Absolute Configuration of Aflastatin A, a Specific Inhibitor of Aflatoxin Production by *Aspergillus parasiticus*

Hiroyuki Ikeda,[1a] Nobuaki Matsumori,[1b,e] Makoto Ono,[1c] Akinori Suzuki,[1a] Akira Isogai,[1d] Hiromichi Nagasawa,[1a] and Shohei Sakuda*,[1a]

Department of Applied Biological Chemistry and Biotechnology, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, Research Institute, Morinaga and Co. Ltd., Tsurumi-ku, Yokohama 230-8504, Japan, and Graduate School of Biological Science, Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan

Received August 11, 1999

Aflastatin A (**1**) is a specific inhibitor of aflatoxin production by *Aspergillus parasiticus*. It has the novel structure of a tetramic acid derivative with a long alkyl side chain. The absolute configurations of 29 chiral centers contained in **1** were chemically elucidated in this study. First, four small fragment molecules were prepared from **1** or its methyl ether (**2**), and their absolute structures were assigned as *N*-methyl-D-alanine, (2*S*,4*R*)-2,4-dimethyl-1,6-hexanediol dibenzoate, (*R*)-3-hydroxydodecanoic acid, and (*R*)-1,2,4-butanetriol tribenzoate. Next, an acyclic fragment molecule **3** with 13 chiral centers was obtained from **1** by $NaIO_4$ oxidation, and its relative stereochemistry was elucidated by *J*-based configuration analysis. By analyzing coupling constants of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ and ROE data, the relative configuration of **3** was verified. Finally, by further *J*-based configuration analysis using a fragment molecule **7** prepared from **2** with 28 chiral carbons, all relative configurations in the alkyl side chain of **1** were clarified. By connecting these relative configurations with the absolute configurations of first four fragment molecules, the absolute stereochemistry of **1** was fully determined.

Aflatoxins belong to a group of mycotoxins produced by some strains of *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus tamarii*. Since they have extremely potent carcinogenicity toward mammals and are contaminants in a wide variety of food commodities, they are generally recognized not only as toxic contaminants in foods and feeds, but also as a certain risk factor for liver cancer in humans.[2] Therefore, the control and management of aflatoxins have been issues of concern.[3]

A specific inhibitor for aflatoxin biosynthesis may be a good candidate for a useful drug to protect foods and feeds from aflatoxin contamination. It is expected to depress aflatoxin contamination without rapid spread of drug-resistant strains since, unlike fungicides, it does not kill the producer of aflatoxin. Until now, some natural products and synthetic pesticides have been known to have inhibitory activity toward aflatoxin production.[4] However, none of them have been used in protecting agricultural products from aflatoxin contamination. Recently, during the course of our screening, aflastatin A (**1**) was isolated from the mycelia of *Streptomyces* sp. MRI142 as an inhibitor of aflatoxin production.[5a] Aflastatin A inhibits aflatoxin production by *Aspergillus parasiticus* at low concentrations without essentially affecting the growth of *A. parasiticus*. We have reported the planar structure of **1**.[5b,c] It is a novel tetramic acid derivative with a long alkyl side chain. The side chain is polyhydroxylated and acyclic except for a tetrahydropyran ring moiety. There are 29 chiral centers in **1**. Determination of the absolute configuration of **1** is very important for further studies such as chemical synthesis, structure−activity relationship, and its mode of action. However, there has been no information regarding the stereochemistry of **1** except for a relative stereochemistry around the tetrahydropyran ring. In this paper, we describe the elucidation of the absolute configuration of **1**.

Since crystals of **1** or its derivative suitable for X-ray analysis have not been obtained, we tried to determine its absolute configuration chemically. It is known that three fragment molecules, **3**−**5**, were obtained from **1** (Figure 1).[5c,6] These three and two other fragment molecules, **6** and **7**, were used for determination of the absolute configuration of **1**. Our strategy was as follows: (1) absolute configurations at C5′, C4 and C6, C33, and C39 of **1** should be clarified by determination of the absolute structures of **4**−**6**, (2) since **3** was acyclic and all chiral carbons in **3** were present in a 1,2- or 1,3-methine system, the relative configuration of **3** should be determined by the *J*-based configuration analysis,[7] which could afford the relative configurations from C10

* To whom correspondence should be addressed. E-mail: asakuda@mail.ecc.u-tokyo.ac.jp FAX: +81-3-5841-8022

(1) (a) Department of Applied Biological Chemistry, The University of Tokyo. (b) Department of Biotechnology, The University of Tokyo. (c) Morinaga and Co., Ltd. (d) Nara Institute of Science and Technology. (e) Present address: Department of Chemistry, Osaka University, 1-16 Machikaneyama, Toyonaka, Osaka 560-0043, Japan.

(2) Massey, T. E.; Stewart, R. K.; Daniels, J. M.; Liu, L. *Proc. Exp. Biol. Med.* **1995**, *208*, 213−227.

(3) Goldblatt, L. A. *Aflatoxin*; Academic Press: New York, 1969; pp 13−46.

(4) (a) Zaika, L. L.; Buchanan, R. L. *J. Food Prot.* **1987**, *50*, 691−708. (b) Wheeler, M. F.; Bhatnagar, D. *Pestic. Biochem. Physiol.* **1995**, *52*, 109−115. (c) Wheeler, M. H.; Bhatnagar, D.; Rojas, M. G. *Pestic. Biochem. Physiol.* **1989**, *35*, 315−320.

(5) (a) Ono, M.; Sakuda, S.; Suzuki, A.; Isogai, A. *J. Antibiot.* **1997**, *50*, 111−118. (b) Sakuda, S.; Ono, M.; Furihata, K.; Nakayama, J.; Suzuki, A.; Isogai, A. *J. Am. Chem. Soc.* **1996**, *118*, 7855−7856. (c) Ono, M.; Sakuda, S.; Ikeda, H.; Furihata, K.; Nakayama, J.; Suzuki, A.; Isogai, A. *J. Antibiot.* **1998**, *51*, 1019−1029.

(6) Sakuda, S.; Ono, M.; Ikeda, H.; Inagaki, Y.; Nakayama, J.; Suzuki, A.; Isogai, A. *Tetrahedron Lett.* **1997**, *38*, 7399−7402.

