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# Lipopeptides from the Tropical Marine Cyanobacterium *Symploca* sp.

Emily Mevers,[†,‡] F. P. Jake Haeckl,[‡] Paul D. Boudreau,[†] Tara Byrum,[†] Pieter C. Dorrestein,[‡,§] Frederick A. Valeriote,[⊥] and William H. Gerwick*[,†,§]

[†]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, United States

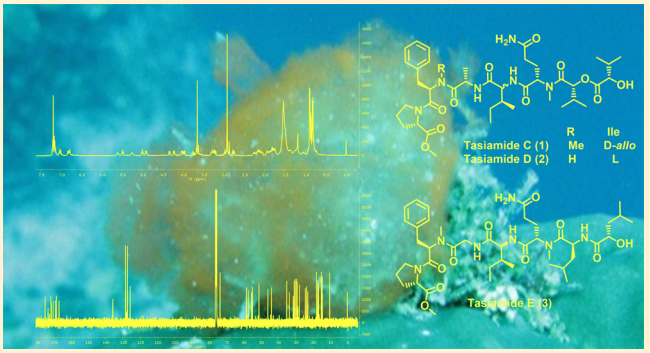
[‡]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California 92093, United States

[§]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California 92093, United States

[⊥]Division of Hematology and Oncology, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan 48202, United States

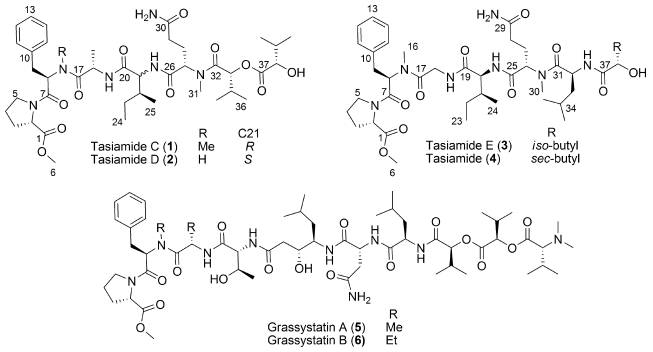
Supporting Information

**ABSTRACT:** A collection of the tropical marine cyanobacterium *Symploca* sp., collected near Kimbe Bay, Papua New Guinea, previously yielded several new metabolites including kimbeamides A–C, kimbelactone A, and tasihalide C. Investigations into a more polar cytotoxic fraction yielded three new lipopeptides, tasiamides C–E (**1**–**3**). The planar structures were deduced by 2D NMR spectroscopy and tandem mass spectrometry, and their absolute configurations were determined by a combination of Marfey's and chiral-phase GC-MS analysis. These new metabolites are similar to several previously isolated compounds, including tasiamide (**4**), grassystatins (**5**, **6**), and symplocin A, all of which were isolated from similar filamentous marine cyanobacteria.




Recent sequencing efforts of marine cyanobacterial genomes have revealed their exceptional capacity to produce a large diversity of intriguing secondary metabolites, representing a range of distinct and unrelated biosynthetic pathways.[1,2] This has also been observed from chemical investigations of such cyanobacterial species as *Moorea bouillonii* and *Moorea producens*, formerly known as *Lyngbya bouillonii* and *Lyngbya majuscula*, respectively.[3] Both of these latter organisms are known to produce a plethora of metabolites, including the lyngbyabellins,[4] apratoxins,[5] laingolide,[6] lyngbyaloside,[7] apramide A,[8] and palau'imide[9] from *M. bouillonii* and lyngbyatoxin A,[10] the jamaicamides,[11] carmabin,[12] various malyngamides,[13] and barbamide[14] from *M. producens*. These examples of highly productive strains and improved genomic insights into cyanobacterial biosynthetic capacity demonstrate the utility of rigorously examining collections of cyanobacteria for novel natural products, even when a given strain has already been extensively investigated.

In the present investigation into a filamentous tuft-forming cyanobacterium from Kimbe Bay, Papua New Guinea, which already yielded kimbeamides A–C, kimbealactone A, and tasihalide C,[15] another chromatography fraction exhibited strong cytotoxicity against several cancer cell lines and was thus chosen for further evaluation. A subsequent NMR-guided fractionation process yielded three new lipopeptides, tasiamides C–E (**1**–**3**), two (C and D) of which were evaluated for cytotoxicity and found to be inactive. Both the planar and absolute configurations of these metabolites were determined and have led to some intriguing insights into the biosynthetic capability of this particular collection.




## RESULTS AND DISCUSSION

Orange tufts of a tropical marine *Symploca* sp. were collected in approximately 20 m of water near Kimbe Bay, Papua New Guinea, in July 2007. The preserved collection was repetitively

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extracted (2:1 $CH_2Cl_2$/MeOH) and fractionated using normal-phase vacuum liquid chromatography (VLC). Previously, a middle polarity fraction (60% hexanes/40% EtOAc) of this extract yielded several biologically active metabolites, including kimbeamides A–C, kimbelactone A, and tasihalide C.[15] Additionally, a relatively polar fraction eluting with 25% MeOH/EtOAc exhibited cytotoxic activity against H-460 human lung cancer cells (81% toxicity at 3 $\mu$g/mL). Further chromatography of this fraction using normal-phase solid-phase extraction (SPE) and reversed-phase HPLC yielded 1.9 mg of tasiamide C (**1**), 2.5 mg of tasiamide D (**2**), and 0.7 mg of tasiamide E (**3**).

HR-ESITOFMS of **1** yielded an $[M + Na]^+$ at $m/z$ 839.4541, giving a molecular formula of $C_{41}H_{64}N_6O_{11}$, with 13 degrees of unsaturation. The IR spectrum featured absorptions indicative of the presence of *N*H or *O*H protons and the presence of amide or ester carbonyls (3371 and 1737 $cm^{-1}$, respectively). Further evidence of ester or amide carbonyls was present as eight downfield signals in the $^{13}C$ NMR spectrum ($\delta_C$ 167.8, 169.2, 169.9, 170.8, 172.1, 172.5, 173.7, and 175.7). Additionally, the presence of a monosubstituted phenyl group was evident from four downfield carbon signals, two of which were composed of two carbons each as indicated by their relative peak height ($\delta_C$ 126.4, 128.0 × 2, 129.2 × 2, and 136.4). Further analysis of the $^1H$ NMR spectrum revealed the presence of three singlet methyls at shifts indicative of an *O*-methyl ($\delta_H$ 3.67) and two *N*-methyl groups ($\delta_H$ 2.93 and 3.03), along with two broad downfield signals suggestive of *N*H protons ($\delta_H$ 6.69 and 6.78).

