

Studies on the Constituents of *Broussonetia* Species. V. Two New Pyrrolidine Alkaloids, Broussonetines K and L, as Inhibitors of Glycosidase, from *Broussonetia kazinoki* SIEB.

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Received October 8, 1998 ; accepted December 28, 1998

Two new pyrrolidine alkaloids, broussonetines K and L, were isolated from the branches of *Broussonetia kazinoki* SIEB. (Moraceae). Broussonetines K and L were formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*)-1-hydroxy-10-oxo-13-(β -D-glucopyranosyloxy) tridecyl] pyrrolidine (**1**) and (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*)-1-hydroxy-9-oxo-13-(β -D-glucopyranosyloxy) tridecyl] pyrrolidine (**2**), respectively, by spectroscopic and chemical methods. **1** and **2** inhibited β -glucosidase, β -galactosidase and β -mannosidase.

Key words pyrrolidine alkaloid; glycosidase inhibitor; *Broussonetia kazinoki*; broussonetine K; broussonetine L; Moraceae

Recently, we reported the structures of ten pyrrolidine alkaloids, broussonetines A–H and broussonetinines A and B as glycosidase inhibitors and two pyrrolidinyl piperidine alkaloids, broussonetines I and J from *Broussonetia kazinoki* SIEB. (Moraceae).^{1–4} In our latest work, we obtained two new pyrrolidine alkaloids, broussonetines K (**1**) and L (**2**) (Fig. 1), from the same tree. The present report deals with the isolation, structural elucidation including absolute stereostructures, and inhibitory activity towards several glycosidases.

The branches of this tree were extracted with hot water and the alkaloid constituents were concentrated as described in the Experimental section and summarized in Fig. 2. Compounds **1** and **2** were isolated by preparative HPLC of the concentrated alkaloids.

Compound **1** was obtained as a colorless powder, $[\alpha]_D^{20} +20.1^\circ$ (MeOH, $c=0.26$), showing a yellowish spot on TLC when sprayed with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction). The molecular formula was determined as $C_{24}H_{45}NO_{11}$ on the basis of positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) (m/z : 524.3073, $[M+H]^+$, error, 0.5 mmu). The IR spectrum showed a strong OH and NH band at 3317 cm^{-1} and a carbonyl band at 1701 cm^{-1} .

The ¹H- and ¹³C-NMR spectra of **1** showed the presence of a β -glucopyranosyl moiety (H-1': δ 4.81 (d, $J=7.8\text{ Hz}$); C-1': δ 104.63, and a number of other protons and carbons: Table 1). Hydrolysis of **1** with 1 N HCl provided a genuine aglycone (**1a**) and D-glucose ($[\alpha]_D^{20} +46.0^\circ$). The ¹H-NMR spectrum of **1a** suggested the presence of eight methylene groups [δ 1.15–2.00 (16H, m)], two oxymethylene groups [δ 4.22 (2H, m), δ 3.90 (2H, t, $J=7.3\text{ Hz}$)], three oxymethine groups [δ 4.96 (1H, t, $J=6.4\text{ Hz}$), δ 4.72 (1H, t, $J=6.4\text{ Hz}$), δ 4.15 (1H, m)], two methylene groups attached to a carbonyl group [δ 2.71 (2H, t, $J=7.3\text{ Hz}$), δ 2.61 (2H, t, $J=7.3\text{ Hz}$)], and two methine groups attached to a nitrogen atom [δ 3.85 (1H, m), δ 3.68 (1H, m)]. These data and ¹³C-NMR spectra were coincident with those of broussonetine E.² Thus, the structure of **1** was concluded to be 13'-O- β -D-glucopyranosylbroussonetine E since the glucosylation shift was 7.36 ppm between the C-13' of **1** and that of **1a** (Table 1), and the heteronuclear multiple bond correlation (HMBC) spectrum

of **1** showed long-range correlations between H-13' and an anomeric carbon, and an anomeric proton and C-13'.

Compound **2** was obtained as a colorless powder, $[\alpha]_D^{20} +21.3^\circ$ (MeOH, $c=0.56$), showing a yellowish spot on TLC with ninhydrin reaction, and the molecular formula was determined as $C_{24}H_{45}NO_{11}$ on the basis of pos. HR-SI-MS (m/z : 524.3074, $[M+H]^+$, error, 0.6 mmu). The IR spectrum showed a strong OH and NH band at 3398 cm^{-1} and a carbonyl band at 1703 cm^{-1} . The ¹H-NMR spectrum was strikingly similar to that of **1** and showed an anomeric proton [δ 4.78 (1H, d, $J=7.8\text{ Hz}$)]. Hydrolysis of **2** with 1 N HCl provided broussonetine F² (**2a**) and D-glucose ($[\alpha]_D^{20} +40.6^\circ$). The structure of **2** was concluded to be 13'-O- β -D-glucopyranosylbroussonetine F since the glucosylation shift was 7.66 ppm between the C-13' of **2** and that of **2a** (Table 1) and the HMBC spectrum showed long-range correlations between H-13' and an anomeric carbon (δ 104.42), and an anomeric proton and C-13'.

