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TETRAHEDRON

Bromotyrosine-derived Metabolites from the Sponge *Aiolochroia crassa*

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Abstract: A chemical investigation of secondary metabolites from the sponge *Aiolochroia crassa* has been performed and five bromotyrosine derivatives (**1-5**) were identified. Their structures have been assigned on the basis of spectroscopic analysis, of which araplysillin III (**2**) and hexadellin C (**3**) possess new structures. The absolute configurations of dibromotyrosine moieties of **2** were determined by HPLC analysis of derivatized constituent amino acids obtained from acid hydrolysis. The stereochemistry of the spirocyclohexadienylisoxazoline moiety of compounds **1-3** was deduced from spectroscopic comparison of the same moiety of aerothionin, of which the absolute configuration was previously assigned by X-ray and CD analysis. © 1999 Elsevier Science Ltd. All rights reserved.

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Marine sponges of the Order Verongida have attracted considerable attention because an extraordinary number of bromotyrosine-derived metabolites have been isolated from sponges belonging to this Order[1] (genera *Aplysina*, *Verongula*, *Pseudoceratina*, *Psammaplysilla*, *Suberea* and *Ianthella*). An investigation of the common Caribbean sponge *Aiolochroia crassa* (Hyatt, 1875) was initiated to identify potential antiinfective leads and to optimize active marine natural product scaffolds by semisynthesis[2] or microbial transformation[3].

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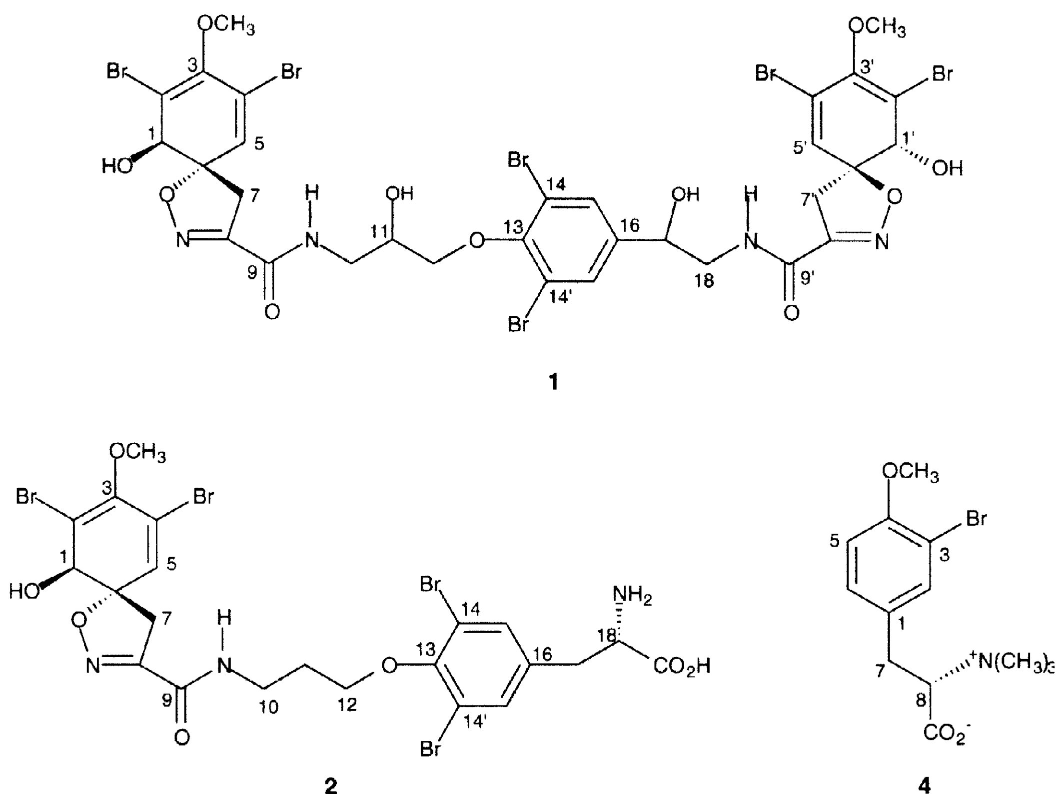
The sponge *Aiolochroia crassa* (Hyatt, 1875) is a Verongida species which has been reported in the marine literature under a number of different names, such as *Suberea crassa* (Hyatt, 1875), *Ianthella ianthella* (De Laubenfels, 1949), and *Ianthella ardis* (De Laubenfels, 1950)[4]. Several bromotyrosine-derived metabolites have been reported from this species, such as aeroplysinin-1,2[5], hexadellin[6] and 2-(3,5-dibromo-1-hydroxy-4,4-dimethoxy-2,5-cyclohexadien-1-yl) ethanamide[7], ianthellin[8], ethyl 3,5-dibromo-4-(3'-N,N-dimethylamino-propyloxy) cinnamate and its free acid[9], and N, N, N-trimethyl bromotyrosines[4].