Analysis of the 2D NMR data (COSY, TOCSY, ROESY, HSQC, and HMBC) enabled the assignment of eight COSY spin systems consisting of five amino and two hydroxy acid residues [proline methyl ester (Pro-Me ester), *N*-MePhe, Ala, Ile, *N*-MeGln, and two 2-hydroxy-3-methylbutyric acids (Hmba)], accounting for all 13 degrees of unsaturation and thereby signifying an overall linear arrangement (Figure 1). HMBC correlations from the *N*H and *N*-Me groups to the carbonyl of the neighboring residues allowed for the assignment of connections between *N*-MePhe and Ala (C-16 to C-17), *N*-MeGln and Hmba-1 (C-31 to C-32), Ala and Ile (*N*H-1 to C-20), and Ile and *N*-MeGln (*N*-H-2 to C-26), leading to three partial structures, Pro-Me ester, *N*-MePhe–Ala–Ile–*N*-MeGln–Hmba-1, and Hmba-2. Key ROESY correlations revealed further connections between these fragments, one between H-5a/b ($\delta$ 3.34/3.15) and H-8 ($\delta$ 5.52) connecting Pro-Me ester to *N*-MePhe and the other between H-34 ($\delta$ 2.15) and H-38 ($\delta$ 4.09), making the final connection between the two Hmba residues. Thus, tasiamide C was deduced to have an overall linear structure consisting of Pro-Me ester–*N*-MePhe–Ala–Ile–*N*-MeGln–Hmba-1–Hmba-2. This planar constitution was supported by tandem mass spectroscopy analysis (Figure 2).

The absolute configurations of several of the amino acids (Pro, *N*-MePhe, Ala, and *N*-MeGln) were determined by Marfey's analysis. Authentic D and L standards for Pro, *N*-MePhe, and Ala were each derivatized with D-(1-fluoro-2,4-dinitrophenyl-5-D-alanine amide) (D-FDAA). Unfortunately, authentic D-*N*-MeGlu was unavailable; therefore, chromatographic standards were prepared by derivatizing L-*N*-MeGlu with both D- and L-FDAA (upon acidic hydrolysis, *N*-MeGln becomes *N*-MeGlu). Compound **1** was hydrolyzed and derivatized with D-FDAA and analyzed by LC-MS in comparison with the retention times of authentic standards.

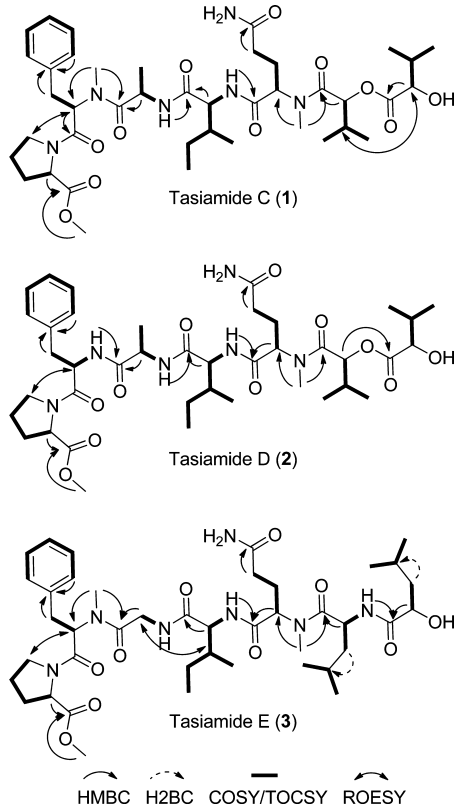


**Figure 1.** Select 2D NMR data for tasiamides C–E (**1**–**3**).

From this analysis it was clear that three of the four amino acids (Pro, Ala, and *N*-MeGln) were of the L configuration, while the *N*-MePhe residue was of the D configuration (Supporting Information).

The absolute configuration of the Ile residue was determined by chiral-phase GC-MS comparison of *N*-Boc, *O*-Me-derivatized authentic standards against the similarly derivatized Ile residue released by acid hydrolysis of **1**. The four protected standards of L-Ile, L-*allo*-Ile, D-Ile, and D-*allo*-Ile were prepared by first synthesizing the *N*-Boc-protected amino acids, followed by methyl esterification of the carboxylic acid using diazomethane. From retention time comparison and co-injection experiments it was clear that the Ile residue was of the D-*allo* configuration (Supporting Information).

The absolute configuration of the final two Hmba stereocenters in **1** presented a challenge. Comparison of authentic standards of *S*- and *R*-Hmba with the methylated hydrolysate revealed that the natural product contained both *S*- and *R*-Hmba residues. A similar situation has been previously reported in closely related metabolites of this compound family.[16] Following mild base treatment (1:1 0.5 N NaOH(aq)/MeOH), only the terminal Hmba residue was released; this residue was subsequently methyl esterified using diazomethane[16] and, by retention time comparison and co-injections with authentic standards, was identified as the *S* configuration. The penultimate Hmba must therefore have the *R* configuration. In summary, the above experiments established that tasiamide C (**1**) possessed a 2*S*, 8*R*, 18*S*, 21*R*, 22*S*, 27*S*, 33*R*, and 38*S* absolute configuration.

