Cyclodepsipeptides, Sesquiterpenoids, and Other Cytotoxic Metabolites from the Filamentous Fungus *Trichothecium* sp. (MSX 51320)

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S *Supporting Information*

ABSTRACT: Two new cyclodepsipeptides (**1** and **2**), two new sesquiterpenoids (**3** and **4**), and the known compounds guangomide A (**5**), roseotoxin S, and three simple trichothecenes were isolated from the cytotoxic organic extract of a terrestrial filamentous fungus, *Trichothecium* sp. The structures were determined using NMR spectroscopy and mass spectrometry. Absolute configurations of the cyclodepsipeptides were established by employing chiral HPLC, while the relative configurations of **3** and **4** were determined via NOESY data. The isolation of guangomide A was of particular interest, since it was reported previously from a marine-derived fungus.
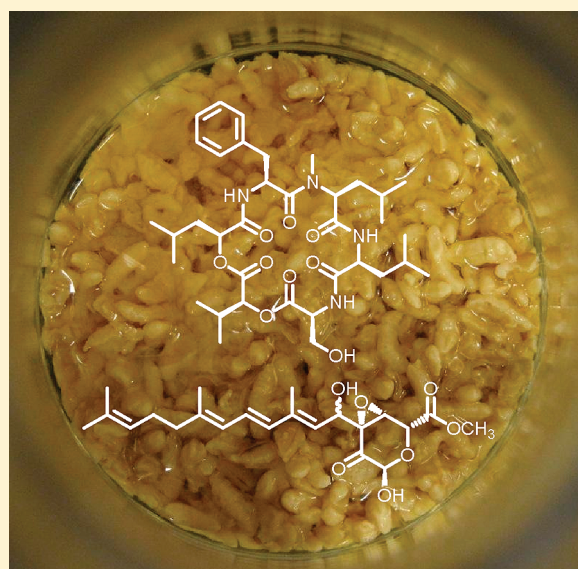
Through our ongoing investigation of diverse organisms for anticancer drug leads,[1,2] an extract of the fungus *Trichothecium* sp., from the Mycosynthetix library of filamentous fungi, displayed promising cytotoxicity, with less than 20% survival of human tumor cells when treated with 20 μg/mL of crude extract. Subsequent bioactivity-directed fractionation studies led to the isolation and characterization of nine secondary metabolites, four of which were new. The isolates could be subclassified as four depsipeptides (compounds **1** and **2** being new) and five sesquiterpenoids (compounds **3** and **4** being new). Furthermore, one of the known cyclodepsipeptides was guangomide A (**5**), which was described previously from an unidentified marine-derived, rather than in this case a terrestrial, fungus.[3] Species of the genus *Trichothecium* have been a rich source of secondary metabolites.[4]

As just one example, at least 29 secondary metabolites have been described from *T. roseum*,[4−12] ranging from depsipeptides such as the destruxins, roseotoxins, and roseocardin, to terpenoids such as eremophilanes, rosenoic acids, and trichothecenes, indicative of the vast biosynthetic potential of this, and perhaps related, fungi.

Compound **1** was assigned the molecular formula $C_{36}H_{56}N_4O_9$ via HRESIMS data (m/z 711.3923 for $[M + Na]^+$), establishing an index of hydrogen deficiency (IHD) of 11. The ^{1}H and ^{13}C NMR spectra suggested that **1** was a depsipeptide-type compound on the basis of chemical shifts and multiplicities typical for α protons and carbons (Table 1). In general, cyclodepsipeptides

