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Blasticidin A as an Inhibitor of Aflatoxin Production by *Aspergillus parasiticus*

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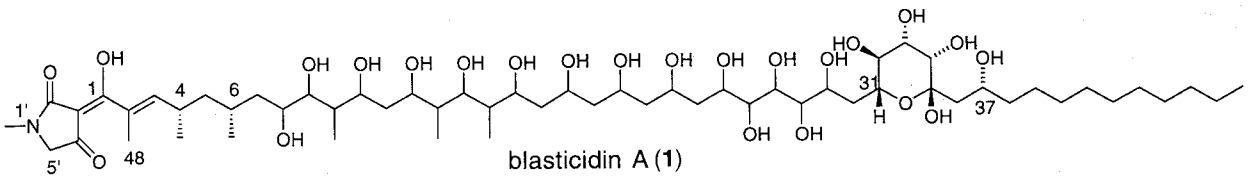
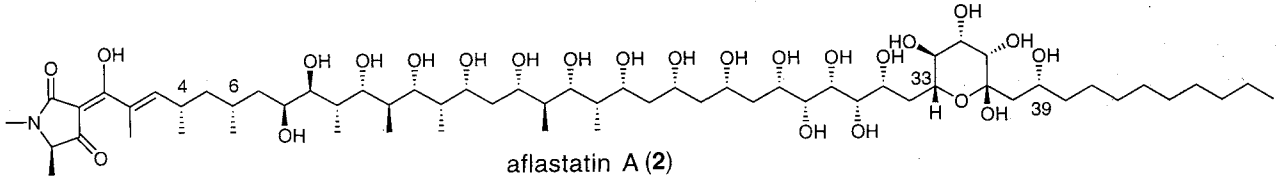
Blasticidin A, an antibiotic, showed strong inhibitory activity toward aflatoxin production by *Aspergillus parasiticus*. Its structure was characterized by NMR and chemical degradation experiments as **1**, which is a tetramic acid derivative with a highly oxygenated long alkyl chain similar to aflastatin A (**2**). Absolute configurations of the eight chiral centers at C-4, 6, 31, 32, 33, 34, 35 and 37 of **1** were chemically determined. Blasticidin A almost completely inhibited aflatoxin production at 0.5 μM.

Aflatoxins, a group of mycotoxins, are potent carcinogens in mammals and can be found as contaminants in a wide variety of food and feed commodities.[1] 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 incurring rapid spread of drug-resistant strains. Recently, in the course of our screening, aflastatin A (**2**) and B (*N*-demethyl derivative of **2**) were isolated from *Streptomyces* sp. MRI142 as strong inhibitors of aflatoxin production by *Aspergillus parasiticus*.[2,3] We have reported their structures and the total absolute configuration of **2**.[3~5]

During the course of our study on the chemical structure of aflastatin A, we discovered blasticidin A in the literature. Blasticidin A was an antibiotic found in 1955 in the culture broth of *Streptomyces griseochromogenes*.[6] Subsequently, KONO *et al.* reported its isolation and physicochemical properties in 1968,[7] but its structure has not been determined yet. Since close homology was observed between the physicochemical properties of blasticidin A and aflastatin A, the biological activity of blasticidin A was reexamined. It became clear that blasticidin A inhibits aflatoxin production by *A. parasiticus* similarly to aflastatin