The absolute stereochemistry of the pyrrolidine ring moiety of **2a**, which has not been established, was determined to be (2*R*, 3*R*, 4*R*, 5*R*) using a benzoate chirality method, as follows. Cyclic carbamate (**2b**) was prepared from **2a** by reaction with phenyl chloroformate in tetrahydrofuran (THF): H₂O (7:3), and monoacetate (**2c**) and diacetate (**2d**) were prepared from **2b** with acetic anhydride in pyridine at room temperature, and dibenzoate (**2e**) was obtained by benzola-

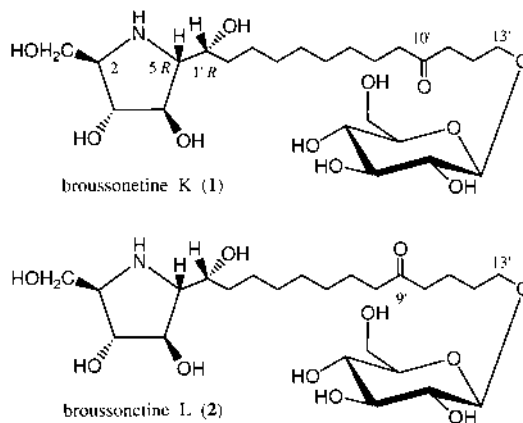
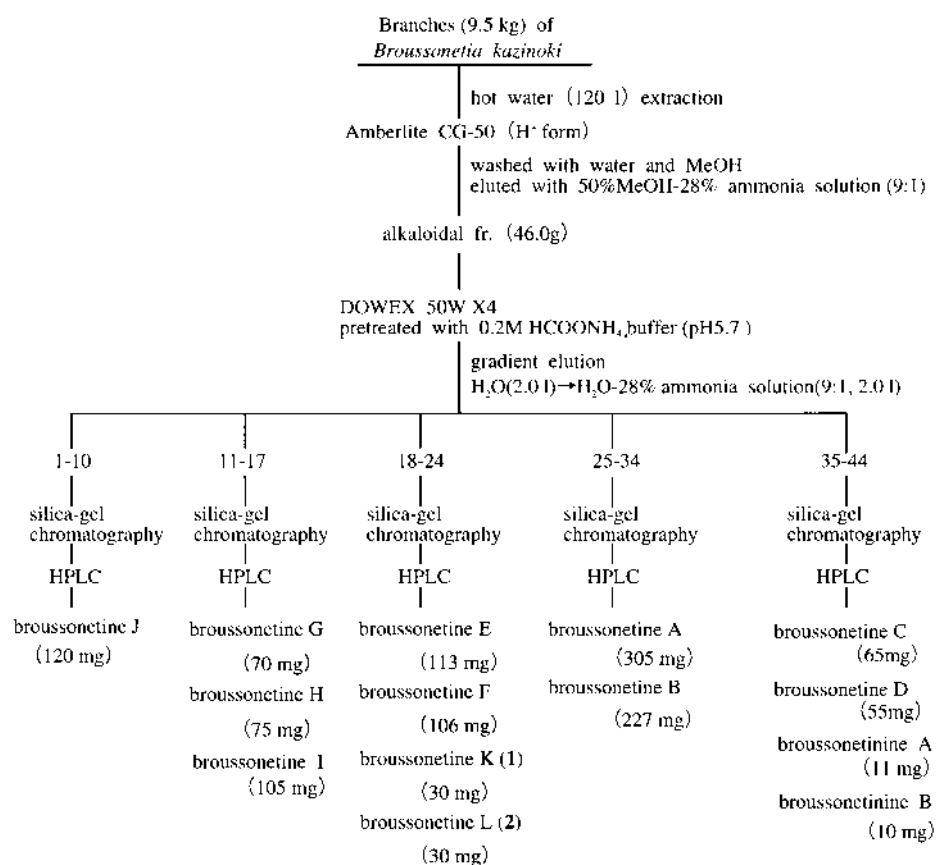


Fig. 1. Structures of **1** and **2**

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Fig. 2. Isolation of **1** and **2**Table 1. ¹H- and ¹³C-NMR Spectral Data for **1** and **2**

