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CHEMOENZYMATIC SYNTHESIS OF NATURALLY OCCURRING PHENETHYL (1→6)-β-D-GLUCO-PYRANOSIDES

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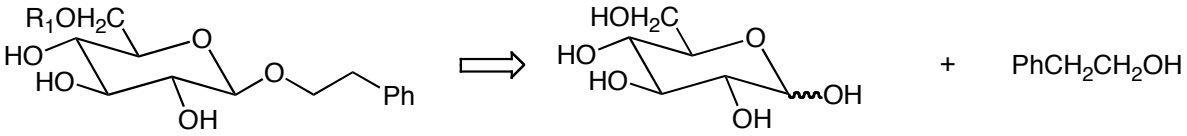
Abstract- Direct β-glucosidation between phenethyl alcohol and D-glucose (**5**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a phenethyl β-D-glucoside (**1**) in 34% yield. The coupling of the phenethyl *O*-β-D-glucopyranoside congener (**8**) and methylthio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**9**), 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl bromide (**11**), and methylthio 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**13**) afforded the coupled products (**10**, **12**, and **14**), respectively. Deprotection of the coupled products (**10**, **12**, and **14**) afforded the synthetic phenethyl *O*-β-D-xylopyranosy-(1→6)- β-D- glucopyranoside (**2**), phenethyl *O*-α-L-arabinopyranosy-(1 → 6)-β-D-glucopyranoside (**3**), and phenethyl *O*-α-L-rhamnopyranosy- (1→6)-β-D-glucopyranoside (**4**), respectively.

Phenylethanoid glycosides are a group of water soluble natural products widely distributed in the plant kingdom.[1] The biological activity of some compounds have been undergone and they are reported to indicate antibacterial activity, cytotoxic and antioxidant properties, enzyme inhibition, and

immunomodulatory properties.[1] Among them, three kinds of naturally occurring phenethy (1→6)-β-D-glucopyranoside congeners, phenethyl *O*-α-D-xylopyranosy-(1→6)-β-D-glucopyranoside (**2**),[2] phenethyl *O*-α-L-arabinopyranosy-(1→6)-β-D-glucopyranoside (**3**)[3] and phenethyl *O*-α-L-rhamnopyranosy-(1→6)-β-D-glucopyranoside (**4**)[4] were isolated from a methanol extract of *Rehmannia glutinosa* var. *purpurea*,[2a] *Rhodiola sacra*[3b] and *Citrus unshi*,[4] respectively. For the purpose of investigation of pharmacological activity of these β-D-glucopyranoside congeners, the synthesis of the above-mentioned β-D-glucopyranoside congeners has aroused our interest. In this paper, we describe the synthesis of phenethyl β-D-glucopyranoside (**1**) and its naturally occurring phenethyl (1→6)-β-D-glucopyranoside congeners (**2**, **3** and **4**) based on the selective β-glycosidation between D-glucose (**5**) and phenethyl alcohol catalyzed by the immobilized β-glucosidase (EC 3.2.1.21) from almonds.

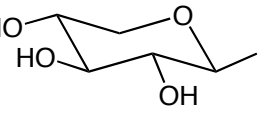


Phenethyl *O*-β-D-glucopyranoside (**1**) **5**

R_1 = H

Phenethyl *O*-α-D-xylopyranosy-(1→6)-β-D-glucopyranoside (**2**)

R_1 =



Phenethyl *O*-α-L-arabinopyranosy-(1→6)-β-D-glucopyranoside (**3**)

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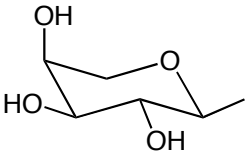
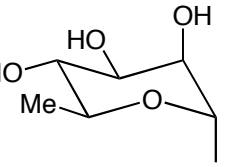


Phenethl *O*-α-L-rhamnopyranosy-(1→6)-β-D-glucopyranoside (**4**)

R_1 =



Scheme 1

Enzymatic β-glycosidation

In case of the direct β-glycosidation between D-glucose (**5**) and primary alcohols using β-glucosidase (EC 3.2.1.21) from almonds under thermodynamic conditions, a high concentration of alcohol or a medium with low water activity is reported to be effective.[5] Meanwhile, the synthesis of **1** using 4-nitrophenyl β-D-glucopyranoside as a glycosyl donor was reported previously by us.[6] On the other hand, we reported the effectiveness of immobilization of β-glucosidase (EC 3.2.1.21) from almonds with a photocross-linkable resin prepolymer (ENTP-4000) in the direct β-glucosidation between

D-glucose (**5**) and 1,8-octanediol.[7] Then we examined the direct β-glucosidation between D-glucose (**5**) and phenethyl alcohol using the reported immobilized β-glucosidase (EC 3.2.1.21)[7] from almonds. When a large amount of phenethyl alcohol (24.7 equivalent) was used as an acceptor for D-glucose (**5**) in the presence of the immobilized β-glucosidase, a 34% yield of phenethyl *O*-β-D-glucopyranoside (**1**) was obtained. Moreover, the same β-glucosidation using the recovered immobilized enzyme afforded **1** in 22% yield. In this case, the partial deactivation of the immobilized enzyme was recognized.