The sponge was collected from the reef slope at a depth of about 25 m using SCUBA along the coast of South Water Key, Belize. The ethanol extract was chromatographed using a silica gel column and a step gradient from hexane, EtOAc, MeOH to H_2O. Fractions were purified by HPLC using C_{18} reverse phase, cyano, and diol columns to obtain compounds **1**, **2**, **3**, **4** and **5**, of which araplysillin III (**2**) and hexadellin C (**3**) possess new structures.



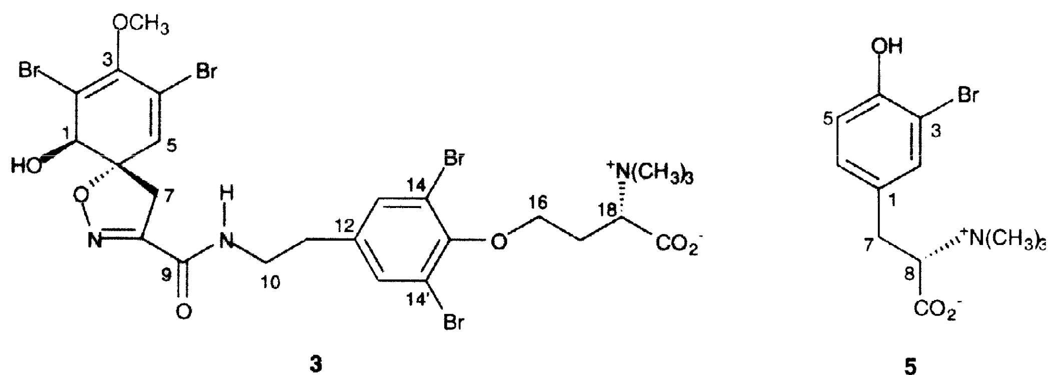


Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data of Araplysillin III (2) and Hexadellin C (3) in DMSO-d_6

2			3		
Position	δ_H	δ_C	Position	δ_H	δ_C
1	3.95 (s)	73.7	1	3.93 (d, J = 8.3 Hz)	73.7
2		121.0	2		121.1
3		147.2	3		147.3
4		113.4	4		113.3
5	6.55 (s)	131.4	5	6.56 (s)	131.3
6		90.4	6		90.4
7	3.21 (d, J = 18.2 Hz) 3.67 (d, J = 18.2 Hz)	39.5	7	3.17 (d, J = 18.1 Hz) 3.60 (d, J = 18.1 Hz)	39.5
8		154.6	8		154.5
9		159.2	9		159.1
10	3.41 (bdd)	36.4	10	3.36 (m)	39.9
11	1.99 (q, J = 6.6)	29.5	11	2.74 (t, J = 6.9 Hz)	33.3
12	3.93 (m)	71.3	12		139.0
13		151.2	13, 13'	7.49 (s)	133.2
14, 14'		117.5	14, 14'		117.3
15, 15'	7.55 (s)	133.9	15		151.1
16		136.8	16	3.94 (m), 4.08 (m)	71.3
17	2.90 (m), 3.08 (bd)	35.3	17	2.23 (m), 2.38 (m)	27.8
18	3.56 (m)	55.0	18	3.56 (m)	74.8
19		169.9	19		167.1
3-OCH$_3$	3.63 (s)	59.8	3-OCH$_3$	3.63 (s)	59.8
9-NH	8.57 (t, J = 5.6 Hz)		9-NH	8.57 (t, J = 5.8 Hz)	
			18-$^+$N(CH$_3$)$_3$	3.15 (s)	51.0

