Cycloaspeptides F and G, Cyclic Pentapeptides from a *Cordyceps*-Colonizing Isolate of *Isaria farinosa*

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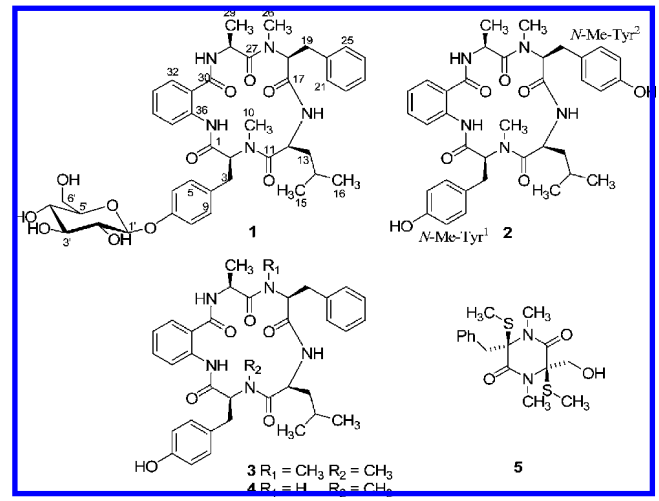
Cycloaspeptides F (**1**) and G (**2**), two new cyclic pentapeptides, and the known cycloaspeptides A (**3**), C (**4**), and bisdethiodi(methylthio)hyalodendrin (**5**) have been isolated from the crude extract of the fungus *Isaria farinosa* that colonizes *Cordyceps sinensis*. The structures of **1** and **2** were elucidated primarily by NMR and MS methods. The absolute configuration of **1** was assigned using Marfey's method on its acid hydrolysate. Compounds **1** and **2** showed cytotoxic effects against HeLa and MCF7 cell lines, displaying the same magnitude of activity toward the MCF7 cells as the positive control 5-fluorouracil.

Cordyceps sinensis (Berk.) Sacc. (Anamorph: *Hirsutella sinensis*)[1] is known as Chinese caterpillar fungus. The combination of the fungus and the dead caterpillars of the moth *Hepilus* spp., "DongChongXiaCao" (winter-worm, summer-grass), is a famous fungal traditional Chinese medicine (FTCM) that has been widely used as a tonic and/or medicine for hundreds of years in the Orient. *Cordyceps* is a unique black, blade-shaped fungus found primarily at high altitude on the Qinghai-Tibetan plateau and endophytically parasitizes dead caterpillars of the moth *Hepilus* spp. Chemical studies of *C. sinensis* have shown that the species can produce different bioactive compounds.[2] However, due to its growing popularity, the natural fungus has been overharvested to the extent that it is an endangered species, and the scarcity of the natural material has limited more systematic chemical investigations of the fungus as a source of bioactive metabolites.

Applications of fungal ecology in the search for new bioactive natural products have proven effective.[3] On the basis of ecological considerations, we initiated chemical investigations of the fungal species that colonize the fruiting body of *C. sinensis*,[4−6] which we named *Cordyceps*-colonizing fungi because of their ill-defined relationships with *C. sinensis*.[7] Despite claimed medical benefits including antitumor activity of *C. sinensi*, whether these effects were originated from its own metabolites or those produced by its colonizing fungi remains to be clearly defined. Our long-term goal is to explore the living strategy of the *Cordyceps*-colonizing fungi and understand their relationships with *C. sinensis* using bioactive secondary metabolites as probes. During the course of our continuing search for new bioactive natural products from this unique source, the fungus *Isaria farinosa* (XJC04-CT-303) isolated from a sample of *C. sinensis* collected in Linzhi, Tibet, People's Republic of China, was grown in a solid-substrate fermentation culture. Its organic solvent extract displayed cytotoxicity against two human tumor cell lines, HeLa and MCF7. Fractionation of the extract led to the isolation of two new cyclic pentapeptides, which we named cycloaspeptides F (**1**) and G (**2**), together with the known cycloaspeptides A (**3**) and C (**4**)[8,9] and bisdethiodi(methylthio)hyalodendrin (**5**).[10,11] Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

