Absolute Stereochemistry of Gastric Antisecretory Compound P371A1 and Its Congener P371A2 from *Streptomyces* Species P371

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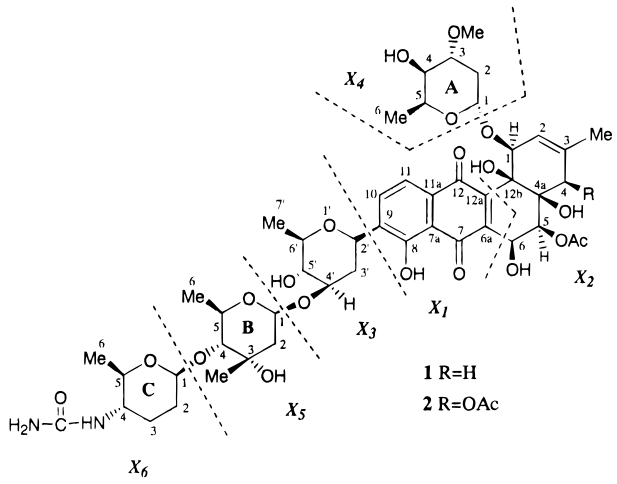
Received October 20, 1999

Absolute configurations of the gastric antisecretory compound P371A1 (**1**) and its congener P371A2 (**2**) from *Streptomyces* sp. P371 were determined on the basis of identification of the methyl glycosides **9**, **10**, and **11** obtained by the acid degradation of **1** and **2**, as well as application of the modified Mosher method to the P371A2 *C*-glycoside MTPA esters **7** and **8** and observation of the excitation chiralities in the P371A2 *C*-glycoside benzoate derivatives **5** and **6**.

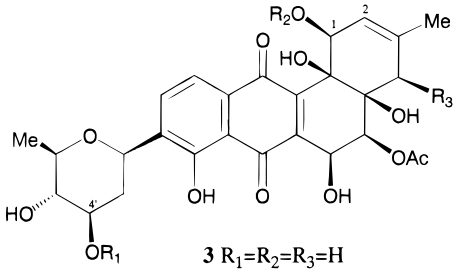
In the preceding paper,[1] we presented the relative structure of the gastric antisecretory compound P371A1 (**1**) (including the absolute configuration of sugar segments X_4 and X_5) from *Streptomyces* strain P371 on the basis of 2D NMR and FABMS techniques. This compound was found to have not only antigastrin, but also gastric mucosal protective activities. The present paper deals with the stereochemistry and determination of the absolute configurations of P371A1 (**1**) and its congener P371A2 (**2**).



Results and Discussion

The relative stereochemistry of **1** and **2** was disclosed by analyzing 1D and 2D NMR (DEPT, $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, HMBC, and NOESY) spectra and FABMS. Full assignments of the proton and carbon signals of **1** and **2**, together with the HMBC and NOE correlations of the signals, are listed in Tables 1 and 2, respectively. The NMR spectral evidence of both compounds indicated that they consist of six segments X_1, X_2, X_3, X_4, X_5, and X_6. The connectivities of these segments were clarified by inspection of the HMBC spectrum as well as the MS/MS spectrum of the parent ion peak appearing at the positive FABMS.[1]

P371A1 (**1**) and P371A2 (**2**) were degraded with methanolic HCl to give *C*-glycosides **3** and **4** as orange powders, respectively, along with three methyl glycosides **9**, **10**, and **11**. Methyl glycosides **9** and **10** were identified as methyl 2,3,6-trideoxy-3-*O*-methyl-β-L-xylohexopyranoside[2] (derivative of X_4) and methyl 2,6-dideoxy-3-*C*-methyl-α-D-ribohexopyranoside[3,4] (derivative of X_5), respectively, by comparison of their physical and spectroscopic properties with the reported data. Methyl glycoside **11** was elucidated to have a chair conformation in which the C-1 methoxy group is axial ($J_{1,2ax}$, $J_{1,2eq} < 2.0$ Hz), whereas both C-4 ureido and C-5 methyl groups are equatorial ($J_{4,3ax} = 9.6$ Hz, $J_{4,3eq} = 4.8$ Hz, and $J_{4,5} = 9.6$ Hz) by the $^1H-^1H$ decoupling experiments in $CDCl_3$. These values substantiated that **11** presents a stable C1 ($^4C_1$) conformation. Thus, methyl glycoside **11** was designated methyl 2,3,4,6-tetradeoxy-4-ureido-α-ribohexopyranoside[1] (derivative of X_6), whose absolute configuration is left to be resolved. The modes of the glycoside linkages in **1** and **2** were specified as α (X_4), β (X_5), and β (X_6) from the $^1H-^1H$ coupling constants of the anomeric protons: δ 4.64 ($J = 4.7$ Hz in **1**) and 4.69 ($J = 4.1$ Hz in **2**) at H-1A (X_4); δ 4.63 ($J = 9.2$ Hz in **1**) and 4.65 ($J = 9.2$ Hz in **2**) at H-1B (X_5); δ 4.47 ($J = 1.7$ and 9.5 Hz in **1**) and 4.46 ($J = 9.2$ Hz in **2**) at H-1C (X_6), together with those of other protons on the pyranose rings. The absolute stereochemistry at X_4 and X_5, as well as the relative stereochemistry at X_6, were thus established.