Synthesis of phenethyl *O*-β-D-xylopyranosy-(1→6)-β-D-glucopyranoside (2)

Tritylation of **1** gave a trityl ether (**6**; 65% yield), which was subjected to acetylation to give an acetate (**7**) in 99% yield. Hydrogenolysis of **7** using 20% Pd-C to provide a mixture (92% yield) of the desired **8** in 97% yield. By applying the reported procedure,[8] coupling reaction of phenethyl β-D-glucopyranoside congener (**8**) and methylthio 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**9**)[9] in the presence of silver triflate (AgOTf) and phenylselenochloride (PhSeCl) gave the coupled product (**10**) in 44% yield. In this case, an inseparable mixture of starting material (**8**) and the migrated product (phenethyl 2, 3, 6, 2', 3', 4'-*O*-hexaacetyl-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside) as a by-product could be obtained. Finally, treatment of **10** with K_2CO_3 in MeOH provided the synthetic phenethyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**) in 70% yield. The spectral data (^{13}C-NMR) and specific rotation ($[\alpha]_D^{27}$ –50.0° (c=0.3, MeOH)) of the synthetic (**2**) were identical with those (^{13}C-NMR and $[\alpha]_D^{28}$ –52.6° (c=0.37, MeOH)) of natural product (**2**).[2a]

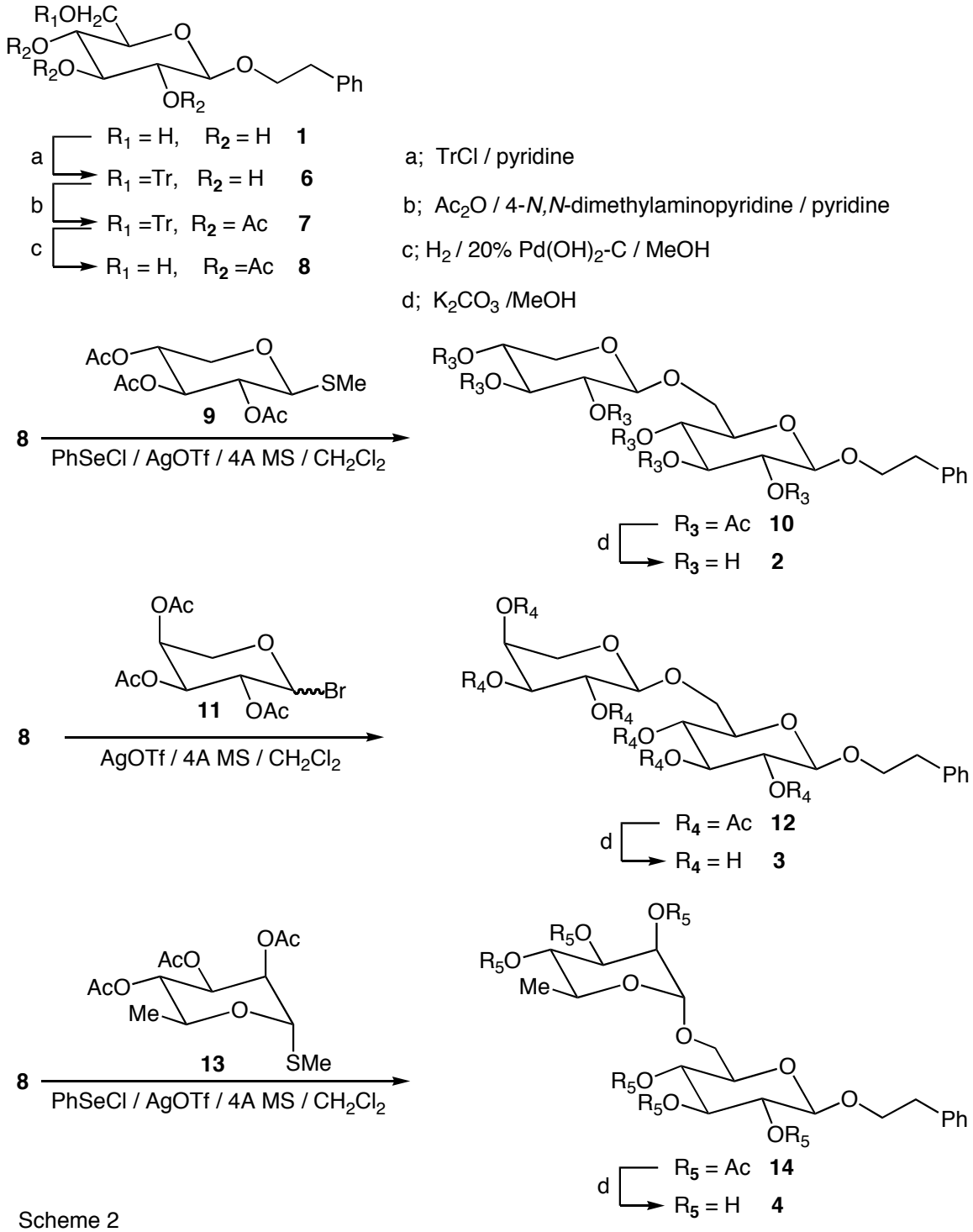
Synthesis of phenethyl *O*-β-L-arabinopyranosy-(1→6)-β-D-glucopyranoside (3)