HR-ESITOFMS of **2** yielded an $[M + Na]^+$ at $m/z$ 825.4371 for a molecular formula of $C_{40}H_{62}N_6O_{11}$. The IR and $^1H$ and $^{13}C$ NMR spectra were similar to those of **1**; however, the molecular formula indicated a reduction of 14 amu (e.g., a $CH_2$


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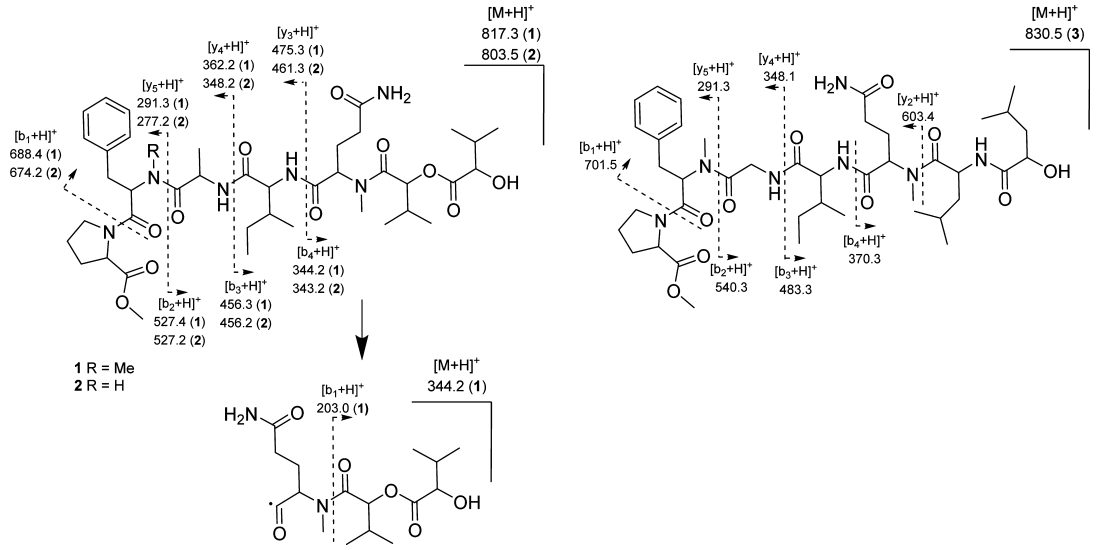


**Figure 2.** Select low-resolution MS fragmentation cleavages for tasiamides C–E (**1**–**3**).

unit) relative to tasiamide C. Inspection of the $^{1}$H NMR spectrum revealed the presence of only two singlet methyl groups (e.g., one *N*-methyl at $\delta_H$ 3.02 and one *O*-methyl at $\delta_H$ 3.58) and three *N*H protons ($\delta_H$ 6.77, 6.89, and 6.98). From the 2D NMR data, it was clear that the only modification between **1** and **2** was the loss of the *N*-methyl on the Phe residue, thus yielding a planar constitution of Pro-Me ester–Phe–Ala–Ile–*N*-MeGln–Hmba-1–Hmba-2 for tasiamide D (**2**). This assembly was corroborated by tandem mass spectrometry analysis (Figure 2).

The absolute configurations of the residues in compound **2** were determined in an identical fashion to that described above for compound **1**. Analysis of the retention times of the Marfey's derivatized hydrolysate and authentic standards by LC-MS revealed that three of the four amino acids (Pro, Ala, and *N*-MeGln) were of the L configuration and that the Phe residue was of the D configuration. Chiral-phase GC-MS analysis of the Ile residue, *N*-Boc and *O*-methylated as with **1**, showed by retention time comparison and co-injections that this residue was of the L configuration. Chiral-phase GC-MS analysis of the methylated hydrolysate of **2** exhibited peaks matching both *S*- and *R*-Hmba. Analysis of the methylated mild base hydrolysate confirmed that, as with **1**, the terminal Hmba was of *S* configuration, and so the penultimate Hmba was of *R* configuration, establishing the absolute configuration of tasiamide D (**2**) as 2*S*, 8*R*, 17*S*, 20*S*, 21*S*, 26*S*, 32*R*, and 37*S*.

HR-ESITOFMS of **3** yielded an $[M + Na]^+$ at *m*/*z* 852.4842 in agreement with a molecular formula of $C_{42}H_{67}N_7O_{10}$, requiring 13 degrees of unsaturation. The IR and $^{1}$H and $^{13}$C NMR spectra again featured similarities to **1** and **2**; however, the molecular weight was 13 and 27 amu greater than **1** and **2**, respectively, and thus could not be readily attributed to a single modification. Closer inspection of the $^{1}$H NMR spectrum revealed the presence of three singlet methyls ($\delta_H$ 2.98, 2.99, and 3.72), similar to **1**, and three amide protons ($\delta_H$ 6.78, 6.82, and 7.01), as seen in **2**, suggesting that one of the hydroxy acids was replaced by an amino acid. Further evidence of this was seen by comparison of the $^{13}$C NMR spectra of **1** and **3**; in tasiamide C there were two $\alpha$-carbons with shifts indicative of hydroxy acids at $\delta_C$ 74.6 and 76.4, whereas there was only one such peak in tasiamide E at $\delta_C$ 71.1.

Further analysis of 1D and 2D NMR data ($^{1}$H, $^{13}$C, COSY, TOCSY, ROESY, HSQC, HMBC, and H2BC[17]) confirmed the presence of seven residues, six amino acids, and one hydroxy acid [Pro-Me ester, *N*-MePhe, Gly, Ile, *N*-MeGln, Leu, and 2-hydroxy-4-methylpentanoic acid (H4mpa)[18]]. In a similar fashion to **1**, each of the connections between residues in **3** were revealed by key HMBC and ROESY correlations. HMBC correlations from both *N*-methyl groups to the carbonyls on the neighboring residues allowed for the assignment of connections between *N*-MePhe to Gly (C-16 to C-17) and *N*-MeGln to Leu (C-30 to C-31). Correspondingly, HMBC correlations from amide protons to adjacent carbonyls allowed for the assignment of connections between Ile and *N*-MeGln (*N*H-2 to C-25) and between Leu and H4mpa (*N*H-3 to C-37), leading to three partial structures, Pro-Me ester, *N*-MePhe–Gly, and Ile–*N*-MeGln–Leu–H4mpa. Two ROESY correlations provided the final connections between these fragments, one between H-5a/b ($\delta$ 3.36/3.31) and H-8 ($\delta$ 5.55), connecting Pro-Me ester and *N*-MePhe, and the other between H-18a/b ($\delta$ 4.05/3.81) and H-21 ($\delta$ 1.82), making the final connection between Gly and Ile. Thus, tasiamide E was deduced to have a planar linear structure consisting of Pro-Me ester–*N*-MePhe–Gly–Ile–*N*-MeGln–Leu–H4mpa, and this was supported by tandem mass spectrometry analysis (Figure 2).