The known metabolites isolated from the crude extract were identified as cycloaspeptides A (**3**) and C (**4**) and bisdethiodi(methylthio)hyalodendrin (**5**) by comparison of their NMR and MS data with those reported.[8−11] Cycloaspeptides A (**3**) and C (**4**) are members of the anthranilic acid (ABA)-containing cyclic pentapeptides initially isolated from *Aspergillus* sp. NE-45,[8,9] whereas bisdethiodi(methylthio)hyalodendrin (**5**) is a diketopiperazine metabolite first reported from an unidentified fungus NRRL 3888.[10]



Cycloaspeptide F (**1**) was obtained as a colorless powder. It was assigned a molecular formula of $C_{42}H_{53}N_5O_{11}$ on the basis of its HRESIMS (*m*/*z* 826.3634 $[M + Na]^+$; Δ −2.5 mmu). Analysis of the ^{1}H, ^{13}C, and HMQC NMR spectroscopic data of **1** (Table 1) revealed the presence of a sugar moiety, three amide *N*-H protons (δ_H 7.95, 8.17, and 12.14, respectively), five methyl groups (including two *N*-methyls), three methylenes, five methines (four of which are heteroatom-bonded), three phenyl rings, and five carboxylic carbons. These data accounted for all the ^{1}H and ^{13}C NMR resonances for **1** and are consistent with the molecular formula $C_{42}H_{53}N_5O_{11}$.

Interpretation of the ^{1}H, ^{13}C, and 2D NMR data of **1**, especially HMBC, revealed its peptidic nature and structural similarity to those of **3** and **4**. Five ^{13}C NMR resonances, at δ_C 174.0, 170.5, 169.9, and 169.1 (2), were characteristic of amide carbonyls with four methine peaks at δ_H/δ_C 5.43/63.8, 4.68/49.0, 4.56/45.0, and 4.06/69.2 being indicative of four amino acid α-CH groups. The ^{1}H NMR resonances for a cluster of five aromatic protons at δ_H 7.23−7.30 suggested the presence of a monosubstituted benzene ring, which was connected to C-19 by HMBC correlations from H_2-19 to C-20, C-21, and C-25. Correlations from H-18 to C-17 and C-19, from H-18 to C-26, and from H_3-26 to C-18 established the *N*-methylphenylalanine (*N*-Me-Phe) unit, like that found in cycloaspeptide A (**3**). Analysis of the ^{1}H−^{1}H coupling patterns for

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Table 1. NMR Spectroscopic Data of Cycloaspeptide F (**1**) in Acetone-d_6