By following the reported procedure,[8] coupling reaction of **8** and 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**11**)[10] in the presence of silver triflate (AgOTf) and tetramethylurea (TMU) gave the coupled product (**12**) in 73% yield. Finally, treatment of **12** with K_2CO_3 in MeOH provided the synthetic phenethyl *O*-β-L-arabinopyranosy-(1→6)-β-D-glucopyranoside (**3**, $[\alpha]_D^{29}$ –25.0° (c=0.1, MeOH))) in 86% yield. The spectral data (^{1}H- and ^{13}C-NMR) of the synthetic **3** were identical with those of natural product (**3**).[3b]

Synthesis of phenethyl *O*-β-L-rhamnopyranosy- (1→6)-β-D-glucopyranoside (4)

Methylthio 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranoside (**13**) was synthesized in 57% yield by applying the reported method[11] based on the $BF_3{\cdot}Et_2O$ catalyzed reaction of methylthiotrimethylsilane and tetra-*O*-acetyl-β-L-rhamnopyranoside obtained by acetylation of β-L-rhamnose. By applying the reported procedure,[8] coupling reaction of phenethyl β-D-glucopyranoside congener (**8**) and **13** in the presence of silver triflate (AgOTf) and phenylseleno chloride (PhSeCl) gave the coupled product (**14**) in 81% yield. Finally, treatment of **14** with K_2CO_3 in MeOH provided the synthetic phenethyl

O-β-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**4**) in 85% yield. The spectral data (^{13}C-NMR) of the synthetic **4** were similar to those (^{13}C-NMR in Pyridine-d_5) of natural product (**4**).[4] The specific rotation ($[\alpha]_D^{28}$ –96.0° (c=0.1, MeOH)) of the synthetic **4** were consistent with those ($[\alpha]_D^{23}$ –101.2° (c=0.1, MeOH)) of natural product (**4**).[4]



Scheme 2

Conclusion

In conclusion, direct β-glucosidation between phenethyl alcohol and D-glucose (**5**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a phenethyl *O*-β-D-glucoside (**1**) in 34% yield. The coupling of the phenethyl *O*-β-D-glucopyranoside

congener (**8**) and methylthio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**9**), 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl bromide (**11**), and methylthio 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**13**) gave the coupled products (**10**, **12**, and **14**), respectively. Deprotection of the coupled products (**10**, **12**, and **14**) afforded the synthetic phenethyl *O*-β-D-xylopyranosy-(1→6)-β-D-glucopyranoside (**2**), phenethyl *O*-α-L-arabinopyranosy-(1→6)-β-D-glucopyranoside (**3**), and phenethyl *O*-α-L-rhamnopyranosy- (1→6)-β-D-glucopyranoside (**4**), respectively.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL EX 400 spectrometer (Tokyo, Japan). Spectra were recorded with 5-10% (w/v) solution in $CDCl_3$ with Me_4Si as an internal reference. Melting points were determined on a Yanaco MP-3S micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. The FAB MS spectra were obtained with a JEOL JMS-AX 500 (matrix; glycerol) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrophotometer. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

Immobilization of β-D-glucosidase using a prepolymer

β-D-Glucosidase (EC 3.2.1.21) from almonds was purchased from Sigma Chemical Co. (G-0395, 2.5-3.6 U/mg). Immobilization of β-D-glucosidase from almonds on the photocross-linkable resin prepolymer (ENTP-4000) was carried out using the following procedure. One gram of ENTP-4000 was mixed with 10 mg of a photosensitizer, benzoin ethyl ether, and 110 mg of β-D-glucosidase from almonds (3.4 units/mg). The mixture was layered on a sheet of transparent polyester film (thickness, *ca*. 0.5 mm). The layer was covered with transparent thin film and then illuminated with chemical lamps (wavelength range, 300-400 nm) for 3 min. The gel film thus obtained was cut into small pieces (0.5 X 5 X 5 mm) and used for the bioconversion reaction.