Chart 1
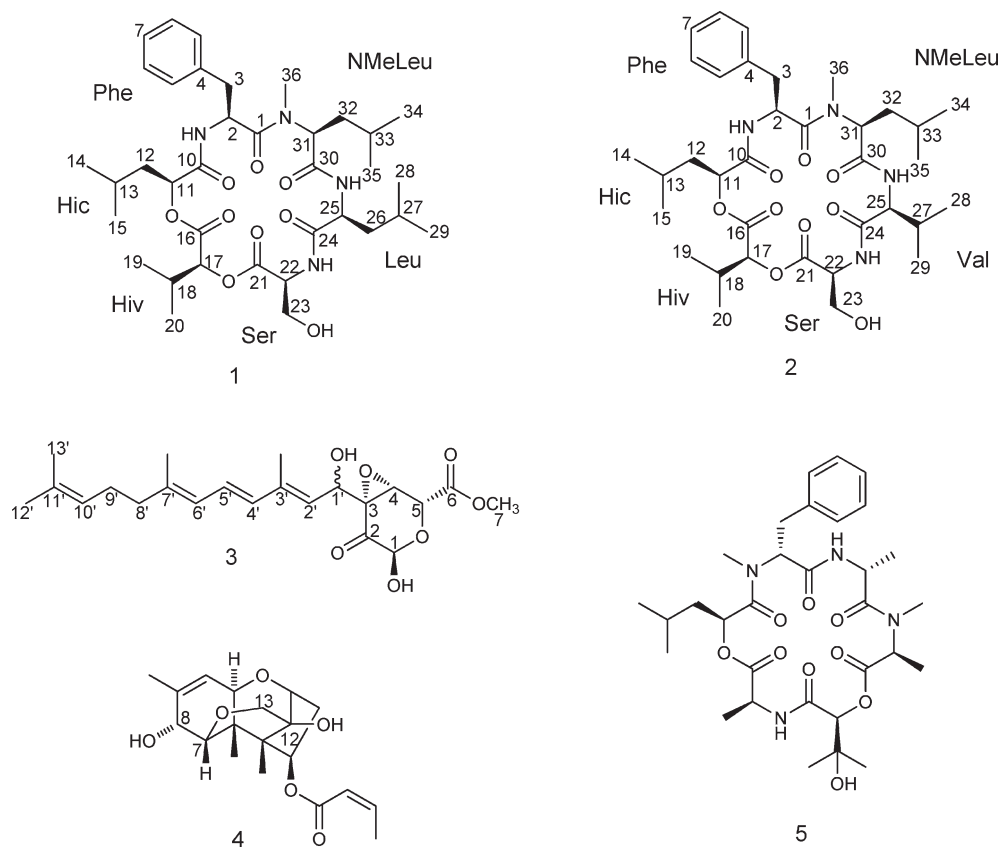
are secondary metabolites that contain amino acids and at least one hydroxy acid linked through ester and amide bonds to form a cyclic structure. ^{1}H NMR data (Table 1) revealed the presence of five aromatic protons (δ_H 7.19−7.26 for H-5 to H-9), suggesting a phenylalanine (Phe) residue. Six α protons were also evident (δ_H 5.45, 5.29, 4.73, 4.65, 4.52, and 3.45 for H-11, H-2, H-22, H-17, H-25, and H-31, respectively). Moreover, three NH protons (δ_H 7.93, 7.46, and 5.92) and an N-methyl group (δ_H 3.06) were observed, as were signals evident of an O−CH$_2$ moiety (δ_H 4.21 and 4.05, H$_2$-23). Most other resonances were found in the upfield region of the ^{1}H NMR spectrum, including those for both methylene and methine protons (δ_H 2.98, 2.84, 1.95, 1.34, and a multiplet from 1.71 to 1.53) and eight doublet methyl resonances (δ_H 0.91, 0.90, 1.02, 1.01, 0.87, 0.93, 0.81, and 0.78, for H$_3$-14, H$_3$-15, H$_3$-19, H$_3$-20, H$_3$-28, H$_3$-29, H$_3$-34, and H$_3$-35, respectively). HSQC data further confirmed the presence of five CH$_2$ resonances (H$_2$-3, H$_2$-12, H$_2$-26, H$_2$-32), including an oxymethylene (H$_2$-23), and eight methyl groups. Data from the ^{13}C and DEPT-135 NMR spectra indicated 36 carbon resonances, consistent with the molecular formula, including six ester and/or amide carbonyl carbons (δ_C 172.4, 171.9, 170.5, 170.2, 169.7, and 167.8, for C-1, C-24, C-30, C-21, C-10, and C-16, respectively), six aromatic carbons (δ_C 135.8, 129.4 × 2, 128.5 × 2, and 127.1, for positions C-4 to C-9), three oxygenated carbons (δ_C 79.0, 73.5, and 63.4, for C-17, C-11, and C-23, respectively), and four nitrogen-bearing carbons (δ_C 65.1, 54.9, 51.7, and 50.1, for C-31, C-22, C-25, and C-2, respectively). COSY data (Figure 1) were key in the establishment of spin systems, leading to the identification of the hydroxy and amino

