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# Veraguamides A-G, Cyclic Hexadepsipeptides from a Dolastatin 16-Producing Cyanobacterium *Symploca* cf. *hydnoides* from Guam

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# **Abstract**

Cytotoxicity-directed purification of a Symploca cf. hydnoides sample from Cetti Bay, Guam, afforded seven new cyclic depsipeptides, veraguamides A-G (1-7), together with the known compound dolastatin 16. The planar structures of 1-7 were elucidated using NMR and MS experiments, while enantioselective HPLC and Mosher's analysis of acid and base hydrolysates, respectively, were utilized to assign the absolute configurations of the stereocenters. Veraguamides A–G (1–7) are characterized by the presence of an invariant proline residue, multiple N-methylated amino acids, an  $\alpha$ -hydroxy acid, and a  $C_8$ -polyketide derived  $\beta$ -hydroxy acid moiety with a characteristic terminus as either an alkynyl bromide, alkyne, or vinyl group. These compounds and a semisynthetic analog (8) showed moderate to weak cytotoxic activity against HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cell lines. Preliminary structure-activity relationship analysis identified several sensitive positions in the veraguamide scaffold that affect the cytotoxic activity of this compound class. Dolastatin 16 showed only weak cytotoxic activity on both cell lines tested. The complete stereostructure of dolastatin 16 was proposed for the first time through degradation followed by a combination of advanced Marfey's analysis and modified Mosher's analysis using phenylglycine methyl ester as a chiral anisotropic reagent.

Marine cyanobacteria have provided both structurally diverse and potent cytotoxic compounds with varying mechanisms of action as lead structures for drug discovery. Most of these natural products are peptide–polyketide hybrids and are derived from the genus *Lyngbya*, marked by *N*-methylation, modified amino acids, α- and β-hydroxy acids, and fatty acid-type moieties. We have had particular success with our drug discovery efforts based on the genus *Symploca*. From Micronesian collections of *Symploca* spp. we previously isolated the linear peptides dolastatin 10 and its analog symplostatin 1.<sup>3,4</sup> These compounds are microtubule disrupting agents that interfere with mitosis and have pico- to nanomolar IC<sub>50</sub> values against cancer cells. From a Floridian *Symploca* sp. collection we recently discovered the HDAC inhibitor, largazole, which showed strong cytotoxic activity against cancer cells with superior selectivity over nontransformed cells. <sup>6,7</sup> In this light, we screened several *Symploca* sp. collections from Guam and Florida to find new cytotoxic agents. In this paper, we present the cytotoxicity-directed fractionation of a *S*. cf. *hydnoides* collection

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from Cetti Bay, Guam, which afforded the known compound dolastatin 16,8 together with seven new cyclic depsipeptides, given the trivial names veraguamides A–G (1–7).9

#### **Results and Discussion**

The freeze-dried cyanobacterium was extracted with EtOAc–MeOH (1:1) and subsequently solvent-partitioned into hexanes-, n-BuOH, and  $H_2O$ -soluble fractions. The n-BuOH soluble fraction showed biological activity at  $10~\mu g/mL$  in an initial cytotoxicity screen using the HT29 colorectal adenocarcinoma cell line. This fraction was further purified by silica column chromatography, with the cytotoxic activity concentrated in the fraction eluting with 20% i-PrOH in CH<sub>2</sub>Cl<sub>2</sub>. Reversed-phase HPLC purification of this silica fraction yielded dolastatin 16 and veraguamides A–G (1–7).

HRESIMS of the major compound in this series, veraguamide A (1), showed the distinctive 1:1 isotopic cluster for a Br-containing compound for the  $[M + H]^+$  peak at m/z767.3675/769.3660, suggesting a molecular formula of  $C_{37}H_{59}BrN_4O_8$ . The <sup>1</sup>H NMR spectrum of 1 displayed characteristic peptide resonances for a secondary amide proton ( $\delta_H$ 6.25), two tertiary amide N-CH<sub>3</sub>s ( $\delta_H$  3.00,  $\delta_H$  2.94) and several  $\alpha$ -protons ( $\delta_H$  3.85 – 4.95). 2D NMR analysis (Table 1) in CDCl<sub>3</sub> using HSQC, COSY, TOCSY, and HMBC established the presence of four amino acids (Pro, Val,  $2 \times N$ -Me-Val) and an  $\alpha$ -hydroxy acid [(2-hydroxy-3-methylpentanoic acid (Hmpa)]. The last spin system consisted of a CH<sub>3</sub> doublet ( $\delta_H$  1.25) that showed a COSY correlation to a methine ( $\delta_H$  3.11) and HMBC correlations to a carbonyl ( $\delta_C$  170.8) and an oxymethine ( $\delta_C$  76.4). Further extension of this unit using HMBC and COSY established the presence of a 8-bromo-3-hydroxy-2-methyl-7octynoic acid (Br-Hmoya) moiety in 1. This was supported by HMBC correlations of the methylene ( $\delta_{C-6}$  19.2/ $\delta_{H_2-6}$  2.23) with two quaternary carbons at  $\delta_C$  38.4 and  $\delta_C$  79.3 and by the large difference in chemical shifts between these quaternary carbons characteristic for an alkynyl bromide. <sup>10</sup> The linear sequence of *N*-Me-Val-1–Pro–Hmpa–*N*-Me-Val-2–Val–Br-Hmoya was established based on HMBC correlations between α-protons and carbonyl groups (Table 1) and was verified by MS/MS fragmentation (Figure 1). The deshielded C-3 methine of the Br-Hmoya unit suggested acylation with the carbonyl of N-Me-Val-1 to form a cyclic hexadepsipeptide, corroborated by HMBC and consistent with the molecular formula requirements based on HRESIMS.

Veraguamide B (2) showed a 1:1 isotopic pattern for the pseudomolecular ion  $[M+H]^+$  at m/z 753.3517/755.3508, suggesting the presence of a Br as in 1 with a negative difference of 14 amu corresponding to one less  $CH_2$  unit and thus a molecular formula of  $C_{36}H_{57}BrN_4O_8$ . Comparison of the  $^1H$  NMR spectrum of 1 and 2 showed differences in the splitting pattern in the  $CH_3$  region at $\delta_H$  0.93 ppm and the chemical shift of the  $\alpha$ -proton ( $\delta_H$  4.85) of the  $\alpha$ -hydroxy acid (Table 2). The vicinal methine ( $\delta_H$  2.17) of the  $\alpha$ -hydroxy acid showed COSY correlations to two methyl groups ( $\delta_H$  0.93,  $\delta_H$  1.02) instead of COSY correlations to methylene and methyl protons in 1. Therefore, 2 possesses a 2-hydroxyisovaleric acid (Hiva) instead of the Hmpa unit as in 1.

The HRESIMS spectrum of veraguamide C (3) showed a negative deviation of 79 amu compared with 1 which indicated the lack of Br and a molecular formula of  $C_{37}H_{60}N_4O_8$ . This was supported by the absence of the 1:1 isotopic pattern for the  $[M+H]^+$  peak when compared to 1. The  $^1H$  NMR spectrum of 3 showed an additional triplet at  $\delta_H$  1.93 with  $J_{H,H}$  = 2.5 Hz (Table 2); otherwise it was virtually identical to that of 1. This proton correlated to a methine at  $\delta_C$  68.8 and a quaternary C ( $\delta_C$  83.6) in the HSQC and HMBC spectra, respectively. These signals are indicative of a terminal alkyne; hence 3 had to bear a 3-hydroxy-2-methyl-7-octynoic acid (Hmoya) moiety in lieu of the Br-Hmoya present in 1 and 2.

Veraguamide D (4) appeared closely related to 3 as its  $^{1}$ H NMR spectrum showed the acetylenic proton at  $\delta_{H}$  1.93 (Table 3). In comparison to 3, the HRESIMS spectrum of 4 showed a positive difference of 14 amu, corresponding to an additional CH<sub>2</sub> unit and in agreement with a molecular formula of  $C_{38}H_{62}N_{4}O_{8}$ . The  $^{1}$ H and  $^{13}$ C NMR spectra of 4 showed a high-field CH<sub>3</sub> at  $\delta_{C}$  11.4/ $\delta_{H}$  0.93, characteristic of an Ile or Ile-derived moiety. COSY ( $\delta_{H}/\delta_{H}$  0.93/1.46, 1.46/2.02, 2.02/4.01) and ( $\delta_{C}/\delta_{H}$  15.8/4.01, 28.7/4.01) correlations established that the *N*-Me-Val-1 residue is replaced by an *N*-Me-Ile in veraguamide D (4).

Compound **5** ( $C_{39}H_{64}N_4O_8$ ) exhibited a close relationship to both **3** and **4**, showing a positive deviation of 28 amu and 14 amu, respectively, and also having the Hmoya moiety. The NMR data of **5** (Table 3) indicated the presence of two high-field methyl ( $\delta_C$  11.7/ $\delta_H$  0.96,  $\delta_C$  11.5/ $\delta_H$  0.85) and additional methylene ( $\delta_C$  26.6/ $\delta_H$  1.54, 1.06;  $\delta_C$  23.9/ $\delta_H$  1.47, 1.05) groups in comparison with **3**, which suggested that two isopropyl groups in the latter are replaced with *sec*-butyl groups in the former. This is further corroborated by HMBC and COSY correlations which established the replacement of Val and *N*-Me-Val-2 moieties with lle and *N*-Me-Ile, respectively, in veraguamide E (**5**). The *N*-Me-Val residue that was replaced by *N*-Me-Ile was located at different positions in **4** and **5**; with *N*-Me-Val-1 replaced in the former and *N*-Me-Val-2 in the latter. This NMR result was verified by MS/MS fragmentation of both **4** and **5** (Figure 1).