Enzymatic synthesis of phenethyl *O*-β-D-glucopyranoside (1)

1) A mixture of D-glucose (**5**) (1.1 g, 6.1 mmol), phenethyl alcohol (18.4 g, 150.9 mmol), water (2 mL), and the immobilized β-glucosidase was incubated for 4 days at 50°C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give phenethyl alcohol (16.8 g, 91% recovery, oil) from the $CHCl_3$ eluent and β-glucoside (**1**, 597 mg, 34%) as colorless crystals (mp 38-40°C) from the HCl_3/MeOH = 10:1 eluent. The NMR (^{1}H- and ^{13}C-NMR) spectral data of β-glucoside (**1**) were identical with those of the reported β-glucoside (**1**).[6]

2) A mixture of D-glucose (**5**) (1.1 g, 6.1 mmol), phenethyl alcohol (18.4 g, 150.9 mmol), water (2 mL), and the recovered immobilized β-glucosidase was incubated for 4 days at 50°C. The reaction

mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give phenethyl alcohol (17.1 g, 93% recovery, oil) from the $CHCl_3$ eluent and β-glucoside (**1**, 382 mg, 22%) as colorless crystals from the $CHCl_3$/MeOH =10:1 eluent.

Phenethyl 6-*O*-trityl-β-D-glucopyranoside (6)

A mixture of **1** (2.15 g, 7.57 mmol) and TrCl (2.12 g, 7.6 mmol) in pyridine (4 mL) was stirred for 24 h at rt. The reaction mixture was diluted with toluene (100 mL) and evaporated under reduced pressure to give a residue, which was chromatographed on silica gel (45 g) to afford **6** (2.59 g, 65%) as a colorless syrup from $CHCl_3$/MeOH (20:1) eluent and starting material (**1**, 0.72 g, 33% recovery) from $CHCl_3$/MeOH (9:1) eluent. **6**: $[\alpha]_D^{26}$ –40.7° (c=0.55, $CHCl_3$); IR (KBr): 3387, 3059, 2928 cm^{-1}, ^{1}H-NMR ($CDCl_3$): 1.72 (2H, br s), 2.68 (1H, br s), 3.03 (2H, t, *J*=7.4 Hz), 3.23-3.36 (4H, m), 3.41-3.47 (2H, m), 3.89 (1H, dt, *J*=7.2, 10.2 Hz), 4.14 (1H, dt, *J*=7.2, 10.2 Hz), 4.38 (1H, d, *J*=8 Hz), 7.14-7.27 (15H, m), 7.46-7.48 (5H, m); ^{13}C-NMR ($CDCl_3$): δ 36.1, 64.1, 70.5, 71.9, 73.4, 73.8, 76.0, 86.9, 102.5, 126.1, 126.5, 126.9, 127.0, 127.4, 127.6[6C], 127.7, 128.2[2C], 128.3[6C], 128.6[2C], 138.0, 143.2; *Anal.* Calcd for $C_{33}H_{34}O_6$: C, 75.26; H, 6.51%. Found: C, 74.81; H, 6.51%.

Phenethyl 2, 3, 4-*tri*-*O*-acetyl-6-*O*-trityl-β-D-glucopyranoside (7)

To a solution of **6** (2.59 g, 4.92 mmol) in pyridine (5 mL) were added Ac_2O (3.52 g, 34.5 mmol) and 4-*N*,*N*-dimethylaminopyridine (DMAP; 10 mg, 0.08 mmol) at 0°C, and the whole was stirred for 1 h at rt. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with 10% aqueous HCl and brine. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed on silica gel (30 g, *n*-hexane/AcOEt (5:1)) to afford **7** (3.21 g, 99%) as a colorless oil. **7**: $[\alpha]_D^{27}$ +23.4° (c=0.38, $CHCl_3$); IR (KBr): 1753 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 1.72 (3H, s), 1.92 (3H, s), 1.97 (3H, s), 2.96 (2H, t, *J*=6.9 Hz), 3.11 (1H, dd, *J*=4.8, 10.4 Hz), 3.25 (1H, dd, *J*=2.0, 10.4 Hz), 3.53-3.57 (1H, m), 3.77 (1H, dt, *J*=7.4, 9.8 Hz), 4.18 (1H, dt, *J*=6.4, 9.8 Hz), 4.50 (1H, d, *J*=8.0 Hz), 5.05 (1H, dd, *J*=8.0, 9.6 Hz), 5.12 (1H, t, *J*=8.0 Hz), 5.16 (1H, t, *J*=8.0 Hz), 7.19-7.29 (15H, m), 7.44-7.46 (5H, m); ^{13}C-NMR ($CDCl_3$): δ 20.2, 20.4, 20.6, 36.1, 61.9, 68.7, 70.2, 71.3, 73.0, 73.2, 86.4, 100.6, 126.0, 126.7[3C], 127.5[6C], 128.1[2C], 128.4[6C], 128.7[2C], 138.4, 143.3[3C], 168.6, 169.0, 170.0 ; *Anal.* Calcd for $C_{39}H_{40}O_9$: C, 71.76; H, 6.18%. Found: C, 71.48; H, 6.22%.

