Purealidins J—R, New Bromotyrosine Alkaloids from the Okinawan Marine Sponge *Psammaplysilla purea*

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Nine new bromotyrosine alkaloids, purealidins J—R (1—9), have been isolated from the Okinawan marine sponge *Psammaplysilla purea* and the structures were elucidated on the basis of spectroscopic data. A hydroxy group at C-1 of purealidins M—O (4—6) may be biosynthetically derived from ring-opening of a spirocyclohexadienylisoxazole unit of purealidin J (1), aerophobin-1 (10), and purealidin L (3), respectively. Purealidins N (5), P (7), and Q (8) were cytotoxic to tumor cell lines, while purealidins J (1), K (2), P (7), and Q (8) showed moderate inhibitory activity against epidermal growth factor (EGF) receptor kinase.

Key words sponge; *Psammaplysilla purea*; bromotyrosine alkaloid; purealidin; cytotoxic; epidermal growth factor receptor kinase

Marine sponges of the order Verongidae have been found to contain a number of bromotyrosine alkaloids[1] such as aeroplysinin 1,[2] fistularin 3,[3] and bastadin 1.[4] In our search for bioactive substances from marine organisms,[5] a series of bromotyrosine alkaloids have been isolated from a Verongid marine sponge *Psammaplysilla purea*.[6] Further examination of the extract of *P. purea* resulted in isolation of nine new bromotyrosine-derived alkaloids, purealidins J—R (**1**—**9**), which might be closely related to one another on biogenesis of them. This paper describes the isolation and structure elucidation of **1**—**9**.

EtOAc-soluble material of the methanolic extract of the sponge *P. purea* collected off Ishigaki island, Okinawa, was separated on silica gel and C_{18} columns, and C_{18} HPLC to yield purealidins J (**1**, 0.004% wet weight), K (**2**, 0.002%), L (**3**, 0.0002%), M (**4**, 0.002%), N (**5**, 0.0003%), O (**6**, 0.0001%), P (**7**, 0.0008%), Q (**8**, 0.0004%), and R (**9**, 0.0004%), which were obtained as the trifluoroacetic acid (TFA) salts, except for **5** and **9**, together with known related compounds, aerophobin-1[7] (**10**, 0.005%), purealin[6a] (**11**), and purealidin B.[6d]

Purealidins J—L (**1**—**3**) revealed pseudomolecular ions in the ratio of 1:2:1 at *m/z* 490, 492, and 494 (**1**), at *m/z* 506, 508, and 510 (**2**), and at *m/z* 494, 496, and 498 (**3**), respectively, in the FAB-MS spectra, indicating the presence of two bromine atoms in each molecule. HR-FAB-MS data of **1**—**3** revealed the molecular formulae, $C_{15}H_{17}Br_2N_5O_4$ (*m/z* 491.9682, $M^+ + 2 + H$, $\Delta -2.3$ mmu), $C_{15}H_{17}Br_2N_5O_5$ (*m/z* 507.9672, $M^+ + 2 + H$, $\Delta +1.8$ mmu), and $C_{15}H_{21}Br_2N_5O_4$ (*m/z* 496.0035, $M^+ + 2 + H$, $\Delta +1.7$ mmu), respectively. IR absorptions at 3400 and 1680—1660 cm^{-1} in **1**—**3** were attributed to NH/OH and amide carbonyl groups, respectively. Comparison of the ^{1}H- and ^{13}C-NMR data (Table I) of **1**—**3** with those of aerophobin-1 (**10**) indicated that **1**—**3** possessed a common spirocyclohexadienylisoxazole unit with different structures for each C-10—C-14 segment.

Observation that purealidin J (**1**, $[\alpha]_D^{21}$ +24° (c=0.98, MeOH)) was positive to Sakaguchi test suggested the presence of guanidine group(s). The ^{1}H-NMR spectrum of **1** containing an NH_2 (δ_H 7.40, 2H) and an olefin proton (δ_H 6.62) signals was similar to that of the 2-aminohistamine unit in purealin (**11**) or purealidin A.[6c] The carbon chemical shifts (Table I) of C-12, C-13, and C-14 of **1** were coincident with those (δ_C 124.11, 109.00, and 146.74, respectively) of the corresponding 2-

TABLE I. ^{13}C-NMR Data for Purealidins J—O and R (**1**—**6** and **9**) and Aerophobin-1 (**10**)

