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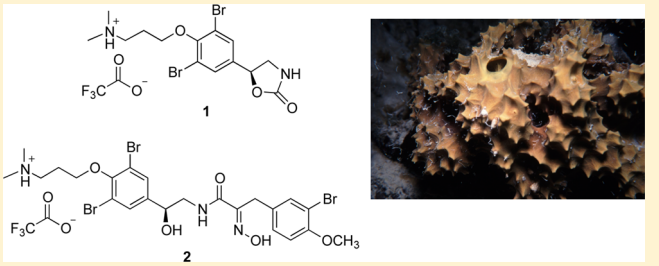
# Bromotyrosine Alkaloids from the Australian Marine Sponge *Pseudoceratina verrucosa*

Trong D. Tran, Ngoc B. Pham, Gregory Fechner, John N. A. Hooper, and Ronald J. Quinn*

Eskitis Institute, Griffith University, Brisbane, Queensland 4111, Australia

Supporting Information

**ABSTRACT:** Two new bromotyrosine alkaloids, pseudoceralidinone A (**1**) and aplysamine 7 (**2**), along with three known compounds were isolated from the Australian sponge *Pseudoceratina verrucosa*. Their structures were characterized by NMR and MS data and the synthetic route. Their cytotoxicity was evaluated against cancer cell lines (HeLa and PC3) and a noncancer cell line (NFF).

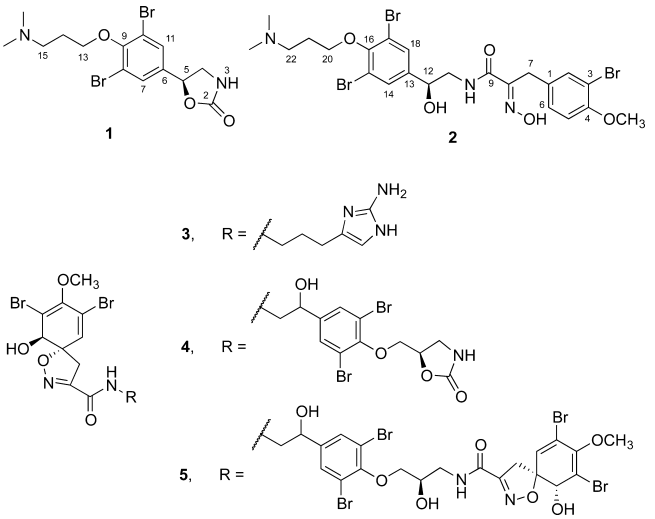


The chemistry of sponges of the order Verongida has been investigated for the past three decades, with hundreds of bromotyrosine alkaloids being isolated.[1] Over 90% of the 280 reported bromotyrosines are derived from four sponge families: Aplysinidae, Aplysinellidae, Ianthellidae, and Pseudoceratinidae.[2] Bromotyrosines, which have been considered as distinct chemotaxonomic markers for the verongida sponges, are further classified into six categories including simple bromotyrosine derivatives, oximes, bastadins, spirocyclohexadienylisoxazolines, spirooxepinisoxazolines, and other structural classes (geodiamolides, jaspamides, polyandrocarpamides, polycitones, polycitrins, and chelonins).[3] These marine alkaloids are well-known for possessing a wide range of bioactivities including histamine $H_2$ and $H_3$ and adrenergic $\alpha_{1D}$ and $\alpha_{2A}$ receptor antagonists,[4,5] anti-human immunodeficiency virus 1 (HIV-1),[6] antiangiogenic,[7] antibacterial,[8,9] antifouling,[10] isoprenylcysteine carboxyl methyltransferase[11,12] and mycothiol-*S*-conjugate amidase[11,12] inhibitory, cytotoxic,[13] and anticancer[14−16] activities.

In the course of our continuing studies on marine natural products, the Australian sponge *Pseudoceratina verrucosa* was found to contain two new bromotyrosine alkaloids, pseudoceralidinone A (**1**) and aplysamine 7 (**2**), together with three known compounds, aerophobin 2[17] (**3**), fistularin 2[18] (**4**), and fistularin 3[14,18] (**5**). Herein the isolation and structure elucidation of the two new compounds (**1** and **2**) are described. Cytotoxicities of all isolated compounds toward human cancer cell lines (HeLa and PC3) and a noncancer cell line (NFF) are also reported.

## RESULTS AND DISCUSSION

The freeze-dried *P. verrucosa* sponge was sequentially extracted with *n*-hexane, $CH_2Cl_2$, and MeOH. The $CH_2Cl_2$/MeOH extracts were then combined and chromatographed using $C_{18}$ bonded silica HPLC (MeOH/$H_2O$/0.1% TFA) to yield two new alkaloids, **1** and **2**, in TFA salt forms, along with three known bromotyrosine derivatives, **3**−**5**.



Pseudoceralidinone A (**1**) was purified as a colorless, amorphous solid. The (+)-LRESIMS spectrum of **1** displayed an isotopic cluster of ions $[M + H]^+$ at *m*/*z* 421, 423, and 425 in the ratio 1:2:1, indicating the presence of two bromines. A pseudomolecular ion peak in the (+)-HRESIMS spectrum at *m*/*z* 420.9746 allowed the molecular formula $C_{14}H_{18}Br_2N_2O_3$ to be assigned to **1**. Analysis of the $^{13}C$ NMR and HSQCAD spectra indicated the molecule contained five quaternary carbons, three methines, four methylenes, and two methyl groups (Table 1). The $^1H$ NMR and COSY spectra demonstrated the presence of two spin systems, −CH−$CH_2$−NH− (moiety **a**, Figure 1) and $(CH_3)_2$−NH−$CH_2$−$CH_2$−$CH_2$− (moiety **c**, Figure 1). A two-proton singlet signal

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**Table 1. NMR Data for Pseudoceralidinone A (1) in DMSO-$d_6$ at 30 °C[a]**

| position | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | gCOSY | gHMBC |
|---|---|---|---|---|
| 2 | 158.2, C | | | |
| 3 | | 7.75, s | 4 | 2, 4, 5 |
| 4 | 47.0, $CH_2$ | $H_a$ 3.85, m; $H_b$ 3.36, t (9.0) | 3, 4b, 5; 3, 4a, 5 | 2, 5, 6; 2, 5, 6 |
| 5 | 74.4, CH | 5.59, t (7.8) | 4a, 4b | 2, 4, 6, 7, 11 |
| 6 | 139.0, C | | | |
| 7, 11 | 130.4, CH | 7.71, s | | 5, 8, 9, 10 |
| 8, 10 | 117.8, C | | | |
| 9 | 152.0, C | | | |
| 13 | 70.3, $CH_2$ | 4.04, t (6.0) | 14 | 9, 14, 15 |
| 14 | 24.8, $CH_2$ | 2.18, m | 13, 15 | 13, 15 |
| 15 | 54.3, $CH_2$ | 3.36, m | 14, 16 | 13, 14, N-$CH_3$ |
| 16 | | 9.63, brs | 15, N-$CH_3$ | |
| N-$CH_3$ | 42.3, $CH_3$ | 2.84, d (4.2) | 16 | 15 |

[a]Referenced to residual DMSO-$d_6$ at $\delta_C$ 39.52 ppm and $\delta_H$ 2.50 ppm.

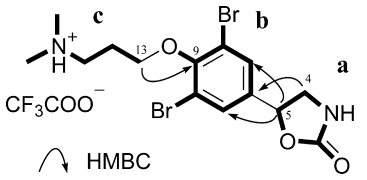


**Figure 1.** Partial structures **a**, **b**, and **c** (in bold) and key HMBC correlations of **1**.

at $\delta_H$ 7.71 ppm correlating with carbons at $\delta_C$ 117.8, 139.0, and 152.0 ppm was assigned to two symmetrical aromatic protons in a 3,5-dibromotyrosine skeleton (moiety **b**, Figure 1).[15] A 5-substituted oxazolidinone ring (moiety **a**) was deduced from the system −CH−$CH_2$−NH− due to the HMBC correlations from H-3 ($\delta_H$ 7.75 ppm), H-4 ($\delta_H$ 3.36 and 3.85 ppm), and H-5 ($\delta_H$ 5.59 ppm) to the same relatively upfield resonance C-2 ($\delta_C$ 158.2 ppm). HMBC correlations from H-5 to C-7 ($\delta_C$ 130.4 ppm) and C-11 ($\delta_C$ 130.4 ppm) and from H-4 to C-6 ($\delta_C$ 139.0 ppm) indicated the connection of moieties **a** and **b** at C-5 and C-6 (Figure 1). The HMBC correlation from H-13 ($\delta_H$ 4.04 ppm) to C-9 ($\delta_C$ 152.0 ppm) supported the assemblage of moieties **b** and **c** at O-12 (Figure 1). Pseudoceralidinone A was elucidated as **1**.

