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The Relationship between the CD Cotton Effect and the Absolute Configuration of FD-838 and Its Seven Stereoisomers

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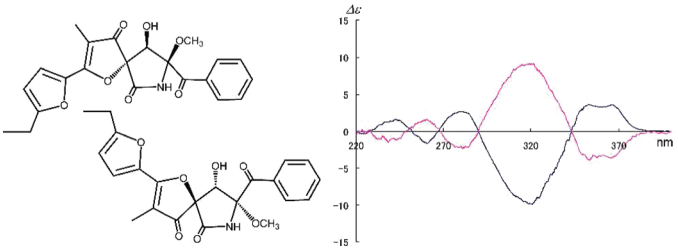

Three new metabolites having a spiro-heterocyclic γ-lactam core, cephalimysins B−D (**1**−**3**), as well as FD-838 (**4**) were isolated from a culture broth of *Aspergillus fumigatus* that was originally separated from the marine fish *Mugil cephalus*. Compounds **1**−**3** are the diastereomers of **4**. Compounds **2** and **3** exhibit an opposite absolute configuration at a spiro carbon to that of other known naturally occurring spiro-heterocyclic γ-lactams. In addition, we succeeded in the chemical transformation of the four natural products (**1**−**4**) into their epimers (**1′**−**4′**) at C-8 to afford all the stereoisomers of FD-838 (**4**) with three stereogenic centers. Consequently, the relationship between the absolute configuration at stereogenic centers and the CD Cotton effects for these compounds could be unambiguously established. All of the compounds except **1** moderately inhibited the growth of cultured P388 and HL-60 cell lines.

Introduction

A variety of natural products with a spiro-heterocyclic γ-lactam structure have been isolated from diverse microorganisms, including (−)-pseurotin A from *Psudeurotium ovalis*,[1] (+)-synerazol from *Aspergillus fumigatus*,[2] and azaspirene from *Neosartorya* sp.[3] We also reported the structure of novel spiro-heterocyclic γ-lactams isolated from parasitic fungi of marine animals in our search for active antitumor compounds,[4] such as cephalimysin A from *A. fumigatus*.[5] The absolute configuration of most of these γ-lactams was determined by asymmetric total synthesis;[6] however, that of pseurotin[1a] was determined by X-ray diffraction analysis of a dibromo derivative,[1a] while the absolute configurations of synerazol[2b] and cephalimycin A[5] were confirmed using the modified Mosher's method after the derivation of hydroxy derivatives.[2b,5] Although one of the most effective means of

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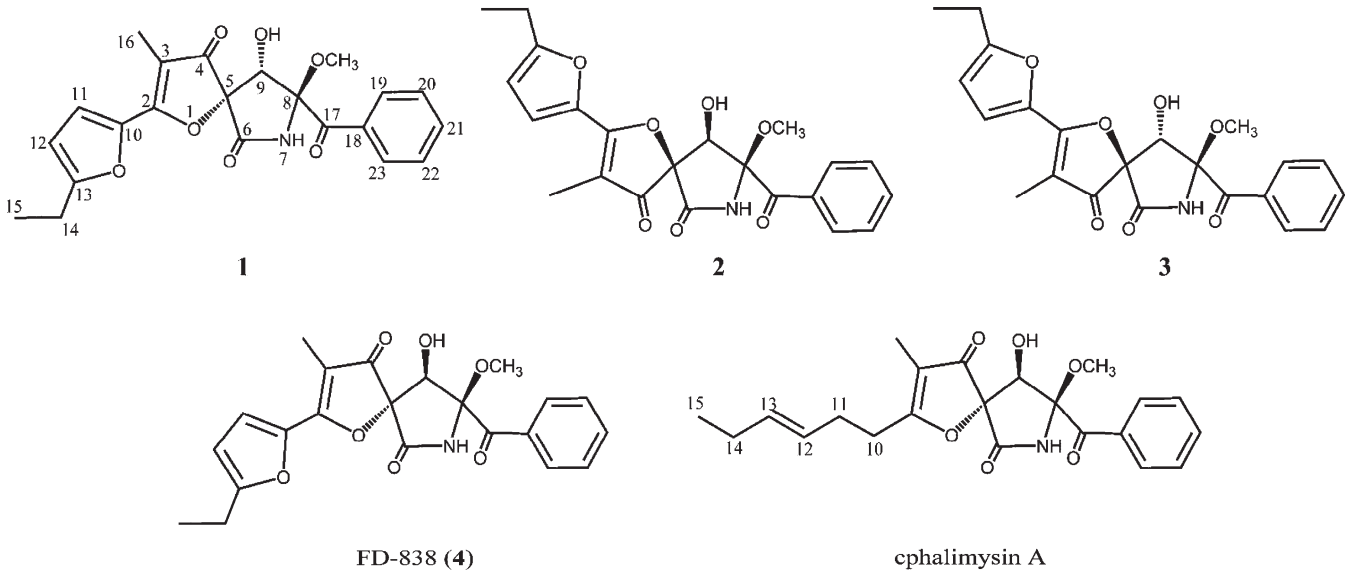

FIGURE 1. Structures of natural products from *A. fumigatus*.

determining the absolute configuration of organic compounds, CD spectroscopy has never been employed to confirm the absolute configuration of spiro-heterocyclic γ-lactams.

Meanwhile, we succeeded in the isolation of all four diastereoisomers of FD-838, a spiro-heterocyclic γ-lactam having three stereogenic centers, from culture broth of *A. fumigatus* OUPS-T106B-5, which was originally obtained from the marine fish *Mugil cephalus*. Moreover, we prepared a set of four unnatural forms of FD-838 by chemical treatment of FD-838 and its naturally occurring diastereomers. With all eight stereoisomers in hand, CD spectra could be analyzed precisely. We herein report the structural determination of these stereoisomers using CD spectra as well as ^{1}H and ^{13}C NMR spectra. In addition, we describe their cytotoxic activity against the murine P388 leukemia, human HL-60 leukemia, murine L1210 leukemia, and human KB epidermoid carcinoma cell lines.

Results and Discussion

Bioassay-directed fractionation (cytotoxicity in P388 cells) of an ethyl acetate extract of the culture broth of *A. fumigatus* OUPS-T106B-5 as reported previously,[5] employing stepwise a combination of Sephadex LH-20 and silica gel column chromatographies, followed by reversed-phase HPLC, afforded cephalimysin B−D (**1**−**3**) together with FD-838 (**4**) (Figure 1).[7]

Cephalimysin B (**1**) had the molecular formula $C_{22}H_{21}NO_7$, as established from the $[M + H]^+$ peak in HRFABMS. Its IR spectrum exhibited bands at 3304, 2932, 1732, 1695, 1621, 1568, and 1509 cm^{-1}, characteristic of a hydroxy group and an amide. Close inspection of the ^{1}H and ^{13}C NMR spectra (Table 1) of **1** using DEPT and ^{1}H−^{13}C correlation spectroscopy (HSQC) revealed the presence of one primary methyl (C-15), one olefinic methyl (C-16), one methoxy group (8-OCH$_3$), one sp^3-hybridized methylene (C-14), one oxygen-bearing sp^3-methine (C-9), two oxygen-bearing quaternary sp^3-carbons (C-5 and C-8), seven sp^2-methines (C-11, C-12, C-19, C-20, C-21, C-22, and C-23), five quaternary sp^2-carbons (C-2, C-3, C-10, C-13, and C-18) including three oxygen-bearing quaternary carbons (C-2, C-10, and C-13), two conjugated carbonyl groups (C-4 and C-17), one amido (C-6 and N-7), and one hydroxy group (9-OH).

