

Chemoenzymatic Synthesis of Naturally Occurring Benzyl 6-*O*-Glycosyl- β -D-glucopyranosides

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Direct β -glucosidation between benzyl alcohol and D-glucose (**5**) using the immobilized β -glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a benzyl β -D-glucoside (**1**) in 53% yield. The coupling of the benzyl β -D-glucopyranoside congener (**8**) derived from **1** with phenyl 2,3,4-tri-*O*-acetyl-1-thio- β -D-xylopyranoside (**9**), ethyl 2,3,4-tri-*O*-acetyl-1-thio- α -L-rhamnopyranoside (**13**), and 2,3,4-tri-*O*-acetyl- α -L-arabinopyranoside (**15**) afforded **10**, **14**, and **16**, respectively, as coupled products. Deprotection of **10**, **14**, and **16** provided the synthetic benzyl β -D-xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**2**), benzyl α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**3**), and benzyl α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**4**), respectively.

Key words β -glucosidase; β -glucosidation; natural product synthesis; benzyl β -D-glucopyranoside

Prunus mume SIEB. *et* ZUCC. (Rosaceae) has been used as medicinal food in Japan for a long time and is reported to have many pharmacological properties, such as the inhibitory effects on bradykinin and prostaglandin E₂ production in the abdominal cavities of mice, and the effects on angiotensin-converting enzyme, aldosterone, and corticosterone levels in rat plasma.¹⁾ Moreover, it has been reported that the methanolic extract of *P. mume* exhibited inhibitory effects on rat lens aldose reductase and platelet aggregation.²⁾ Benzyl β -D-glucopyranoside (**1**) is one of the pharmacologically active constituents of *P. mume*. On the other hand, three kinds of naturally occurring benzyl 6-*O*-glycosyl- β -D-glucopyranoside congeners, benzyl β -D-xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**2**),^{3,4)} benzyl α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**3**)⁵⁾ and benzyl α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**4**)⁶⁾ were isolated from *Alangium chinense*,⁴⁾ *Margyricarpus setosus*,⁵⁾ and *Lycopersicon esculentum*,⁶⁾ respectively. For the purpose of investigation of pharmacological activity of these β -D-glucopyranoside congeners in comparison to **1**, the synthesis of the above-mentioned β -D-glucopyranoside congeners has aroused our interest. In this paper, we describe the synthesis of benzyl β -D-glucopyranoside (**1**) and its naturally occurring benzyl 6-*O*-glycosyl- β -D-glucopyranoside congeners **2**, **3** and **4**, based on the selective β -glycosidation of benzyl alcohol with D-glucose (**5**) catalyzed by the immobilized β -glucosidase (EC 3.2.1.21) from almonds.

Enzymatic β -Glycosidation In the case of the direct β -glycosidation of primary alcohols with D-glucose (**5**) 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,⁷⁾ and this method is applied to the synthesis of **1**. Meanwhile, the synthesis of **1** using 4-nitrophenyl β -D-glucopyranoside as a glycosyl donor was reported previously by us.⁸⁾ 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 of 1,8-octanediol with D-glucose (**5**).⁹⁾ Then we examined the direct β -glucosidation of benzyl alcohol

with D-glucose (**5**) using the reported immobilized β -glucosidase (EC 3.2.1.21)⁹⁾ from almonds. When a large amount of benzyl alcohol (23.4 eq) was used as an acceptor for D-glucose (**5**) in the presence of the immobilized β -glucosidase, benzyl β -D-glucopyranoside (**1**) was obtained in 53% yield. Moreover, the same β -glucosidation using the recovered immobilized enzyme afforded **1** in 52% yield.