The absolute configurations of the residues in **3** were determined as described above for compounds **1** and **2**. Analysis of the Marfey's derivatized hydrolysate with the retention times of authentic standards on LC-MS revealed that three of the four amino acids (Pro, Leu, and *N*-MeGln) were of the L configuration, while the *N*-MePhe residue was of the D configuration. As for the chiral GC-MS analysis of the Ile residue, retention time comparison and co-injections confirmed that it was also of the L configuration. The absolute configuration of the H4mpa was determined using chiral-phase GC-MS, both comparing retention time and co-injections with authentic standards, thus confirming its *S* configuration and establishing the absolute configuration of tasiamide E (**3**) as 2*S*, 8*R*, 20*S*, 21*S*, 26*S*, 32*S*, and 38*S*.

Tasiamides C–E (**1**–**3**) are of close structural relation to several families of known metabolites, the grassystatins, tasiamides, and symplocin A. In this regard, **1**–**3** are structurally most similar to tasiamide (**4**), with **3** varying only in the


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**Table 1. NMR Data for Tasiamide C (1) in $CDCl_3$**

| residue | position | $\delta_C$[c], type | $\delta_H$ ($J$ in Hz)[a] | HMBC[b] | COSY[b] |
|---|---|---|---|---|---|
| Pro-Me Ester | 1 | 172.1, C | | | |
| | 2 | 59.5, CH | 4.37, dd (7.6, 7.5) | 1, 3 | 3a, 3b |
| | 3a | 28.7, $CH_2$ | 2.16, m | 1, 2, 4 | 2, 3b, 4a |
| | 3b | | 1.86, m | 1, 2, 4, 5 | 2, 3a, 4b |
| | 4a | 25.2, $CH_2$ | 1.92, m | 5 | 3a, 4b, 5a, 5b |
| | 4b | | 1.75, m | 3, 5 | 3b, 4a, 5a, 5b |
| | 5a | 47.1, $CH_2$ | 3.34, m | 3 | 4a, 4b, 5b |
| | 5b | | 3.15, m | 3, 4 | 4a, 4b, 5a |
| | 6 | 52.3, $CH_3$ | 3.67, s | 1 | |
| *N*-MePhe | 7 | 167.8, C | | | |
| | 8 | 55.9, CH | 5.52, dd (9.8, 6.1) | 7, 9, 16 | 9a, 9b |
| | 9a | 34.7, $CH_2$ | 3.14, m | 7, 8, 10, 11, 15 | 8, 9b |
| | 9b | | 2.87, dd (14.3, 9.7) | 8, 10, 11, 15 | 8, 9a |
| | 10 | 136.4, C | | | |
| | 11/15 | 129.2, CH | 7.10, m | 9, 10, 12, 14 | 12, 14 |
| | 12/14 | 128.0, CH | 7.15, m | 10, 11, 13, 15 | 11, 13, 15 |
| | 13 | 126.4, CH | 7.11, m | 12, 14 | 12, 14 |
| | 16 | 30.5, $CH_3$ | 2.93, s | 8, 17 | |
| Ala | 17 | 172.5, C | | | |
| | 18 | 45.1, CH | 4.67, dq (7.4, 7.3) | 17, 19, 20 | 19 |
| | 19 | 17.4, $CH_3$ | 0.76, d (7.1) | 17, 18 | 18 |
| | NH-1 | | 6.69, d (8.6) | 20 | 18 |
| Ile | 20 | 169.9, C | | | |
| | 21 | 58.0, CH | 4.14, dd (8.2, 7.3) | 20, 22, 23, 25 | 22 |
| | 22 | 36.1, CH | 1.91, m | 21, 23, 24, 25 | 21, 25 |
| | 23a | 24.7, $CH_2$ | 1.30, m | 25 | 23b, 24 |
| | 23b | | 1.02, m | 21, 22, 24 | 23a, 24 |
| | 24 | 11.1, $CH_3$ | 0.78, t (7.4) | 22, 23 | 23a, 23b |
| | 25 | 15.4, $CH_3$ | 0.74, d (6.8) | 21, 22, 23 | 22 |
| | NH-2 | | 6.78, d (7.5) | 26 | 21 |
| *N*-MeGlu | 26 | 169.2, C | | | |
| | 27 | 56.7, CH | 4.93, dd (8.0, 7.0) | 26, 28, 29, 32 | 28a, 28b |
| | 28a | 24.5, $CH_2$ | 2.35, m | 26, 27, 29, 30 | 27, 28b |
| | 28b | | 1.86, m | 26, 27, 29, 30 | 27, 28a, 29a |
| | 29a | 32.7, $CH_2$ | 2.26, m | 27, 28a, 28, 30 | 28b, 29b |
| | 29b | | 2.10, m | 30 | 29a |
| | 30 | 173.7, C | | | |
| | $NH_2$a | | 6.62, bs | | |
| | $NH_2$b | | 6.46, bs | | |
| | 31 | 31.2, $CH_3$ | 3.03, s | 27, 32 | |
| Hmba-1 | 32 | 170.8, C | | | |
| | 33 | 76.5, CH | 4.85, d (7.9) | 32, 34, 35, 36, 37 | 34 |
| | 34 | 30.0, CH | 2.15, m | 33, 35, 36 | 33, 35, 36 |
| | 35 | 18.3, $CH_3$ | 0.99, d (6.7) | 33, 34, 36 | 34 |
| | 36 | 18.2, $CH_3$ | 0.93, d (7.0) | 33, 34, 35 | 34 |
| Hmba-2 | 37 | 175.7, C | | | |
| | 38 | 74.6, CH | 4.09, d (3.7) | 37, 39 | 39 |
| | 39 | 31.7, CH | 2.07, m | 38, 40, 41 | 38, 40, 41 |
| | 40 | 18.9, $CH_3$ | 0.97, d (6.9) | 39, 41 | 39 |
| | 41 | 16.2, $CH_3$ | 0.83, d (6.9) | 39, 40 | 39 |

[a]600 MHz for $^1$H NMR. [b]500 MHz for HMBC and COSY. [c]125 MHz for $^{13}$C NMR.