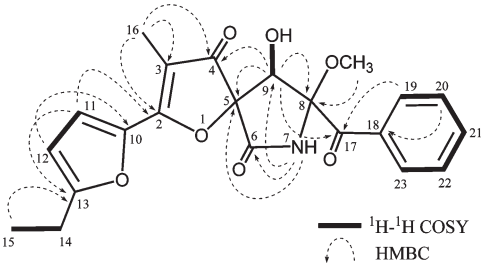

FIGURE 2. Selected ^{1}H−^{1}H COSY and HMBC correlations of **1**.

^{1}H−^{1}H COSY analysis of **1** gave four partial structural units as shown by bold lines in Figure 2. The connection of these units with the remaining functional groups was determined on the basis of HMBC correlations summarized in Figure 2. The above data revealed that the planar structure of **1**, elucidated as shown in Figure 2, is the same as that of FD-838 (**4**), which was identified by comparison with data in the literature.[7] The general features of the ^{1}H and ^{13}C NMR spectra of **1** closely resembled those of **4** except for a chemical shift of the proton signal for H-9 (δ_H 4.87) and the carbon signals for C-8 (δ_C 94.9) and C-9 (δ_C 77.0). In NOESY experiments, no correlation that demonstrated the difference in stereochemistry between **1** and **4** was observed.

Cephalimysins C (**2**) and D (**3**) were assigned the same molecular formula as **1** and **4** based on deduction made from HRFABMS data, and analyses of HMBC correlations confirmed that **2** and **3** had the same planar structure as the above cephalimysins. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) showed features closely resembling those of **4** except for a difference in the chemical shifts of the carbon signals for C-4 (δ_C 198.1) and C-5 (δ_C 86.6). On the other

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TABLE 1. NMR Data for 1−4 in $CDCl_3$

	1			2			3			4		
position	δ_H[a]	*J*/Hz	δ_C	δ_H[a]	*J*/Hz	δ_C	δ_H[a]	*J*/Hz	δ_C	δ_H[a]	*J*/Hz	δ_C
1												
2			170.1 (s)			171.2 (s)			172.8 (s)			172.5 (s)
3			108.1 (s)			109.3 (s)			109.3 (s)			107.8 (s)
4			195.8 (s)			198.1 (s)			199.5 (s)			195.6 (s)
5			87.9 (s)			86.6 (s)			85.6 (s)			89.6 (s)
6			168.0 (s)			167.1 (s)			167.9 (s)			166.2 (s)
7	7.57 s			7.42 s			7.40 s			7.44 s		
8			94.9 (s)			92.4 (s)			96.5 (s)			91.5 (s)
9	4.87 d	3.9	77.0 (d)	4.59 d	12.4	73.4 (d)	4.90 d	4.1	79.1 (d)	4.69 d	12.7	74.2 (d)
10			143.1 (s)			143.4 (s)			143.3 (s)			143.3 (s)
11	7.02 d	3.4	117.9 (d)	7.10 d	3.4	117.7 (d)	7.17 d	3.4	118.9 (d)	7.04 d	3.4	118.3 (d)
12	6.19 d	3.4	107.7 (d)	6.26 d	3.4	107.6 (d)	6.29 d	3.4	107.9 (d)	6.23 d	3.4	107.9 (d)
13			163.8 (s)			163.8 (s)			164.5 (s)			163.7 (s)
14	2.74 q	7.6	21.8 (t)	2.79 q	7.6	21.8 (t)	2.80 q	7.6	21.9 (t)	2.75 q	7.6	21.8 (t)
15	1.28 t	7.6	11.7 (q)	1.32 t	7.6	11.7 (q)	1.32 t	7.6	11.7 (q)	1.28 t	7.6	11.8 (q)
16	2.03 s		6.2 (q)	2.02 s		6.2 (q)	2.04 s		6.0 (q)	2.01 s		6.2 (q)
17			193.3 (s)			193.8 (s)			192.4 (s)			194.6 (s)
18			133.9 (s)			133.0 (s)			133.9 (s)			132.4 (s)
19	8.26 d	8.7	129.8 (d)	8.32 d	8.5	130.6 (d)	8.23 d	8.5	129.4 (d)	8.32 d	8.5	130.6 (d)
20	7.48 t	7.6	128.6 (d)	7.48 t	7.7	128.6 (d)	7.49 t	7.6	128.8 (d)	7.49 t	7.6	128.7 (d)
21	7.61 t	7.6	134.0 (d)	7.63 t	7.7	134.5 (d)	7.62 t	7.6	134.0 (d)	7.64 t	7.6	134.5 (d)
22	7.48 t	7.6	128.6 (d)	7.48 t	7.7	128.6 (d)	7.49 t	7.6	128.8 (d)	7.49 t	7.6	128.7 (d)
23	8.26 d	8.7	129.7 (d)	8.32 d	8.5	130.6 (d)	8.23 d	8.5	129.4 (d)	8.32 d	8.5	130.6 (d)
8-OCH_3	3.35 s		51.4 (q)	3.37 s		51.7 (q)	3.30 s		51.4 (q)	3.40 s		51.6 (q)
9-OH	2.98 d	3.9		3.37 d	12.4		5.36 d	4.1		4.13 d	12.7	

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* /Hz). Numbers in parentheses indicate the proton coupling with that position.

