Immunomodulatory Constituents from an Ascomycete, *Chaetomium seminudum*

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A screening study focusing on immunomodulatory activity of the EtOAc extract of an Ascomycete, *Chaetomium seminudum*, has afforded a known epipolythiodioxopiperazine, chetomin (**1**), together with three new chetomin-related metabolites named chetoseminudins A (**2**), B (**3**), and C (**4**). Among these four metabolites, **1** and **2** have been deduced as the immunosuppressive features of this fungus.

In our screening project on immunomodulatory constituents from fungi, nine 2-pyrones, a hexaketide, a macrocyclic sesterterpenetriol, and three 2-furanones from four *Gelasinospora* fungi,[1] a 2-pyrone and two macrocyclic diesters from a *Diplogelasinospora* fungus,[2] two anthraquinones, two octaketides, and two dioxopiperazines from a *Microascus* fungus,[3] four sesterterpenes and a sesquiterpenetriol diester from an *Emericella* fungus,[4] and two sesquiterpene-related metabolites from an *Eupenicillium* fungus[5] have been isolated as immunosuppressive constituents. Successively, we found that the EtOAc extract of an Ascomycete, *Chaetomium seminudum* Ames, appreciably suppressed the proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partitions followed by repeated chromatographic fractionations of the extract, monitored by immunomodulatory activity, afforded four compounds tentatively named CS-A (**1**), -B (**2**), -C (**3**), and -D (**4**), among which **1** and **2** were the immunosuppressive features of this fungus. This paper deals with the structures and immunosuppressive activities of these four metabolites.

The EtOAc extract of *C. seminudum* 72-S-204-1[6] cultivated on sterilized rice medium suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 91% at 2 μg/mL. The extract was partitioned between *n*-hexane and water into an *n*-hexane layer and an aqueous suspension. The aqueous suspension was further partitioned between EtOAc and water into an EtOAc layer and an aqueous layer [yields (%) of the *n*-hexane, EtOAc, and aqueous layers after evaporation of the solvents from the EtOAc extract: 44.5, 41.9, and 7.1, respectively]. The *n*-hexane, EtOAc, and aqueous layers suppressed the Con A-induced proliferation by −5, 95, and 6% at 2 μg/mL, respectively. Repeated chromatographic fractionation of the EtOAc layer monitored by the immunomodulatory activity afforded four components, CS-A (**1**), -B (**2**), -C (**3**), and -D (**4**) [yields (%) of **1**, **2**, **3**, and **4** from the EtOAc extract: 9.57, 2.08, 0.062, and 0.18, respectively].

The molecular formula of CS-A (**1**) was determined to be $C_{31}H_{30}N_6O_6S_4$ by HRFABMS. The UV spectrum of **1** gave the absorption of an indole system (276, 287, and 295 nm), and the IR spectrum showed the absorption of OH (3424 cm^{-1}) and amide >C═O groups (1685 and 1680 cm^{-1}). The physicochemical data and the ^{1}H NMR and ^{13}C NMR data of **1** including two-dimensional COSY, HMQC, and HMBC data (Table 1) suggested that **1** was quite similar to one of the known fungal epipolythiodioxopiperazines, chetomin, which had been isolated from *Chaetomium cochliodes* and *C. globosum* as an antimicrobial feature[7] and from *C. subglobosum* and *C. tenuissimum* as a cytotoxic feature against HeLa cells.[8] On acetylation with acetic anhydride in pyridine, **1** gave diacetate **5**, whose ^{1}H NMR spectrum was identical with that of authentic chetomin diacetate mentioned in the literature.[7a] Accordingly, it was finally concluded that CS-A was identical with chetomin (**1**), as shown in Figure 1.