	Broussonetine K (1)		Aglycone (1a)		Broussonetine L (2)		Aglycone (2a)
	Proton (500 MHz)	Carbon (125 MHz)	Carbon (75 MHz)		Proton (500 MHz)	Carbon (125 MHz)	Carbon (75 MHz)
2	3.85 m	65.61	65.99	2	3.81 m	65.36	65.66
3	4.72 t (6.4)	80.08	80.72	3	4.70 t (6.4)	79.85	80.36
4	4.98 t (6.4)	79.66	80.37	4	4.93 t (6.4)	79.64	80.19
5	3.72 ^a t (6.4)	67.23	67.68	5	3.67 ^a t (6.4)	66.99	67.32
1'	4.19 ^a m	75.91	74.14	1'	4.09 ^a m	73.18	73.78
2'—7'	1.14—1.32	34.84, 26.66 30.08, 29.79 29.67, 29.43	35.15, 29.95 30.39, 29.71 30.06, 28.10	2'—6'	1.14—1.96	29.82, 29.62 29.50, 29.30 26.48	34.79, 29.92 29.72, 29.40 26.61
8'	1.50 ^a m	24.08	24.42	7'	1.50 ^a m	24.00	24.04
9'	2.30 t (7.3)	42.78	43.06	8'	2.28 t (7.3)	42.54	42.60
10'		210.39	210.98	9'		210.63	210.57
11'	2.59 t (7.3)	39.37	39.74	10'	2.37 t (7.3)	42.23	42.48
12'	2.02 ^a m	24.50	26.94	11'	1.71 ^a m	20.72	20.85
13'	3.73 ^a , 4.10	68.95	61.59	12'	1.92 m	34.62	32.99
CH ₂ OH	4.19 dd (5.4, 15.3) 4.24 dd (4.2, 15.3)	62.90	63.68	13'	3.69 ^a , 4.09	69.34	61.68
1''	4.81 d (7.8)	104.63		CH ₂ OH	4.19 dd (15.3, 5.4) 4.24 dd (15.3, 4.2)	62.62	63.33
2''	4.02 dd (7.8, 8.5)	75.16		1''	4.78 d (7.8)	104.42	
3''	4.21 ^a dd (8.5, 8.5)	78.45		2''	3.98 dd (7.8, 8.5)	74.94	
4''	4.17 ^a dd (8.5, 8.5)	71.65		3''	4.21 ^a dd (8.5, 8.5)	78.22	
5''	3.95 m	78.49		4''	4.17 ^a dd (8.5, 8.5)	71.50	
6''	4.39 dd (5.3, 11.8) 4.54 dd (2.4, 11.8)	62.78		5''	3.91 m	78.30	
				6''	4.33 dd (5.3, 11.8) 4.52 dd (2.4, 11.8)	62.71	

a) Overlapped signal. ppm (Hz)

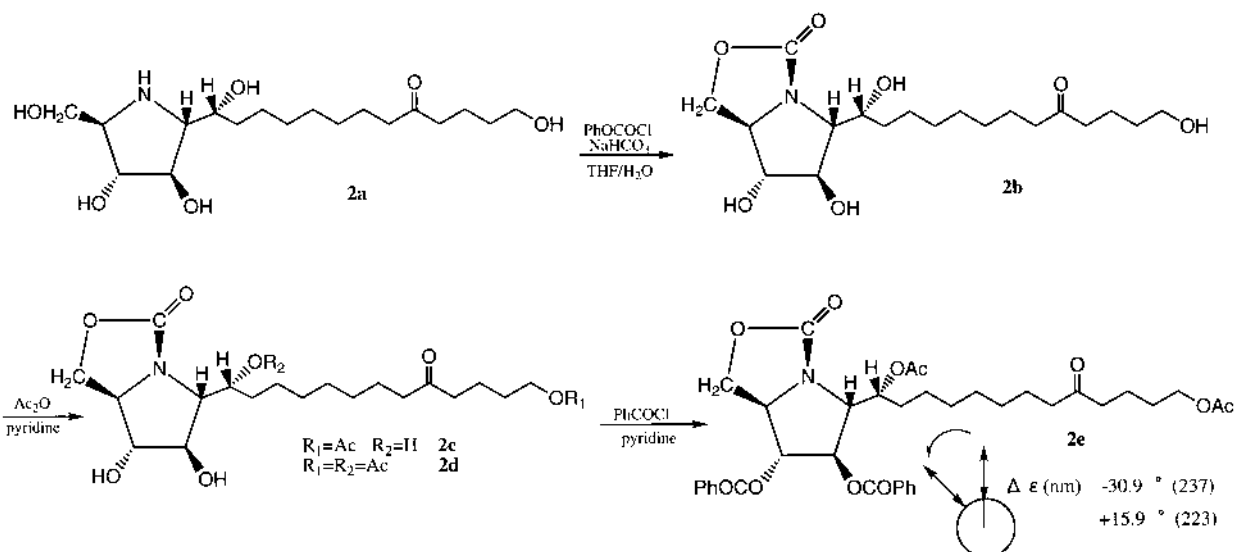
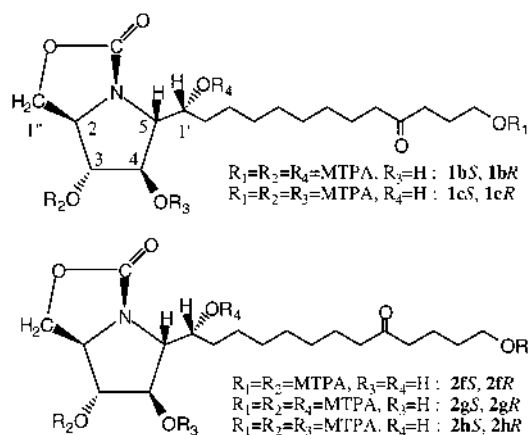