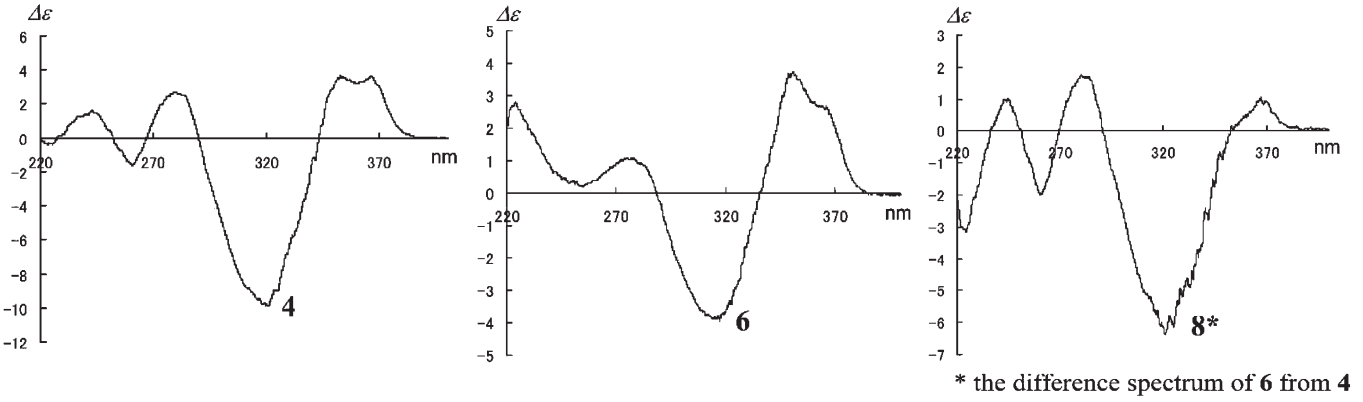


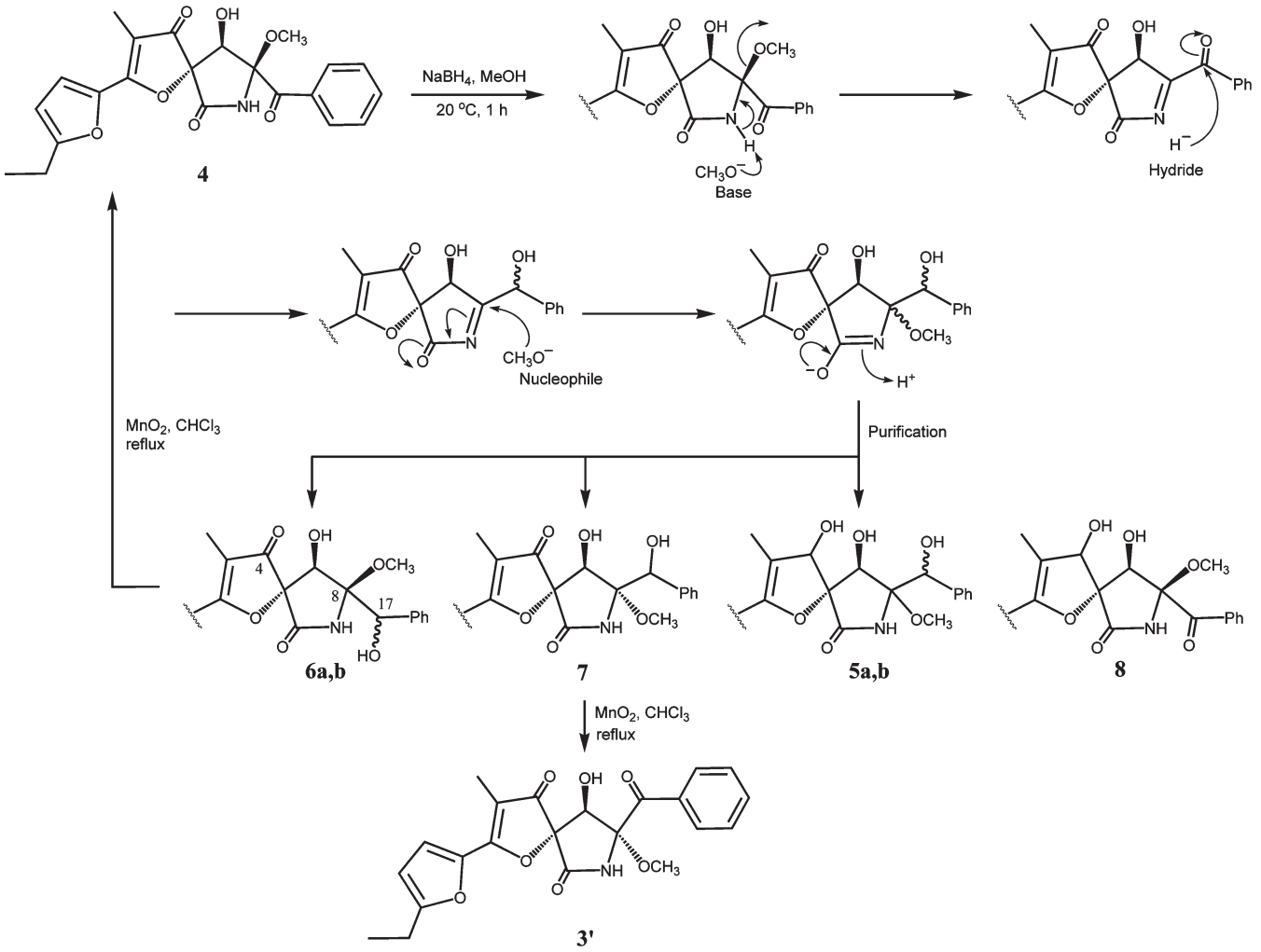
FIGURE 3. CD spectra of **4**, **6**, and **8**.

hand, the difference in chemical shift between **3** [the proton signal: H-9 (δ_H 4.90); the carbon signals: C-4 (δ_C 199.5), C-5 (δ_C 85.6), C-8 (δ_C 96.5) and C-9 (δ_C 79.1)] and **4** [the proton signal: H-9 (δ_H 4.69); the carbon signals: C-4 (δ_C 195.6), C-5 (δ_C 89.6), C-8 (δ_C 91.5) and C-9 (δ_C 74.2)] was the same as that of both **1** and **2** as described above.

The spectroscopic study suggested **1**−**3** to be the diastereomers of FD-838 (**4**), whose absolute configuration was recently confirmed by enantioselective synthesis.[6e] Thus, assignment of the CD spectrum of FD-838 (**4**) (Figure 3) was attempted. Compound **4** was reduced with sodium borohydride to afford **5a**,**b** (20.2%), in which the C-4 and C-17 carbonyl groups were reduced to alcohols, **6a**,**b** (39.9%), and **7** (7.8%), where the C-17 carbonyl group was converted into a benzyl alcohol moiety (Scheme 1).

The reduction products **6a**,**b** could be reconverted to **4** by oxidation with manganese dioxide in dichloromethane, while the oxidation of **7** gave the product **3′**, the ^1H and ^{13}C NMR spectra of which were identical with those of **3**. On the basis of these results, **6a**,**b** and **7** having the same planar structure were identified as stereoisomers at the C-8 position, i.e., the 8β-OCH_3 and 8α-OCH_3 derivatives, respectively (vide infra). A plausible mechanism for this reaction is shown in Scheme 1. The 8-OCH_3 group was eliminated as a result of the abstraction of an amide proton by the methoxide anion, and then, the methoxide anion attacked C-8 as a nucleophile, accompanying the synchronous reduction of the carbonyl group at C-17. In principle, the CD spectrum of **6a** or **6b** should show the absorption band attributable to the enone moiety, while that of **8** would exhibit the absorption band ascribed to the benzophenone moiety of **4** (Figure 3). Since, in practice, **8** was not obtained in the reduction of **4**, the CD spectrum of **8** was determined by subtracting the CD spectrum of **6** from that of **4**. In conclusion, the positive ($\Delta\varepsilon_{352}$ +3.7) and negative ($\Delta\varepsilon_{320}$ −10.1) Cotton effects in the CD spectrum of **4** indicate *S* configuration at C-5 and C-8 (Figure 3).