acid residues. Some of the prominent spin systems include a Phe moiety (from NH at δ 7.46 to H-2 and H-3 and from H-5 to H-9), a hydroxyisocaproic acid (Hic) moiety (from H-11 to H$_3$-15), a hydroxyisovaleric acid (Hiv) moiety (from H-17 to H$_3$-20), a serine (Ser) moiety (from NH at δ 7.93 to H-22 and H-23), a leucine (Leu) moiety (from NH at δ 5.92 to H-25 through H$_3$-29), and a second leucine (Leu) moiety (from H-31 to H-35). The lack of COSY correlations from any NH signal to the α proton of the latter Leu system suggested an NMe group, which was supported by the upfield chemical shift of this proton (δ_H 3.45; H-31);[3] indeed, an HMBC correlation from an NMe group (H$_3$-36) to C-31 confirmed this assignment. HMBC correlations from H$_2$-3 to the aromatic carbons C-4, C-5, and C-9 revealed the presence of Phe, while correlations from each α proton and NH proton to each carbonyl carbon helped establish the connectivities in the core of the structure (Figure 1). HMBC correlations from H-2 to C-1, C-3, and C-10, from the NH proton at δ 7.46 to C-10 and C-2, and from H-11 to C-10 suggested that Hic acylates Phe; correlations from H-11, H-17, and H-18 to C-16 suggested that Hiv acylates Hic; correlations from H-17, H-22, and H-23 to C-21 suggested that Ser acylates Hiv; correlations from the NH proton at δ 7.93 and H-25 to C-24 suggested that Leu acylates Ser; correlations from the NH proton at δ 5.92 and H-31 to C-30 suggested that NMeLeu acylates Leu; and correlations from H$_2$-3 and H$_3$-36 to C-1 suggested that Phe acylates the NMeLeu. These connectivities confirmed the cyclic hexadepsipeptide nature of compound **1**, thereby satisfying the IHD.

Compound **2** was assigned the molecular formula C$_{35}$H$_{54}$N$_4$O$_9$ on the basis of HRESIMS data (m/z 697.3770 for [M +

Table 1. NMR Data for Compounds 1 and 2[a]

	compound 1		compound 2	
position	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)	δ_C
1		172.4		172.8
2	5.29, ddd (5, 9, 10)	50.1	5.27, ddd (5, 9, 10)	50.4
3	2.84, dd (10, 13)	39.5	2.83, dd (10, 13)	39.7
	2.98, dd (5, 13)		3.05, d (5)	
4		135.8		135.8
5	7.19–7.26, m	129.4	7.22–7.28, m	129.5
6	7.19–7.26, m	128.5	7.22–7.28, m	128.7
7	7.19–7.26, m	127.1	7.22–7.28, m	127.2
8	7.19–7.26, m	128.5	7.22–7.28, m	128.7
9	7.19–7.26, m	129.4	7.22–7.28, m	129.5
NH	7.46, d (9)		7.42, d (9)	
10		169.7		169.9
11	5.45, dd (5, 8)	73.5	5.45, dd (5, 9)	73.5
12	1.53–1.71, m	42.0	1.51–1.71, m	42.0
13	1.53–1.71, m	24.4	1.51–1.71, m	24.5
14	0.91, d (7)	23.0	0.93, d (7)	23.2
15	0.90, d (6)	22.0	0.92, d (6)	22.1
16		167.8		168.0
17	4.65, d (5)	79.0	4.66, d (5)	79.1
18	2.26, m	30.4	2.26, m	30.5
19	1.02, d (6)	17.5	1.03, d (7)	17.6
20	1.01, d (7)	18.8	1.03, d (7)	19.0
21		170.2		170.2
22	4.73, dt (8, 2)	54.9	4.71, d (10)	54.6
23	4.05, dt (12, 2)	63.4	4.03, br d (12)	64.1
	4.21, dt (2, 12)		4.26, dt (2, 12)	
NH	7.93, d (8)		7.86, d (9)	
OH	4.87, dd (12, 2)		4.84, dt (9, 2)	
24		171.9		171.3
25	4.52, ddd (4, 9, 10)	51.7	4.41, dd (4, 9)	58.5
26[b]	1.95, ddd (4, 9, 14)	39.1		
	1.53–1.71, m			
27	1.53–1.71, m	25.0	2.60, m	28.0
28	0.87, d (7)	21.3	0.86, d (7)	20.2
29	0.93, d (7)	23.3	0.97, d (7)	17.2
NH	5.92, d (9)		6.16, d (9)	
30		170.5		170.3
31	3.45, dd (5, 10)	65.1	3.47, dd (7, 9)	65.7
32	1.34, ddd (5, 10, 14)	36.8	1.33, ddd (5, 9, 14)	37.0
			1.51–1.60, m	
	1.53–1.71, m			
33	1.53–1.71, m	24.5	1.51–1.60, m	24.7
34	0.81, d (7)	21.6	0.86, d (7)	22.0
35	0.78, d (6)	23.6	0.81, d (6)	23.4
36	3.06, s	40.9	3.03, s	41.0

[a] 500 MHz for ^{1}H, 125 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, CDCl$_3$. [b] Position 26 is present only in compound 1.