The  $^1H$  NMR spectrum of veraguamide F (6) (Table 4) showed additional resonances for aromatic protons at  $\delta_H$  7.2 – 7.4 ppm, upfield-shifted N-Me protons to  $\delta_H$  2.60 ppm presumably due to shielding by the aromatic ring, and a low-field  $\alpha$ -proton of the hydroxy acid ( $\delta_H$  5.47), with the acetylenic proton still present ( $\delta_H$  1.93). COSY correlations of  $\delta_H$  5.47 to diastereotopic CH<sub>2</sub> protons at  $\delta_H$  3.17/ $\delta_H$  2.91, together with HMBC correlations of the latter to aromatic carbons at  $\delta_C$  136.2,  $\delta_C$  129.3 (Table 4) established the presence of phenyllactic acid (Pla) as the  $\alpha$ -hydroxy acid in 6. These NMR-derived conclusions fulfilled the molecular formula requirements for  $C_{40}H_{58}N_4O_8$  based on HRESIMS of 6.

Veraguamide G (7) lacked the acetylenic signal ( $\delta_C$  68.8/ $\delta_H$  1.93) observed for **3–6** and instead showed downfield resonances of a terminal methylene ( $\delta_C$  114.9/ $\delta_H$  4.97) and a methine ( $\delta_C$  138.2/ $\delta_H$  5.74). These signals indicated that the terminal alkyne group of the C<sub>8</sub>-polyketide derived moiety is replaced by a terminal vinyl group. This conclusion was further supported by the positive deviation of 2 amu compared to **3** and a molecular formula of C<sub>37</sub>H<sub>62</sub>N<sub>4</sub>O<sub>8</sub>. Hence,the Hmoya unit present in **3–6** was replaced by 3-hydroxy-2-methyl-7-octenoic acid (Hmoea) in **7**.

Enantioselective HPLC analysis coupled with mass spectrometry or UV detection of the acid hydrolysates of 1-7 allowed us to assign the absolute configuration of all the amino acids and  $\alpha$ -hydroxy acid components as L and S, respectively. To determine the absolute configuration at C-2 and C-3 of the Br-Hmoya unit, veraguamide A (1) was subjected to methanolysis to yield the linear fragment 9 (Figure 2). The observed coupling constant of 3.2 Hz was characteristic for a syn configuration, whereas a coupling constant near 6.3 Hz would have been expected for the anti configuration. 11 The absolute configuration at C-3 and consequently for C-2 of the Br-Hmoya unit of 9 was determined using Mosher's analysis. The derived  $\Delta\delta$  values (Figure 2) predicted an R configuration at C-3 and hence from the relative configuration, C-2 should have an S configuration. Of note, comparison of the  ${}^{3}J_{H,H}$  values of H-2 and H-3 with a model system to assign the relative configuration could only be applied when C-3 bears a free hydroxy group. This moiety is involved in intramolecular H-bonding with the adjacent carbonyl group, thus hindering free bond rotation across C-2 and C-3. 12,13 Accordingly, the corresponding MTPA-esters (10, 11) did not show the same  ${}^3J_{\rm H,H}$  values for H-2 and H-3 as that of **9**. The same absolute configuration at C-2 and C-3 for Hmoya, Hmoea and 3-hydroxy-2-methyl-octanoic acid

(Hmoaa) is expected based on virtually identical <sup>13</sup>C NMR shifts and specific rotations observed for **1–8**.

To gain insight into structure–activity relationships, veraguamide A (1) was partially (Lindlar catalyst, H<sub>2</sub>) and fully (Pd/C, H<sub>2</sub>) hydrogenated to yield the semisynthetic veraguamide G (7) and tetrahydroveraguamide A (8), respectively. The cytotoxic activities of 1–8, semisynthetic veraguamide G (7), and dolastatin 16 were evaluated for effects on viability of HT29 colorectal and HeLa cervical adenocarcinoma cells (Table 6). The IC<sub>50</sub> values of the natural and semisynthetic veraguamide G (7) were comparable suggesting the activities of these compounds were not likely due to traces of highly biologically active impurities. The most active in this series of compounds are veraguamides D (4) and E (5), with IC<sub>50</sub> values more than 5-fold lower than those for their related congener veraguamide C (3). This suggested that increased hydrophobicity of specific units (II, IV, V, VI) increased the cytotoxicity of this compound class, with the position having minimal effect on the bioactivity as 4 and 5 showed comparable IC<sub>50</sub>s. However, modification with bulkier groups is detrimental to the activity, as exemplified by a close to 10-fold decrease in the cytotoxic activity of 6 (Table 6) compared to 3, where a phenyllactic acid (Pla) moiety is introduced at position IV of the former instead of the Hmpa unit. The C<sub>8</sub>-polyketide derived moiety also plays a role in the cytotoxicity of these compounds. Comparing the biological activities of related compounds 1, 3, 7, and 8, weaker cytotoxicity was observed for compounds with a Br-Hmoya or Hmoaa unit, while compounds with Hmoya or Hmoea were about equally potent. This then suggests the importance of a  $\pi$ -system combined with the presence of acetylenic or vinylic protons in this moiety for cytotoxic activity.

The veraguamides are reminiscent of other cyanobacterial compounds such as hantupeptins,  $^{13,14}$  antanapeptins,  $^{15}$  and trungapeptins.  $^{16}$  These compounds are also cyclic hexadepsipeptides with the characteristic  $C_8$ -polyketide derived units as Hmoya, Hmoea, or Hmoaa. Veraguamide F (**6**) is a constitutional isomer of antanapeptin D,  $^{15}$  where an *N*-Me-Phe and Hiva are present in the latter instead of Pla and *N*-Me-Val as in **6**. It is interesting that subtle changes in structure of these compounds have a profound effect on the cytotoxicity. Antanapeptins A–D (brine shrimp) as well as trungapeptin A (KB and LoVo cells) did not display cytotoxicity at the reported concentrations ( $10~\mu g/mL$ ),  $^{15,16}$  while hantupeptins A–C were cytotoxic against MOLT-4 leukemia and MCF7 breast cancer cells, with hantupeptin A being the most active in this series with IC50s of 32 nM and 4.0  $\mu$ M, respectively.  $^{13}$  Trungapeptins B and C were not tested for cytotoxicity.  $^{16}$ 

Although dolastatin 16 has been isolated previously from sea hares<sup>8</sup> and cyanobacteria. 15,17 its stereostructure has been only partially assigned. Therefore we analyzed the remaining dolaphenvaline (Dpv) and dolamethylleucine (Dml) units. The absolute configuration of the Dpv unit in dolastatin 16 was determined as 2S,3R using enantioselective HPLC-MS analysis of the FDLA-derivatized acid hydrolysate in comparison with that for pitiprolamide. <sup>18</sup> The configuration of the Dpv unit in dolastatin 16 is the same as in pitiprolamide and kulokekahilide-1 determined from X-ray crystallography and chemical synthesis, respectively. <sup>18,19</sup> To determine the configuration of the stereocenters in the Dml unit, we carried out acid and base hydrolysis of dolastatin 16 to yield the Dml unit and the linear fragment 12, respectively. The Dml unit is related to the 3-amino-2-methylpentanoic acid (Map) unit, present in dolastatin 12<sup>20,21</sup> and majusculamide C,<sup>22</sup> as well as the 3amino-2-methylhexanoic acid (Amha) unit in kulokekahilide-1, <sup>19</sup> lyngbyastatin 1, <sup>21</sup> and the ulongamides.<sup>23</sup> For all these α-substituted β-amino acids, the corresponding Marfey's adducts of the two 3R isomers (2S,3R and 2R,3R) consistently eluted later by reversed-phase C<sub>18</sub> HPLC than those of the two 3S isomers (2S,3S and 2R,3S), while the C-2 stereoisomers are poorly resolved and variable. <sup>19–23</sup> Extrapolation to the elution order we obtained for the Dml suggested the presence of 3R in the Dml unit of dolastatin 16.24 To assign the

configuration at C-2 of Dml, modified Mosher's analysis using phenylglycine methyl ester (PGME) $^{25}$  derivatization of **12** (Figure 3) suggested a 2R configuration. The applicability of this chiral derivatization technique has been demonstrated for  $\alpha$ - and  $\beta$ - substituted carboxylic acids, $^{25}$  but may not have been widely used for  $\alpha$ , $\beta$ -disubstituted carboxylic acids. From the  $\Delta\delta$  values that we obtained (Figure 3), it is predicted that there is some deviation from the presumed conformation of the PGME amide, but nonetheless a systematic arrangement of positive and negative  $\Delta\delta$  values was obtained. Therefore, we propose a 2R,3R configuration for the Dml unit of dolastatin 16. The  $^{13}$ C NMR chemical shifts for the Dpv and Dml unit in both dolastatin 16 and homodolastatin 16 were similar, suggesting the latter would also have a (2S,3R)-Dpv and (2R,3R)-Dml units. $^{8,26}$  Dolastatin 16 showed weak cytotoxic activity with IC $_{50}$  of 78  $\mu$ M and 58  $\mu$ M for HT29 and HeLa cell lines, respectively. These values are significantly higher than those reported by Pettit et al. against a different panel of cell lines.