Positn.	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[b]	**6**[b]	**9**[b]	**10**[a]
1	73.6	73.6	76.3	152.8	155.0	155.2	75.5	73.5
2	113.0	113.1	114.9	107.6	108.8	108.2	114.2	113.1
3	147.1	147.2	150.1	152.7	154.3	153.6	149.3	147.1
4	120.8	120.9	123.5	105.5	107.4	108.2	122.7	120.9
5	131.2	131.3	133.0	132.2	134.6	135.4	132.3	131.2
6	90.2	90.4	93.2	122.0	122.7	123.7	92.6	90.3
7	39.2[d]	39.5[d]	40.1[d]	27.0	25.5	26.4	40.0	39.4
8	154.3	154.3	159.5	150.3	151.5	152.6	155.2	154.4
9	158.9	157.9	162.5	164.7	167.3	167.8	163.6	159.1
10	37.3	35.0	40.5	37.6	39.2	40.7		37.7
11	24.2	30.2	28.3	24.7	25.8	29.0		24.1
12	124.1	56.9	28.0	124.2	132.2	28.4		130.8
13	109.4	174.8	42.9	109.3	117.7	42.9		161.1
14	146.9	159.2	156.1	146.7	134.9	156.4		133.6
3-OCH_3	59.6	59.7	61.2	60.0	60.8	61.7	60.4	59.6

a) In DMSO-d_6. *b*) In MeOH-d_4. *c*) In DMSO-d_6 with a drop of 1 N HCl. *d*) This carbon signal overlapped the DMSO signal.

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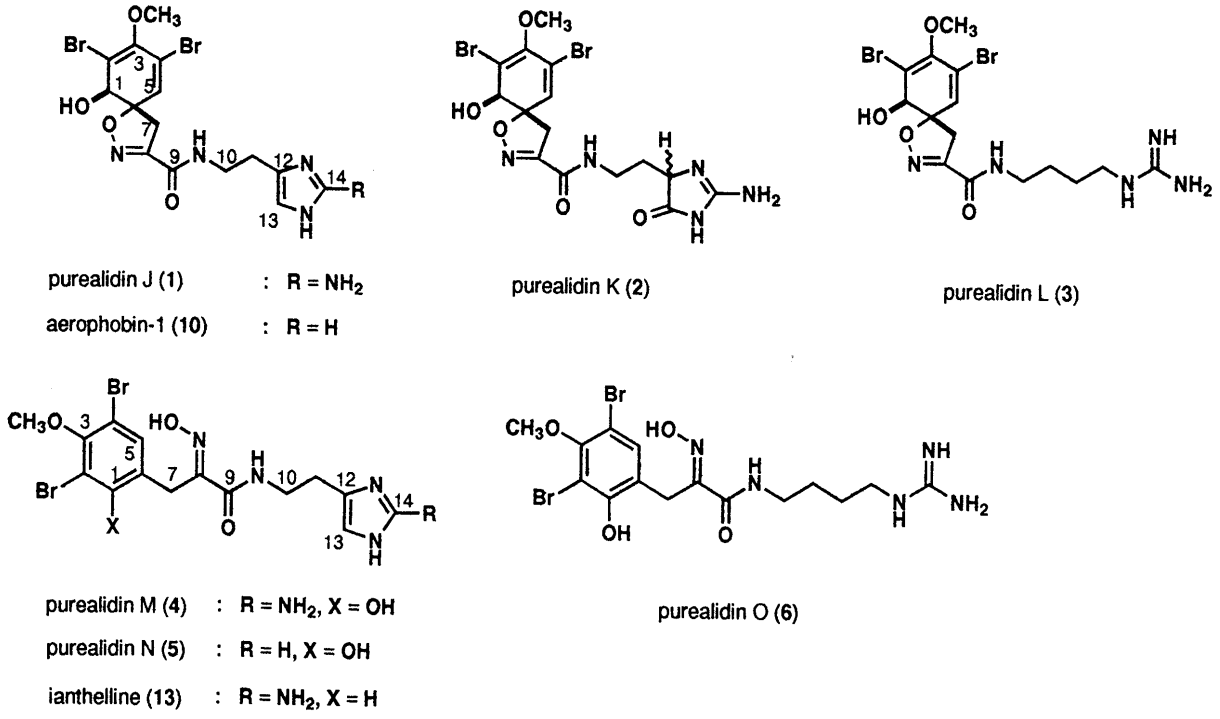