Aplysamine 7 (**2**) was obtained as a colorless, amorphous solid. The (+)-LRESIMS spectrum of **2** exhibited an isotopic cluster of ions $[M + H]^+$ at $m/z$ 664, 666, 668, and 670 in the ratio 1:3:3:1, indicating the presence of three bromine atoms. The molecular formula of **2** was determined to be $C_{23}H_{28}Br_3N_3O_5$ by a pseudomolecular ion peak in the (+)-HRESIMS spectrum at $m/z$ 663.9662. The $^1$H NMR spectrum of **2** indicated the presence of a 1,3,4-trisubstituted aromatic ring containing H-2 ($\delta_H$ 7.37, d, 1.8 Hz), H-5 ($\delta_H$ 6.99, d, 8.4 Hz), and H-6 ($\delta_H$ 7.12, dd, 8.4 and 1.8 Hz) (Table 2). Two substituents, a methoxy group ($\delta_H$ 3.80, $\delta_C$ 56.1 ppm) and a bromine atom, were assigned to positions 4 and 3 of the aromatic ring by HMBC correlations. The upfield chemical shift of C-3 ($\delta_C$ 110.2 ppm) is due to the shielding effect of the bromine atom on the chemical shift of an aromatic carbon.[3] HMBC correlations from H-7 ($\delta_H$ 3.71 ppm) to aromatic carbons ($\delta_C$ 112.5, 130.3, and 132.9 ppm) and two downfield quaternary carbons ($\delta_C$ 151.3 and 163.0 ppm) indicated the

**Table 2. NMR Data for Aplysamine 7 (2) in DMSO-$d_6$ at 30 °C[a]**

| position | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | gCOSY | ROESY | gHMBC |
|---|---|---|---|---|---|
| 1 | 130.3, C | | | | |
| 2 | 132.9, CH | 7.37, d (1.8) | 6 | 7 | 3, 4, 6, 7 |
| 3 | 110.2, C | | | | |
| 4 | 153.7, C | | | | |
| 5 | 112.5, CH | 6.99, d (8.4) | 6 | O-$CH_3$ | 1, 3, 4 |
| 6 | 129.1, CH | 7.12, dd (8.4, 1.8) | 2, 5 | 7 | 2, 4, 5, 7 |
| 7 | 27.6, $CH_2$ | 3.71, s | | 2, 6, N-OH | 1, 2, 6, 8, 9 |
| 8 | 151.3, C | | | | |
| 9 | 163.0, C | | | | |
| 10 | | 7.79, t (6.0) | 11 | 11, 12 | 9, 11 |
| 11 | 46.1, $CH_2$ | 3.39, m; 3.27, m | 10, 12; 10, 12 | | 9, 12, 13; 9, 12, 13 |
| 12 | 69.4, CH | 4.67, t (6.0) | 11 | 10, 14, 18 | 11, 13, 14, 18 |
| 13 | 143.0, C | | | | |
| 14, 18 | 130.4, CH | 7.56, s | | 11, 12 | 12, 15, 16, 17 |
| 15, 17 | 117.0, C | | | | |
| 16 | 150.8, C | | | | |
| 20 | 70.1, $CH_2$ | 3.99, t (6.0) | 21 | 22 | 16, 21, 22 |
| 21 | 24.8, $CH_2$ | 2.16, m | 20, 22 | | 20, 22 |
| 22 | 54.3, $CH_2$ | 3.35[b] | 21, 23 | | 20, 21, N-$CH_3$ |
| 23 | | 9.43, brs | 22, N-$CH_3$ | | |
| O-$CH_3$ | 56.1, $CH_3$ | 3.80, s | | 5 | 4 |
| N-OH | | 11.94, s | | 2, 6, 7 | 8 |
| N-$CH_3$ | 42.4, $CH_3$ | 2.84, d (4.2) | 16 | 22 | 22 |

[a]Referenced to residual DMSO-$d_6$ at $\delta_C$ 39.52 ppm and $\delta_H$ 2.50 ppm. [b]Obscured by $H_2O$ peak.

isolated methylene carbon C-7 to be a $\beta$-carbon in a 3-(3-bromo-4-methoxyphenyl)-2-(hydroxyimino)propanoic acid residue (moiety **a**, Figure 2), which was also found in the

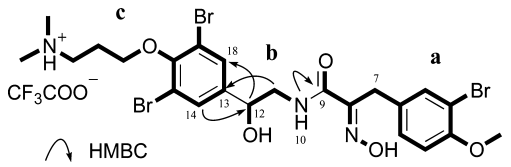


**Figure 2.** Partial structures **a**, **b**, and **c** (in bold) and key HMBC correlations of **2**.

psammaplin and purpuramine skeletons.[3] This assignment was supported by an HMBC correlation of a hydroxy signal in an oxime group ($\delta_H$ 11.94 ppm) to C-8 ($\delta_C$ 151.3 ppm). The upfield resonance of C-7 at $\delta_C$ 27.6 ppm suggested an *E* configuration for the oxime functionality, as the corresponding value for a *Z*-geometry would be >35 ppm.[16,19,20] COSY and HMBC correlations also led to the establishment of 1-substituted-2-aminoethoxy and 3,5-dibromo-4-(3-dimethylamino)propyloxyphenyl residues (moieties **b** and **c**, Figure 2). The HMBC correlation from an exchangeable NH proton ($\delta_H$ 7.79 ppm) to a carbonyl carbon ($\delta_C$ 163.0 ppm) established the formation of an amide bond between moieties **a** and **b** (Figure 2). HMBC correlations from H-11 ($\delta_H$ 3.27 and 3.39 ppm) to C-13 ($\delta_C$ 143.0 ppm) and from H-12 ($\delta_H$ 4.67 ppm) to C-14 and C-18 ($\delta_C$ 130.4 ppm) confirmed the


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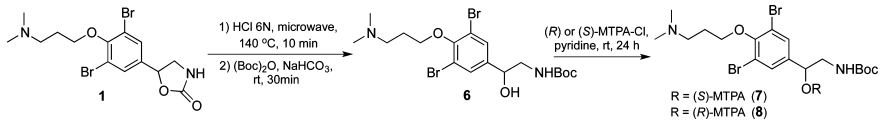
**Scheme 1. Modification of Pseudoceralidinone A (1) to Determine the Absolute Configuration**



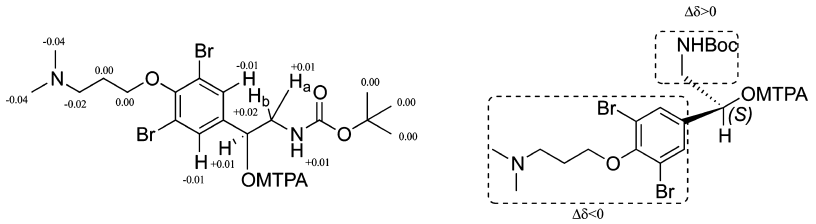
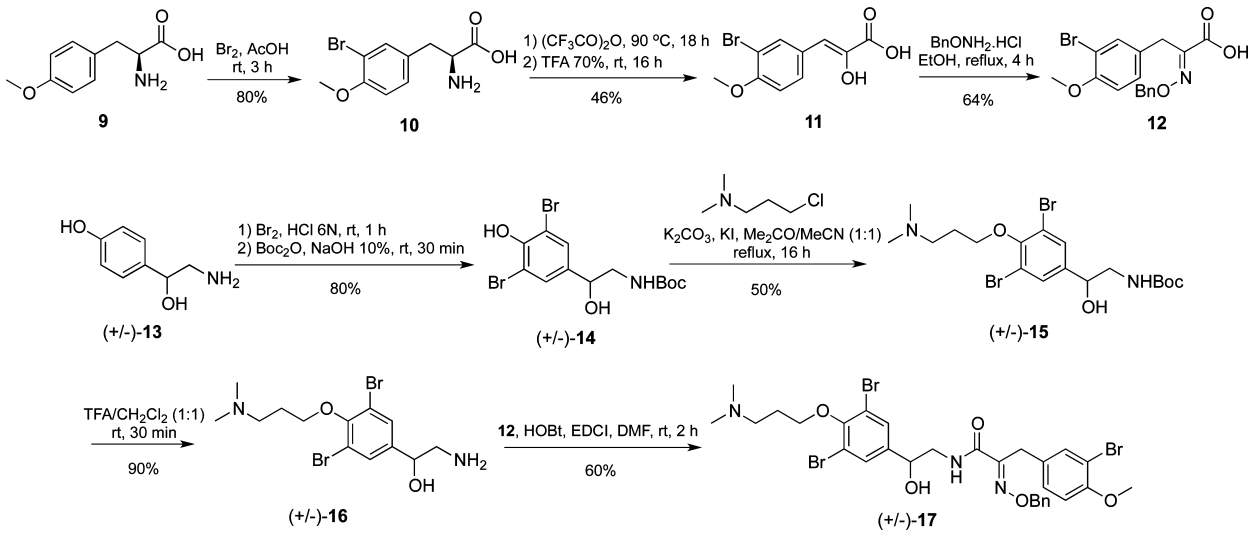




**Figure 3.** $\Delta\delta^{SR}$ values for MTPA derivatives (**7** and **8**) and their configurational correlation model.