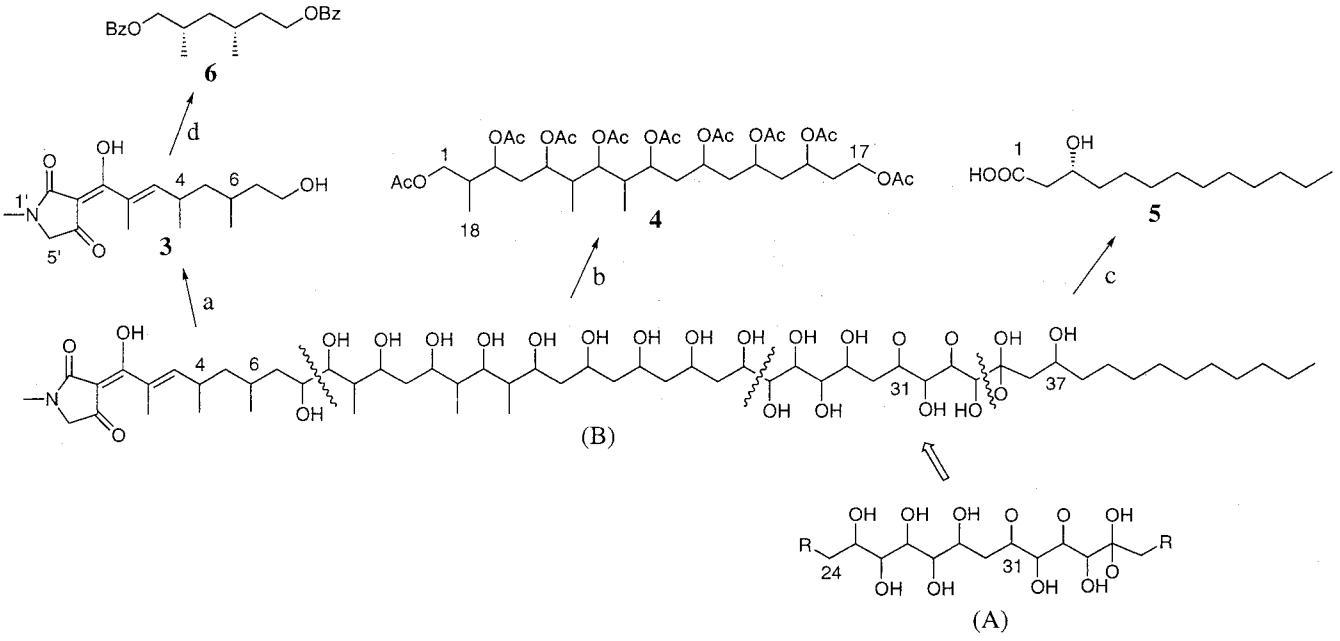


blasticidin A (**1**)



aflastatin A (**2**)

Fig. 1. Partial structures A and B, and degradation experiments of **1**.



(a) $NaIO_4$; $NaBH_4$, (b) $NaIO_4$; $NaBH_4$; Ac_2O, pyridine, (c) $NaIO_4$, (d) O_3; $NaBH_4$, BzCl, pyridine.

A. These facts prompted us to elucidate the structure of blasticidin A. We have reported a preliminary structural elucidation of blasticidin A.[8] This paper describes the detailed structural elucidation of blasticidin A, including determination of the absolute configurations of the eight chiral centers at C-4, 6, 31, 32, 33, 34, 35 and 37, and its biological activity as an inhibitor of aflatoxin production.

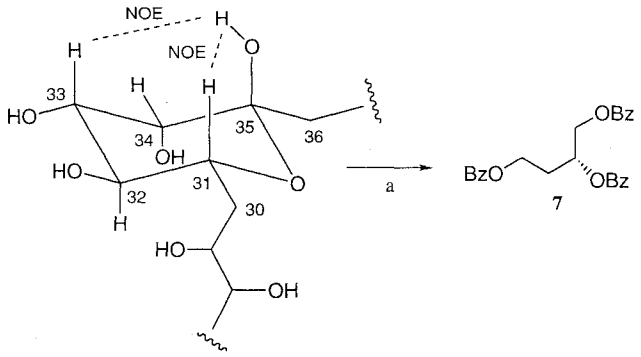
S. griseochromogenes was cultured at 27°C for 7 days in a medium reported by KONO *et al.*[7] Blasticidin A (**1**) was isolated as a white powder from a methanolic extract of the mycelia by *n*-BuOH extraction, precipitation from $CHCl_3$/MeOH (3 : 1) and from THF, and finally by reverse-phase HPLC under basic conditions.

Analysis of the HR-FABMS spectrum and NMR spectra showed the molecular formula of **1** was $C_{58}H_{107}NO_{23}$, which is smaller than that of **2** by C_4H_8O. The UV spectrum of **1** was very close to that of **2**, indicating that **1** and **2** have a similar chromophore. The presence of a tetramic acid moiety in **1** was suggested by the UV and NMR spectra. All NMR spectra of **1** were measured as diethylamine salt of **1** as in the case of **2** because the NMR signals of the carbons involved in the chromophore moiety of **1** were broadened when measured as the free acid of **1**. ^1H and ^{13}C NMR spectra of **1** closely resembled those of **2**, and the presence of a common partial structure (A) (Fig. 1) was clarified by analyzing the DQF-COSY, DQF-relayed COSY, HMQC, and HMBC spectra of **1** (Table 1). From the NMR spectra, it was noted that the remainder of the structure of **1** also resembled that of the counterpart of **2**. However, it was difficult to determine the total structure of **1** by further NMR analysis with the intact molecule. Therefore, oxidation of **1** with $NaIO_4$ was performed to obtain fragment molecules according to the method with slight modification used for the preparation of fragments of **2**.