Figure 1. Selected HMBC ($\rightarrow$) and COSY (—) correlations for 1.

were 14 amu less than 1. Further analyses of the 2D NMR data revealed that 2 contained the amino acid valine (Val), instead of the Leu unit present in 1, which acylates Ser. This was apparent from the signals for the isopropyl unit (H-27 to H-29) connected to the α carbon at C-25, as observed via HMBC and COSY correlations.

The absolute configurations of the α carbons of compounds 1 and 2 were determined via chiral HPLC analyses performed on the hydrolysis products.[13–15] For the organic extracts of the acid hydrolysates, comparison of retention times with those of standard hydroxy acids revealed the Hiv and Hic units of 1 and 2 to both be of the L-configuration. All four amino acids were determined to be of the L-configuration in both 1 and 2 by a similar analysis of the aqueous extracts of the acid hydrolysates. The closest known compound to these new cyclodepsipeptides was hirsutatin A, which was isolated from the pathogenic fungus *Hirsutella nivea*.[13] However, hirsutatin A differs due to a L-threonine unit instead of L-Leu (1) or L-Val (2) units. The new isolates were ascribed the trivial names trichodepsipeptide A (1) and trichodepsipeptide B (2) due to their isolation from *Trichothecium* sp.

Compound 3 was ascribed a molecular formula of $C_{22}H_{32}O_8$ on the basis of HRESIMS data (m/z 447.1981 for [M + Na]$^+$), indicating an IHD of 7. The ^{1}H NMR data (see Experimental Section) showed the presence of five olefinic protons (δ_H 6.47, 6.11, 5.85, 5.28, and 5.06 for H-5′, H-4′, H-6′, H-2′, and H-10′, respectively), four oxygenated methine protons (δ_H 5.23, 5.19, 4.63, and 3.82 for H-1, H-1′, H-5, and H-4, respectively), one methoxy group (δ_H 3.74, H-7), four methylene protons (δ_H 2.08 for H$_2$-8′ and H$_2$-9′), and four vinyl methyl groups (δ_H 1.92, 1.77, 1.66, and 1.58 for CH$_3$-3′, CH$_3$-7′, CH$_3$-13′, and CH$_3$-12′, respectively). The ^{13}C and DEPT-135 data revealed 22 carbons, consistent with the molecular formula and indicative of two carbonyl carbons, likely a ketone and an ester (δ_C 193.6 and 170.8, for C-2 and C-6, respectively), eight olefinic carbons (δ_C 140.6, 140.1, 134.2, 132.0, 126.4, 125.7, 125.1, and 124.0 for C-7′, C-3′, C-4′, C-11′, C-5′, C-2′, C-6′, and C-10′, respectively), six oxygenated carbons, including that of a methoxy signal (δ_C 99.3, 61.5, 64.4, 65.9, 56.8, and 56.1 for C-1, C-3, C-1′, C-5, C-7, and C-4, respectively), two methylenes (δ_C 40.3 and 26.8 for C-8′ and C-9′, respectively), and four vinylic methyl groups (25.9, 17.9, 17.1, and 13.6 for C-13′, C-12′, CH$_3$-7′, and CH$_3$-3′, respectively). COSY data (Figure 2) identified four spin systems, for example, those from H$_2$-8′ to H-10′, from H-4′ to H-6′, from H-1′ to H-2′, and from H-4 to H-5; the J value of 15 Hz for coupling between H-4′ and H-5′ verified a *trans* double bond. A sesquiterpenoid side chain was apparent from HMBC correlations from H-1′ to H-13′ (Figure 2). The point of attachment for the side chain was evident from HMBC correlations from

Na]$^+$), establishing an IHD of 11. It had a spectroscopic profile similar to 1, but differences were apparent in the ^{13}C and DEPT-135 NMR data, where 2 displayed one less methylene than 1. This finding was supported by the HRESIMS data of 2, which
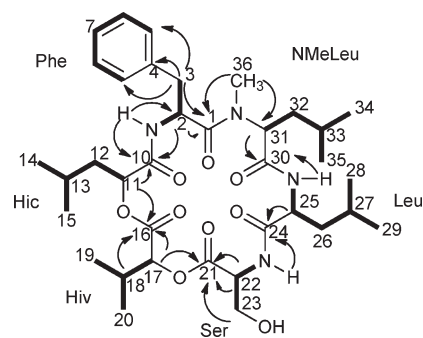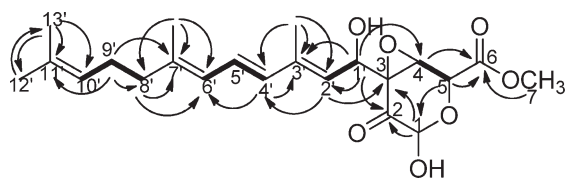
Figure 2. Selected HMBC ($\rightarrow$) and COSY (—) correlations for **3**.