**Scheme 2. Total Synthesis of Oxime-Protected Aplysamine 7 (17)**



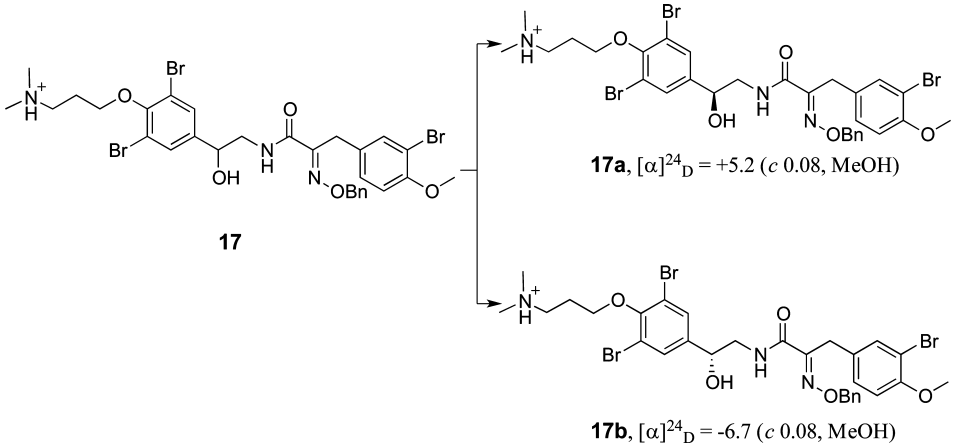




**Figure 4.** Stereochemical structures of two enantiomers (**17a** and **17b**) separated from **17**.

connectivity of **b** and **c** (Figure 2). Thus, aplysamine 7 was assigned as **2**.

CD spectra have been recorded for **1** and **2**; however no characteristic curves were observed. Here we used a synthetic route to determine the absolute configurations for C-5 of **1** and C-12 of **2**.

The absolute configuration of **1** was examined by Mosher's analysis (Scheme 1). Compound **1** was hydrolyzed with 6 N hydrochloric acid using microwave-assisted conditions at 140 °C for 10 min, followed by Boc-protection in aqueous sodium bicarbonate for 30 min at room temperature (rt) to produce **6**. The hydrolysis conditions should not change the configurations at C-5.[21,22] It was noticed that the specific rotation of **6** ($[\alpha]^{25}_{D}$ +3.1 (*c* 0.1, MeOH)) had the same sign compared with that of **1** ($[\alpha]^{25}_{D}$ +3.5 (*c* 0.1, MeOH)). Mosher's analysis was then performed on **6** to determine its absolute configuration. An *S*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configuration was deduced due to the $\Delta\delta^{SR}$ values obtained by subtracting the chemical shift ($\delta_R$) of a proton of the (*R*)-MTPA ester (**8**) from that ($\delta_S$) of the (*S*)-MTPA ester (**7**) (Figure 3). This result led to the assignment of the *S* configuration for **1**.

The absolute configuration of **2** was addressed by a total synthesis (Scheme 2). The alpha-protected 3-bromo-4-methoxyphenylpyruvic acid oxime (**12**) was prepared from *O*-methyl-L-tyrosine (**9**). Compound **9** was converted to 3-bromo-4-methoxyphenylpyruvic acid (**11**) via an azlactone in three steps.[23] Compound **11** was treated with *O*-benzylhydroxylamine in EtOH at reflux for 4 h[24] to give **12** with a yield of 64%. A racemate of commercially available octopamine (**13**) was chosen as a starting material for synthesis of **16**. Dibromination of **13** with bromine in hydrochloric acid at rt gave the 3,5-dibromo derivative, which was then protected by $(Boc)_2O$ under basic conditions to give **14** with an overall yield of 80% for the two steps. *O*-Alkylation of **14** with *N*-(3-chloropropyl)-*N,N*-dimethylamine hydrochloride using potassium carbonate and potassium iodide in $(Me)_2CO$/MeCN (1:1) at reflux[25] produced **15** (50%). Removal of the Boc group in **15** was achieved with 50% trifluoroacetic acid (TFA) in $CH_2Cl_2$ to obtain **16** (90%). This compound was coupled with **12** using EDCI/HOBt[24,26] to give **17** with a yield of 60%.

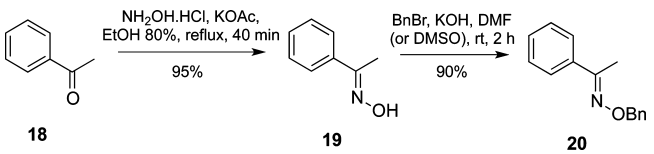
The racemic mixture of **17** was subjected to a chiral-phase HPLC column and resolved into two enantiomers. The absolute configurations of these two stereoisomers were then assigned by Mosher's ester analysis. The results indicated that **17a** with a specific rotation of +5.2 (*c* 0.08, MeOH) was determined to have an *S* configuration, and its stereoisomer (**17b**), with a specific rotation of −6.7 (*c* 0.08, MeOH), had an *R* configuration (Figure 4).

Attempts at hydrogenolysis of the *O*-benzyl oxime-protecting group on **17a** and **17b** to determine the absolute configuration of **2** were unsuccessful. Hydrogenolysis with palladium black in AcOH/dioxane (1:1)[27] or Pd/C in EtOH[28] led to decomposition. Attempted deprotection using HBr/AcOH (1:3)[29] resulted in decomposition; neat TFA[30] or TFA/toluene (1:1)[31] resulted in no conversion. The difficulty of deprotection of the *O*-benzyl group was also reported in a synthetic route to purealidin, a bromotyrosine natural product.[32] Since we could not deprotect the *O*-benzyl group of **17a** (*S* configuration) and **17b** (*R* configuration), we converted the natural product **2** to the oxime-protected compound (**21**) and used the specific rotation to assign the configuration for **21** and thus **2**. Oxime ethers can be made under anhydrous conditions using strong bases such as NaH[33,34] or sodium alkoxides.[35,36] However, strong bases could also benzylate the oxygen of the alcohol group C-12 in **2**.[37,38] Therefore, model reactions were conducted to determine the least basic conditions to *O*-benzylate an oxime (Scheme 3).

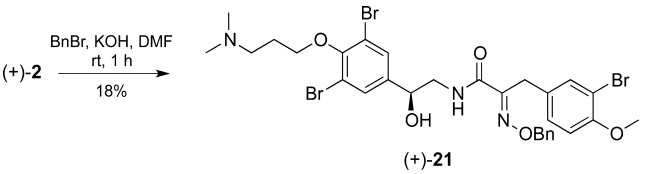
Model oxime **19** was made from acetophenone (**18**) and hydroxylamine in the presence of potassium acetate in 80% aqueous EtOH at reflux for 40 min (95%).[39] The *O*-benzyl product **20** was formed with a yield of 90% using KOH in dry DMF or DMSO at rt for 2 h.[40] Other basic conditions with $Na_2CO_3$, $K_2CO_3$, or $CsCO_3$ gave no or very low yield. Treatment of **17** with benzyl bromide using KOH in DMF at rt monitored by LC/MS showed that the alcohol group was not benzylated. Therefore, KOH in DMF was chosen for the conversion of **2** to **21**.

Reaction between **2** and benzyl bromide using KOH in DMF at rt for 1 h was successful in selectively benzylating the oxime (**21**) with a yield of 18% (Scheme 4). Compounds **21** and **17a** displayed the same retention time by chiral-phase HPLC at 14.4 min, while **17b** had a retention time of 15.3 min, and the measured specific rotation of **21** of +5.8 (*c* 0.04, MeOH) confirmed an *S* configuration for **21**. Therefore, the *S* configuration was assigned for **2**.