The molecular formula of CS-B (**2**) was determined to be $C_{31}H_{30}N_6O_6S_5$ by HRFABMS. The UV and IR spectra of **2** were very similar to those of **1**. Comparison of the ^{13}C NMR spectrum of **2** with that of **1** showed that only the signals of C-1, C-3, C-4, C-11, and C-11a and *C*H_2OH-3 were shifted significantly (see Table 1). In comparison of the ^{1}H NMR spectrum of **2** with that of **1**, the signals of H_2-11 and C*H*$_2$OH-3 were shifted significantly. These findings suggested that the disulfide (−S−S−) bridge at ring D in **1** might be replaced with a trisulfide (−S−S−S−) one in **2**. The stereochemistry at positions 3 and 11a of **2** was supposed to be identical with that of **1**, because the optical rotation of **2** was quite similar to that of **1**, and also **2** was isolated together with **1** from the same fungus. Thus, the structure of CS-B was deduced to be **2**, as shown in Figure 1. To our knowledge, this is the first time that CS-B (**2**) has been isolated from a natural source. Accordingly, we propose to call CS-B chetoseminudin A (**2**).

The molecular formula of CS-C (**3**) was determined to be $C_{17}H_{21}N_3O_3S_2$ by HRFABMS. The UV spectrum of **3** gave the absorption of an indole system (274, 281, and 290 nm), and the IR spectrum of **3** showed the absorption of OH (3410 cm^{-1}) and amide >C═O groups (1655 and 1638 cm^{-1}). Comparison of the molecular formula and the ^{1}H NMR and ^{13}C NMR spectral data of **3** (Table 2) with those of **1** showed that **3** was built up by a combination of a 3-methylindole and dithiodioxopiperazine moieties. In the NMR spectra of **3**, the three CH_3 signals at δ_H/δ_C 2.16/12.9, 2.40/14.5, and 2.99/29.1 ppm were assigned to *C*H_3−S, *C*H_3−S, and *C*H_3−N, respectively,[7d,8c] and the two CH_2 signals at δ_H/δ_C 3.26,3.90/36.6 and 3.33,3.85/63.5 ppm were assigned to *C*H_2-7 and *C*H_2-3, respectively, by the aid of HMBC data. In the HMBC spectrum of **3**, some long-range couplings between proton and carbon with J_2 and/or J_3 [H/C], namely, [C*H*$_2$(δ_H 3.26, 3.90)/C(δ_C 65.9), C(107.5), C(125.9), C(166.9)], [C*H*$_3$−S(2.40)/C(65.9)], [C*H*$_3$−N(2.99)/C(73.5), C(166.9)], [C*H*$_3$−S(2.16)/ C(73.5)], [C*H*$_2$(3.33, 3.85)/C(73.5), C(165.7)], and [N*H*(7.24)/C(73.5), C(166.9)], were observed significantly, indicating that **3** possessed a dethiodimethylthiodioxopiperazine moiety (ring C), as shown in Figure 2. Thus, the structure of CS-C was deduced to be **3**. To our knowledge, this is the first time that CS-C (**3**)

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Table 1. NMR Data for Chetomin (**1**), Chetomin Diacetate (**5**), and Chetoseminudin A (**2**) in $CDCl_3$, δ (ppm), from TMS as an Internal Standard [multiplicities and coupling constants (in Hz) in parentheses]