H-2$'$ to C-3 and from H-1$'$ to C-2, C-4, and C-3, suggesting that the quaternary carbon C-3 was connected directly to C-1$'$ and flanked by the ketone carbonyl C-2 and the oxygenated C-4. HMBC correlations from H-1 to C-2, C-3, and C-5 and from H-5 to C-1 suggested that the anomeric proton (H-1) was connected to C-2 and C-5; the latter was via an ether linkage, thereby satisfying the oxygen atoms required by the molecular formula. Finally, correlations from H-4 to C-3, C-5, and C-6, from H-5 to C-6, and from H$_3$-7 to C-6 established the six-membered ring with the carboxymethyl group attached at C-5. The sesquiterpenoid chain (C-1$'$ to C-12$'$) with a conjugated triene was similar to the oligosporon group of compounds, which were isolated from the nematode-trapping fungus *Arthrobotrys oligospora*.[16] However, compound **3** had a unique ring portion that was derived from a sugar unit (hexanose). Compound **3** was ascribed the trivial name trichothosporon A.

The relative configuration of **3** was determined via a NOESY experiment. This revealed that H-5 and H-4 were on the same face of the ring. Moreover, a correlation between H-1 and the methoxy moiety of the ester showed these were on the opposite face. Unfortunately, it was not possible to determine the relative configuration at H-1$'$ unambiguously through the same experiment. Several unsuccessful reactions were attempted, resulting in decomposition of compound **3**. Thus, the absolute configuration remains undetermined at this time.

Compound **4** ($C_{19}H_{26}O_6$ by HRMS) was a new trichothecene analogue, which was related in structure to a previously reported (and unnamed) synthetic intermediate.[17] On the basis of NMR data, compound **4** retained the core of the trichothecene structure, but key signals for the epoxide moiety were replaced by a methylene unit (H$_2$-13). The geminal coupling constant of these protons ($J = 12$ Hz) was too large for an epoxide moiety (typically 4 Hz), suggesting a larger ring system. HMBC data indicated the presence of either a tetrahydropyran ring (connecting C-7 to C-13 and the formation of a tertiary alcohol at C-12) or a tetrahydrofuran ring (connecting C-7 to C-12 with a free CH$_2$OH at C-13). Chemical shifts of the methylene group at C-13 were consistent with the former.[17] Furthermore, acetylation with pyridine—acetic anhydride resulted in the incorporation of only one acetyl group, indicating that **4** had only one OH group susceptible to the reaction (i.e., position 8). NOESY data showed correlations between H-11 and H$_2$-13, which suggested that these protons were on the same face of the molecule, while the broad singlet signals for H-7 and H-8, together with their NOESY correlations, suggested that H-7 and H-8 were on the opposite face of the molecule from H-11.

Five other known compounds, guangomide A (**5**),[3] roseotoxin S,[18] crotocin,[17,19] trichothecinol B,[20] and trichothecin,[4,19] were isolated and elucidated; in all cases, the structural data were in agreement with the literature. The identification of the cyclodepsipeptide **5** was of particular interest, as it was described initially from an unidentified fungal strain isolated from an *Ianthella* sponge.[3] The NMR data of **5** were identical to the literature (see Supporting Information), although comparison of the optical rotation showed opposite signs (literature: −44.6 vs compound **5**: +6.5). Crystals of **5** suitable for X-ray crystallographic analysis were generated, which were identical to the literature as well (data not shown). This could mean that **5** was either guangomide A or its enantiomer. Amino acid analysis and Marfey's derivatization of the acid hydrolysis products of **5** showed the same amino acid residues as reported for guangomide A, particularly L-NMeAla and D-NMePhe (see Supporting Information for UPLC chromatograms of the amino acid analyses). Thus, compound **5** was presumed to be guangomide A;[3] the difference in optical rotation values cannot be explained at this time. To the best of our knowledge, this is the first description of **5** from a terrestrial organism. Crews and colleagues reported weak antibacterial activity for **5** vs *Staphylococcus epidermidis* and *Enterococcus durans* (MIC values of 100 μg/mL in both assays).[3] The same authors reported **5** as inactive against murine and human tumor cell lines in a disk diffusion assay. In the human tumor panel, **5** displayed EC$_{50}$ values of approximately 15 μM against MCF-7, H460, and SF268 cell lines.