Synthesis of Benzyl β -D-Xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (2**)** Silylation of **1** gave a silyl ether (**6**; 95% yield), which was subjected to acetylation to afford an acetate (**7**) in 99% yield. Deprotection of silyl group of **7** using 1 M IBr solution in CH₂Cl₂ gave a primary alcohol (**8**) in 76% yield. When 2% I₂ in MeOH solution or *N*-bromosuccinimide (NBS) instead of 1 M IBr solution in CH₂Cl₂ was applied for desilylation of **7**, the yield of **8** was improved to 90% or 98%, respectively. By the following reported procedure,¹⁰⁾ the coupling reaction of benzyl β -D-glucopyranoside congener (**8**) and phenyl 2,3,4-tri-*O*-acetyl-1-thio- β -D-xylopyranoside (**9**)¹¹⁾ in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) gave an inseparable mixture (**10**:**11**=3:2) of the coupled products **10** and **11** in 40% yield. Finally, treatment of this mixture with K₂CO₃ in MeOH provided the synthetic benzyl β -D-xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**2**, 55% yield) and benzyl β -D-xylopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside (**12**, 37% yield). The spectral data (¹H- and ¹³C-NMR) and specific rotation ([α]_D -48.3° (MeOH)) of the synthetic **2** were identical with those (¹H- and ¹³C-NMR and [α]_D -48.0° (MeOH)) of the natural product **2**.⁴⁾ Where, **11** was formed by glycosylation of benzyl 2,3,6-tri-*O*-acetyl- β -D-glucopyranoside (**8a**), which was formed from **8** by 4 $\rightarrow$ 6 acyl migration in the reaction medium. When **8** was treated with the same coupling reaction medium without **9**, **8a** was partly formed in the reaction mixture. The structure of **12** was determined by the HMBC correlation between H-1" (δ _H 4.33)/C-4' (δ _C 80.8). The low yield of the desired compound (**2**) could be explained by the following reason. In the coupling process between **8** and **9**, partial 4 $\rightarrow$ 6 acyl migration in the substrate (**8**) under acidic condition (TfOH) might be occurred to afford the undesired coupled product (**11**). Low yield is pre-

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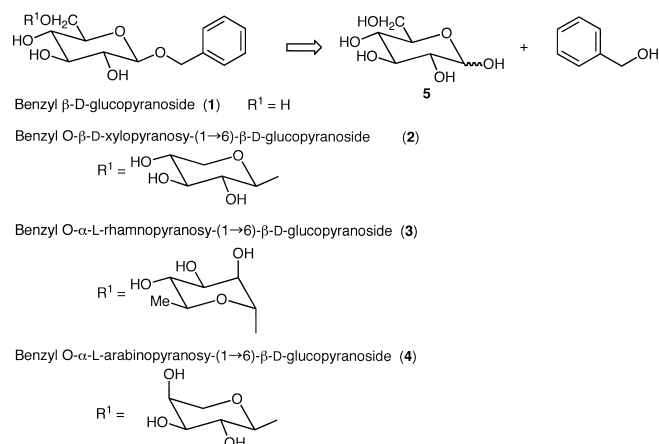


Chart 1

sumably due to low reactivity of **9** as a glycosyl donor. To overcome this low yield process, the usage of 2,3,4-tri-*O*-benzoyl-α-D-xylopyranosyl bromide is presumably effective.

Synthesis of Benzyl α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside (3) Ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**13**) was synthesized by applying the reported method¹²⁾ based on the $BF_3 \cdot Et_2O$ catalyzed reaction of ethane thiol and tetra-*O*-acetyl-α-L-rhamnopyranoside obtained by acetylation of α-L-rhamnose. By the following reported procedure,¹⁰⁾ the coupling reaction of benzyl β-D-glucopyranoside congener (**8**) and ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**13**) in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) gave the coupled product (**14**) in 76% yield. Finally, treatment of **14** with K_2CO_3 in MeOH provided the synthetic benzyl β-D-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**) 85% yield. The spectral data (1H - and ^{13}C -NMR) and specific rotation ($[\alpha]_D -52.8^\circ$ (MeOH)) of the synthetic **3** were identical with those (1H - and ^{13}C -NMR and $[\alpha]_D -40.0^\circ$ (MeOH)) of the natural product **3**.⁵⁾

Synthesis of Benzyl α-L-Arabinopyranosyl-(1→6)-β-D-glucopyranoside (4) By following the reported procedure,¹³⁾ the coupling reaction of benzyl β-D-glucopyranoside congener (**8**) and 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl bromide (**15**) in the presence of silver triflate ($AgOTf$) and 4A molecular sieves gave the coupled product (**16**) in 40% yield. Finally, treatment of **16** with K_2CO_3 in MeOH provided quantitatively the synthetic benzyl α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**4**). The spectral data (1H - and ^{13}C -NMR) and specific rotation ($[\alpha]_D -33.6^\circ$ (MeOH)) of the synthetic **4** were identical with those (1H - and ^{13}C -NMR and $[\alpha]_D -39.0^\circ$ (MeOH)) of the natural product **4**.⁶⁾ The low yield of **16** is presumably overcome by the usage of 2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl bromide instead of **15**.

