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CHEMOENZYMATIC SYNTHESIS OF NATURALLY OCCURRING (*Z*)-3-HEXENYL 6-*O*-GLYCOSYL-β-D-GLUCOPYRANOSIDES

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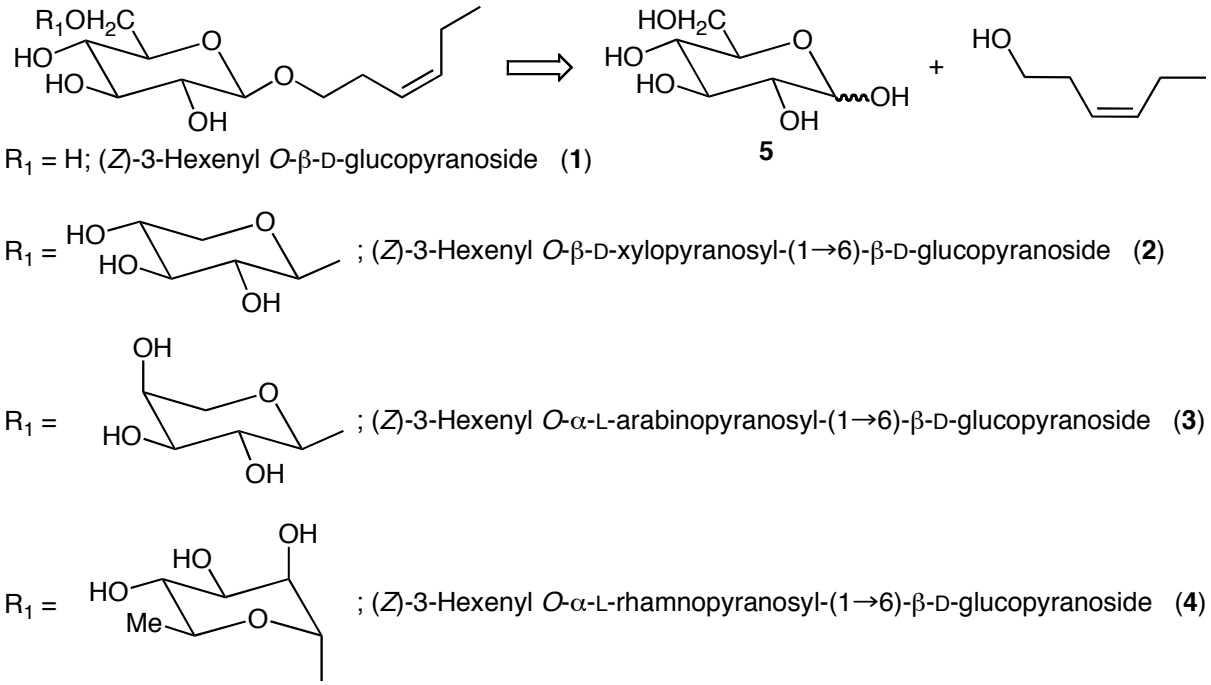
Abstract- Direct β-glucosidation between *cis*-3-hexen-1-ol and D-glucose (**5**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave (*Z*)-3-hexenyl β-D-glucoside (**1**) in 17% yield. The coupling of the (*Z*)-3-hexenyl *O*-β-D-glucopyranoside congener (**7**) with 2,3,4-tri-*O*-benzoyl-α-D-xylopyranosyl bromide (**8**) gave the coupled product (**11**). Similarly the coupling of **7** with 2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl bromide (**9**), and that of **7** with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**10**) afforded the coupled products (**12** and **13**), respectively. Deprotection of the products (**11**, **12**, and **13**) afforded (*Z*)-3-hexenyl *O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**), (*Z*)-3-hexenyl *O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**3**), and (*Z*)-3-hexenyl *O*-α-L-rhamnopyranosyl- (1→6)-β-D-glucopyranoside (**4**), respectively.