terminal residue, while compounds **1** and **2** possess other relatively simple modifications. In 2008, Li and co-workers revised the original configuration of the *N*-MeGln residue in **4** from L to D based on the comparison of analytical data ($^{13}$C NMR and specific rotation) of the natural product with four stereoisomers obtained from a total synthesis (containing both L and D stereoisomers of *N*-MeGln and Leu; Table 2).[19] However, the original assignments for all of the residues in **4** are consistent with that of **3**, and a comparison of $^{13}$C shifts revealed no significant differences between **3** and **4**, along with the four additional synthetic stereoisomers (**A**–**D**, Table 2) (Supporting Information).[20] Furthermore, the opposite specific rotation signs for tasiamides C and D suggest a structural variance between these two metabolites (**3**, $[\alpha]^{25}_D$ −22.2; **4**, $[\alpha]^{21}_D$ +15.0). Therefore, a more detailed analysis of the $^{13}$C NMR data for tasiamide and the four synthetic analogues was conducted using DP4 probability calculations and revealed that the carbon data alone are insufficient to deduce the correct configuration in tasiamide (**4**).[21] The probability of the original experimental data matching each analogue was calculated as 1.3% for analogue **A** (**4**), 56.0% for analogue **B**, 3.0% for analogue **C**, and 39.7% for analogue **D**. Because analogues **B** and **D** have the same sign and magnitude of specific optical rotation and are indistinguishable by DP4 calculations, it is not possible to unequivocally assign the configuration of **4** based solely on these data. This also suggests that the misassignment in tasiamide (**4**) may involve residues other than just the *N*-MeGln, and thus, a broader investigation into the configuration of **4** will be necessary to clarify its correct absolute configuration.

It is interesting to note that there appears to be considerable biosynthetic flexibility in this family of metabolites. Insights are thus provided by comparing the amino or hydroxy acid residues in each member of this family (Table 2). The first three residues (A, B, and C) starting from the carboxy terminus of these molecules are rather well conserved, with each analogue containing a Pro-Me ester followed by the incorporation of D-Phe, which is *N*-methylated in all but one analogue, and then the incorporation of an Ala, Aba (2-aminobutyric acid), or Gly as the third residue. Residues E, H, and J are also conserved residues (statine, Hmba, and an *N*,*N*-dimethylated amino acid, respectively), but they occur only in a subset of the natural products. Residues F and I are slightly less conserved, with three different polar amino acids (*N*-MeGln, Asn, and Ser) incorporated into residue F and four different hydroxy acids (Hmpa, H4mpa, Hpa, and H3mpa)[18] in residue I. Also observed are variations in the absolute configurations of these residues. For example, residues D and J in eight of nine metabolites incorporate an L residue, while a single metabolite has a D-*allo* residue at this position. At residue F all of the


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**Table 2. Residue Sequence Comparisons of the Tasiamide/Grassystatin/Symplocin Superfamily of Metabolites**

| compound | A | B | C | D | E | F | G | H[d] | I[d] | J | $[\alpha]_D$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| grassystatin A (**5**)[16] | L-*O*Me-Pro | D-*N*Me-Phe | L-Ala | L-Thr | (*S*,*S*)-Sta | L-Asn | L-Leu | D-Hmba | L-Hmba | L-*N*,*N*-diMe-Val | −4.4 (*c* 0.08, MeOH) |
| grassystatin B (**6**)[16] | L-*O*Me-Pro | D-*N*Me-Phe | L-Aba | L-Thr | (*S*,*S*)-Sta | L-Asn | L-Leu | D-Hmba | L-Hmba | L-*N*,*N*-diMe-Val | −5.0 (*c* 0.1, MeOH) |
| grassystatin C[16] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | (*S*,*S*)-Sta | L-*N*Me-Gln | L-Leu | | D-*allo*-H3mpa | | −21.9 (*c* 0.04, MeOH) |
| symplocin A[23] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Val | (*R*,*S*)-Sta | L-Ser | L-Tyr | D-Hmba | | D-*N*,*N*-diMe-Ile | +16 (*c* 2.18, MeOH) |
| tasiamide (**4**)[20 a] | *O*Me-Pro | *N*Me-Phe | Gly | Ile | | *N*Me-Gln | Leu | | Hmba | | +15 (*c* 0.4, $CHCl_3$) |
| tasiamide B[24 a] | *O*Me-Pro | *N*Me-Phe | Ala | Leu | Ahppa | *N*Me-Gln | Val | | Hpi | | −28 (*c* 0.4, $CHCl_3$) |
| tasiamide C (**1**) | L-*O*Me-Pro | D-*N*Me-Phe | L-Ala | D-*allo*-Ile | | L-*N*Me-Gln | | D-Hmba | L-Hmba | | −37 (*c* 1.17, $CHCl_3$) |
| tasiamide D (**2**) | L-*O*Me-Pro | D-Phe | L-Ala | L-Ile | | L-*N*Me-Gln | | D-Hmba | L-Hmba | | −85 (*c* 1.67, $CHCl_3$) |
| tasiamide E (**3**) | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | | L-*N*Me-Gln | L-Leu | | L-H4mpa | | −22 (*c* 0.53, $CHCl_3$) |
| tasiamide synthetic analogue A (**4**)[19 b] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | | L-*N*Me-Gln | L-Leu | | L-Hmba | | −12.6 (*c* 0.4, $CHCl_3$) |
| tasiamide synthetic analogue B[19 c] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | | D-*N*Me-Gln | L-Leu | | L-Hmba | | +15 (*c* 0.4, $CHCl_3$) |
| tasiamide synthetic analogue C[19] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | | L-*N*Me-Gln | D-Leu | | L-Hmba | | −15.6 (*c* 0.4, $CHCl_3$) |
| tasiamide synthetic analogue D[19] | L-*O*Me-Pro | D-*N*Me-Phe | Gly | L-Ile | | D-*N*Me-Gln | D-Leu | | L-Hmba | | +18.5 (*c* 0.4, $CHCl_3$) |

