $[\alpha]^{24}_D$ = −92 (*c* 0.03, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 878 (32), 980 (68), 982 (100), 984 (68), 986 (38) [M + Na]$^+$; (+)-HRESIMS m/z 982.0123 [M + Na]$^+$ (calcd for $C_{35}H_{49}Br_4N_3O_8Na$, 982.0104; Δ −1.9 ppm).

Psammaplysin R (**15**): colorless glass; $[\alpha]^{22}_D$ = −88 (*c* 0.09, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 1006 (24), 1008 (64), 1010 (100), 1012 (68), 1014 (30) [M + Na]$^+$; (+)-HRESIMS m/z 1006.0460 [M + Na]$^+$ (calcd for $C_{37}H_{53}Br_4N_3O_8Na$, 1006.0458; Δ −0.2 ppm).

Psammaplysin S (**16**): colorless glass; $[\alpha]^{22}_D$ = −98 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 5; (+)-LRESIMS m/z (rel int) 1004 (24), 1006 (58), 1008 (100), 1010 (66), 1012 (20) [M + Na]$^+$; (+)-HRESIMS m/z 1008.0637 [M + Na]$^+$ (calcd for $C_{38}H_{55}Br_4N_3O_7Na$, 1008.0625; Δ −1.2 ppm).

19-Hydroxypsammaplysin S (**17**): colorless glass; $[\alpha]^{22}_D$ = −117 (*c* 0.18, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1020 (16), 1022 (54), 1024 (100), 1026 (48), 1028 (16) [M + Na]$^+$; (+)-HRESIMS m/z 1020.0619 [M + Na]$^+$ (calcd for $C_{38}H_{55}Br_4N_3O_8Na$, 1020.0615; Δ −0.4 ppm).

Psammaplysin T (**18**): colorless glass; $[\alpha]^{22}_D$ = −90 (*c* 0.04, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1032 (14), 1034 (50), 1036 (100), 1038 (58), 1040 (14) [M + Na]$^+$; (+)-HRESIMS m/z 1052.0679 [M + Na]$^+$ (calcd for $C_{40}H_{59}Br_4N_3O_7Na$, 1052.0677; Δ −0.2 ppm).

19-Hydroxypsammaplysin T (**19**): colorless glass; $[\alpha]^{22}_D$ = −133 (*c* 0.13, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1048 (14), 1050 (74), 1052 (100), 1054 (72), 1056 (22) [M + Na]$^+$; (+)-HRESIMS m/z 1048.0924 [M + Na]$^+$ (calcd for $C_{40}H_{59}Br_4N_3O_8Na$, 1048.0928; Δ 0.3 ppm).

Psammaplysin U (**20**): colorless glass; $[\alpha]^{22}_D$ = −9.2 (*c* 0.17, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1002 (18), 1004 (58), 1006 (100), 1008 (60), 1010 (34) [M + Na]$^+$; (+)-HRESIMS m/z 1006.0492 [M + Na]$^+$ (calcd for $C_{38}H_{53}Br_4N_3O_7Na$, 1006.0468; Δ −2.4 ppm).

19-Hydroxypsammaplysin U (**21**): colorless glass; $[\alpha]^{22}_D$ = −69 (*c* 0.19, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1018 (14), 1020 (70), 1022 (100), 1024 (80), 1026 (24) [M + Na]$^+$; (+)-HRESIMS m/z 1022.0463 [M + Na]$^+$ (calcd for $C_{38}H_{53}Br_4N_3O_8Na$, 1022.0417; Δ −4.4 ppm).

Psammaplysin V (**22**): colorless glass; $[\alpha]^{22}_D$ = −22 (*c* 0.03, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 988 (14), 990 (72), 992 (100), 994 (82), 996 (18) [M + Na]$^+$; (+)-HRESIMS m/z 1008.0093 [M + K]$^+$ (calcd for $C_{37}H_{51}Br_4N_3O_7K$, 1008.0051; Δ −4.1 ppm).

Psammaplysin W (**23**): colorless glass; $[\alpha]^{22}_D$ = −43 (*c* 0.04, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1030 (20), 1032 (64), 1034 (100), 1036 (60), 1038 (20) [M + Na]$^+$; (+)-HRESIMS m/z 1034.0767 [M + Na]$^+$ (calcd for $C_{40}H_{57}Br_4N_3O_7Na$, 1034.0781; Δ 2.3 ppm).

19-Hydroxypsammaplysin W (**24**): colorless glass; $[\alpha]^{22}_D$ = −84 (*c* 0.04, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 4 and Table 5; (+)-LRESIMS m/z (rel int) 1046 (10), 1048 (50), 1050 (100), 1052 (54), 1054 (14) [M + Na]$^+$; (+)-HRESIMS m/z 1046.0773 [M + Na]$^+$ (calcd for $C_{40}H_{57}Br_4N_3O_8Na$, 1046.0771; Δ −0.1 ppm).