(*Z*)-3-Hexenyl β-D-glucoside (**1**) is widely distributed in the plant,[1] and was isolated from the leaves of *Pertya glabrescens*,[1a] *Epimedium grandiflorum* var. *thunbergium*,[1b] the leaves of *Celosia argentea*,[1f] and leaves of *Thymus vulgaris*.[1l] Moreover, three kinds of naturally occurring (*Z*)-3-hexenyl 6-*O*-glycosyl-β-D-glucopyranoside congeners, (*Z*)-3-hexenyl *O*-β-D-xylopyranosyl-(1

→ 6)-β-D-glucopyranoside[2] (**2**), (*Z*)-3-hexenyl *O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside[3] (**3**) and (*Z*)-3-hexenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside[1f] (**4**) were isolated from a methanolic extract of leaves of *Alangium platanifolium* var. *trilobim*,[2a] *Hippophae rhamnoides*[3] and *African Celosia argentea*,[1f] respectively. Interestingly, compound (**2**) was found to exhibit growth promotive activity of lettuce, whereas *cis*-3-hexen-1-ol, which is the aglycone of **2**, inhibited the germination of lettuce.[1f] To investigate their pharmacological activities, the synthesis of the above-mentioned β-D-glucopyranoside congeners has aroused our interest. In this paper, we describe the synthesis of (*Z*)-3-hexenyl β-D-glucopyranoside (**1**) and its naturally occurring (*Z*)-3-hexenyl 6-*O*-glycosyl-β-D-glucopyranoside congeners (**2**, **3** and **4**) based on the selective β-glucosidation between D-glucose (**5**) and *cis*-3-hexen-1-ol catalyzed by the immobilized β-glucosidase (EC 3.2.1.21) from almonds.



Scheme 1

Enzymatic β-glucosidation

In case of the direct β-glucosidation between D-glucose (**5**) and primary alcohols using β-glucosidase (EC 3.2.1.21) from almonds under thermodynamic conditions, a high concentration of a primary alcohol or a medium with low water activity is reported to be effective.[4] Meanwhile, the synthesis of mono-β-D-glucopyranoside using 4-nitrophenyl β-D-glucopyranoside as a glycosyl donor was reported previously by us.[5] 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.[6] Then we examined the direct β-glucosidation between D-glucose (**5**) and *cis*-3-hexen-1-ol using the reported immobilized β-glucosidase (EC 3.2.1.21)[6] from almonds. When a large amount of *cis*-3-hexene-1-ol (25 equivalents) was used as an acceptor for D-glucose (**5**) in the presence of the immobilized β-glucosidase, a 17% yield of (*Z*)-3-hexenyl *O*-β-D-glucopyranoside (**1**) was obtained. Moreover, the same β-glucosidation using the recovered immobilized enzyme afforded **1** in 17% yield.

Synthesis of (*Z*)-3-hexenyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (2)

The *tert*-butyldimethylsilyl (TBDMS) protection of **1** gave a silyl ether (**6**) in 60 % yield, which was subjected to consecutive benzoylation and deprotection of TBDMS group to give the desired (*Z*)-3-hexenyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**7**) in 80 % yield (2 steps). On the other hand, 2,3,4-tri-*O*-benzoyl-α-D-xylopyranosyl bromide (**8**) was prepared by literature procedures.[7] The alcohol (**7**) was treated with 2 equivalents of bromide (**8**) in the presence of silver triflate (AgOTf) and tetramethylurea (TMU) in CH_2Cl_2 to give the corresponding coupling product (**11**) in 69% yield. Finally, treatment of **11** with NaOMe in MeOH-THF provided the synthetic (*Z*)-3-hexenyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**) in 85% yield. The spectral data (^{13}C-NMR) and specific rotation ($[\alpha]_D^{26}$ –56.8° (c=0.90, MeOH)) of the synthetic **2** were identical with those (^{13}C-NMR and $[\alpha]_D^{20}$ –56.7° (c=0.90, MeOH)) of natural product (**2**).[2a]

Synthesis of (*Z*)-3-hexenyl *O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (3)

