The First Cyclic Phenolic Acid Glycoside Dimer and New α-Tetralone and Triterpenoid Glucosides from *Gentiana loureirii*

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Further chemical investigation of the whole plants of *Gentiana loureirii* led to the isolation and characterization of three new compounds, cyclic 4-*O*-β-D-glucopyranosylcaffeic acid dimer (1), a new α-tetralone, 4-hydroxy-6-methyl-α-tetralone 4-*O*-β-D-glucopyranoside (2), and 3β,30-dihydroxy-12-ursen-28-oic acid 28-*O*-β-D-glucopyranosyl ester (3), along with five known compounds. To the best of our knowledge, compound 1 is the first cyclic phenolic acid glycoside dimer.

Key words *Gentiana loureirii*; *Gentiana*; phenolic acid glycoside; α-tetralone; triterpenoid

Gentiana loureirii Griseb. (Gentianaceae), a perennial herb widely distributed in South China,[1] is a Chinese folk medicine used for the treatment of various disorders related to inflammation and infections.[2] We previously reported the isolation of iridoids, flavonoids, and oleanolic acid as its predominant constituents.[3] In further investigation on the minor constituents of this plant, a novel phenolic acid glycoside, cyclic 4-*O*-β-D-glucopyranosylcaffeic acid dimer (**1**), a new α-tetralone, 4-hydroxy-6-methyl-α-tetralone 4-*O*-β-D-glucopyranoside (**2**), and a new triterpenoid saponin, 3β,30-dihydroxy-12-ursen-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**), were isolated along with five known compounds, rotundic acid, tormentic acid, 2α,3α,23-trihydroxy-12,20(30)-ursadien-28-oic acid, 1α,2α,3α,24-tetrahydroxy-12-oleanen-28-oic acid, and 1-*O*-feruloylglucose. Herein, we report the isolation and structure elucidation of these new compounds.

Results and Discussion

The EtOH extract of the whole plants of *Gentiana loureirii* was fractionated with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. Separation by a combination of silica gel, octadecyl silica (ODS) column chromatography, and preparative HPLC afforded three new compounds, **1**—**3**, and five known compounds, rotundic acid,[4] tormentic acid,[5] 2α,3α,23-trihydroxy-12,20(30)-ursadien-28-oic acid,[6] 1α,2α,3α,24-tetrahydroxy-12-oleanen-28-oic acid,[7] and 1-*O*-feruloylglucose.[8] The structures of the known compounds were determined by interpretation of their spectroscopic data as well as by comparison with reported data.

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined as $C_{30}H_{32}O_{16}$ by HR-electrospray ionization (ESI)-MS and NMR data. The ^{1}H- and ^{13}C-NMR spectra in combination with ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) spectra showed the presence of a β-glucopyranose moiety (δ_H 3.97—5.66; δ_C 65.3—102.3) and a caffeic acid residue (δ_H 6.71—7.88; δ_C 116.5—166.7). The D-form of the glucose moiety was determined by alkaline and acid hydrolysis. In the heteronuclear multiple bond correlation (HMBC) spectrum, correlations between the anomeric proton [δ_H 5.66 (1H, d, J=7.8 Hz, H-1′)] and the aromatic quaternary carbon at δ_C 148.8 (C-4) and between the protons at δ_H 5.18 (1H, dd, J=11.3, 2.1 Hz, H-6′a) and 4.67 (1H, dd, J=11.3, 10.3 Hz, H-6′b) and the carbonyl carbon at δ_C 166.7 (C-9) were observed, indicating attachments of the glucose moiety to C-4 of the caffeic acid residue *via* a glycosidic linkage and the caffeoyl group to C-6′ of the glucose moiety *via* an ester linkage. These findings in the NMR spectra in combination with the molecular formula suggested a symmetrical structure composed of two 4-*O*-β-D-glucopyranosylcaffeic acid units which were connected with each other by ester linkages between each C-6′ in one unit and the carbonyl carbon (C-9) in the other unit to form a 26-membered macrolide ring. The large coupling constant (J=10.3 Hz) observed between H-5′ and H-6′b in the ^{1}H-NMR spectrum was in accord with the energy-minimized structure (Fig. 1) obtained by MOPAC in CS Chem3D, which showed a dihedral angle of near 180° between H-5′ and H-6′b bonds. Therefore compound **1**, tentatively named cyclic 4-*O*-β-D-glucopyranosylcaffeic acid dimer, was established to have the