	1		**5**	**2**	
position	δ_H	δ_C	δ_H	δ_H	δ_C
1		165.5 (s)[a]			168.3 (s)
2-CH_3	3.20 (3H, s)	27.5 (q)[b]	3.17 (3H, s)[c]	3.28 (3H, s)	27.5 (q)
3		74.8 (s)			75.4 (s)
3-CH_2OH	4.29, 4.35 (each d, 12.6)	60.6 (t)	4.76, 4.99 (each d, 12.7)	4.03, 4.09 (each d, 12.5)	62.2 (t)
4		163.2 (s)			165.4 (s)
5	6.21 (s)	80.1 (d)	6.23 (s)	6.12 (s)	79.4 (d)
6	5.35 (s)		5.41 (s)	5.48 (s)	
6a		148.4 (s)			149.7 (s)
7	7.34 (d, 7.6)	125.0 (d)	7.23−7.31 (m)	7.13 (d, 7.6)	124.9 (d)
8	7.31 (t, 7.6)	131.4 (d)	7.23−7.31 (m)	7.26 (m)	131.8 (d)
9	6.95 (t, 7.6)	120.4 (d)	6.93 (t, 7.8)	6.82 (t, 7.6)	120.4 (d)
10	6.80 (d, 7.6)	111.2 (d)	6.79 (d, 7.8)	6.84 (d, 7.6)	111.2 (d)
10a		126.6 (s)			125.8 (s)
10b		73.8 (s)			72.0 (s)
11	3.10, 4.42 (each d, 15.4)	42.7 (t)	3.10, 4.41 (each d, 15.6)	3.38, 4.08 (each d, 14.3)	49.1 (t)
11a		73.6 (s)			78.3 (s)
1′		165.6 (s)[a]			165.5 (s)
2′-CH_3	3.17 (3H, s)	27.4 (q)[b]	3.13 (3H, s)[c]	3.19 (3H, s)	27.7 (q)
3′		76.1 (s)			74.8 (s)
3′-CH_2OH	4.27, 4.32 (each d, 12.6)	61.2 (t)	4.74, 4.95 (each d, 12.7)	4.30, 4.35 (each d, 12.5)	61.3 (t)
4′		166.9 (s)			166.9 (s)
5′-CH_3	2.96 (3H, s)	28.2 (q)	2.98 (3H, s)	2.98 (3H, s)	28.3 (q)
6′		76.5 (s)			76.6 (s)
7′	3.71, 3.88 (each d, 15.4)	27.1 (t)	3.74, 3.89 (each d, 15.6)	3.74, 3.87 (each d, 15.4)	27.1 (t)
8′		107.7 (s)			108.0(s)
9′	7.19 (s)	127.3 (d)	7.24 (s)	7.26 (s)	126.7 (d)
10a′		134.1 (s)			134.5 (s)
11′	7.31 (br d, 6.7)	111.4 (d)	7.23−7.31 (m)	7.38 (dd, 7.6, 3.0)	111.6 (d)
12′	7.23 (m)	120.6 (d)	7.23−7.31 (m)	7.18 (m)	120.5 (d)
13′	7.23 (m)	122.8 (d)	7.23−7.31 (m)	7.18 (m)	122.9 (d)
14′	7.66 (d, 6.7)	119.2 (d)	7.67 (m)	7.63 (dd, 6.3, 3.0)	18.8 (d)
14a′		130.4 (s)			130.1 (s)
-$COCH_3$			2.17, 2.19 (each 3H, s)		

[a,b,c] Assignments with the same superscript in the same column may be interchangeable.

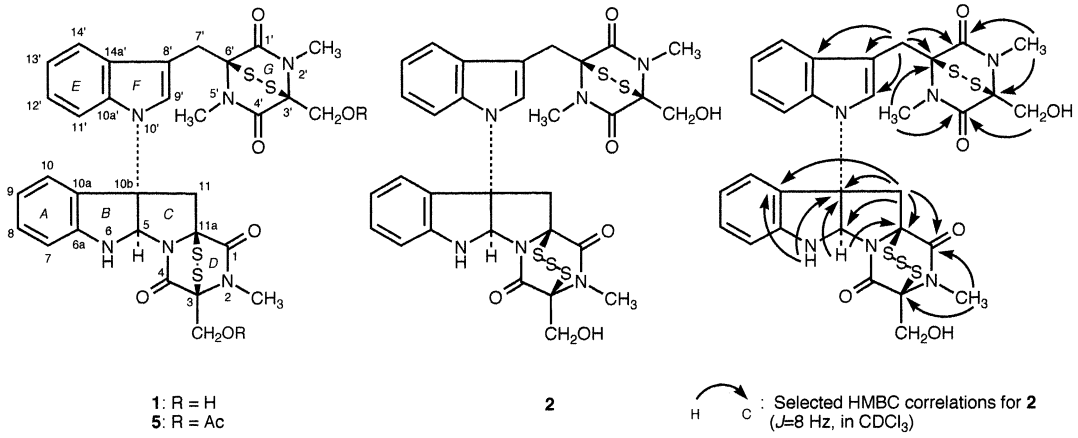

Figure 1.

has been isolated from a natural source. Accordingly, we propose to call CS-C chetoseminudin B (**3**).