residue	position	δ_H[a] (J in Hz)	δ_C[b], mult.	HMBC (H → C#)
N-Me-Tyr	1		169.1, qC	
	2	4.06, m	69.2, CH_3	
	3	3.35, m; 3.45, m	32.8, CH_2	2, 4, 5, 9
	4		133.4, qC	
	5	7.14, d (8.5)	131.0, CH	3, 4, 6, 7, 9
	6	7.05, d (8.5)	117.5, CH	4, 5, 7, 8
	7		157.6, qC	
	8	7.05, d (8.5)	117.5, CH	4, 6, 7, 9
	9	7.14, d (8.5)	131.0, CH	3, 4, 5, 7, 8
	10	2.78, s	39.2, CH_3	2, 11
Leu	11		170.5, qC	
	12	4.68, t (8.0)	49.0, CH	11, 14, 17
	13	1.35, m; 1.76, m	42.2, CH_2	11
	14	1.65, m	25.4, CH	
	15	0.97, d (6.5)	22.7, CH_3	13, 14, 16
	16	0.97, d (6.5)	23.5, CH_3	13, 14, 15
	*N*H	8.17, d (8.0)		
N-Me-Phe	17		169.1, qC	
	18	5.43, dd (12, 3.0)	63.8, CH	17, 19, 26
	19	2.97, dd (14, 3.0); 3.48, dd (14, 12)	34.8, CH_2	18, 20, 21, 25
	20		139.5, qC	
	21	7.30, m	130.5, CH	19, 20, 22, 23, 25
	22	7.29, m	129.6, CH	20, 21, 23, 24
	23	7.23, t (7.0)	127.5, CH	21, 22, 24, 25
	24	7.29, m	129.6, CH	22, 23, 25, 20
	25	7.30, m	130.5, CH	19, 20, 21, 23, 24
	26	2.83, s	30.6, CH_3	18, 27
Ala	27		174.0, qC	
	28	4.56, q (6.5)	45.0, CH	29
	29	0.37, d (6.5)	16.4, CH_3	27, 28
	*N*H	7.95, br s		
ABA	30		169.9, qC	
	31		117.0, qC	
	32	7.89, d (8.5)	128.9, CH	30, 34, 36
	33	7.01, t (8.5)	122.5, CH	31, 32, 34, 35
	34	7.49, t (8.5)	134.0, CH	32, 36
	35	8.89, d (8.5)	120.3, CH	31, 33
	36		142.4, qC	
	*N*H	12.14, s		
Glu	1′	4.94, d (7.0)	102.2, CH	7
	2′	3.47, m	74.6, CH	1′, 3′
	3′	3.51, m	77.8, CH	2′, 4′
	4′	3.49, m	71.2, CH	2′, 5′
	5′	3.51, m	77.6, CH	4′, 6′
	6′	3.88, dd (12,2.0); 3.72, dd (12, 5.0)	62.5, CH_2	4′

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz.

the aromatic protons H-32 (d; J = 8.5 Hz), H-33 (t; J = 8.5 Hz), H-34 (t; J = 8.5 Hz), and H-35 (d; J = 8.5 Hz) revealed a substructure for an *ortho*-substituted aryl ring, which was supported by relevant $^1H-^1H$ COSY and HMBC correlations. An HMBC cross-peak from H-32 to C-30 led to the connection of C-30 to C-31, completing the anthranilic acid (ABA) unit. Similarly, interpretation of the $^1H-^1H$ COSY and HMBC data for **1** established the structures of leucine (Leu), alanine (Ala), and *N*-methyltyrosine (*N*-Me-Tyr) residues. Due to the lack of HMBC correlation from H-1 and/or H_2-3 to C-1, the C-1 amide carbon was assigned to *N*-Me-Tyr by default. The sugar moiety in **1** was determined as glucose (Glc) on the basis of its ^1H and ^{13}C NMR data (Table 1). A key HMBC correlation from the anomeric proton of Glc (H-1′; δ_H 4.94) to the oxygenated aromatic carbon of *N*-Me-Tyr (C-7; δ_C 157.6) connected the Glc unit to *N*-Me-Tyr at C-7. Upon extensive analysis of these data, cycloaspeptide F (**1**) was assigned as a cyclic pentapeptide containing one equivalent each of Leu, Ala, *N*-Me-Phe, ABA, and *N*-Me-Tyr glycoside.

The sequence of **1** was determined by HMBC correlations of the *N*-methyl groups and various α- and β-protons with neighboring carboxylic carbons and was supported by relevant NOESY correlations (Figure 1). The *N*-methyl proton of *N*-Me-Phe (H_3-26; δ_H 2.83) showed an HMBC correlation to the carboxylic carbon of Ala (C-27; δ_C 174.0), indicating that it is acylated by Ala, and this connection was also supported by NOESY correlation of H-18 with H-28. An HMBC cross-peak from the *N*-methyl proton of *N*-Me-Tyr (H_3-10; δ_H 2.78) to the carboxyl carbon of Leu (C-11; δ_C 170.5) led to the connection of these two residues, which was supported by NOESY correlations of H_3-10 with H-2 and H-12. The α-proton of Leu (H-12; δ_H 4.68) was correlated to the carboxylic carbon of *N*-Me-Phe (C-17; δ_C 169.1). These correlations enabled assignment of the partial sequence Ala → *N*-Me-Phe → Leu → *N*-Me-Tyr. Although no further sequence-relevant HMBC correlations were observed, the only remaining ABA unit must be placed between *N*-Me-Tyr and Ala, thereby completing the sequence of **1** as shown.