Araplysillin III (**2**) showed an isotope pattern of 1:4:6:4:1 in the ESI-FTMS spectrum for the [M+H]$^+$ peaks at m/z 758, 760, 762, 764 and 766, indicative of the presence of four bromine atoms in the molecule and the center peak [M+H]$^+$ at 761.8410 indicating a molecular formula of $C_{22}H_{24}Br_4N_3O_7$ (calcd 761.8307 for $C_{22}H_{24}{}^{79}Br_2{}^{81}Br_2N_3O_7$, Δ +10.3 mmu). The ^{1}H-NMR spectrum exhibited signals of a spirocyclohexadienylisoxazoline ring at δ 3.95 (1H, s, H-1), 6.55 (1H, s, H-5) and 3.21, 3.67 (two d, 1H each, J = 18.2 Hz, H$_2$-7). This partial structure was further supported by the ^{13}C-NMR data for carbons 1-9[10,11] (Table 1). The other signals at δ 8.57 (1H, 9-NH), 3.41 (2H, H$_2$-10), 1.99 (2H, H$_2$-11) and 3.93 (2H,

H_2-12), correlated by a ^{1}H-^{1}H COSY experiment, suggested the partial structure -CO-NH-CH_2-CH_2-CH_2-O-. In the COSY spectrum the methine proton bearing the amino group (δ 3.56, 1H, H-18) was coupled to benzylic methylene protons (δ 2.90, 3.08, 2H, H_2-17). The remaining ^{1}H-NMR signals could be assigned as two isolated aromatic protons (δ 7.55, s, 2H, H-15, H-15'). The HMBC correlations between the two isolated methines (δ_C 133.9, δ_H 7.55, C-15, 15') and the methylene group (δ_C 35.3, δ_H 2.90, 3.08, C-17) and correlation of the methine proton (δ 3.56, H-18) to the carboxyl carbon (δ 169.9) showed the presence of a 3,5-dibromotyrosine derivative. The HMBC correlation between C-13 and H-12 shows that C-13 was linked to C-12 via an oxygen atom. These data indicated that araplysillin III had the proposed structure **2**.

The relative stereochemistry of the spirocyclohexadienylisoxazoline moiety was established to be trans between the hydroxyl group at C-1 and the oxygen atom in the isoxazoline unit by comparing the proton and carbon chemical shifts and coupling constants the same moiety of aerothionin. The absolute stereochemistry of aerothionin was assigned by X-ray analysis in combination with circular dichroism[10]. The absolute configuration at C-18 was shown to be L by comparing the (1-fluoro-2, 4-dinitrophen-5-yl)-L-alanine-amide (FDAA) derivatized 3,5-dibromotyrosine from the acid hydrolysate of **2** with the same derivatives of standard D,L-3, 5-dibromotyrosine by HPLC according to Marfey's procedure[12].

Hexadellin C (**3**) exhibited an isotope pattern of five $[M+H]^+$ ions separated by two mass units (ratio 1:4:6:4:1) centered at $[M+H]^+$ 803.8504 in ESI-FTMS, indicating the presence of four bromine atoms. The molecular formula was established to be $C_{25}H_{29}Br_4N_3O_7$ (calcd 803.8777 for $C_{25}H_{30}{}^{79}Br_2{}^{81}Br_2N_3O_7$, Δ -27.3 mmu). The ^{1}H-NMR spectrum in DMSO-d_6 (Table 1) contained signals at δ 3.17 (1H, d, J = 18.1 Hz, H-7), 3.60 (1H, d, J = 18.1 Hz, H-7'), 3.93 (1H, d, J = 8.3 Hz, H-1), 6.56 (1H, s, H-5) ascribable to dibromospirocyclohexadienylisoxazoline[10]. The signal at δ 3.63 (3H, s) indicated the presence of a methoxyl group. The ^{1}H-NMR and ^{1}H-^{1}H COSY resonances showed a spin system consisting of δ 8.57 (1H, t, J = 5.8 Hz, 9-NH), 3.36 (2H, m, H-10), and 2.74 (2H, t, J = 6.9 Hz, H-11). HMBC correlations between the NH proton (δ 8.57, 9-NH) and the carboxyl carbon (δ 159.1), as well as between the methylene protons (δ 2.74, H_2-11) and two aromatic (quaternary) carbons revealed the partial structure -CO-NH-CH_2-CH_2-Ar-. HMQC and HMBC experiments were used to assign the substitution pattern on the aromatic ring. The HMBC correlations between the C-13 aromatic methine proton and the C-11 methylene group indicated that both protons (δ 7.49, s, H-13) were ortho to the C-12 alkyl group. The structure of the aromatic ring was 2, 6-dibromo-4-alkyl-benzene. The N-methyl signal at δ 3.15 in the ^{1}H-NMR spectrum integrated for nine protons. The HMBC experiment showed correlations of a methine proton at δ 3.56 (1H, m, H-18) to the N-methyl carbons (δ 51.0) and to the carboxyl carbon (167.1, C-19). The ^{1}H-^{1}H COSY provided correlations between the methylene group (δ 4.08, 3.94, 2H, m, H_2-16), to the C-17 methylene protons (δ 2.38, 2.23, 2H, m, H_2-17) and the nitrogen-bearing methine (δ 3.56, 1H, m, H-18), suggesting the