Degradation experiments of **1** are summarized in Figure 1. First, the chromophoric fragment of **1** was obtained by oxidation with $NaIO_4$, followed by $NaBH_4$ reduction. This oxidation reaction was stopped when the amount of product **3** reached a maximum since the tetramic acid moiety of the chromophore was labile to excess $NaIO_4$. The molecular formula of **3**, whose UV spectrum is very close to that of **1**, was determined as $C_{16}H_{25}NO_4$ from its HR-FABMS spectrum. The structure of **3** was determined by analysis of the NMR spectra. The 3-acyltetramic acid rings of **3** and other compounds in this paper are represented as one of the possible tautomers according to Royles' representation.[9]

Next, the polyol fragment **4** was obtained by $NaIO_4$ oxidation followed by $NaBH_4$ reduction and acetylation. The HR-FABMS spectrum of **4** indicated that the molecular formula was $C_{38}H_{60}O_{18}$. By detailed analysis of COSY,

Fig. 2. Stereochemistry of tetrahydropyran ring and degradation experiment of the ring.



$J_{H31,H32}$=9.5 Hz, $J_{H32,H33}$=9.5 Hz, and $J_{H33,H34}$=3.0 Hz were observed in the spectrum obtained in DMSO-d_6+3% D_2O. (a) 5% HCl-MeOH; $NaIO_4$; 3 N HCl; BzCl, pyridine.

Table 1. NMR Assignments of **1**.[a]

C-No.	δ_C	δ_H	C-No.	δ_C	δ_H	HMBC (H→C)[f]	H-H COSY[f]	Relayed H-H COSY[f]	C-No.	δ_C	δ_H
1	191.2		24	40.9	1.85, 1.35	C-25		H-25,26	37	68.5	3.88
2	135.2		25	69.7	3.62	C-26	25-OH, H-26	H-24, 26	38	38.2	1.30
3	139.2	5.46 d (9)	25-OH		4.66		H-25		39	24.9[d]	1.25
4	29.9	2.52	26	74.3	3.25	C-24,25	26-OH, H-25,27	H-24,25,27,28	40	28.7[d]	1.23
5	44.6	1.33, 0.94	26-OH		4.57		H-26		41	29.0[d]	1.23
6	26.2	1.86	27	69.4	3.82	C-25,29	27-OH, H-26,28	H-26,28	42	29.1[d]	1.23
7	42.4	1.23	27-OH		4.11		H-27		43	29.1[d]	1.23
8	68.0	3.56	28	72.5	3.44	C-26,27	28-OH, H-27,29	H-26,27,29,30	44	29.2[d]	1.23
9	74.6	3.27	28-OH		4.14		H-28		45	31.3	1.23
10	39.6	1.61	29	68.6	3.82	C-30	29-OH. H-28	H-28,30,31	46	22.1	1.23
11	71.6	3.89	29-OH		4.11		H-29		47	14.0	0.84 t (6.5)
12	34.8[b]	1.48, 1.33	30	35.8	2.05, 1.48			H-28,29,31,32	48	13.2	1.69
13	71.0	3.93	31	70.2	3.62	C-29,30,32	H-32	H-29,30,32,33	49	21.4	0.88 d (6.5)
13-OH		4.73	32	71.2	3.18	C-30,31,33	32-OH, H-31,33	H-29,30,32,33	50	20.8	0.86 d (6.5)
14	41.7	1.63	32-OH		4.57		H-32		51	8.7	0.83 d (7)
15	76.0	3.44	33	70.7	3.56	C-32	H-32,34	H-31,32,34	52	10.5	0.68 d (6.5)
15-OH		4.57	34	73.0	3.41	C-32,33	34-OH, H-33	H-32,33	53	5.9	0.79 d (6.5)
16	38.2	1.51	34-OH		4.45		H-34		2'	174.4	
17	73.4	3.78	35	98.4					3'	99.7	
17-OH		4.70[e]	35-OH		6.11	C-34,35,36			4'	189.4	
18	41.3[b]	1.55	36	41.6	1.82, 1.42	C-35			5'	55.9	3.31
19	67.5[c]	3.87							6'	28.4	2.71
20	41.9[b]	1.55									
21	67.8[c]	3.78									
21-OH		4.73[e]									
22	44.5[b]	1.53									
23	67.8[c]	3.80									
23-OH		4.70									