[a]Stereochemical assignments for tasiamide and tasiamide B intentionally omitted due to unresolved stereochemical questions (see discussion). [b]Matches original configuration proposed by Williams et al.[20] [c]Proposed revised structure of tasiamide according to Ma et al.[19] [d]Abbreviations developed based on IUPAC nomenclature of each residue.[18]

amino acids were originally deduced as L; however, in tasiamide and tasiamide B these residues were reassigned as D based on total synthesis. Although non-ribosomal peptide synthetase (NRPS)-derived peptides can show variations in the incorporated amino acid, it is difficult to imagine a single biosynthetic pathway that would be capable of producing all of these metabolites, especially those of varying absolute configurations, which usually requires an epimerase.[22]

Both the grassystatins and symplocin A were reported to possess exceptional inhibitory activity toward cathepsin E (grassystatin A: $IC_{50}$ = 886 pM, symplocin A: $IC_{50}$ = 300 pM);[16,23] however, this activity is likely due to the presence of a statine residue, which is absent in the tasiamides. Additionally, tasiamide and tasiamide B both showed moderate cytotoxicity against KB cells, with $IC_{50}$ values of 0.48 and 0.8 $\mu$M, respectively.[19,24] Due to limited isolated quantities of tasiamide E (**3**), only tasiamides C (**1**) and D (**2**) were evaluated for their cytotoxicity against the HCT-116 colon cancer cell line and were found to be inactive (tasiamide C, $IC_{50}$ > 25 $\mu$M; tasiamide D, $IC_{50} \approx 25$ $\mu$M).

## CONCLUSION

The new metabolites tasiamides C−E (**1**−**3**) were isolated from a Kimbe Bay, Papua New Guinea, collection of the tropical marine cyanobacterium *Symploca* sp. These metabolites add to the growing family of structurally homologous natural products [tasiamide (**4**), grassystatins (**5**, **6**), and symplocin A] that have all been isolated from similar tuft-forming cyanobacteria. This particular *Symploca* sp. is a prolific producer of secondary metabolites, as several structurally diverse natural products were isolated from this collection including kimbeamides A−C, kimbelactone, tasihalide C, and the three tasiamides reported herein.[15]

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-2000 polarimeter, UV spectra on a Beckman Coulter DU-800 spectrophotometer, and IR spectra on a Nicolet IR-100 FT-IR spectrophotometer using KBr plates. NMR spectra were recorded with chloroform as an internal standard ($\delta_C$ 77.0, $\delta_H$ 7.26 for $CHCl_3$) on a Varian Unity 500 MHz spectrometer (500 and 125 MHz for $^1H$ and $^{13}C$ NMR, respectively), a Varian Unity 300 MHz spectrometer (300 and 75 MHz for $^1H$ and $^{13}C$ NMR, respectively), a Varian VNMRS (Varian NMR System) 500 MHz spectrometer equipped with a cold probe (500 and 125 MHz for $^1H$ and $^{13}C$ NMR), and a Bruker 600 MHz spectrometer equipped with a 1.7 mm MicroCyroProbe (600 and 150 MHz for $^1H$ and $^{13}C$ NMR). LR- and HR-ESIMS data were obtained on ThermoFinnigan LCQ Advantage Max and Thermo Scientific LTQ Orbitrap-XL mass spectrometers, respectively. Tandem mass spectroscopy experiments were run with a Biversa Nanomate (Advion Biosystems) electrospray source for a Finnigan LTQ-FTICR-MS instrument (Thermo-Electron Corporation) running Tune Plus software version 1.0. HPLC was carried out using a Waters 515 pump system with a Waters 996 PDA detector. GC-MS was conducted with a Thermo Electron Corp. DSQ/TRACE-GC-Ultra GCMS system. All solvents were either distilled or of HPLC quality. Acid hydrolysis was performed using a Biotage (Initiator) microwave reactor equipped with high-pressure vessels.

**Cyanobacterial Collection.** The tasiamides C−E-producing cyanobacterium (collection code: PNG 07/14/07-6) was collected by hand on reefs 20 m deep from approximately eight nearby sites located in Kimbe Bay off the north coast of New Britain, Papua New Guinea (S 5°26.292′, E 150°40.813′). Field notes identified the puffballs as a consortium of *Schizothrix* sp. with a minor amount of *Lyngbya* sp. present. The collected samples were stored in 1:1 EtOH/seawater in the field, and the supernatant was decanted and discarded before shipment. As was previously reported, a portion of the cyanobacterial biomass was used for 16*S* rRNA sequencing, which revealed it cladded with "tropical marine *Symploca*" (acc. no. JQ388599).[15]


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**Extraction and Isolation.** The cyanobacterial biomass (101.7 g dry wt) was extracted with 2:1 $CH_2Cl_2$/MeOH to afford 1.8 g of dried extract. A portion of the extract was fractionated by silica gel VLC using a stepwise gradient solvent system of increasing polarity starting from 100% hexanes to 100% MeOH (nine fractions, A–I). The fraction eluting with 25% MeOH/75% EtOAc (fraction H) was separated further using RP HPLC [4 $\mu$ Synergi Hydro, isocratic 65% MeCN/35% $H_2O$] to yield pure tasiamide C (**1**, 1.9 mg), tasiamide D (**2**, 2.5 mg), and tasiamide E (**3**, 0.7 mg).

*Tasiamide C (**1**):* white, amorphous solid; $[\alpha]^{25}_D$ −37 (*c* 1.2, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 211.0 (3.84); IR (neat) $\nu_{max}$ 3371, 2965, 2933, 2877, 1737, 1644, 1521, 1453, 1263, 1201, 1178, 1098, 1031 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) and $^{13}C$ NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m*/*z* 839.4541 $[M + Na]^+$ (calcd for $C_{41}H_{64}N_6O_{11}Na$, 839.4525).