3 $R_1=R_2=R_3=H$

4 $R_1=R_2=H$, $R_3=OAc$

5 $R_1=Bz$, $R_2=H$, $R_3=OAc$

6 $R_1=R_2=Bz$, $R_3=OAc$

7 $R_1=(S)$-(-)-MTPA, $R_2=H$, $R_3=OAc$

8 $R_1=(R)$-(+)-MTPA, $R_2=H$, $R_3=OAc$

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10.1021/np990533p CCC: $19.00
Published on Web 04/29/2000

Table 1. NMR Spectral Data for P371A1 (**1**) in $CDCl_3$

C/H no.	^{1}H (d, *J* in Hz)	^{13}C	HMBC[a]	NOEY[b]
C-glycoside				
1	4.20, d (4.3)	80.7, d	H-1A	H-5, H-1A
2	5.65, br d	118.9, d		H-1, Me-3
3		135.9, s	H-1, H-4, Me-3	
4	2.13, 2.15, m	35.9, t		
4a		74.2, s	H-1, H-4, H-6	
5	5.86, d (6.7)	74.1, d		H-1, H-6
6	4.94, d (6.7)	66.0, d		H-5
6a		145.9, s	H-6	
7		188.6, s	H-11	
7a		113.9, s	H-11	
8	12.5, s	157.6, s	H-10	
9		138.9, s	H-2′, H-11	
10	7.86, d (7.8)	132.8, d		H-11
11	7.63, d (7.8)	119.4, d		H-10
11a		130.1, s	H-10	
12		187.7, s	H-11	
12a		140.6, s	H-6	
12b		77.4, s	H-1, H-4	
Me-3	1.72, s	23.1, q		H-2
MeCO-5	2.27, s	20.8, q		
MeCO-5		170.5, s	H-5	
2′	4.84, d (10.5)	71.0, d		H-4′, H-6′
3′	1.47, 2.45, m	37.6, t		
4′	3.71, m	83.1, d	H-1B	H-2′, H-1B
5′	3.18, t (8.7)	75.3, d		Me-6′
6′	3.48, m	76.2, d		H-2′
7′	1.45, d (7.1)	18.2, q		H-5′
sugar A				
1A	4.64, d (4.7)	98.9, d		H-1
2A	ca. 1.32, 1.70, m	30.0, t		
3A	3.38, br s	76.2, d	MeO-3A	
4A	3.52, m	73.8, d		MeO-3A
5A	4.35, m	72.7, d		MeO-3A
6A	1.21, d (6.7)	16.2, q		
MeO-3A	3.29, s	57.1, q		H-4A, H-5A
sugar B				
1B	4.63, br d (9.2)	99.4, d		H-4′, Me-3B, H-5B
2B	ca. 1.67, 1.95, m	44.1, t	Me-3B, H-4B	
3B		69.7, s	Me-3B	
4B	3.14, d (9.6)	89.5, d	H-1C	H-1C
5B	3.50, m	70.6, d		H-1B, Me-3B
6B	1.34, d (6.1)	18.0, q		
Me-3B	1.27, s	22.0, q		H-1B, H-5B
sugar C				
1C	4.47, dd (1.7, 9.5)	103.1, d		H-4B, H-5C
2C	ca. 1.70, 1.90, m	30.1, t		
3C	ca. 1.45, 2.17, m	27.7, t		
4C	4.35, m	61.7, d		
5C	3.53, m	65.7, d		
6C	1.25, d (5.8)	17.6, q		
NH_2CONH-4C		155.9, s		

[a] Proton showing long-range correlations with indicated carbon. [b] Proton showing NOE correlations with indicated proton.

The relative stereochemistry at X_2 and X_3 was established by inspection of the NOE correlation peaks and the coupling constants between the protons concerned in the following way. In the NOESY spectrum of **1**, the cross-peaks were observed between H-2′ (δ 4.84)/H-4′ (δ 3.71); H-2′/H-6′ (δ 3.48), and H-5′ (δ 3.18)/Me-6′ (δ 1.45) (Table 1). These data, as well as the coupling constant (t, $J = 8.7$ Hz) of H-5′, clearly defined that X_3 assumes the C1 conformation in which H-2′, H-4′, H-5′, and H-6′ are all axially oriented, only H-5′ being on the opposite side of the pyranose ring. Additionally, the NOESY spectrum showed cross-peaks between H-5 (δ 5.86) and H-6 (δ 4.94) and beween H-5 and H-1 (δ 4.20), strongly indicating that all three protons are situated on the concave side of the Decalin ring of X_2. These findings led us to define the relative stereochemistry at X_2 and X_3 of **1** and **3** as shown in the formulas. The ^{1}H and ^{13}C NMR spectra of **2** and **4** were very similar to those of **1** and **3**, except for signals due to a methine (δ_H 5.34 and δ_C 68.6 in **2**; δ_H 5.32 and δ_C 68.5 in **4**) bearing an acetoxy group in place of the C-4 methylene (δ_H 2.13, 2.15 and δ_C 35.9 in **1**; δ_H 2.12, 2.35 and δ_C 36.0 in **3**) in **1** and **3**. Furthermore, the NOESY spectrum of **2** showed the cross-peak between H-4 (δ 5.34) and H-5 (δ 5.81), besides those between H-5 and H-6 (δ 5.01) and between H-5 and H-1 (δ 4.32) (Table 2). It was, therefore, deduced that X_2 and X_3 of **2** and **4** have the same relative stereochemistry as do those of **1** and **3**, except for the C-4 center.

The absolute structure in the sugar segment X_3 was clarified by applying the modified Mosher method[5] to the α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters of *C*-glycoside **4** as follows: *C*-glycoside **4** derived from the