Chart 1



	1b	1c	2f	2g	2h
1''	-0.043	+0.061	+0.030	-0.042	+0.061
2	-0.160	-0.011		-0.154	-0.009
3	-0.328	-0.100	+0.100	-0.332	-0.099
4	-0.161	-0.217	-0.039	-0.161	-0.218
5	-0.037	-0.053	-0.019	-0.037	-0.052
1'	-0.061	+0.075	0.000	-0.060	+0.076
2'	+0.015	+0.032	+0.020	+0.013	+0.035
3'	+0.054	+0.255	0.000	+0.050	+0.251
4'		+0.004	0.000		+0.002
5'					
3''				+0.145	

Fig. 3. Relevant $\Delta\delta$ Values ($\Delta\delta = \delta S - \delta R$) Obtained for the MTPA Esters (1b, 1c, 2f, 2g, 2h)

tion of **2d** and purification of the products by preparative HPLC. The circular dichroism (CD) curve of **2e** showed negative Cotton effect ($\Delta\epsilon$ 237 -30.9°) and a positive effect ($\Delta\epsilon$ 223 $+15.9^\circ$) to establish the chiral arrangements in a counter-clockwise manner (Chart 1).

Additionally, a new version of Mosher's method was applied to determine the absolute configuration of C-1' in **2a**. The di (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)-acetic acid (MTPA) esters (**2fS**, **2fR**) and tri (*S*)- and (*R*)-MTPA esters (**2gS**, **2gR**, **2hS**, **2hR**) prepared from **2b** were assigned by $^1\text{H}-^1\text{H}$ correlation spectroscopy (COSY) (500 MHz) and the $\Delta\delta$ ($=\delta S - \delta R$) values were measured. These values thereby established the (*R*) configuration at C-1' of **2a**

Table 2. Concentration of Inhibitor Required to Produce 50% Inhibition of Enzyme Activity

	Inhibitor				
	1	2	DNJ	DGJ	DMJ
α -Glucosidase (from yeast)	NI	NI	0.93	—	—
β -Glucosidase (from sweet almond)	0.026	0.017	0.58	—	—
β -Galactosidase (from bovine liver)	0.005	0.004	—	0.13	—
α -Mannosidase (from Jack beans)	NI	NI	—	—	0.94
β -Mannosidase (from snail acetone powder)	0.30	0.20	—	—	0.81

NI: up to 100 μM or no inhibition. (μM)

by comparison between di-MTPA esters (**2fS**, **2fR**) and tri-MTPA esters (**2gS**, **2gR**) (Fig.3).

Thus, the structure of **2** was formulated as (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*)-1-hydroxy-9-oxo-13-(β -D-glucopyranosyloxy) tridecyl] pyrrolidine, and **1** was concluded to be (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*)-1-hydroxy-10-oxo-13-(β -D-glucopyranosyloxy) tridecyl] pyrrolidine by comparison of the $\Delta\delta$ values patterns of tri-MTPA esters (**1bS**, **1bR**, **1cS**, **1cR**) and those of **2gS**, **2gR**, **2hS**, and **2hR** (Fig.3).

The inhibitory activities of **1**, **2**, 1-deoxyojirimycin (DNJ),^{8,9} 1-deoxygalactonojirimycin (DGJ), and 1-deoxymannojirimycin (DMJ)^{8,9} against α -glucosidase, β -glucosidase, β -galactosidase, α -mannosidase and β -mannosidase were assayed by the methods described in the Experimental section and the results are summarized in Table 2.

Broussonetines K (**1**) and L (**2**) inhibited β -glucosidase, β -galactosidase and β -mannosidase. Broussonetines E and F,² the aglycones of **1** and **2**, inhibited α -glucosidase, but **1** and **2** did not.

Experimental

General The instruments used in this work were: a JASCO digital polarimeter (for specific rotation, measured at 25 $^\circ$); a Perkin-Elmer 1720X-

FTIR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); a Varian Mercury 300, unity Inova-500 (for NMR spectra, measured in pyridine- d_5 , on the δ scale using tetramethylsilane as an internal standard); a Shimadzu spectrophotometer UV 1200 (for enzyme assay).

Column chromatography was carried out on ion exchange resin (Amberlite CG-50, Amberlite IRA-67/Orugano Company and Dowex 50W-X4/Dow Chemical Company), and silica gel (Chromatorex DM1020/Fuji Silysia Chemical Ltd.). HPLC was conducted on a Gilson 305 pump or a JASCO PU 980 equipped with a JASCO 830-RI or UV-970 as a detector. Silica gel 60 F₂₅₄ (Merck) precoated TLC plates were used, developed with a CHCl₃-MeOH-AcOH-H₂O (20:10:7:5) solvent system, and detection was carried out by ninhydrin reagent followed by heating.