**Scheme 3. Protection of the *O*-Benzyl Group in the Model System**




**Scheme 4. Synthesis of *O*-Benzylaplysamine 7 (21)**




The known compounds (**3**−**5**) were characterized by comparison of their spectroscopic data (UV, NMR, and MS) and specific rotations with the values in the literature.[17,18]

The cytotoxicities of the isolated compounds were evaluated against three cell lines, cervical adenocarcinoma cells (HeLa), prostate adenocarcinoma cells (PC3), and human neonatal foreskin fibroblast cells (noncancer cells, NFF) (Table 4). The new compound aplysamine 7 (**2**) showed potency against PC3 cells with an $IC_{50}$ of 4.9 $\mu$M and was inactive ($IC_{50}$ > 10 $\mu$M) against HeLa cells and NFF cells, while pseudoceralidinone A (**1**) and the other known bromotyrosines (**3**−**5**) displayed no cytotoxicity against all three cell lines.

In summary, two new marine metabolites, pseudoceralidinone A (**1**) and aplysamine 7 (**2**), were isolated from the Australian sponge *P. verrucosa*. Their absolute configurations were determined by spectroscopic analysis and chemical synthesis. Compound **2** showed cytotoxic activity against the human prostate cancer cell line PC3.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-1020 polarimeter (10 cm cell). UV and IR spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer and Bruker Tensor 27 FT-IR spectrometer, respectively. NMR spectra were recorded at 30 °C on Varian Inova 600 and 500 MHz spectrometers. The $^1H$ and $^{13}C$ chemical shifts were referenced to the DMSO-$d_6$ solvent peaks at $\delta_H$ 2.50 and $\delta_C$ 39.52 ppm. Standard parameters were used for the 2D NMR spectra obtained, which included gCOSY, HSQCAD ($^1J_{CH}$ = 140 Hz), gHMBC ($^nJ_{CH}$ = 8.3 Hz), and ROESY. Low-resolution mass spectra were acquired using a Mariner TOF mass spectrometer (Applied Biosystems Pty Ltd.). High-resolution mass measurement was acquired on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an Apollo API source. A Betasil $C_{18}$ column (5 $\mu$m, 21.2 × 150 mm) and Hypersil BDS $C_{18}$ column (5 $\mu$m, 10 × 250 mm) were used for semipreparative HPLC. A Phenomenex Luna $C_{18}$ column (3 $\mu$m, 4.6 × 50 mm) was used for LC/MS controlled by MassLynx 4.1 software. All solvents used for extraction and chromatography were Omnisolv HPLC grade, and the $H_2O$ used was Millipore Milli-Q PF


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**Table 3. NMR Data for Compounds 2 and 17 in DMSO-$d_6$ at 30 °C[a]**

| | 2 | | 17 | |
|---|---|---|---|---|
| position | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) |
| 1 | 130.3, C | | 129.6, C | |
| 2 | 132.9, CH | 7.37, d (1.8) | 133.1, CH | 7.36, m |
| 3 | 110.2, C | | 110.3, C | |
| 4 | 153.7, C | | 154.0, C | |
| 5 | 112.5, CH | 6.99, d (8.4) | 112.6, CH | 6.97, d (8.4) |
| 6 | 129.1, CH | 7.12, dd (8.4, 1.8) | 129.2, CH | 7.08, d (8.4) |
| 7 | 27.6, $CH_2$ | 3.71, s | 28.6, $CH_2$ | 3.72, s |
| 8 | 151.3, C | | 152.2, C | |
| 9 | 163.0, C | | 162.2, C | |
| 10 (NH) | | 7.79, t (6.0) | | 7.96, t (6.0) |
| 11 | 46.1, $CH_2$ | 3.39, m; 3.27, m | 46.3, $CH_2$ | 3.36, m; 3.27, m |
| 12 | 69.4, CH | 4.67, t (6.0) | 69.4, CH | 4.67, t (6.0) |
| 13 | 143.0, C | | 143.1, C | |
| 14, 18 | 130.4, CH | 7.56, s | 130.4, CH | 7.57, s |
| 15, 17 | 117.0, C | | 117.2, C | |
| 16 | 150.8, C | | 150.8, C | |
| 20 | 70.1, $CH_2$ | 3.99, t (6.0) | 70.2, $CH_2$ | 3.98, t (6.0) |
| 21 | 24.8, $CH_2$ | 2.16, m | 24.8, $CH_2$ | 2.16, m |
| 22 | 54.3, $CH_2$ | 3.35[b] | 54.4, $CH_2$ | 3.35, m |
| 23 (NH) | | 9.43, brs | | 9.47, brs |
| *O*-$CH_3$ | 56.1, $CH_3$ | 3.80, s | 56.2, $CH_3$ | 3.80, s |
| *N*-$CH_3$ | 42.4, $CH_3$ | 2.84, d (4.2) | 42.4, $CH_3$ | 2.84, d (4.2) |
| *O*-$CH_2$-Ph | | | 76.6, $CH_2$ | 5.25, s |
| Ph (OBn) | | | 136.8, C | |
| | | | 128.0, CH | 7.33, d (7.2) |
| | | | 128.5, CH | 7.36, m |
| | | | 128.1, CH | 7.36, m |
| *N*-OH | | 11.94, s | | |

[a]Referenced to residual DMSO-$d_6$ at $\delta_C$ 39.52 ppm and $\delta_H$ 2.50 ppm. [b]Obscured by $H_2O$ peak.

**Table 4. Cytotoxicity Evaluation for Compounds 1−5**

| | $IC_{50}$ ± SD ($\mu$M) or % inhibition ± SD[a] | | |
|---|---|---|---|
| compound | HeLa | PC3 | NFF |
| **1** | 44 ± 14% | 35 ± 6.7% | 29 ± 2.5% |
| **2** | 19 ± 5.2 | 4.9 ± 1.8 | 16 ± 7.2 |
| **3** | 20 ± 4.7% | 66 ± 4.8% | 25 ± 8.1% |
| **4** | 40 ± 3.4 | 73 ± 12% | 68 ± 3.4% |
| **5** | 46 ± 14% | 60 ± 20% | 48 ± 12% |
| vincristine sulfate | 0.0358 ± 0.0035 | 0.0153 ± 0.0011 | 0.0169 ± 0.0032 |

[a]Each $IC_{50}$ or % inhibition at 100 $\mu$M was determined as the mean ± SD of two independent experiments with triplicate determinations for each concentration.

filtered. A Phenomenex chiral-phase column, Lux 5 $\mu$m, Amylose-2, 4.6 × 250 mm, was used for separation of compounds **17a** and **17b** and confirmation of the absolute configuration of compound **21**.

**Animal Material.** A specimen of *P. verrucosa* was collected at the depth of 9.4 m, at Hook Reef Lagoon, Queensland, Australia, in 1995. It was identified as *Pseudoceratina verrucosa* Bergquist (phylum Porifera, class Demospongiae, order Verongida, family Aplysinellidae). A voucher specimen (QMG315237) has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

**Extraction and Isolation.** A freeze-dried sample of *P. verrucosa* (5 g) was extracted exhaustively with hexane (250 mL), $CH_2Cl_2$ (250 mL), and MeOH (2 × 250 mL), respectively. The $CH_2Cl_2$ and MeOH extracts were combined, and solvents were then evaporated to yield a yellow residue (1.5 g). This extract was preadsorbed onto $C_{18}$ (1.0 g) and packed dry into a small cartridge, which was connected to a $C_{18}$ preparative HPLC column (5 $\mu$m, 21.2 × 150 mm). A linear gradient from 100% $H_2O$ (0.1% TFA) to 100% MeOH (0.1% TFA) was performed over 60 min at a flow rate of 9 mL/min, and 60 fractions (1.0 min each) were collected. Fistularin 3 (**5**, 40.0 mg, 0.8% dry wt) was obtained from fractions 44−48. Fractions 23−37 were combined and loaded onto a $C_{18}$ preparative HPLC column (5 $\mu$m, 21.2 × 150 mm) with a 60 min linear gradient from 20% MeOH (0.1% TFA)−80% $H_2O$ (0.1% TFA) to 90% MeOH (0.1% TFA)−10% $H_2O$ (0.1% TFA). A new bromotyrosine, pseudoceralidinone A (**1**, 12 mg, 0.24% dry wt), and two known compounds, aerophobin 2 (**3**, 18 mg, 0.36% dry wt) and fistularin 2 (**4**, 1.5 mg, 0.03%), were isolated respectively. Further purification for fractions 37 to 39 on the same reversed-phase HPLC column with a linear gradient from 20% MeOH (0.1% TFA)−80% $H_2O$ (0.1% TFA) to 80% MeOH (0.1% TFA)−20% $H_2O$ (0.1% TFA) in 60 min yielded the new aplysamine 7 (**2**, 4.5 mg, 0.09% dry wt).