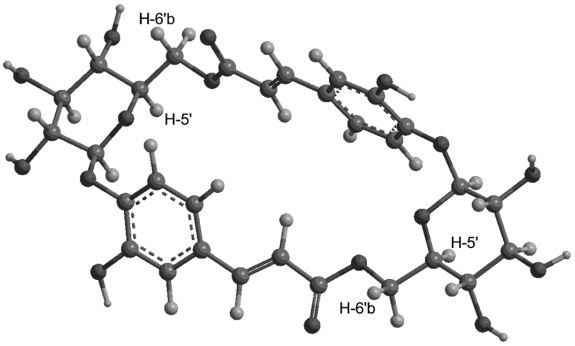
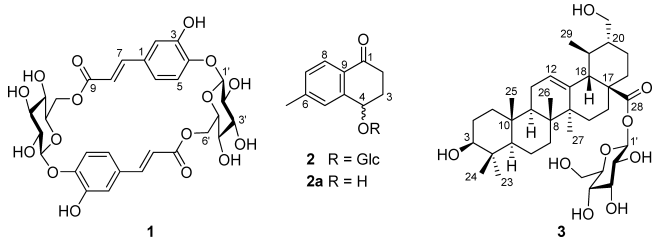


Fig. 1. The Energy-Minimized Structure of **1**, Showing a Dihedral Angle of Near 180° between H-5′ and H-6′b



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structure as shown. To the best of our knowledge, this compound is the first cyclic phenolic acid glycoside dimer.

Compound **2**, obtained as a white amorphous powder, had the molecular formula $C_{17}H_{22}O_7$ as determined by combined analysis of HR-ESI-MS and NMR data. The UV spectrum exhibited absorption maxima at 219, 257, and 291 (sh) nm. The ^{1}H- and ^{13}C-NMR spectra of **2** showed the presence of a 1,2,4-trisubstituted benzene ring [δ_H 8.12 (1H, d, J=7.9 Hz, H-8), 7.89 (1H, br s, H-5), 7.13 (1H, br d, J=7.9 Hz, H-7); δ_C 129.6—143.5], two methylenes [δ_H 3.01 (H-3ax), 2.52 (H-3eq), and 2.41 (H_2-2); δ_C 35.2 (C-2), and 31.2 (C-3)], an oxymethine [δ_H 5.20 (1H, t, J=5.1 Hz, H-4); δ_C 74.9 (C-4)], and a ketone carbonyl group [δ 197.0 (C-1)]. These data were in accord with a structure of 4-hydroxy-α-tetralone.[9] In addition, the spectra exhibited resonances indicating the presence of a β-glucopyranose moiety and a methyl group [δ_H 2.16 (3H, s); δ_C 21.6]. The presence of D-glucose moiety was also indicated by acid hydrolysis. In the HMBC spectrum, the presence of correlations from the anomeric proton at δ_H 5.05 (1H, d, J=7.7 Hz, H-1′) to C-4 (δ_C 74.9), from H-4 to the anomeric carbon (C-1′), and from the methyl protons (δ_H 2.16) to C-5, C-6, and C-7 showed that the sugar moiety was bound to C-4 by a glycosidic linkage and the methyl group was linked to C-6. To determine the absolute configuration of the chiral center at C-4, compound **2** was hydrolyzed with β-glucosidase to give aglycone **2a**. The circular dichroism (CD) spectrum of **2a** was similar to that of (4*S*)-4-hydroxy-α-tetralone,[10] but with much lower intensities of all cotton effects. This in combination with its small positive optical rotation value (+3.0°) aroused us to assume that **2a** was an enantiomeric mixture with slightly more (4*S*)-epimer. To verify our assumption, **2a** was subjected to Mosher esterification.[11] As predicted, the reaction afforded a mixture of two isomeric esters in approximately 5 : 3 ratio, which was estimated from the intensity of proton signals in the ^{1}H-NMR spectrum. Thus compound **2** was determined to be a mixture of (4*S*)- and (4*R*)-epimers (*ca.* 5 : 3) of 4-hydroxy-6-methyl-α-tetralone 4-*O*-β-D-glucopyranoside.