Isolation of 1 and 2 Dried branches of *Broussonetia kazinoki* (9.5 kg, collected in Takatsuki City (Osaka) in 1995) were cut finely and then extracted with hot water (40 l×3) for 2 h. The extracted solution was chromatographed on an Amberlite CG-50 (H⁺-form) column (8 l, 6.5 i.d.×30 cm, repeated 8 times). After washing the column with water and then 50% MeOH, the adsorbed material was eluted with 50% MeOH-28% ammonia solution (9:1). The eluted fraction was concentrated *in vacuo* to give a basic fraction (46.0 g). This fraction was chromatographed on a Dowex 50W-X4 column (200-400 mesh, 500 ml, 5.0 i.d.×30 cm) pretreated with formic acid-ammonium formate buffer (0.2 M ammonia formate, adjusted to pH 5.7 with 1 N formic acid), with gradient elution (H₂O (2.0 l)→H₂O-28% ammonia solution (9:1, 2.0 l)). The fraction containing **1** and **2** was rechromatographed on silica gel (Chromatorex DM1020) using CHCl₃ and MeOH, followed by preparative HPLC [column, Asahipak ODP 5E (10 i.d.×250 mm); solvent, CH₃CN-H₂O (14:86), adjusted to pH 12.0 with ammonia solution; flow rate, 1.5 ml/min; column temperature, ambient]. **1** (30 mg) and **2** (30 mg) were finally obtained.

Broussonetine K (1): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), [α]_D²⁰ +20.1° (MeOH c =0.26), C₂₄H₄₅NO₁₁. pos. HR-SI-MS m/z : 524.3073 ([M+H]⁺) error: 0.5 mmu. IR ν (KBr) cm⁻¹: 3359 (OH, NH), 1701 (CO), ¹H- and ¹³C-NMR (pyridine- d_5): Table 1.

Broussonetine L (2): Colorless powder, ninhydrin reaction: positive (a yellow spot on TLC), [α]_D^{21.3} (MeOH c =0.56), C₂₄H₄₅NO₁₁. pos. HR-SI-MS m/z : 524.3074 ([M+H]⁺) error: 0.6 mmu. IR ν (KBr) cm⁻¹: 3398 (OH, NH), 1703 (CO), ¹H- and ¹³C-NMR (pyridine- d_5): Table 1.

Hydrolysis of **1** and **2** with 1 N HCl: **1** (18 mg) was dissolved in 1 N HCl (10 ml) and the solution was refluxed on a water bath for 4 h. After cooling, the reaction mixture was passed through an Amberlite IRA-67 (OH⁻ form) column (2.0 i.d.×5.0 cm) to neutralize it. The resulting solution was chromatographed on a Sep-Pak C-18 column (Waters), and elution with water afforded D-glucose (3 mg), [α]_D²⁰ +46.0° (c =0.30, H₂O), which was identified by HPLC (t_R =12.2 min) [column, Shodex NH2 P-50 4E (4.6 i.d.×250 mm); solvent, CH₃CN-H₂O (75:25); flow rate, 0.8 ml/min; detection, RI; column temperature, 40°C], ¹H-NMR and ¹³C-NMR. Elution with MeOH afforded the aglycone, broussonetine E (**1a**) (13 mg) as a colorless powder (identified by comparison of ¹H-NMR, ¹³C-NMR and HPLC data²⁾). Similarly, D-glucose (3.2 mg), [α]_D²⁰ +40.6° (c =0.32, H₂O) and an aglycone, broussonetine F (**2a**) (7 mg) were obtained from **2** (12 mg).

Carbamate (**2b**): **2a** (35 mg) was treated with phenyl chloroformate (1.5 ml) in THF-H₂O (7:3) (10 ml) and NaHCO₃ (0.5 g) at 2°C for 3 h followed by warming to room temperature for 36 h. The reaction products were subjected to HPLC [column, Asahipak ODP-5E (6.0 i.d.×250 mm); solvent, CH₃CN-H₂O (19:81), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient]. Carbamate (**2b**) was obtained as a colorless oil (30 mg). **2b**: C₁₉H₃₃NO₇. ¹H-NMR (pyridine- d_5) δ : 2.35 (2H, t, J =6.4 Hz, 9'-H), 2.46 (2H, t, J =6.4 Hz, 11'-H), 3.84 (2H, t, J =7.0 Hz, 13'-H), 4.28*—4.40* (3H, m, 1'-H, 2-H, 3-H), 4.47 (1H, m, 5-H), 4.51* (2H, m, CH₂O), 5.19 (1H, t, J =5.5 Hz, 4-H). *: overlapped signals.