presence of a N, N, N-trimethylhomoserine moiety. The HMBC correlation between the methylene proton (δ 3.94, 1H, H-16) and the aromatic carbon (δ 151.1, C-15) indicated that the aromatic ring was attached to N, N, N-trimethylhomoserine by an ether bond at C-15. These data allowed the structure to be assigned as **3**.

The relative stereochemistry of the spirocyclohexadienylisoxazoline ring system was shown to be trans configuration between the hydroxyl group at C-1 and the oxygen atom in the isoxazoline unit by comparing the proton and carbon chemical shifts and coupling constants to those reported for aerothionin[10]. The absolute configuration of the N,N,N-trimethylhomoserine moiety was established as L by comparing the optical rotation value with those of D- and L- N,N,N-trimethylhomoserine standards. The $[\alpha]_D$ values of D- and L-amino acid standards were determined to be +5.1° and -5.2°, respectively. The $[\alpha]_D$ value of the amino acid obtained from acid hydrolysis of **3** was shown to be -14.1°. The weight of hydrolyzed amino acid is only 0.17 mg, so a low degree of accuracy is likely. However, the negative value of $[\alpha]_D$ indicates the amino acid moiety of **3** has an L-configuration.

Fistularin-3 (**1**) was identified by comparison of its spectral data with that reported in the literature[11]. Moreover, the relative stereochemistry of the spirocyclohexadienylisoxazoline moiety was also the same: a trans relationship between the hydroxyl group at C-1/C-1' and the oxygen atom in the isoxazoline unit. The absolute stereochemistry of the spirocyclohexadienylisoxazoline was deduced from the CD spectrum: (MeOH) $(\vartheta)_{287}$ +106,000 $(\vartheta)_{273}$ +91,000, which was comparable to aerothionin.[8] The absolute configurations of the two chiral centers at C-11 and C-17 remain to be determined.

The absolute configuration of both **4** and **5** were established as L by comparing optical rotation with those of D- and L-N,N,N-trimethyl-3,5-dibromotyrosine standards. Compounds **4** and **5** were transformed into dibrominated forms by bromination for comparison to the dibromo amino acid standards. The $[\alpha]_D$ values of D- and L-amino acid standards were measured as +3.5° and -4.0°, respectively. The $[\alpha]_D$ value of dibrominated **4** and **5** were measured as -7.6° and -2.7°, respectively. Therefore, the absolute configurations of both **4** and **5** were determined to be L. These two secondary metabolites were isolated from the Caribbean sponge *Pseudoceratina crassa* (the same species as *Aiolochroia crassa*) in 1994[4]. However, this is the first report of these two compounds that presents absolute configurations.

Compounds **2**, **3**, **4** and **5** were isolated from the same sponge *A. crassa* and the absolute configuration of brominated tyrosine and homoserine moieties of each compound is L. Compound **4** is methylated **5**, and the tyrosine moiety of **2** is dibrominated as opposed to the monobrominated **5**. For this reason it appears that **5** could be the biosynthetic precursor of **2** and **4**. It could provide important clues to the biosynthetic origin of these molecules.

Compounds **1** and **2** were assayed for activity against tumor cell lines P-388, A-549, HT-29 and MEL-28, *Plasmodium falciparum* and *Mycobacterium tuberculosis*. Fistularin-3 (**1**) did not exhibit cytotoxicity or antimalaria activity but is active against TB in vitro with an MIC <12.5μg/mL (99% inhibition). Araplysillin III (**2**) was inactive in the same assays including TB. The biological data reveals the differential activity of Fistularin-3 for *M.*

tuberculosis. It also shows that both the spirocyclohexadienylisoxazoline moieties are essential for the activity against TB.