Typical Procedure for Preparation of Fatty Acid Methyl Ester Derivatives of Psammaplysins. A sample of psammaplysin U (0.5 mg) was treated with HCl in MeOH (6.0 M, 2 mL) in a 4 mL round-bottom flask, equipped with a condenser and drying tube, and refluxed for 16 h at 70 °C. The mixture was taken to dryness under a stream of N_2 gas before addition of toluene (0.5 mL), which was then also removed by a stream of N_2 gas. The resulting transmethylated fatty acid ester was dissolved in 1 mL of hexanes before passing through a short silica column (0.2 g silica, 1 cm height) eluting with hexanes (10 mL). The eluted FAME was evaporated to dryness prior to GC-MS analysis. A similar procedure was used to prepare FAME esters of psammaplysins N (**9**), O (**10**), Q (**13**), and R (**15**) and 19-hydroxypsammaplysin U (**21**).

(Z)-Methyl 15-methylhexadec-10-enoate (iso 17:1n−10).[25] GC-MS m/z 282 [M$^+$] (1), 74 (100), 43 (66), 69 (42), 87 (26), 250 (17), 98 (13), 227 (7), 123 (6), 152 (5), 208 (5), 195 95), 166 (4), 137 (3), 177 (3), 252 (1), 217 (0.9).

Pyrrolidide Derivative of the FAME Product of ***21***. The fatty acid methyl ester resulting from the FAME reaction was dried under N_2 gas in a 4 mL screw-capped vial before adding fresh distilled pyrrolidine (0.5 mL) and 2 drops of Ac_2O. The mixture was saturated with N_2 gas, and a single crystal of BHT was added; then the vial was placed in a 100 °C oil bath for 1 h. The mixture was then cooled to 0 °C, Et_2O (3 mL) was added, and the product was transferred into a 20 mL vial. The 4 mL vial was rinsed with Et_2O (3 × 3 mL), and the combined organic extracts were washed with 5% HCl (3 × 5 mL) followed by H_2O (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered through a 1 cm NP silica column using hexanes as eluent, and prepared for GC-MS analysis.

(Z)-15-Methyl-1-(pyrrolidin-1-yl)hexadec-10-en-1-one. GC-MS m/z 321 [M$^+$] (3), 43 (23), 55 (19), 69 (6), 70 (15), 85 (6), 98 (12), 113 (100), 126 (19), 140 (2), 154 (1), 168 (2), 182 (2), 196 (1), 222 (0.7), 236 (0.8), 250 (0.9), 278 (1), 306 (1), 321 (3).

Acetylation, Hydrolysis, and MPA Ester Formation of Psammaplysin B (2). Acetylation of Psammaplysin B (2). A sample of psammaplysin B (19.3 mg) in pyridine (0.5 mL) was cooled in an ice bath. Ac_2O (0.5 mL) was added, and the solution was warmed to room temperature and stirred for 1.5 h. Toluene (1 mL) was added, and the resulting mixture was evaporated in vacuo. The reaction mixture was then filtered through a short NP silica column eluted with hexanes before purification using RP HPLC (40 → 90% MeOH−H_2O over 30 min) to give psammaplysin B acetamide diacetate (**25**) (26.4 mg). A similar procedure was used to prepare the acetamide acetate derivative of psammaplysin A.

Psammaplysin B acetamide diacetate (**25**)[23]: colorless glass; $[\alpha]^{24}_D$ = −58.4 (*c* 0.35, MeOH); ^{1}H NMR (methanol-d_4, 500 MHz) δ_H 7.19 (1H, s, H-1), 3.07 (1H, d, J = 16.2 Hz, H-5a), 3.24 (1H, d, J = 16.2 Hz, H-5b), 6.31 (1H, s, H-7), 3.59 (2H, t, J = 7.7 Hz, H-10), 2.12

(2H, m, H-11), 4.12 (2H, t, J = 6.0, H-12), 7.59 (2H, s, H-15 and H-17), 5.73 (1H, dd, J = 4.5 Hz, 7.6 Hz, H-19), 3.44 (1H, dd, J = 7.7 Hz, 14.1 Hz, H-20a), 3.54 (1H, dd, J = 7.7 Hz, 14.1 Hz, H-20a), 3.63 (3H, s, $-OCH_3$); (+)-LR-ESIMS m/z (rel int) 893.8 (1), 895.8 (3), 897.8 (5), 899.8 (3), 901.8 (1) $[M + Na]^+$.