Table 2. NMR Spectral Data for P371A2 (**2**) in $CDCl_3$

C/H no.	^{1}H (d, *J* in Hz)	^{13}C	HMBC[a]	NOESY[b]
C-glycoside				
1	4.32, d (5.89)	79.3, d	H-1A	H-5, H-1A
2	5.98, d (4.8)	124.0, d		H-1, Me-3
3		133.9, s	H-1, H-4, Me-3	
4	5.34, s	68.6, d		H-5, Me-3
4a		74.5, s	H-1, H-4, H-6	
5	5.81, d (6.5)	74.6, d		H-1, H-4, H-6
6	5.01, d (6.5)	68.7, d		H-5
6a		145.8, s	H-6	
7		188.3, s	H-11	
7a		113.9, s	H-11	
8	12.10, s	157.7, s	H-10	
9		139.2, s	H-2′, H-11	
10	7.87, d (7.8)	132.9, d		H-11
11	7.63, d (7.8)	119.6, d		H-10
11a		130.1, s	H-10	
12		187.0, s	H-11	
12a		140.0, s	H-6	
12b		77.8, s	H-1, H-4	
Me-3	1.77, s	20.9, q		H-2, H-4
MeCO-4	2.16, s	20.7, q		
MeCO-4		170.9, s	H-4	
MeCO-5	2.30, s	20.8, q		
MeCO-5		171.0, s	H-5	
2′	4.84, d (11.1)	71.7, d		H-4′, H-6′
3′	ca. 1.50, 2.46, m	37.7, t		
4′	3.70, m	83.2, d	H-1B	H-2′, H-1B
5′	3.17, t (8.7)	75.9, d		Me-6′
6′	3.49, m	76.9, d		H-2′
7′	1.42, d (6.0)	18.2, q		H-5′
sugar A				
1A	4.69, d (4.1)	99.2, d		H-1
2A	ca. 1.42, 1.88, m	29.9, t		
3A	3.40, br s	76.7, d	MeO-3A	
4A	3.56, m	74.5, d		MeO-3A
5A	4.36, m	73.3, d		MeO-3A
6A	1.21, d (6.7)	16.3, q		
MeO-3A	3.28, s	57.2, q		H-4A, H-5A
sugar B				
1B	4.65, br d (9.2)	99.4, d		H-4′, Me-3B, H-5B
2B	ca. 1.75, 2.01, m	44.2, t	Me-3B, H-4B	
3B		70.2, s	Me-3B	
4B	3.14, d (9.4)	89.6, d	H-1C	H-1C
5B	3.51,[c] m	71.2, d		H-1B, Me-3B
6B	1.32, d (6.0)	18.0, q		
Me-3B	1.26, s	22.2, q		H-1B, H-5B
sugar C				
1C	4.46, br d (9.2)	103.1, d		H-4B, H-5C
2C	ca. 1.90, m	30.1, t		
3C	ca. 1.55, 2.21, m	27.7, t		
4C	4.32, m	61.8, d		
5C	3.53,[c] m	65.7, d		
6C	1.24, d (6.8)	17.6, q		
NH_2CONH-4C		155.8, s		

[a] Proton showing long-range correlations with indicated carbon. [b] Proton showing NOE correlations with indicated proton. [c] Chemical shifts with superscript within a given column are interchangeable.

major component P371A2 (**2**) was divided into two halves and esterified with (*R*)-(−)-MTPA chloride and (*S*)-(+)-MTPA chloride in the presence of pyridine, affording (*S*)-(−)-MTPA ester (**7**) (in a 57.7% yield) and (*R*)-(+)-MTPA ester (**8**) (in a 54.0% yield) as powdery orange crystals, respectively. Because the C-4′ protons in **7** and **8** were shifted downfield (δ 5.28 and δ 5.30), respectively, relative to that (δ 3.72) in **4**, the MTPA groups were introduced at the C-4′ hydroxy group in **4**. It was presumed that the sterically less crowded C-4′ hydroxy group, compared with other secondary hydroxy groups in **4**, led to the selective esterification by the bulky MTPA chloride at the C-4′ position. The MTPA esters thus obtained were subjected to 400 MHz NMR measurements, and $\Delta\delta$ (ppm) = (*S*)-(−)-MTPA − (*R*)-(+)-MTPA was calculated for each proton around the C-4′ MTPA ester group. The $\Delta\delta$ values and their signs shown in Figure 1 pointed out unequivocally that the absolute stereochemistry at C-4′ is *R*.[5] Because the relative stereochemistry at the segment X_3 in **4** has already been settled, the absolute configuration at the asymmetric centers of X_3 in **4** has therefore been elucidated.

Second, the absolute configuration (X_2) in the angucycline moiety of *C*-glycoside **4** was elicited from application of the CD exciton chirality method for its benzoate derivatives in the following way. An attempted selective benzoylation at the C-1 hydroxy group after protection of other hydroxy groups of **4**, including an acetonide formation between C-4′ and C-5′, failed. Thus, *C*-glycoside **4** was

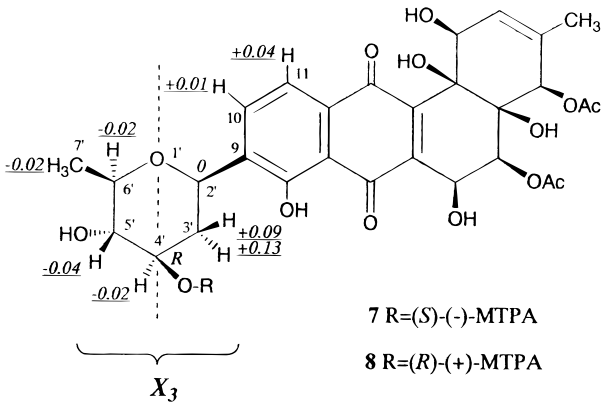


Figure 1. Determination of the absolute stereochemistry of the sugar segment X_3 of *C*-glycoside **4** by the modified Mosher method.