Fig. 1

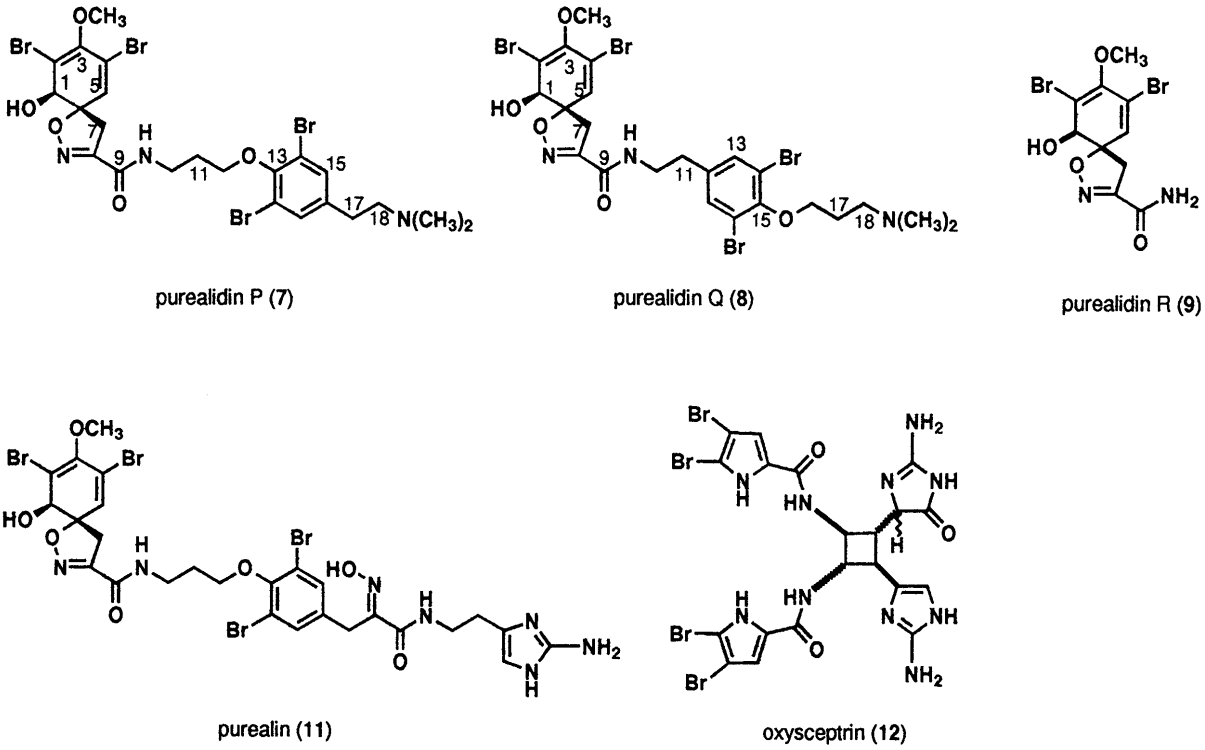


Fig. 2

aminoimidazole ring in **11**. Thus the structure of purealidin J was elucidated to be **1**.

The molecular weight of purealidin K (**2**, $[\alpha]_D^{24}$ +26° (c=0.38, MeOH)) was larger than that of **1** by 16 Da. The signals due to NH (δ_H 9.64) and a carbonyl carbon (δ_C 174.8) observed in the ^{1}H- and ^{13}C-NMR spectra, respectively, were indicative of the presence of an additional amide carbonyl group. The position of the amide carbonyl (C-13) was assigned by HMBC correlations for H-11/C-12, H-11/C-13 and 13-NH/C-13. The chemical shifts at C-12, C-13, and C-14 in **2** were close to those (δ_C 61.7, 174.1, and 160.5, respectively) of the aminoimidazolone ring in oxysceptrin[8] (**12**). Thus the structure of purealidin K was assigned to be **2**. Purealidin K (**2**) was subjected to ozonolysis followed by oxidation with H_2O_2 and subsequently acid hydrolysis. Standard amino acid analysis and chiral HPLC analyses of the hydrolysate revealed D- and L-2,4-diaminobutyric acid in the ratio of 1 : 1, so

that C-12 in **2** was racemic.

The ^{13}C-NMR data (Table I) of purealidin L (**3**, $[\alpha]_D^{24}$ +27° (c=0.18, MeOH)) showed characteristic resonances due to four sp^3 methylenes at δ_C 42.9, 40.5, 28.3, and 28.0 and an sp^2 quaternary carbon at δ_C 156.1. The sp^2 carbon chemical shift at C-14 as well as positive coloration in the Sakaguchi test implied the presence of a guanidino group. An agmatine (4-(aminobutyl)guanidine) moiety in **3** was assigned by the following ^{1}H–^{1}H COSY cross-peaks: 9-NH/H_2-10, H_2-10/H_2-11, H_2-11/H_2-12, H_2-12/H_2-13, and H_2-13/13-NH. Thus the structure of purealidin L was elucidated to be **3**.