# **Experimental Section**

# **General Experimental Procedures**

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on SpectraMax M5 (Molecular Devices).  $^1H$  and 2D NMR spectra were recorded in CDCl3 on a Bruker Avance II 600 MHz spectrometer equipped with a 5-mm TXI cryogenic probe using residual solvent signals ( $\delta_H$  7.26;  $\delta_C$  77.0) as internal standards. HSQC and HMBC experiments were optimized for  $^1J_{CH}$  = 145 and  $^nJ_{CH}$  = 7 Hz, respectively. TOCSY experiments were done using a mixing time of 100 ms.  $^{13}C$  NMR spectra were recorded on Varian 400 MHz or Bruker 500 MHz NMR spectrometers. HRESIMS data was obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector. LRESIMS measurements and MS/MS fragmentation were done on an ABI 3200Q TRAP.

# **Biological Material**

The *Symploca* cf. *hydnoides* cyanobacterium was collected by hand while snorkeling in the shallow waters of the southern fore-reef (1–3 m) of Cetti Bay, Guam on April 17, 2009. A voucher specimen, which is preserved in 100% EtOH, is deposited in the University of Guam Herbarium (accession no. GUAM-GH11446). A voucher specimen is also retained at the Smithsonian Marine Station, Fort Pierce, FL.

# **Extraction and Isolation**

The freeze-dried cyanobacterium (142.0 g) was extracted with EtOAc–MeOH (1:1) to yield the nonpolar extract (11.6 g). This was partitioned between hexanes and 20% aqueous MeOH, the latter concentrated under reduced pressure and further partitioned between n-BuOH and H<sub>2</sub>O. The n-BuOH fraction was concentrated to dryness (2.84 g) and chromatographed on Si gel eluting first with CH<sub>2</sub>Cl<sub>2</sub>, followed by increasing concentrations of i-PrOH, while after 100% i-PrOH, increasing gradients of MeOH were used. The 20% i-PrOH fraction was subjected to a C<sub>18</sub> SPE eluting with 25%, 50%, 75%, and 100% MeOH in H<sub>2</sub>O. The 100% MeOH fraction was purified by semipreparative reversed-phase HPLC (Phenomenex Synergi-Hydro RP, 250 × 10 mm, 4  $\mu$ m; flow rate, 2.0 mL/min) using a linear gradient of MeOH–H<sub>2</sub>O (70%–100% MeOH in 60 min and then 100% MeOH for 10 min) to yield dolastatin 16 (t<sub>R</sub> 32.6 min, 21.6 mg), semipure veraguamide C (t<sub>R</sub> 36.4 min, 15.1 mg), semipure veraguamide F (t<sub>R</sub> 37.4 min, 10.0 mg), a mixture of veraguamides B and D (t<sub>R</sub> 40.0 min, 25.0 mg), veraguamide A (1) (t<sub>R</sub> 42.6 min, 25.9 mg), and a mixture of veraguamides E and G (t<sub>R</sub> 43.7 min, 9.0 mg).

The final purification of the semipure veraguamide C (3) was achieved using semipreparative HPLC (Phenomenex Phenyl-hexyl,  $250 \times 10$  mm, 4 µm; flow rate, 2.0 mL/min) using a linear gradient of MeOH–H<sub>2</sub>O (85%–100% MeOH in 40 min and then 100% MeOH for 5 min) to yield veraguamide C (3) ( $t_R$  19.9 min, 10.7 mg). Using the same chromatographic conditions, purification of the semipure veraguamide F yielded veraguamide F (6) ( $t_R$  21.7 min, 6.8 mg). The mixture of veraguamides B and D was resolved using the same chromatographic condition with a different linear gradient (70%–100% MeOH in 45 min and then 100% MeOH for 10 min) to yield veraguamide D (4) ( $t_R$  36.6 min, 4.0 mg) and veraguamide B (2) ( $t_R$  37.3 min, 11.5 mg). The mixture of veraguamides E and G was further purified using the same chromatographic conditions to yield veraguamide G (7) ( $t_R$  39.9 min, 4.4 mg) and veraguamide E (5) ( $t_R$  40.5 min, 3.6 mg).

## Hydrogenation of 1

A catalytic amount of 10% Pd/C was added to a methanolic solution of 1 (1.8 mg/mL). The reaction was left to stir for 6 h under a hydrogen balloon. The catalyst was filtered through a Celite pad, and the filtrate, upon concentration, was purified by semipreparative HPLC (Phenomenex Phenyl-hexyl,  $250 \times 10$  mm, 4 µm; flow rate, 2.0 mL/min) using a linear gradient of MeOH–H<sub>2</sub>O (70%–100% MeOH in 45 min and then 100% MeOH for 10 min) to yield 8 ( $t_R$  40.9 min, 1.2 mg).

Partial hydrogenation of **1** was carried out with Lindlar catalyst, using the same reaction and chromatographic conditions stated above. This afforded the semisynthetic veraguamide G ( $t_R$  39.9 min, 1.7 mg). The LRESIMS and <sup>1</sup>H NMR spectra of the semisynthetic veraguamide G were in good agreement with the spectra for the natural product (**7**).

**Veraguamide A (1)**—colorless, amorphous solid;  $[\alpha]^{20}_D$  –44 (c 0.44, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 202 (6.29); IR (film)  $\nu_{max}$  3428, 2966, 2877, 2361, 2334, 1736, 1647, 1190 cm<sup>-1</sup>; <sup>1</sup>H NMR , <sup>13</sup>C NMR, COSY, and HMBC data, see Table 1; HRESIMS m/z 767.3675 [M + H]<sup>+</sup> (calcd for  $C_{37}H_{60}^{79}BrN_4O_8$ , 767.3594), m/z [M + H]<sup>+</sup> 769.3660 (calcd for  $C_{37}H_{60}^{81}BrN_4O_8$ , 769.3574) (100:100 [M + H]<sup>+</sup> ion cluster).

**Veraguamide B (2)**—colorless, amorphous solid;  $[\alpha]^{20}_D$  –40c 0.16, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 202 (4.30); IR (film)  $\nu_{max}$  3378, 2966, 2886, 1738, 1657, 1190 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table 2; HRESIMS m/z 753.3517 [M + H]<sup>+</sup> (calcd for  $C_{36}H_{58}^{79}$ BrN<sub>4</sub>O<sub>8</sub>, 753.3438), m/z [M + H]<sup>+</sup> 755.3508 (calcd for  $C_{36}H_{58}^{81}$ BrN<sub>4</sub>O<sub>8</sub>, 755.3418) (100:100 [M + H]<sup>+</sup> ion cluster).

**Veraguamide C (3)**—colorless, amorphous solid;  $[\alpha]^{20}_D$  –44 (c 0.31, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 202 (4.17); IR (film) v 3429, 2965, 2871, 1732, 1650, 1190 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table 2; HRESIMS m/z 689.4486 [M + H]<sup>+</sup> (calcd for  $C_{37}H_{61}N_4O_8$ , 689.4490).

**Veraguamide D (4)**—colorless, amorphous solid;  $[α]^{20}_D$  –57 (c 0.11, MeOH); UV (MeOH);  $λ_{max}$  (log ε) 202 (4.30); IR (film)  $ν_{max}$  3378, 2958, 2878, 1732, 1647 cm<sup>-1</sup>;  $^1$ H NMR and  $^{13}$ C NMR data, see Table 3; HRESIMS m/z 703.4639 [M + H]<sup>+</sup> (calcd for  $C_{38}H_{63}N_4O_8$ , 703.4646).

**Veraguamide E (5)**—colorless, amorphous solid;  $[\alpha]^{20}_D$  –56 (c 0.22, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 202 (4.30); IR (film)  $\nu_{max}$  3320, 2958, 2877, 1733, 1647 cm<sup>-1</sup>;  $^1$ H NMR and  $^{13}$ C NMR data, see Table 3; HRESIMS m/z 717.4799 [M + H]<sup>+</sup> (calcd for  $C_{39}H_{65}N_4O_8$ , 717.4802).

**Veraguamide F (6)**—colorless, amorphous solid; [α] $^{20}_D$  –41 (c 0.13, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 206 (4.33); IR (film)  $\nu_{max}$  3450, 3306, 2965, 2871, 1737, 1661, 1647, 1490 cm $^{-1}$ ;  $^1$ H NMR and  $^{13}$ C NMR data, see Table 4; HRESIMS m/z 723.4411 [M + H] $^+$  (calcd for  $C_{40}H_{59}N_4O_8$ , 723.4333).

**Veraguamide G (7)**—colorless, amorphous solid;  $[\alpha]^{20}_D$  –41 (*c* 0.17, MeOH); UV (MeOH);  $\lambda_{max}$  (log ε) 202 (4.26); IR (film)  $\nu_{max}$  3436, 2965, 2879, 1740, 1661, 1646 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table 5; HRESIMS m/z 691.4649 [M + H]<sup>+</sup> (calcd for C<sub>40</sub>H<sub>59</sub>N<sub>4</sub>O<sub>8</sub>, 691.4646).

**Tetrahydroveraguamide A (8)**—colorless, amorphous solid;  $[α]^{20}D^{-43}$  (c 0.05, MeOH); UV (MeOH);  $λ_{max}$  (log ε) 202 (4.33);  ${}^{1}H$  NMR and  ${}^{13}C$  NMR data, see Table 5; HRESIMS m/z 693.4791  $[M + H]^{+}$  (calcd for  $C_{37}H_{65}N_4O_8$ , 693.4802).