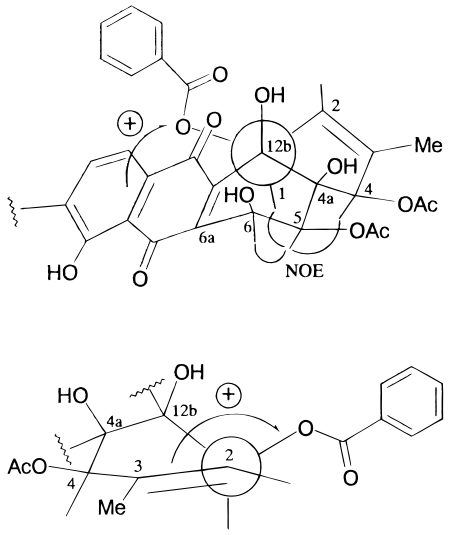
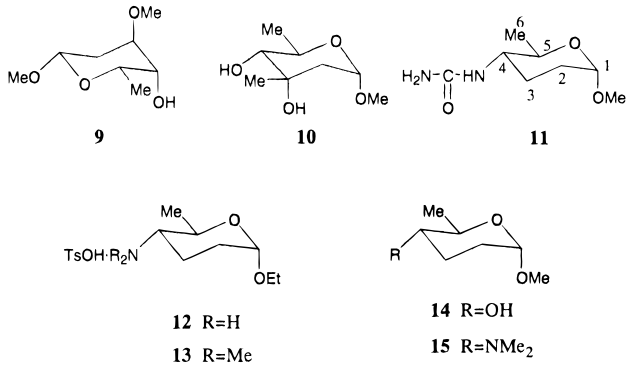


Figure 2. Exciton chiralities of C-1 benzoyloxy and naphthoquinone chromophores and of allylic benzoate system in **6**.

treated with 2 equiv of benzoyl chloride in the presence of pyridine in CH_2Cl_2 to furnish the more polar benzoate **5** (23.4% yield) and the less polar dibenzoate **6** (7.0% yield) as orange powders. The ^{1}H NMR spectrum of **5** showed a significant deshielding ($\Delta\delta$ 1.49) of the C-4′ proton as compared with that in **4**, whereas the spectrum of **6** exhibited downfield shifts of both C-1 and C-4′ protons (δ 5.85 and δ 5.16) relative to those (δ 4.54 and 3.72) in **4**. These findings clearly demonstrated the presence of benzoyloxy groups at C-4′ for **5** and at C-1 and C-4′ for **6**. The CD spectra of **5** and **6** measured each at the concentration of 0.74 mM in EtOH showed positive first Cotton effects ($\Delta\epsilon$: +29.4 and +35.4) at the 231−232 nm and negative second Cotton effects ($\Delta\epsilon$: −13.4 and −22.0) around 216 nm, respectively. Thus, it follows that the exciton chirality between the axes of the C-4′ benzoyloxy and naphthoquinone moieties in the benzoates is positive. Furthermore, the $\Delta\epsilon$ value of the first Cotton effect for dibenzoate **6** was larger than that for benzoate **5** by 6.0, whereas the absolute value of the second Cotton effect for **6** was larger than for **5** by 8.6. This finding led us to surmise that the exciton chirality between the axes of the C-1 benzoyloxy and naphthoquinone moieties in dibenzoate **6** is also positive, and we propose that these two moieties are clockwise-oriented in the Newman projection (Figure 2) in which the following proton pairs are mutually in proximity: H-5/H-1, H-5/H-4, and H-5/H-6. It is also considered that the positive exciton chirality due to the allylic benzoate[6] (the C-1 benzoyloxy and Δ^2 double-bond axes) would contribute to the further magnification of the Cotton effect around 231 nm. Thus, it was elicited that the C-1 stereocenter has *S*-chirality, and, hence, the angucycline moiety (X_2) of *C*-glycoside **4** has the absolute stereochemistry shown in Figure 1. Thus, the absolute stereochemistry of X_2 and X_3 of **4** was established. The segments X_2 and X_3 of *C*-glycoside **3** were also concluded to have the same absolute stereochemistry as those of **4**, except for the C-4 center, in view of the similar CD spectra of **3** and **4**.

The remaining problem, the absolute stereochemistry of X_6, was deduced from the ^{1}H−^{1}H NMR coupling constants as well as from the signs of the specific rotatory dispersion at 589.3 nm ($[\alpha]_D$) of methyl glycoside **11** in the following way. According to the report[7] by Listowsky et al. on general structural relationships deduced from an analysis of the dispersion curves of the methyl D-glycopyranoside series, all of C-1 axial methoxy group and C-4 and C-5 equatorial components in a C1 conformation contribute to a positive direction for the rotatory dispersion in either of the far-ultraviolet (200 nm) and the visible near-ultraviolet regions (600−250 nm). Thus, the positive $[\alpha]_D$ (+162.9° in MeOH and +165.8° in $CHCl_3$) of **11**, possessing the C1 conformation, can be explained by assuming the contribution of the C-1 axial methoxy-, the C-4 equatorial ureido-, and the C-5 methyl-groups. This finding was supported by the fact that the reported glycosides **12**,[8] **13**,[8] **14**,[9] and **15**[9] having the same absolute configuration at the C-1, C-4, and C-5 as does the proposed structure of **11**, exhibited the following positive $[\alpha]_D$ values: **12**, +77.2° (MeOH); **13**, +91.1° (MeOH); **14**, +142 ±1° (H_2O); **15**, +174 ± 2° ($CHCl_3$). Methyl glycoside **11** is, therefore, designated methyl 2,3,4,6-tetradeoxy-4-ureido-α-D-ribohexopyranoside. In conclusion, P371A1 (**1**) has the absolute stereochemistry (1*S*, 4a*S*, 5*S*, 6*S*, 12b*S*, 2′*R*, 4′*R*, 5′*R*, 6′*R*, 1A*S*, 3A*R*, 4A*R*, 5A*S*, 1B*S*, 3B*S*, 4B*R*, 5B*R*, 1C*S*, 4C*S*, 5C*R*), whereas P371A2 (**2**) has (1*S*, 4*R*, 4a*S*, 5*S*, 6*S*, 12b*S*, 2′*R*, 4′*R*, 5′*R*, 6′*R*, 1A*S*, 3A*R*, 4A*R*, 5A*S*, 1B*S*, 3B*S*, 4B*R*, 5B*R*, 1C*S*, 4C*S*, 5C*R*).



Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto MP-32 micromelting point appartus and were uncorrected. Recycling preparative HPLC was carried out using Japan Analytical Industry model LC-908. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. CD spectra were measured with a JASCO J-720 spectropolarimeter using a cell (1 mm in length) in a stream of N_2 (12−15 L/min) at 25 °C. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer and a Hitachi model 260-30 IR spectrophotometer. HRFABMS were recorded on an instrument of EB geometry equipped with direct inlet system. LRFABMS were measured on a JEOL JMS-HX 100 and Finnigan-Mat TSQ-700 instruments. NMR spectra were recorded using Bruker AX-300, Bruker-ARX-400, Bruker-AM-600, and JEOL JNM-GX 500 instruments. Chemi-

cal shifts are expressed in δ (ppm) values with tetramethylsilane (TMS) as an internal reference in $CDCl_3$, and coupling constants are expressed in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dq = doublet of quadruplets, qd = quadruplet of doublets, br s = broad singlet. COSY, HMBC, and NOESY spectra were recorded using phase cycling for coherence pathway selection.

Organisms and Culture Conditions. *Streptomyces* strain P371 was obtained from a soil sample collected at Mt. Fuji, Shizuoka Prefecture, Japan.[1] A slant culture of strain P371 was inoculated into five flasks (500 mL) containing a seed medium (100 mL). The flasks were then shaken on the rotary shaker (250 rpm) at 30 °C for 2 days. The resultant seed cultures were inoculated into a 30-L jar fermenter containing the above medium (25 L). The fermentation was carried out at 30 °C under aeration of 20 L/min and agitation of 200 rpm for 2 days. The medium used for fermentation consisted of starch (20 g/L), glucose (1 g/L), yeast extract (5 g/L), peptone (3 g/L), meat extract (3 g/L), $CaCO_3$ (4 g/L), a 1-L H_2O solution of 1 g each of $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $CoCl_2 \cdot 6H_2O$ (5 mL/L), and distilled water, pH 7.0. The culturing on a 25-L scale was repeated six times.

Extraction and Isolation. The culture medium (25 L), after separating the mycelium, was extracted with ethyl acetate (10 L × 3). The combined ethyl acetate extracts were evaporated to dryness (12.5 g residue). This residue was first applied to Si gel column (3.5 × 37 cm) chromatography with $CHCl_3$/MeOH as an eluent. The eluate with $CHCl_3$/MeOH (30:1) was concentrated in vacuo to give a residue (1.4 g) containing P371A1 (**1**) and A2 (**2**). This crude product was further subjected to Si gel column (3 × 16 cm) chromatography with $CHCl_3/Me_2CO$ as an eluent. The eluate with $CHCl_3/Me_2CO$ (4:1) afforded a crude mixture (416 mg) of **1** and **2**, which was finally separated by a combination of ODS column (RP_{18}, 3 × 35 cm) chromatography with 70% MeOH as an eluent and GPC column (GS-310, 2 × 60 cm) chromatography (recycling preparative HPLC) with $CHCl_3$ as an eluent, yielding P371A1 (**1**) (83.0 mg) and A2 (**2**) (155.7 mg) each as an orange powder. The extraction and isolation using 25 L of the culture medium was repeated six times.

P371A1 (1): orange powder; $[\alpha]^{20}_D$ +51.5° (*c* 0.40, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 250.8 (3.88), 275.6 (3.86), 426.0 (3.65) nm; IR (KBr) ν_{max} 3448, 2976, 2936, 1734, 1636, 1438, 1388, 1283, 1240, 1062, 903, 788 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; HRFABMS *m/z* 993.4423 ($[M + 2H + H]^+$, calcd for $C_{48}H_{69}N_2O_{20}$, 993.4444).

P371A2 (2): orange powder; $[\alpha]^{20}_D$ +61.7° (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 251.6 (3.89), 273.6 (3.85), 426.4 (3.66) nm; IR (KBr) ν_{max} 3446, 2976, 2936, 1734, 1637, 1438, 1381, 1240, 1061, 1013, 904, 783, 762 cm^{-1}; ^1H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRFABMS *m/z* 1051.4490 ($[M + 2H + H]^+$, calcd for $C_{50}H_{71}N_2O_{22}$, 1051.4498).

Acid Degradation of P371A1 (1) with 2 N HCl/MeOH. P371A1 (**1**) (19.2 mg) was dissolved in a mixture of 2 N HCl (1 mL) and MeOH (1 mL). After stirring for 4 h at room temperature, the reaction mixture was diluted with ethyl acetate and then washed with saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was subjected to preparative TLC with $CHCl_3$/MeOH (10:1) as a developing solvent, yielding *C*-glycoside **3** (6.8 mg) as an orange powder.

***C*-Glycoside 3:** $[\alpha]^{27}_D$ +175° (*c* 0.10, EtOH); UV (EtOH) λ_{max} (log ϵ) 218.2 (4.25), 252.6 (3.78), 275.8 (3.74), 431.8 (3.52) nm; IR (KBr) ν_{max} 3448, 2926, 1718, 1636, 1438, 1375, 1252, 1090, 877, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.39 (3H, d, *J* = 6.1 Hz, Me-6′), ca. 1.60 & 2.25 (2H, m, H_2-3′), 1.72 (3H, s, Me-3), ca. 2.12 & 2.35 (2H, m, H_2-4), 2.25 (3H, s, 5-OCOMe), 3.00 (1H, t, *J* = 8.5 Hz, H-5′), 3.43 (1H, m, H-6′), 3.75 (1H, m, H-4′), 4.48 (1H, br s, H-1), 4.81 (1H, d, *J* = 10.7 Hz, H-2′), 5.15 (1H, dd, *J* = 3.2 and 7.4 Hz, H-6), 5.57 (1H, br s, H-2), 5.61 (1H, dd, *J* = 1.7 and 7.4 Hz, H-5), 7.66 (1H, d, *J* = 7.8 Hz, H-11), 7.83 (1H, d, *J* = 7.8 Hz, H-10); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.1 (q, C-7′), 20.9 (q, 5-OCO*Me*), 23.1 (q, Me-3), 36.0 (t, C-4), 39.1 (t, C-3′), 69.0 (d, C-6), 71.1 (d, C-2′), 72.4 (d, C-1), 72.6 (d, C-4′), 72.8 (d, C-5), 73.6 (s, C-4a), 76.2 (C-6′), 77.7 (d, C-5′), 78.2 (s, C-12b), 114.2 (s, C-7a), 119.3 (d, C-2), 120.0 (d, C-11), 130.6 (s, C-11a), 133.4 (d, C-10), 136.1 (s, C-3), 139.0 (s, C-9), 141.6 (s, C-12a), 144.0 (s, C-6a), 157.9 (s, C-8), 170.9 (s, 5-OCOMe), 186.7 (s, C-12), 190.5 (s, C-7); negative FABMS *m/z* 546 ($[M]^-$); HRFABMS *m/z* 546.1768 ($[M]^-$, calcd for $C_{27}H_{30}O_{12}$ 546.1785).