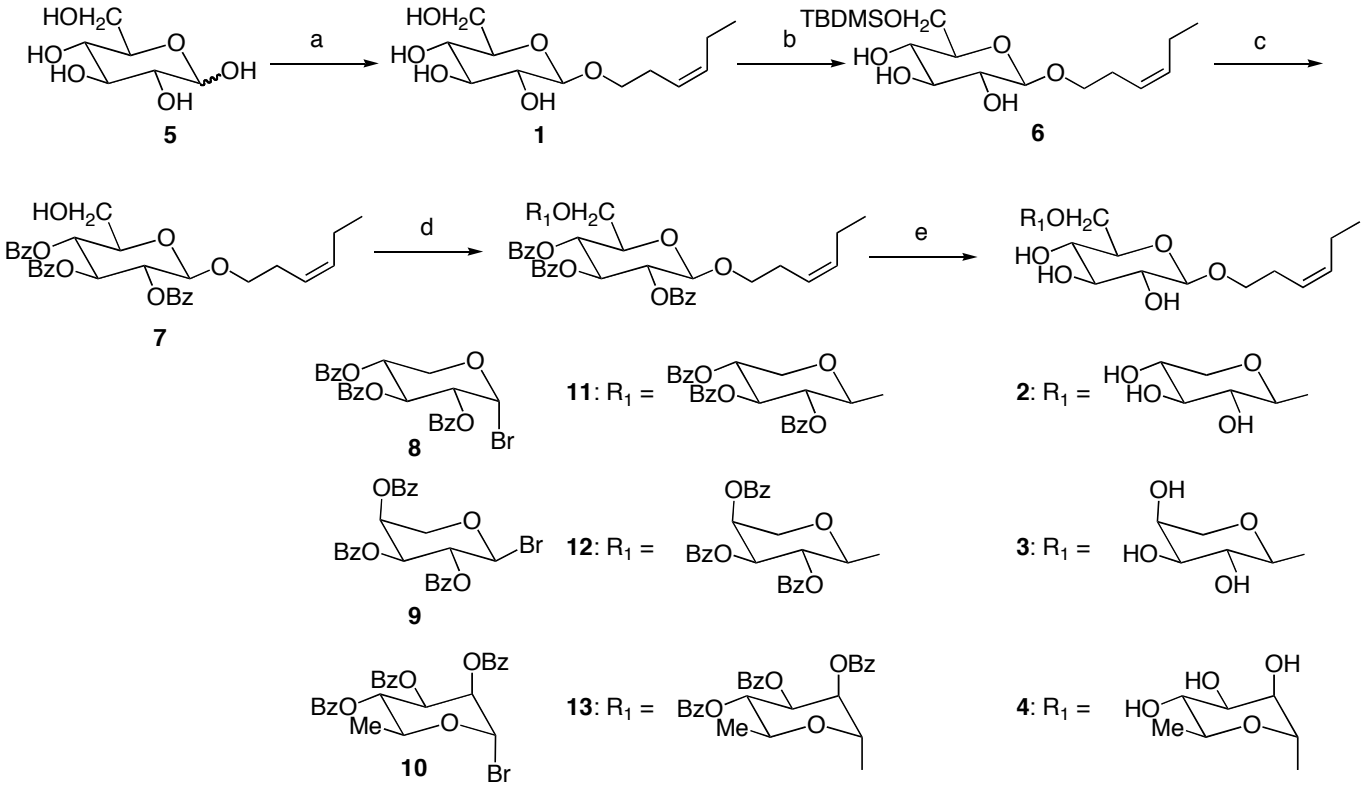
The coupling reaction of **7** and 2 equivalents of the known 2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl bromide[8] (**9**) in the presence of silver triflate (AgOTf) and tetramethylurea (TMU) in CH_2Cl_2 gave the coupled product (**12**) in 68% yield. Finally, treatment of **12** with NaOMe in MeOH-THF provided the synthetic (*Z*)-3-hexenyl *O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**, $[\alpha]_D^{23}$ –31.0° (c=0.74, MeOH)) in 83% yield. The spectral data (^{1}H- and ^{13}C-NMR) of the synthetic **3** were identical with those of natural product (**3**).[3]

Synthesis of (*Z*)-3-hexenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (4)

The coupling reaction of **7** with 2 equivalents of the reported 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide[9] (**10**) in the presence of silver triflate (AgOTf) and tetramethylurea (TMU) in CH_2Cl_2 gave the coupled product (**13**) in 56% yield. Finally, treatment of **13** with NaOMe in MeOH-THF provided the synthetic (*Z*)-3-hexenyl *O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**) in 96% yield. The spectral data (^{13}C-NMR) of the synthetic **4** was identical with that (^{13}C-NMR in MeOH-d_4) of natural product (**4**).[1f] The specific rotation ($[\alpha]_D^{25}$ –53.9° (c=0.63, MeOH)) of the synthetic **4** was consistent with that ($[\alpha]_D^{20}$ –48.26° (c=0.5, MeOH)) of natural product (**4**).[1f]