10.1021/jo991284c CCC: $19.00
Published on Web 12/31/1999

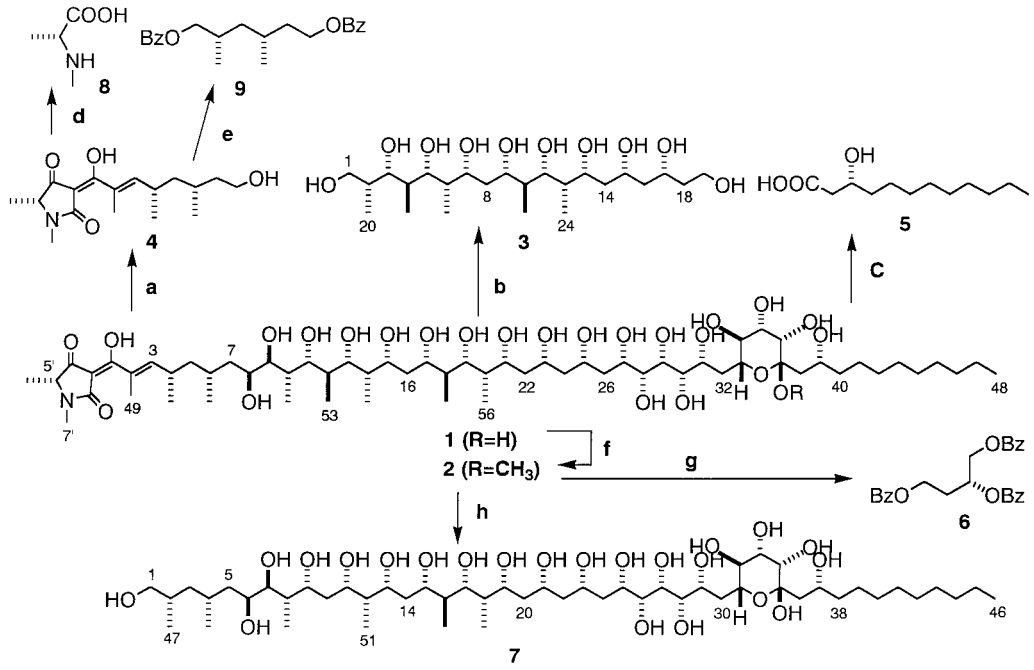
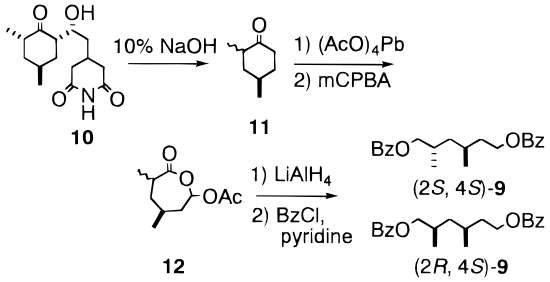


Figure 1. Procedures for the degradation of aflastatin A (**1**). (a) $NaIO_4$; $NaBH_4$. (b) $NaIO_4$; $NaBH_4$; Ac_2O, pyridine; NaOMe. (c) $NaIO_4$. (d) $NaIO_4$; 3 N HCl. (e) O_3; Me_2S; $LiAlH_4$; BzCN, tri-*n*-butylamine. (f) 5% HCl-MeOH. (g) $NaIO_4$; $NaBH_4$; 3 N HCl; BzCl, pyridine. (h) O_3; $NaBH_4$; Ac_2O, pyridine; NaOMe; Dowex-50W (H^+).

to C25 of **1**, (3) the remaining relative configurations from C6 to C10 and from C25 to C33 of **1** should be clarified by the determination of those of the counterparts in **7**, which could also be analyzed by the *J*-based method, and (4) by connecting the absolute configurations at C6 and C33 of **1** with the relative configurations from C6 to C33 and from C33 to C37 of **1**, the complete absolute configuration of **1** should be determined.

First, **4** was degraded to afford *N*-methylalanine (**8**) and 2,4-dimethyl-1,6-hexanediol dibenzoate (**9**). The absolute configuration of **8** was determined as D by Marfey's method.[8] To determine the absolute configuration of **9**, optically active (2*S*,4*S*)- and (2*R*,4*S*)-**9** were prepared

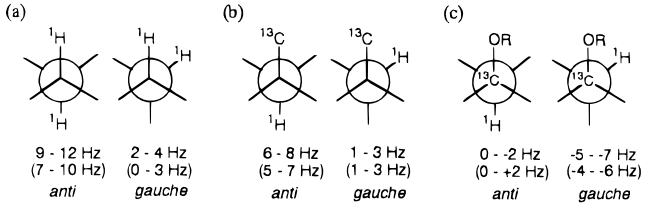
Scheme 1



from cycloheximide (**10**) according to Scheme 1. Alkaline degradation of **10** afforded (2*S*,4*R*)- and (2*R*,4*R*)-2,4-dimethylcyclohexanone (**11**).[9] Compound (2*S*,4*R*)-**11** was acetoxylated and converted to lactone (2*S*,4*S*)-**12**. $LiAlH_4$ reduction of this lactone, followed by benzoylation, afforded (2*S*,4*S*)-**9**. Similarly, (2*R*,4*S*)-**9** was obtained from (2*R*,4*R*)-**11**. The ^1H NMR spectrum of natural **9** was identical with that of (2*R*,4*S*)-**9**, indicating that natural **9** had a *syn* stereochemistry. Comparison of the CD spectrum of natural **9** with that of (2*R*,4*S*)-**9** showed that they were enantiomers. Thus, the configuration of natural **9** was assigned as (2*S*,4*R*). From the configurations of **8** and **9**, the absolute configurations at C5′, C4, and C6 of **1** were determined as shown in Figure 1.

Next, **1** was converted to its methyl glycoside **2**. This was oxidized with $NaIO_4$, followed by $NaBH_4$ reduction, acid hydrolysis, and benzoylation, to afford 1,2,4-butanetriol tribenzoate (**6**), in which the configuration at C33 of **1** was maintained. The absolute configuration of **6** was assigned as *R* by comparison of its CD spectrum with that of an authentic sample, which was prepared from a commercially available (*R*)-1,2,4-butanetriol. Since the relative configuration of the tetrahydropyran ring has been verified, the absolute configurations from C33 to

(7) (a) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876. In the *J*-based method, the configuration of a 1,2- or 1,3-methine system is deduced from the result of its conformation analysis with the *J* values and NOE (ROE) data. In the case of a 1,3-methine system separated by a methylene group, both conformations for the two methine−methylene systems are analyzed, respectively. The following *J* values are used for the *J*-based configuration analysis in general.



a: $^3J_{H,H}$. b: $^3J_{C,H}$. c: $^2J_{C,H}$. The figures in parentheses represent the values of 1,2-dioxygenated systems.

This method has been applied to determination of the configurations of some natural products such as maitotoxin,[7b−d] dysiherbaine,[7e] and amphidinol 3.[7f] (b) Matsumori, N.; Nonomura, T.; Sasaki, M.; Murata, M.; Tachibana, K.; Satake, M.; Yasumoto, T. *Tetrahedron Lett.* **1996**, *37*, 1269−1272. (c) Sasaki, M.; Matsumori, N.; Maruyama, T.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1672−1675. (d) Nonomura, T.; Sasaki, M.; Matsumori, M.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1675−1678. (e) Sakai, R.; Kamiya, H.; Murata, M.; Shimamoto, K. *J. Am. Chem. Soc.* **1997**, *119*, 4112−4116. (f) Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999**, *121*, 870−871.

(8) Marffey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(9) Bernard, C. L. *J. Am. Chem. Soc.* **1962**, *84*, 239−244.