Phenethyl 2, 3, 4-*tri*-*O*-acetyl-β-D-glucopyranoside (8)

A mixture of **7** (3.29 g, 5.1 mmol) and 20% $Pd(OH)_2$-C (0.9 g) in MeOH (120 mL) was subjected to catalytic hydrogenolysis at ambient temperature and ordinary hydrogen pressure, and the reaction mixture was filtered with the aid of celite to give the filtrate. Evaporation of the filtrate gave a residue, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt (1:1)) to give **8** (2.0 g, 97%) as a colorless oil. **8**: $[\alpha]_D^{28}$ –23.33° (c=0.59, $CHCl_3$); IR (KBr): 3500, 2950, 2884, 1752 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 1.90 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 2.88 (2H, t, *J*=6.8 Hz), 3.49 (1H, ddd, *J*=2.6, 6.4,

9.6 Hz), 3.56-3.61 (1H, m), 3.66-3.75 (2H, m), 4.09-4.12 (1H, m), 4.51 (1H, d, *J*=8.0 Hz), 4.96 (1H, dd, *J*=8.0, 9.6 Hz), 5.02 (1H, t, *J*=9.6 Hz), 5.21 (1H, t, *J*=9.6 Hz), 7.18-7.30 (5H, m); ^{13}C-NMR ($CDCl_3$): □ 20.5, 20.6, 20.8, 36.0, 61.3, 68.8, 70.6, 71.3, 72.7, 74.1, 100.7, 126.3, 128.3[2C], 129.0[2C], 138.5, 169.3, 170.1, 170.3; *Anal.* Calcd for $C_{20}H_{26}O_9 \cdot H_2O$: C, 56.07; H, 6.59%. Found: C, 55.74; H, 6.16%. FAB-MS (NBA) *m/z*: 449 $(M+K)^+$.

Phenethyl 2, 3, 4, 2', 3', 4'-*O*-hexaacetyl-□-D-xylopyranosyl-(1→6)-□-D-glucopyranoside (10)

To a mixture of PhSeCl (0.10 g, 0..52 mmol) and 4A molecular sieves (0.6 g) in 1,2-dichloroethane (2 mL) was added silver triflate (AgOTf; 0.134 g, 0.052 mmol) with stirring at 0°C for 10 min under argon atmosphere. To the above-mentioned reaction mixture was added a solution of **8** (0.108 g, 0.265 mmol) and **9** (0.13 g, 0.424 mmol) in 1,2-dichloroethane (2 mL) and the whole was stirred for 1 h at the same temperature. The reaction mixture was cooled at 0°C and quenched with AcOEt (15 mL) and 7% aqueous $NaHCO_3$ solution (6 mL). The reaction mixture was filtered with the aid of celite and the filtrate was extracted with AcOEt and dried over $MgSO_4$. Evaporation of the organic solvent gave a residue, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt (2:1)) to afford **15** (0.077g, 44%) as a colorless amorphous. **10**: $[□]_D^{27}$ –38.0° (c=0.2, $CHCl_3$); IR (KBr): 1754 cm^{-1}, ^{1}H-NMR ($CDCl_3$): □ 1.90 (3H, s), 1.96 (3H, s), 1.98 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 2.05 (3H, s), 2.88 (2H, t, *J*=6.4 Hz), 3.33 (1H, dd, *J*=9.0, 11.9 Hz), 3.56-3.70 (3H, m), 3.83 (1H, d, *J*=9.0 Hz), 4.12 (2H, dt, *J*=6.4, 9.0 Hz), 4.44 (1H, d, *J*=8.1 Hz), 4.52 (1H, d, *J*=6.9 Hz), 4.87-4.96 (4H, m), 5.14 (2H, dd, *J*=8.1, 9.1 Hz), 7.18-7.29 (5H, m); ^{13}C-NMR ($CDCl_3$): □ 20.7[3C], 20.8[2C], 20.9, 35.9, 61.9[2C], 67.7, 68.7, 69.0, 70.4, 70.5, 71.2, 72.8, 73.2, 100.4, 100.4, 126.2, 128.2[2C], 128.8[2C], 138.3, 169.1, 169.1, 169.3, 169.6, 169.8, 170.7; FAB MS *m/z*: 691 $(M+Na)^+$. *Anal.* Calcd for $C_{31}H_{40}O_{16}$; C, 55.69; H, 6.03%. Found: C, 55.25; H, 6.00%. HRMS (FAB) (NBA) *m/z*: Calcd for $C_{31}H_{41}O_{16}$: 669.2394, Found: 669.2388 $(M+1)^+$.