Conclusion

Direct β-glucosidation between benzyl alcohol and D-glucose (**5**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a benzyl β-D-glucoside (**1**) in 53% yield. The coupling of the benzyl β-D-glucopyranoside congener (**8**) derived from **1** and phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (**9**), ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**13**), and 2,3,4-tri-

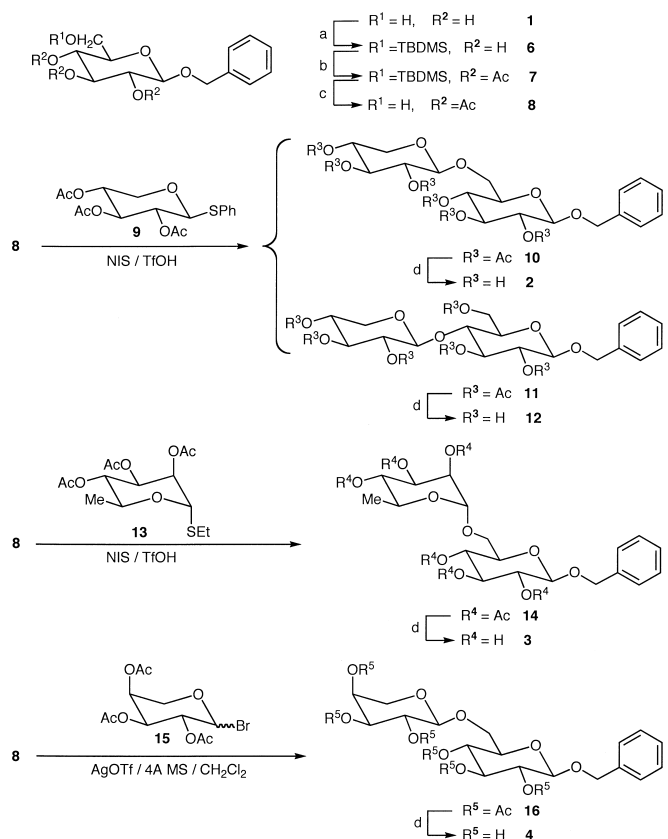


Chart 2

O-acetyl-α-L-arabinopyranosyl bromide (**15**) afforded the coupled products **10**, **14**, and **16**, respectively. Deprotection of the coupled products **10**, **14**, and **16** afforded the synthetic benzyl β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**), benzyl α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**), and benzyl α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**4**), respectively.

Experimental

1H - and ^{13}C -NMR spectra were recorded on a JEOL JNM-LA 500 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 mass spectra were obtained with a JEOL JMS-AX 500 (matrix; *m*-nitrobenzyl alcohol (NBA)) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. 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 (0.5×5×5 mm) and used for the bioconversion reaction.

Enzymatic Synthesis of Benzyl β-D-Glucopyranoside (1) 1) A mixture of D-glucose **5** (1.1 g, 6.1 mmol), benzyl alcohol (18.8 g, 173.9 mmol), water (2 ml), and the immobilized β-glucosidase was incubated for 4 d at

50 °C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give benzyl alcohol (16.9 g, 90% recovery) from the CHCl_3 eluent and β -glucoside (**1**, 0.875 g, 53% yield) as colorless solid from the $\text{CHCl}_3/\text{MeOH}=10:1$ eluent. The NMR (^1H - and ^{13}C -NMR) data of β -glucoside **1** were identical with those of the reported β -glucoside **1**.^{7,8)}

2) A mixture of D-glucose **5** (1.1 g, 6.1 mmol), benzyl alcohol (18.8 g, 173.9 mmol), water (2 ml), and the recovered immobilized β -glucosidase was incubated for 4 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give benzyl alcohol (17.1 g, 91% recovery) from the CHCl_3 eluent and β -glucoside (**1**, 0.858 g, 52% yield) as colorless solid from the $\text{CHCl}_3/\text{MeOH}=10:1$ eluent.