*Tasiamide D (**2**):* white, amorphous solid; $[\alpha]^{25}_D$ −85 (*c* 1.6, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 220.0 (3.86) nm; IR (neat) $\nu_{max}$ 3318, 2965, 2931, 1739, 1648, 1526, 1453, 1203, 1033 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.17 (2H, m), 7.13 (1H, m), 7.09 (2H, m), 6.98 (1H, d, *J* = 6.8), 6.89 (1H, d, *J* = 7.7), 6.77 (1H, d, *J* = 6.7), 5.98 (1H, bs), 5.62 (1H, bs), 5.08 (1H, dd, *J* = 7.6, 6.7), 4.85 (1H, d, *J* = 7.5), 4.76 (1H, q, *J* = 6.6), 4.43 (1H, dq, *J* = 8.0, 6.8), 4.13 (1H, m), 4.12 (1H, m), 4.11 (1H, m), 3.58 (3H, s), 3.47 (1H, dd, *J* = 8.7, 5.1), 3.02 (3H, s), 2.94 (2H, m), 2.68 (1H, m), 2.48 (1H, m), 2.32 (1H, m), 2.25 (1H, m), 2.14 (1H, m), 2.07 (1H, m), 1.97 (1H, m), 1.81 (1H, m), 1.78 (2H, m), 1.35 (2H, m), 1.26 (3H, d, *J* = 7.2), 1.07 (2H, m), 0.99 (3H, d, *J* = 6.1), 0.97 (3H, d, *J* = 6.2), 0.93 (3H, d, *J* = 7.0), 0.82 (3H, t, *J* = 6.8), 0.82 (6H, d, *J* = 6.3); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 175.8, 174.6, 172.2, 171.8, 170.7, 170.4, 136.2, 129.1, 128.5, 127.0, 76.7, 75.1, 59.4, 59.1, 57.1, 52.5, 52.2, 48.8, 46.9, 39.0, 35.9, 33.0, 32.1, 31.1, 29.9, 29.0, 24.7, 24.6, 24.0, 18.7, 18.4, 18.1, 16.1, 15.7, and 11.5; HRESIMS *m*/*z* 825.4371 $[M + Na]^+$ (calcd for $C_{40}H_{62}N_6O_{11}Na$, 825.4369).

*Tasiamide E (**3**):* white, amorphous solid; $[\alpha]^{25}_D$ −22 (*c* 0.5, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 207.0 (4.10) nm; IR (neat) $\nu_{max}$ 3329, 2926, 2958, 1743, 1645, 1521, 1454, 1281, 1177 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) $\delta$ 7.26 (2H, m), 7.22 (2H, m), 7.20 (1H, m), 7.01 (1H, d, *J* = 9.3), 6.82 (1H, d, *J* = 9.4), 6.78 (1H, d, *J* = 4.4), 5.61 (1H, bs), 5.55 (1H, t, *J* = 7.4), 5.25 (1H, bs), 5.06 (1H, t, *J* = 7.4), 4.95 (1H, q, *J* = 7.9), 4.39 (1H, dd, *J* = 8.1, 5.2), 4.29 (1H, dd, *J* = 8.8, 6.3), 4.09 (1H, dd, *J* = 9.1, 4.5), 4.05 (1H, dd, *J* = 17.7, 3.3), 3.81 (1H, dd, *J* = 17.7, 3.3), 3.72 (3H, s), 3.36 (1H, m), 3.31 (1H, m), 3.27 (1H, dd, *J* = 13.6, 8.2), 2.99 (3H, s), 2.98 (3H, s), 2.82 (1H, dd, *J* = 13.6, 6.9), 2.31 (1H, m), 2.24 (1H, m), 2.18 (1H, m), 2.12 (1H, m), 1.99 (1H, m), 1.93 (1H, m), 1.87 (2H, m), 1.86 (1H, m), 1.82 (1H, m), 1.80 (1H, m), 1.60 (2H, m), 1.54 (2H, m), 1.43 (1H, m), 1.11 (1H, m), 0.96 (6H, d, *J* = 6.6), 0.95 (3H, d, *J* = 6.4), 0.92 (3H, d, *J* = 6.4), 0.88 (3H, d, *J* = 7.2), 0.87 (3H, t, *J* = 7.7); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 175.2, 174.4, 174.0, 172.5, 171.5, 169.7, 167.8, 136.8, 129.4, 128.4, 126.8, 71.1, 58.9, 57.6, 56.9, 56.2, 52.3, 46.8, 43.1, 41.2, 37.2, 35.1, 31.9, 31.0, 29.7, 28.8, 25.0, 24.8, 24.7, 24.6, 23.4, 23.0, 22.7, 22.3, 21.4, 15.6, 11.3; HRESIMS *m*/*z* 852.4842 $[M + Na]^+$ (calcd for $C_{42}H_{67}N_7O_{10}Na$, 852.4842).

**Acid Hydrolysis and Marfey's Analysis of Tasiamides C–E (1–3).** Tasiamides C–E (**1**–**3**, 0.2 mg) were treated separately with 400 $\mu$L of 6 N HCl in a microwave reactor at 160 °C for 5 min. An aliquot of the reaction product was dissolved in 500 $\mu$L of a 1 mg/mL solution of D-FDAA (1-fluoro-2,4-dinitrophenyl-5-D-alanine amide) in acetone followed by the addition of 20 $\mu$L of 1 N $NaHCO_3$. The solution was maintained at 40 °C for 1 h, at which time the reaction was quenched by the addition of 40 $\mu$L of 1 N HCl. The reaction mixture was then dried down under $N_2$(g) and resuspended in 200 $\mu$L of 50% $H_2O$/50% MeCN, and 10 $\mu$L of the solution was analyzed by LC-ESIMS.