SCHEME 1. Plausible Mechanism for the Reduction of 4



Compound **3′**, the oxidation product of **7**, was identical to the natural product **3** in terms of IR, ^{1}H, and ^{13}C NMR data; however, its specific rotation and CD spectrum (Figure 4) were the reverse of those of **3**. This finding revealed that **3** was the enantiomer of **3′**, being the diastereomer of **4** at C-5 and C-9. Next, as expected, treatment of **4** with conc. H_2SO_4 in MeOH also gave the product **3′** at a constant ratio (14.6%) as shown in Scheme 2. The same acid treatment was applied to **3** to produce **4′**, which was identified as the enantiomer of **4** on the basis of accordance of all spectroscopic data expect the CD data (Figure 4). The above evidence revealed the absolute configuration of **3** and **4′**.

Split Cotton effects (λ_{max} ~350 and 320 nm) were observed for the 5*S*,8*S* isomer **4**, i.e., positive and negative Cotton

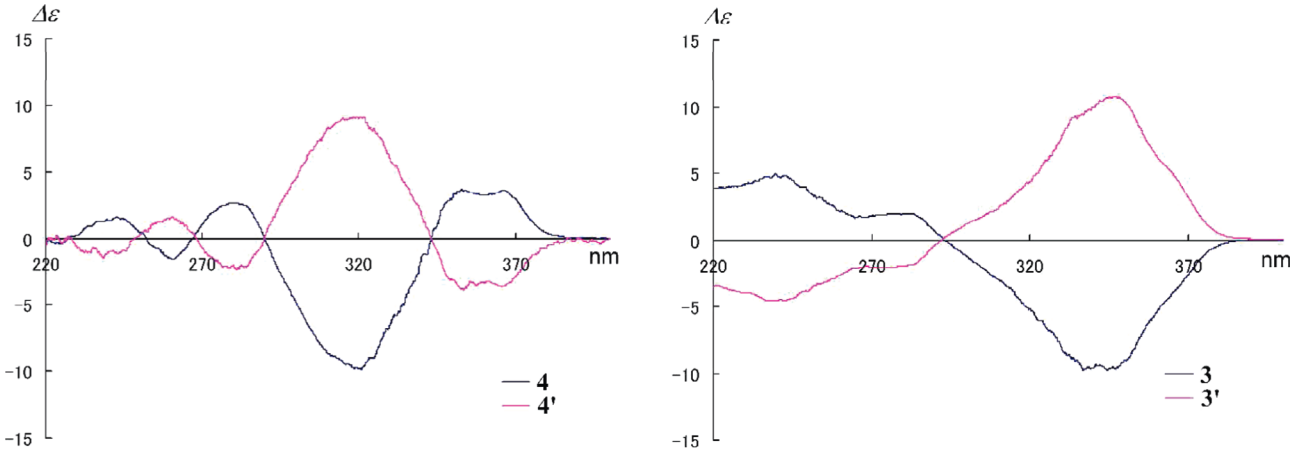
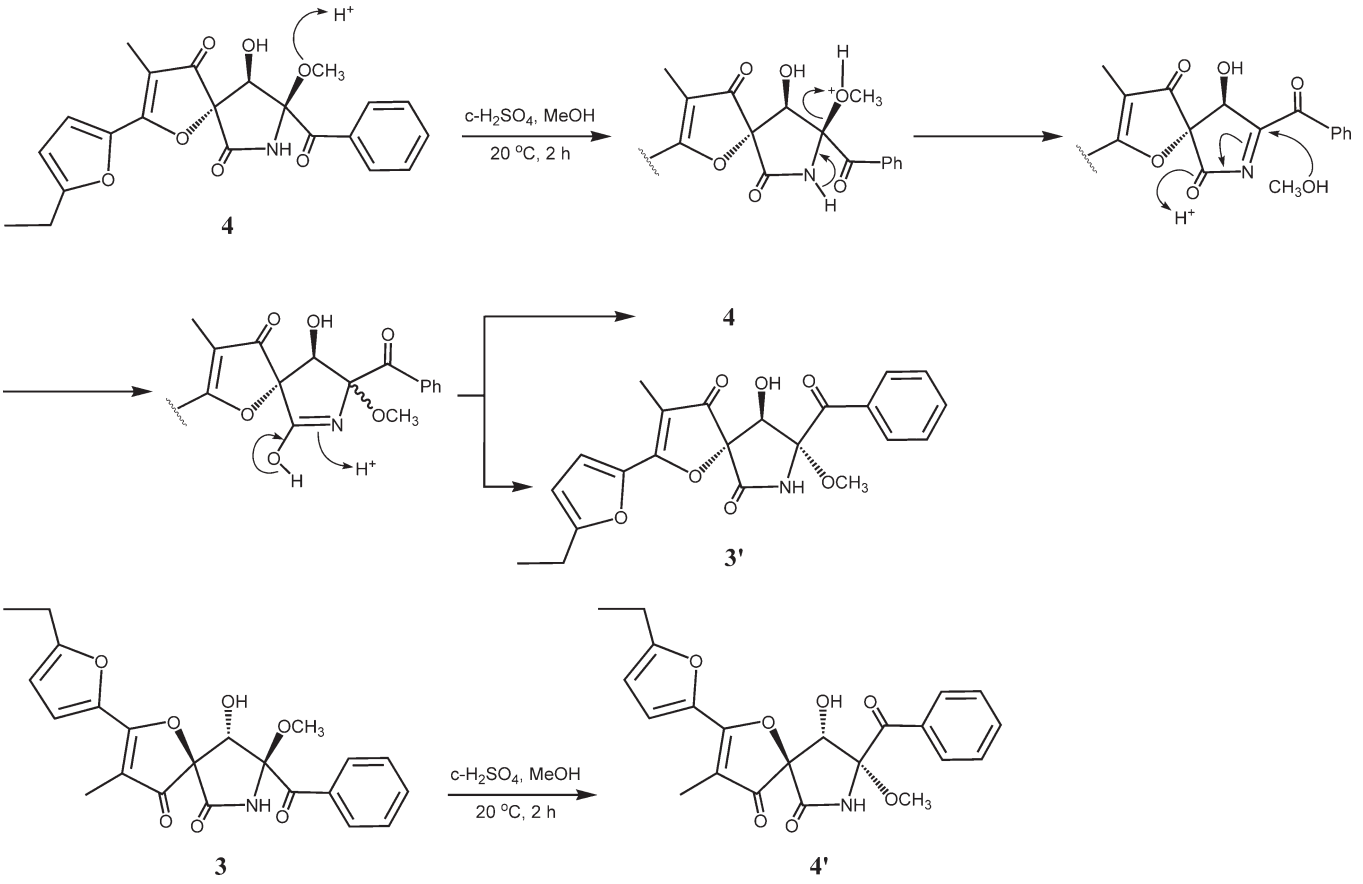


FIGURE 4. CD spectra of **4**, **4′**, **3**, and **3′**.

SCHEME 2. Acid-Catalyzed Treatment of Compound 4



effects. The 5*R*,8*R* isomer **4′** showed negative and positive Cotton effects, while the 5*R*,8*S* isomer **3** and the 5*S*,8*R* isomer **3′** showed a negative Cotton effect and a positive Cotton effect (λ_{max} ~345 nm), respectively (Figure 4).