Benzyl 6-O-tert-Butyldimethylsilyl- β -D-glucopyranoside (6) To a solution of **1** (1.00 g, 3.70 mmol) in pyridine (30 ml) was added TBDMSCl (1.10 g, 7.33 mmol) and 4-*N,N*-dimethylaminopyridine (DMAP; 10 mg, 0.08 mmol) at 0 °C, and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was condensed and the resulting residue diluted with water and extracted with AcOEt. The organic layer was washed with 10% aqueous HCl, 7% aqueous NaHCO_3 , and brine. The organic layer was dried over MgSO_4 and evaporated to give a residue, which was chromatographed on silica gel (50 g, $\text{CHCl}_3/\text{MeOH}$ (30:1)) to afford **6** (1.35 g, 95%) as a colorless amorphous powder. **6**: $[\alpha]_D^{21} -58.8^\circ$ ($c=0.873$, CHCl_3); IR (KBr): 3423 (br), 3032, 2954, 2927, 2881, 2855 cm^{-1} ; ^1H -NMR (CDCl_3): δ 0.00 (6H, s), 0.82 (9H, s), 3.13 (1H, m), 3.25–3.38 (3H, m), 3.66 (1H, dd, $J=6.0$, 11.2 Hz), 3.81 (1H, dd, $J=2.4$, 11.2 Hz), 4.17 (1H, d, $J=7.2$ Hz), 4.25 (1H, brs), 4.44 (1H, brs), 4.47 (1H, d, $J=11.6$ Hz), 4.73 (1H, d, $J=11.6$ Hz), 5.20 (1H, brs), 7.12–7.26 (5H, m); ^{13}C -NMR (CDCl_3): δ -4.77, -4.75, 18.7, 26.3 [3C], 63.9, 70.7, 71.2, 73.6, 76.1, 76.7, 101.3, 127.9, 128.4 [2C], 128.5 [2C], 137.3; *Anal.* Calcd for $\text{C}_{19}\text{H}_{32}\text{O}_6\text{Si}$: C, 59.34; H, 8.39%. Found: C, 58.91; H, 8.86%.

Benzyl 2,3,4-Tri-O-acetyl-6-O-tert-butyldimethylsilyl- β -D-glucopyranoside (7) To a solution of **6** (1.35 g, 3.51 mmol) in pyridine (5 ml) was added Ac_2O (4 ml) and 4-*N,N*-dimethylaminopyridine (DMAP; 10 mg, 0.08 mmol) at 0 °C, and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with 0.5 M aqueous HCl, 7% aqueous NaHCO_3 , and brine. The organic layer was dried over MgSO_4 and evaporated to give a residue, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt (8:1)) to afford **7** (1.79 g, 99%) as a colorless amorphous powder. **7**: $[\alpha]_D^{22} -33.2^\circ$ ($c=1.08$, CHCl_3); IR (KBr): 1759 cm^{-1} ; ^1H -NMR (CDCl_3): δ 0.00 (6H, s), 0.83 (9H, s), 1.92 (3H, s), 1.93 (3H, s), 3.44 (1H, m), 3.64 (2H, m), 4.44 (1H, d, $J=8.0$ Hz), 4.55 (1H, d, $J=12.4$ Hz), 4.80 (1H, d, $J=12.4$ Hz), 4.95 (2H, m), 5.08 (1H, t, $J=9.2$ Hz), 7.18–7.29 (5H, m); ^{13}C -NMR (CDCl_3): δ -5.17 [2C], 18.5, 20.8, 20.8, 20.8, 25.9 [3C], 62.5, 69.1, 70.3, 71.5, 73.2, 74.8, 98.9, 127.7 [2C], 127.8, 128.3 [2C], 136.7, 169.2, 169.2, 170.2; *Anal.* Calcd for $\text{C}_{25}\text{H}_{38}\text{O}_9\text{Si}$: C, 58.80; H, 7.50%. Found: C, 58.87; H, 7.56%.