The UV spectrum of CS-D (**4**) gave the absorption of an indole system, and the IR spectrum showed the absorption of OH and amide >C═O groups. On acetylation with acetic anhydride in pyridine, **4** gave monoacetate **6** [δ_H 1.45 ppm (3H, s, CH_3CO) in CD_3OD], whose molecular formula was determined to be $C_{18}H_{21}N_3O_4S_2$ by HRFABMS. Thus, the molecular formula of **4** was calculated to be $C_{16}H_{19}N_3O_3S_2$. Comparison of the ^{1}H NMR and ^{13}C NMR spectra of **4** (Table 2) with those of **3** suggested that the CH_3−N group at position 2 (δ_H/δ_C 2.99/29.1 ppm) in **3** was replaced with an H−N group in **4**. Therefore, the structure of CS-D was deduced to be **4**, as shown in Figure 2. To our knowledge, this is the first time that CS-D (**4**) has been isolated from a natural source. Accordingly, we propose to call CS-D chetoseminudin C (**4**).

Antimicrobial[7] and cytotoxic activities[8] have already been reported for chetomin (**1**). This time, the immunosuppressive activities (IC_{50} values) of **1**−**4** were calculated against Con A-induced (T-cells) and LPS-induced (B-cells) proliferations of mouse splenic lymphocytes, as shown in Table 3. Among these four natural metabolites, **1**, which possessed two epidithiodioxopiperazine moieties, and **2**,

Table 2. NMR Data for Chetoseminudins B (**3**), C (**4**), and C Monoacetate (**6**), δ (ppm) from TMS as an Internal Standard [multiplicities and coupling constants (in Hz) in parentheses]

	3 in $CDCl_3$		**4** in CD_3OD		**6** in CD_3OD
position	δ_H	δ_C	δ_H	δ_C	δ_H
1		166.9 (s)		169.0 (s)	
2-CH_3	2.99 (3H, s)	29.1 (q)			
3		73.5 (s)		68.1 (s)	
3-CH_2OH	3.33, 3.85 (each d, 11.9)	63.5 (t)	2.89, 3.23 (each d, 11.6)	66.7 (t)	3.76, 3.96 (each d, 11.3)
3-SCH_3	2.16 (3H, s)	12.9 (q)	2.07 (3H, s)	13.4 (q)	2.20 (3H, s)
4		165.7 (s)		167.8 (s)	
5	7.24 (s)				
6		65.9 (s)		69.3 (s)	
6-SCH_3	2.40 (3H, s)	14.5 (q)	2.23 (3H, s)	13.9 (q)	2.39 (3H, s)
7	3.26, 3.90 (each d, 14.6)	36.6 (t)	3.13, 3.63 (each d, 14.3)	36.9 (t)	3.18, 3.80 (each d, 14.3)
8		107.5 (s)		108.5 (s)	
9	7.11 (s)	125.9 (d)	7.04 (s)	126.5 (d)	7.13 (s)
10	8.50 (br s)				
10a		135.9 (s)		137.6 (s)	
11	7.31 (d, 7.6)	111.4 (d)	7.23 (d, 7.6)	112.1 (d)	7.26 (d, 7.6)
12	7.18 (t, 7.6)	122.2 (d)	6.97 (td, 7.6, 0.9)	122.4 (d)	7.04 (t, 7.6)
13	7.11 (t, 7.6)	119.0 (d)	6.89 (td, 7.6, 0.9)	120.1 (d)	6.97 (t, 7.6)
14	7.65 (d, 7.6)	119.8 (d)	7.54 (dd, 7.6, 0.9)	120.1 (d)	7.68 (d, 7.6)
14a		127.3 (s)		129.1 (s)	
$-COCH_3$					1.45 (3H, s)

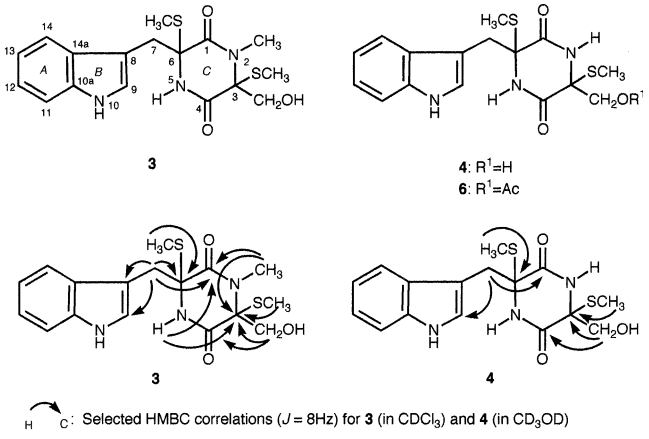

Figure 2.