**Dolastatin 16**—colorless, amorphous solid;  $[α]^{20}_D$  +22 (c 0.18, MeOH) {lit.  $^8$   $[α]^{20}_D$  +15.5 (c 0.20, MeOH)}; UV (MeOH);  $λ_{max}$  (log ε) 202 (4.51); IR (film)  $ν_{max}$  3407, 2965, 2871, 1733, 1647 cm<sup>-1</sup>;  $^1$ H NMR and  $^{13}$ C NMR data are identical with literature values  $^8$  (Supporting Information); LRESIMS m/z 879.6 [M + H]<sup>+</sup>, 901.3 [M + Na]<sup>+</sup>.

# Acid Hydrolysis of Veraguamides and Enantioselective Analysis

Portions of 1–7 (100 µg) were acid-hydrolyzed (200 µL 6 N HCl, 110 °C, 20 h), the product mixtures dried, reconstituted in 100 µL H<sub>2</sub>O and analyzed by enantioselective HPLC-UV and enantioselective HPLC-MS. The absolute configurations of the amino acids N-Me-Ile, Ile, N-Me-Val, Val, and Pro were determined by enantioselective HPLC-MS [column, Chirobiotic TAG (250 × 4.6 mm), Supelco; solvent, MeOH–10 mM NH<sub>4</sub>OAc (40:60, pH 5.30); flow rate, 0.5 mL/min; detection by ESIMS in positive ion mode (MRM scan)]. The acid hydrolysates of 1-4, 6, and 7 showed retention times at 7.8, 11.6, and 13.6 min corresponding to L-Val, N-Me-L-Val, and L-Pro, respectively. The acid hydrolysate of 4 in addition showed a retention time at 12.4 min corresponding to N-Me-L-Ile. The acid hydrolysate of 5 showed retention times at 8.4, 11.6, 12.4, and 13.6 min, corresponding to L-Ile, N-Me-L-Val, N-Me-L-Ile, and L-Pro, respectively. The retention times (t<sub>R</sub>, min; MRM ion pair) of the authentic amino acids were as follows: NMe-L-Val (11.6; 132 \to 86), N-Me-D-Val (34.3), L-Val (7.8;  $118 \rightarrow 72$ ), D-Val (13.7), N-Me-L-Ile (12.4;  $146 \rightarrow 100$ ), N-Me-Lallo-Ile (15.0), N-Me-D-Ile (49.0), N-Me-D-allo-Ile (51.0), L-Ile (8.4; 132→86), L-allo-Ile (8.6), D-allo-Ile (17.6), D-Ile (20.2), L-Pro (13.6; 116→70), D-Pro (36.0). Compounddependent parameters used were as follows: N-Me-Val: DP 29.4, EP 4.2, CE 17.4, CXP 2.7, CEP 10.6; Val: DP 5.7, EP 9.0, CE 40.0, CXP 8.0, CEP 10.0; N-Me-Ile: DP 35.0, EP 7.0, CE 17.0, CXP 2.0, CEP 10.0; Ile: DP 40.0, EP 9.0, CE 15.0, CXP 3.0, CEP 8.0; Pro: DP 35.0, EP 7.7, CE 22.7, CXP 5.0, CEP 10.3. Source gas parameters used were as follows: CUR 40, CAD Medium, IS 4500, TEM 750, GS1 65, GS2 65.

The absolute configurations of the  $\alpha$ -hydroxy acids [2-hydroxyisovaleric acid (Hiva), 2-hydroxy-3-methylpentanoic acid (Hmpa), and phenyllactic acid (Pla)] were determined using enantioselective HPLC [column, CHIRALPAK MA (+) (50 × 4.6 mm); solvent, CH<sub>3</sub>CN-2 mM CuSO<sub>4</sub> (10:90); flow rate, 1.0 mL/min; detection by UV (254 nm)]. The acid hydrolysates of **1**, **3**–**5**, and **7** each showed peaks at 33.0 min corresponding to (2*S*,3*S*)-Hmpa. The acid hydrolysate of **2** contained a component that had a retention time at 10.0 min corresponding to (2*S*)-Hiva, while **6** gave a peak at 51.0 min corresponding to (2*S*)-Pla. The retention times of the authentic standards were as follows: (2*R*)-Hiva (6.0), (2*S*)-Hiva (10.0), (2*R*,3*S*)-Hmpa (16.0), (2*R*,3*R*)-Hmpa (19.0), (2*S*,3*R*)-Hmpa (26.0), (2*S*,3*S*)-Hmpa (33.0), (2*R*)-Pla (33.5), (2*S*)-Pla (51.0). All other amino acid units eluted within less than 5.0 min using this chromatographic condition.

### Methanolysis of 1

Compound **1** (5.0 mg) was dissolved in 2.0 mL of 5% (w/w) methanolic KOH solution and stirred for 24 h at room temperature. The solvent was evaporated and the residue was partitioned between  $CH_2Cl_2$  and  $H_2O$ . The organic layer was collected, dried over anhydrous  $MgSO_4$  and concentrated to dryness under nitrogen. The crude methanolysis product was further purified by semipreparative reversed-phase HPLC (Phenomenex Synergi-Hydro RP,  $250 \times 10$  mm, 4  $\mu$ m; flow rate, 2.0 mL/min) using a linear gradient of MeOH– $H_2O$  (70%–100% MeOH in 60 min and then 100% MeOH for 10 min) to yield **9** ( $t_R$  19.3 min, 1.4 mg).

**9**—colorless, amorphous solid;  ${}^{1}H$  NMR (CDCl<sub>3</sub>)  $\delta$  6.30 (d, J = 8.1 Hz, 1H), 4.92 (d, J = 10.5 Hz, 1H), 4.76 (dd, J = 9.1, 6.6 Hz, 1H), 3.78 (ddd, J = 8.6, 4.4, 3.2 Hz, 1H), 3.49 (s, 3H), 3.08 (s, 3H), 2.42 (qd, J = 6.8, 3.2 Hz, 1H), 2.23 (m, 2H), 2.03 (m, 1H), 1.74 (m, 2H), 1.56 (m, 1H), 1.46 (m, 2H), 1.01 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); HRESIMS m/z 497.1640 [M + Na]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>35</sub><sup>79</sup>BrN<sub>2</sub>O<sub>5</sub>Na, 497.1627), m/z [M + Na]<sup>+</sup> 499.1616 (calcd for C<sub>21</sub>H<sub>35</sub><sup>81</sup>Br N<sub>2</sub>O<sub>5</sub>Na, 499.1607) (100:100 [M + Na]<sup>+</sup> ion cluster).

#### **Preparation of MTPA Esters**

The methanolysis product **9** was dissolved in 50  $\mu$ L CDCl<sub>3</sub>, and was divided into two equal portions and to each was added 0.75 mL triethylamine. To one portion was added 10  $\mu$ L of (*R*)-MTPA-Cl and to another was added 10  $\mu$ L of (*S*)-MTPA-Cl to give the (*S*)-MTPA ester (**10**) and (*R*)-MTPA ester (**11**), respectively. Each reaction was allowed to stir for 24 h and 10  $\mu$ L of *N*,*N*-dimethylaminopropylamine was added to quench the reactions. The reaction products were dried under N2 and applied onto silica SPE eluting with EtOAc – hexanes (1:1). The semipure product was further purified by semipreparative HPLC (Phenomenex Phenyl-hexyl, 250 × 10 mm, 4  $\mu$ m; flow rate, 2.0 mL/min) using a linear gradient of MeOH-H<sub>2</sub>O (70%–100% MeOH in 45 min and then 100% MeOH for 10 min) to yield **10** ( $t_R$  38.0 min, 0.1 mg) or **11** ( $t_R$  37.8 min, 0.1 mg).

**10**—colorless, amorphous solid;  $^1\text{H}$  NMR (CDCl<sub>3</sub>)  $\delta$  7.57 (dd, J = 6.4, 2.7 Hz, 2H), 7.41 (m, 3H), 6.14 (d, J = 9.2 Hz, 1H), 5.28 (q, J = 6.9 Hz, 1H), 4.93 (d, J = 10.6 Hz, 1H), 4.68 (dd, J = 9.1, 7.6 Hz, 1H), 3.69 (s, 3H), 3.58 (s, 3H), 3.06 (s, 3H), 2.46 (quintet, J = 7.1 Hz, 1H), 2.21 (m, 1H), 2.15 (t, J = 6.8 Hz, 2H), 1.95 (m, 1H), 1.68 (m, 1H), 1.47 (m, 2H), 1.09 (d, J = 7.0 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H), 0.86 (d, J = 6.9 Hz, 3H), 0.81 (d, J = 6.9 Hz, 3H); HRESIMS m/z 729.1746 [M + K]<sup>+</sup> (calcd for C<sub>31</sub>H<sub>42</sub><sup>81</sup>BrF<sub>3</sub>N<sub>2</sub>O<sub>7</sub>K, 729.1759), m/z [M + K]<sup>+</sup> 731.1747 (calcd for C<sub>31</sub>H<sub>42</sub><sup>81</sup>BrF<sub>3</sub>N<sub>2</sub>O<sub>7</sub>K, 731.1755) (100:100 [M + K]<sup>+</sup> ion cluster); LRESIMS m/z 691/693 (100:100 [M + H]<sup>+</sup> ion cluster), 713/715 (100:100 [M + Na]<sup>+</sup> ion cluster).