Compound **3**, a white amorphous powder, was determined to have the molecular formula $C_{36}H_{58}O_9$ by HR-ESI-MS and NMR data. Its ^{1}H-NMR spectrum exhibited distinguishable signals for five tertiary methyl groups (δ_H 1.21, 1.20, 1.19, 1.02, 0.93), a secondary methyl group [δ_H 1.12 (d, J=6.6 Hz)], an olefinic methine [δ_H 5.50 (t, J=3.0 Hz)], an oxymethine [δ_H 3.43 (J=11.4, 4.4 Hz)], an oxymethylene [δ_H 3.92 (1H, dd, J=10.8, 2.5 Hz) and 3.87 (1H, dd, J=10.8, 5.8 Hz)], three methines [δ_H 2.65 (1H, d, J=11.5 Hz), 1.60 (1H, dd, J=11.6, 6.5 Hz), and 0.82 (1H, br d, J=11.2 Hz)], and a β-glucopyranose moiety [δ_H 6.30 (1H, d, J=7.9 Hz, H-1′) and δ_H 4.45—4.03]. The ^{13}C-NMR data (see Experimental), assigned on the basis of distortionless enhancement by polarization transfer (DEPT), HSQC, and HMBC, indicated, besides resonances for the β-glucopyranose moiety, the presence of thirty carbons, including six methyls, ten sp^3 methylenes, six sp^3 methines, five sp^3 quaternary carbons, one sp^2 methine, one sp^2 quaternary carbon, and one ester carbonyl carbon. The above spectral data suggested that this compound was an urs-12-ene type of triterpenoid glucoside. Comparison of the ^{1}H- and ^{13}C-NMR data for the aglycone with those of analogues[12—14] unambiguously assigned the aglycone as 3β,30-dihydroxyurs-12-en-28-oic acid. The downfield shift of the anomeric proton (δ_H 6.30) and upfield shifts of the anomeric carbon (δ_C 96.6) and C-28 carbonyl carbon (δ_C 176.1), as well as the presence of a correlation between H-1′ and C-28 in HMBC spectrum indicated that the glucose moiety was bound to C-28 by an ester glycosidic linkage. This was supported by alkaline hydrolysis which yielded D-glucose. Therefore compound **3** was established as 3β,30-dihydroxy-12-ursen-28-oic acid 28-*O*-β-D-glucopyranosyl ester.

Experimental

General Procedures Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH and C_5H_5N as solvent. The UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 25 UV–vis spectrophotometer. The CD spectra were recorded in MeOH on a JASCO J-810 spectropolarimeter. The ^{1}H (600 MHz), ^{13}C (125 MHz), and 2D NMR spectra were recorded on a Bruker AVANCE 600 instrument using TMS as internal reference. HR-ESI-MS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer. ESI-MS data were collected on an MDS SCIEX API 2000 LC/MS/MS instrument. Preparative HPLC was conducted with a Shimadzu LC-6A pump and a Shimadzu RID-10A refractive index detector using an XTerra prep MS C_{18} column (10 μm, 300×19 mm). For column chromatography, silica gel 60 (100—200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Develosil ODS (75 μm, Nomura Chemical Co. Ltd., Japan) were used.

Plant Material The whole plants of *G. loureirii* were purchased at Guangzhou Qingping Professional Market for Traditional Chinese Medicine in August 2006; they were collected from Shaoguan, the north part of Guangdong province, and authenticated by Prof. Xing Fuwu (South China Botanical Garden, Chinese Academy of Sciences). A voucher specimen (No. 395775) has been deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation The powered dried whole plants (2.8 kg) were extracted with 95% EtOH three times at room temperature. The EtOH extract was suspended in water, then sequentially extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH to yield petroleum ether- (83.17 g), $CHCl_3$- (62.66 g), EtOAc- (54.77 g), and *n*-BuOH-soluble (93.08 g) fractions.