Table 3. Immunosuppressive Effects of Chetomin (**1**), Chetoseminudins A (**2**), B (**3**), and C (**4**), and Other Compounds on Con A-Induced and LPS-Induced Proliferations of Mouse Splenic Lymphocytes[a]

	IC_{50} (μg/mL)	
compound	Con A-induced	LPS-induced
1	0.17	0.09
2	0.18	0.13
3	24	n.t.[b]
4	>50	n.t.
azathioprine	2.7	2.7
cyclosporin A	0.04	0.07
FK506 (tacrolimus)	1.5×10^{-5}	1.6×10^{-3}

[a] The IC_{50} value of each sample was calculated from the correlation curve between the sample concentration (horizontal axis) and the cell proliferation (vertical axis). The curve of each sample was drawn with 7 points, each of which represented the mean of 3 experiments on each correlation between 7 different concentrations and cell proliferations. [b] n.t.: not tested.

which possessed one epidithio- and one epitrithiodioxopiperazine moiety, displayed comparatively high immunosuppressive activity. Meanwhile, **3** and **4**, which possessed dethio-dimethylthiodioxopiperazine moieties, displayed very low activity. Accordingly, the immunosuppressive activity of **1** and **2** might result from their epipolythiodioxopiperazine moiety in their molecules. This seems to be similar to the result already obtained on other fungal epipolythiodioxopiperazines, sporidesmin and gliotoxin, by Muellbacher et al.[9] Here, the inhibitory activity of **1** and **2** against the proliferation of immune cells may be an aspect of widely cytotoxic activities of these compounds. The investigation on the mode of action of the inhibitory activity of these compounds against immune cells is now in progress.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP 140 digital polarimeter. UV and IR spectra were recorded on Hitachi U-3200 and JASCO FT/IR-230 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were measured with JEOL JNM-A400 (^1H, 399.65; ^{13}C, 100.40 MHz) and -A500 (^1H, 500.00; ^{13}C, 125.65 MHz) spectrometers using chemical shift, δ(ppm), values from TMS as an internal standard. FABMS and HRFABMS spectra were recorded on a JEOL JMS-AX505 HA spectrometer using *m*-nitrobenzyl alcohol (*m*-NBA) as a matrix and a JEOL JMS-HX110A spectrometer using *m*-NBA as a matrix, respectively. Column chromatography was performed with Fuji Silysia Si gel PSQ-100B and Fuji Silysia Chromatorex octadecyl Si gel (ODS). HPLC was performed using Senshu SSC-3100, Waters 600-E, and JASCO PU-980 flow systems equipped with Senshu SSC-UV5200, Waters UV/VIS-486, and JASCO UV-970 detectors, and Senshu ODS 5251-N, Senshu Pegasil ODS, and Nomura Develosil ODS UG-5 columns at 220 nm.

Evaluation of Effects of Samples on Mitogen-Induced and Mitogen-Free Proliferations of Mouse Splenic Lymphocytes.[1b,10] Preparation of a suspension of lymphocytes in cRPMI medium [fetal bovine serum (FBS)/RPMI medium] at a concentration of 8.0×10^6 cells/mL from the spleens of three BALB/c mice (male, 7−11 weeks old, purchased from Nippon SLC Co. Ltd., Hamamatsu, Japan) was executed in the same way as described in our previous report.[10a] Con A (Sigma) and LPS (from *E. coli*, Difco) were dissolved in cRPMI medium to prepare the mitogen solutions. As the stimulative concentration of Con A or LPS, 70% of the optimum stimulative concentration of Con A or LPS, which was calculated from a dose−response curve between concentration of Con A or LPS and proliferative response of the lymphocytes, was employed. Each sample was dissolved in 1.0% EtOH in cRPMI medium to prepare a sample solution. The sample solution (100 μL) was incubated with 50 μL of the cell suspension and 50 μL of