Compound **1** showed a CD spectrum resembling that of **4** (Figure 5). Since the rule concerning the Cotton effect did not involve the configuration at C-9, the similarity of the CD spectra suggested **1** to be an epimer of **4** at C-9; i.e., **1** possessed the 5*S*,8*S*,9*S* absolute configuration. Treatment of **1** with concd H_2SO_4 in MeOH gave compound **2′** (Scheme 3), which was identical with **2** in all spectroscopic data expect the CD data (Figure 5) and had a $[\alpha]_D$ value opposite that of **2**. This evidence showed that the natural product **2** was the enantiomer of the reaction product **2′** obtained from **1**. As expected, the epimerization at C-8 of **2** with concd H_2SO_4 gave the product **1′**, the enantiomer of **1**. Thus, the absolute configurations of **1′**, **2**, and **2′** could be deduced as (5*R*,8*R*,9*R*), (5*R*,8*S*,9*R*), and (5*S*,8*R*,9*S*), respectively.

We succeeded in the isolation of all stereoisomers of FD-838 (**4**) including four reaction products and, therefore, could establish the relationship between the absolute configurations at C-5 and C-8 in the spirofuranone−lactam skeleton and the CD Cotton effects. In addition, we found that the coupling constants of H-9 and 9-OH in the ^{1}H NMR spectra

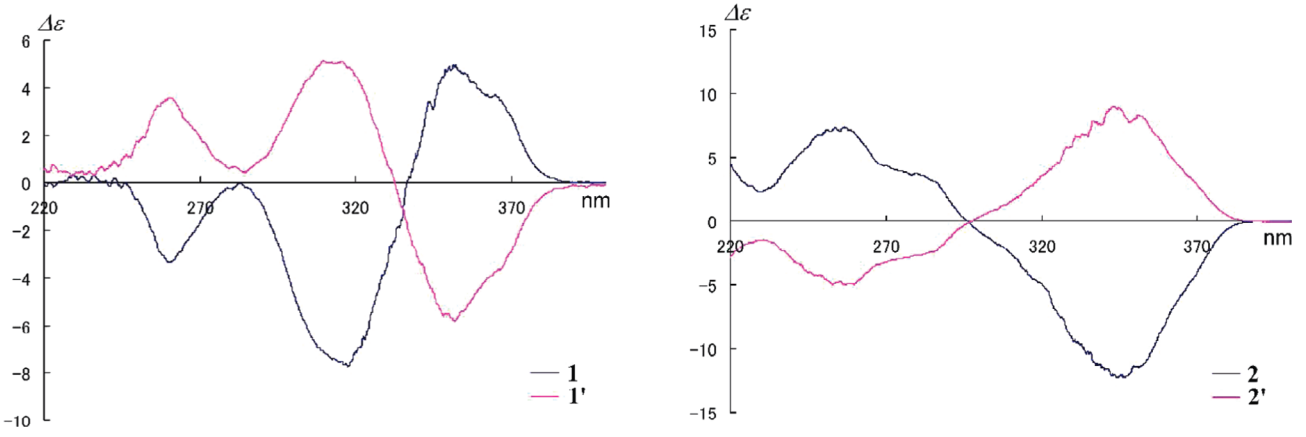


FIGURE 5. CD spectra of **1**, **1′**, **2**, and **2′**.

SCHEME 3. Acid Treatment of 1 and 2

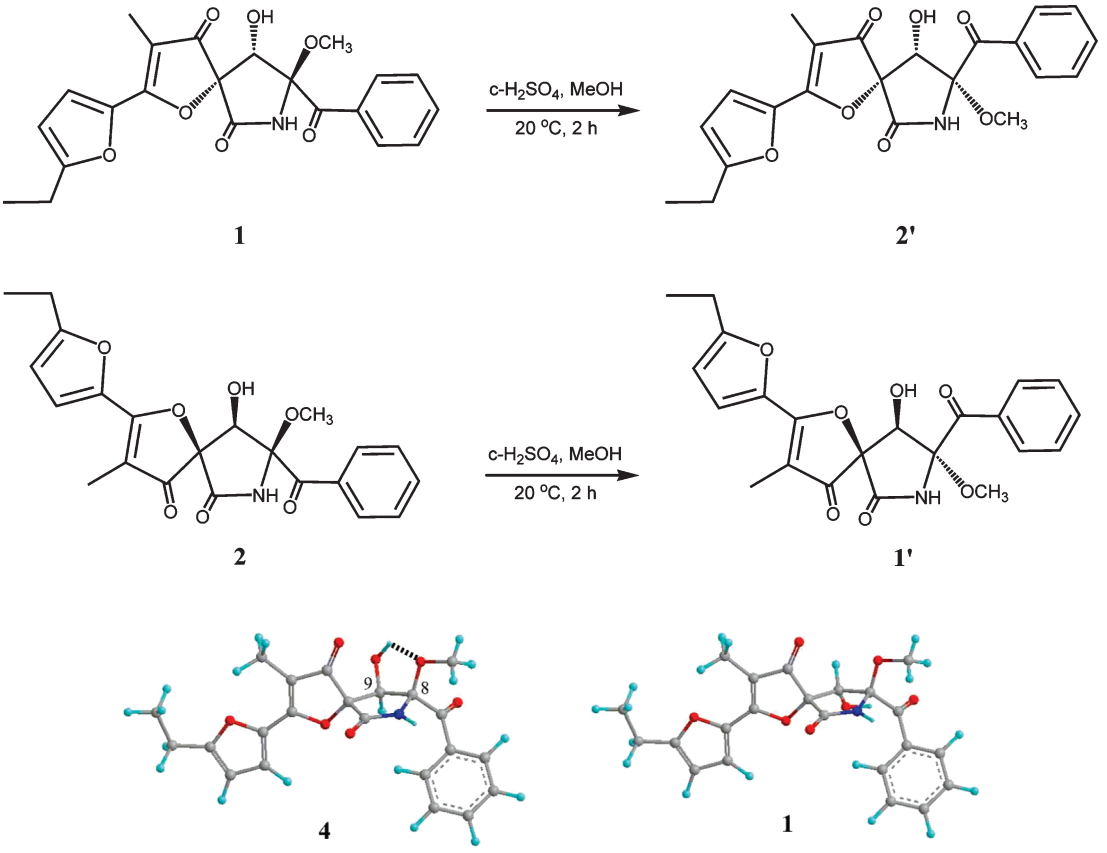


FIGURE 6. Hydrogen-bond interactions in **4**.