Dibenzoate (**2e**): **2b** (12 mg) was treated with acetic anhydride (20 mg) in pyridine at room temperature, and after the usual treatment and HPLC purification [column, Asahipak ODP-5E (6.0 i.d.×250 mm); solvent, CH₃CN-H₂O (30:70), adjusted to pH 12.0 with ammonia solution; flow rate, 1.0 ml/min; detection, RI; column temperature, ambient], monoacetate (**2c**) (5 mg) and diacetate (**2d**) (7 mg) were obtained as colorless oils. **2d** (7 mg) was dissolved in pyridine (2 ml) and benzoyl chloride (50 μ l) was added and the solution stirred at room temperature for 36 h. The reaction products were subjected to HPLC [column, Cosmosil C18-AR-II (10 i.d.×250 mm); solvent, CH₃CN-H₂O (20:80→100:0, 50 min); flow rate, 1.5 ml/min; detection, UV 254 nm; column temperature, 40°C]. Dibenzoate (**2e**) was obtained as a colorless oil (6.5 mg). **2e**: C₃₇H₄₅NO₁₁. pos. SIMS m/z : 702 (M+Na⁺), 105 (base peak). ¹H-NMR (CDCl₃) δ : 2.35 (2H, t, J =6.5 Hz, 9'-H), 2.42

(2H, t, J =6.5 Hz, 11'-H), 4.05* (2H, m, 13'-H), 4.10* (1H, m, 2-H), 4.25 (1H, dd, J =5.0, 2.0 Hz, 5-H), 4.78 (2H, m, CH₂O), 5.20* (1H, m, 3-H), 5.85 (1H, t, J =2.0 Hz, 4-H), 7.40—7.70 (6H, m, phenyl H), 8.00—8.15 (4H, m, phenyl H). *: overlapped signals. CD (c =6.8×10⁻⁵, MeOH) $\Delta\epsilon$ ²⁵: +15.9 (223), -30.9 (237).

(S)-(-)-MTPA Esters (**2fS**, **2gS**, **2hS**, **1bS**, **1cS**): **2b** (6 mg) was treated with (R)-(-)-MTPA-Cl (20 μ l) in pyridine (300 μ l) at room temperature overnight, and then *N,N*-dimethyl-1,3-propanediamine was added. The reaction products were subjected to HPLC [column, Cosmosil C18-AR-300 (4.6 i.d.×150 mm); solvent, CH₃CN-H₂O (20:80→100:0, 40 min); flow rate, 1.0 ml/min; detection, UV 230 nm; column temperature, 40°C]. **2fS**, **2gS**, and **2hS** were obtained as colorless oils (2.0, 1.5, 2.5 mg). **2fS**: C₃₉H₄₇NO₁₁F₆. pos. SIMS m/z : 820 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.650* (CH₂), 1.289* (3'-H), 1.483* (2'-H), 2.351 (2H, t, J =7.0 Hz, 8'-H), 2.398 (2H, t, J =7.0 Hz, 10'-H), 3.545, 3.518 (each 3H, s, OCH₃), 3.700 (1H, t, J =5.0 Hz, 5-H), 3.748 (1H, m, 1'-H), 4.018 (1H, ddd, J =7.5, 7.5, 7.5 Hz, 2-H), 4.312 (2H, m, 13'-H), 4.580 (2H, m, CH₂O), 4.621 (1H, m, 4-H), 4.916 (1H, dd, J =7.5, 5.5 Hz, 3-H), 7.390—7.530 (10H, m, MTPA-Ar H). **2gS**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.435* (3'-H), 1.750* (2'-H), 2.350 (2H, t, J =7.0 Hz, 8'-H), 2.410 (2H, t, J =7.0 Hz, 10'-H), 2.920 (1H, m, 2-H), 3.537, 3.500, 3.482 (each 3H, s, OCH₃), 3.852 (1H, dd, J =5.5, 4.0 Hz, 5-H), 4.300 (2H, m, 13'-H), 4.066 (1H, dd, J =10.0, 8.0 Hz, CH₂O), 4.346 (1H, dd, J =10.0, 4.5 Hz, CH₂O), 4.373 (1H, dd, J =8.5, 5.5 Hz, 4-H), 4.645 (1H, dd, J =8.5, 6.5 Hz, 3-H), 5.283 (1H, ddd, J =6.5, 6.5, 4.0 Hz, 1'-H), 7.320—7.520 (15H, m, MTPA-Ar H). **2hS**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.520*, 1.838* (2'-H), 2.350 (2H, t, J =7.0 Hz, 8'-H), 2.412 (2H, t, J =7.0 Hz, 10'-H), 3.530, 3.502, 3.480 (each 3H, s, OCH₃), 3.702 (1H, m, 1'-H), 3.904 (1H, dd, J =5.5, 4.0 Hz, 5-H), 4.100 (1H, m, 2-H), 4.305 (2H, m, 13'-H), 4.416 (1H, dd, J =10.0, 4.5 Hz, CH₂O), 4.581 (1H, dd, J =10.0, 8.5 Hz, CH₂O), 4.940 (1H, dd, J =6.5, 3.5 Hz, 3-H), 5.838 (1H, dd, J =4.0, 3.5 Hz, 4-H), 7.320—7.520 (15H, m, MTPA-Ar H). *: overlapped signals. Similarly, **1bS** (1.5 mg) and **1cS** (3.0 mg) were obtained from **1a** (10 mg). **1bS**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.435* (3'-H), 1.754* (2'-H), 2.331 (2H, t, J =7.0 Hz, 9'-H), 2.418 (2H, t, J =7.0 Hz, 11'-H), 2.922 (1H, m, 2-H), 3.537, 3.498, 3.481 (each 3H, s, OCH₃), 3.849 (1H, dd, J =5.5, 4.0 Hz, 5-H), 4.300 (2H, m, 13'-H), 4.060 (1H, dd, J =10.0, 8.0 Hz, CH₂O), 4.345 (1H, dd, J =10.0, 4.5 Hz, CH₂O), 4.375 (1H, dd, J =8.5, 5.5 Hz, 4-H), 4.649 (1H, dd, J =8.5, 6.5 Hz, 3-H), 5.285 (1H, ddd, J =6.5, 6.5, 4.0 Hz, 1'-H), 7.320—7.520 (15H, m, MTPA-Ar H). **1cS**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.525*, 1.840* (2'-H), 2.331 (2H, t, J =7.0 Hz, 9'-H), 2.418 (2H, t, J =7.0 Hz, 11'-H), 3.535, 3.500, 3.478 (each 3H, s, OCH₃), 3.700 (1H, m, 1'-H), 3.905 (1H, dd, J =5.5, 4.0 Hz, 5-H), 4.102 (1H, m, 2-H), 4.305 (2H, m, 13'-H), 4.418 (1H, dd, J =10.0, 4.5 Hz, CH₂O), 4.581 (1H, dd, J =10.0, 8.5 Hz, CH₂O), 4.940 (1H, dd, J =6.5, 3.5 Hz, 3-H), 5.837 (1H, dd, J =4.0, 3.5 Hz, 4-H), 7.320—7.520 (15H, m, MTPA-Ar H). *: overlapped signals.