mitogen solution (in the case of mitogen-induced proliferation) or 50 μL of cRPMI medium (in the case of mitogen-free proliferation) in a 96-well microtiter plate (Inter Med, Nunclon) at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 72 h using a CO_2 incubator (Sanyo, MCO-96). Then, 20 μL of the MTT/PBS(−) solution, which was prepared in advance by dissolution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma) in phosphate-buffered saline without $Ca^{2+}Mg^{2+}$ [PBS(−)] (Nacalai tesque) at 5.0 mg/mL, was added to the culture, and the culture was further incubated at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 4 h. The culture was centrifuged at 1000 rpm for 5 min at room temperature. After removal of the supernatant by an aspirator, 100 μL of DMSO was added to the precipitated cells to extract formazan, and the DMSO solution was vibrated to complete extraction with a microplate mixer for a few minutes. The absorbance of each DMSO solution was measured at 570 (a test wavelength) and 630 nm (a reference wavelength) with a microplate reader (Bio Rad, model 550). The effect of each sample on mitogen-induced and mitogen-free proliferations of the lymphocytes was evaluated in triplicate and expressed as a percentage of the absorbance of formazan, which was proportional to the cell proliferation, formed from MTT in the incubation with the sample to that without the sample (control).

Fungal Material. *Chaetomium seminudum* 72-S-204-1 was deposited earlier at Research Institute for Chemobiodynamics, Chiba University (present name: Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University). The voucher specimen was also deposited in Laboratory of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University. This fungus was cultivated on sterilized moistened rice in Roux flasks (200 g/flask × 85) at 25 °C for 24 days to give white-yellowish-green moldy rice.

Isolation of CS-A (1)−D (4) from *C. seminudum*. The moldy rice was extracted with EtOAc (11 L) with shaking at room temperature for 6 h twice to give an EtOAc solution (ca. 22 L), which gave, after evaporation in vacuo, an EtOAc extract (61.2 g). The IC_{50} value of the EtOAc extract against the proliferation of mouse splenic lymphocytes induced with Con A (T-cells) was 0.7 μg/mL. A portion of the EtOAc extract (57.8 g) was suspended in H_2O (1.0 L). The suspension was partitioned with *n*-hexane (1.0 L) twice into an *n*-hexane layer (after evaporation in vacuo, 25.7 g) and an aqueous suspension. The aqueous suspension was further partitioned with EtOAc (1.0 L) twice into an EtOAc layer (24.2 g) and an aqueous layer (4.1 g). The IC_{50} values of the *n*-hexane, the EtOAc, and the aqueous layers against the proliferation of the T-cells were 29.8, <0.4, and 45.6 μg/mL, respectively. A part of the EtOAc layer (8.1 g) was subjected to chromatography on a Si gel column (Fuji Silysia PSQ-100B) with *n*-hexane−EtOAc (1:1, v/v), (1:2), (1:4), (1:4), EtOAc, and MeOH to give fractions 1a−1f (0.62, 1.10, 4.09, 0.18, 0.20, 1.25 g), respectively. Fraction 1c was treated with MeOH (20 mL) to give a MeOH-insoluble part (2.97 g). A portion of the MeOH-insoluble part (65 mg), whose IC_{50} value against the proliferation of the T-cells was <0.4 μg/mL, was chromatographed on an HPLC ODS column with 60% CH_3CN at a flow rate of 8.0 mL/min to afford CS-A (**1**) (40.5 mg) and CS-B (**2**) (8.8 mg). Fraction 1d was chromatographed on a Si gel column (PSQ-100B) with $CHCl_3$−MeOH (50:1), (40:1), (30:1), (20:1), (10:1), and MeOH to give fractions 2a−2f (27, 57, 32, 12, 18, and 16 mg), respectively. Fraction 2c was further chromatographed on an HPLC ODS column with 60% CH_3CN at a flow rate of 8.0 mL/min to afford CS-C (**3**) (12.0 mg). Fraction 2e was further chromatographed on an HPLC ODS column with 30% CH_3CN at a flow rate of 2.0 mL/min to afford CS-D (**4**) (2.2 mg). Fraction 2d was chromatographed in a similar way as for the purification of **4** from fraction 2e to afford further **4** (1.0 mg). Fraction 1e was further chromatographed on an ODS column (Waters C_{18} Sep-Pak cartridge, 16 × 50 mm) with 30% CH_3CN to afford **4** (32 mg).