TABLE 2. Cytotoxicity Assay against P388, HL-60, L1210, and KB Cells

	IC_{50} (μM)[a]			
compd	cell line P388	cell line HL-60	cell line L1210	cell line KB
1	> 200	> 200	> 200	> 200
2	53.5	58.4	> 200	> 200
3	51.1	48.7	> 200	> 200
4	56.0	60.8	70.5	73.0
1′	53.5	56.0	> 200	> 200
2′	60.8	51.1	184.2	> 200
3′	70.6	53.5	150.9	> 200
4′	58.4	60.8	111.9	> 200
5-fluorouracil[b]	2.5	2.2	2.1	7.7

[a]DMSO was used as vehicle. [b]Positive control.

show the orientations of 9-OH and 8-OCH_3, i.e., 9-OH oriented *cis* to 8-OCH_3 for a large coupling constant (J = 12 Hz) and *trans* to 8-OCH_3 for a small coupling constant (J = 4 Hz). This would occur by a hydrogen bond, which held the conformation of 9-hydroxy as shown in Figure 6. Hayashi and co-workers had reported that the hydrogen bond prevented the racemization at C-9 in the last step, the elimination of the protecting group, of the synthesis process of synerazol.[6d] Such findings are useful for examining the stereochemistry of spirofuranone−lactams.

As a primary screening for antitumor activity, cancer cell growth inhibitory properties of the natural products **1**−**4** and their epimers **1′**−**4′** were examined using the murine P388 leukemia, the human HL-60 leukemia, the murine L1210 leukemia, and the human KB epidermoid carcinoma cell lines. All the compounds except **1** exhibited moderate activity against the P388 and HL-60 cell lines (Table 2). The cancer cell growth inhibitory properties did not imply a structure−activity relationship, and so molecular target screening for inhibitory activities of histone deacetylase, protein kinase, telomerase, and fenecyltransferase are in progress.

Conclusion

All diastereomers of FD-838 (**4**), referred to as cephalimysins B−D (**1**−**3**), were obtained from the broth of *A. fumigatus*. Since acid treatment of **1**−**4** led to the epimerization of the C-8 acetal carbon to afford all stereoisomers of **4**, the CD cotton effects could be studied comprehensively. The results concerning the relationship between CD spectra and spiro-hetero γ-lactams will be valuable for determining the absolute configuration of related compounds.

Experimental Section

General Methods. NMR spectra were recorded at 27 °C with TMS as internal reference. Liquid chromatography over silica gel (mesh 230−400) was performed at medium pressure and detected with TLC [the solvent system CH_2Cl_2−MeOH (90:10)], and compounds were viewed under a UV lamp, sprayed with 10% H_2SO_4, and heated. ODS HPLC was detected with a differential refractometer.

Culture and Isolation of Metabolites. A strain of *A. fumigatus* was initially isolated from the marine fish *M. cephalus* captured

in Katsuura Bay, Japan, in October 2000. The fish was disinfected with EtOH and its gastrointestinal tract applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *A. fumigatus*. The fungal strain was cultured at 27 °C for 6 weeks in a liquid medium (75 L) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4. The culture was filtered under suction, and the culture filtrate was extracted three times with EtOAc. The combined extracts were evaporated in vacuo to afford a mixture of crude metabolites (18.8 g) that exhibited cytotoxicity against P388 cell line (IC_{50} < 1 μg/mL). The EtOAc extract was passed through a Sephadex LH-20 column using $CHCl_3$−MeOH (1: 1) as the eluent. The second fraction (14.6 g), exhibiting strong activity, was chromatographed on a silica gel column with $CHCl_3$−MeOH gradient as the eluent. The 100% $CHCl_3$ eluate (1.2 g) was purified by ODS HPLC using MeOH−H_2O (70: 30) as the eluent to afford fraction 1 (20.3 mg) and fraction 2 (100.7 mg) exhibiting cytotoxicity. Fraction 1 was further purified by ODS HPLC using MeCN−H_2O (45: 55) as the eluent to afford **1** (7.4 mg) and **2** (8.2 mg). Fraction 2 was further purified by ODS HPLC using MeCN−H_2O (45: 55) as the eluent to afford **4** (64.0 mg) and **3** (1.1 mg).

Cephalimysin B (1): pale yellow oil; $[\alpha]^{22}_D$ +129.7 (*c* 0.09, EtOH); IR (liquid) ν_{max} 3304, 2932, 1732, 1695, 1621, 1568, 1509 cm^{-1}; UV (EtOH) λ_{max} (log ε) 210 (3.85), 249 (3.95), 318 (3.85), 355 (3.85) nm; NMR data, see Table 1 and Table S1 (Supporting Information); FABMS *m*/*z* (rel int) 412 ($[M + H]^+$, 52.2), 380 ($[M - OCH_3]^+$, 100); HRFABMS *m*/*z* 412.1394 $[M + H]^+$ (calcd for $C_{22}H_{22}NO_7$ 412.1397); CD (*c* 2.37 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 386 (0), 352 (5.5), 337 (0), 318 (−7.7), 283 (0) nm.

Cephalimysin C (2): pale yellow oil; $[\alpha]^{22}_D$ −209.2 (*c* 0.09, EtOH); IR (liquid) ν_{max} 3347, 2933, 1724, 1704, 1617, 1566, 1507 cm^{-1}; UV (EtOH) λ_{max} (log ε) 212 (3.79), 249 (3.88), 318 (3.79), 358 (3.76) nm; NMR data, see Table 1 and Table S2 (Supporting Information); FABMS *m*/*z* (rel. int. %) 412 ($[M+H]^+$, 100), 380 ($[M-OCH_3]^+$, 13.2); HRFABMS *m*/*z* 412.1395 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_7$: 412.1397); CD (*c* 2.90 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 391 (0), 346 (−12.7), 296 (0) nm.

Cephalimysin D (3): pale yellow oil; $[\alpha]^{22}_D$ −236.6 (*c* 0.11, EtOH); IR (liquid) ν_{max} 3289, 2937, 1732, 1691, 1612, 1563, 1507 cm^{-1}; UV (EtOH) λ_{max} (log ε) 210 (3.85), 248 (3.95), 315 (3.87), 356 (3.83) nm; NMR data, see Table 1 and Table S3 (Supporting Information); FABMS *m*/*z* (rel int) 412 ($[M + H]^+$, 100), 380 ($[M - OCH_3]^+$, 44.2); HRFABMS *m*/*z* 412.1399 $[M + H]^+$ (calcd for $C_{22}H_{22}NO_7$ 412.1397); CD (*c* 2.55 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 389 (0), 344 (−10.2), 293 (0) nm.

FD-838 (4): pale yellow oil; $[\alpha]^{22}_D$ +40.5 (*c* 0.10, EtOH); IR (liquid) ν_{max} 3433, 2921, 1734, 1681, 1619, 1566, 1506 cm^{-1}; UV (EtOH) λ_{max} (log ε) 210 (3.82), 252 (3.92), 319 (3.86), 356 (3.83) nm; NMR data, see Table 1; FABMS *m*/*z* (rel int) 412 ($[M + H]^+$, 100), 380 ($[M - OCH_3]^+$, 32.5); HRFABMS *m*/*z* 412.1398 $[M + H]^+$ (calcd for $C_{22}H_{22}NO_7$ 412.1397); CD (*c* 2.47 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 385 (0), 352 (3.7), 343 (0), 320 (−10.1), 290 (0) nm.