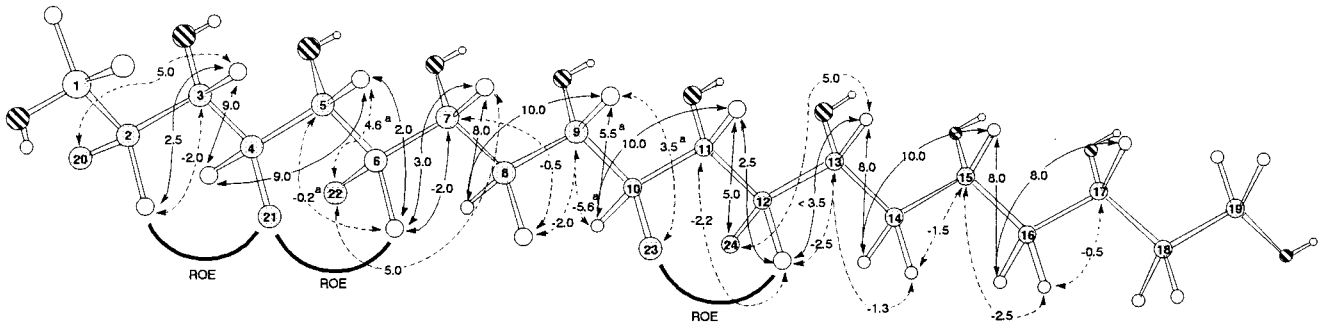


Figure 2. Configurations and conformation of **3** established on the basis of $^{3}J_{H,H}$, $^{2,3}J_{C,H}$, and key ROEs. Arrows, broken lines with arrows, and bold curves show $^{3}J_{H,H}$, $^{2,3}J_{C,H}$, and ROE, respectively. C9−C10 portion is depicted with a zigzag-type conformation. Spectra were measured in CD_3OD or pyridine-d_5: CD_3OD (3:1). [a] Measured in pyridine-d_5:CD_3OD (3:1).

C37 of **1** were assigned as shown in Figure 1 based on the configuration at C33 determined. From the optical rotation value of **5**, its absolute configuration was assigned as *R*,[10] indicating that the configuration of C39 of **1** was *R*.

To elucidate the relative configuration of **3** by the *J*-based method, $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ values were obtained from E.COSY, HETLOC,[11,7a] and phase-sensitive HMBC[12,7a] spectra. Two different solvents, CD_3OD and CD_3OD−pyridine-d_5 (3:1), were used for the NMR measurement to overcome the signal overlappings. As shown in Figure 2, dominant conformations for C2−C3, C3−C4, C4−C5, C5−C6, C6−C7, C7−C9, C10−C11, C11−C12, C12−C13, C13−C15, and C15−C17 of **3** were unambiguously assigned from the typical *anti* or *gauche* *J* values and ROEs,[7a] which lead to the establishment of configuration as *threo*, *erythro*, *erythro*, *threo*, *threo*, *syn*, *erythro*, *threo*, *threo*, *syn*, and *syn*, respectively. For the remaining C9−C10 bond in **3**, the medium value of $^{3}J_{H-9,H-10}$ (5.5 Hz) was observed, indicating that an alternating conformational change occurs around the bond. From the $^{3}J_{C23,H-9}$ (3.5 Hz) and $^{2}J_{C9,H-10}$ (−5.6 Hz) values, a pair of alternating conformers with the *erythro* configuration (Figure 3a) was easily assigned for C9−C10 according to the *J*-based method.[7a] Thus, the total relative configuration of **3** was determined as shown in Figure 2, which afforded relative configurations from C10 to C25 of **1**.

This relative configuration of **3** was partly confirmed by using the acetonide method.[13] Pentaacetonide of **3** (**13**) was prepared, and its ^{13}C NMR spectrum was analyzed. Figure 4 shows the δ value of each carbon involved in the five acetonide moieties, which was assigned by analysis of COSY, FG-HMQC, FG-HMBC, and NOESY spectra of **13**. Since all these values were typical for the case of the 1,3-diol system with *syn* relationship, all relative configurations for C5−C7, C9−C11, and C13−C15 were assigned as *syn*, which were completely consistent with those obtained by the *J*-based method mentioned above.

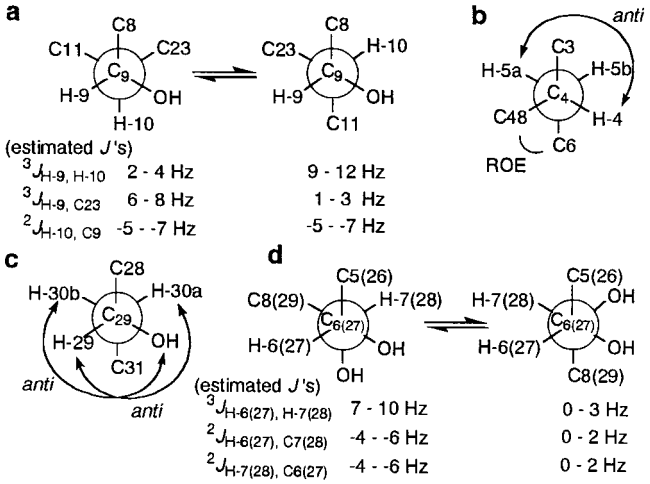


Figure 3. Rotamers for C9−C10 of **3** (a), C4−C5 of **7** (b), C29−C30 of **7** (c), and C6−C7 and C27−C28 of **7** (d).

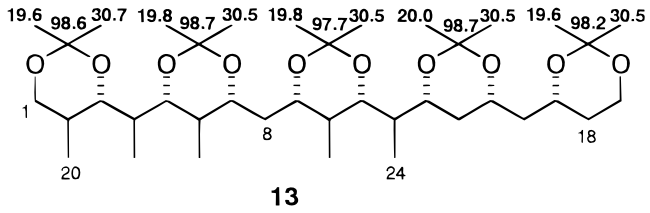


Figure 4. Structure of **13** and ^{13}C assignments of its acetonide moieties. Bold-faced numbers indicate δ_C values in benzene-d_6.

Finally, to determine the remaining configurations from C6 to C10 and from C25 to C33 of **1**, a large fragment molecule (**7**) was prepared from **2** by ozonolysis and subsequent reactions as shown in Figure 1. The *J* values and ROEs used for determination of the configurations from C4 to C8 and from C23 to C31 of **7** are summarized in Table 1. Each configuration for C7−C8, C23−C25, C25−C26, C26−C27, and C28−C29 of **7** was assigned as *erythro*, *syn*, *threo*, *threo*, and *threo*, respectively, from each dominant conformation determined by the typical *J* values and ROE data. For the 1,3-methine system of C4−C6, by analyzing the *J* values measured at room temperature (Table 1), the conformation for C5−C6 was easily determined, but it was difficult to assign the conformation for C4−C5 because no dominant rotamer or a pair of alternating rotamers satisfied the *J* values around the bond. However, when the spectra were taken at 0 °C with the expectation of increasing the population of a dominant conformer, the *anti* relationship

(10) Vining, L. C.; Taber, W. A. *Can. J. Chem.* **1962**, *40*, 1579−1584.
(11) (a) Otting, G.; Wuthrich, K. *Quart. Rev. Biophys.* **1990**, *23*, 39−96. (b) Willborn, U.; Leibfritz, D. *J. Magn. Reson.* **1992**, *98*, 142−146. (c) Kurz, M.; Schmieder, P.; Kessler, H. *Angew. Ed. Engl.* **1991**, *30*, 1329−1331.
(12) (a) Zhu, G.; Bax, A. *J. Magn. Reson.* **1993**, *104A*, 353−357. (b) Zhu, G.; Live, D.; Bax, A. *J. Am. Chem. Soc.* **1994**, *116*, 8370−8371. (c) Boyd, J.; Soffe, N.; John, B.; Plant, D.; Hurd, R. *J. Magn. Reson.* **1992**, *98*, 660−664. (d) Davis, A. L.; Keeler, J.; Laue, E. D.; Moskau, D. *J. Magn. Reson.* **1992**, *98*, 207−216.
(13) Rychnovsky, S. D.; Griesgraber, G.; Schlegel, R. *J. Am. Chem. Soc.* **1995**, *117*, 197−210.