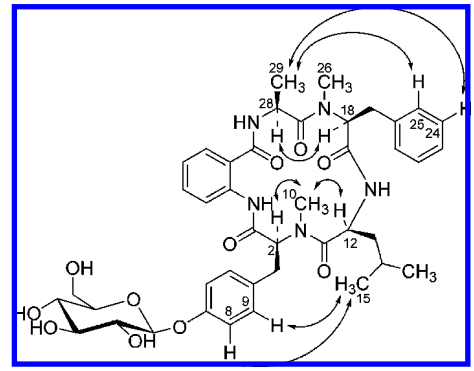

Figure 1. Selected NOESY correlations for cycloaspeptide F (**1**).

The relative configurations between the α-protons of Ala and *N*-Me-Phe, and Leu and *N*-Me-Tyr glycoside were assigned by analysis of NOESY correlations (Figure 1). In the NOESY spectrum of **1**, the α-protons of *N*-Me-Phe (H-18; δ_H 5.43) and Ala (H-28; δ_H 4.56) were correlated, indicating that these protons have the same orientations with respect to the macrocycle, whereas correlations of the β-protons of Ala (H_3-29; δ_H 0.37) with H-24 (δ_H 7.29) and H-25 (δ_H 7.30) of *N*-Me-Phe were used to place them on the opposite face. The *N*-methyl signal of *N*-Me-Tyr glycoside (H_3-10; δ_H 2.78) was correlated to the α-protons of Leu (H-2; δ_H 4.06) and itself (H-12; δ_H 4.68), indicating that these protons are all cofacial, and this assignment was supported by correlations from H_3-15 (δ_H 0.97) to H-8 (δ_H 7.05) and H-9 (δ_H 7.14).

Marfey's method[12] was applied to assign the absolute configuration of the Ala and Leu residues resulting from acid hydrolysis of cycloaspeptide F (**1**). HPLC analysis of the 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) derivatives of the acid hydrolysate of **1** gave the same retention times as those prepared from samples of authentic L-Ala and L-Leu. Therefore, the Ala and Leu residues in **1** were both assigned the L-configuration. Considering the relative configurations established between Ala and *N*-Me-Phe, and Leu and *N*-Me-Tyr glycoside by NOESY data, *N*-Me-Phe and *N*-Me-Tyr were both deduced to have the L-configuration.

The Glc unit in **1** was connected to the *N*-Me-Tyr via a β-linkage on the basis of the downfield chemical shift of the anomeric carbon (C-1′; δ_C 102.2), as well as the $^1H-^1H$ coupling constant (7.0 Hz) observed for the anomeric proton (H-1′). Acid hydrolysis was performed to separate the Glc unit from the *N*-Me-Tyr glycoside portion of **1**. Treatment of the liberated Glc with L-cysteine methyl ester, followed by trimethylsilylation, afforded a derivative matching that of the D-Glc by GC-MS analysis in comparison with the D- and L-standards.[13,14]

The molecular formula of cycloaspeptide G (**2**) was determined to be $C_{36}H_{43}N_5O_7$ on the basis of its HRESIMS (*m/z* 680.3047 [M + Na]$^+$; Δ −0.8 mmu). Analysis of the ^1H and ^{13}C NMR spectroscopic data of **2** (Table 2) revealed structural features similar as those found in **1**, except that the signals for the Glc moiety were absent, and those for the *N*-Me-Phe unit were replaced by an *N*-Me-