Reduction of FD-838 (4) to 5−7. $NaBH_4$ (4.0 mg) was added to a MeOH solution (1.5 mL) of FD-838 (**4**) (41.2 mg, 0.1 mmol), and the reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with water and extracted with $CHCl_3$, and the $CHCl_3$ was evaporated under reduced pressure. The residue was purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford fraction 1 (20.5 mg) and **6** (**6a**: 6.3 mg and **6b**: 10.2 mg). Fraction 1 was purified by HPLC using MeCN−H_2O (35: 65) as the eluent to afford **5** (**5a**: 5.1 mg and **5b**: 3.3 mg) and **7** (3.8 mg).

5a: pale yellow oil; $[\alpha]^{22}_D$ −158.6 (*c* 0.10, EtOH); IR (liquid) ν_{max} 3433, 2938, 1721, 1614, 1619, 1530, 1508 cm^{-1}; UV (EtOH) λ_{max} (log ε) 216 (3.96), 274 (3.97), 334 (3.71) nm; FABMS *m*/*z* (rel int) 415 ($[M]^+$, 55.6); HRFABMS $[M]^+$ *m*/*z* 415.1639 ($C_{22}H_{25}NO_7$, calcd $[M]^+$ 415.1631); ^1H NMR δ ppm ($CDCl_3$) 1.23 (3H, t, *J* = 7.5 Hz, H-15), 1.98 (3H, s, H-16), 2.04 (1H, d, *J* = 11.8 Hz, 4-OH), 2.45 (1H, d, *J* = 4.2 Hz, 17-OH), 2.61 (1H, d, *J* = 7.8 Hz, 9-OH), 2.66 (2H, q, *J* = 7.5 Hz, H-14), 3.35 (3H, s, 8-OCH_3), 4.63 (1H, d, *J* = 7.8 Hz, H-9), 4.87 (1H, d, *J* = 4.2 Hz, H-17), 5.10 (1H, d, *J* = 11.8 Hz, H-4), 6.02 (1H, d, *J* = 3.0 Hz, H-12), 6.47 (1H, d, *J* = 3.0 Hz, H-11), 6.68 (1H, br s, H-7), 7.37−7.47 (5H, m, Ar-H).

5b: pale yellow oil; $[\alpha]^{22}_D$ −150.5 (*c* 0.09, EtOH); IR (liquid) ν_{max} 3376, 2939, 1721, 1612, 1563, 1532 cm^{-1}; UV (EtOH) λ_{max} (log ε) 220 (3.97), 274 (3.95), 335 (3.81) nm; FABMS *m*/*z* (rel int) 415 ($[M]^+$, 52.6); HRFABMS $[M]^+$ *m*/*z* 415.1629 ($C_{22}H_{25}NO_7$, calcd $[M]^+$ 415.1631); ^1H NMR δ ppm ($CDCl_3$) 1.22 (3H, t, *J* = 7.5 Hz, H-15), 1.98 (3H, s, H-16), 2.09 (1H, d, *J* = 11.8 Hz, 4-OH), 2.58 (1H, br s, 17-OH), 2.65 (2H, q, *J* = 7.5 Hz, H-14), 2.74 (1H, d, *J* = 7.6 Hz, 9-OH), 3.50 (3H, s, 8-OCH_3), 4.39 (1H, d, *J* = 7.6 Hz, H-9), 4.96 (1H, d, *J* = 4.2 Hz, H-17), 5.15 (1H, d, *J* = 11.8 Hz, H-4), 5.99 (1H, d, *J* = 3.0 Hz, H-12), 6.40 (1H, d, *J* = 3.0 Hz, H-11), 6.65 (1H, br s, H-7), 7.36−7.43 (5H, m, Ar-H).

6a: pale yellow oil; $[\alpha]^{22}_D$ −78.4 (*c* 0.10, EtOH); IR (liquid) ν_{max} 3467, 2940, 1730, 1705, 1614, 1567, 1508 cm^{-1}; UV (EtOH) λ_{max} (log ε) 216 (4.00), 284 (3.90), 317 (3.98), 355 (3.95) nm; FABMS *m*/*z* (rel int) 414 ($[M + H]^+$, 31.1); HRFABMS $[M + H]^+$ *m*/*z* 414.1561 ($C_{22}H_{24}NO_7$, calcd $[M + H]^+$ 414.1553); ^1H NMR δ ppm ($CDCl_3$) 1.30 (3H, d, *J* = 7.4 Hz, H-15), 1.97 (3H, s, H-16), 2.40 (1H, br s, 17-OH), 2.77 (2H, q, *J* = 7.4 Hz, H-14), 3.20 (3H, s, 8-OCH3), 3.61 (1H, d, *J* = 11.3 Hz, 9-OH), 4.85 (1H, br s, H-17), 4.97 (1H, d, *J* = 11.3 Hz, H-9), 6.24 (1H, d, *J* = 3.6 Hz, H-12), 6.60 (1H, br s, H-7), 7.09 (1H, d, *J* = 3.6 Hz, H-11), 7.38−7.44 (3H, m, Ar-H), 7.46−7.49 (2H, m, Ar-H); ^{13}C NMR δ ppm ($CDCl_3$) 6.1 (C-16), 11.8 (C-15), 21.8 (C-14), 52.6 (8-CH_3), 73.4 (C-9), 74.3 (C-17), 87.5 (C-5), 91.6 (C-8), 107.7 (C-12), 108.0 (C-3), 118.0 (C-11), 127.2 (C-19, C-23), 128.7 (C-20, C-22), 129.0 (C-21), 137.8 (C-18), 143.6 (C-10), 163.5 (C-13), 167.5 (C-6), 172.3 (C-2), 196.0 (C-4).

6b: pale yellow oil; $[\alpha]^{22}_D$ +145.6 (*c* 0.10, EtOH); IR (liquid) ν_{max} 3418, 2941, 1730, 1698, 1616, 1568, 1509 cm^{-1}; UV (EtOH) λ_{max} (log ε) 215 (3.86), 284 (3.75), 312 (3.85), 357 (3.82) nm; FABMS *m*/*z* (rel int) 414 ($[M + H]^+$, 17.9); HRFABMS $[M + H]^+$ *m*/*z* 414.1554 ($C_{22}H_{24}NO_7$, calcd $[M + H]^+$ 414.1553); ^1H NMR δ ppm ($CDCl_3$) 1.27 (3H, d, *J* = 7.7 Hz, H-15), 1.95 (3H, s, H-16), 2.73 (2H, q, *J* = 7.7 Hz, H-14), 2.93 (1H, br s, 17-OH), 3.52 (1H, d, *J* = 11.0 Hz, 9-OH), 3.58 (3H, s, 8-OCH_3), 4.64 (1H, d, *J* = 11.0 Hz, H-9), 4.89 (1H, br s, H-17), 6.20 (1H, d, *J* = 3.6 Hz, H-12), 7.01 (1H, d, *J* = 3.6 Hz, H-11), 7.05 (1H, br s, H-7), 7.36 − 7.42 (5H, m, Ar-H); ^{13}C NMR δ ppm ($CDCl_3$) 6.1 (C-16), 11.7 (C-15), 21.7 (C-14), 52.8 (8-CH_3), 73.8 (C-9), 75.9 (C-17), 88.0 (C-5), 91.5 (C-8), 107.6 (C-12), 108.0 (C-3), 118.0 (C-11), 127.2 (C-19, C-23), 128.5 (C-20, C-22), 128.8 (C-21), 137.6 (C-18), 143.5 (C-10), 163.6 (C-13), 168.1 (C-6), 172.2 (C-2), 195.8 (C-4).