The new compounds (**1**−**4**) were not active in the human tumor panel (i.e., IC$_{50}$ values > 10 μM against all cell lines). Thus, roseotoxin S and the trichothecene analogues likely account for the cytotoxicity observed with the crude extract. Our team reported recently a procedure to dereplicate macrocyclic trichothecenes,[21] and studies are ongoing to augment those methods for simple trichothecenes. Regardless, the biosynthetic potential of this fungus was quite intriguing, given the variety of structural types isolated on a nonoptimized culture that was collected over two decades ago.

■ EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra and optical rotations were acquired on a Varian Cary 100 Bio UV—vis spectrophotometer and a Rudolph Research Autopol III polarimeter, respectively. NMR spectra were recorded on a JEOL ECA-500, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. HPLC was carried out using a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector, with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2). For preparative HPLC, a YMC ODS-A (5 μm; 250 × 20 mm) column was used with a 10 mL/min flow rate; for the semipreparative HPLC, a YMC ODS-A (5 μm; 250 × 10 mm) column was used with a 5 mL/min flow rate; for analytical HPLC, YMC ODS-A (5 μm; 150 × 4.6 mm) columns were used with a 1 mL/min flow rate (all from Waters). For analytical HPLC, MetaTherm HPLC column temperature controllers (Varian) maintained these columns at 30 °C. Reversed-phase chiral HPLC was carried out using a Chirex 3126 D-penicillamine (5 μm; 150 × 4.6 mm) column at 1 mL/min flow rate (Phenomenex). UPLC was carried out on a Waters Acquity UPLC system (Waters; Milford, MA, USA) using a Waters HSS T3 C18 column (1.8 μm; 2.1 × 100 mm) maintained at 40 °C. HRESIMS on compounds **1**−**3** were performed by direct injection coupled to QtofMS using a Waters SYNAPT MS system; HRMALDITOFMS data for compounds **4** and **5** were acquired with an Applied Biosystems TOF/TOF mass spectrometer using conditions described previously.[22] Flash chromatography was conducted using a CombiFlash Rf system using RediSep Rf Si gel columns (both from Teledyne-Isco).

Producing Organism and Fermentation. Mycosynthetix fungal strain 51320 was isolated by Dr. Barry Katz of MYCOsearch in September 1990 from leaf litter collected in Leon County, approximately 2 miles east southeast of Tallahassee, FL, USA. DNA analyses

were performed by MIDI Laboratories, Inc. (Newark, DE, USA), and the D2 variable region of the large subunit (LSU) rRNA was sequenced and compared to their database. This analysis suggested that this fungus was related to *Trichothecium* sp. (order Hypocreales); these data were deposited in Genbank (accession no. JF930284). The culture was stored on a malt extract slant and was transferred periodically. A fresh culture was grown on a similar slant, and a piece was transferred to a medium containing 2% soy peptone, 2% dextrose, and 1% yeast extract (YESD media). Following incubation (7 d) at 22 °C with agitation, the culture was used to inoculate 50 mL of a rice medium, prepared using rice to which was added a vitamin solution and twice the volume of rice with H_2O, in a 250 mL Erlenmeyer flask. This was incubated at 22 °C until the culture showed good growth (approximately 14 d). The scale-up culture was grown in a 2.8 L Fernbach flask containing 150 g of rice and 300 mL of H_2O and was inoculated using a seed culture grown in YESD medium. This was incubated at 22 °C for 14 d.

Extraction and Isolation. To the large-scale solid fermentation was added 500 mL of 1:1 MeOH−$CHCl_3$. The mixture was shaken for 24 h, then filtered, and the solvent was evaporated (waxy yellow solid). This crude extract was partitioned between $CHCl_3$−MeOH−H_2O (4:1:5), and the organic soluble material was dried and further partitioned between hexanes and CH_3CN (1:1). The CH_3CN partition (4 g) was fractionated via flash chromatography using silica gel via a hexanes−$CHCl_3$−MeOH gradient to afford four fractions. Fraction 2 (1.5 g), which eluted with 0 to 10% MeOH in $CHCl_3$ over 14 min, was separated further by reversed-phase HPLC (20−100% CH_3CN−H_2O over 100 min) to obtain 18 fractions. Fraction 13 yielded guangomide A (4; 91 mg). Fraction 14 (11.4 mg) was purified by semipreparative reversed-phase HPLC (20−100% CH_3CN−H_2O over 15 min) to yield trichothosporon A (3, 3 mg). Fraction 16 (9.4 mg) was separated further by semipreparative reversed-phase HPLC (20−100% CH_3CN−H_2O over 15 min) to obtain trichodepsipeptide B (2; 4 mg), while similar separation conditions were utilized on fraction 17 (18 mg) to yield trichodepsipeptide A (1; 7 mg). An initial sample of this same fungus was processed in the same fashion to yield a CH_3CN partition (672 mg), which was subjected to flash chromatography using silica gel via a hexanes−$CHCl_3$−MeOH gradient to afford seven fractions. Fraction 5 (490 mg) was separated further by preparative reversed-phase HPLC (20−100% CH_3CN−H_2O over 100 min) to yield a new trichothecene analogue (4; 17 mg), trichothecin (6 mg), crotocin (7 mg), a mixture of roseotoxin S and trichothecinol B (16 mg), guangomide A (5; 22 mg), and trichothosporon A (3; 22 mg).