EXPERIMENTAL SECTION

General procedures. NMR spectra were measured on Bruker Avance DPX-300, DRX-400 and 500 spectrometers. ^{1}H- and ^{13}C-NMR spectra were measured and reported in ppm by using the residual solvent peak as an internal standard. ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. IR spectra were recorded on an AATI Mattson Genesis Series FTIR spectrometer. UV spectra were recorded on a Perkin-Elmer Lambda 3B UV/Vis spectrometer. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. The CD spectra were recorded in MeOH on a JASCO J-715 spectropolarimeter. HPLC was carried out on a Waters 510 model system.

Sponge Collection and Taxonomy. The sponge was collected from the reef slope at a depth of about 25 m using SCUBA during March 1997 along the coast of South Water Key (16^0,49',08''N; 88^0,04',87''W), Belize. The sponge formed massive tubes with blunt conules on the surface and a sphincter-like opening at the apical end. The texture was smooth and elastic, and fleshy to the touch, the color in life is brilliant yellow with tinges of green and blue. The sponge changes to deep royal blue upon collection. The skeleton is sparse in the sponge, each fiber formed of thick bark and a substantial pith component. The sponge is *Suberea crassa* (Hyatt 1875) (Order Verongida, Family Aplysinellidae), also known as *Pseudoceratina crassa* in recent literature. A voucher specimen has been deposited in the Natural History Museum, London.

Extraction and isolation. 1.01 kg (wet) of frozen sponge was cut into small pieces, and extracted with 95% EtOH (4 times, overnight each time). The combined extracts, after filtration, were concentrated *in vacuo* until dried. The residue (22.88 g) was powdered and then dispersed in hexane to give a suspension, and then chromatographed using a silica gel column, first with hexane, then with EtOAc, MeOH and finally H_2O (600 ml each) to give 13 step gradient-eluted fractions. Fractions 7 (2.64 g) and 9 (4.52 g) were chromatographed by HPLC using C_{18} reverse phase, cyano, and diol columns to obtain compounds **1** (8 mg), **2** (6 mg), **3** (0.8 mg), **4** (6 mg) and **5** (2 mg).

Fistularin-3 (1). White amorphous solid; physical data: CD (MeOH) $(\vartheta)_{287}$ +106,000 $(\vartheta)_{273}$ +91,000; MS: ESI-FTMS *m/z* $[M+H]^+$ centered at 1114.7033 (calcd 1114.7027 for $C_{31}H_{31}{}^{79}Br_3{}^{81}Br_3N_4)_{11}$, Δ +0.5 mmu); ^{1}H-NMR (DMSO-d_6): δ 8.43 (1H, t, J = 5.8 Hz, 9-NH), 8.39 (1H, t, J = 5.7 Hz, 9'-NH), 7.57 (2H, s, H-15, 15'), 6.58 (1H, s, H-5), 6.56 (1H, s, H-5'), 6.39, 6.37 (2H, d, J = 7.9 Hz, 1, 1'-OH), 5.75 (1H, d, J = 4.6 Hz, 17-OH), 5.29 (1H, d, J = 5.4 Hz, 11-OH), 4.68 (1H, m, J = 5.2 Hz, H-17), 4.06 (1H, m, H-11), 3.93, 3.91 (2H, d, J = 7.9 Hz, H-1, 1'), 3.89 (1H, m, H-12), 3.82 (1H, m H-12), 3.64 (6H, s,

3, 3'-OCH3), 3.61 (1H, d J = 18.8 Hz, H-7), 3.59 (2H, submerged by other signal, H-7'), 3.47 (1H, m, H-10), 3.33 (2H, d, J = 5.3 Hz, H_2-18), 3.29 (1H, m, H-10), 3.24 (1H, d, J = 18.8 Hz, H-7), 3.19 (1H, t-like, J = 18.6 Hz, H-7), 3.15 (1H, submerged by other signal, H-7'). ^{13}C-NMR (DMSO-d_6): δ 159.2 (C-9), 159.1 (C-9'), 154.5 (C-8), 154.5 (C-8'), 151.3 (C-13), 147.2 (C-3, 3'), 142.7 (C-16), 131.3 (C-5), 131.2 (C-5'), 130.5 (C-15, 15'), 121.1 (C-2), 121.1 (C-2'), 117.3 (C-14, 14'), 113.2 (C-4, 4'), 90.4 (C-6, 6'), 75.4 (C-12), 73.6 (C-1), 73.6 (C-1'), 69.4 (C-17), 68.1 (C-11), 59.7 (3, 3'-OCH3), 46.4 (C-18), 42.6 (C-10), 39.4 (C-7), 39.4 (C-7').