[a] Spectra were obtained in DMSO-d_6 on a JEOL GX-500. [b,c,d,e] May be interchanged. [f] Correlations observed in partial structure A. Coupling constants in Hertz are given in parentheses

HMQC and HMBC spectra, its structure was determined as **4** (Fig. 1). Finally, the β-hydroxycarboxylic acid (**5**) was obtained only by oxidation with $NaIO_4$. It was characterized as **5** by analysis of the MS and NMR spectra.

Since all carbon atoms of **1** were involved in structure A or fragments **3**, **4** or **5**, its total carbon skeleton could easily be reconstructed yielding a large partial structure (B) (Fig. 1). From structure B and the molecular formula of **1**, the only problem was the determination of the position of an ether linkage. The formation of a tetrahydropyran ring by the ether linkage between C-31 and C-35 was revealed by the J values and NOEs around the ring protons as shown in Figure 2. Thus, the total structure of blasticidin A was determined as **1**. The assignments of protons and carbons in the NMR spectra of **1** are summarized in Table 1.

To determine the absolute configurations at C-4 and C-6

Table 2. Effect of blasticidin A on aflatoxin production by *Aspergillus parasiticus* in liquid culture (A), and on agar plate (B).

A)

Conc. of Blasticidin A (μM)	Mycelial dry weight (m±S.D., mg/10ml)[b]	Aflatoxin conc.[a] (m±S.D., μg/ml)[b]
0	24.1±0.7	20.7±2.0
0.25	24.3±0.3	1.1±0.1
0.5	23.2±0.6	0.5±0.1

B)

	Blasticidin A		Aflastatin A[d]	
Conc. (μg/ml)	Diameter of colony (m±S.D., mm)[b]	Aflatoxin conc.[a] (m±S.D., μg/ml)[b]	Diameter of colony (m±S.D., mm)[b]	Aflatoxin conc.[a] (m±S.D., μg/ml)[b]
0	81±1	13.8±0.8	81±1	13.8±0.8
0.03	79±2	7.2±1.9	78±2	7.1±2.2
0.125	71±5	1.1±0.7	65±2	0.8±0.6
0.5	40±3	N.D.[c]	55±3	N.D.[c]

[a] Total amount of aflatoxin B_1, B_2, G_1 and G_2.
[b] n = 3.
[c] <0.1 μg/ml.
[d] Assayed at the same time to compare the effects of the two compounds precisely.

of **1**, fragment **3** was degraded to afford 2,4-dimethyl-1,6-hexanediol dibenzoate (**6**). We have previously prepared (2*S*,4*S*)- and (2*R*,4*S*)-**6** to determine the absolute configuration of the corresponding part of **2**.[5] The ^{1}H NMR spectrum of natural **6** was identical with that of (2*R*,4*S*)-**6**, indicating that natural **6** had a *syn* stereochemistry. Comparison of the CD spectrum of natural **6** with that of authentic (2*R*,4*S*)-**6** showed that they were enantiomers. Thus, the configuration of natural **6** was assigned as (2*S*,4*R*). The absolute configuration at C-37 of **1** was assigned as *R* from the optical rotation value of **5**.[10]

Next, 1,2,4-butanetriol tribenzoate (**7**) was prepared from the methyl glycoside of **1** by $NaIO_4$ oxidation, followed by $NaBH_4$ reduction, acid hydrolysis, and benzoylation (Fig. 2).[5] The absolute configuration at C-31 of **1** was maintained in **7**. By comparison of the CD spectrum of **7** with that of an authentic sample, the configuration of **7** was assigned as *R*, which afforded the absolute configuration at C-31 of **1**. Based on this configuration and the relative stereochemistry of the tetrahydropyran ring, the absolute configurations from C-31 to C-35 of **1** were assigned as shown in Figure 2.