**11**—colorless, amorphous solid;  $^{1}$ H NMR (CDCl<sub>3</sub>)  $\delta$  7.56 (dd, J = 4.3, 3.6 Hz, 2H), 7.41 (m, 3H), 6.27 (d, J = 8.3 Hz, 1H), 5.27 (q, J = 6.0 Hz, 1H), 4.94 (d, J = 10.4 Hz, 1H), 4.73 (dd, J = 9.1, 7.5 Hz, 1H), 3.69 (s, 3H), 3.57 (s, 3H), 3.07 (s, 3H), 2.54 (quintet, J = 6.7 Hz, 1H), 2.22 (m, 1H), 2.08 (td, J = 7.2, 2.9 Hz, 2H), 2.00 (m, 1H), 1.63 (m, 1H), 1.31 (m, 2H), 1.19 (d, J = 6.7 Hz, 3H), 1.01 (d, J = 6.3 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 0.83 (d, J = 6.5 Hz, 3H); HRESI/APCIMS m/z 691.2202 [M + H]<sup>+</sup> (calcd for  $C_{31}H_{43}^{79}BrF_{3}N_{2}O_{7}$ , 691.2206), m/z [M + H]<sup>+</sup> 693.2186 (calcd for  $C_{31}H_{43}^{81}BrF_{3}N_{2}O_{7}$ , 693.2186) (100:100 [M + H]<sup>+</sup> ion cluster).

#### Advanced Marfey's Analysis of Dpv Unit in Dolastatin 16

To liberate the authentic (2S,3R)-Dpv standard, pitiprolamide (500 µg) was hydrolyzed (400 μL of 6 N HCl, 110 °C, 20 h). The mixture was dried and reconstituted in 400 μL H<sub>2</sub>O. Half of this sample was used to epimerize the α-amino acids in this mixture (40 μL Et<sub>3</sub>N and 40  $\mu$ L Ac<sub>2</sub>O, heating at 60 °C for 1 h)<sup>27</sup> to yield a mixture of (2S,3R)-Dpv and (2R,3R)-Dpv standards. These standards were derivatized with 1% (w/v) Marfey's reagent (L-FDLA and DL-FDLA). 28 Dolastatin 16 was hydrolyzed and derivatized with 1% (w/v) L-FDLA according to the procedure stated above. The absolute configuration of the Dpv unit was determined by reversed-phase HPLC-MS [column, Phenomenex Synergi Hydro-RP (150  $\times$ 4.6 mm) using a linear gradient of 0.1% HCOOH in MeOH- 0.1% aqueous HCOOH (40%-100% for 45 min and then 100% MeOH for 10 min); flow rate, 0.5 mL/min; detection by ESIMS in negative ion mode (MRM scan)] of the FDLA adducts. The acid hydrolysate of dolastatin 16 showed a peak at 36.1 min corresponding to (2S,3R)-Dpv. Coinjection of the acid hydrolysate with the DL-FDLA derivatized epimerization product showed enrichment of the peak corresponding to (2S,3R)-Dpv. The retention times  $(t_R, \min; MRM \text{ ion pair})$  of the authentic amino acid L-FDLA adducts were as follows: (2S,3R)-Dpv  $(36.1;486\rightarrow191)$ , (2S,3S)-Dpv [(36.6); (= D-FDLA derivatized (2R,3R)-Dpv)], (2R,3S)-Dpv [(43.5); (= D-FDLA derivatized (2R,3R)-Dpv)] FDLA derivatized (2S,3R)-Dpv], (2R,3R)-Dpv (44.3). Compound-dependent parameters used were as follows: DP -95.0, EP -6.0, CE -31.0, CXP -2.0, CEP -26.0. Source gas parameters used were as follows: CUR 40, CAD High, IS 4500, TEM 450, GS1 40, GS2 40.

#### Advanced Marfey's Analysis of β-Amino Acid Unit in Dolastatin 12 and Dolastatin 16

Samples of dolastatin 12 and dolastatin 16 (0.2 mg each) were hydrolyzed (200 µL of 6 N HCl, 110 °C, 20 h) to liberate the β-amino acids Map and Dml, respectively. Another portion of dolastatin 12 and dolastatin 16 (0.2 mg each) was first heated with 3 N NaOH (80 °C, 3 h), the resulting product mixture acidified with 5% (v/v) HCl, dried under N<sub>2</sub>, and hydrolyzed (200  $\mu$ L of 6 N HCl, 110 °C, 20 h) to liberate the partially C-2 epimerized  $\beta$ amino acid mixture. The reaction products were derivatized using L-FDLA and DL-FDLA.<sup>28</sup> The order of elution of the β-amino acids Dml and Map liberated from dolastatin 16 and dolastatin 12, respectively, was determined by reversed-phase HPLC-MS [column, Phenomenex Synergi Hydro-RP (150 × 4.6 mm) using a linear gradient of 0.1% HCOOH in MeOH – 0.1% aqueous HCOOH (25%–80% for 50 min and then 100% MeOH for 5 min); flow rate, 0.5 mL/min; detection by ESIMS in negative ion mode (MRM scan)] of the FDLA adducts. The enantiomers bearing the 3R configuration consistently eluted later than those with the 3S configuration. The retention times ( $t_R$ , min; MRM ion pair) of the Dml-<sub>L</sub>-FDLA adducts were as follows: (2S,3S)-Dml [ $(29.3;438.2\rightarrow310.3); (= p$ -FDLA derivatized (2R,3R)-Dml)], (2R,3S)-Dml [(30.8); (= D-FDLA derivatized (2S,3R)-Dml)], (2R,3R)-Dml (33.7), (2S,3R)-Dml (35.6). The retention times ( $t_R$ , min; MRM ion pair) of the Map-L-FDLA adducts were as follows: (2R,3S)-Map [ $(27.5;424.5\rightarrow310.4)$ ; (= D-FDLA derivatized (2S,3R)-Map], (2S,3S)-Map [(29.6); (= D-FDLA derivatized (2R,3R)-Dml)], (2R,3R)-Dml (31.0), (2S,3R)-Dml (32.5). Compound-dependent parameters used were as follows: DP -95.0, EP -6.0, CE -31.0, CXP -2.0, CEP -26.0. Source gas parameters used were as follows: CUR 40, CAD Medium, IS 4500, TEM 450, GS1 40, GS2 40.

#### **Base Hydrolysis of Dolastatin 16**

Dolastatin 16 (4.0 mg) was hydrolyzed using 2 N KOH–MeOH (1:1) for 3 h at 80 °C. The base hydrolysate was neutralized with 2 N HCl, partitioned between EtOAc: $H_2O$ , the organic layer collected and dried under  $N_2$ . The crude base hydrolysate product was purified by semipreparative HPLC (Phenomenex Synergi Hydro-RP,  $250 \times 10$  mm, 4 µm; flow rate, 2.0 mL/min) using a linear gradient of ACN– $H_2O$  (40%–100% MeOH in 45 min) to yield 12 ( $t_R$  22.7 min, 1.0 mg).

**12**—colorless, amorphous solid;  ${}^{1}\text{H}$  NMR (CDCl<sub>3</sub>)  $\delta$  7.13 – 7.26 (m), 6.81 (d, J = 9.0 Hz, 1H), 6.65 (d, J = 10.4 Hz, 1H), 4.83 (d, J = 10.6 Hz, 1H), 4.73 (dd, J = 8.7, 3.8 Hz, 1H), 4.59 (m, 2H), 4.48 (m, 1H), 4.28 (m, 1H), 3.79 (m, 1H), 3.56 – 3.69 (m, 2H), 3.50 (m, 1H), 3.36 (m, 1H), 3.16 (s, 3H), 2.70 (m, 2H), 2.24 – 2.48 (m, 6H), 1.94 – 2.19 (m, 2H), 1.77 – 1.94 (m), 1.14 (d, J = 6.8 Hz, 3H), 1.03 (d, J = 7.3 Hz, 3H), 0.96 (m, 6H), 0.90 (m, 6H), 0.80 (m, 6H); HRESI/APCIMS m/z 750.4407 [M + Na]<sup>+</sup> (calcd for  $C_{39}H_{61}N_{5}O_{8}Na$ , 750.4418).

#### Modified Mosher's Analysis Using PGME

The base hydrolysate product was dissolved in 600  $\mu$ L DMF- $d_7$  and divided into two portions. To one portion was added 0.7 mg (S)-PGME, and the solution cooled and stirred at 0 °C before the successive addition of PyBop (1.8 mg), HOBt (0.5 mg), and N-Me morpholine (5.0  $\mu$ L). The reaction was left to stir for another 3 h at room temperature and quenched with the addition of EtOAc. The resulting solution was successively washed with 5% HCl, saturated NaHCO<sub>3</sub>, and brine. The organic layer was collected and dried over anhydrous MgSO<sub>4</sub> before drying under N<sub>2</sub> to yield **13** (0.8 mg). The same procedure was used to prepare the (R)-PGME derivatized product **14** (0.6 mg). <sup>1</sup>H NMR chemical shifts of the Dml spin system were assigned by COSY and TOCSY analyses.