The $CHCl_3$-soluble fraction was subjected to a silica gel CC eluted with a gradient of $CHCl_3$–MeOH (100 : 0—80 : 20), to give 18 fractions (C_1—C_{18}). Fraction C_{10} (11.00 g), obtained on elution with $CHCl_3$–MeOH (90 : 10), was chromatographed on silica gel using petroleum ether–acetone (80 : 20 to 60 : 40) to afford six subfractions ($C_{10\text{-}1}$—$C_{10\text{-}6}$). Subfraction $C_{10\text{-}6}$ was further separated by HPLC using 75% MeOH to afford rotundic acid (17 mg), tormentic acid (6 mg), and 2α,3α,23-trihydroxy-12,20(30)-ursadien-28-oic acid (6 mg).

The *n*-BuOH-soluble fraction was subjected to silica gel CC and eluted with $CHCl_3$–MeOH of increasing polarities (95 : 5—60 : 40), to yield 14 fractions (B_1—B_{14}). Fraction B_3 (1.31 g), obtained on elution with $CHCl_3$–MeOH (95 : 5), was further chromatographed on an ODS column eluted with MeOH–H_2O mixtures of decreasing polarities (2 : 8—4 : 6) to give six subfractions ($B_{3\text{-}1}$—$B_{3\text{-}6}$). Subfraction $B_{3\text{-}6}$ (27 mg) was separated by HPLC using 75% MeOH to afford 1α,2α,3α,24-tetrahydroxy-12-oleanen-28-oic acid (3 mg). Fraction B_4 (1.62 g), obtained on elution with $CHCl_3$–MeOH (90 : 10), was further chromatographed on an ODS column eluted with MeOH–H_2O mixtures of decreasing polarities (2 : 8—4 : 6) to yield seven subfractions ($B_{4\text{-}1}$—$B_{4\text{-}7}$). Subfraction $B_{4\text{-}6}$ (122 mg) was separated by HPLC using 40% MeOH to afford compound **2** (7 mg). Fraction B_5 (5.39 g), obtained on elution with $CHCl_3$–MeOH (90 : 10), was further applied to ODS CC using MeOH–H_2O mixtures of decreasing polarities (2 : 8—3 : 7) to obtain four subfractions ($B_{5\text{-}1}$—$B_{5\text{-}4}$). Subfraction $B_{5\text{-}2}$ (167 mg) was separated by HPLC using 30% MeOH to afford 1-*O*-feruloylglucose (9 mg). Subfraction $B_{5\text{-}4}$ (96 mg) was separated by HPLC using 40% MeOH to afford compound **3** (7 mg). Fraction B_7 (4.57 g), obtained on elution with $CHCl_3$–MeOH (80 : 20), was further separated by ODS CC using MeOH–H_2O mixtures of decreasing polarities (3 : 7 to 5 : 5) to give six subfractions ($B_{7\text{-}1}$—$B_{7\text{-}6}$). Subfraction $B_{7\text{-}6}$, when adding MeOH, yielded precipitates which were collected by filtration and washed with MeOH to afford compound **1** (6 mg).

Cyclic 4-*O*-β-D-Glucopyranosylcaffeic Acid Dimer (**1**): Amorphous solid, $[\alpha]_D^{20}$ +80.6° (c=0.25, C_5H_5N); UV (MeOH) λ_{max} (log ε) nm: 219 (4.31), 242 (sh), 286 (4.23), 330 (sh); positive ESI-MS *m/z*: 671 $[M+Na]^+$; nega-