Blasticidin A and aflastatin A have a similar structure. There are a few differences between structures **1** and **2** as follows. A methyl group in the tetramic acid moiety of **2** is not present in the corresponding part of **1**. The length of the carbon chain and the number or position of methyl or hydroxyl groups in the part corresponding to the polyol fragment **4** are different from those in the counterpart of **2**. The length of the hydrocarbon end of **1** is longer than that of **2** by one carbon unit. With respect to the stereochemistry of **1** and **2**, the relative stereochemistry and absolute configuration of the tetrahydropyran ring of **1** are the same as those of **2**. The absolute configurations at C-4, C-6 and C-31 of **1** are also the same as those of the corresponding part of **2**. Work to determine the absolute configurations of the remaining part of **1** is now in progress.

The biological activity of **1** toward the aflatoxigenic fungus, *A. parasiticus* NRRL2999, was examined by both serial broth and agar dilution methods, and the results are summarized in Table 2. Blasticidin A reduced the amount of aflatoxin produced by the fungus in a dose-dependent manner in both methods. In the case of the broth dilution method, it almost completely inhibited the aflatoxin

production at 0.5 μM (Table 2A). This level of inhibition was comparable to that of aflastatin A.[3] At the same concentration, the mycelial weight of the fungus was not significantly affected by **1**. On the other hand, **1** inhibited the aflatoxin production at a concentration of 0.5 μg/ml on the agar plate (Table 2B). In this case, however, **1** reduced the hyphal extension rate of the fungus more strongly than **2** at the same concentration. At that time, **1** caused some morphological changes to afford dense and elevated colonies like the case of **2**.[3] Totally, the activity of **1** toward the aflatoxin production was almost the same as that of **2**, while **1** affected the fungal growth on the agar plate more significantly than **2**.

Experimental

Production and Isolation of Blasticidin A

Spores of *Streptomyces griseochromogenes* IFO13413 were inoculated into a medium (80 ml) reported by KONO *et al.* for preculture in 500-ml Erlenmeyer flasks. The flasks were incubated at 27°C and 160 rpm on a rotary shaker for 44 hours. These cultures (240 ml) were transferred into the same medium (5 liters) in a 10-liter jar fermenter for the main culture. Incubation was carried out at 27°C and 400 rpm under aeration of 5 liters/minute for 7 days.

Isolation of blasticidin A was performed according to KONO's method[7] with slight modifications. The culture broth (4.3 liters) was filtered and the mycelial cake obtained was extracted three times with MeOH (1.2 liters) at 65°C. The extract was evaporated *in vacuo* to give a slurry (118 g), which was dissolved in *n*-BuOH saturated with water (600 ml) and the solution was washed twice with 0.5% $NaHCO_3$ (300 ml) and once with water (300 ml) successively. The organic layer was evaporated to give a brownish oily material (113 g). Chloroform - MeOH (3 : 1) solution (400 ml) was poured onto this oily material and stirred vigorously, and the mixture was allowed to stand in the dark at room temperature overnight to obtain an amorphous precipitate. The precipitate was suspended into tetrahydrofuran (300 ml), and the insoluble matter was washed with a small amount of MeOH and dried to afford crude blasticidin A (9.89 g). The crude sample was further purified by HPLC (column: Capcell Pak C_{18}, 15×250 mm, Shiseido; mobile phase: MeOH - 0.5% diethylamine in water, 65 : 35; flow rate: 5 ml/minute). Due to poor solubility of blasticidin A in the mobile phase (0.9~1.2 mg/ml), this HPLC was repeated and the fractions containing blasticidin A were combined. After lyophilization of the combined fraction, diethylamine salt of blasticidin A (33.5 mg) was obtained from crude blasticidin A (**1**, 40.4 mg). **1**: HR-FABMS (positive, glycerol matrix) *m/z* 1208.7153 $(M+Na)^+$ (Calcd for $C_{58}H_{107}NO_{23}Na$, 1208.7132); $[\alpha]_D^{22}$ +10.8° (*c* 1.0, DMSO); UV λ_{max} nm (ε), (MeOH - H_2O, 1 : 1): 299 (5,800), 246 (11,500); (MeOH - 0.01 N NaOH, 1 : 1): 299 (5,800), 246 (11,500); (MeOH - 0.01 N HCl, 1 : 1): 314 (6,400), 237 (9,000). Co-elution experiment on HPLC with our sample and KONO's sample of blasticidin A confirmed that they are identical.