(R)-(+)-MTPA Esters (**2fR**, **2gR**, **2hR**, **1bR**, **1cR**): **2b** (6 mg) was treated with (S)-(+)-MTPA-Cl (20 μ l) in pyridine (300 μ l) at room temperature overnight, then *N,N*-dimethyl-1,3-propanediamine was added. The reaction products were subjected to HPLC [column, Cosmosil C18-AR-300 (4.6 i.d.×150 mm); solvent, CH₃CN-H₂O (20:80→100:0, 40 min); flow rate, 1.0 ml/min; detection, UV 230 nm; column temperature, 40°C]. **2fR**, **2gR**, and **2hR** were obtained as colorless oils (2.0, 1.5, 2.5 mg). **2fR**: C₃₉H₄₇NO₁₁F₆. pos. SIMS m/z : 820 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.720* (CH₂), 1.289* (3'-H), 1.483* (2'-H), 2.351 (2H, t, J =7.0 Hz, 8'-H), 2.396 (2H, t, J =7.0 Hz, 10'-H), 3.543, 3.541 (each 3H, s, OCH₃), 3.700 (1H, t, J =5.5 Hz, 5-H), 3.729 (1H, m, 1'-H), 3.918 (1H, m, 2-H), 4.310 (2H, m, 13'-H), 4.550 (2H, m, CH₂O), 4.640 (1H, m, 4-H), 4.955 (1H, dd, J =7.5, 5.5 Hz, 3-H), 7.390—7.530 (10H, m, MTPA-Ar H). **2gR**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.290* (3'-H), 1.700* (2'-H), 2.386 (2H, t, J =7.0 Hz, 8'-H), 2.412 (2H, t, J =7.0 Hz, 10'-H), 3.252 (1H, m, 2-H), 3.538, 3.532, 3.510 (each 3H, s, OCH₃), 3.912 (1H, dd, J =5.5, 4.5 Hz, 5-H), 4.302 (2H, m, 13'-H), 4.220 (1H, dd, J =10.0, 8.0 Hz, CH₂O), 4.388 (1H, dd, J =10.0, 4.5 Hz, CH₂O), 4.410 (1H, dd, J =7.0, 4.5 Hz, 4-H), 4.806 (1H, dd, J =7.0, 6.5 Hz, 3-H), 5.270 (1H, m, 1'-H), 7.360—7.520 (15H, m, MTPA-Ar H). **2hR**: C₄₉H₅₄NO₁₃F₉. pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.269*, 1.836* (2'-H), 2.386 (2H, t, J =7.0 Hz, 8'-H), 2.412 (2H, t, J =7.0 Hz, 10'-H), 3.532, 3.519, 3.502 (each 3H, s, OCH₃), 3.667 (1H, m, 1'-H), 3.828 (1H, dd, J =5.0, 4.0 Hz, 5-