tive ESI-MS *m/z*: 1295 $[2M-H]^-$, 683 $[M+Cl]^-$, 647 $[M-H]^-$; ^{1}H-NMR (600 MHz, C_5D_5N) δ: 7.88 (1H, d, J=16.0 Hz, H-7), 7.71 (1H, d, J=8.4 Hz, H-5), 7.49 (1H, d, J=2.0 Hz, H-2), 7.44 (1H, dd, J=8.4, 2.0 Hz, H-6), 6.71 (1H, d, J=16.0 Hz, H-8), 5.66 (1H, d, J=7.8 Hz, H-1′), 5.18 (1H, dd, J=11.3, 2.1 Hz, H-6′a), 4.67 (1H, dd, J=11.3, 10.3 Hz, H-6′b), 4.41—4.30 (2H, m, H-3′, 5′), 4.27 (1H, m, H-2′), 3.97 (1H, t, J=9.2 Hz, H-4′); ^{13}C-NMR (125 MHz, C_5D_5N) δ: 130.0 (C-1), 116.5 (C-2), 149.2 (C-3), 148.8 (C-4), 117.7 (C-5), 121.1 (C-6), 144.9 (C-7), 117.1 (C-8), 166.7 (C-9), 102.3 (C-1′), 74.6 (C-2′), 75.5 (C-3′), 72.5 (C-4′), 78.9 (C-5′), 65.3 (C-6′); HR-ESI-MS *m/z*: 671.1583 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_{16}Na$, 671.1588).

4-Hydroxy-6-methyl-α-tetralone 4-*O*-β-D-Glucopyranoside (**2**): Amorphous powder, $[\alpha]_D^{20}$ −35.3° (c=0.36, MeOH); UV (MeOH) λ_{max} (log ε) nm: 219 (3.86), 257 (3.84), 291 (sh); positive ESI-MS *m/z*: 715 $[2M+K]^+$, 699 $[2M+Na]^+$, 377 $[M+K]^+$, 361 $[M+Na]^+$; negative ESI-MS *m/z*: 675 $[2M-H]^-$, 373 $[M+Cl]^-$, 338 $[M-H]^-$; ^{1}H-NMR (600 MHz, C_5D_5N) δ: 8.12 (1H, d, J=7.9 Hz, H-8), 7.89 (1H, br s, H-5), 7.13 (1H, br d, J=7.9 Hz, H-7), 5.20 (1H, t, J=5.1 Hz, H-4), 5.05 (1H, d, J=7.7 Hz, H-1′), 4.64 (1H, dd, J=11.6, 1.6 Hz, H-6′a), 4.43 (1H, dd, J=11.6, 5.6 Hz, H-6′b), 4.24—4.27 (2H, m, H-3′, 5′), 4.11 (1H, m, H-2′), 3.01 (1H, m, H-3ax), 2.52 (1H, m, H-3eq), 2.41 (2H, m, H-2), 2.16 (3H, s, CH_3).^{13}C-NMR (125 MHz, C_5D_5N) δ: 197.0 (C-1), 35.2 (C-2), 31.2 (C-3), 74.9 (C-4), 129.9 (C-5), 144.5 (C-6), 129.6 (C-7), 127.3 (C-8), 130.2 (C-9), 143.5 (C-10), 21.6 (CH_3), 104.3 (C-1′), 75.4 (C-2′), 78.7 (C-3′), 71.8 (C-4′), 78.8 (C-5′), 62.9 (C-6′); HR-ESI-MS *m/z*: 361.1258 $[M+Na]^+$ (Calcd for $C_{17}H_{22}O_7Na$, 361.1263).