7: pale yellow oil; $[\alpha]^{22}_D$ +52.9 (*c* 0.11, EtOH); IR (liquid) ν_{max} 3348, 2939, 1729, 1698, 1625, 1569, 1509 cm^{-1}; UV (EtOH) λ_{max} (log ε) 212 (3.92), 284 (3.79), 316 (3.93), 358 (3.91) nm; FABMS *m*/*z* (rel int) 414 ($[M + H]^+$, 100); HRFABMS $[M + H]^+$ *m*/*z* 414.1554 ($C_{22}H_{24}NO_7$, calcd $[M + H]^+$ 414.1553); ^1H NMR δ ppm ($CDCl_3$) 1.31 (3H, d, *J* = 7.6 Hz, H-15), 2.05 (3H, s, H-16), 2.78 (2H, q, *J* = 7.6 Hz, H-14), 3.35 (1H, br s, 17-OH), 3.37 (3H, s, 8-OCH_3), 4.52 (1H, br s, 9-OH), 5.02 (1H, s, H-9), 5.44 (1H, br s, H-7), 5.90 (1H, br s, H-17), 6.26 (1H, d, *J* = 3.7 Hz, H-12), 7.11 (1H, d, *J* = 3.7 Hz, H-11), 7.35−7.40 (3H, m, Ar-H), 7.52−7.44 (2H, m, Ar-H); ^{13}C NMR δ ppm ($CDCl_3$) 6.2 (C-16), 11.8 (C-15), 21.8 (C-14), 49.3 (8-CH_3), 74.7 (C-9), 76.1 (C-17), 89.7 (C-5), 91.2 (C-8), 107.8 (C-12), 108.1 (C-3), 118.3 (C-11), 128.3 (C-19, C-23), 128.5 (C-20, C-21, C-22), 137.0 (C-18), 143.3 (C-10), 163.8 (C-13), 165.4 (C-6), 172.2 (C-2), 196.4 (C-4).

Oxidation of 6a to 4. MnO_2 (4.4 mg) was added to a CH_2Cl_2 solution (4.0 mL) of the reduction product **6a** (2.5 mg, 0.005 mmol), and the reaction mixture was refluxed overnight. The solution of the mixture in CH_2Cl_2 was evaporated under reduced pressure. The residue was purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford **4** (0.3 mg, yield 15.0%).

Oxidation of 6b to 4. Using the same procedure as above with **6a**, a solution of **6b** (1.8 mg, 0.004 mmol) in CH_2Cl_2 (4.0 mL) was treated with MnO_2 (3.9 mg) and purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford 4 (0.2 mg, yield 12.1%).

Oxidation of 7 to 3′. Using the same procedure as above with compound **6a**, a solution of **7** (3.5 mg, 0.009 mmol) in CH_2Cl_2 (4.0 mL) was treated with MnO_2 (7.4 mg) and purified by HPLC using MeCN−H_2O (45:55) as the eluent to afford **3′** (0.7 mg, yield 20.3%). The optical rotation and CD data of **3′** are described later.

Transformation of 1 to 2′. To a solution of cephalimysin B (**1**) (18.5 mg, 0.05 mmol) in MeOH (1.0 mL) was added concd H_2SO_4 (0.01 mL), and the reaction mixture was left at room temperature for 8 h. The mixture was diluted with water and extracted with $CHCl_3$, the extract was evaporated under reduced pressure, and then the residue was purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford **1** (8.0 mg) and **2′** (1.2 mg).

2′: pale yellow oil; $[\alpha]^{22}_D$ +201.7 (*c* 0.12, EtOH); CD (*c* 2.99 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 385 (0), 345 (9.7), 298 (0) nm.

Transformation of 2 to 1′. Using the same procedure as above with **1**, a solution of cephalimysin C (**2**) (10.0 mg, 0.02 mmol) in MeOH (1.0 mL) was treated with concd H_2SO_4 (0.01 mL) and purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford **2** (1.2 mg) and **1′** (0.4 mg).

1′: pale yellow oil; $[\alpha]^{22}_D$ −133.6 (*c* 0.08, EtOH); CD (*c* 1.50 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 389 (0), 352 (−6.0), 333 (0), 315 (5.3), 284 (0.4) nm.

Transformation of 4 to 3′. Using the same procedure as above with **1**, a solution of FD-838 (**4**) (12.4 mg, 0.03 mmol) in MeOH (1.0 mL) was treated with concd H_2SO_4 (0.01 mL) and purified by HPLC using MeCN−H_2O (45: 55) as the eluent to afford **4** (2.1 mg) and **3′** (1.8 mg).

3′: pale yellow oil; $[\alpha]^{22}_D$ +233.4 (*c* 0.16, EtOH); CD (*c* 3.19 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 388 (0), 346 (10.7), 292 (0) nm.

Transformation of 3 to 4′. Using the same procedure as above with **1**, a solution of cephalimysin D (**3**) (0.8 mg, 0.002 mmol) in MeOH (1.0 mL) was treated with concd H_2SO_4 (0.01 mL) and purified by HPLC using MeCN−H_2O (45:55) as the eluent to afford **3** (0.15 mg) and **4′** (0.05 mg).

4′: pale yellow oil; $[\alpha]^{22}_D$ −39.7 (*c* 0.05, EtOH); CD (*c* 1.22 × 10^{-4} M, EtOH) λ ($\Delta\varepsilon$) 386 (0), 353 (−3.8), 343 (0), 321 (9.7), 290 (0) nm.

Assay for Cytotoxicity. Cytotoxic activities of cephalimysins B−D (**1**−**3**) and FD-838 (**4**) and epimers (**1′**−**4′**) were examined with the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method. P388, HL-60, L1210, and KB cells were cultured in Eagle's Minimum Essential Medium (10% fetal calf serum) at 37 °C in 5% CO_2. The test material was dissolved in DMSO to give a concentration of 10 mM, and the solution was diluted with the Essential Medium to yield concentrations of 200, 20, and 2 μM, respectively. Each solution was combined with each cell suspension (1×10^5 cells/mL) in the medium, respectively. After incubation at 37 °C for 72 h in 5% CO_2, grown cells were labeled with 5 mg/mL of MTT in phosphate-buffered saline (PBS), and the absorbance of formazan dissolved in 20% sodium dodecyl sulfate (SDS) in 0.1 N HCl was measured at 540 nm with a microplate reader. Each absorbance value was expressed as percentage relative to that of the control cell suspension that was prepared without the test substance using the same procedure as that described above. All assays were performed three times, semilogarithmic plots were constructed from the averaged data, and the effective dose of the substance required to inhibit cell growth by 50% (IC_{50}) was determined.

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Supporting Information Available: Copies of spectra and chromatograms for these metabolites and the reaction products. This material is available free of charge via the Internet at http://pubs.acs.org.