Preparation of Fragments **3**, **4** and **5**

A solution of 0.2 M $NaIO_4$ (1.6 ml) in water was mixed with a solution of **1** (50 mg) in MeOH (8 ml). The solution was stirred for 5 hours at room temperature in the dark. After decomposing excess $NaIO_4$ by adding ethylene glycol (24 μl), 4.5 M $NaBH_4$ in 1.2 ml of 0.1 M NaOH was added to the solution in an ice bath, and stirred in an ice bath for 1 hour. The reaction mixture was purified by HPLC (column: Capcell Pak C_{18}, 10×250 mm, Shiseido; mobile phase: gradient elution of 20~50% MeOH in water containing 0.05% diethylamine in 15 minutes; flow rate: 3 ml/minute) to afford 5 mg of **3**. **3**: HR-FABMS (positive, NBA matrix) *m/z* 318.1687 $(M+Na)^+$ (Calcd for $C_{16}H_{25}O_4NNa$, 318.1681); UV λ_{max} (nm) (ε) (MeOH - H_2O, 1 : 1): 299 (7,300), 246 (14,400); (MeOH - 0.01 N NaOH, 1 : 1): 298 (6,300), 245 (12,300); (MeOH - 0.01 N HCl, 1 : 1): 314 (6,800), 236 (8,600); $[\alpha]_D^{18}$ +37.6° (*c* 0.1, MeOH); δ_H (CD_3OD, 500 MHz) 5.67 (dq, *J*=9.5, 1.5 Hz, H-3), 3.58 (H-8), 3.55 (H-5′), 2.87 (H-6′), 2.64 (H-4), 1.82 (d, *J*=1.5 Hz, H-9), 1.69 (H-6), 1.49 (H-7a), 1.34 (H-7b), 1.34 (H-5a), 1.11 (H-5b), 0.98 (d, *J*=6.5 Hz, H-10), 0.90 (d, *J*=6.5 Hz, H-11); δ_C (CD_3OD, 500 MHz) 195.8 (C-1), 192.7 (C-4′), 176.8 (C-2′), 143.1 (C-3), 137.3 (C-2), 102.5 (C-3′), 60.9 (C-8), 57.1 (C-5′), 46.2 (C-5), 41.7 (C-7), 31.7 (C-4), 28.9 (C-6′), 28.2 (C-6), 21.1 (C-10), 20.4 (C-11), 13.5 (C-9); HMBC correlations ($^nJ_{CH}$=8 Hz): H-3 to C-1, 4, 5, 9 and 10, H-5 to C-3, 4, 6 and 10, H-7 to C-5, 6, 8 and 11, H-8 to C-6 and 7, H-9 to C-1, 2 and 3, H-10 to C-3, 4 and 5, H-11 to C-5, 6 and 7, H-5′ to C-2′ and 4′, H-6′ to C-2′ and 5′.

A solution of $NaIO_4$ (340 mg) in water (8 ml) was mixed with a solution of **1** (250 mg) in MeOH (40 ml). The solution was stirred for 24 hours at room temperature in the dark. After decomposing excess $NaIO_4$ by adding ethylene glycol (120 μl), $NaBH_4$ (1.0 g) in 6 ml of 0.1 M NaOH was added to the solution in an ice bath, and stirred in an ice bath for 1 hour and then for 1 hour at room temperature. The reaction solution was neutralized with 3 N HCl and concentrated. The residue obtained was extracted with MeOH and the extract was concentrated. A mixture of