H), 4.001 (1H, m, 2-H), 4.305 (2H, m, 13'-H), 4.425 (1H, dd, $J=10.0$, 4.5 Hz, CH₂O), 4.520 (1H, dd, $J=10.0$, 8.5 Hz, CH₂O), 5.158 (1H, dd, $J=7.0$, 4.5 Hz, 3-H), 5.890 (1H, dd, $J=4.5$, 3.5 Hz, 4-H), 7.360—7.520 (15H, m, MTPA-Ar H). *: overlapped signals. Similarly, **1bR** (1.5 mg) and **1cR** (2.0 mg) were obtained from **1a** (10 mg). **1bR**: C₄₉H₅₄NO₁₃F₉, pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.300* (3'-H), 1.700* (2'-H), 2.331 (2H, t, $J=7.0$ Hz, 9'-H), 2.416 (2H, t, $J=7.0$ Hz, 11'-H), 3.250 (1H, m, 2-H), 3.536, 3.517, 3.506 (each 3H, s, OCH₃), 3.910 (1H, dd, $J=5.5$, 4.5 Hz, 5-H), 4.300 (2H, m, 13'-H), 4.220 (1H, dd, $J=10.0$, 8.0 Hz, CH₂O), 4.388 (1H, dd, $J=10.0$, 4.5 Hz, CH₂O), 4.412 (1H, dd, $J=7.0$, 4.5 Hz, 4-H), 4.810 (1H, dd, $J=7.0$, 6.5 Hz, 3-H), 5.270 (1H, m, 1'-H), 7.360—7.520 (15H, m, MTPA-Ar H). **1cR**: C₄₉H₅₄NO₁₃F₉, pos. SIMS m/z : 1036 (M+H)⁺, 189 (base peak). ¹H-NMR (CDCl₃) δ : 1.200*—1.550* (CH₂), 1.270*, 1.836* (2'-H), 2.330 (2H, t, $J=7.0$ Hz, 9'-H), 2.416 (2H, t, $J=7.0$ Hz, 11'-H), 3.534, 3.519, 3.504 (each 3H, s, OCH₃), 3.668 (1H, m, 1'-H), 3.830 (1H, dd, $J=5.0$, 4.0 Hz, 5-H), 4.002 (1H, m, 2-H), 4.305 (2H, m, 13'-H), 4.429 (1H, dd, $J=10.0$, 4.5 Hz, CH₂O), 4.520 (1H, dd, $J=10.0$, 8.5 Hz, CH₂O), 5.157 (1H, dd, $J=7.0$, 4.5 Hz, 3-H), 5.890 (1H, dd, $J=4.5$, 3.5 Hz, 4-H), 7.360—7.520 (15H, m, MTPA-Ar H). *: overlapped signals.

Enzyme Assays Materials α -Glucosidase (from Bakers yeast, lot 83H8000), β -galactosidase (from bovine liver, lot 54H7025), α -mannosidase (from Jack beans, lot 48F95454), and β -mannosidase (from snail acetone powder, lot 45H3826) were obtained from Sigma Chemical Company (St. Louis, U.S.A.), and β -glucosidase (from sweet almond, lot 34552) was obtained from Toyobo Company (Osaka, Japan). *p*-Nitrophenyl α -D-glucopyranoside, β -D-glucopyranoside, α -D-mannopyranoside, and β -D-galactopyranoside were obtained from Nacalai Tesque, Inc. (Osaka, Japan), *p*-nitrophenyl β -D-mannopyranoside from Sigma Chemical Company, and DNJ, DGJ, and DMJ from Funakoshi Company (Tokyo, Japan).

Assay of β -Glucosidase Inhibition The reaction mixture consisted of 475 μ l 0.1 M acetate buffer (pH 5.0), 250 μ l 250 mM *p*-nitrophenyl β -D-glucopyranoside and 250 μ l β -glucosidase solution (A stock solution of 1.0 mg/ml in 50 mM Tris-HCl-buffer, pH 7.8 was diluted 200 times with the 10 mM phosphate buffer, pH 7.0, just before assay), with the substrates **1**, 2

or DNJ (25 μ l solution, concentration: 20—0.1 mg/ml). After incubation for 20 min at 37 °C, the reaction was interrupted by the addition of 1 ml 0.5 M sodium carbonate, and the amount of *p*-nitrophenol liberated was measured colorimetrically at 400 nm (ODtest). The inhibition rates (%) were calculated from the formula $100 - 100 \times (\text{ODtest} - \text{ODblank}) / (\text{control ODtest} - \text{control ODblank})$ and the IC₅₀ values were obtained from the inhibition curves. The IC₅₀ values were 0.026 μ M for **1** and 0.017 μ M for **2**, while that of DNJ was 0.58 μ M. Assays for α -glucosidase, β -galactosidase, β -mannosidase and α -mannosidase were carried out as above using *p*-nitrophenyl α -D-glucopyranoside, β -D-galactopyranoside, β -D-mannopyranoside and α -D-mannopyranoside as substrates. The IC₅₀ values are shown in Table 2.

Acknowledgements The authors are grateful to Mr. Minoura for 500 MHz NMR spectral measurement and Mrs. Fujitake for mass spectral measurement at Osaka University of Pharmaceutical Sciences.

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