Trichodepsipeptide A (1): white powder; $[\alpha]^{25}_D$ −45.6 (c 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (2.97); ^1H, ^{13}C NMR, and HMBC data, see Table 1; HRESIMS m/z 711.3923 [M + Na]$^+$ calcd for $C_{36}H_{56}N_4O_9Na$, 711.3945.

Trichodepsipeptide B (2): white powder; $[\alpha]^{25}_D$ −79.0 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (2.67); ^1H, ^{13}C NMR and HMBC data, see Table 1; HRESIMS m/z 697.3770 [M + Na]$^+$, calcd for $C_{35}H_{54}N_4O_9Na$, 697.3788.

Trichothosporon A (3): white powder; $[\alpha]^{25}_D$ −8.8 (c 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.23 (s, H-1), 3.82 (d, J = 3, H-4), 4.63 (d, J = 3, H-5), 3.74 (s, H-7), 5.19 (d, J = 9, H-1′), 5.28 (d, J = 9, H-2′), 6.11 (d, J = 15, H-4′), 6.47 (dd, J = 15, 11, H-5′), 5.85 (d, J = 11, H-6′), 2.08 (m, H-8′), 2.08 (m, H-9′), 5.06 (m, H-10′), 1.58 (s, H-12′), 1.66 (s, H-13′), 1.92 (d, J = 1, 3′-CH₃), 1.77 (s, 7′-CH₃); ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.3 (C-1), 193.6 (C-2), 61.5 (C-3), 56.1 (C-4), 65.9 (C-5), 170.8 (C-6), 56.8 (C-7), 64.4 (C-1′), 125.7 (C-2′), 140.1 (C-3′), 134.2 (C-4′), 126.4 (C-5′), 125.1 (C-6′), 140.6 (C-7′), 40.3 (C-8′), 26.8 (C-9′), 124.0 (C-10′), 132.0 (C-11′), 17.9 (C-12′), 25.9 (C-13′), 13.6 (3′-CH₃), 17.1 (7′-CH₃); HRESIMS m/z 447.1981 [M + Na]$^+$, calcd for $C_{22}H_{32}O_8Na$, 447.1995.

Trichothecene analogue (4): colorless film; $[\alpha]^{25}_D$ −11.0 (c 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.35 (dq, J = 11.5, 7.5, H-3′), 5.77 (dq, J = 11.5, 2, H-2′), 5.58 (d, J = 6, H-10), 5.51 (dd, J = 8, 3.4,

H-4), 4.20 (d, J = 5, H-2), 3.95 (br s., H-8), 3.92 (d, J = 12, Ha-13), 3.83 (br s., H-7), 3.63 (d, J = 7, H-11), 3.61 (d, J = 12, Hb-13), 2.39 (dd, J = 15.5, 8.6, Ha-3), 2.12 (m, Hb-3), 2.11 (dd, J = 7.5, 2, H-4′), 1.84 (s, H₃-16), 1.03 (s, H₃-15), 0.92 (s, H₃-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9 (C-1′), 146.7 (C-3′), 136.8 (C-9), 121.4 (C-10), 120.2 (C-2′), 82.6 (C-2), 77.0 (C-7), 74.8 C-4), 74.6 (C-12), 72.5 (C-8), 69.7 (C-11), 66.7 (C-13), 50.1 (C-5), 38.9 (C-6), 37.3 (C-3), 20.7 (C-16), 15.8 (C-15), 15.7 (C-4′), 6.7 (C-14); HMBC data, H-2 → C-5, C-11, C-12; H₂-3 → C-2, C-4; H-4 → C-1′, C-2, C-5, C-6, C-12; H-7 → C-5, C-8, C-7; H-8 → C-6, C-7, C-9, C-10, C-16; H-10 → C-6, C-8, C-11, C-16; H-11 → C-2, C-9, C-10; H₂-13 → C-2, C-5, C-12; H₃-14 → C-4, C-5, C-6; H₃-15 → C-5, C-6; H₃-16 → C-8, C-9, C-10; H-2′ → C-1′, C-4′; H-3′ → C-1′, C-4′; H₃-4′ → C-1′, C-2′; HRESIMS m/z 373.1620 [M + Na]$^+$, calcd for $C_{19}H_{26}O_6Na$, 373.1622.