CONCLUSION

In conclusion, direct β-glucosidation between *cis*-3-hexen-1-ol and D-glucose (**5**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a (*Z*)-3-hexenyl *O*-β-D-glucoside (**1**) in 17% yield. The coupling of the (*Z*)-3-hexenyl *O*-β-D-glucopyranoside congener (**7**) and 2,3,4-tri-*O*-benzoyl-α-D-xylopyranosyl bromide (**8**), 2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl bromide (**9**), and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**10**) gave the coupled products (**11**, **12**, and **13**), respectively. Deprotection of the coupled products (**11**, **12**, and **13**) afforded the synthetic (*Z*)-3-hexenyl *O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**), (*Z*)-3-hexenyl *O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**), and (*Z*)-3-hexenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**4**), respectively.



a; *cis*-3-hexen-1-ol / H_2O / immobilized β-glucosidase with ENTP-4000, 50°C, 7 d, 17 %
b; TBDMSCl / pyridine / DMF, rt, 12 h, 60%
c; (i) BzCl / cat. DMAP/ pyridine, rt, overnight (ii) 1N HCl / THF, rt, 12 h, 2 steps 80%
d; **8** or **9** or **10** / AgOTf / tetramethylurea / CH_2Cl_2, 0°C-rt, 12 h, 56-69%
e; 25% NaOMe in MeOH / MeOH / THF, rt, 1 h, 83-96%

Scheme 2

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL EX 400 spectrometer (Tokyo, Japan) or a Bruker 400 spectrometer. Spectra were recorded with 5-10% (w/v) solution in $CDCl_3$ or methanol-d_4, 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 and *m*-nitrobenzyl alcohol) 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 and for flash column chromatography, silica gel (Silica Gel 60N, spherical, neutral, 40-50 μm) 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 74 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 (*Z*)-3-hexenyl *O*-β-D-glucopyranoside (1)

1) A mixture of D-glucose (**5**) (1.10 g, 6.1 mmol), *cis*-3-hexen-1-ol (18 mL, 15.3 g, 153 mmol), water (2.0 mL), and the immobilized β-glucosidase (250 units) was incubated for 7 days at 50°C. The reaction mixture was filtered off and the immobilized β-glucosidase was washed with AcOEt (3.0 mL). The combined filtrate was directly chromatographed on silica gel (25 g) to give *cis*-3-hexen-1-ol (9.73 g, 64% recovery) from the CH_2Cl_2/MeOH = 25:1 eluent and β-glucoside (**1**, 0.271g, 17%) as a white solid from the CH_2Cl_2/MeOH = 10:1 eluent. **1**: mp 79.4-80.3 °C; $[\alpha]_D^{23}$ –32.1 ° (c = 1.10, MeOH){lit. $[\alpha]_D^{21}$ –35.5° (c = 2.75, MeOH)[1b], $[\alpha]_D^{29}$ –43.8° (c = 0.73, MeOH) [1g], $[\alpha]_D^{26}$ –40.6° (c = 0.19, MeOH) [1j]}; IR (KBr) 3346, 2930, 1374, 1163, 1079, 1033 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz) δ: 5.49-5.34 (m, 2H), 4.27 (d, 1H, *J* = 7.8 Hz), 3.90-3.84 (m, 2H), 3.69-3.64 (m, 1H), 3.54 (dt, 1H, *J* = 9.6, 6.8 Hz), 3.37-3.23 (m, 3H), 3.17 (dd, 1H, *J* = 7.8, 8.8 Hz), 2.38 (td, 2H, *J* = 6.8, 6.8 Hz), 2.07 (dq, 2H, *J* = 7.1, 7.6 Hz), 0.96 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR (methanol-d_4, 100 MHz) δ: 134.5, 125.9, 104.3, 78.1, 77.9, 75.1, 71.6, 70.5, 62.8, 28.9, 21.5, 14.6; *Anal.* Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.70; H, 8.33.