3β,30-Dihydroxy-12-ursen-28-oic Acid 28-*O*-β-D-Glucopyranosyl Ester (**3**): Amorphous powder, $[\alpha]_D^{20}$ +7.7° (c=0.14, MeOH); positive ESI-MS *m/z*: 1307 $[2M+K]^+$, 1291 $[2M+Na]^+$, 673 $[M+K]^+$, 657 $[M+Na]^+$; negative ESI-MS *m/z*: 1303 $[2M+Cl]^-$, 669 $[M+Cl]^-$, 633 $[M-H]^-$; ^{1}H-NMR (600 MHz, C_5D_5N) δ: 6.30 (1H, d, J=7.9 Hz, H-1′), 5.50 (1H, t, J=3.0 Hz, H-12), 4.45 (1H, dd, J=12.0, 4.5 Hz, H-6′a), 4.40 (1H, dd, J=12.0, 1.8 Hz, H-6′b), 4.37 (1H, t, J=9.0 Hz, H-4′), 4.30 (1H, t, J=9.0 Hz, H-3′), 4.22 (1H, t, J=9.0 Hz, H-2′), 4.03 (1H, m, H-5′), 3.92 (1H, dd, J=10.8, 2.5 Hz, H-30a), 3.87 (1H, dd, J=10.8, 5.8 Hz, H-30b), 3.43 (1H, dd, J=11.4, 4.4 Hz, H-3), 2.65 (1H, d, J=11.5 Hz, H-18), 1.60 (1H, dd, J=11.6, 6.5 Hz, H-9), 1.21 (3H, s, H_3-23), 1.20 (3H, s, H_3-27), 1.19 (3H, s, H_3-26), 1.12 (3H, d, J=6.6 Hz, H_3-29), 1.02 (3H, s, H_3-24), 0.93 (3H, s, H_3-25), 0.82 (1H, br d, J=11.2 Hz, H-5); ^{13}C-NMR (600 MHz, C_5D_5N) δ: 40.0 (C-1), 26.3 (C-2), 78.9 (C-3), 40.4 (C-4), 56.8 (C-5), 19.6 (C-6), 34.4 (C-7), 41.0 (C-8), 48.9 (C-9), 38.1 (C-10), 24.6 (C-11), 127.2 (C-12), 139.3 (C-13), 43.3 (C-14), 29.6 (C-15), 25.6 (C-16), 49.2 (C-17), 54.2 (C-18), 34.6 (C-19), 47.9 (C-20), 28.9 (C-21), 37.6 (C-22), 29.6 (C-23), 17.5 (C-24), 16.6 (C-25), 18.9 (C-26), 24.7 (C-27), 177.3 (C-28), 18.1 (C-29), 66.2 (C-30), 96.6 (C-1′), 74.9 (C-2′), 79.8 (C-3′), 72.2 (C-4′), 80.2 (C-5′), 63.3 (C-6′); HR-ESI-MS *m/z*: 669.3744 $[M+Cl]^-$ (Calcd for $C_{36}H_{58}O_9Cl$, 669.3769).

Enzymatic Hydrolysis of 2 Hydrolysis of compound **2** was carried out by the method described by Liu *et al.*[9] with minor modifications. Briefly, a solution of **2** (2.12 mg) in 1.0 ml phosphate buffer (pH 4.1) was treated with β-glucosidase (Oriental Yeast Co., 3.7 mg) then the reaction mixture was stirred at 40 °C for 24 h. The reaction mixture was extracted three times with EtOAc. The EtOAc solution was concentrated *in vacuo* to give **2a** (1.6 mg).

4-Hydroxy-6-methyl-α-tetralone (**2a**): Amorphous powder, $[\alpha]_D^{20}$ +3.0° (c=0.13, MeOH); UV (MeOH) λ_{max} (log ε) nm: 202 (3.96), 256 (3.61), 291 (sh); CD (c=1.45×10^{-4} M, MeOH), $[\theta]_{290}$ −958.0, $[\theta]_{252}$ −738.4, $[\theta]_{220}$ −505.0, $[\theta]_{204}$ +8138.1; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.94 (1H, d, J=7.9 Hz, H-8), 7.41 (1H, br s, H-5), 7.24 (1H, br d, J=7.9 Hz, H-7), 4.96 (1H, dd, J=7.9, 3.8 Hz, H-4), 4.11 (1H, m, H-2′), 2.92 (1H, ddd, J=17.3, 7.5, 4.5 Hz, H-3eq), 2.60 (1H, ddd, J=17.3, 9.6, 4.5 Hz, H-3ax), 2.43 (3H, s, CH_3), 2.40 (1H, ddd, J=12.3, 7.5, 4.0 Hz, H-2eq), 2.18 (1H, m, H-2ax); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.1 (C-1), 35.1 (C-2), 32.2 (C-3), 68.0 (C-4), 128.9 (C-5), 145.3 (C-6), 127.4 (C-7), 127.4 (C-8), 129.3 (C-9), 145.1 (C-10), 21.9 (CH_3).