The Marfey's derivatives of the hydrolysate and standards reacted with D-FDAA (Ala, Phe, *N*-MePhe, Pro, and Leu) were analyzed by RP HPLC using a Phenomenex Luna 5 $\mu$m $C_{18}$ column (4.6 × 250 mm). The HPLC conditions began with 10% MeCN/90% $H_2O$ acidified with 0.1% formic acid (FA) (aqueous) followed by a gradient profile to 50% MeCN/50% $H_2O$ acidified with 0.1% FA(aq) over 85 min at a flow of 0.4 mL/min, monitoring from 200 to 600 nm. Because authentic D-*N*MeGlu was not available, L-*N*MeGlu standard was derivatized with both the L-FDAA and D-FDAA. The *N*MeGlu residue was analyzed by RP HPLC using a Kinetex 5 $\mu$ C18 100A column (4.6 × 100 Å mm). The HPLC condition began with 5% MeOH/95% $H_2O$ acidified with 0.1% FA(aq) followed by a gradient profile to 45% MeOH/55% $H_2O$ acidified with 0.1% FA(aq) over 125 min at a flow of 0.4 mL/min, monitoring from 200 to 600 nm. The retention times of the derivatives of the authentic amino acids when analyzing for **1** were D-Ala (64.8 min), L-Ala (71.1 min), D-Pro (66.6 min), L-Pro (69.4 min), D-*N*MePhe (78.4 min), L-*N*MePhe (79.7 min), L-*N*MeGlu reacted with L-FDAA (93.3 min), and L-*N*MeGlu reacted with D-FDAA (91.9 min); the derivatives of the hydrolysate product of **1** gave peaks with retention times of 71.5, 69.8, 78.3, and 91.8 min, according to L-Ala, L-Pro, D-*N*MePhe, and L-*N*MeGlu, respectively. The retention times of the derivatives of the authentic amino acids when analyzing for **2** were D-Ala (59.3 min), L-Ala (63.6 min), D-Pro (60.4 min), L-Pro (62.6 min), D-Phe (78.7 min), L-Phe (83.8 min), L-*N*MeGlu reacted with L-FDAA (93.3 min), and L-*N*MeGlu reacted with D-FDAA (91.9 min); the derivatives of the hydrolysate product of **2** gave peaks with retention times of 64.0, 62.6, 83.8, and 91.7 min, according to L-Ala, L-Pro, D-Phe, and L-Glu. The retention times of the derivatives of the authentic amino acids when analyzing for **3** were D-Leu (68.4 min), L-Leu (85.8 min), D-Pro (60.4 min), L-Pro (62.6 min), D-*N*MePhe (78.6 min), L-*N*MePhe (79.2 min), L-*N*MeGlu reacted with L-FDAA (107.6 min), and L-*N*MeGlu reacted with D-FDAA (103.9 min); the derivatives of the hydrolysate product of **3** gave peaks with retention times of 85.7, 62.2, 78.2, and 104.5 min, according to L-Leu, L-Pro, D-*N*MePhe, and L-*N*MeGlu.

**Preparation and GC-MS Analysis of Isoleucine (Ile) in Tasiamides C–E (1–3).** An aliquot (~0.1 mg) of the above hydrolysate product was dissolved in 100 $\mu$L of $H_2O$ and then treated with 0.26 mg (0.43 $\mu$mol) of $NaHCO_3$ followed by 0.94 mg (0.43 $\mu$mol) of di-*tert*-butyl dicarbonate. After 16 h at room temperature (rt), the reaction mixture was quenched by 500 $\mu$L of 5% $KHSO_4$ and back extracted with 3 × 1 mL of EtOAc, and the combined organic layers were dried over $MgSO_4$. The reaction product was then treated with freshly prepared diazomethane in diethyl ether. Each of the four Ile standards were prepared in a similar fashion.

The derivatized hydrolysate product and standards were analyzed by chiral-phase GC-MS using a Chirasil-Val (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 40 °C, kept for 2 min, followed by a ramp from 40 to 75 °C at a rate of 10 °C/min, kept for 5 min, followed by another ramp to 110 °C, at a rate of 0.5 °C/min, followed by a final ramp to 200 °C, at a rate of 25 °C/min, kept for 2 min. The retention times for the four authentic standards when analyzing **1** were D-*allo*-Ile (49.6 min), L-*allo*-Ile (49.9), D-Ile (51.5 min), and L-Ile (51.7 min); the derivatized hydrolysis product of **1** yielded a peak at 49.6 min, according to D-*allo*-Ile. The retention times for the four authentic standards when analyzing **2** and **3** were D-*allo*-Ile (47.7 min), L-*allo*-Ile (48.2), D-Ile (50.1 min), and L-Ile (50.5 min); the derivatized hydrolysis products of **2** and **3** each yielded a peak at 50.6 min, according to L-Ile.

**Base Hydrolysis to Determine Configuration of Hmba Units in Tasiamides C and D (1 and 2).** Tasiamides C (**1**) and D (**2**) (0.15 mg) were treated separately with 150 $\mu$L of 1:1 0.5 N NaOH(aq)/MeOH (1:1) solution at room temperature for 72 h.[16] The reaction mixture was neutralized by the addition of 40 $\mu$L of 1 N HCl(aq) and back extracted with 3 × 1 mL of EtOAc, and the combined organic layers were dried over $MgSO_4$. The product was then treated with freshly prepared diazomethane in diethyl ether for 5 min at rt. The derivatized acid hydrolysate was analyzed by chiral-phase GC-MS using a Cyclosil B column (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temp was 35 °C, kept for 15 min, followed by a ramp to 60 °C, at a rate of 1.5 °C, followed by a ramp to 170 °C, at a rate of 5 °C/min, kept for 5 min. The retention times for the authentic standards were *R*-Hmba (34.9 min) and *S*-Hmba (35.8 min); the derivatized hydrolysis product for **1** and **2** each exhibited a single peak at 35.8 and 35.9 min, respectively, according to *S*-Hmba.


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**GC-MS Analysis of H4mpa in Tasiamide E (3).** The derivatized hydrolysate product used in the Ile analysis from **3** was also used in the H4mpa analysis. Authentic standards were synthesized following a previously published method.[25] The derivatized hydrolysis product and authentic standards were analyzed by chiral-phase GC-MS using a Cyclosil B column under the following conditions: the initial oven temp was 40 °C, kept for 15 min, followed by a ramp to 90 °C, at a rate of 1.5 °C/min, followed by a ramp to 200 °C, at a rate of 10 °C/min, kept for 5 min. The retention times for the authentic standards were *R*-H4mpa (43.2 min) and *S*-H4mpa (43.8 min); the derivatized hydrolysis product yielded a peak at 43.7 min, consistent with *S*-H4mpa.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1$H NMR, $^{13}$C NMR, COSY, ROESY, TOCSY, HSQC, HMBC, and H2BC (only for **3**) spectra in $CDCl_3$ for tasiamides C−E (**1**−**3**) and complete NMR data tables for tasiamides D and E (**2** and **3**). LC-MS and GC-MS chromatograms for chiral-phase analyses, MS fragmentation data for tasiamides C−E (**1**−**3**), and descriptions of both the H-460 and HCT-116 cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Tel: 858-534-0578. Fax: 858-534-0576. E-mail: wgerwick@ucsd.edu.

### Notes

The authors declare no competing financial interest.

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