Phenethyl *O*-□-D-xylopyranosyl-(1→6) -□-D-glucopyranoside (2)

A mixture of **10** (0.10 g, 0.15 mmol) and K_2CO_3 (0.138 g, 0.15 mmol) in MeOH (3 mL) was stirred for 30 min at rt. The reaction mixture was condensed to give a residue, which was chromatographed on silica gel (10 g, $CHCl_3$/MeOH (4:1)) to afford **2** (0.045 g, 70%) as a colorless amorphous. **2**: $[□]_D^{27}$ –50.0° (c=0.3, MeOH); IR (KBr): 3367, 2925, 1458 cm^{-1}, ^{1}H-NMR (CD_3OD): □ 2.93 (2H, t, *J*=8.0 Hz), 3.15-3.22 (3H, m), 3.28-3.37 (3H, m), 3.41-3.44 (1H, m), 3.48 (1H, ddd, *J*=5.3, 8.6, 11.0 Hz), 3.74 (1H, dd, *J*=5.9, 11.0 Hz), 3.76 (1H, dd, *J*=8.6, 10.0 Hz), 3.85 (1H, dd, *J*=5.2, 11.5 Hz), 4.07 (1H, dd, *J*=2.6, 10.0 Hz), 4.08 (1H, dd, *J*=2.6, 11.5 Hz), 4.30 (1H, d, *J*=7.8 Hz), 4.31 (1H, d, *J*=7.3 Hz), 7.17 (1H, m), 7.25-7.26 (5H, m); ^{13}C-NMR (CD_3OD): □ 37.3, 66.9, 69.7, 71.1, 71.4, 71.8, 74.8, 75.0, 76.9, 77.6, 77.9, 104.3, 105.4, 127.0, 129.2[2C], 129.8[2C], 139.9; HR FAB-MS (NBA) *m/z*: Calcd for $C_{19}H_{28}O_{10}K$: 455.1320 $(M+K)^+$. Found: 455.1271.

Phenethyl 2, 3, 4, 2', 3', 4'-*O*-hexaacetyl-β-L-arabinopyranosyl-(1→6) - β-D-glucopyranoside (12)

To a solution of **8** (0.29 g, 0.7 mmol) and 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl bromide (**11**, 0.478 g, 1.41 mmol) in CH_2Cl_2 (1 mL) was added tetramethylurea (TMU, 0.246 g, 2.1 mmol) at 0°C under argon atmosphere. AgOTf (0.36 g, 1.4 mmol) was added to the above-mentioned reaction mixture at 0°C under argon atmosphere. The whole was covered with aluminum foil and stirred for 2.5 h at rt. The reaction mixture was cooled at 0°C and quenched with AcOEt (15 mL) and 7% aqueous $NaHCO_3$ solution (20 mL). The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent gave a residue, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt (3:1)) to afford **12** (0.347 g, 73% yield) as a colorless amorphous. **12**: $[\alpha]_D^{22}$ –5.91° (c=0.44, $CHCl_3$); IR (KBr): 1749,1058 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 1.89 (3H, s), 1.96 (3H, s), 1.98 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 2.12 (3H, s), 2.88 (1H, t, *J*=6.2 Hz), 3.56-3.61 (2H, m), 3.66-3.70 (2H, m), 3.88 (2H, dd, *J*=1.6, 11.0 Hz), 4.00 (1H, dd, *J*=3.2, 13.2 Hz), 4.13 (1H, dt, *J*=6.2, 10.0 Hz), 4.46 (1H, d, *J*=7.6 Hz),), 4.47 (1H, d, *J*=6.8 Hz), 4.90-4.96 (2H, m), 5.02 (1H, dd, *J*=3.6, 9.2 Hz), 5.14-5.19 (2H, m), 5.23-5.26 (1H, m), 7.19-7.30 (5H, m); ^{13}C-NMR ($CDCl_3$): δ 20.6 [5C], 20.9, 35.9, 63.1, 67.5, 67.8, 69.0, 69.1, 70.0, 70.4, 71.2, 72.8, 73.2, 100.5, 100.8, 126.3, 128.3[2C], 129.0[2C], 138.5, 169.3, 169.4, 169.5, 170.1, 170.2[2C]; *Anal.* Calcd for $C_{31}H_{40}O_{16}$: C, 55.69; H, 6/03%. Found: C, 55.64; H, 6.03%.