2) A mixture of D-glucose (**5**) (1.10 g, 6.1 mmol), *cis*-3-hexen-1-ol (18 mL, 15.3 g, 153 mmol), water (2 mL), and the recovered immobilized β-glucosidase was incubated for 7 days at 50°C. The reaction mixture was worked up in the same way as 1) to give *cis*-3-hexen-1-ol (14.5 g, 95% recovery) and β-glucoside (**1**, 0.274 g, 17%).

(*Z*)-3-Hexenyl 6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (6)

A mixture of **1** (2.56 g, 9.8 mmol) and pyridine (1.16 g, 14.7 mmol) in DMF (10 mL) was treated with *tert*-butyldimethylsilyl chloride (7.77 g, 11.7 mmol) at 0°C and stirred at rt for 18 h. The reaction mixture was recooled at 0°C and treated with *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.30 g, 2.0 mmol). After stirred at 0-5 °C for 2 h, the reaction mixture was diluted with 1N HCl (20 mL) and extracted with AcOEt. The organic layer was washed with water (100 mL), saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash column chromatography on silica gel (120 g, CH_2Cl_2/MeOH (20:1-15:1)) to afford **6** (2.20 g, 60%) as a colorless solid. **6**: mp 78.5-80.5 °C (CH_2Cl_2/MeOH); $[\alpha]_D^{27}$ -38.3° (c = 0.89, $CHCl_3$); IR (KBr) 3472, 2930, 2856, 1251, 1148, 1059, 837, 775 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.51-5.44 (m, 1H), 5.36-5.29 (m, 1H), 4.28 (d, 1H, *J* = 7.5 Hz), 3.91-3.82 (m, 5H), 3.59-3.48 (m, 3H), 3.38-3.32 (m, 3H), 2.37 (td, 2H, *J* = 6.0, 6.0 Hz), 2.00 (dq, 2H, *J* = 6.0, 7.6 Hz), 0.95 (t, 3H, *J* = 7.6 Hz), 0.90 (s, 9H), 0.093 (s, 3H), 0.087 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 134.1, 124.2, 102.4, 76.2, 74.6, 73.4, 72.4, 69.4, 64.4, 27.7, 25.8, 20.6, 18.2, 14.2, -5.4; *Anal.* Calcd for $C_{18}H_{38}O_6Si$: C, 57.41; H, 9.64. Found: C, 57.13; H, 9.51.

(*Z*)-3-Hexenyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (7)

A mixture of **6** (61 mg, 0.16 mmol), benzoyl chloride (100 mg, 0.71 mmol) and 4-*N*,*N*-dimethylaminopyridine (DMAP, 2 mg) in pyridine (0.8 mL) was stirred at rt for 16 h. The reaction mixture was diluted with 1N HCl and extracted with AcOEt. The organic layer was washed with 1N HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and evaporated to give a crude (*Z*)-3-hexenyl 2,3,4-tri-*O*-benzoyl-6-*tert*-butyldimethylsilyl-β-D-glucopyranoside. The crude product was mixed with 1N HCl (0.5 mL) in THF (1.0 mL) and stirred at rt for 12 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash column chromatography on silica gel (5 g, *n*-hexane /AcOEt (5:1-2:1)) to afford **7** (74 mg, 80%) as a colorless syrup. **7**: $[\alpha]_D^{27}$ –7.8° (c= 0.57, $CHCl_3$); IR (KBr) 3508, 2962, 1732, 1262, 1094, 1028, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.98-7.92 (m, 4H), 7.86-7.82 (m, 2H), 7.55-7.50 (m, 2H), 7.45-7.36 (m, 5H), 7.30-7.26 (m, 2H), 5.93 (t, 1H, *J* = 9.8 Hz), 5.53-5.46 (m, 2H), 5.31-5.25 (m, 1H), 5.22-5.17 (m, 1H), 4.85 (d, 1H, *J* = 7.8 Hz), 3.94 (dt, 1H, *J* = 6.8, 9.6 Hz), 3.87 (d, 1H, *J* = 10.9 Hz), 3.82-3.72 (m, 2H), 3.55 (dt, 1H, *J* = 7.1, 9.6 Hz), 2.65 (br s, 1H), 2.29 (td, 2H, *J* = 7.1, 7.1 Hz), 1.93 (dq, 2H, *J* = 7.3, 7.6 Hz), 0.87 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.0, 165.9, 165.0, 134.0, 133.7, 133.2, 133.1, 129.9, 129.74, 129.73, 129.4, 128.9, 128.6, 128.5, 128.3, 123.9, 101.3, 74.6, 72.8, 71.9, 69.9, 69.6, 61.4, 27.6, 20.5, 14.1; *Anal.* Calcd for $C_{33}H_{34}O_9$: C, 68.82; H, 6.00. Found: C, 68.98; H, 5.96.