Benzyl 2,3,4-Tri-O-acetyl- β -D-glucopyranoside (8) 1) Method 1: To a solution of **7** (2.11 g, 4.13 mmol) in MeOH (35 ml) was added 1 M IBR in CH_2Cl_2 solution (6.20 g) at room temperature, and the whole mixture was stirred for 3 h at the same temperature. The reaction mixture was diluted with saturated sodium thiosulfate solution and extracted with CHCl_3 . 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 (50 g, *n*-hexane/AcOEt (2:1)) to afford **8** (1.25 g, 76%) as a colorless amorphous powder. **8**: $[\alpha]_D^{23} -44.3^\circ$ ($c=0.75$, CHCl_3); IR (KBr): 3522, 2962, 1734 cm^{-1} ; ^1H -NMR (CDCl_3): δ 1.99 (6H, s), 2.02 (3H, s), 3.51 (1H, m), 3.60 (1H, dd, $J=11.6$, 4.8 Hz), 3.73 (1H, d, $J=11.6$ Hz), 4.59 (1H, d, $J=8.0$ Hz), 4.64 (1H, d, $J=12.4$ Hz), 4.88 (1H, d, $J=12.4$ Hz), 5.04 (2H, m), 5.21 (1H, t, $J=9.6$ Hz), 7.27–7.35 (5H, m); ^{13}C -NMR (CDCl_3): δ 20.4 [2C], 20.4, 61.0, 68.5, 70.7, 71.2, 72.6, 73.9, 99.2, 127.3 [2C], 127.6, 128.0 [2C], 136.5, 168.9, 169.5, 169.8; *Anal.* Calcd for $\text{C}_{19}\text{H}_{24}\text{O}_9$: C, 57.57; H, 6.10%. Found: C, 57.37; H, 6.12%.

2) Method 2: To a solution of **7** (0.79 g, 1.55 mmol) in MeOH (10 ml) was added 2% I_2 in MeOH solution (10 ml) at room temperature, and the whole mixture was stirred for 4 d at the same temperature. The reaction mixture was worked up in the same way as for method 1 to give **8** (0.55 g, 90%) as a colorless amorphous powder.

3) Method 3: To a solution of **7** (0.015 g, 0.03 mmol) in 95% aqueous DMSO (1.0 ml)/THF (0.5 ml)/ H_2O (28.5 ml) solution was added NBS (0.003 g, 0.033 mmol) at room temperature, and the whole mixture was stirred for 17 h at the same temperature. The reaction mixture was worked up in the same way as for method 1 to give **8** (0.012 g, 98%) as a colorless amorphous powder.

A Mixture of Benzyl 2,3,4,2',3',4'-O-Hexaacetyl- β -D-xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (10) and Benzyl 2,3,6,2',3',4'-O-Hexaacetyl- β -D-xylopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside (11) A mixture of **8** (0.067 g, 0.168 mmol), **9** (0.109 g, 0.451 mmol), and NIS (0.120 g, 0.533 mmol) in CH_2Cl_2 (5 ml) was stirred for 30 min at 0 °C. To the above mentioned mixture was added methane sulfonic acid (0.048 g, 0.50 mmol) at 0 °C and the whole mixture was stirred for 3 h at the same temperature. The reaction mixture was filtered with the aid of celite and the filtrate was condensed to afford a residue, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt (3:1)) to give a mixture (**10**:**11**=3:2, 0.044 g, 40%) as a colorless oil. Major **10**: IR (KBr): 1751 cm^{-1} ; ^1H -NMR (CDCl_3): δ 1.59–2.14 (18H, m), 3.34 (1H, dd, $J=8$, 12 Hz), 3.64 (1H, m), 3.85 (1H, d, $J=10$ Hz), 4.13 (1H, dd, $J=4$, 12 Hz), 4.51 (1H, d, $J=8$, 12.0 Hz), 4.55 (1H, d, $J=6.4$ Hz), 4.63 (1H, d, $J=12$ Hz), 4.87–5.04 (5H, m), 5.15 (2H, m), 7.25–7.35 (5H, m); ^{13}C -NMR (CDCl_3): δ 20.7, 20.7, 20.7, 20.8, 20.8, 20.8, 62.0, 67.7, 68.7, 69.0, 70.4, 70.5, 71.2, 71.3, 72.8, 73.2, 76.7, 99.0, 100.5, 127.4, 127.8, 128.3, 136.6, 169.1, 169.1, 169.2, 169.6, 169.8, 170.0; HR-FAB-MS (NBA) m/z : Calcd for $\text{C}_{30}\text{H}_{38}\text{O}_{16}\text{Na}$ 677.2031 ($\text{M}+\text{Na}$)⁺. Found: 677.2058.