The molecular formula, $C_{15}H_{16}Br_2N_4O_4$, of purealidin M (**4**) was established by the HR-FAB-MS (m/z 476.9564, M^++2+H, Δ−3.2 mmu). Though the ^{1}H- and ^{13}C-NMR data (Table I) of **4** were similar to those of ianthelline[9] (**13**), differences were found for the aromatic ring (C-1—C-6): **13** possessed a symmetrical tetrasubstituted benzene ring, while the ^{1}H-NMR spectrum of **4** showed signals due to a pentasubstituted benzene ring (δ_H 7.26, 1H) and an additional phenol hydroxy group (δ_H 10.48). Assignment of C-1—C-6 was based on HMBC correlations for H-5/C-1, H-5/C-3, H-5/C-4, H-5/C-6, H_2-7/C-1, H_2-7/C-5, H_2-7/C-6, and 3-OMe/C-3. Thus the structure of purealidin M was determined to be **4**. *E*-Geometry of the oxime at C-8 of **4**—**6** was inferred from the carbon chemical shift of C-7 (δ_C 25.5—27.0).[10]

The molecular formulae, $C_{15}H_{17}Br_2N_5O_4$ and $C_{15}H_{21}Br_2N_5O_4$, of purealidins N (**5**) and O (**6**) were the same as those of aerophobin-1 (**10**) and purealidin L (**3**), respectively. Comparison of the ^{1}H- and ^{13}C-NMR data (Table I) of **5** and **6** with those of purealidin M (**4**) suggested that **5** and **6** had the same pentasubstituted bromotyrosine moiety (C-1—C-9) as **4**. The carbon resonances of C-10—C-14 in **5** were similar to those of the histamine unit in **10**, while those for **6** corresponded to the agmatine unit in **3**. The structures of purealidins N and O were, therefore, concluded to be **5** and **6**, respectively.

Purealidin P (**7**) showed the pseudomolecular ions at m/z 742, 744, 746, 748, and 750 (1:4:6:4:1) in the FAB-MS spectrum, and the molecular formula, $C_{23}H_{27}Br_4N_3O_5$, was established by the HR-FAB-MS (m/z 745.8776, M^++4+H, Δ+5.5 mmu). The ^{1}H- and ^{13}C-NMR data for **7** were almost same as those of purealidin B[6d] except for an *N*-methyl resonance (δ_C 59.2), which was shifted to higher field than that (δ_C 64.2) of purealidin B. A 6H singlet proton signal resonated at δ_H 2.78 was assigned to be a dimethylamino group. NOEs for H_2-15/H_2-17 and H_2-18/18-NMe_2 in addition to the above data established the structure of purealidin P to be **7**.

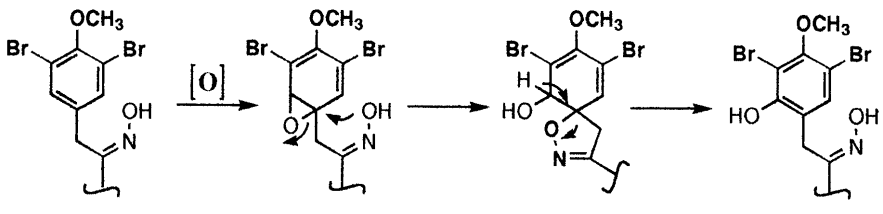
Purealidin Q (**8**) possessed the same molecular formula as that of **7**, and the ^{1}H- and ^{13}C-NMR data suggested that **7** and **8** had a common partial structure. Analyses of the ^{1}H–^{1}H COSY spectrum of **8** revealed proton connectivities of 9-NH—H_2-11 and H_2-16—H_2-18, and the NOE observed for H_2-18/18-NMe_2 suggested that an *N*-dimethyl group was attached to a C_3 unit. Thus the structure of purealidin Q was assigned to be **8**.

Purealidin R (**9**, $[\alpha]_D^{24}$ +86° (c=0.19, MeOH)) showed molecular ions at m/z 380, 382, and 384 (1:2:1) in the EI-MS spectrum. The molecular formula, $C_{10}H_{10}Br_2N_2O_4$, was determined by HR-EI-MS (m/z 381.8901, M^++2, Δ+0.3 mmu). The ^{1}H- and ^{13}C-NMR data (Table I) revealed the presence of a spirocyclohexadienylisoxazole unit and a primary amide NH_2, thus suggesting that the structure of purealidin R is **9**.

Stereochemistry at C-1 and C-6 of the spiroisoxazole ring in purealidins J (**1**), K (**2**), L (**3**), P (**7**), Q (**8**), and R (**9**), and aerophobin-1 (**10**) was deduced to be *trans* from the proton chemical shift (*ca.* δ_H 4.05) of H-1 in CD_3OD.[11] These compounds were found to be dextrorotatory from the signs of optical rotations and Cotton effects at 248 and 284 nm in the CD spectra, indicating that absolute configurations at C-1 and C-6 are 1*R* and 6*S*.[6a,6d,12] The $[\alpha]_D$ value (+68°) of compound **10** is smaller than that ($[\alpha]_D$ +139°) in the literature,[7] indicating that aerophobin-1 (**10**) isolated from this sponge may be an enantiomeric mixture containing an excess of the dextrorotamer (*ca.* 50% ee).