*Pseudoceralidinone A (**1**):* colorless, amorphous solid; $[\alpha]^{25}_D$ +3.5 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 257 (3.4), 362 (2.7) nm; IR (film) $\nu_{max}$ 3444, 1748, 1682, 1203 $cm^{-1}$; $^1H$ (600 MHz) and $^{13}C$ (150 MHz) NMR data are summarized in Table 1; (+)-HRESIMS *m/z* 420.9746 $[M + H]^+$ (calcd for $[C_{14}H_{19}{}^{79}Br_2N_2O_3]^+$, 420.9757, Δ −2.6 ppm).

*Aplysamine 7 (**2**):* colorless, amorphous solid; $[\alpha]^{24}_D$ +8.1 (*c* 0.08, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 282 (3.5) nm; IR (film) $\nu_{max}$ 3405, 1673, 1204 $cm^{-1}$; $^1H$ (600 MHz) and $^{13}C$ (150 MHz) NMR data are summarized in Table 2; (+)-HRESIMS *m/z* 663.9662 $[M + H]^+$ (calcd for $[C_{23}H_{29}{}^{79}Br_3N_3O_5]^+$, 663.9652, Δ 1.5 ppm).

*Compound **6**.* Compound **1** (4.6 mg, 0.011 mmol) was hydrolyzed in 200 $\mu$L of 6 N HCl using microwave-assisted conditions at 140 °C for 10 min. The solvent was removed under reduced pressure. The residue was then basified with aqueous saturated $NaHCO_3$ (1 mL) followed by Boc-protection with $Boc_2O$ (3 mg, 0.014 mmol) for 30 min at rt. The crude product was concentrated *in vacuo* and separated by RP-HPLC to obtain **6** as a colorless, amorphous solid (2.5 mg, 46% yield in two step): $[\alpha]^{25}_D$ +3.1 (*c* 0.1, MeOH); $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.51 (2H, s, H-7 and H-11), 6.67 (1H, t, *J* = 5.4 Hz, NH-3), 4.54 (1H, t, *J* = 6.0, H-5), 3.95 (2H, t, *J* = 6.6 Hz, H-13), 3.08 (2H, m, H-4), 2.41 (2H, t, *J* = 6.6 Hz, H-15), 2.14 (6H, s, 2 × $NCH_3$), 1.91 (2H, pentet, *J* = 6.6 Hz, H-14), 1.32 (9H, s, 3 × $CH_3$-Boc); $^{13}C$ NMR (150 MHz, DMSO-$d_6$, obtained from correlations observed in HSQCAD and gHMBC spectra) $\delta$ 155.7 (C, C-2), 151.5 (C, C-9), 143.2 (C, C-6), 130.3 (CH, C-7 and C-11), 117.3 (C, C-8 and C-10), 77.7 (C, C-Boc), 71.5 ($CH_2$, C-13), 69.8 (CH, C-5), 55.4 ($CH_2$, C-15), 47.0 ($CH_2$, C-4), 44.9 ($CH_3$, 2 × $NCH_3$), 27.8 ($CH_3$, 3 × $CH_3$-Boc), 27.4 ($CH_2$, C-14); (+)-LRESIMS *m/z* 495, 497, 499 (in the ratio 1:2:1).

**Preparation of MTPA Esters (7 and 8).** (*R*)- or (*S*)-MTPA-Cl (2 $\mu$L, 0.01 mmol) was added to **6** (0.5 mg, 0.001 mmol) in anhydrous pyridine (150 $\mu$L) and stirred at rt. Reactions were monitored by LC/MS and stopped after 24 h. An aliquot was then dried under dry nitrogen. The residue was partitioned with the solvent system $H_2O$/$CH_2Cl_2$ (1:1). The $CH_2Cl_2$ fraction was evaporated to dryness to yield Mosher's esters **7** and **8**. The $^1H$ and COSY NMR experiments were performed on the Mosher's esters (**7** and **8**) to obtain the $\delta_S$ and $\delta_R$ values, which were used to determine the absolute configuration at position C-5.

*(S)-MTPA ester of **6** (**7**):* $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.62 (2H, s, H-7 and H-11), 7.49 (2H, m, MTPA-ArH), 7.46 (3H, m, MTPA-ArH), 7.13 (1H, t, *J* = 5.5 Hz, NH-3), 5.96 (1H, t, *J* = 6.0 Hz, H-5), 4.01 (2H, t, *J* = 6.0 Hz, H-13), 3.49 (3H, s, MTPA-$OCH_3$), 3.45 (1H, m, H-4a), 3.32 (1H, m, H-4b), 3.31 (2H, m, H-15), 2.79 (6H, s, 2 × $NCH_3$), 2.24 (2H, m, H-14), 1.32 (9H, s, Boc-H); (+)-LRESIMS *m/z* 711, 713, 715 (in the ratio 1:2:1).

*(R)-MTPA ester of **6** (**8**):* $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.63 (2H, s, H-7 and H-11), 7.50 (2H, m, MTPA-ArH), 7.46 (3H, m, MTPA-ArH), 7.12 (1H, t, *J* = 5.5 Hz, NH-3), 5.95 (1H, t, *J* = 6.0 Hz, H-5), 4.01 (2H, t, *J* = 6.0 Hz, H-13), 3.49 (3H, s, MTPA-$OCH_3$), 3.44


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(1H, m, H-4a), 3.30 (1H, m, H-4b), 3.33 (2H, m, H-15), 2.83 (6H, s, 2 × $NCH_3$), 2.24 (2H, m, H-14), 1.32 (9H, s, Boc-H); (+)-LRESIMS *m/z* 711, 713, 715 (in the ratio 1:2:1).

*3-Bromo-O-methyl-L-tyrosine (**10**).* To a cooled solution (5 °C) of *O*-methyl-L-tyrosine (**9**, 261 mg, 1.3 mmol) in glacial acetic acid (8.0 mL, 0.14 mol) was added $Br_2$ (0.12 mL, 2.3 mmol), and the mixture stirred for 3 h at rt. The reaction was then quenched with saturated $Na_2S_2O_3$ solution, and the solvent was removed under vacuum. The reaction mass was extracted with EtOAc. The organic layer was dried prior to being purified by RP-HPLC to give **10** as a colorless, amorphous solid (285 mg, 80%): IR (film) $\nu_{max}$ 3411, 1625, 1255, 1024 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 8.31 (3H, brs), 7.47 (1H, d, *J* = 1.8 Hz), 7.23 (1H, dd, *J* = 9.0, 1.8 Hz), 7.07 (1H, d, *J* = 8.4 Hz), 4.17 (1H, brs), 3.83 (3H, s), 3.05 (2H, m); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 170.3 (C), 154.7 (C), 133.8 (CH), 130.1 (CH), 128.5 (C), 112.7 (CH), 110.6 (C), 56.2 ($OCH_3$), 53.1 (CH), 34.5 ($CH_2$); (+)-LRESIMS *m/z* 274, 276 (in the ratio 1:1).

*3-Bromo-4-methoxyphenylpyruvic acid (**11**).* A solution of **10** (161 mg, 0.6 mmol) in $(CF_3CO)_2O$ (3.0 mL, 21.2 mmol) was heated at 90 °C for 18 h. The solvent was removed under reduced pressure. The residue was again dissolved in 70% aqueous TFA and allowed to stand for 16 h at rt. The product was chromatographed on RP-HPLC to yield **11** (74 mg, 46%): IR (film) $\nu_{max}$ 3410, 1650, 1254, 1054 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 9.28 (1H, brs), 8.10 (1H, d, *J* = 1.8 Hz), 7.66 (1H, dd, *J* = 8.4, 1.8 Hz), 7.10 (1H, d, *J* = 8.4 Hz), 3.85 (3H, s); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 166.2 (C), 154.3 (C), 141.1 (C), 133.2 (CH), 130.2 (CH), 129.2 (C), 112.5 (CH), 110.5 (C), 108.2 (CH), 56.2 ($OCH_3$); (−)-LRESIMS *m/z* 271, 273 (in the ratio 1:1).

*2-(Benzyloxyimino)-3-(3-bromo-4-methoxyphenyl)propanoic acid (**12**).* To a solution of **11** (33 mg, 0.12 mmol) in EtOH (2 mL) was added *O*-benzylhydroxylamine (56 mg, 0.35 mmol), and the mixture refluxed for 4 h. The crude product was purified by RP-HPLC to afford **12** (29 mg, 64%): IR (film) $\nu_{max}$ 3420, 2940, 1625, 1599, 1541, 1397, 1255, 1022, 806 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.42 (1H, d, *J* = 1.8 Hz); 7.32 (2H, d, *J* = 7.8 Hz), 7.29 (1H, d, *J* = 7.2 Hz), 7.25 (2H, d, *J* = 7.2 Hz), 7.19 (1H, dd, *J* = 8.4, 1.8 Hz), 6.96 (1H, d, *J* = 8.4 Hz), 5.05 (2H, s), 3.79 (3H, s), 3.72 (2H, s); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 164.7 (C), 158.2 (C), 153.5 (C), 138.2 (C), 133.1 (CH), 131.5 (C), 129.6 (CH), 128.2 (2 × CH), 127.54 (2 × CH), 127.50 (CH), 112.3 (CH), 110.0 (C), 74.8 ($CH_2$), 56.2 ($OCH_3$), 30.8 ($CH_2$); (+)-LRESIMS *m/z* 378, 380 (in the ratio 1:1).