Acid Degradation of P371A2 (2) with 2 N HCl/MeOH. P371A2 (**2**) (200 mg) was treated with a mixture of 2 N HCl (4 mL) and MeOH (4 mL), and the resultant product was subjected to preparative TLC in the same way as in the case of **1**, giving *C*-glycoside **4** (74.0 mg) as an orange powder.

***C*-Glycoside 4:** $[\alpha]^{24}_D$ +187° (*c* 0.52, EtOH); UV (EtOH) λ_{max} (log ϵ) 217.6 (4.35), 253.2 (3.94), 276.0 (sh), 432.0 (3.68) nm; IR (KBr) ν_{max} 3448, 2925, 1718, 1637, 1438, 1376, 1244, 1020, 908, 877, 764 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 1.30 (3H, d, *J* = 5.9 Hz, Me-6′), 1.70 (3H, s, Me-3), ca. 1.87 & 2.60 (2H, m, H_2-3′), 2.10 (3H, s, 4-OCOMe), 2.23 (3H, s, 5-OCOMe), 3.22 (1H, br t, H-5′), 3.50 (1H, m, H-6′), 3.72 (1H, m, H-4′), 4.54 (1H, d, *J* = 3.8 Hz, H-1), 4.64 (1H, d, *J* = 9.0 Hz, H-2′), 5.05 (1H, d, *J* = 7.1 Hz, H-6), 5.32 (1H, s, H-4), 5.53 (1H, d, *J* = 7.0 Hz, H-5), 5.84 (1H, d, *J* = 3.3 Hz, H-2), 7.60 (1H, d, *J* = 7.8 Hz, H-11), 7.71 (1H, d, *J* = 7.8 Hz, H-10); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 18.1 (q, C-7′), 20.6 (q, 5-OCO*Me* or 4-OCO*Me*), 20.8 (q, 4-OCO*Me* or 5-OCO*Me*), 21.0 (q, 3-Me), 38.5 (t, C-3′), 68.5 (each d, C-4 and C-6), 70.8 (d, C-1), 71.0 (d, C-2′), 71.7 (d, C-4′), 73.6 (d, C-5), 76.0 (d, C-6′), 76.1 (d, C-5′), 78.8 (d, C-12b), 114.1 (s, C-7a), 119.9 (d, C-11), 125.0 (d, C-2), 132.9 (s, C-11a), 133.3 (d, C-10), 134.0 (s, C-3), 139.1 (s, C-9), 141.0 (s, C-12a), 144.1 (s, C-6a), 157.4 (s, C-8), 171.1 (s, 5-O*C*OMe or 4-O*C*OMe), 171.8 (s, 4-O*C*OMe or 5-O*C*OMe), 186.7 (s, C-12), 189.5 (s, C-7); negative FABMS *m/z* 603.0 ($[M - H]^-$); HRFABMS *m/z* 604.1807 ($[M]^-$, calcd for $C_{29}H_{32}O_{14}$, 604.1827).

Formation of Methyl Glycosides 9−11 by Acid Degradation of P371A2 (2) with 5% HCl in MeOH. P371A2 (**2**) (200 mg) was treated with 5% HCl in MeOH (2 mL) for 30 min at room temperature. The mixture was diluted with ethyl acetate (150 mL), washed successively with saturated $NaHCO_3$ and saturated NaCl, and then concentrated in vacuo. The resulting residue (240 mg) was chromatographed over a Si gel column with $CHCl_3$/MeOH with an increasing MeOH content. Elution with $CHCl_3$ gave a crude mixture of **9** and **11** (50 mg), whereas elution with $CHCl_3$/MeOH (5:1) afforded crude **10** (32 mg). The former mixture was then separated by chromatography over a Si gel column with benzene/EtOAc/HOAc (100:5:1) as eluent, yielding first **11** (19.9 mg) and then **9** (19.8 mg). Furthermore, the latter was purified by a combination of Sepak C_{18} column chromatography with 20% MeOH, Si gel column chromatography with benzene/EtOAc (2:1), and Si gel column chromatography with $CHCl_3$/MeOH/HOAc (100:1:0.1), giving **10** (17.0) mg. The $[\alpha]_D$ and ^1H NMR data of **9** were in agreement with those[2] reported for methyl 2,3,6-trideoxy-3-*O*-methyl-β-L-xylohexopyranoside, whereas the data of **10** were in accord with those[3,4] reported for methyl 2,6-dideoxy-3-*C*-methyl-α-D-ribohexopyranoside.