Benzyl β -D-Xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (2) and Benzyl β -D-Xylopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside (12) A mixture of the above-mentioned mixture (**10**, **11**, 0.026 g, 0.27 mmol) and K_2CO_3 (0.005 g) in MeOH (2 ml) was stirred for 12 h at room temperature. The reaction mixture was condensed to give a residue, which was chromatographed on silica gel (10 g) to afford **2** (0.009 g, 55%) as a colorless amorphous from $\text{CHCl}_3/\text{MeOH}$ (4:1) eluent and by-product **12** (0.006 g, 37%) as a colorless amorphous from $\text{CHCl}_3/\text{MeOH}$ (4:1). **2**: $[\alpha]_D^{19} -48.3^\circ$ ($c=0.76$, MeOH); IR (KBr): 3348 (br), 2925, 2872 cm^{-1} ; ^1H -NMR (CD_3OD): δ 3.09–3.41 (8H, m), 3.68 (1H, d, $J=12$ Hz), 3.77 (1H, dd, $J=5$, 12 Hz), 4.02 (1H, d, $J=10$ Hz), 4.26 (2H, d, $J=8$ Hz), 4.56 (1H, d, $J=12$ Hz), 4.82 (1H, d, $J=12$ Hz), 7.16–7.34 (5H, m); ^{13}C -NMR (CD_3OD): δ 66.9, 69.8, 71.1, 71.5, 71.9, 74.8, 75.0, 77.0, 77.7, 77.9, 103.2, 105.5, 128.5, 129.1 [2C], 129.1 [2C], 138.8; HR-FAB-MS (NBA) m/z : Calcd for $\text{C}_{18}\text{H}_{27}\text{O}_{10}$: 403.1604 ($\text{M}+1$)⁺. Found: 403.1579. By-product **12**: $[\alpha]_D^{19} -38.6^\circ$ ($c=0.79$, MeOH); IR (KBr): 3348 (br), 2925, 2872 cm^{-1} ; ^1H -NMR (CDCl_3): δ 3.17–3.56 (8H, m), 3.82–3.93 (3H, m), 4.33 (1H, d, $J=8$ Hz), 4.37 (1H, d, $J=7.6$ Hz), 4.65 (1H, d, $J=12$ Hz), 4.90 (1H, d, $J=12$ Hz), 7.24–7.41 (5H, m); ^{13}C -NMR (CD_3OD): δ 61.8, 67.1, 70.9, 71.8, 74.8, 74.9, 76.1, 76.4, 77.8, 80.8, 103.0, 105.3, 128.5, 129.0 [2C], 129.1 [2C], 138.8; HR-FAB-MS (NBA) m/z : Calcd for $\text{C}_{18}\text{H}_{27}\text{O}_{10}$: 403.1604 ($\text{M}+1$)⁺. Found: 403.1558.

Ethyl 2,3,4-O-Triacetyl-1-thio- α -L-rhamnopyranoside (13) To a solution of 1,2,3,4-O-tetraacetyl- α -L-rhamnopyranose (10.0 g, 30.1 mmol) in CHCl_3 (50 ml) was added ethane thiol (2.35 g, 39.1 mmol) and 47% $\text{BF}_3 \cdot \text{Et}_2\text{O}$ complex (22.4 g, 74 mmol) at 0 °C and the whole mixture was stirred for 6 h at room temperature. The reaction mixture was diluted with water and the organic layer was washed with 7% aqueous NaHCO_3 , and brine. The organic layer was dried over MgSO_4 and evaporated to give a residue, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt (5:1)) to afford a mixture of α - and β -isomers **13** (8.82 g, 88%). Crystallization of this mixture from ether gave a isomers **13** (4.05 g, 40%) as a colorless needles. **13**: mp 135 °C; $[\alpha]_D^{23} +69.1^\circ$ ($c=1.06$, CHCl_3); IR (KBr): 1749 cm^{-1} ; ^1H -NMR (CDCl_3): δ 1.29 (3H, d, $J=6$ Hz), 1.99 (3H, s), 2.06 (3H, s), 2.16 (3H, s), 2.58–2.69 (2H, m), 4.24 (1H, m), 5.10 (1H, t, $J=10$ Hz), 5.20 (1H, d, $J=1.6$ Hz), 5.24 (1H, dd, $J=10$, 3.2 Hz), 5.34 (1H, dd, $J=1.6$, 3.2 Hz); ^{13}C -NMR (CDCl_3): δ 14.9, 17.7, 20.6, 20.6, 20.8, 25.6, 70.4, 70.8, 71.9, 74.9, 81.9, 169.8, 170.2, 170.3; *Anal.* Calcd for $\text{C}_{14}\text{H}_{22}\text{O}_7\text{S}$: C, 50.29; H, 6.63%. Found: C, 50.43; H, 6.64%.