Purealidins M (**4**), N (**5**) and O (**6**) are the second examples[13] of bromotyrosines with a phenolic hydroxy group at C-1 from natural origin.[14] Biosynthetically compounds **1**—**9** may be closely related to one another, presumably being generated through condensation of bromotyrosine units (C-1—C-9) with various amines such as agmatine, brominated tyramine with C_3 units, ammonia, or histamine. The spirocyclohexadienylisoxazole ring may be derived from an arene oxide intermediate (**a**) as proposed by Andersen and Faulkner[15] (Chart 1). Ring-opening of the spirocyclohexadienylisoxazole units of purealidins J (**1**), L (**3**), and aerophobin-1 (**10**) may generate purealidins M (**4**), O (**6**), and N (**5**), respectively, with a hydroxy group at C-1. The aminohistamine units of **1** and **4** may be biogenetically derived from the agmatine units of **3** and **6**. Cyclization between C-12 and 14-N in **3** may afford **1**, which is probably oxygenated to yield **2**.

Purealidins N (**5**), P (**7**), and Q (**8**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50} values: 0.07, 2.8, and 0.95 μg/ml, respectively) and human epidermoid carcinoma KB cells (IC_{50}: 0.074, 7.6, and 1.2 μg/ml, respectively) *in vitro*, while purealidins J—M (**1**—**4**), O (**6**) and R (**9**), and aerophobin-1 (**10**) had no cytotoxicity (IC_{50}



a

Chart 1. Plausible Biogenetic Path of Bromotyrosines with a Spirocyclohexadienylisoxazole Unit and/or a Hydroxy Group at C-1

$>10\,\mu g/ml$). Purealidins J (**1**), K (**2**), P (**7**), and Q (**8**) showed inhibitory activity against epidermal growth factor (EGF) receptor kinase[16] (IC_{50}: 23, 14, 18, and 11 $\mu g/ml$, respectively).

Experimental

General Methods UV and IR spectra were taken on a JASCO Ubest-35 and a JASCO IR Report-100 spectrometer, respectively. ^1H- and ^{13}C-NMR spectra were conducted with a JEOL EX-400 and GSX-270 spectrometer in CD_3OD and DMSO-d_6. The resonances of MeOH at δ_H 3.30 and δ_C 49.0 were used as internal references for ^1H- and ^{13}C-NMR spectra, respectively. The resonances of residual DMSO at δ_H 2.50 and δ_C 39.5 were used as internal references for ^1H- and ^{13}C-NMR spectra, respectively. FAB-MS spectra were recorded employing a JEOL HX-110 spectrometer by using glycerol as a matrix. EI-MS spectra were obtained on a JEOL DX-303 spectrometer operating at 70 eV.

Collection, Extraction, and Isolation The dark brown sponge, *Psammaplysilla purea* Carter, was collected off Ishigaki Island, Okinawa and kept frozen until needed. The sponge (1.5 kg, wet weight) was extracted with MeOH (1.3 l and then 1 l). After evaporation under reduced pressure the residue (55.6 g) was partitioned between EtOAc (500 ml × 3) and H_2O (500 ml). The EtOAc soluble material (3.30 g) was subjected to a silica gel column with $CHCl_3$–*n*-BuOH–AcOH–H_2O (1.5 : 6 : 1 : 1). The fraction (134 mg, 760—940 ml) was chromatographed on a C_{18} column (Develosil LOP ODS 24S, Nomura Chemical, 30 × 300 mm) with CH_3CN–H_2O–CF_3CO_2H (35 : 65 : 0.1) and then MeOH to give two fractions. The fraction (34 mg, 45—100 ml) was separated by C_{18} HPLC (YMC Pack AM323 ODS, 10 × 250 mm; eluent, CH_3CN–H_2O–CF_3CO_2H, 35 : 75 : 0.1; flow rate, 2.5 ml/min; UV detection at 254 nm) to afford purealidins J (**1**, 0.004%, t_R 10.8 min), K (**2**, 0.002%, t_R 10.2 min) and L (**3**, 0.0002%, t_R 11.5 min). The other fraction (36.4 mg) eluted with MeOH from the C_{18} column was subjected to C_{18} HPLC (YMC Pack AM323 ODS, 10 × 250 mm; eluent, CH_3CN–H_2O–CF_3CO_2H, 42 : 58 : 0.1; flow rate, 2.5 ml/min; UV detection at 254 nm) to afford purealidins N (**5**, 0.0003%, t_R 12.0 min), O (**6**, 0.0001%, t_R 14.0 min), and R (**9**, 0.0004%, t_R 19.0 min). The other fraction (219.2 mg) eluted at 950—1080 ml from the first silica gel column was subjected to a C_{18} column (Develosil LOP ODS 24S, 30 × 300 mm; eluent, CH_3CN–H_2O–CF_3CO_2H, 45 : 55 : 0.1) followed by purification by C_{18} HPLC (YMC Pack AM323 ODS, 10 × 250 mm; eluent, CH_3CN–H_2O–CF_3CO_2H, 45 : 55 : 0.1; flow rate, 2.5 ml/min; UV detection at 254 nm) to afford purealidins M (**4**, 0.002%, t_R 8.4 min), P (**7**, 0.0008%, t_R 17.4 min), and Q (**8**, 0.0004%, t_R 16.2 min) together with aerophobin-1 (**10**, 0.005%).