Table 1. $^3J_{H,H}$ and $^2J_{C,H}$ Values (Hz) and Key ROEs Used for Determination of the Configurations from C4 to C8 and from C23 to C31 of 7[a]

bond	coupled nuclei	$^3J_{H,H}$	coupled nuclei	$^2J_{C,H}$	ROE
C4−C5	H-4,H-5a	7.5 (10.0[b])			H-6,H-48[b]
	H-4,H-5b	5.0			
C5−C6	H-5a,H-6	4.5 (4.5[b])	H-5a,C6	−2.5	
	H-5b,H-6	9.0 (9.0[b])	H-5b,C6	−5.5	
C6−C7	H-6,H-7	4.0	H-6,C7	−2.0	
			H-7,C6	−1.2	
C7−C8	H-7,H-8	8.0			H-6,H-49
C23−C24	H-23,H-24a	3.0	H-24a,C23	0.0	
	H-23,H-24b	8.0	H-24b,C23	−5.9	
C24−C25	H-24a,H-25	3.0	H-24a,C25	0.0	
	H-24b,H-25	8.0	H-24b,C25	−5.0	
C25−C26	H-25,H-26	7.5			H-24,H-27
C26−C27	H-26,H-27	3.0	H-26,C27	1.0	
			H-27,C26	0.0	
C27−C28	H-27,H-28	5.0	H-27,C28	−1.5	
			H-28,C27	−2.0	
C28−C29	H-28,H-29	3.0	H-28,C29	0.0	
			H-29,C28	0.0	
C29−C30	H-29,H-30a	8.0 (9.0[b])	H-30a,C29	−4.8 (−6.0[b])	
	H-29,H-30b	6.0 (5.0[b])	H-30b,C29	−5.0 (−3.0[b])	
C30−C31	H-30a,H-31	3.5 (3.5[b])	H-30a,C31	−2.0	
	H-30b,H-31	10.0 (10.0[b])	H-30b,C31	−6.0	

[a] Spectra were obtained in C_5D_5N at 27 °C or 0 °C. [b] Measured at 0 °C.

between H-4 and H-5a was shown by the $^3J_{H-4,H-5a}$ value (10 Hz). In addition to this *J* value, the observation of ROE between H-6 and H-48 confirmed the conformation for C4−C5 at 0 °C as shown in Figure 3b. Since the $^3J_{H-6,H-5a}$ and $^3J_{H-6,H-5b}$ values measured at 0 °C were identical with those at room temperature, it was shown that the conformation for C5−C6 was not changed by lowering the temperature. Thus, the configuration for C4−C6 was assigned as *anti*. Similarly, the conformation for C29−C30 could not be deduced from the *J* values measured at room temperature, but it could be assigned by those obtained at 0 °C, as shown in Figure 3c. Since the conformation for C30−C31 was clearly determined by the *J* values, and both of the $^3J_{H-30a,H-31}$ and $^3J_{H-30b,H-31}$ values at 0 °C were the same as those at room temperature, the configuration of C29−C31 was assigned as shown in Figure 1.

With respect to the C6−C7 diol system of **7**, the medium values of $^3J_{H-6,H-7}$ (4.0 Hz), $^2J_{H-6,C7}$ (−1.2 Hz), and $^2J_{C6,H-7}$ (−2.0 Hz) were obtained. Because these *J* values are observed in the case of the pair of rotamers (Figure 3d),[7a] in which H-6/7-OH and H-7/6-OH alternated *anti* and *gauche* conformation, the configuration of C6−C7 was assigned as *threo*. In the same manner, the configuration for the C27−C28 diol system with the *J* values of $^3J_{H-27,H-28}$ (5.0 Hz), $^2J_{H-27,C28}$ (−1.5 Hz), and $^2J_{C27,H-28}$ (−2.0 Hz) was also assigned as *threo* (Figure 3d). Thus, all the configurations from C4 to C8 and from C23 to C31 of **7** were determined. By connecting this result with the configuration of **3**, the relative stereochemistry of **7** was assigned as shown in Figure 1, affording the relative configurations from C6 to C33 of **1**. These relative configurations were further connected with the absolute configuration at C6 and C33 of **1** to give their absolute configurations. Since the absolute configurations from C8 to C31 of **1** obtained from the configuration at C6 agreed with those based on that at C33, the validity of the relative configurations from C6 to C33 of **1** determined by *J*-based method was confirmed.

From the results obtained above, the absolute configuration of aflastatin A was determined as **1**. We have recently obtained some information about the mode of action of **1**; **1** inhibits the expression of mRNA of some genes coding for aflatoxin biosynthetic enzymes.[14] The stereochemistry of **1** is very important for the study of the interaction between **1** and its target molecule. Work to investigate the molecular mechanism of inhibition of aflatoxin production by **1** is now in progress.

Experimental Section

General Methods. NMR spectra were recorded at 27 °C or 0 °C. $^3J_{H,H}$ values were extracted from 1D ^{1}H NMR and 2D E. COSY spectra. $^{2,3}J_{C,H}$ values were obtained from phase-sensitive (P)HETLOC,[11b] CH−HETLOC,[11b] and phase-sensitive (P)FG-HMBC[12c,d] spectra, which were measured under the following conditions. PHETLOC: BIRD delay, 275 ms or 500 ms; spin-lock period, 30 ms for $^2J_{C,H}$ or 60 ms for $^3J_{C,H}$ with each 2.5 ms trim pulses; Δ, 3.45 ms. CH−HETLOC: BIRD delay, 275, 350, or 500 ms; spin-lock period, 30 ms for $^2J_{C,H}$ or 60 ms for $^3J_{C,H}$ with each 1.0 ms trim pulses; Δ, 3.45 ms. PFG-HMBC: delay, 40 ms or 50 ms; FG, grad1 (44), grad2 (4), grad3 (20), and grad4 (−20). Aflastatin A (**1**) was purified from the mycelial methanol extracts of *Streptomyces* sp. MRI142 according to the procedure previously reported.[5a] *N*-Methyl-D- and *N*-methyl-L-alanine were purchased from Sigma, and (*R*)- and (*S*)-isomers of 1,2,4-butanetriol were purchased from Aldrich and Fluka, respectively.

Fragments 3 and 4. The degradation procedure to obtain **4** and the decaacetate of **3** and their spectral data have been reported previously.[5b,c] Compound **3** was prepared from its decaacetate as follows. To a solution of **3** decaacetate (28.5 mg) in absolute MeOH (3.0 mL) was added a small piece of Na, the mixture was stirred at room temperature for 40 min. After the mixture was passed through a Dowex-50W (H^+) column (18 ∅ × 40 mm), the solvent was removed under reduced pressure to afford **3** (11.5 mg).