acetic anhydride (8 ml), pyridine (8 ml) and 4-dimethylaminopyridine (20 mg) was added to the residue, and the reaction mixture was stirred for 24 hours at room temperature, then was poured into water (140 ml). After adjusting pH to 2.0 with conc. HCl, the solution was extracted with ethyl acetate (80 ml×3). The ethyl acetate layer was concentrated and chromatographed on a Sephadex LH-20 column (2.6×70 cm) packed in and eluted with MeOH. A crude **4** (87 mg) was eluted from the column at the eluting volume from 150 ml to 200 ml. After evaporating the MeOH, the residue was purified by HPLC (column: Capcell Pak C_{18}, 10×250 mm, Shiseido; mobile phase: gradient elution of 60~75% CH_3CN in water in 30 minutes; flow rate: 3 ml/minute) to afford 8.5 mg of **4**. **4**: HR-FABMS (positive, glycerol matrix) *m/z* 827.3677 $(M+Na)^+$ (Calcd for $C_{38}H_{60}O_{18}Na$, 827.3677); $[\alpha]_D^{18}$ +7.1° (*c* 0.1, MeOH); δ_H (CD_3OD, 500 MHz) 4.99 (H-3), 4.99 (H-15), 4.93 (H-13), 4.90 (H-5), 4.88 (H-11), 4.84 (H-9), 4.82 (H-7), 4.07 (H-17), 3.91 (H-1), 2.11 (H-6), 2.05 (H-8), 2.02 (H-2), 1.96 (H-16a), 1.93 (H-10a), 1.92 (H-4a), 1.92 (H-12), 1.82 (H-14), 1.82 (H-10b), 1.81 (H-16b), 1.81 (H-4b), 0.95 (d, *J*=7 Hz, H-18), 0.92 (d, *J*=7 Hz, H-19), 0.91 (d, *J*=7 Hz, H-20), 2.10, 2.04, 2.03, 2.03, 2.03, 2.02, 2.02, 2.01 and 2.01 (Ac); δ_C (CD_3OD, 500 MHz) 76.0 (C-7), 73.7 (C-9), 73.0 (C-5), 72.8 (C-3), 70.2 (C-11), 69.9 (C-13), 69.6 (C-15), 66.8 (C-1), 61.7 (C-17), 40.0 (C-6), 39.9 (C-14), 39.7 (C-12), 39. 1(C-8), 38.1 (C-10), 36.9 (C-2), 34.2 (C-16), 32.2 (C-4), 11.4 (C-19), 11.0 (C-18), 8.9 (C-20), 21 and 172 (Ac).; HMBC correlations ($^nJ_{CH}$=8 Hz): H-1 to C-2, 3 and 18, H-2 to C-18, H-3 to C-1, 2, 4 and 5, H-5 to C-6, H-6 to C-19, H-7 to C-5, 6, 8, 9 and 20, H-8 to C-20, H-9 to C-7, 8, 10, 11 and 20, H-11 to C-9, 10, 12 and 13, H-13 to C-11, 12, 14 and 15, H-15 to C-14, 16 and 17, H-17 to C-15 and 16, H-18 to C-1, 2 and 3, H-19 to C-5, 6 and 7, H-20 to C-7, 8 and 9.

A solution of 0.5 M $NaIO_4$ (3.2 ml) in water was mixed with a solution of **1** (50 mg) in MeOH (8 ml). The solution was stirred for 5 hours at room temperature in the dark. After decomposing excess $NaIO_4$ by adding ethylene glycol (120 μl), the reaction mixture was extracted with CH_2Cl_2 (40 ml×3). The CH_2Cl_2 layer was concentrated and the obtained residue was purified by HPLC (column: Capcell Pak C_{18}, 10×250 mm, Shiseido; mobile phase: 40% CH_3CN in water; flow rate: 3 ml/minute) to afford **5**. **5**: HR-FABMS (positive, NBA matrix) *m/z* 253.1764 $(M+Na)^+$ (Calcd for $C_{13}H_{26}O_3Na$, 253.1780); $[\alpha]_D^{22}$ −13.8° (*c* 0.05, $CHCl_3$) [Lit.10 $[\alpha]_D$ −15° (*c* 2.0, $CHCl_3$)]; δ_H (CD_3OD, 500 MHz) 3.86 (H-3), 2.31 (dd, *J*=15, 4.5 Hz, H-2), 2.22 (dd, *J*=15, 8.5 Hz, H-2), 1.43 (H-4 and 5a), 1.28 (H-5b, H-6~H-12), 0.89 (t, *J*=6.8 Hz, H-13); δ_C (CD_3OD, 500 MHz) 180.8 (C-1), 70.4 (C-3), 45.4 (C-2), 38.0 (C-4), 33.1 (C-11), 30.8, 30.5 and 26.7 (C-5, 6, 7, 8, 9, 10), 23.7 (C-12), 14.4 (C-13).