Preparation of Mosher's Ester of 2a A solution of compound **2a** (1.3 mg, 7.8 μmol) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) (9.1 g, 39.0 μmol) in the presence of EDC-HCl (7.5 mg, 39.0 μmol) and 4-dimethylaminopyridine (DMAP) (4.8 mg, 39.0 μmol); the mixture was allowed to stand at room temperature for 30 h. The reaction solution was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and H_2O then dried over $MgSO_4$ and filtered. Evaporation of the solvent from the filtrate furnished a residue, which was purified by preparative TLC (silica gel GF_{254} plates, developed with $CHCl_3$) to give a mixture of a pair of (*R*)-MTPA esters (0.5 mg).

(*R*)-MTPA Esters of **2a**: ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.98/7.92 (peak intensity ratio: *ca.* 3 : 5) (1H, d, J=8.0 Hz, H-8), 7.52—7.28 (7H, m, Ar-H), 6.30/6.17 (peak intensity ratio: 5 : 3) (1H, dd, J=7.0, 3.7 Hz, H-4), 3.54/3.50 (peak intensity ratio: *ca.* 5 : 3) (3H, s, OCH_3), 2.87—2.48 (2H, m, H_2-3), 2.40/2.31 (peak intensity ratio: *ca.* 3 : 5) (3H, s, 5-Me), 2.40—2.27 and 2.06—2.00 (2H, m, H_2-3); HR-ESI-MS *m/z*: 415.1128 $[M+Na]^+$ (Calcd for $C_{21}H_{19}F_3O_4Na$, 415.1133).

Alkaline and/or Acid Hydrolysis of 1—3 Compound **1** (2 mg) in 1 N NaOH–dioxane (1 : 1, 1 ml) was heated at 80 °C for 4 h. The reaction mixture was neutralized with 1 N HCl then extracted with EtOAc (1 ml×3). The EtOAc layer was concentracted. To the residue, 2 N HCl–dioxane (1 : 1, 1 ml) was added and heated at 80 °C for 4 h. The reaction mixture was extracted with EtOAc (1 ml×3). The H_2O layer was concentrated to dryness to obtain the monosaccharide portion. Compound **2** (2 mg) in 2 N HCl–dioxane (1 : 1, 1 ml) was treated as described for acid hydrolysis of **1** to afford the monosaccharide portion of **2**. Compound **3** (3 mg) in 1 N NaOH–dioxane (1 : 1, 1 ml) was heated at 80 °C for 4 h. The reaction mixture was neutralized by passing through an Amberlite 200 (hydrogen form, 200 mg) column then extracted with $CHCl_3$. The H_2O layer was concentrated to obtain the monosaccharide portion of **3**.

Each monosaccharide portion was dissolved in MeOH (0.2 ml) then analyzed by TLC on a silica gel HPTLC plate (Merk Kieselgel 60GF254) using *n*-BuOH–*i*-PrOH–H_2O (10 : 5 : 4) as development and anisaldehyde-H_2SO_4 as detection and compared with an authentic glucose sample. All residues showed a spot (Rf=0.50) identical with that of glucose. The remaining MeOH solution was subjected to preparative HPLC (column: XTerra prep MS C_{18}, 19 mm i.d.×300 mm; detection: Shimadzu RID-10A refractive index detector; flow rate: 4 ml/min) using H_2O and the peak (t_R=10.3 min) corresponding to glucose was collected and concentrated. The residue was dissolved in MeOH (1.2 ml) then subjected to optical rotation measurement. The glucose from **1**—**3** gave $[\alpha]_D^{20}$ values of +36.7 (c=0.03), +42.1 (c=0.02), and +30.4 (c=0.04), respectively. The presence of D-glucose was therefore confirmed.

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