*(±)-tert-Butyl-2-(3,5-dibromo-4-hydroxyphenyl)-2-hydroxyethylcarbamate (**14**).* To a solution of racemic octopamine (**13**, 384 mg, 2.0 mmol) in distilled $H_2O$ (3 mL) was added 6 N HCl (3 mL), and the mixture was cooled to 5 °C. Bromine (0.35 mL, 6.8 mmol) was then injected into the stirred solution. The reaction was performed in 1 h and then dried *in vacuo*. It was then basified with NaOH 10% (5 mL), and $Boc_2O$ (500 mg, 2.2 mmol) was added with stirring at rt for 30 min. The solvent was removed *in vacuo*, and the residue was subjected to RP-HPLC to give **14** (654 mg, 80% yield in two steps): IR (film) $\nu_{max}$ 3411, 2977, 2932, 1693, 1468, 1250, 1170, 1055 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.10 (2H, s), 6.64 (1H, t, *J* = 5.4 Hz), 4.29 (1H, dd, *J* = 7.2, 5.4 Hz), 3.00 (1H, m), 2.92 (1H, m), 1.36 (9H, s); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 160.8 (C), 155.7 (C), 128.8 (2 × CH), 123.9 (C), 114.4 (2 × C), 77.5 (C), 70.7 (CH), 48.3 ($CH_2$), 28.3 (3 × $CH_3$); (+)-LRESIMS *m/z* 410, 412, 414 (in the ratio 1:2:1).

*(±)-tert-Butyl-2-(3,5-dibromo-4-(3-(dimethylamino)propoxy)-phenyl)-2-hydroxyethyl carbamate (**15**).* To a racemic mixture of compound **14** (327 mg, 0.8 mmol), $K_2CO_3$ (0.90 g, 6.5 mmol), and KI (1.08 g, 6.5 mmol) in dry $Me_2CO$/MeCN (1:1, 5.0 mL) was added 3-dimethylamino-1-propyl chloride hydrochloride (406 mg, 2.5 mmol), and the mixture was refluxed for 16 h. The solvent was evaporated *in vacuo*, and crude product was extracted with EtOAc. The organic layer was concentrated and purified by RP-HPLC to obtain **15** (198 mg, 50%): IR (film) $\nu_{max}$ 3336, 2974, 2823, 1698, 1456, 1253, 1168, 1042 $cm^{-1}$; $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.52 (2H, s), 6.76 (1H, brs), 4.55 (1H, t, *J* = 6.0 Hz), 3.95 (2H, t, *J* = 6.5 Hz), 3.08 (2H, t, *J* = 6.5 Hz), 2.41 (2H, t, *J* = 7.0 Hz), 2.14 (6H, s), 1.91 (2H, m), 1.32 (9H, s); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta$ 155.5 (C), 151.3 (C), 142.7 (C), 130.4 (2 × CH), 117.0 (2 × C), 77.6 (C), 71.7 ($CH_2$), 70.0 (CH), 55.6 ($CH_2$), 47.3 ($CH_2$), 45.1 (2 × $NCH_3$), 28.1 (3 × $CH_3$), 27.7 ($CH_2$); (+)-LRESIMS *m/z* 495, 497, 499 (in the ratio 1:2:1).

*(±)-2-Amino-1-(3,5-dibromo-4-(3-(dimethylamino)propoxy)-phenyl)ethanol (**16**).* Compound **15** (158 mg, 0.3 mmol) was taken in 2 mL of $CH_2Cl_2$/TFA (1:1) and stirred for 30 min. After the solvent was removed, the crude product was purified by RP-HPLC to obtain free amine **16** (114 mg, 90%): IR (film) $\nu_{max}$ 3408, 3033, 2739, 1676, 1202, 1133 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 8.10 (2H, brs), 7.68 (2H, s), 4.82 (1H, dd, *J* = 9.0, 3.0 Hz), 4.00 (2H, t, *J* = 6.6 Hz), 3.36 (2H, t, *J* = 7.2 Hz), 3.10 (1H, d, *J* = 12.0 Hz), 2.89 (1H, t, *J* = 11.6 Hz), 2.84 (6H, s), 2.19 (2H, m); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 151.4 (C), 141.4 (C), 130.5 (2 × CH), 117.6 (2 × C), 70.3 ($CH_2$), 67.6 (CH), 54.3 ($CH_2$), 45.1 ($CH_2$), 42.3 (2 × $NCH_3$), 24.8 ($CH_2$); (+)-LRESIMS *m/z* 395, 397, 399 (in the ratio 1:2:1).

*(±)-2-(Benzyloxyimino)-3-(3-bromo-4-methoxyphenyl)-N-(2-(3,5-dibromo-4-(3-(dimethylamino)propoxy)phenyl)-2-hydroxyethyl)-propanamide (**17**).* To a solution of **16** (30 mg, 0.076 mmol) in dry dimethylformamide (DMF, 3.5 mL) was added *N*-hydroxybenzotriazole (HOBt, 20 mg, 0.15 mmol), and the reaction mixture was stirred for 15 min at rt. The reaction mixture was then cooled to 0 °C, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI, 28.7 mg, 0.15 mmol) was added, and stirring continued for 30 min at 0 °C. To this mixture was then added compound **12** (28 mg, 0.076 mmol), and the mixture was stirred for 2 h at rt. The crude product was concentrated *in vacuo* and separated by RP-HPLC (MeOH, $H_2O$, 0.1% TFA) to yield a racemic mixture of **17** (34 mg, 60%): IR (film) $\nu_{max}$ 3395, 2940, 1673, 1496, 1204 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 9.47 (1H, brs), 7.96 (1H, t, *J* = 6.0 Hz), 7.57 (2H, s), 7.36 (3H, m), 7.33 (2H, d, *J* = 7.2 Hz), 7.08 (1H, d, *J* = 8.4 Hz), 6.97 (1H, d, *J* = 8.4 Hz), 5.25 (2H, s), 4.67 (1H, t, *J* = 6.0 Hz), 3.98 (2H, t, *J* = 6.0 Hz), 3.80 (3H, s), 3.72 (2H, s), 3.36 (1H, m), 3.35 (2H, m), 3.27 (1H, m), 2.84 (6H, d, *J* = 4.2 Hz), 2.16 (2H, m); $^{13}C$ NMR (150 MHz, DMSO-$d_6$) $\delta$ 162.2 (C), 154.0 (C), 152.2 (C), 150.8 (C), 143.1 (C), 136.8 (C), 133.1 (CH), 130.4 (2 × CH), 129.6 (C), 129.2 (CH), 128.5 (2 × CH), 128.1 (CH), 128.0 (2 × CH), 117.2 (2 × C), 112.6 (CH), 110.3 (C), 76.6 ($CH_2$), 70.2 ($CH_2$), 69.4 (CH), 56.2 ($OCH_3$), 54.4 ($CH_2$), 46.3 ($CH_2$), 42.4 (2 × $NCH_3$), 28.6 ($CH_2$), 24.8 ($CH_2$); (+)-LRESIMS *m/z* 754, 756, 758, 760 (in the ratio 1:3:3:1).

*Isolation of Two Enantiomers from a Synthetic Racemic Oxime-Protected Aplysamine 7 Mixture (**17**).* Compound **17** was further purified by HPLC on a chiral-phase HPLC column (Phenomenex, Lux 5 μm, Amylose-2, 4.6 × 250 mm) with an isocratic condition of 30% MeCN (0.1% TFA)−70% $H_2O$ (0.1% TFA) in 15 min at a flow rate of 0.8 mL/min. Two enantiomers (**17a** and **17b**) eluted at 7.5 and 9.8 min, respectively.