Table 2. NMR Spectroscopic Data of Cycloaspeptide G (**2**) in Acetone-d_6

residue	position	δ_H[a] (*J* in Hz)	δ_C[b], mult.	HMBC (H → C#)
N-Me-Tyr[1]	1		169.1, qC	
	2	4.02, m	70.1, CH	
	3	3.35, m; 3.36, m	32.7, CH_2	5, 9
	4		129.7, qC	
	5	7.03, d (8.5)	131.1, CH	3, 6, 7, 9
	6	6.81, d (8.5)	116.1, CH	
	7		157.6, qC	
	8	6.81, d (8.5)	116.1, CH	
	9	7.03, d (8.5)	131.1, CH	3, 5, 7, 8
	10	2.78, s	39.1, CH_3	2, 11
Leu	11		170.6, qC	
	12	4.66, t (8.0)	54.0, CH	
	13	1.35, m; 1.82, m	42.2, CH_2	11
	14	1.36, m	25.3, CH	
	15	0.96, d (7.0)	22.6, CH_3	13, 14, 16
	16	0.96, d (7.0)	23.4, CH_3	13, 14, 15
	*N*H	8.17, d (8.0)		
N-Me-Tyr[2]	17		169.2, qC	
	18	5.34, dd (12, 3.0)	64.0, CH	17
	19	2.97, m; 3.30, m	33.9, CH_2	21, 25
	20		130.3, qC	
	21	7.09, d (8.5)	131.4, CH	19, 25
	22	6.77, d (8.5)	116.3, CH	20, 24
	23		157.2, qC	
	24	6.77, d (8.5)	116.3, CH	20, 22
	25	7.09, d (8.5)	131.4, CH	19, 21
	26	2.83, s	30.5, CH_3	18, 27
Ala	27		174.1, qC	
	28	4.59, q (7.0)	45.2, CH	
	29	0.50, d (7.0)	16.3, CH_3	27, 28
ABA	30		171.8, qC	
	31		118.5, qC	
	32	7.91, d (8.5)	128.9, CH	30, 34, 36
	33	7.02, t (8.5)	122.5, CH	35
	34	7.49, t (8.5)	134.0, CH	32, 36
	35	8.89, d (8.5)	120.3, CH	31, 33
	36		142.1, qC	
	*N*H	12.14, s		

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz.

Table 3. Cytotoxic Effects of Compounds **1**−**5**

compound	GI_{50} (μM) HeLa	GI_{50} (μM) MCF7
1	49.8	18.7
2	30.4	15.2
3	31.2	23.4
4	>63.4	31.9
5	>112.9	112.9
5-fluorouracil	10.0	15.0

Tyr in the spectra of **2**. These observations were confirmed by relevant $^1H-^1H$ COSY and HMBC correlations. Therefore, the gross structure of cycloaspeptide G was established as shown. The absolute configuration of **2** was deduced by analogy to **1**.

Cycloaspeptides F (**1**) and G (**2**) and the known compounds cycloaspeptides A (**3**) and C (**4**) and bisdethiodi(methylthio)hyalodendrin (**5**) were evaluated for cytotoxic activity against two human tumor cell lines, HeLa and MCF7 (Table 3). Cycloaspeptides F (**1**) and G (**2**) showed inhibitory effects on the growth of MCF7 cells that are comparable to the positive control 5-fluorouracil, with GI_{50} values of 18.7 and 15.2 μM, respectively (5-Fu showed a GI_{50} value of 15.0 μM). Compounds **1** and **2** also displayed modest cytotoxic effects against the HeLa cells, with GI_{50} values of 49.8 and 30.4 μM, respectively (5-Fu showed a GI_{50} value of 10.0 μM).

Cycloaspeptides are a class of ABA-containing cyclic pentapeptides isolated from fungi. Examples include cycloaspeptides A−C from *Aspergillus* sp. NE-45,[8] cycloaspeptide D from the psychrotolerant fungus *Penicillium ribeum*,[9] and cycloaspeptide E from several *Penicillia* and a *Tricothecium* strain.[15] Cycloaspeptides F (**1**) and G (**2**) are new members of this class of metabolites, and the presence of a Glc unit in **1** is unprecedented. Cycloaspeptide G (**2**) is closely related to C (**3**), but differs in having an *N*-Me-Tyr unit rather than an *N*-Me-Phe moiety.