Purealidin J (1) TFA Salt Colorless oil, $[\alpha]_D^{21}$ +24° (c = 0.98, MeOH). IR (KBr): 3400, 2930, 2845, 1680, 1540, 1430, 1200, 1135 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 277 (1700), 284 (1400). CD ($c = 0.64 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +2.2 (255), +2.1 (287). ^1H-NMR (DMSO-d_6) δ: 2.61 (2H, t, J = 6.4 Hz, H_2-11), 3.19 (1H, d, J = 18.6 Hz, H-7), 3.37 (2H, br t, J = 6.4 Hz, H_2-10), 3.61 (1H, d, J = 18.6 Hz, H-7), 3.64 (3H, s, 3-OMe), 3.91 (1H, d, J = 6.4 Hz, H-1), 6.37 (1H, d, J = 6.4 Hz, 1-OH), 6.58 (1H, s, H-5), 6.62 (1H, br s, H-13), 7.40 (2H, s, 14-NH_2), 8.63 (1H, t, J = 5.6 Hz, 9-NH), 11.71 (1H, br s, 12-NH), 12.13 (1H, br s, 13-NH). ^{13}C-NMR: see Table I. FAB-MS m/z: 490, 492, 494 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{19}Br_2N_5O_4$ $(M+2+H)^+$: 491.9705. Found: 491.9682.

Purealidin K (2) TFA Salt Colorless oil, $[\alpha]_D^{24}$ +26° (c = 0.38, MeOH). IR (KBr): 3400, 2920, 2850, 1670, 1540, 1430, 1200, 1135, 1125 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 231 (6300), 284 (2400). CD ($c = 0.73 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +2.3 (255), +2.1 (289). ^1H-NMR (DMSO-d_6) δ: 1.86 (1H, m, H-11), 1.96 (1H, m, H-11), 3.19 (1H, d, J = 18.0 Hz, H-7), 3.35 (2H, m, H_2-10), 3.62 (1H, d, J = 18.0 Hz, H-7), 3.65 (3H, s, 3-OMe), 3.92 (1H, d, J = 6.3 Hz, H-1), 4.30 (1H, dd, J = 4.9, 7.8 Hz, H-12), 6.36 (1H, d, J = 6.3 Hz, 1-OH), 6.58 (1H, s, H-5), 8.63 (1H, t, J = 5.1 Hz, 9-NH), 8.96 (2H, s, 14-NH_2), 9.64 (1H, br s, 13-NH), 12.5 (1H, br, 12-NH). ^{13}C-NMR: see Table I. FAB-MS m/z: 506, 508, 510 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{18}Br_2N_5O_5$ $(M+2+H)^+$: 507.9654. Found: 507.9672.

Purealidin L (3) TFA Salt Colorless oil, $[\alpha]_D^{24}$ +27° (c = 0.18, MeOH). IR (KBr): 3400, 2920, 1660, 1520, 1470, 1210 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 228 (8600), 290 (3400). CD ($c = 1.4 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +2.3 (248), +1.9 (284). ^1H-NMR (DMSO-d_6) δ: 1.45 (2H, m, H_2-12), 1.47 (2H, m, H_2-11), 3.09 (2H, m, H_2-13), 3.16 (2H, m, H_2-10), 3.19 (1H, d, J = 18.2 Hz, H-7), 3.62 (1H, d, J = 18.2 Hz, H-7), 3.64 (3H, s, 3-OMe), 3.91 (1H, d, J = 7.9 Hz, H-1), 6.34 (1H, d, J = 7.9 Hz, 1-OH), 6.57 (1H, s, H-5), 7.1—7.3 (4H, br, 14-C(–NH_2)NH_2), 7.44 (1H, br s, 13-NH), 8.54 (1H, t, J = 5.8 Hz, 9-NH). ^{13}C-NMR see Table I. FAB-MS m/z: 494, 496, 498 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{22}Br_2N_5O_4$, $(M+2+H)^+$: 496.0018. Found: 496.0035.

Purealidin M (4) TFA Salt Colorless oil. IR (KBr): 3400, 2920, 1680, 1520, 1470, 1200, 1135, 1120 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 277 (1700), 284 (1400). ^1H-NMR (DMSO-d_6) δ: 2.63 (2H, t, J = 6.9 Hz, H_2-11), 3.40 (2H, dt, J = 5.9, 6.9 Hz, H_2-10), 3.70 (2H, s, H_2-7), 3.74 (3H, s, 3-OMe), 6.60 (1H, s, H-13), 7.26 (1H, s, H-5), 7.33 (2H, s, 14-NH_2), 8.55 (1H, t, J = 5.9 Hz, 9-NH), 10.48 (1H, br s, 1-OH), 11.55 (1H, br s, 12-NH), 11.99 (1H, br s, 13-NH), 12.19 (1H, s, 8-NOH). ^{13}C-NMR: see Table I. FAB-MS m/z: 490, 492, 494 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{18}Br_2N_5O_4$ $(M+2+H)^+$: 491.9705. Found 491.9740.