3: HRFABMS (positive, glycerol matrix) *m*/*z* 499.3481 (M + H)$^+$ (calcd for $C_{24}H_{51}O_{10}$, 499.3482); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.02 (H-9), 4.01 (H-7), 3.96 (H-15), 3.95 (H-13), 3.93 (H-17), 3.83 (H-5), 3.76 (H-3), 3.69 (H-19a,b), 3.65 (H-11), 3.56 (H-1b), 3.47 (H-1a), 1.86 (H-2), 1.80 (H-10), 1.79 (H-4), 1.78 (H-6), 1.75 (H-8a), 1.73 (H-18a), 1.71 (H-14a), 1.68 (H-12), 1.63 (H-18b), 1.62 (H-14b), 1.62 (H-16a,b), 1.51 (H-8b), 0.95 (H-22), 0.93 (H-24), 0.87 (H-20), 0.81 (H-23), 0.78 (H-21); ^{13}C NMR

(14) Sakurada, M.; Okamoto, S.; Ono, M.; Tsukigi, H.; Suzuki, A.; Nagasawa, H.; Sakuda, S. Unpublished data.

(CD_3OD, 125 MHz) δ 81.4 (C-5), 78.6 (C-11), 77.6 (C-3), 77.0 (C-7), 75.8 (C-13), 73.7 (C-9), 70.4 (C-15), 68.8 (C-17), 66.5 (C-1), 60.0 (C-19), 45.4 (C-16), 43.1 (C-10), 42.6 (C-14), 41.0 (C-18), 40.1 (C-12), 39.9 (C-6), 39.4 (C-4), 38.8 (C-2), 36.6 (C-8), 13.4 (C-21), 11.4 (C-23), 9.7 (C-20), 6.5 (C-22), 6.2 (C-24); ^{1}H NMR ($C_5D_5N-CD_3OD$ 3:1, 500 MHz) δ 4.33 (H-9), 4.28 (H-15), 4.27 (H-7), 4.26 (H-17), 4.24 (H-13), 4.00 (H-3), 3.95 (H-5), 3.94 (H-19b), 3.89 (H-19a), 3.86 (H-11), 3.82 (H-1b), 3.70 (H-1a), 2.05 (H-10), 1.95 (H-2), 1.90 (H-4,8a), 1.89 (H-14a), 1.88 (H-18a,b), 1.86 (H-16a), 1.85 (H-6), 1.81 (H-14b), 1.78 (H-8b), 1.78(H-16b), 1.77 (H-12), 1.06 (H-22), 1.04 (H-24), 0.97 (H-20), 0.84 (H-23), 0.69 (H-21); ^{13}C NMR ($C_5D_5N-CD_3OD$ 3:1, 125 MHz) δ 80.9 (C-5), 78.2 (C-11), 76.7 (C-3), 76.4 (C-7), 75.4 (C-13), 73.3 (C-9), 70.1 (C-15), 68.4 (C-17), 66.5 (C-1), 59.2 (C-19), 44.9 (C-16), 42.2 (C-10), 42.2 (C-14), 40.7 (C-18), 39.2 (C-12), 39.0 (C-6), 38.4 (C-4), 38.0 (C-2), 36.3 (C-8), 12.7 (C-21), 11.0 (C-23), 9.3 (C-20), 5.9 (C-22), 5.6 (C-24); $[\alpha]^{23}_D$ −4.03° (*c* 0.6, EtOH).

3-Hydroxydodecanoic Acid (5). A solution of **1** (80 mg) in MeOH (20 mL) was added to 0.5 M $NaIO_4$ aq solution (6.4 mL), and the mixture was stirred for 20 h at room temperature. After being quenched with ethylene glycol (0.24 mL), the reaction mixture was filtered and the solvent was removed under reduced pressure. The product was purified by reverse-phase HPLC (mobile phase: 50% CH_3CN in H_2O; column: PEGASIL ODS, 10 ∅ × 250 mm) to afford **5** (1.4 mg).

5: HRFABMS (positive, NBA matrix) *m*/*z* 239.1617 (M + Na)$^+$ (calcd for $C_{12}H_{24}O_3Na$, 239.1623); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.36 (H-6a,b), 3.96 (H-3), 2.43 (H-2a, dd, J = 4.5, 15 Hz), 2.36 (H-2b, dd, J = 8.5, 15 Hz), 1.46 (H-4 and H-5a), 1.30 (H-5b, H-6, H-7, H-8, H-9, H-10 and H-11), 0.89 (H-12, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.8 (C-1), 69.4 (C-3), 43.3 (C-2), 38.1 (C-4), 33.1 (C-10), 30.7, 30.5, and 26.7 (C-5, C-6, C-7, C-8 and C-9), 23.7 (C-11), 14.4 (C-12); $[\alpha]^{28}_D$ −16.0° (*c* 0.1, $CHCl_3$) [lit.[10] $[\alpha]^{25}_D$ −15.2° (*c* 1.6, $CHCl_3$)].

Natural *N*-Methylalanine and Procedure of Marfey's Method. A solution of **4** (1.0 mg) in MeOH (0.5 mL) was added to 0.04 M $NaIO_4$ aq solution (1.5 mL), and the mixture was stirred for 16 h at room temperature. After being quenched with ethylene glycol (80 μL), the reaction solution was concentrated in vacuo. The obtained residue was dissolved in 6 N HCl (3.0 mL), and the solution was refluxed for 6 h. After the reaction solution was concentrated to dryness, the residue was dissolved in distilled water (1.5 mL). Ten microliters of this solution was mixed with 1 M $NaHCO_3$ solution (40 μL), a solution of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (FDAA) in acetone (40 μL), and distilled water (910 μL), and the reaction mixture was maintained at 40 °C for 1 h. After cooling, this solution (50 μL) was subjected to reverse-phase HPLC (column: SSC-ODS-1151-N, 4.6 ∅ × 150 mm; flow rate: 1 mL/min; mobile phase: gradient elution of 10−50% CH_3CN in 10 mM AcONa buffer pH 5.5 in 35 min). The retention times of FDAA derivatives of *N*-methyl-L-alanine and *N*-methyl-D-alanine, and natural sample, were 19.0, 20.4, and 20.4 min, respectively.

Natural 2,4-Dimethyl-1,6-hexanediol Dibenzoate (9). Ozone was passed through a solution of **4** (7.9 mg) in absolute MeOH (1.0 mL) at −78 °C for 30 min. After removal of excess O_3 in the solution by passage of N_2, the reaction flask was allowed to reach room temperature. Dimethyl sulfide (1.0 mL) was added to the solution, and the resulting mixture was kept at room temperature for 30 min. The product was extracted with EtOAc, and the EtOAc solution was washed with brine and distilled water and dried. After removal of the solvent, the resulting residue was treated with $LiAlH_4$ (5.0 mg) in Et_2O (2.0 mL) at room temperature for 1.5 h. After adding distilled water (10 mL) and 3 N HCl (3.0 mL) to the reaction solution, the product was extracted with Et_2O. The ether layer was washed with distilled water, dried, and evaporated. The resulting residue was dissolved in dry CH_3CN (1.0 mL). Tri-*n*-butylamine (20 mL) and benzoyl cyanide (6.2 mg) were added to the solution, and the mixture was stirred at room temperature for 2 h. The reaction was stopped by adding distilled water (10 mL), and the solution was stirred for 30 min. The product was extracted with CH_2Cl_2, and the CH_2Cl_2 solution was washed with brine, sat $NaHCO_3$ solution, and distilled water and dried. After removal of the solvent, the resulting residue was purified by normal-phase HPLC (mobile phase: hexane−EtOAc, 100:1; column: Senshu pak Silica, 4.6 ∅ × 250 mm) to afford **9** (1.2 mg).