The species *I. farinosa* (formerly classified as *Paecilomyces farinosus*) is a well-known entomopathogenic fungus, which was regarded as a biocontrol agent against *Rhizoecus kondonis* (Homoptera), *Dendrolimus pini* (Lepidoptera), *Lymantria dispar* (Lepidoptera), *Hyloicus pinastri* (Lepidoptera), and *Bupalus piniarius* (Lepidoptera), as well as various pest insects, plant diseases, and nematodes.[16−18] A variety of metabolites have been reported from different strains of the fungus as antioxidative, antitumor, and antifungal agents.[19−22] In this work, the discovery of new ABA-containing cycloaspeptides from a *Cordyceps*-colonizing strain of *I. farinosa* further expanded structural diversity of the secondary metabolites produced by this fungal species.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Bruker Avance-500 and -600 spectrometers using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.0) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using a Bruker APEX III 7.0 T spectrometer. GC-MS (70 eV) data were acquired on a Shimadzu GCMS-QP2010 instrument using a DB-5 ms capillary column (30 m × 0.25 mm, i.d.; J & W Scientific) with He as carrier gas and a detector and an injection temperature of 250 °C. The initial temperature was maintained at 50 °C for 2 min and then raised to 250 °C at the rate of 15 °C/min.

Fungal Material. The culture of *I. farinosa* was isolated by Dr. Mu Wang from a sample of *C. sinensis* (Berk.) Sacc. collected in Linzhi, Tibet, in June 2004. The fungus was identified by one of the authors (B.S.) based on sequence (Genbank Accession No. AB233337.1) analysis of the ITS region of the rDNA and assigned the Accession No. XJC04-CT-303 in X.L's culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. The agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. Fermentation was carried out in four 500 mL Fernbach flasks each containing 75 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was extracted repeatedly with EtOAc (3 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (3.3 g). The extract was fractionated by Si gel vacuum liquid chromatography (VLC) using *n*-hexanes−CH_2Cl_2−MeOH gradient elution. The fraction (110 mg) eluted with CH_2Cl_2−MeOH (100:2) was fractionated by Sephadex LH-20 column chromatography (CC) using CH_3OH as eluent. The subfraction (25 mg) was further separated by semipreparative reversed-phase HPLC (RP HPLC; Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 20% CH_3OH in H_2O for 5 min, and followed by 20−100% CH_3OH in H_2O for 25 min; 2 mL/min) to afford cycloaspeptide A (**3**; 4.0 mg, t_R 23.3 min) and bisdethiodi(methylthio)hyalodendrin (**5**; 1.5 mg, t_R 17.5 min). The fractions (135 mg) eluted with 100:4 CH_2Cl_2−CH_3OH were combined and separated again by Sephadex LH-20 CC eluting with CH_3OH. Purification of the resulting subfractions by RP HPLC (20% CH_3OH in H_2O for 5 min, followed by 20−100% CH_3OH in H_2O for 40 min) afforded cycloaspeptides G (**2**; 1.5 mg, t_R 36.6 min) and C (**4**; 2.5 mg, t_R 38.0 min). The fractions (225 mg) eluted with 100:6 and 100:8 CH_2Cl_2−CH_3OH were combined and separated

again by Sephadex LH-20 CC using CH_3OH as eluent. Further purification of the subfractions by RP HPLC (40% CH_3CN in H_2O for 5 min, followed by 40−60% CH_3CN in H_2O for 25 min) afforded cycloaspeptide F (**1**; 4.0 mg, t_R 12.5 min).

Cycloaspeptide F (1): white powder; $[\alpha]_D$ −18 (*c* 0.4, CH_3OH); UV (CH_3OH) λ_{max} (ε) 211 (100 100), 253 (17 600) nm; IR (neat) ν_{max} 3323 (br), 2929, 2868, 1682, 1637, 1444, 1228, 1177, 1075 cm^{-1}; ^1H, ^{13}C NMR, and HMBC data see Table 1; NOESY correlations (acetone-d_6, 600 MHz) H-18 ↔ H-28; H_3-10 ↔ H-2, H-12; H_3-15 ↔ H-8, H-9; H_3-29 ↔ H-24, H-25; HRESIMS *m*/*z* 826.3634 (calcd for $C_{42}H_{53}N_5O_{11}Na$, 826.3659).