Methyl glycoside 11: colorless needles; mp 141.1−146.2° (EtOH−H_2O); $[\alpha]^{23}_D$ +162.9° (*c* 0.1, MeOH); $[\alpha]^{23}_D$ +165.8° (*c* 0.24, $CHCl_3$); IR (KBr) ν_{max} 3428, 3292, 3201, 2940, 2833, 1700, 1611, 1400, 1364, 1340, 1126, 1057, 1014, 988, 932, 824, 603, 559 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.20 (3H, d, *J* = 6.3 Hz, Me-5), 1.76−1.96 (4H, m, H_2-2 and H_2-3), 3.36 (3H, s, MeO-1), 3.73 (1H, qd, *J* = 6.3, 9.6 Hz, H-5), 4.38 (1H, dt, *J* = 4.8, 9.6 Hz, H-4), 4.64 (1H, br s, H-1); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 17.8 (q, Me-5), 24.3 (t, C-3), 29.1 (t, C-2), 54.5 (q, MeO-1), 66.6 (d, C-4), 74.1 (d, C-5), 97.4 (d, C-1); positive FABMS *m/z* 158 ($[M + H - OMe]^+$); HRFABMS *m/z* 158.0955 ($[M + H - OMe]^+$ calcd for $C_8H_{14}O_3$, 158.0943).

Methyl glycoside 9: a viscous syrup; $[\alpha]^{25}_D$ +52.0° (*c* 0.45, $CHCl_3$) (lit.[2] $[\alpha]^{20}_D$ +58°) (*c* 1.2 $CHCl_3$); IR (film) ν_{max} 3421, 2937, 1718, 1654, 1637, 1448, 1389, 1330, 1169, 1093, 1040, 1005, 961, 873, 815, 743 cm^{-1}; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.3 (q, Me-5), 30.5 (t, 2), 56.2 (q, MeO-3 or MeO-1), 56.9 (q,

MeO-1 or MeO-3), 68.1 (d, C-5), 69.1 (d, C-3), 78.2 (d, C-4), 99.8 (d, C-1); positive FABMS *m/z* 158 ([M − H_2O]$^+$), 145 ([M + H − MeOH]$^+$); HRFABMS *m/z* 158.0954 ([M − H_2O]$^+$ calcd for $C_8H_{14}O_3$, 158.0943), 145.0882 ([M + H − MeOH]$^+$ calcd for $C_7H_{13}O_3$, 145.0865).

Methyl glycoside 10: a viscous solid; [α]$^{25}_D$ +139.3° (*c* 0.2, $CHCl_3$) [(lit.[3] [α]$^{20}_D$ +136°) (*c* 1.0, $CHCl_3$); lit.[4] [α]$_D$ +138° ($CHCl_3$)]; IR (film) ν_{max} 3420, 2935, 1717, 1654, 1560, 1388, 1340, 1200, 1129, 1057, 980, 929, 906, 861, 840, 761 cm^{-1}; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.0 (q, Me-5), 22.1 (q, Me-3), 43.2 (t, C-2), 54.8 (q, MeO-1), 66.6 (d, C-5), 71.6 (s, C-3), 79.7 (d, C-4), 98.4 (d, C-1); positive FABMS *m/z* 158 ([M − H_2O]$^+$), 145.1 ([M + H − MeOH]$^+$); HRFABMS *m/z* 158.0959 ([M − H_2O]$^+$ calcd for $C_8H_{14}O_3$, 158.0943).

Formation of Methyl Glycosides 9−11 by Acid Degradation of P371A1(1) with 5% HCl in MeOH. P371A1 (**1**) (50 mg) was treated with 5% HCl in MeOH (0.5 mL) in the same way as in the case of **2**, yielding **9** (2.5 mg), **10** (1.3 mg), and **11** (1.7 mg), respectively.

Esterification of *C*-Glycoside 4 with (*R*)-(−)-MTPA Chloride. To a solution of *C*-glycoside **4** (10 mg) in CH_2Cl_2 (3 mL) were added (*R*)-(−)-MTPA chloride (10 μL) and Py (10.3 μL). After stirring for 2 h, the mixture was washed successively with 2 N HCl, saturated $NaHCO_3$, and saturated NaCl; dried over Na_2SO_4, and concentrated. The product was purified by preparative TLC with $CHCl_3$/MeOH (40:1, × 2), yielding (*S*)-(−)-MTPA ester **7** (7.8 mg) as powdery orange crystals.

(*S*)-(−)-MTPA ester 7: mp 182−183.5° (EtOH); [α]$^{25}_D$ +192.0° (*c* 0.094, $CHCl_3$); IR (KBr) ν_{max} 3448, 2934, 1734, 1636, 1610, 1438, 1376, 1244, 1169, 1020, 912, 876, 764, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (3H, d, *J* = 6.0 Hz, Me-6′), 1.53 (1H, m, Hax-3′), 1.75 (3H, s, Me-3), 2.16 (3H, s, 4-OCOMe), 2.29 (3H, s, 5-OCOMe), 2.61 (1H, m, Heq-3′), 3.35 (1H, t, *J* = 9.1 Hz, H-5′), 3.51 (3H, s, MeO), 3.54 (1H, m, H-6′), 4.61 (1H, d, *J* = 4.0 Hz, H-1), 4.99 (1H, d, *J* = 10.3 Hz, H-2′), 5.19 (1H, d, *J* = 7.3 Hz, H-6), 5.28 (1H, m, H-4′), 5.39 (1H, s, H-4), 5.64 (1H, d, *J* = 7.1 Hz, H-5), 5.82 (1H, d, *J* = 4.5 Hz, H-2), ca. 7.39 (3H, m, arom. H_3 of MTPA), ca. 7.51 (2H, m, arom. H_2 of MTPA), 7.66 (1H, d, *J* = 7.8 Hz, H-11), 7.85 (1H, d, *J* = 7.8 Hz, H-10), 12.27 (1H, s, H-8); negative FABMS *m/z* 819.9 ([M]$^-$).

Esterification of *C*-Glycoside 4 with (*S*)-(+)-MTPA Chloride. *C*-Glycoside **3** (10 mg) was treated with (*S*)-(+)-MTPA chloride and Py in the same way as above to give (*R*)-(+)-MTPA ester **8** (7.4 mg) as powdery orange crystals.