Natural-**9**: HRFABMS (positive, NBA matrix) *m*/*z* 355.1898 (M + H)$^+$ (calcd for $C_{22}H_{27}O_4$, 355.1909); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.36 (H-6a,b), 4.21 (H-1a, dd, J = 5.5, 11 Hz), 4.09 (H-1b, dd, J = 6.5, 11 Hz), 2.07 (H-2), 1.84 (H-4, 5a), 1.55 (H-5b), 1.50 (H-3a), 1.16 (H-3b), 1.03 (H-7, CH_3, d, J = 6.5 Hz), 1.01 (H-8, CH3, d, J = 6.5 Hz), 8.00 (Bz-*meta*), 7.52 (Bz-*para*), 7.40 (Bz-*ortho*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.6 (C-1), 63.2 (C-6), 41.2 (C-3), 35.3 (C-5), 30.1 (C-2), 27.3 (C-4), 20.1 (C-8), 17.7 (C-7), 166.7 (Bz-ester), 132.8 (Bz-*para*), 130.4 (Bz), 129.5 (Bz-*meta*), 128.3 (Bz-*ortho*); CD (CH_3CN) $\Delta\epsilon_{230}$ = +1.52; $[\alpha]^{26}_D$ +8.5° (*c* 0.1, $CHCl_3$).

(2*R*,4*R*)- and (2*S*,4*R*)-2,4-Dimethylcyclohexanone (11). (2*R*,4*R*)- and (2*S*,4*R*)-**11** were prepared according to the literature procedure.[9] In brief, a solution of **10** (5.0 g) in 10% NaOH solution (50 mL) was stirred at −4 °C for 8 h and then at −18 °C for 14 h. The petroleum ether extract of the reaction mixture was purified by normal-phase HPLC (mobile phase: hexane−EtOAc, 100:1; column: SSC-silica 4251-N, 10 ∅ × 250 mm) to afford (2*R*,4*R*)-**11** (730 mg) and (2*S*,4*R*)-**11** (69.5 mg) as a colorless oil. (2*R*, 4R)-(**11**): FABMS (positive, NBA matrix) *m*/*z* 149 (M + Na)$^+$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.35 (H-2), 2.27 (H-6), 1.98 (H-5eq), 1.95 (H-3eq), 1.89 (H-4), 1.28 (H-5ax), 1.05 (H-3ax), 0.91 (H-7, CH_3, d, J = 6.5 Hz), 0.90 (H-8, CH3, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.7 (C-1), 44.5 (C-3), 44.3 (C-2), 41.2 (C-6), 35.9 (C-5), 32.0 (C-4), 21.2 (C-8), 14.5 (C-7); $[\alpha]^{23}_D$ −7.6° (*c* 1.7, $CHCl_3$).

(2*S*,4*R*)-(**11**): FABMS (positive, NBA matrix) *m*/*z* 149 (M + Na)$^+$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.52 (H-2a, b), 2.34 (H-3eq, 6eq), 2.06 (H-4), 1.90 (H-5eq), 1.70 (H-3ax), 1.65 (H-6ax), 1.58 (H-5ax), 1.08 (H-7, CH_3, d, J = 6.5 Hz), 1.07 (H-8, CH3, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 215.0 (C-1), 41.8 (C-2), 41.5 (C-3), 37.4 (C-6), 33.9 (C-5), 26.5 (C-4), 19.4 (C-7), 16.0 (C-8); $[\alpha]^{23}_D$ +64.2° (*c* 0.44, $CHCl_3$).

(2*S*,4*S*)-2,4-Dimethyl-1,6-hexanediol Dibenzoate (9). A mixture of (2*S*,4*R*)-**11** (61.6 mg) and $Pb(OAc)_4$ (160 mg) in benzene (1.0 mL) was heated under reflux for 8 h. After Et_2O (150 mL) was added to the reaction mixture, the organic solution was washed with brine and distilled water, dried, and evaporated. The resulting residue was chromatographed on a silica gel column (hexane−EtOAc, 9:1). Crude acetoxylated product (32.9 mg) that eluted from the column was dissolved in CH_2Cl_2 (1.3 mL), and NaH_2PO_4 (140 mg) and *m*CPBA (90 mg) were added to the solution. The mixture was stirred at room temperature for 24 h. After CH_2Cl_2 (50 mL) was added to the reaction mixture, the organic solution was washed with brine, sat $NaHCO_3$ solution, and distilled water, dried, and evaporated. Without purifying the lactone (2*S*,4S)-**12**, the resulting crude product (82.3 mg) was treated with $LiAlH_4$ (31 mg) in Et_2O (5.0 mL) at room temperature for 2 h. After cooled distilled water (10 mL) and 3 N HCl (4.0 mL) were added to the reaction solution, the product was extracted with Et_2O (50 mL). The ether layer was washed with distilled water, dried, and evaporated. The resulting residue (228 mg) was dissolved in dry pyridine (7.0 mL), and BzCl (3.5 mL) was added to the solution. After being stirred at room temperature for 24 h, the reaction was stopped by adding distilled water (5.0 mL). The product was extracted with CH_2Cl_2 (50 mL), and the organic layer was washed with sat $NaHCO_3$ solution, brine, and distilled water, dried, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane−EtOAc, 7:3) and normal-phase HPLC (mobile phase: hexane−EtOAc, 100:1; column: SSC-silica 4251-N, 10 ∅ × 250 mm) to afford (2*S*,4*S*)-**9** (13.6 mg, 7.9%) as a colorless oil.

(2*S*,4*S*)-(**9**): HRFABMS (positive, NBA matrix) *m*/*z* 355.1900 (M + H)$^+$ (calcd for $C_{22}H_{27}O_4$, 355.1909); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.36 (H-6a, b), 4.18 (H-1a, dd, J = 6.5, 11 Hz), 4.10 (H-1b, dd, J = 6.5, 11 Hz), 2.05 (H-2), 1.80 (H-4, 5a), 1.64 (H-5b), 1.31 (H-3a, b), 1.00 (H-7, CH3, d, J = 7.0 Hz), 0.96 (H-8, CH_3, d, J = 6.5 Hz), 8.02 (Bz-*meta*), 7.53 (Bz-*para*), 7.41 (Bz-*ortho*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 70.2 (C-1), 63.3 (C-6),

40.8 (C-3), 36.3 (C-5), 30.2 (C-2), 27.3 (C-4), 19.3 (C-8), 16.7 (C-7), 166.6 (Bz-ester), 132.8 (Bz-*para*), 130.4 (Bz), 130.2 (Bz), 129.5 (Bz-*meta*), 128.3 (Bz-*ortho*); CD (CH_3CN) $\Delta\epsilon_{237} = -1.39$; $[\alpha]^{23}_D$ −9.7° (*c* 0.18, $CHCl_3$).

(2*R*,4*S*)-2,4-Dimethyl-1,6-hexanediol Dibenzoate (9). Crude lactone (2*R*,4*S*)-**12** was prepared from (2*R*,4*R*)-**11** in the same manner as crude (2*S*,4*S*)-**12** was prepared from (2*S*,4*R*)-**11**. When this crude (2*R*,4*S*)-**12** was used for the next $LiAlH_4$ reduction as in the case of the preparation of (2*S*,4*S*)-**9**, pure (2*R*,4*S*)-**12** was not obtained by the final HPLC because of contamination of byproducts. Thus, crude (2*R*,4*S*)-**12** (153.5 mg) was purified by normal-phase HPLC (mobile phase: hexane−2-PrOH, 150:1; column: SSC-silica 4251-N, 10 ∅ × 250 mm) to afford (2*R*,4*S*)-**12** (56.7 mg) before the reduction.