Benzyl 2,3,4,2',3',4'-O-Hexaacetyl- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (14) A mixture of **8** (0.155 g, 0.39 mmol), **13** (0.13 g, 0.39 mmol), molecular sieves 4A (0.5 g) and NIS (0.187 g, 0.83 mmol) in CH_2Cl_2 (5 ml) was stirred for 30 min at 0 °C. To the above mentioned mixture was added methane sulfonic acid (0.098 g, 1.02 mmol) at 0 °C and the whole mixture was stirred for 3 h at the same temperature. The reaction mixture was filtered with the aid of celite and the filtrate was condensed to afford a residue, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt (3:1)) to give **14** (0.20 g, 76%) as a colorless amorphous powder. **14**: $[\alpha]_D^{21} -71.5^\circ$ ($c=1.08$, CHCl_3); ^1H -NMR (CDCl_3): δ 1.22 (3H, d, $J=4.4$ Hz), 2.00 (3H, s), 2.00 (3H, s), 2.01 (3H, s), 2.05 (3H, s), 2.05 (3H, s), 2.12 (3H, s), 3.65–3.75 (3H, m), 3.85–3.93 (1H, m), 4.56 (2H, d, $J=8$ Hz), 4.63 (2H, d, $J=12$ Hz), 4.86 (1H, d, $J=0.8$ Hz), 4.87 (2H, d, $J=12$ Hz), 4.96 (1H, t, $J=9.6$ Hz), 5.04 (1H, dd, $J=9.6$, 8.0 Hz), 5.05–5.10 (1H, m), 5.28–5.31 (2H, m), 5.78 (1H, t, $J=9.6$ Hz), 7.26–7.37 (5H, m); ^{13}C -NMR (CDCl_3): δ 17.2, 20.4, 20.4, 20.45, 20.5, 20.6, 20.65, 66.4, 66.7, 68.8, 69.2, 69.4, 70.4, 70.8, 71.1, 72.6, 73.3, 90.0, 99.0, 127.5 [2C], 127.7,

128.3 [2C], 136.6, 169.1, 169.3, 169.7, 169.75, 169.8, 170.0; *Anal.* Calcd for $C_{33}H_{40}O_{16}$: C, 55.68; H, 6.03%. Found: C, 55.44; H, 5.92%.

Benzyl α -L-Rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (3) A mixture of **13** (0.493 g, 0.73 mmol) and K_2CO_3 (0.005 g) in MeOH (2 ml) was stirred for 12 h at room temperature. The reaction mixture was condensed to give a residue, which was chromatographed on silica gel (10 g, $CHCl_3$ /MeOH (4:1)) to afford **3** (0.26 g, 85%) as a colorless oil **3**: $[\alpha]_D^{19}$ -52.8° ($c=1.37$, MeOH); IR (KBr): 3375 (br), 2926 cm^{-1} ; 1H -NMR (CD_3OD): δ 1.24 (3H, d, $J=8$ Hz), 3.22–3.40 (3H, m), 3.55–3.71 (3H, m), 3.87 (1H, dd, $J=1$, 3 Hz), 3.94 (1H, d, $J=7.6$ Hz), 4.29 (1H, d, $J=8$ Hz), 4.58 (1H, d, $J=12$ Hz), 4.83 (1H, d, $J=12$ Hz), 7.20–7.38 (5H, m); ^{13}C -NMR (CD_3OD): δ 18.11, 67.95, 69.60, 71.42, 71.61, 71.95, 72.17, 73.76, 74.78, 76.59, 77.08, 101.92, 102.81, 128.50, 129.03 [2C], 129.04 [2C], 138.41; HR-FAB-MS (NBA) m/z : Calcd for $C_{19}H_{28}O_{10}Na$; 439.1580 ($M+Na$) $^+$. Found: 439.1601.