Araplysillin III (2). Colorless solid; physical data: $[\alpha]_D$ +96.3 (c 0.19, MeOH); IR neat (NaCl): 3251 (s, br), 2935 (s), 1658 (s), 1543 (s), 1456 (s), 1257 (s), 989 (s) cm^{-1}; UV (EtOH): λmax 208 nm (ε 8100), 284 (1200); MS: ESI-FTMS *m/z* $[M+H]^+$ centered at 761.8410 (calcd 761.8307 for $C_{22}H_{24}{}^{79}Br_2{}^{81}Br_2N_3O_7$, Δ +10.3 mmu); ^{1}H-NMR (DMSO-d_6): δ 8.57 (1H, t, J = 5.6 Hz, 9-NH), 7.55 (2H, s, H-15, 15'), 6.55 (1H, s, H-5), 3.95 (1H, s, H-1), 3.93 (2H, m, H-12), 3.67 (1H, d, J = 18.2 Hz, H-7), 3.63 (3H, s, 3-OCH_3), 3.56 (1H, s, H-18), 3.41 (2H, bdd, H-10), 3.21 (1H, d, J = 18.2 Hz, H-7'), 3.08 (1H, bd, H-17), 2.90 (1H, m, H-17'), 1.99 (2H, q, J = 6.6 Hz, H-11); ^{13}C-NMR (DMSO-d_6): δ 169.9 (C-19), 159.2 (C-9), 154.6 (C-8), 151.2 (C-13), 147.2 (C-3), 136.8 (C-16), 133.9 (C-15, 15'), 131.4 (C-5), 121.0 (C-2), 117.5 (C-14, 14,), 113.4 (C-4), 90.4 (C-6), 73.7 (C-1), 71.3 (C-12), 59.8 (3-OCH_3), 55.0 (C-18), 39.5 (C-7), 36.4 (C-10), 35.3 (C-17), 29.5 (C-11).

Hexadellin C (3). Colorless solid; physical data: $[\alpha]_D$ +102.0° (c 0.067, MeOH); IR neat (NaCl): 3255 (s, br), 3058 (br), 2933 (m), 1771 (w), 1631 (s), 1542 (s), 1456 (s), 1257 (s), 1024 (s), 987 (s) cm^{-1}; UV (EtOH): λmax 207 nm (ε 8300), 284 (1200); MS: ESI-FTMS *m/z* $[M+H]^+$ centered at 803.8504 (calcd 803.8777 for $C_{25}H_{30}{}^{79}Br_2{}^{81}Br_2N_3O_7$, Δ -27.3 mmu); ^{1}H-NMR (DMSO-d_6): δ 8.57 (1H, t, J = 5.8 Hz, 9-NH), 7.49 (2H, s, H-13, 13'), 6.56 (1H, s, H-5), 4.08 (2H, m, H-16), 3.94 (1H, m, H-16'), 3.93 (1H, d, J = 8.3 Hz, H-1), 3.63 (3H, s, 3-OCH_3), 3.60 (1H, d, J = 18.1 Hz, H-7), 3.56 (1H, m, H-18), 3.36 (2H, m, H-10), 3.17 (1H, d, J = 18.1 Hz, H-7'), 3.15 [9H, s, 18-$^+N(CH_3)_3$], 2.74 (2H, t, J = 6.9 Hz, H-11), 2.38 (1H, m, H-17), 2.23 (1H, m, H-17'); ^{13}C-NMR (DMSO-d_6): δ167.1 (C-19), 159.1 (C-9), 154.5 (C-8), 151.1 (C-15), 147.3 (C-3), 139.0 (C-12), 133.2 (C-13, 13'), 131.3 (C-5), 121.1 (C-2), 117.3 (C-14, 14'), 113.3 (C-4), 90.4 (C-6), 74.8 (C-18), 73.7 (C-1), 71.3 (C-16), 59.8 (3-OCH_3), 51.0 [18-$^+N(CH_3)_3$], 39.9 (C-10), 39.5 (C-7), 33.3 (C-11), 27.8 (C-17).