(2*R*,4*S*)-**12**: FABMS (positive, NBA matrix) *m*/*z* 201 $(M + H)^+$, ^1H NMR ($CDCl_3$, 500 MHz) δ 6.45 (H-6), 2.63 (H-2), 2.01 (H-5a), 1.96 (H-4), 1.71 (H-5b), 1.66 (H-3a), 1.33 (H-3b), 1.19 (H-7, CH_3), 0.99 (H-8, CH_3), 2.10 (Ac CH_3).

Compound (2*R*,4*S*)-**12** was converted to (2*R*,4*S*)-**9** by $LiAlH_4$ reduction and benzoylation using the same method as that used to convert crude (2*S*,4*S*)-**12** to (2*S*,4*S*)-**9**. Final HPLC purification (mobile phase: hexane−EtOAc, 100:1; column: SSC-silica 4251-N, 10 ∅ × 250 mm) afforded (2*R*,4*S*)-**9** (13.5 mg) as a colorless oil.

(2*R*,4*S*)-(**9**): HRFABMS (positive, NBA matrix) *m*/*z* 355.1896 $(M + H)^+$ (calcd for $C_{22}H_{27}O_4$, 355.1909); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.36 (H-6a, b), 4.21 (H-1a, dd, J = 5.5, 11 Hz), 4.09 (H-1b, dd, J = 6.5, 11 Hz), 2.07 (H-2), 1.84 (H-4, 5a), 1.55 (H-5b), 1.50 (H-3a), 1.16 (H-3b), 1.03 (H-7, CH_3, d, J = 6.5 Hz), 1.01 (H-8, CH3, d, J = 6.5 Hz), 8.00 (Bz-*meta*), 7.52 (Bz-*para*), 7.40 (Bz-*ortho*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.6 (C-1), 63.2 (C-6), 41.2 (C-3), 35.3 (C-5), 30.1 (C-2), 27.3 (C-4), 20.1 (C-8), 17.7 (C-7), 166.6 (Bz-ester), 132.8 (Bz-*para*), 130.4 (Bz), 129.5 (Bz-*meta*), 128.3 (Bz-*ortho*); CD (CH_3CN) $\Delta\epsilon_{230} = -1.96$; $[\alpha]^{26}_D$ −8.9° (*c* 0.57, $CHCl_3$).

Natural 1,2,4-Butanetriol Tribenzoate (6). A solution of **1** (80 mg) in 5% HCl−MeOH (1.0 mL) was stirred at room temperature for 2 h. The reaction mixture was purified by reverse-phase HPLC (mobile phase: 63% MeOH in 0.5% diethylamine; column: Capcell pak C_{18}, 10 ϕ × 250 mm) to afford **2** (33 mg). **2**: FABMS (positive, glycerol matrix) *m*/*z* 1316 $(M + 2Na - H)^+$.

A mixture of **2** (16 mg) in MeOH (4.0 mL) and 0.2 M $NaIO_4$ aq solution (1.0 mL) was stirred at room temperature for 3 h, and the reaction was stopped by adding ethylene glycol (0.1 mL). The reaction mixture was filtered with a glass filter, and the solvent was removed under reduced pressure. The resulting residue was dissolved in MeOH (1.0 mL), and $NaBH_4$ (26 mg) was added to the solution. The reaction mixture was stirred at room temperature for 2.5 h, and the reaction was quenched by addition of acetic acid (120 μL). After removal of the solvent and boron, the resulting residue was dissolved in 3 N HCl (3.0 mL). The solution was heated at 80 °C for 3.5 h and evaporated. The residue was dissolved in dry pyridine (4.0 mL), and 4-(dimethylamino)pyridine (2.0 mg) and benzoyl chloride (1.5 mL) were added to the solution. After being stirred at room temperature for 24 h, the reaction was stopped by adding cooled distilled water (3.0 mL). The product was extracted with EtOAc, and the EtOAc solution was washed with brine, sat $NaHCO_3$ solution, and distilled water, dried, and evaporated. The obtained residue was purified by reverse-phase HPLC (mobile phase: gradient elution of 20−90% CH_3CN in H_2O; column: Capcell pak C_{18}, 10 ∅ × 250 mm) to afford **6** (1.3 mg).

Natural-**6**: HRFABMS (positive, NBA matrix) *m*/*z* 419.1491 $(M + H)^+$ (calcd for $C_{25}H_{23}O_6$, 419.1495); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.72 (H-2), 4.65 (H-1a, dd, J = 3.5, 12 Hz), 4.56 (H-4a), 4.53 (H-1b), 4.45 (H-4b), 2.34 (H-3), 8.03−7.98 (Bz-*meta*), 7.55−7.49 (Bz-*para*), 7.41−7.36 (Bz-*ortho*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.5 (C-2), 65.5 (C-1), 61.0 (C-4), 30.4 (C-3), 166.4, 166.2, 165.9 (Bz ester), 133.2, 133.0 (Bz-*meta*), 129.7, 129.6 (Bz-*para*), 128.4 (Bz-*ortho*); CD (CH_3CN) $\Delta\epsilon_{230} = +3.8$; $[\alpha]^{19}_D$ +63.0° (*c* 0.1, $CHCl_3$).

(*R*)- and (*S*)-1,2,4-Butanetriol Tribenzoate (6). Authentic (*R*)- and (*S*)-1,2,4-butanetriol tribenzoate were prepared from (*R*)- and (*S*)-1,2,4-butanetriol, respectively, by using the same benzoylation method as that used to prepare natural-**6**.

(*R*)-**6**: HRFABMS (positive, NBA matrix) *m*/*z* 419.1501 $(M + H)^+$ (calcd for $C_{25}H_{23}O_6$, 419.1495); CD (CH_3CN) $\Delta\epsilon_{230} = +4.8$; $[\alpha]^{18}_D$ +45.3° (c 2.3, $CHCl_3$).

(*S*)-**6**: HRFABMS (positive, NBA matrix) *m*/*z* 419.1477 $(M + H)^+$ (calcd for $C_{25}H_{23}O_6$, 419.1495); CD (CH_3CN) $\Delta\epsilon_{230} = -3.8$; $[\alpha]^{18}_D$ −50.9° (*c* 1.2, $CHCl_3$).

Pentaacetonide of Fragment 3 (13). 2,2-Dimethoxypropane (0.5 mL) and camphorsulfonic acid (25 mg) were added to a solution of **3** (8.4 mg) in dry acetone (1.5 mL), and the solution was stirred at room temperature for 24 h. After adding triethylamine (0.3 mL), the reaction solution was concentrated under reduced presssure. The resulting residue was purified by silica gel column chromatography (hexane−EtOAc, 8:2) and normal-phase HPLC (mobile phase: hexane−2-PrOH, 400:1; column: PEGASIL silica, 4.6 ∅ × 250 mm) to afford **13** (0.8 mg).