Phenethyl *O*-β-D-arabinopyranosyl-(1→6) -β-D-glucopyranoside (3)

A mixture of **12** (0.10 g, 0.15 mmol) and K_2CO_3 (0.021 g, 0.15 mmol) in MeOH (5 mL) was stirred for 15 min at rt. The reaction mixture was condensed to give a residue, which was chromatographed on silica gel (8 g, CH_2Cl_2/EtOH (7:1)) to afford **3** (0.054 g, 86%) as a colorless amorphous. **3**: $[\alpha]_D^{29}$ –25.0° (c=0.1, MeOH); IR (KBr): 3360, 2925, 1459 cm^{-1}, ^{1}H-NMR (CD_3OD): δ 2.93 (2H, t, *J*=7.1 Hz), 3.17-3.19 (1H, m), 3.33-3.35 (2H, m), 3.43-3.61 (4H, m), 3.71-3.79 (3H, m), 3.86 (1H, dd, *J*=3.2, 12.4 Hz), 4.07 (2H, dt, *J*=2.0, 7.1 Hz), 4.299 (1H, d, *J*=6.8 Hz), 4.301 (1H, d, *J*=8.0 Hz), 7.13-7.26 (5H, m); ^{13}C-NMR (CD_3OD): δ 37.2, 66.6, 69.3 [2C], 71.5, 71.7, 72.2, 74.0, 74.9, 76.8, 77.8, 104.2, 104.9, 126.9, 129.1[2C], 129.8[2C], 139.8; HR FAB-MS (NBA) *m/z*: Calcd for $C_{19}H_{29}O_{10}$: 417.1761 $(M+1)^+$. Found: 417.1770.

Methylthio 2, 3, 4-*O*-triacetyl β-L-rhamnopyranoside (13)

To a solution of 1, 2, 3, 4-*O*-tetraacetyl α-L-rhamnopyranoside (0.58 g, 2.56 mmol) in CH_2Cl_2 (20 mL) was added methylthiotrimethylsilane (0.9 mL, 8.82 mmol) and 47% $BF_3{\cdot}Et_2O$ complex (174 μL, 1.2 mmol) at 0°C and the whole was stirred for 1 h at rt. The reaction mixture was diluted with 7% aqueous $NaHCO_3$ (15 mL) and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a residue, which was chromatographed

on silica gel (17 g, *n*-hexane/AcOEt (10:1)) to afford **13** (0.443g, 57%) as a colorless oil. **13**: $[\alpha]_D^{23}$ –124.4° (c=0.62, $CHCl_3$); IR (KBr): 1770 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 1.25 (2H, d, *J*=6.4 Hz), 1.99 (3H, s), 2.05 (3H, s), 2.15 (3H, s), 2.16 (3H, s), 4.20 (1H, dd, *J*=6.4, 10.0 Hz), 5.09 (1H, d, *J*=1.4 Hz), 5.10 (1H, t, *J*=10.0 Hz), 5.25 (1H, dd, *J*=3.2, 10.0 Hz), 5.35 (1H, dd, *J*=1.4, 3.2 Hz); ^{13}C-NMR ($CDCl_3$): δ 13.8, 17.4, 20.7, 20.8, 20.9, 66.9, 69.5, 71.2, 71.3, 83.4, 170.0[3C]; *Anal.* Calcd for $C_{13}H_{20}O_7S$; C, 48.74; H, 6.29%. Found: C, 48.61; H, 6.32%.

Phenethyl 2, 3, 4, 2', 3', 4'-*O*-hexaacetyl α-L-rhamnopyranosyl-(1→6) - β-D-glucopyranoside (14)

To a mixture of PhSeCl (0.257 g, 1.34 mmol) and 4A molecular sieves (2 g) in 1,2-dichloroethane (4 mL) was added silver triflate (AgOTf; 0.343 g, 1.34 mmol) with stirring at 0°C for 10 min under argon atmosphere. To the above-mentioned reaction mixture was added a solution of **8** (0.347 g, 0.913 mmol) and **13** (0.44 g, 1.37 mmol) in 1,2-dichloroethane (7 mL) and the whole mixture was stirred for 1 h at the same temperature. The reaction mixture was cooled at 0°C and quenched with 7% aqueous $NaHCO_3$ solution (20 mL). The reaction mixture was filtered with the aid of celite and the filtrate was extracted with AcOEt and dried over $MgSO_4$. Evaporation of the organic solvent gave a residue, which was chromatographed on silica gel (25 g, *n*-hexane/AcOEt (3:1)) to afford **14** (0.505 g, 81%) as a colorless amorphous. **14**: $[\alpha]_D^{26}$ –52.3° (c=0.80, $CHCl_3$); IR (KBr): 1752 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 1.21 (3H, d, *J*=6.4 Hz), 1.88 (3H, s), 1.98 (3H, s), 1.99 (3H, s), 2.04 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.87 (2H, t, *J*=6.0 Hz), 3.61-3.72 (4H, m), 3.83-3.90 (1H, m), 4.09-4.15 (1H, m), 4.47 (1H, d, *J*=8.0 Hz), 4.81 (1H, s), 4.93 (1H, dd, *J*=6.0, 9.6 Hz), 4.96 (1H, t, *J*=4.8 Hz), 5.05 (1H, t, *J*=9.6 Hz), 5.17 (1H, t, *J*=9.6 Hz), 5.23-5.24 (2H, m), 7.17-7.28 (5H, m); ^{13}C-NMR ($CDCl_3$): δ 17.4, 20.5, 20.6[2C], 20.7, 20.8[2C], 35.8, 66.6, 67.0, 69.0, 69.5, 69.6, 70.5, 71.0, 71.2, 72.8, 73.3, 98.2, 100.6, 126.2, 128.3[2C], 130.0[2C], 138.6, 169.3, 169.5, 169.9, 170.0[2C], 170.3; FAB MS *m/z*: 682 (M^+).