**13**—colorless, amorphous solid;  ${}^{1}\text{H}$  NMR (CDCl<sub>3</sub>)  $\delta$  7.27 – 7.38 (m, 12H, incl. NH Dml at 7.27), 5.57 (dd, J = 14.0, 7.4 Hz, 1H), 5.02 (d, J = 10.2 Hz, 1H), 4.42 (m, 2H), 4.24 (d, J = 2.6 Hz, 1H), 3.74 (s, 3H), 3.56 – 3.65 (m, 3H, incl. H-3 Dml at 3.62), 3.45 (m, 2H), 3.29 (m, 1H), 2.99 (s, 3H), 2.75 (m, 2H, incl. H-2 Dml), 2.30 – 2.40 (m, 5H), 2.02 – 2.09 (m, 4H), 1.97 – 2.00 (m, 4H), 1.45 (m, 1H, H-5 Dml), 1.19 (d, J = 7.2 Hz, 3H, H<sub>3</sub>-7 Dml), 1.04 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H, H<sub>3</sub>-6 Dml), 0.85 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H, H<sub>3</sub>-5 Dml), 0.80 (d, J = 6.6 Hz, 6H); HRESI/APCIMS m/z 897.5099 [M + Na]+ (calcd for  $C_{48}H_{70}F_{3}N_{6}O_{9}Na$ , 897.5102).

**14**—colorless, amorphous solid;  $^1\text{H}$  NMR (CDCl<sub>3</sub>)  $\delta$  7.30 – 7.40 (m, 10H), 7.14 – 7.24 (m, 4H, incl. NH Dml at 7.21), 5.46 (d, J = 7.3 Hz, 1H), 4.99 (d, J = 10.2 Hz, 1H), 4.52 (dd, J = 6.4, 2.3 Hz, 1H), 4.42 (t, J = 6.4 Hz, 1H), 4.27 (d, J = 2.0 Hz, 1H), 4.01, (m, 1H), 3.72 (s, 3H), 3.56 – 3.67 (m, 3H, incl. H-3 Dml at 3.64), 3.02 (s, 3H), 2.65 – 2.68 (m, 2H, incl. H-2 Dml at 2.68), 2.30 – 2.40 (m, 5H), 1.89 – 2.02 (m, 8H), 1.62 (m, 1H, H-5 Dml), 1.13 (d, J = 6.8 Hz, 3H, H<sub>3</sub>-7 Dml), 1.03 (d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H, H<sub>3</sub>-6 Dml), 0.96 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H, H<sub>3</sub>-5 Dml), 0.86 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 6.7 Hz, 6H); HRESI/APCIMS m/z 897.5101 [M + Na]<sup>+</sup> (calcd for C<sub>48</sub>H<sub>70</sub>F<sub>3</sub>N<sub>6</sub>O<sub>9</sub>Na, 897.5102).

#### **Cell Viability Assay**

HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone) under a humidified environment with 5%  $\rm CO_2$  at 37 °C. HeLa (3,000) and HT29 (12,500) cells were seeded in 96-well plates and treated with varying concentrations of **1–8**, and dolastatin 16, positive control (paclitaxel), and solvent control (DMSO) after 24 h of seeding. The cells were incubated for an additional 48 h before the addition of the MTT reagent. Cell viability was measured according to the manufacturer's instructions (Promega).  $\rm IC_{50}$  values for positive control were 3.0 and 2.6 nM for HT29 and HeLa cells, respectively.  $\rm IC_{50}$  calculations were done by GraphPad Prism 5.03 based on duplicate experiments.

# Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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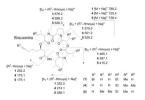


Figure 1.
MS/MS fragmentation of veraguamide A (1), veraguamide D (4), and veraguamide E (5).

Figure 2. Assignment of absolute configuration of veraguamide A (1) using methanolysis and subsequent Mosher's analysis.  $\Delta \delta = \delta(S\text{-MTPA ester}) - \delta(R\text{-MTPA ester})$ .

**Figure 3.** Assignment of absolute configuration dolastatin 16 by base hydrolysis and modified Mosher's analysis using PGME.  $\Delta \delta = \delta(S\text{-PGME amide}) - \delta(R\text{-PGME amide})$ .

II N-Me-Val-1/, I Br-Hmoya/Hmoya/	R <sup>1</sup>		Pro N 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	R <sup>2</sup>	)3. <sub>R5</sub>	IV pa/Hiva/ g3 V V√al-2/N	
Veraguamides		R <sup>1</sup>	$R^2$	$\mathbb{R}^3$	$R^4$	$R^5$	$R^6$
Α	(1)	{Br	H	Н	Et	Me	Н
В	(2)	{- <u>-</u> Br	Н	Н	Ме	Me	Н
С	(3)	<b>}</b> —	Н	Н	Et	Me	Н
D	(4)	<b>}</b> —	Н	Н	Et	Me	Me
E	(5)	<b>}</b> —	Me	Me	Et	Me	Н
F	(6)	<b>}</b> —	Н	Н	Ph	Н	Н
G	(7)	3	Н	Н	Et	Me	Н
semisynthetic	(8)	3	Н	Н	Et	Me	Н

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Table 1

NMR Data for Veraguamide A (1) in CDCl<sub>3</sub>

		δ c <sup>a</sup>	$\delta H (J \text{ in Hz})^{D}$	$_{a}$ XSOO	$\text{HMBC}^{o}$
Br-Hmoya	1	170.8, C			
	2	42.4, CH	3.11, br q (7.4)	$H-3, H_3-9$	1, 3, 4, 9
	3	76.4, CH	4.85, dt (10.2, 2.5)	H-2, H-4a, H-4b	1, 1 (N-Me-Val-1)
	4a	27.4, CH <sub>2</sub>	2.06, m	H-3, H-4b, H-5a, H-5b	5,6
	4b		1.59, m	H-3, H-4a, H-5a, H-5b	
	5a	$25.0, CH_2$	1.60, m	$H-4a$ , $H-4b$ , $H-5b$ , $H_2-6$	7,8
	5b		1.41, m	H-4a, H-4b, H-5a, H <sub>2</sub> -6	6,8
	9	$19.2, CH_2$	2.23, m	H-5a, H-5b	7,8
	7	38.4, C			
	∞	79.3, C			
	6	14.1, $CH3$	1.25, d (7.4)	Н-2	1, 2, 3
N-Me-Val-1	1	170.6, C			
	2	65.0, CH	3.93, d (10.3)	H-3	1, 3, 4, N-Me, 1 (Pro)
	3	28.3, CH	2.30, m	H-2, H <sub>3</sub> -4, H <sub>3</sub> -5	2, 4, 5
	4	$19.56, CH_3$	0.98, d (6.6)	Н-3	2
	5	$19.51$ , $CH_3$	0.91, d (6.6)	Н-3	2
	<i>N</i> -Me	$28.6$ , $CH_3$	3.00, s		2, 1 (Pro)
Pro	1	172.1, C			
	2	57.3, CH	4.94 dd (8.9, 4.5)	H-3a, H-3b	1, 3, 4, 1 (Hmpa)
	3a	$29.5, CH_2$	2.30, m	H-2, H-3b, H-4a	1, 2, 5
	3b		1.79, m	H-2, H-3a, H-4a	1, 2, 5
	4a	$24.9, CH_2$	2.03, m	H-3a, H-3b, H-4b, H-5a, H-5b	2, 3, 5
	4b		1.98, m	H-4a, H-5a, H-5b	2, 3, 5
	5a	47.3, CH <sub>2</sub>	3.84, dt (-17.0, 7.1)	H-4a, H-4b, H-5b	2, 3, 4
	5b		3.61, dt (-17.0, 7.1)	H-4a, H-4b, H-5a	3,4
Hmpa	_	165.9, C			
	2	76.1, CH	4.90, d (9.1)	Н-3	1, 3, 4, 5, 1 (N-Me-Val-2)

unit	C/H no	δ <sub>C</sub> <sup>a</sup>	$\delta H (J \text{ in Hz})^b$	${ m COSY}_p$	$\mathrm{HMBC}^b$
	3	35.7, CH	1.97, m	H-2, H <sub>3</sub> -6	
	4a	$24.81,\mathrm{CH}_2$	1.54, m	$H-4b$ , $H_3-5$	
	4p		1.13, m	H-4a, H <sub>3</sub> -5	
	5	$10.5, \mathrm{CH}_3$	0.87, t (7.3)	H-4a, H-4b	3,4
	9	13.8, CH <sub>3</sub>	1.01, d (6.8)	H-3	2, 3, 4
N-Me-Val-2	1	169.6, C			
	2	66.0, CH	4.15, d (9.5)	H-3	1, 3, 5, N-Me, 1(Val)
	3	28.5, CH	2.27, m	H-2, H <sub>3</sub> -4, H <sub>3</sub> -5	1
	4	$20.4$ , $CH_3$	1.00, d (7.0)	H-3	2
	5	$20.2, \mathrm{CH}_3$	1.11, d (7.0)	H-3	2,3
	<i>N</i> -Me	$30.0, \mathrm{CH}_3$	2.94, s		2, 1 (Val)
Val	1	173.4, C			
	2	52.8, CH	4.70, dd (8.7, 6.5)	H-3, NH	1, 3, 4, 5, 1 (Br-Hmoya)
	3	32.1, CH	1.96, m	$H-2$ , $H_3-4$ , $H_3-5$	5
	4	$20.3$ , $CH_3$	0.95, d (6.7)	H-3	2, 3, 5
	\$	17.5, CH <sub>3</sub>	0.88, d (6.7)	H-3	2,3
	NH		6.25, d (8.7)	Н-2	1, 1 (Br-Hmoya)