(*Z*)-3-Hexenyl 2,3,4,2',3',4'-*O*-hexabenzoyl-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (11)

A 50-mL three-necked round-bottom flask covered with aluminum foil, was charged with **7** (0.29 g, 0.50 mmol), 2,3,4-*tri*-*O*-benzoyl-α-D-xylopyranosyl bromide[7] (**8**) (0.52 g, 1.0 mmol), tetramethylurea (0.17 g, 1.5 mmol) and CH_2Cl_2 (2.0 mL). To this solution was added AgOTf (0.26 g, 1.0 mmol) at 0 °C under argon atmosphere and the mixture was stirred for 24 h at rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel (20 g, *n*-hexane/AcOEt (4:1-5:2)) to afford **11**, which was recrystallized from acetone/*n*-hexane to give a pure **11** (0.35 g, 69%) as a colorless solid. **11**: mp 202.8-203.5 °C; $[\alpha]_D^{27}$ –29.7° (c= 0.59, $CHCl_3$); IR (KBr) 1730, 1261, 1177, 1101, 1029, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.01-7.97 (m, 6H), 7.94-7.88 (m, 4H), 7.79-7.76 (m, 2H), 7.55-7.47 (m, 5H), 7.43-7.31 (m, 11H), 7.28-7.23 (m, 2H), 5.83 (t, 1H, *J* = 9.8 Hz), 5.74 (t, 1H, *J* = 7.0 Hz), 5.45-5.36 (m, 3H), 5.27-5.16 (m, 2H), 5.12-5.06 (m, 1H), 4.92 (d, 1H, *J* = 5.3 Hz), 4.70 (d, 1H, *J* = 7.8 Hz), 4.40 (dd, 1H, *J* = 4.3, 12.4 Hz), 4.07-4.98 (m, 2H), 3.82 (dd, 1H, *J* = 6.6, 11.1 Hz), 3.73-3.65 (m, 2H), 3.28 (dt, 1H, *J* = 6.8, 9.4 Hz), 2.10 (td, 2H, *J* = 6.8, 6.8 Hz), 1.87 (dq, 2H, *J* = 7.3, 7.6 Hz), 0.84 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.8, 165.5, 165.34, 165.27, 165.01, 164.98, 133.7, 133.44, 133.38, 133.3, 133.2, 133.13, 133.05, 129.86, 129.73, 129.5, 129.3, 129.2, 129.1, 128.84, 128.79, 128.5, 128.41, 128.38, 128.3, 128.24, 128.22, 124.2, 101.1, 100.3, 73.9, 72.9, 71.9, 70.1, 70.0, 69.70, 69.68, 69.0, 67.9, 61.0, 27.4, 20.5, 14.1; *Anal*. Calcd for $C_{59}H_{54}O_{16}$: C, 69.29; H, 5.38. Found: C, 69.54; H, 5.34.

(*Z*)-3-Hexenyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (2)