*Hydrolysis of Psammaplysin B Acetamide Diacetate (**25**).* Psammaplysin B acetamide diacetate (**25**) (12.1 mg) was dissolved in dry MeOH (1.0 mL) along with K_2CO_3 (5.0 mg) and stirred at 40 °C overnight. The resulting cloudy solution was neutralized with 1 M HCl (1.0 mL) and partitioned between $CHCl_3$ (10 mL) and H_2O (3 × 5 mL). The organic phase was washed with saturated Na_2CO_3 and H_2O, dried over $MgSO_4$, and evaporated to dryness before being purified by NP HPLC using a mobile phase of EtOAc−hexanes (70:30) to return the carbamate **26** (6.4 mg).

*N-[3-(4-(2-Acetamido-1-hydroxyethyl)-2,6-dibromophenoxy)-propyl]-2-methoxyacetamide (**26**):* colorless glass; $[\alpha]^{24}_D$ = +0.7 (c 0.13, MeOH); ^{1}H NMR (methanol-d_4, 500 MHz) δ_H 7.59 (2H, s, H-15 and H-17), 4.68 (1H, dd, J = 5.0, 7.5 Hz, H-19), 4.04 (2H, t, J = 6.0 Hz, H-12), 3.63 (s, 3H, OCH_3), 3.39 (t, 2H, J = 7.5 Hz, H-10), 3.25 (1H, dd, J = 7.5, 13.5 Hz, H-20a), 3.40 (1H, signal under H-10, H-20b), 2.04 (p, 2H, J = 7.0 Hz, H-11), 1.93 (s, 3H, NHMe); (+)-LRESIMS m/z (rel int) 637 (1), 639 (3), 641 (1) $[M + Na]^+$; (+)-HRESIMS m/z 488.9643 $[M + Na]^+$ (calcd for $C_{15}H_{20}Br_2N_2O_5Na$, 488.9631; Δ −2.5 ppm).

*Preparation of (R)-MPA Ester of **26**.* N-[3-(4-(2-Acetamido-1-hydroxyethyl)-2,6-dibromophenoxy)propyl]-2-methoxyacetamide (2.0 mg) was dissolved in dry DCM (0.2 mL), to which (R)-MPA (2 equiv, 0.83 mg) was added, followed by DCC (2 equiv, 1.03 mg) and DMAP (2 equiv, 0.61 mg). The reaction was stirred at room temperature for 16 h and filtered through a cotton wool plug. The solvent was removed by rotary evaporation, and the residue passed through a silica column eluting with DCM−MeOH (8:2) to give an (R)-MPA ester mixture (0.8 mg).

*Diastereomeric mixture of (R)-MPA esters of **26**:* colorless oil; ^{1}H NMR (methanol-d_4, 500 MHz) δ_H 7.59 (2H, s, H-15 and H-17), 7.34−7.41 (5H, m, MPA phenyl protons), 5.70 (1H, dd, J = 4.5, 8.5 Hz, H-19) and 5.67 (0.8H, brd, J = 7.5 Hz, H-19), 4.04 (2H, t, J = 6.2 Hz, H-12), 3.69 (1H, s, C*H* of MPA), 3.63 (s, 3H, OCH_3), 3.39 (t, 2H, J = 7.5 Hz, H-10), 3.37 (3H, s, OMe of MPA), 3.51 (1H, dd, J = 9.1, 14.4 Hz, H-20b), 3.34 (1H, dd, J = 4.4, 13.7 Hz, H-20a), 2.04 (t, 2H, J = 6.0 Hz, H-11), 1.93 (s, 3H, NHMe); (+)-LRESIMS m/z (rel int) 637 (1), 639 (3), 641 (1) $[M + Na]^+$.

***P. falciparum* Growth Inhibition Assay.** *P. falciparum* in vitro growth inhibition assays were carried out using an isotopic microtest, modified from the previously described method.[26] Briefly, asynchronous *P. falciparum*-infected erythrocytes (1% parasitemia and 1% hematocrit) were added in triplicate wells into 96-well tissue culture plates containing control (chloroquine; Sigma Aldrich, C6628) or test compounds, and then 0.5 μCi [^{3}H]-hypoxanthine was added per well. Plates were incubated under standard *P. falciparum* culture conditions for 48 h; then cells were harvested onto 1450 MicroBeta filter mats (Wallac), and [^{3}H] incorporation was determined using a 1450 MicroBeta liquid scintillation counter. Percentage growth inhibition compared to DMSO (0.5%) and background controls was determined. Compounds were initially tested at 10 μM in triplicate wells, in two independent experiments, and only compounds showing >∼50% inhibition in these primary tests were further investigated to determine IC_{50} values. IC_{50} (±SD) values were calculated using linear interpolation of inhibition curves[27] for three independent experiments, each carried out in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1−S30. ^{1}H and selected 2D NMR data for compounds **4**−**24** and enantioselective HPLC traces for **25** and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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