*Compound **17a**:* colorless, amorphous solid; $[\alpha]^{24}_D$ +5.2 (*c* 0.08, MeOH); $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 9.56 (1H, brs, NH-23), 7.96 (1H, t, *J* = 6.0 Hz, NH-10), 7.57 (2H, s, H-14 and H-18), 7.36 (3H, m, H-2 and H-Ph), 7.33 (3H, d, *J* = 7.2 Hz, H-Ph), 7.08 (1H, d, *J* = 8.4 Hz, H-6), 6.97 (1H, d, *J* = 8.4 Hz, H-5), 5.25 (2H, s, $OCH_2$-Ph), 4.67 (1H, t, *J* = 6.0 Hz, H-12), 3.98 (2H, t, *J* = 6.0 Hz, H-20), 3.80 (3H, s, $OCH_3$), 3.72 (2H, s, H-7), 3.36 (1H, m, H-11a), 3.35 (2H, m, H-22), 3.27 (1H, m, H-11b), 2.84 (6H, d, *J* = 4.2 Hz, 2 × $NCH_3$), 2.16 (2H, m, H-21); (+)-LRESIMS *m/z* 754, 756, 758, 760 (in the ratio 1:3:3:1); (+) HRESIMS *m/z* 754.0086 $[M + H]^+$ (calcd for $[C_{30}H_{35}{}^{79}Br_3N_3O_5]^+$, 754.0121, Δ −4.6 ppm).

*Compound **17b**:* colorless, amorphous solid; $[\alpha]^{24}_D$ −6.7 (*c* 0.08, MeOH); $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ 9.55 (1H, brs, NH-23), 7.96 (1H, t, *J* = 6.0 Hz, NH-10), 7.57 (2H, s, H-14 and H-18), 7.36 (3H, m, H-2 and H-Ph), 7.33 (3H, d, *J* = 7.2 Hz, H-Ph), 7.08 (1H, d, *J* = 8.4 Hz, H-6), 6.97 (1H, d, *J* = 8.4 Hz, H-5), 5.25 (2H, s, $OCH_2$-Ph), 4.67 (1H, t, *J* = 6.0 Hz, H-12), 3.98 (2H, t, *J* = 6.0 Hz, H-20), 3.80 (3H, s, $OCH_3$), 3.72 (2H, s, H-7), 3.36 (1H, m, H-11a), 3.35 (2H, m, H-22), 3.27 (1H, m, H-11b), 2.84 (6H, d, *J* = 4.2 Hz, 2 × $NCH_3$), 2.16 (2H, m, H-21); (+)-LRESIMS *m/z* 754, 756, 758, 760 (in the ratio 1:3:3:1); (+) HRESIMS *m/z* 754.0090 $[M + H]^+$ (calcd for $[C_{30}H_{35}{}^{79}Br_3N_3O_5]^+$, 754.0121, Δ −4.1 ppm).


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**Preparation of MTPA Esters (17a and 17b).** The absolute configuration at C-12 of compounds **17a** and **17b** was assigned using the same procedure as that for compound **6**.

*(S)-MTPA ester of 17a (22a):* $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.99 (1H, t, $J$ = 6.0 Hz, NH-10), 7.54 (2H, s, H-14 and H-18), 5.259 (2H, s, $OCH_2$-Ph), 4.715 (1H, t, $J$ = 6.0 Hz, H-12), 3.26 (1H, m, H-11), 2.79 (6H, s, 2 × $NCH_3$); (+)-LRESIMS $m/z$ 970, 972, 974, 976 (in the ratio 1:3:3:1).

*(R)-MTPA ester of 17a (23a):* $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.97 (1H, t, $J$ = 6.0 Hz, NH-10), 7.55 (2H, s, H-14 and H-18), 5.254 (2H, s, $OCH_2$-Ph), 4.710 (1H, t, $J$ = 6.0 Hz, H-12), 3.25 (1H, m, H-11), 2.80 (6H, s, 2 × $NCH_3$); (+)-LRESIMS $m/z$ 970, 972, 974, 976 (in the ratio 1:3:3:1).

*(S)-MTPA ester of 17b (22b):* $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.97 (1H, t, $J$ = 6.0 Hz, NH-10), 7.543 (2H, s, H-14 and H-18), 5.257 (2H, s, $OCH_2$-Ph), 4.71 (1H, t, $J$ = 6.0 Hz, H-12), 3.24 (1H, m, H-11), 2.80 (6H, s, 2 × $NCH_3$); (+)-LRESIMS $m/z$ 970, 972, 974, 976 (in the ratio 1:3:3:1).

*(R)-MTPA ester of 17b (23b):* $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.98 (1H, t, $J$ = 6.0 Hz, NH-10), 7.535 (2H, s, H-14 and H-18), 5.261 (2H, s, $OCH_2$-Ph), 4.71 (1H, t, $J$ = 6.0 Hz, H-12), 3.25 (1H, m, H-11), 2.79 (6H, s, 2 × $NCH_3$); (+)-LRESIMS $m/z$ 970, 972, 974, 976 (in the ratio 1:3:3:1).

*(E)-Acetophenone oxime (19):* A suspension of acetophenone (**18**) (200 $\mu$L, 1.7 mmol), hydroxylamine hydrochloride (419 mg, 6 mmol), and KOAc (410 mg, 4.2 mmol) in 80% aqueous EtOH (35.0 mL) was heated gently to reflux for 40 min. Following cooling to rt, the solvent was removed *in vacuo* and the residue purified by flash chromatography (9:1 hexane/EtOAc eluent) to provide **19** (218 mg, 95% yield): IR (film) $\nu_{max}$ 3220, 2920, 1447, 1305, 1008, 928, 764 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.51 (1H, brs), 7.65 (2H, m), 7.41 (3H, m), 2.33 (3H, s); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 156.1 (C), 136.7 (C), 129.4 (C), 128.7 (2 × CH), 126.2 (2 × CH), 12.5 ($CH_3$); (+)-LRESIMS $m/z$ 136.

*(E)-Acetophenone O-benzyl oxime (20).* Benzyl bromide (2.1 $\mu$L, 0.0178 mmol) was added to the solution of **19** (2 mg, 0.0148 mmol) and KOH (40 mg) in dry DMF (600 $\mu$L). The reaction mixture was stirred for 2 h at rt. Brine (1 mL) was then added to the reaction followed by extraction with EtOAc (6 mL). The organic layer was washed three times with brine (0.5 mL), and the solvent was removed under dry nitrogen. The residue was purified by flash chromatography (100% hexane eluent) to provide **20** (3 mg, 90% yield): IR (film) $\nu_{max}$ 2929, 1455, 1378, 1304, 1001, 919, 772 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.65 (2H, m), 7.43 (2H, d, $J$ = 7.5 Hz), 7.36 (5H, m), 7.31 (1H, m), 5.25 (2H, s), 2.28 (3H, s); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 155.1 (C), 138.3 (C), 136.8 (C), 129.2 (CH), 128.5 (4 × CH), 128.3 (2 × CH), 127.9 (CH), 126.2 (2 × CH), 76.3 ($CH_2$), 13.0 ($CH_3$); (+)-LRESIMS $m/z$ 226.

*O-Benzylaplysamine 7 (21).* Benzyl bromide (0.64 $\mu$L, 0.0054 mmol) was added to the solution of **2** (3 mg, 0.0045 mmol) and KOH (40 mg) in dry DMF (600 $\mu$L). The reaction mixture was stirred for 1 h at rt and then stopped by adding 1 mL of brine followed by extraction with EtOAc (6 mL). The organic layer was washed three times with brine (0.5 mL), and the solvent was then removed under dry nitrogen. The residue was purified by RP-HPLC (MeOH, $H_2O$) to yield compound **21** (0.6 mg, 18% yield) in a neutral form: $[\alpha]^{26}_D$ +7.2 ($c$ 0.04, MeOH, neutral form); $[\alpha]^{26}_D$ +5.8 ($c$ 0.04, MeOH, TFA salt); $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.90 (1H, t, $J$ = 6.0 Hz, NH-10), 7.54 (2H, s, H-14 and H-18), 7.33−7.36 (6H, m, H-2 and H-Ph), 7.06 (1H, dd, $J$ = 8.4, 1.8 Hz, H-6), 6.97 (1H, d, $J$ = 8.4 Hz, H-5), 5.71 (1H, d, $J$ = 4.8 Hz, 12-OH), 5.25 (2H, s, $OCH_2$-Ph), 4.67 (1H, dd, $J$ = 6.0, 5.4 Hz, H-12), 3.95 (2H, t, $J$ = 6.0 Hz, H-20), 3.80 (3H, s, $OCH_3$), 3.72 (2H, s, H-7), H-11 and H-22 obscured by $H_2O$ peak, 2.15 (6H, s, 2 × $NCH_3$), 1.91 (2H, m, H-21); (+)-LRESIMS $m/z$ 754, 756, 758, 760 (in the ratio 1:3:3:1); (+)-HRESIMS $m/z$ 754.0151 $[M + H]^+$ (calcd for $[C_{30}H_{35}{}^{79}Br_3N_3O_5]^+$, 754.0121, Δ 4.0 ppm).