Purealidin N (5) Colorless oil. IR (KBr): 3400, 2920, 2845, 1680, 1520, 1135, 1120 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 235 (2600), 290 (2800). ^1H-NMR (DMSO-d_6) δ: 2.85 (2H, t, J = 6.7 Hz, H_2-11), 3.48 (2H, dt, J = 5.9, 6.7 Hz, H_2-10), 3.70 (2H, s, H_2-7), 3.74 (3H, s, 3-OMe), 7.26 (1H, s, H-5), 7.40 (1H, s, H-13), 8.59 (1H, t, J = 5.9 Hz, 9-NH), 8.89 (1H, s, H-14), 10.45 (1H, br s, 1-OH), 12.20 (1H, s, 8-NOH), 14.1 (1H, br s, 13-NH). ^{13}C-NMR: see Table I. FAB-MS m/z: 475, 477, 479 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{17}Br_2N_4O_4$ $(M+2+H)^+$: 476.9596. Found: 476.9564.

Purealidin O (6) TFA Salt Colorless oil. IR (KBr): 3400, 2920, 2850, 1680, 1635, 1135 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 235 nm (2600), 287 (700). ^1H-NMR (DMSO-d_6) δ: 1.42 (2H, m, H_2-12), 1.44 (2H, m, H_2-11), 3.09 (2H, m, H_2-13), 3.18 (2H, m, H_2-10), 3.70 (2H, s, H_2-7), 3.74 (3H, s, 3-OMe), 7.1—7.3 (4H, br, 14-C (–NH_2)NH_2), 7.31 (1H, s, H-5), 7.43 (1H, br s, 13-NH), 8.56 (1H, t, J = 5.9 Hz, 9-NH), 10.68 (1H, br s, 1-OH), 12.17 (1H, s, 8-NOH). ^{13}C-NMR: see Table I. FAB-MS m/z: 494, 496, 498 (M^+ + H, 1 : 2 : 1). HR-FAB-MS Calcd for $C_{15}H_{22}Br_2N_5O_4$ $(M+2+H)^+$: 496.0018. Found: 496.0028.

Purealidin P (7) TFA Salt Colorless oil, $[\alpha]_D^{19}$ +6.6° (c = 0.75, MeOH). IR (KBr): 3400, 2940, 2845, 1670, 1520, 1470, 1135, 1120 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 277 (1700), 284 (1400). CD ($c = 0.37 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +0.9 (248), +1.1 (284). ^1H-NMR (DMSO-d_6) δ_H: 2.01 (2H, m, H_2-11), 2.78 (6H, s, 18-NMe_2), 2.93 (2H, t, J = 8.1 Hz, H_2-17), 3.23 (1H, d, J = 18.0 Hz, H-7), 3.35 (2H, m, H_2-18), 3.41 (2H, m, H_2-10), 3.62 (1H, d, J = 18.0 Hz, H-7), 3.65 (3H, s, 3-OMe), 3.92 (1H, d, J = 8.1 Hz, H-1), 3.97 (2H, t, J = 6.3 Hz, H_2-12), 6.36 (1H, d, J = 8.1 Hz, 1-OH), 6.59 (1H, s, H-5), 7.64 (2H, s, H-15, 15′), 8.57 (1H, t, J = 5.8 Hz, 9-NH). ^{13}C-NMR (CD_3OD) δ_C: 30.3 (t, C-17), 30.6 (t, C-11), 37.9 (t, C-10), 40.1 (t, C-7), 43.6 (2C, q, 18-NMe_2), 59.2 (t, C-18), 60.4 (q, 1-OMe), 72.3 (t, C-12), 75.5 (d, C-1), 92.5 (s, C-6), 114.2 (s, C-2), 119.5 (2C, s, C-14, 14′), 122.8 (s, C-4), 132.3 (d, C-5), 134.4 (d, C-15), 134.5 (d, C-15′), 136.5 (s, C-16), 149.4 (s, C-3), 153.7 (s, C-13), 155.3 (s, C-8), 161.6 (s, C-9). FAB-MS m/z: 742, 744, 746, 748, 750 (M^+ + H, 1 : 4 : 6 : 4 : 1). HR-FAB-MS Calcd for $C_{23}H_{28}Br_4N_3O_5$ $(M+4+H)^+$: 745.8721. Found: 745.8776.