Cycloaspeptide G (2): white powder; $[\alpha]_D$ −16 (*c* 0.3, CH_3OH); λ_{max} (ε) 211 (9000), 251 (12 500) nm; IR (neat) ν_{max} 3303 (br), 2926, 2853, 1653, 1627, 1516, 1446, 1260, 1172, 1097 cm^{-1}; ^1H, ^{13}C NMR, and HMBC data see Table 2; NOESY correlations (acetone-d_6, 600 MHz) H-18 ↔ H-28; H_3-10 ↔ H-2, H-12; H_3-15 ↔ H-8, H-9; H_3-29 ↔ H-24, H-25; HRESIMS *m*/*z* 680.3047 (calcd for $C_{36}H_{43}N_5O_7Na$, 680.3055).

Cycloaspeptide A (3): ^1H, ^{13}C NMR, and ESIMS data were consistent with literature values.[8]

Cycloaspeptide C (4): ^1H, ^{13}C NMR, and ESIMS data were consistent with literature values.[8]

Bisdethiodi(methylthio)hyalodendrin (5): ^1H, ^{13}C NMR, and ESIMS data were consistent with literature values.[10,11]

Absolute Configuration of Ala and Leu in 1.[12] Separate solutions of cycloaspeptide F (**1**; 0.5 mg) and G (**2**; 0.5 mg) in 6 N HCl (1.0 mL) were heated at 155 °C for 1 h. Upon removal of excess HCl under vacuum, the hydrolysate was placed in a 1 mL reaction vial and treated with a 1% solution of FDAA (200 μL) in acetone, followed by 1.0 N $NaHCO_3$ (40 μL). The reaction mixtures were heated at 45 °C for 1.5 h, cooled to room temperature, and then acidified with 2.0 N HCl (20 μL). In a similar fashion, standard L-Leu, D-Leu, L-Ala, and D-Ala were derivatized separately. The derivatives of the acid hydrolysate and the standard amino acids were subjected to RP HPLC analysis (Kromasil C_{18} column; 10 μm, 4.6 × 250 mm; 2.0 mL/min) at 25 °C using the following gradient program: solvent A, H_2O (0.1% TFA); solvent B, acetonitrile; linear gradient, 10−50% of B for 40 min with UV detection at 340 nm. The retention times for the FDAA derivatives of L-Ala, D-Ala, L-Leu, and D-Leu were 22.37, 23.94, 31.74, and 35.49 min, respectively, whereas those for the FDAA derivatives of Ala and Leu in the hydrolysate of **1** were 22.43 and 31.74 min, respectively.

Determination of D-Glc.[13,14] A sample of 2.5 mg of cycloaspeptide F (**1**) in 300 μL of acetone was added to 700 μL of 6 N HCl in a hydrolysis tube and heated at 100 °C for 24 h. After evaporation of excess CH_3OH, 1.0 mg of L-cysteine methyl ester hydrochloride in 100 μL of pyridine was added, and the mixture was stirred at 60 °C for 1 h. A 3:1 mixture of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane) was then added (150 μL), and the solution was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a stream of N_2. The residue was partitioned between *n*-hexanes and H_2O, and the hexane layer was directly subjected to GC-MS analysis. The resulting Glc derivative coeluted with a derivatized D-Glc standard (t_R 19.04 min), but not with a derivatized L-Glc standard (t_R 19.25 min).

MTT Assay.[4] In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO or appropriate concentration of test compounds (10 mg/mL as stock solution of a compound in DMSO and serial dilutions). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first and then were allowed to grow for another 48 h after the medium was changed to fresh DMEM. MTT (Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from wells and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well and shaken at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

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Supporting Information Available: ^1H, ^{13}C, and 2D NMR spectra of cycloaspeptides F (**1**) and G (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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