Guangomide A (5): $[\alpha]^{25}_D$ +6.5 (c 0.31, $CHCl_3$); ^1H and ^{13}C NMR data matched the literature;[3] HRMALDITOFMS m/z 641.3163 [M + Na]$^+$, calcd for $C_{31}H_{46}N_4O_9Na$, 641.3157.

Amino Acid Analysis by Chiral HPLC.[13−15] Independently, an aliquot of compounds 1 (2 mg) and 2 (2 mg) were subjected to hydrolysis with 6 N HCl (1 mL) in a sealed tube at 100 °C for 24 h. Upon cooling, the solutions were evaporated to dryness. Each residue was dissolved in H_2O and extracted with diethyl ether (2 × 2 mL). Both organic and aqueous solutions were subjected to chiral HPLC analysis at a flow rate of 1.0 mL/min using four different solvent systems: (1) 5% 2-propanol in 2 mM $CuSO_4$: L-Hiv (t_R = 15 min), D-Hiv (t_R = 27 min), L-Hic (t_R = 49 min), D-Hic (t_R = 68 min), L-NMeLeu (t_R = 12 min), D-NMeLeu (t_R = 17 min); (2) 5% MeOH in 2 mM $CuSO_4$: L-Ser (t_R = 4.9 min), D-Ser (t_R = 5.3 min); (3) 30% MeOH in 2 mM $CuSO_4$: L-Phe (t_R = 25 min), D-Phe (t_R = 35 min), L-Leu (t_R = 15 min), D-Leu (t_R = 18 min); (4) 100% 1 mM $CuSO_4$: L-Val (t_R = 26 min), D-Val (t_R = 47 min). Dilutions were adjusted in each case to approximate the response of the UV detector (λ = 214 nm) with those of hydrolyzed samples.

Amino Acid Analysis of Marfey's Derivatives[23] **by UPLC.** A sample of compound 5 (2.3 mg) was subjected to acid hydrolysis in a manner similar to the above. The dried aqueous solution was resuspended in H_2O (100 μL), and 1% Marfey's reagent in acetone (200 μL) and 1 M $NaHCO_3$ (40 μL) were added. The mixture was heated at 40 °C for 1 h in a shaking water bath, after which it was removed and cooled. The mixture was quenched with 2 M HCl (20 μL), dried, and dissolved in MeOH for UPLC analysis. Amino acid standards (1 mg each) were derivatized with Marfey's reagent in a manner similar to the above. The UPLC analysis was performed using two solvent systems: (1) 10−50% CH_3CN−0.1% TFA in H_2O over 10 min at a flow rate of 0.5 mL/min: L-NMePhe (t_R = 8.334 min), D-NMePhe (t_R = 8.447 min), L-Ala (t_R = 5.281 min), D-Ala (t_R = 6.186 min); (2) 10−70% MeOH−0.1% TFA in H_2O over 10 min at a flow rate of 0.4 mL/min: L-NMeAla (t_R = 7.730 min), D-NMeAla (t_R = 7.682 min), L-Ala (t_R = 6.836 min), D-Ala (t_R = 8.067 min).

Cytotoxicity Assay. The cytotoxicity measurements against MCF-7[24] human breast carcinoma (Barbara A. Karmanos Cancer Center, Detroit, MI, USA), NCI-H460[25] human large-cell lung carcinoma (HTB-177, American Type Culture Collection, Manassas, VA, USA), and SF-268[26] human astrocytoma (NCI Developmental Therapeutics Program, Frederick, MD, USA) cell lines were performed as described previously.[22] Compounds 1 and 2 were also tested against the HT-29[27] human colorectal adenocarcinoma (HTB-38, American Type Culture Collection) and MDA-MB-435[28] human melanoma (HTB-129, American Type Culture Collection) cell lines as described previously.[29]

■ ASSOCIATED CONTENT

S **Supporting Information.** ^1H and ^{13}C NMR spectra of compounds 1−4. These materials are available free of charge via the Internet at http://pubs.acs.org.

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