Purealidin Q (8) TFA Salt Colorless oil, $[\alpha]_D^{19}$ +9.1° (c = 0.39, MeOH). IR (KBr): 3400, 2940, 2850, 1675, 1470, 1200, 1135, 1120 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 277 (1700), 284 (1400). CD ($c = 0.52 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +1.5 (250), +1.4 (284). ^1H-NMR (DMSO-d_6) δ_H: 2.15 (2H, m, H_2-17), 2.76 (2H, t, J = 6.9 Hz, H_2-11), 2.83 (6H, s, 18-NMe_2), 3.18 (1H, d, J = 18.2 Hz, H-7), 3.36 (2H, m, H_2-10), 3.40 (2H, m, H_2-18), 3.60 (1H, d, J = 18.2 Hz, H-7), 3.64 (3H, s, 3-OMe), 3.92 (1H, d, J = 8.2 Hz, H-1), 3.99 (2H, t, J = 6.3 Hz, H_2-16), 6.34 (1H, d, J = 8.2 Hz, 1-OH), 6.57 (1H, s, H-5), 7.54 (2H, s, H-15, 15′), 8.58 (1H, t, J = 5.7 Hz, 9-NH). ^{13}C-NMR (CD_3OD) δ_C: 26.4 (t, C-17), 35.1 (t, C-11), 40.1 (t, C-10), 41.4 (t, C-7), 43.7 (2C, q, 18-NMe_2), 57.2 (t, C-18), 60.4 (q, 1-OMe), 71.2 (t, C-16), 75.5 (d, C-1), 92.4 (s, C-6), 114.2 (s, C-2), 118.8 (2C, s, C-14, 14′), 122.8 (s, C-4), 132.2 (d, C-5), 134.5 (d, C-13), 134.6 (d, C-13′), 140.3 (s, C-12), 149.3 (s, C-3), 152.2 (s, C-15), 155.2 (s, C-8), 161.6 (s, C-9). FAB-MS m/z: 742, 744, 746, 748, 750 (M^+ + H, 1 : 4 : 6 : 4 : 1). HR-FAB-MS Calcd for $C_{23}H_{28}Br_4N_3O_5$ $(M+4+H)^+$: 745.8721. Found: 745.8729.

Purealidin R (9) Colorless oil, $[\alpha]_D^{24}$ +86° (c = 0.19, MeOH). IR (KBr): 3400, 2940, 1675, 1135, 1120 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 228 (8000), 290 (2000). CD ($c = 0.52 \times 10^{-4}$, MeOH) $\Delta\varepsilon^{30}$ (nm): +4.0 (248), +4.3 (284). ^1H-NMR (DMSO-d_6) δ: 3.18 (1H, d, J = 18.2 Hz, H-7), 3.60 (1H, d, J = 18.2 Hz, H-7), 3.65 (3H, s, 3-OMe), 3.92 (1H, d, J = 6.2 Hz, H-1), 6.35 (1H, d, J = 6.2 Hz, 1-OH), 6.58 (1H, s, H-5), 7.58 (1H, br s, NH-9), 7.82 (1H, br s, NH-9). ^{13}C-NMR: see Table I. EI-MS m/z: 380, 382, 384

($M^+ + H$, 1:2:1). HR-EI-MS Calcd for $C_{10}H_{10}Br_2N_2O_4$ $(M+2)^+$: 381.8898. Found: 381.8901.

Determination of the Stereochemistry of C-12 in Purealidin K (2) A solution of purealidin K (**2**, 1.0 mg) in MeOH (100 μl) was bubbled with O_3 at -78 °C for 1 min. After removal of excess O_3 with a stream of N_2, the solvent was evaporated under reduced pressure, and to the residue were added HCO_2H (250 ml) and 35% H_2O_2 (25 μl). The mixture was stirred for 1 h at 0 °C and then for 18 h at room temperature. The solvent was evaporated, and then the residue was dissolved in 6 N HCl (100 μl); this solution was heated at 110 °C for 24 h. Standard amino acid analysis was performed on a Hitachi amino acid autoanalyzer (Model 835, column #2617 4.0 × 250 mm) with Na buffer at a flow rate of 0.275 ml/min and detected at 570 nm. 2,4-Diaminobutyric acid (t_R 96.42 min) was found in the hydrolysate. The absolute stereochemistry of 2,4-diaminobutyric acid was examined by chiral HPLC analysis (Sumichiral OA-6000, Sumika Chemical Analysis Service, Ltd., 4.6 × 150 mm; flow rate, 0.5 ml/min; UV detection at 254 nm; eluent, 1 mM aqueous $CuSO_4$). The retention times of authentic L- and D-2,4-diaminobutyric acid were 30.5 and 29.6 min, respectively. The retention times of the 2,4-diaminobutyric acid in the hydrolysate was found to be 30.5 and 29.6 min (ratio of *ca.* 1:1).

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