13: HRFABMS (positive, NBA matrix) *m*/*z* 721.4835 $(M + Na)^+$ (calcd for $C_{39}H_{70}O_{10}Na$, 721.4867); ^1H NMR (benzene-d_6, 500 MHz) δ 4.49 (H-7), 4.17 (H-3, dd, J = 2.0, 7.0 Hz), 4.13 (H-17, dd, J = 2.5, 6.5 Hz), 4.09 (H-15), 3.98 (H-5, dd, J = 2.0, 9.0 Hz), 3.95 (H-13), 3.93 (H-1a), 3.69 (H-19b), 3.68 (H-19a), 3.64 (H-11, dd, J = 2.0, 8.0 Hz), 3.53 (H-9), 3.46 (H-1b, dd, J = 1.5, 6.5 Hz), 2.30 (H-4), 2.14 (H-16a), 1.90 (H-8a), 1.84 (H-12), 1.80 (H-10), 1.77 (H-8b), 1.70 (H-14a), 1.63 (H-16b), 1.55 (H-6), 1.55 (H-18a), 1.55 (H-27, CH_3), 1.54 (H-30, CH_3), 1.53 (H-39, CH_3), 1.50 (H-36, CH_3), 1.49 (H-33, CH_3), 1.47 (H-14b), 1.39 (H-28, CH_3), 1.36 (H-25, CH_3), 1.36 (H-31, CH_3), 1.34 (H-2), 1.33 (H-18b), 1.33 (H-37, CH_3), 1.30 (H-34, CH_3), 1.24 (H-24, CH_3, d, J = 6.5 Hz), 1.16 (H-20, CH_3, d, J = 7.0 Hz), 1.03 (H-22, CH_3), 0.95 (H-21, CH_3, d, J = 7.5 Hz), 0.61 (H-23, CH_3, d, J = 6.5 Hz); ^{13}C NMR (benzene-d_6, 125 MHz) δ 74.3 (C-5), 73.1 (C-11), 72.0 (C-9), 71.6 (C-3), 71.6 (C-13), 70.2 (C-7), 68.3 (C-1), 65.5 (C-15), 65.5 (C-17), 59.8 (C-19), 44.1 (C-16), 39.8 (C-12), 38.3 (C-4), 36.4 (C-8), 35.0 (C-10), 35.0 (C-14), 32.8 (C-6), 31.5 (C-18), 30.5 (C-2), 13.0 (C-20), 11.6 (C-23), 10.1 (C-21), 9.6 (C-24), 6.1 (C-22), acetonide moieties (Figure 4); $[\alpha]^{23}_D$ −0.4° (*c* 0.1, $CHCl_3$).

Fragment 7. Ozone was passed through a solution of the **2** (40 mg) in absolute MeOH at −78 °C for 20 min. After removal of excess O_3 by passage of N_2, $NaBH_4$ (35 mg) was added to the solution and the reaction flask was allowed to reach room temperature. The reaction mixture was stirred at room temperature for 2 h, and the reaction was stopped by adding acetic acid (0.2 mL). After removal of solvent and boron, a mixture of dry pyridine (6.0 mL), acetic anhydride (3.0 mL), and 4-(dimethylamino)pyridine (3.0 mg) was added to the residue. The reaction mixture was stirred at room temperature for 22 h, and the reaction was stopped by adding cooled distilled water (3.0 mL). The solution was extracted with EtOAc, and the EtOAc layer was washed with brine, sat $NaHCO_3$ solution, and distilled water and dried. After evaporation in vacuo, the resulting residue was purified by reverse-phase HPLC (mobile phase: 70% MeOH in H_2O; column: Capcell pak C_{18}, 10 ∅ × 250 mm) to afford peracetate of **7** (34.0 mg). To a solution of **7** peracetate (34.0 mg) in absolute MeOH (3.0 mL), a small piece of Na was added and stirred at room temperature for 40 min. After being passed through a Dowex-50W (H^+) column (18 ∅ × 40 mm), the solvent was removed under reduced pressure to afford **7** (19.7 mg).

7: HRFABMS (positive, glycerol matrix) *m*/*z* 1117.7025 $(M + Na)^+$ (calcd for $C_{53}H_{106}O_{22}Na$, 1117.7073); ^1H NMR (C_5D_5N, 600 MHz) δ 4.96 (H-27), 4.92 (H-29), 4.67 (H-23 and H-26), 4.64 (H-34), 4.61 (H-15), 4.56 (H-33), 4.49 (H-13, H-21and H-28), 4.42 (H-19), 4.35 (H-25), 4.32 (H-9 and H-32), 4.29 (H-6), 4.24 (H-31), 4.18 (H-37), 4.12 (H-11), 4.05 (H-17), 4.00 (H-7), 3.80 (H-1a), 3.61 (H-1b), 3.17 (H-30a), 2.59 (H-24a), 2.49 (H-30b), 2.45 (H-36a), 2.31 (H-8), 2.19 (H-16 and H-36b), 2.14 (H-4 and H-24b), 2.08 (H-10), 2.05 (H-22a), 2.04 (H-14a and H-20a), 1.98 (H-2 and H-22b), 1.97 (H-12), 1.95 (H-14b), 1.86 (H-18 and H-20b), 1.84 (H-5a), 1.78 (H-3a), 1.75 (H-5b), 1.67 (H-38a), 1.59 (H-38b and H-39a), 1.48 (H-39b), 1.28 (H-49), 1.25 (H-40a,b), 1.23 (H-45a,b and H-51), 1.19 (H-41,H-42,H-43,H-44 and H-53), 1.10 (H-47), 1.07 (H-48), 1.04 (H-3b), 0.97

(H-52), 0.83 (H-46), 0.81 (H-50); ^{13}C NMR (C_5D_5N, 150 MHz) δ 103.2 (C-35), 81.6 (C-11), 78.8 (C-9), 78.8 (C-17), 77.9 (C-7), 77.1 (C-13), 76.2 (C-19), 76.2 (C-25), 75.4 (C-28), 73.9 (C-15), 73.1 (C-23), 72.8 (C-31), 72.6 (C-33), 72.3 (C-32), 72.2 (C-26), 71.5 (C-27), 71.5 (C-34), 70.9 (C-29), 70.8 (C-21), 69.5 (C-6), 67.2 (C-1), 66.9 (C-37), 45.7 (C-14), 42.7 (C-16), 42.7 (C-20), 42.3 (C-5), 42.1 (C-24), 41.6 (C-3), 39.5 (C-18), 39.3 (C-12), 39.3 (C-36), 39.3 (C-38), 38.8 (C-10), 38.1 (C-8), 37.5 (C-30), 36.9 (C-22), 34.0 (C-2), 32.0 (C-44), 30.0 (C-41), 30.0 (C-42), 29.8 (C-40), 29.5 (C-43), 27.8 (C-4), 26.0 (C-39), 22.9 (C-45), 21.7 (C-48), 18.6 (C-47), 14.2 (C-46), 13.2 (C-50), 11.6 (C-52), 8.3 (C-49), 6.4 (C-51), 6.1 (C-53).

Acknowledgment. We thank Dr. M. Murata of Osaka University and Dr. K. Furihata of the University of Tokyo for helpful discussions. This work was supported by a grant from JSPS Program for Research for the Future.

Supporting Information Available: ^{13}C and ^{1}H NMR spectra of **3**, **5**−**7**, **9**, **11**, and **13**, the ^{1}H NMR spectrum of **12**, COSY, FG-HMQC, FG-HMBC, NOESY spectra and summary of NMR data of **13**, and partial HETLOC, sliced PS-HMBC and ROESY spectra of **3** and **7** used for their configuration assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

JO991284C