CS-A (chetomin) (1): white powder (lit.[8c] colorless powder); mp 218−220 °C (lit.[8c] 218−220 °C); $[\alpha]_D^{25}$ +278° (*c* 0.124, $CHCl_3$) (lit.[7b] +257° (*c* 0.1, $CHCl_3$)); UV (EtOH) λ_{max} (log ϵ) 276 (3.80), 287 (3.83), 295 (3.82) nm (lit.[8c] 275 (3.92), 284 (4.01), 294 (3.92)); IR ν_{max} (KBr) 3424, 2925, 1685, 1680, 1458, 1349, 1233, 1067, 745 cm^{-1} (lit.[8c] 3400, 1680, 1672, 1605, 1450, 1347, 1230, 1065, 742); positive FABMS *m/z* 711 [(M + H)$^+$]; HRFABMS *m/z* 711.1177 (calcd for $C_{31}H_{31}N_6O_6S_4$ [(M + H)$^+$], 711.1188); ^{1}H NMR and ^{13}C NMR, see Table 1.

CS-B (chetoseminudin A) (2): white amorphous solid; $[\alpha]_D^{24}$ +279° (*c* 0.052, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 277 (3.88), 285 (3.89), 295 (3.87) nm; IR ν_{max} (KBr) 3423, 2919, 1685, 1678, 1672, 1458, 1354, 1259, 1065, 742 cm^{-1}; positive FABMS *m/z* 743 [(M + H)$^+$]; HRFABMS *m/z* 743.0869 (calcd for $C_{31}H_{31}N_6O_6S_5$ [(M + H)$^+$], 743.0909); ^{1}H NMR and ^{13}C NMR, see Table 1.

CS-C (chetoseminudin B) (3): pale yellow amorphous solid; $[\alpha]_D^{24}$ +40° (*c* 0.341, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 274 (3.73), 281 (3.76), 290 (3.69) nm; IR ν_{max} (KBr) 3410, 2922, 1655, 1638, 1425, 1388, 1065, 742 cm^{-1}; positive FABMS *m/z* 379 (M$^+$); HRFABMS *m/z* 379.1015 (calcd for $C_{17}H_{21}N_3O_3S_2$ (M$^+$), 379.1025); ^{1}H NMR and ^{13}C NMR, see Table 2.

CS-D (chetoseminudin C) (4): pale yellow amorphous solid; UV (EtOH) λ_{max} (log ϵ) 274 (3.72), 281 (3.74), 290 (3.70) nm; IR ν_{max} (KBr) 3410, 2923, 1671, 1665, 1420, 1262, 1054, 744 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2.

Acetylation of CS-A (1). After addition of acetic anhydride (200 μL) to a solution of **1** (6.0 mg) in pyridine (200 μL), the solution was stirred at room temperature for 1 h. After pouring a small volume of cold water into the solution, the reaction mixture was extracted with EtOAc (2 mL) three times. The EtOAc solution was treated as usual to afford **5** (5.7 mg).

CS-A diacetate (5): pale yellow solid; ^{1}H NMR, see Table 1.

Acetylation of CS-D (4). After addition of acetic anhydride (250 μL) to a solution of **4** (6.6 mg) in pyridine (250 μL), the solution was stirred at room temperature for 1 h. After pouring a small volume of cold water into the solution, the reaction mixture was extracted with EtOAc (2 mL) three times. The EtOAc solution was treated as usual to give a crude product, which was then purified on a preparative ODS TLC (Merck RP-18F_{254}) plate developed with 40% CH_3CN followed by extraction with EtOAc to afford **6** (2.7 mg).

CS-D monoacetate (6): pale yellow solid; $[\alpha]_D^{24}$ −204° (*c* 0.08, $CHCl_3$); positive FABMS *m/z* 407 (M$^+$); HRFABMS *m/z* 407.0950 (calcd for $C_{18}H_{21}N_3O_4S_2$ (M$^+$), 407.0973); ^{1}H NMR, see Table 2.

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