(*R*)-(+)-MTPA ester 8: mp 178.8−179.8° (EtOH); [α]$^{25}_D$ +142.7° (*c* 0.27, $CHCl_3$); IR (KBr) ν_{max} 3447, 2935, 1734, 1636, 1610, 1437, 1375, 1244, 1167, 1020, 912, 875, 764, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (3H, d, *J* = 6.0 Hz, Me-6′), 1.44 (1H, m, Hax-3′), 1.72 (3H, s, Me-3), 2.16 (3H, s, 4-OCOMe), 2.30 (3H, s, 5-OCOMe), 2.48 (1H, m, Heq-3′), 3.39 (1H, m, H-5′), 3.54 (1H, m, H-6′), 3.56 (3H, s, MeO), 4.60 (1H, d, *J* = 4.2 Hz, H-1), 4.99 (1H, d, *J* = 9.0 Hz, H-2′), 5.20 (1H, d, *J* = 7.2 Hz, H-6), 5.30 (1H, m, H-4′), 5.38 (1H, s, H-4), 5.65 (1H, d, *J* = 7.2 Hz, H-5), 5.81 (1H, d, *J* = 3.9 Hz, H-2), ca. 7.36 (3H, m, arom. H_3 of MTPA), ca. 7.50 (2H, m, arom. H_2 of MTPA), 7.65 (1H, d, *J* = 7.8 Hz, H-11), 7.81 (1H, d, *J* = 7.8 Hz, H-10), 12.34 (1H, s, H-8); negative FABMS *m/z* 820.2 ([M]$^-$).

Benzoylation of *C*-Glycoside 4. To a solution of *C*-glycoside **4** (28.3 mg) in CH_2Cl_2 (2 mL) were added benzoyl chloride (16.6 μL) and Py (11.5 μL). The mixture was stirred for 6 h and then washed successively with 2 N HCl, saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The products were separated by preparative TLC with $CHCl_3$/MeOH (30:1), furnishing the more polar benzoate **5** (6.1 mg) and the less polar dibenzoate **6** (2.8 mg) both as orange powders.

Benzoate 5: IR (KBr) ν_{max} 3447, 2926, 2853, 1734, 1637, 1610, 1438, 1376, 1239, 1070, 907, 786, 763, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (3H, d, *J* = 6.0 Hz, Me-6′), 1.55 (1H, m, Hax-3′), 1.69 (3H, s, Me-3), 2.09 (3H, s, 4-OCOMe), 2.22 (3H, s, 5-OCOMe), 2.58 (1H, m, Heq-3′), 3.43 (1H, t, *J* = 9.1 Hz, H-5′), 3.58 (1H, m, H-6′), 4.58 (1H, d, *J* = 4.0 Hz, H-1), 4.93 (1H, d, *J* = 10.4 Hz, H-2′), 5.12 (1H, d, *J* = 7.3 Hz, H-6), 5.21 (1H, m, H-4′), 5.33 (1H, s, H-4), 5.57 (1H, d, *J* = 7.3 Hz, H-5), 5.77 (1H, d, *J* = 4.4 Hz, H-2), 7.3−7.4 (6H, m, arom. H_6), 7.5−7.6 (2H, m, arom. H_2), 7.60 (1H, d, *J* = 7.8 Hz, H-11), 7.83 (1H, d, *J* = 7.8 Hz, H-10), 7.9−8.0 (2H, m, arom. H_2), 12.13 (1H, s, H-8); negative FABMS *m/z* 708.2 ([M]$^-$); CD (0.74 mM, EtOH) $\Delta\epsilon_{216}$ −13.4, $\Delta\epsilon_{222}$ 0, $\Delta\epsilon_{231}$ +29.4, $\Delta\epsilon_{274}$ −1.9, $\Delta\epsilon_{288}$ 0, $\Delta\epsilon_{300}$ +1.6.

Dibenzoate 6: IR (KBr) ν_{max} 3447, 2919, 2850, 1718, 1636, 1610, 1438, 1375, 1272, 1070, 960, 764, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, d, *J* = 6.0 Hz, Me-6′), 1.55 (1H, m, Hax-3′), 1.72 (3H, s, Me-3), 2.12 (3H, s, 4-OCOMe), 2.26 (3H, s, 5-OCOMe), 2.60 (1H, m, Heq-3′), 3.43 (1H, t, *J* = 9.0 Hz, H-5′), 3.57 (1H, m, H-6′), 4.89 (1H, d, *J* = 10.8 Hz, H-2′), 5.15 (1H, d, *J* = 7.5 Hz, H-6), 5.16 (1H, m, H-4′), 5.42 (1H, s, H-4), 5.85 (1H, m, H-1), 5.85 (1H, d, *J* = 7.5 Hz, H-5), 5.91 (1H, d, *J* = 5.0 Hz, H-2), 7.22−7.26 (2H, m, arom. H_2), 7.33−7.37 (3H, m, arom. H_3), 7.48−7.50 (1H, m, arom. H), 7.61 (1H, d, *J* = 7.8 Hz, H-11), 7.82 (1H, d, *J* = 7.8 Hz, H-10), 7.91−7.95 (4H, m, arom. H_4), 11.95 (1H, s, H-8); negative FABMS *m/z* 812.2 ([M]$^-$); CD (0.74 mM, EtOH) $\Delta\epsilon_{216}$ −22.0, $\Delta\epsilon_{223}$ 0, $\Delta\epsilon_{232}$ +35.4, $\Delta\epsilon_{273}$ −4.9, $\Delta\epsilon_{289}$ 0, $\Delta\epsilon_{299}$ +1.6.

Acknowledgment. We thank Ms. Masako Akaji (Superconducting NMR Spectrometer Laboratory, Faculty of Pharmaceutical Sciences, Kyoto University) and Ms. Toshie Minematsu (Faculty of Pharmaceutical Sciences, Kinki University) for measurements of NMR spectra. We are also thankful to Dr. Masanori Morita (Research Institute for Technology and Science, Kinki University) for measurements of MS spectra.

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NP990533P