Syntheses of D- and L-3,5-dibromotyrosines. D- and L-3,5-dibromotyrosines were synthesized from D- and L-tyrosines, respectively, because they were not commercially available. The general procedure was as follows[12]: D- or L-tyrosine (1.61 mmol, 291.6 mg) was dissolved in glacial acetic acid (5 ml) and bromine (165 μl, 3.22 mmol) was added. The mixture was stirred at 80 °C for 2 h. The solvent was then removed *in vacuo* and the residue slurried in water. The pH was adjusted to 6 with saturated aqueous $NaHCO_3$ and D- or L-3,5-dibromotyrosine (500 mg, 1.47 mmol, 92%) filtered off as a white solid. D- or L-3,5-dibromotyrosine was further purified by HPLC using reverse phase C_{18} chromatography with linear gradient elution from 100% H_2O to 100% MeOH.

Hydrolysis and derivatization procedures of araplysillin III (2). Araplysillin III (**2**) (0.5 mg, 6.6×10^{-4} mmol) was dissolved in 2 ml 6 N HCl in a glass reaction vial sealed with a Teflon screw cap, and placed in an oven at 100-110 °C for 24 h. After repeated dissolution in H_2O and re-evaporation *in vacuo*, the residue was resuspended in 100 μl H_2O and derivatized with (1-flouro-2, 4-dinitrophen-5-yl) L-alanine amide (FDAA)[13]. To the solution (100 μl, 6.6×10^{-4} mmol) was added 150 μl of a 1% acetone solution of FDAA (0.5 mg, 1.9×10^{-3} mmol). The molar ratio of FDAA to the NH_2 and OH groups of hydrolysate of compound **2** was 3:2, treated by 20 μl of 1 M $NaHCO_3$. The contents were mixed and heated in a water-bath at 30-40 °C for 1 h with frequent stirring. After cooling at room temperature, 10 μl of 2 M HCl was added to the reaction mixture. The contents after mixing were dried *in vacuo*. The residue was dissolved in 130 μl DMSO affording 5.0 mM of the diastereomer of dibromotyrosine. The diastereomers of standard D- and L-dibromotyrosine were synthesized according to the same procedures. HPLC analysis was done using a reverse phase column (Ultracarb C_{18} 250x10 mm) with a linear gradient of CH_3CN in 50 mM triethylammonium phosphate (pH 3.0) from 30% to 70% CH_3CN over 60 min with a flow rate 1.5 ml/min and UV detection at 340 nm. A 30 μl sample (150 nanomoles) of 5.0 mM solution of the FDAA derivatized hydrolysates of **2** and derivatized amino acid standards, D- and L-3, 5-dibromotyrosines, respectively, were used for HPLC analyses.

Syntheses of D- and L-N,N,N-trimethylhomoserines. D- and L-N,N,N-trimethylhomoserines were synthesized from D- and L-homoserines, respectively. The general procedure was as follows[14]: CH_3I (16 mmol, 1 ml), $KHCO_3$ (1 g) and D- or L-homoserine (1.0 mmol, 119 mg) were dissolved in 20 ml MeOH and stirred at room temperature for 48 h. The mixture was evaporated to dryness, and then neutralized with 1M HCl, followed by evaporation and extraction with anhydrous Me_2CO, to give the D- or L-N,N,N-trimethylhomoserines (137 mg, 0.85 mmol, 85%), which were further purified by HPLC using reverse phase C_{18} chromatography with linear gradient elution from 100% H_2O to 100% MeOH.

Syntheses of D- and L-N,N,N-trimethyl-3,5-dibromotyrosines. D- and L-N,N,N-trimethyl-3,5-dibromotyrosines were synthesized from D- and L-3,5-dibromotyrosines, respectively. The procedure is the same as that for the syntheses of D- and L-N,N,N-trimethylhomoserines.

Bromination of N,N,N-trimethyl-3-bromo-4-O-methyl-tyrosine (4) and N,N,N-trimethyl-3-bromo-tyrosine (5). The bromination of **4** and **5** was accomplished by the same procedure used for D- and L-3,5-dibromotyrosines.

Hydrolysis of hexadellin C (3). The hydrolysis of hexadellin C is similar to that of **2**. The hydrolysis temperature and time were adjusted to 80 °C for 36 h to prevent the elimination of N,N,N-trimethylamine from the homoserine moiety which occurred above 100 °C.

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