*LC/MS Analysis of Compound 21.* Compound **21** was loaded on a chiral-phase HPLC column (Phenomenex, Lux 5 $\mu$m, Amylose-2, 4.6 × 250 mm) using a linear gradient from 45% MeOH (0.1% HCOOH)−55% $H_2O$ (0.1% HCOOH) to 100% MeOH (0.1% HCOOH) in 25 min and isocratic at 100% MeOH (0.1% HCOOH) for the next 5 min at a flow rate of 0.8 mL/min. Compound **21** eluted at 14.4 min. Using the same procedure as for compound **17**, the two enantiomers **17a** and **17b** were separated with retention times of 14.4 and 15.3 min, respectively.

**Cytotoxicity Assay.** Human neonatal foreskin fibroblast (non-cancer cells, NFF) was grown in DMEM media supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin. Cervical adenocarcinoma cells (HeLa) and prostate adenocarcinoma cells (PC3) were grown in RPMI media supplemented with 10% FBS, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin. Cells were grown under 5% $CO_2$ in a humidified environment at 37 °C. Forty-five microliters of media containing 1000 cells was added to a 384-well microtiter plate (Falcon black clear 384 TC microtiter plates). Plates were incubated overnight at 37 °C, 5% $CO_2$, and 80% humidity to allow cells to adhere. Stock concentrations of pure compounds were prepared at 10 mM in 100% DMSO. Compounds were then diluted 1 in 10 in media. Five microliters of diluted compound was added to the cells to give a total volume of 50 $\mu$L. The final compound concentration range tested was 100 $\mu$M to 3 nM (final DMSO concentration of 1%). Each concentration in media was tested in triplicate. Vincristine sulfate was used as a positive control. Cells and compounds were then incubated for 72 h at 37 °C, 5% $CO_2$, and 80% humidity. Cell proliferation was measured with the addition of 10 $\mu$L of a 60% Alamar blue solution in media to each well of the microtiter plate to give a final concentration of 10% Alamar blue. The plates were incubated at 37 °C, 5% $CO_2$, and 80% humidity within 24 h. The fluorescence of each well was read at excitation 535 nm and emission 590 nm on a Victor II Wallac plate reader (PerkinElmer). Ten-point concentration response curves were then analyzed using nonlinear regression, and $IC_{50}$ values were determined by using GraphPad Prism 5 software.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1$H and $^{13}$C NMR spectra for **1**, **2**, and other synthetic compounds and also a photograph of the sponge *Pseudoceratina verrucosa* are available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Tel: +61-7-3735-6009. Fax: +61-7-3735-6001. E-mail: r.quinn@griffith.edu.au.

### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55.
(2) Rogers, E. W.; Molinski, T. F. *J. Nat. Prod.* **2007**, *70*, 1191−1194.
(3) Peng, J.; Li, J.; Hamann, M. T. *The Marine Bromotyrosine Derivatives−The Alkaloids*; Elsevier Inc., 2005; Vol. *61*, Chapter 2, pp 59−262.
(4) Kennedy, J. P.; Brogan, J. T.; Lindsley, C. W. *J. Nat. Prod.* **2008**, *71*, 1783−1786.

(5) Swanson, D. M.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Apodaca, R.; Barbier, A. J.; Lovenberg, T. W.; Carruthers, N. I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 897−900.
(6) Ross, S. A.; Weete, J. D.; Schinazi, R. F.; Wirtz, S. S.; Tharnish, P.; Scheuer, P. J.; Hamann, M. T. *J. Nat. Prod.* **2000**, *63*, 501−503.
(7) Kotoku, N.; Tsujita, H.; Hiramatsu, A.; Mori, C.; Koizumi, N.; Kobayashi, M. *Tetrahedron* **2005**, *61*, 7211−7218.
(8) Pick, N.; Rawat, M.; Arad, D.; Lan, J.; Fan, J.; Kend, A. S.; AvGay, Y. *J. Med. Microbiol.* **2006**, *55*, 407−415.
(9) Sepcic, K.; Mancini, I.; Vidic, I.; Franssanito, R.; Pietra, F.; Macek, P.; Turk, T. *J. Nat. Toxins* **2001**, *10*, 181−191.
(10) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 8181−8186.
(11) Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 601−608.
(12) Nicholas, G. M.; Newton, G. L.; Fahey, R. C.; Bewley, C. A. *Org. Lett.* **2001**, *3*, 1543−1545.
(13) Schoenfeld, R. C.; Conova, S.; Rittschof, D.; Ganem, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 823−825.
(14) Rogers, E. W.; Oliveira, M. F. D.; Berlinck, R. G. S.; Konig, G. M.; Molinski, T. F. *J. Nat. Prod.* **2005**, *68*, 891−896.
(15) Kalaitzis, J. A.; Leone, P. D. A.; Hooper, J. N. A.; Quinn, R. J. *Nat. Prod. Res.* **2008**, *22*, 1257−1263.
(16) Xynas, R.; Capon, R. J. *Aust. J. Chem.* **1989**, *42*, 1427−1433.
(17) Cimino, G.; Rosa, S. D.; Stefano, S. D.; Self, R.; Sodano, G. *Tetrahedron Lett.* **1983**, *24*, 3029−3032.
(18) Gopichand, Y.; Schmitz, F. J. *Tetrahedron Lett.* **1979**, *20*, 3921−3924.
(19) Buchanan, M. S.; Carroll, A. R.; Wessling, D.; Jobling, M.; Avery, V. M.; Davis, R. A.; Feng, Y.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 973−975.
(20) Tilvi, S.; Rodrigues, C.; Naik, C. G.; Parameswaran, P. S.; Wahidhulla, S. *Tetrahedron* **2004**, *60*, 10207−10215.
(21) Kunieda, T.; Abe, Y.; Iitaka, Y.; Hirobe, M. *J. Org. Chem.* **1982**, *47*, 4291−4297.
(22) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett.* **1989**, *30*, 6637−6640.
(23) Hoshino, O.; Murakata, M.; Yamada, K. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1561−1562.
(24) Kende, A. S.; Lan, J.; Fan, J. *Tetrahedron Lett.* **2004**, *45*, 133−135.
(25) Rudkevich, D. M.; Chalmers, J. D. M.; Verboom, W.; Ungaro, R.; Jong, F. D.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6124−6125.
(26) Reddy, S. M.; Srinivasulu, M.; Venkateswarlu, Y. *Indian J. Chem.* **2006**, *45B*, 2757−2762.
(27) Murakata, M.; Tamura, M.; Hoshino, O. *J. Org. Chem.* **1997**, *62*, 4428−4433.
(28) Jarowicki, K.; Kocienski, P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4005−4037.
(29) Anbazhagan, M.; Boykin, D. W.; Stephens, C. E. *Tetrahedron Lett.* **2002**, *43*, 9089−9092.
(30) Marsh, J. P.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 2491−2492.
(31) Fletcher, S.; Gunning, P. T. *Tetrahedron Lett.* **2008**, *49*, 4817−4819.
(32) Zhu, G.; Yang, F.; Balachandran, R.; Hook, P.; Vallee, R. B.; Curran, D. P.; Day, B. W. *J. Med. Chem.* **2006**, *49*, 2063−2076.
(33) Itsuno, S.; Nakano, M.; Miyazaki, K.; Masuda, H.; Ito, K.; Hirao, A.; Nakahama, S. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2039−2044.
(34) Huang, X.; Marciales, M. O.; Huang, K.; Stepanenko, V.; Merced, F. G.; Ayala, A. M.; Correa, W.; Jesus, M. D. *Org. Lett.* **2007**, *9*, 1793−1795.
(35) Karabatsos, G. J.; Hsi, N. *Tetrahedron* **1967**, *23*, 1079−1095.
(36) Corey, E. J.; Petrzilka, M.; Ueda, Y. *Helv. Chim. Acta* **1977**, *60*, 2294−2302.
(37) Rahaim, R. J.; Maleczka, R. E. *Org. Lett.* **2011**, *13*, 584−587.
(38) Lee, S. H.; Kim, I. S.; Li, Q. R.; Dong, G. R.; Jeong, L. S.; Jung, Y. H. *J. Org. Chem.* **2011**, *76*, 10011−10019.
(39) Wood, J. L.; Stoltz, B. M.; Goodman, S. N.; Onwueme, K. *J. Am. Chem. Soc.* **1997**, *119*, 9652−9661.
(40) Li, C. B.; Cui, Y.; Zhang, W. Q.; Li, J. L.; Zhang, S. M.; Choi, M. C. K.; Chan, A. S. C. *Chin. Chem. Lett.* **2002**, *13*, 95−96.