Benzyl 2,3,4,2',3',4'-O-Hexaacetyl- α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (16) To a solution of **8** (0.6 g, 1.52 mmol) in CH_2Cl_2 (10 ml) was added 2,3,4-tri-*O*-acetyl- α -L-arabinopyranosyl bromide (**15**, 1.03 g, 3.03 mmol) and tetramethylurea (TMU, 0.704 g, 6.6 mmol) and AgOTf (0.778 g, 3.03 mmol) at 0 $^\circ C$ under argon atmosphere. The whole was covered with aluminum foil and stirred for 3.5 h at room temperature. The reaction mixture was cooled at 0 $^\circ C$ and quenched with AcOEt (100 ml) and 7% aqueous $NaHCO_3$ solution (20 ml). The organic layer was washed with brine and dried over Na_2SO_4 . Evaporation of the organic solvent gave a residue, which was chromatographed on silicagel (20 g, *n*-hexane/AcOEt (4:1)) to afford **16** (0.4 g, 40% yield) as a colorless amorphous. **16**: $[\alpha]_D^{26}$ -32.1° ($c=1.01$, $CHCl_3$); IR (KBr): 1739 cm^{-1} ; 1H -NMR ($CDCl_3$): δ 1.99 (3H, s), 2.01 (3H, s), 2.02 (3H, s), 2.03–2.04 (6H, m), 2.14 (3H, s), 3.57–3.62 (2H, m), 3.65–3.71 (1H, m), 3.90 (1H, dd, $J=1.5$, 10.6 Hz), 4.31 (1H, dd, $J=3.5$, 12.1 Hz), 4.48 (1H, d, $J=7.0$ Hz), 4.52 (1H, d, $J=8.0$ Hz), 4.61 (1H, d, $J=12$ Hz), 4.89 (1H, d, $J=12$ Hz), 4.95 (1H, dd, $J=9.6$, 9.6 Hz), 5.03 (1H, dd, $J=9.6$, 9.6 Hz), 5.02–5.05 (1H, m), 5.15 (1H, d, $J=9.6$ Hz), 5.17–5.26 (2H, m), 7.28–7.38 (5H, m); ^{13}C -NMR ($CDCl_3$): δ 20.69, 20.73 [2C], 20.75, 20.89, 21.01, 63.18, 67.61, 67.99, 69.13, 69.22, 70.12, 70.54, 71.43, 72.97, 73.28, 99.17, 100.91, 127.71 [2C], 128.05, 128.56 [2C], 136.85, 169.45, 169.53, 169.68, 170.21, 170.32, 170.36; HR-FAB-MS (NBA) m/z : Calcd for $C_{30}H_{39}O_{16}$ 655.2238 ($M+1$) $^+$. Found: 655.2241.

Benzyl β -D-Arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (4) A mixture of **16** (0.15 g, 0.23 mmol) and K_2CO_3 (0.032 g) in a mixed solvent (MeOH: CH_2Cl_2 =5:1; 12 ml) was stirred for 15 min at room temperature. The reaction mixture was condensed to give a residue, which was chro-

matographed on silica gel (10 g, CH_2Cl_2 /EtOH (5:1)) to afford **4** (0.062 g, 69%). **4**: $[\alpha]_D^{24}$ -33.6° ($c=0.025$, H_2O); IR (KBr): 3600–3100 cm^{-1} ; 1H -NMR (CD_3OD+D_2O): δ 3.17–3.22 (1H, m), 3.30–3.39 (2H, m), 3.40–3.52 (4H, m), 3.72 (1H, dd, $J=5.0$, 11.6 Hz), 3.76–3.84 (2H, m), 4.04 (1H, dd, $J=2.0$, 11.6 Hz), 4.26 (1H, d, $J=7.0$ Hz), 4.35 (1H, d, $J=7.6$ Hz), 4.60 (1H, d, $J=12$ Hz), 4.83 (1H, d, $J=12$ Hz), 7.21–7.37 (5H, m); ^{13}C -NMR (CD_3OD+D_2O): δ 66.31, 66.99, 69.38, 70.83, 72.01, 72.23, 73.70, 74.52, 76.36, 77.24, 102.88, 104.88, 129.02, 129.32 [2C], 129.46 [2C], 138.26; HR-FAB-MS (NBA) m/z : Calcd for $C_{18}H_{27}O_{10}$ 403.1604 ($M+1$) $^+$. Found: 403.1594.

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