Preparation of 2,4-Dimethyl-1,6-hexanediol Dibenzoate (**6**)

Ozone was passed through a solution of **3** (26.9 mg) in absolute MeOH (5.0 ml) at −78°C for 20 minutes. After removal of excess O_3 in the solution by passage of N_2, $NaBH_4$ (20 mg) was added to the solution. The solution was stirred at room temperature for 2 hours and the reaction was stopped by adding acetic acid (0.2 ml). After removal of the solvent and boron, pyridine (3.0 ml) and benzoyl chloride (1.0 ml) were added to the residue, and stirred at room temperature for 17 hours. The reaction was stopped by adding water (4.0 ml), and the reaction mixture was extracted with ethyl acetate (120 ml). The ethyl acetate layer was washed with brine, sat. $NaHCO_3$ solution and water, and dried. After removal of the solvent, the resulting residue was chromatographed on a silica gel column (10×250 mm). Hexane - EtOAc (7 : 3) eluate from the column was purified by normal-phase HPLC (column: Senshu pak Silica, 10×250 mm; mobile phase: hexane - EtOAc, 60 : 1; flow rate: 4.0 ml/minute) to afford **6** (0.4 mg). **6**: FABMS (positive, NBA matrix) *m/z* 355 $(M+H)^+$; δ_H ($CDCl_3$, 500 MHz) : 4.36 (H-6a, b), 4.21 (dd, *J*=5.5, 11 Hz, H-1a), 4.09 (dd, *J*=6.5, 11 Hz, H-1b), 2.07 (H-2), 1.84 (H-4, 5a), 1.55 (H-5b), 1.50 (H-3a), 1.16 (H-3b), 1.03 (d, *J*=6.5 Hz, H-7), 1.01 (d, *J*=6.5 Hz, H-8), 8.00 (Bz-*meta*), 7.52 (Bz-*para*), 7.40 (Bz-*ortho*); CD (CH_3CN) $\Delta\varepsilon_{230}$=+1.89 {authentic (2*R*,4*S*)-**6**: $\Delta\varepsilon_{230}$=−1.96[5]}.

Preparation of 1,2,4-butanetriol Tribenzoate (**7**)

A solution of **1** (50 mg) in 5% HCl-MeOH (5.0 ml) was stirred at room temperature for 1 hour. The reaction mixture was purified by reverse-phase HPLC (column: Capcell Pak C_{18}, 10×250 mm, Shiseido; mobile phase: gradient elution of 66~90% MeOH in water containing 0.5% diethylamine in 10 minutes; flow rate: 3 ml/minute) to afford methyl glycoside of **1** (26.5 mg). HR-FABMS (positive, NBA matrix) *m/z* 1222.7213 $(M+Na)^+$ (Calcd for $C_{59}H_{109}O_{23}NNa$, 1222.7288).

A mixture of methyl glycoside of **1** (17.3 mg) in MeOH (4.0 ml) and 0.3 M $NaIO_4$ aq. solution (1.0 ml) was stirred at room temperature for 15 hours, and the reaction was stopped by adding ethylene glycol (0.2 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 (4.0 ml), and $NaBH_4$ (40 mg) was added to the solution. The reaction mixture was stirred at

room temperature for 2 hours and the reaction was quenched by addition of acetic acid (0.2 ml). 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 hours and evaporated. The residue was dissolved in dry pyridine (3.0 ml), and benzoyl chloride (1.0 ml) was added to the solution. After stirring at room temperature for 16 hours, the reaction was stopped by adding cooled water (4.0 ml). The product was extracted with EtOAc (80 ml), and the EtOAc solution was washed with 3 M HCl, sat. $NaHCO_3$ solution, brine and water, dried, and evaporated. The obtained residue was purified by reverse-phase HPLC (column: Capcell pak C_{18}, 10× 250 mm, Shiseido; mobile phase: gradient elution of 20~70% CH_3CN in water in 30 minutes; flow rate: 3.0 ml/minute) to afford **7** (0.7 mg). **7**: FABMS (positive, NBA matrix) *m/z* 419 $(M+H)^+$; δ_H ($CDCl_3$, 500 MHz): 5.72 (H-2), 4.65 (dd, J=3.5, 12 Hz, H-1a), 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*); CD (CH_3CN) $\Delta\varepsilon_{230}$= +3.4 (authentic **7**: $\Delta\varepsilon_{230}$= +4.8[5]).

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