Phenethyl *O*-α-L-rhamnopyranosyl-(1→6) -β-D-glucopyranoside (4)

A mixture of **14** (0.10 g, 0.147 mmol) and K_2CO_3 (0.02 g) in MeOH (3 mL) was stirred for 30 min at rt. The reaction mixture was condensed to give a residue, which was chromatographed on silica gel (8 g, $CHCl_3$/MeOH (7:1)) to afford **4** (0.054 g, 85%) as a colorless amorphous. **4**: $[\alpha]_D^{28}$ –96.0° (c=0.1, MeOH); IR (KBr): 3046, 2925, 1612 cm^{-1}, ^{1}H-NMR (CD_3OD): δ 1.25 (3H, d, *J*=6.0 Hz), 2.94 (2H, dt, *J*=2.0, 8.0 Hz), 3.18 (1H, dd, *J*=8.0, 9.0 Hz), 3.25-3.39 (4H, m), 3.61 (1H, dd, *J*=6.0, 11.2 Hz), 3.66-3.69 (2H, m), 3.73-3.79 (1H, m), 3.83 (1H, dd, *J*=1.6, 3.2 Hz), 3.98 (1H, dd, *J*=2.0, 11.2 Hz), 4.03 (1H, dt, *J*=7.6, 9.6 Hz), 4.29 (1H, d, *J*=8.0 Hz), 4.75 (1H, d, *J*=1.6 Hz), 7.14-7.20 (1H, m), 7.25-7.26 (4H, m); ^{13}C-NMR (CD_3OD): δ 18.0, 37.3, 68.1, 69.8, 71.6, 71.8, 72.2, 72.4, 74.0, 75.0, 76.8, 78.0, 102.2, 104.4, 127.2, 129.4[2C], 130.0[2C], 140.0; FAB-MS (NBA) *m/z*: 453 $(M+Na)^+$.

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REFERENCES

1. C. Jimenez and R. Riguera, *Natural Product Reports*, **1994**, 591.
2. a) H. Nishimura, H. Sasaki, T. Morota, M. Chin, and H. Mitsuhashi, *Phytochemistry*, 1990, **29**, 3303. b) H. Otsuka, Y. Takeda, and K. Yamasaki, *Phytochemistry*, 1990, **29**, 3681. c) W. Guo, R. Hosoi, K. Sakata, N. Watanabe, A. Yagi, K. Ina, and S. Luo, *Biosci. Biotech. Biochem.*, 1994, **58**, 1532.
3. a) D. Uhrin, A. Buckova, E. Eisenreichova, M. Haladova, and J. Tomko, *Chem. Papers*, 1989, **43**, 793. b) M. Yoshikawa, H. Shimada, S. Horikawa, T. Murakami, Hi. Shimada, J. Yamahara, and H. Matsuda, *Chem. Pharm. Bull.*, 1997, **45**, 1498.
4. K. Umehara, L. Hattori, T. Miyase, A. Ueno, S. Hara, and C. Kageyama, *Chem. Pharm. Bull.*, 1988, **36**, 5004.
5. G. Vic and D. H. G. Crout, *Tetrahedron: Asymmetry*, 1994, **5**, 2513.
6. K. Kurashima, M. Fujii, Y. Ida, and H. Akita, *Chem. Pharm. Bull.*, 2004, **52**, 270.
7. H. Akita, E. Kawahara, and K. Kato, *Tetrahedron: Asymmetry*, 2004, **15**, 1623.
8. Y. Ito, T. Ogawa, M. Numata, and M. Sugimoto, *Carbohydr. Res.*, 1990, **202**, 165.
9. M. Kishida, M. Nishiuchi, K. Kato, and H. Akita, *Chem. Pharm. Bull.*, 2004, **52**, 1105.
10. K. P. R. Kartha and H. J. Jennings, *Carbohydrate Chemistry*, 1990, **9**, 777.
11. V. Pozsgay and H. J. Jennings, *Tetrahedron Lett.*, 1987, **28**, 1375.