 $a = 100 \, \mathrm{MHz}$   $b = 600 \, \mathrm{MHz}$ 

Table 2

NMR Data for Veraguamides B (2) and C (3) in CDCl<sub>3</sub>

unit	C/H no.	δ c <sup>a</sup>	$\delta_{ m H}  (J  { m in}  { m Hz})^{b}$	δ c <sup>a</sup>	$\delta_{ m H}  (J  { m in}  { m Hz})^{b}$
Br-Hmoya <sup>C</sup> /Hmoya <sup>d</sup>	1	170.8, C		170.8, C	
	2	42.3, CH	3.13, br q (7.4)	42.4, CH	3.10, br q (7.2)
	8	76.4, CH	4.85, d (8.7)	76.4, CH	4.86, dt (10.4, 2.5)
	4a	27.5, $CH2$	2.07, m	27.4, CH <sub>2</sub>	2.07, m
	4b		1.60, m		1.62, m
	5a	$24.93, CH_2$	1.61, m	$25.2$ , $CH_2$	1.62, m
	5b		1.42, m		1.44, m
	9	$19.2, \mathrm{CH}_2$	2.21, m	$18.0, \mathrm{CH}_2$	2.19, m
	7	38.4, C		83.6, C	
	∞	79.4, C		68.8, CH	1.93 t (2.5)
	6	14.6, CH <sub>3</sub>	1.25, d (7.4)	14.5, CH <sub>3</sub>	1.25, d (7.2)
N-Me-Val-1	1	170.7, C		170.7, C	
	2	65.0, CH	3.94, d (10.4)	65.0, CH	3.93, d (11.0)
	8	28.26, CH	2.29, m	28.3, CH	2.28, m
	4	19.57, CH <sub>3</sub>	0.98, d (6.5)	$19.58, CH_3$	0.98, d (6.8)
	S	19.55, CH <sub>3</sub>	0.92, d (6.5)	$19.56, CH_3$	0.91, d (6.8)
	<i>N</i> -Me	$28.7$ , $CH_3$	3.00, s	$28.7$ , $CH_3$	3.00, s
Pro	1	172.1, C		172.2, C	
	2	57.2, CH	4.95 dd (8.6, 4.8)	57.3, CH	4.94 dd (8.4, 5.0)
	3a	$29.4$ , $CH_2$	2.28, m	$29.5, \mathrm{CH}_2$	2.28, m
	3b		1.79, m		1.79, m
	4a	$24.99, CH_2$	2.04, m	$24.89, CH_2$	2.03, m
	4b		1.98, m		1.99, m
	5a	47.3, CH <sub>2</sub>	3.80, dt (-16.4, 6.9)	47.3, CH <sub>2</sub>	3.84, dt (-16.8, 7.1)
	5b		3.60, dt (-16.4, 6.9)		3.60, dt (-16.8, 7.1)
$Hiva^{C}/Hmpa^{d}$	1	165.8, C		165.9, C	

			2		3
unit	C/H no.	δ c <sup>a</sup>	$\delta_{ m H}(J{ m in}{ m Hz})^{b}$	8 c <sup>a</sup>	$\delta_{ m H}$ (J in Hz) $^b$
	2	77.2, CH	4.85, d (8.7)	76.0, CH	4.89, d (9.4)
	8	29.6, CH	2.17, m	35.7, CH	1.98, m
	4	$18.1, CH_3$	1.02, t (6.6)	$24.81$ , $CH_2$	1.54, m
					1.13, m
	ß	$18.5, CH_3$	0.93, d (6.6)	$10.5, CH_3$	0.86, t (7.3)
	9			$13.8, CH_3$	1.01, d (6.7)
N-Me-Val-2	1	169.6, C		169.6, C	
	2	66.1, CH	4.15, d (10.2)	66.0, CH	4.13, d (10.0)
	3	28.34, CH	2.28, m	28.5, CH	2.28, m
	4	$20.3$ , $CH_3$	0.99, d (6.8)	$20.4$ , $CH_3$	0.99, d (6.4)
	S	$20.1, CH_3$	1.11, d (6.8)	$20.2$ , $CH_3$	1.10, d (6.4)
	<i>N</i> -Me	$30.0, \mathrm{CH}_3$	2.94, s	$30.0, \mathrm{CH}_3$	2.93, s
Val	1	173.5, C		173.4, C	
	2	52.8, CH	4.71, dd (8.6, 6.4)	52.8, CH	4.70, dd (8.6, 6.2)
	8	32.2, CH	1.98, m	32.1, CH	1.96, m
	4	$20.3$ , $CH_3$	0.94, d (6.4)	$20.3$ , $CH_3$	0.94, d (6.7)
	S	17.5, CH <sub>3</sub>	0.88, d (6.4)	$17.6$ , $CH_3$	0.87, d (6.7)
	HN		6.26, d (8.6)		6.26, d (8.6)

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 $^c$ Refers to Compound  ${f 2}$ 

 $a_{100\,\mathrm{MHz}}$  $^b_{600\,\mathrm{MHz}}$   $^d$ Refers to Compound 3

Table 3

NMR Data for Veraguamides D (4) and E (5) in CDCl<sub>3</sub>

unit	C/H no.	δ <sub>C</sub> <sup>a</sup>	$\delta_{\mathrm{H}} \left( J \text{ in Hz} \right)^{b}$	δα	$\delta (J \text{ in Hz})^b$
Hmoya	1	170.8, C		170.71, C	
	2	42.4, CH	3.13, br q (7.2)	42.4, CH	3.08, br q (7.4)
	3	76.4, CH	4.86, dt (10.8, 2.6)	76.5, CH	4.85, d (9.0)
	4a	27.5, CH <sub>2</sub>	2.07, m	27.5, CH <sub>2</sub>	2.06, m
	4b		1.63, m		1.62, m
	5a	25.2, $CH2$	1.63, m	25.2, $CH2$	1.61, m
	5b		1.47, m		1.43, m
	9	17.5, CH <sub>2</sub>	2.18, m	$18.0, \mathrm{CH}_2$	2.18, m
	7	83.6, C		83.6, C	
	∞	68.8, CH	1.93, t (2.5)	68.8, CH	1.93, t (2.3)
	6	14.4, CH <sub>3</sub>	1.24, d (7.2)	$14.5$ , $CH_3$	1.23, d (7.4)
N-Me-IIe <sup>C</sup> /N-Me-Val <sup>d</sup>	1	170.7, C		170.69, C	
	2	64.0, CH	4.01, d (10.6)	64.9, CH	3.93, d (10.0)
	3	34.6, CH	2.02, m	28.3, CH	2.29, m
	4	25.7, CH <sub>2</sub>	1.46, m	19.59, CH <sub>3</sub>	0.91, d (6.4)
	5	11.4, CH <sub>3</sub>	0.93, t (6.5)	19.56, CH <sub>3</sub>	0.98, d (6.4)
	9	15.8, CH <sub>3</sub>	0.94, d (6.8)		
	<i>N</i> -Me	$28.7$ , $CH_3$	2.99, s	$28.6, CH_3$	3.00, s
Pro	1	172.7, C		172.2, C	
	2	57.2, CH	4.94 dd (8.9, 4.8)	57.3, CH	4.94 dd (9.0, 5.3)
	3a	28.8, CH <sub>2</sub>	2.26, m	$29.5, CH_2$	2.29, m
	3b		1.78, m		1.78, m
	4a	24.9, CH <sub>2</sub>	2.03, m	24.89, $CH2$	2.01, m
	4b		1.97, m		1.99, m
	5a	$47.2, CH_2$	3.82, dt (-17.0, 7.3)	$47.3$ , $CH_2$	3.86, dt (-17.0, 7.0)
	5b		3.60, dt (-17.0, 7.3)		3.60, dt (-17.0, 7.0)

unit	C/H no.	8 c <sup>a</sup>	$\delta_{ m H}  (J  { m in}  { m Hz})^b$	8 a	$\delta$ ( $J$ in Hz) $^b$
Hmpa	1	165.9, C		166.0, C	
	2	76.0, CH	4.90, d (9.2)	76.1, CH	4.85, d (9.0)
	3	35.7, CH	1.98, m	35.1, CH	1.98, m
	4	24.8, CH <sub>2</sub>	1.53, m	24.86, CH <sub>2</sub>	1.54, m
			1.12, m		1.13, m
	S	$10.5, \mathrm{CH}_3$	0.86, t (7.6)	$10.5$ , $CH_3$	0.86, t (7.0)
	9	13.9, CH <sub>3</sub>	0.99, d (6.9)	13.8, CH <sub>3</sub>	1.01, d (6.8)
N-Me-Val <sup>C</sup> /N-Me-Ile <sup>d</sup>	1	169.6, C		169.7, C	
	2	66.0, CH	4.15, d (9.4)	65.2, CH	4.22, d (9.6)
	3	28.4, CH	2.28, m	35.7, CH	1.98, m
	4	$20.3, \mathrm{CH}_3$	1.10, d (6.8)	$26.6, \mathrm{CH}_2$	1.54, m
					1.06, m
	S	$20.2, CH_3$	0.99, d (6.8)	11.7, CH <sub>3</sub>	0.96, t (7.2)
	9			16.5, CH <sub>3</sub>	1.04, d (6.9)
	<i>N</i> -Me	$30.0, \mathrm{CH}_3$	2.92, s	$30.1, \mathrm{CH}_3$	2.93, s
$Val^C/\Pi e^d$	1	173.4, C		173.5, C	
	2	52.8, CH	4.70, dd (8.6, 6.6)	52.4, CH	4.70, dd (8.4, 6.7)
	3	32.1, CH	1.98, m	38.6, CH	1.69, m
	4	$19.0, \mathrm{CH}_3$	0.94, d (6.6)	$23.9$ , $CH_2$	1.47, m
	5	17.6, CH <sub>3</sub>	0.87, d (6.6)		1.05, m
				11.5, CH <sub>3</sub>	0.85, d (6.6)
	9			$16.3, CH_3$	0.91, d (6.6)
	NH		6.24, d (8.6)		6.26, d (8.4)
<sup>a</sup> 125 MHz					
$^{b}_{600~\mathrm{MHz}}$					
<sup>c</sup> Refers to Compound 4					
-					

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Table 4

NMR Data for Veraguamide F (6) in CDCl<sub>3</sub>

			· · · · · · · · · · · · · · · · · · ·
unit	C/H no.	$\delta_{\rm C}{}^a$	$\delta (J \text{ in Hz})^{b}$
Hmoya	1	170.9, C	
	2	42.2, CH	3.24 br q (7.3)
	3	76.6, CH	4.89, dt (10.6, 2.4)
	4a	27.5, CH <sub>2</sub>	2.08, m
	4b		1.64, m
	5a	25.2, CH <sub>2</sub>	1.65, m
	5b		1.45, m
	6	18.0, CH <sub>2</sub>	2.20, m
	7	83.5, C	
	8	68.9, CH	1.93, t (2.5)
	9	14.6, CH <sub>3</sub>	1.29, d (7.3)
N-Me-Val-1	1	170.7, C	
	2	65.3, CH	3.95, d (10.4)
	3	28.3, CH	2.32, m
	4	19.6, CH <sub>3</sub>	1.00, d (6.5)
	5	19.7, CH <sub>3</sub>	0.93, d (6.5)
	<i>N</i> -Me	28.7, CH <sub>3</sub>	3.06, s
Pro	1	172.3, C	
	2	57.1, CH	4.97, dd (9.0, 4.5)
	3a	29.1, CH <sub>2</sub>	2.27, m
	3b		1.83, m
	4a	25.0, CH <sub>2</sub>	2.07, m
	4b		1.97, m
	5a	47.0, CH <sub>2</sub>	3.62, m
	5b		3.56, m
Pla	1	165.4, C	
	2	72.8, CH	5.47, dd (9.8, 4.0)
	3	36.7, CH <sub>2</sub>	3.17, m
			2.91, m
	4	136.2, C	
	5//9	129.3, CH	7.18, d (8.0)
	6/8	128.5, CH	7.28, m
	7	126.7, CH	7.20, m
N-Me-Val-2	1	168.9, C	
	2	65.8, CH	4.05, d (10.5)
	3	27.4, CH	2.08, m
	4	19.8, CH <sub>3</sub>	0.89, d (6.8)
	5	20.0, CH <sub>3</sub>	0.91, d (6.8)

unit	C/H no.	$\delta_{\rm C}{}^a$	$\delta (J \text{ in Hz})^b$
	<i>N</i> -Me	29.0, CH <sub>3</sub>	2.60, s
Val	1	173.4, C	
	2	52.7, CH	4.76, dd (8.5, 6.1)
	3	32.3, CH	1.98, m
	4	20.3, CH <sub>3</sub>	0.91, d (6.6)
	5	17.7, CH <sub>3</sub>	0.88, d (6.6)
	NH		6.28, d (8.5)

 $<sup>^</sup>a 100~\mathrm{MHz}$ 

 $<sup>^</sup>b_{\rm 600~MHz}$ 

Table 5

NMR Data for Veraguamide G (7) and Tetrahydroveraguamide A (8) in CDCl $_{\rm 3}$ 

			7		8
unit	C/H no.	δ c <sup>a</sup>	$\delta_{ m H}$ (J in Hz) $^b$	$\delta_{\rm c}^{b,c}$	$\delta_{ m H}  (J  { m in}  { m Hz})^b$
$Hmoea^d/Hmoaa^e$		170.9, C		170.7, C	
	2	42.4, CH	3.10, br q (7.4)	42.1, CH	3.08, br q (7.4)
	3	76.8, CH	4.85, dt (10.6, 2.4)	76.8, CH	4.86, dt (10.1, 2.1)
	4a	$27.9$ , $CH_2$	1.98, m	$31.0, \mathrm{CH}_2$	1.21, m
	4b		1.45, m		1.26, m
	5a	25.5, $CH2$	1.48, m	$28.2$ , $CH_2$	1.39, m
	5b		1.30, m		
	<b>6</b> a	$33.2$ , $CH_2$	2.05, m	25.8, CH <sub>2</sub>	1.39, m
	<b>q</b> 9				1.20, m
	7	138.2, CH	5.74, m	$22.2, CH_2$	1.26, m
	∞	114.9, CH <sub>2</sub>	4.97, m	13.6, CH <sub>3</sub>	0.85, t (6.9)
	6	14.4, CH <sub>3</sub>	1.22, d (7.4)	$14.0, CH_3$	1.23, d (7.4)
N-Me-Val-1	1	170.7, C		170.7, C	
	2	65.0, CH	3.93, d (9.8)	64.9, CH	3.93, d (10.7)
	3	28.3, CH	2.28, m	28.3, CH	2.28, m
	4	19.59, CH <sub>3</sub>	0.98, d (6.4)	19.2, CH <sub>3</sub>	0.98, d (6.5)
	5	19.54, CH <sub>3</sub>	0.92, d (6.4)	$19.3, CH_3$	0.91, d (6.5)
	<i>N</i> -Me	$28.6, CH_3$	3.01, s	28.4, CH <sub>3</sub>	3.00, s
Pro	-	172.2, C		172.0, C	
	2	57.3, CH	4.95, dd (8.7, 5.0)	57.1, CH	4.94, dd (9.0, 5.0)
	3a	$29.4, CH_2$	2.29, m	$29.1$ , $CH_2$	2.28, m
	3b		1.79, m		1.79, m
	4a	$24.9, CH_2$	2.03, m	24.6, CH <sub>2</sub>	2.03, m
	4b		1.98, m		1.99, m
	5a	47.3, CH <sub>2</sub>	3.84, dt (-16.7, 7.1)	47.0, CH <sub>2</sub>	3.84, dd (-17.0, 7.3)
	5b		3.61, dt (-16.7, 7.1)		3.60, dd (-17.0, 7.3)

			7		∞
unit	C/H no.	δ <sub>C</sub> <sup>α</sup>	$\delta_{ m H}(J{ m in}{ m Hz})^{b}$	$\delta_{\rm C}^{}b,c$	$\delta_{ m H}(J{ m in}{ m Hz})^{b}$
Hmpa	1	165.9, C		165.7, C	
	2	76.0, CH	4.90, d (8.7)	76.6, CH	4.90, d (8.8)
	3	35.7, CH	1.98, m	35.4, CH	1.98, m
	4	24.8, CH <sub>2</sub>	1.54, m	24.5, CH <sub>2</sub>	1.54, m
			1.13, m		1.13, m
	5	$10.5, \mathrm{CH}_3$	0.86, t (7.3)	$10.2, \mathrm{CH}_3$	0.86, t (7.1)
	9	13.8, CH <sub>3</sub>	1.00, d (6.0)	$13.5, CH_3$	1.00, d (6.4)
N-Me-Val-2	1	169.6, C		169.5, C	
	2	66.0, CH	4.15, d (10.2)	65.8 CH	4.14, d (9.6)
	3	28.6, CH	2.28, m	28.1, CH	2.28, m
	4	$20.4$ , $CH_3$	1.00, d (6.1)	$20.0, \mathrm{CH}_3$	0.99, d (6.6)
	5	$20.2, CH_3$	1.10, d (6.1)	$19.9, CH_3$	1.10, d (6.6)
	N-Me	$30.0, \mathrm{CH}_3$	2.93, s	$29.7, CH_3$	2.93, s
Val	1	173.4, C		173.3, C	
	2	52.7, CH	4.70, dd (8.6, 6.7)	52.5, CH	4.70, dd (8.6, 6.2)
	3	32.1, CH	1.98, m	31.7, CH	1.96, m
	4	$20.3$ , $CH_3$	0.93, d (6.8)	$19.3, CH_3$	0.93, d (6.3)
	S	17.6, CH <sub>3</sub>	0.86, d (6.8)	$17.2, CH_3$	0.87, d (6.3)
	HN		6.23, d (8.6)		6.26, d (8.6)

<sup>a</sup>125 MHz

 $^{b}_{600\,\mathrm{MHz}}$ 

<sup>c</sup>Based on HSQC and HMBC

 $^d$ Refers to Compound 7

 $^e$ Refers to Compound  ${f 8}$ 

 $\label{eq:Table 6} \textbf{Cytotoxic Activity (IC}_{50},\,\mu\text{M}) \ \text{of Natural and Semisynthetic Veraguamides}$ 

Compound	HT29	HeLa
Veraguamide A (1)	26	21
Veraguamide B (2)	30	17
Veraguamide C (3)	5.8	6.1
Veraguamide D (4)	0.84	0.54
Veraguamide E (5)	1.5	0.83
Veraguamide F (6)	49	49
Veraguamide G (7)	2.7	2.3
Tetrahydroveraguamide A (8)	33	48
Dolastatin 16	78	58