Compound (**11**) (0.15 g, 0.15 mmol) was dissolved in 2 mL of methanol/THF (1:1) and 25% NaOMe in MeOH (0.035 g, 0.17 mmol) was added. The solution was stirred for 30 min. A small amount of ion exchange resin (Amberlyst 15, H^+-form) was added to remove sodium ions. The reaction mixture was diluted with methanol and resin was filtered off and washed thoroughly. The filtrate was evaporated with silica gel (0.50 g) to dryness and the residue was purified by flash column chromatography on silica gel (8 g, CH_2Cl_2/MeOH (5:1)) to afford **2** (0.049 g, 85 %) as a colorless amorphous solid. **2**: mp 74.0-75.0 °C; $[\alpha]_D^{26}$ –56.8° (c= 0.90, MeOH){lit. $[\alpha]_D$ –56.7° (c= 0.90, MeOH)[2a] }; IR (KBr) 3386, 2878, 1370, 1167, 1042 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz) δ: 5.49-5.35 (m, 2H), 4.31 (d, *J* = 7.3 Hz, 1H), 4.26 (d, 1H, *J* = 7.8 Hz), 4.08 (dd, 1H, *J* = 2.0, 11.4 Hz), 3.86 (dd, 1H, *J* = 5.6, 11.6 Hz), 3.84 (td, 1H, *J* = 7.1, 9.4 Hz), 3.74 (dd, 1H, *J* = 5.5, 11.4 Hz), 3.54 (td, 1H, *J* = 7.3, 9.4 Hz), 3.48 (ddd, 1H, *J* = 5.3, 8.6, 10.1 Hz), 3.44-3.40 (m, 1H), 3.35-3.28 (m, 3H), 3.22-3.15 (m, 3H), 2.38 (td, 2H, *J* = 6.8, 6.8 Hz), 2.08 (dq, 2H, *J* = 7.6, 7.6 Hz), 0.97 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR (methanol-d_4, 100 MHz) δ: 134.5 (C-4), 125.9 (C-3), 105.5 (Xyl-C1), 104.4 (Glc-C1), 78.0 (Glc-C3), 77.7 (Xyl-C3), 77.0 (Glc-C5), 75.0 (Xyl-C2), 74.9

(Glc-C2), 71.4 (Glc-C4), 71.2 (Xyl-C4), 70.6 (C1), 69.7 (Glc-C6), 66.9 (Xyl-C5), 28.8 (C-2), 21.5 (C-5), 14.6 (C-6); HR FAB-MS *m/z*: Calcd for $C_{17}H_{30}O_{10}$: 395.1917 $(M+1)^+$. Found: 395.1905.

(*Z*)-3-Hexenyl 2,3,4,2',3',4'-*O*-hexabenzoyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (12)

A 50-mL three-necked round-bottom flask covered with aluminum foil, was charged with **7** (0.57 g, 1.0 mmol), 2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl bromide[8] (**9**) (1.04 g, 2.0 mmol), tetramethylurea (0.34 g, 3.0 mmol) and CH_2Cl_2 (4.0 mL). To this solution was added AgOTf (0.52 g, 2.0 mmol) at 0 °C under argon atmosphere and the mixture was stirred for 24 h at rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel (80 g, *n*-hexane/AcOEt (3:1-2:1)) to afford **12** (0.79 g, 78 %), which was recrystallized from ether to give a highly pure **12** (0.56 g, 55%) as a colorless solid. **12**: mp 136.8-138.5 °C; $[\alpha]_D^{27}$ +67.4° (c= 0.59, $CHCl_3$); (KBr) 1731, 1452, 1261, 1095, 1029, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.01 (d, 2H, *J* = 7.3 Hz), 7.95-7.87 (m, 6H), 7.78-7.76 (m, 2H), 7.59-7.54 (m, 1H), 7.53-7.46 (m, 4H), 7.43-7.34 (m, 11H), 7.28-7.24 (m, 2H), 5.81 (t, 1H, *J* = 9.8 Hz), 5.72 (dd, 1H, *J* = 6.1, 8.4 Hz), 5.67-5.64 (m, 1H), 5.60 (dd, 1H, *J* = 3.5, 8.6 Hz), 5.39 (dd, 1H, *J* = 2.3, 9.8 Hz), 5.36 (dd, 1H, *J* = 4.0, 9.9 Hz), 5.19-5.13 (m, 1H), 5.08-5.01 (m, 1H), 4.84 (d, 1H, *J* = 6.1 Hz), 4.67 (d, 1H, *J* = 7.8 Hz), 4.26 (dd, 1H, *J* = 4.3, 12.9 Hz), 4.08 (dd, 1H, *J* = 1.6, 11.4 Hz), 4.04-3.98 (m, 1H), 3.86 (dd, 1H, *J* = 2.2, 12.6 Hz), 3.82 (dd, 1H, *J* = 7.3, 11.1 Hz), 3.61 (td, 1H, *J* = 6.6, 9.6 Hz), 3.22 (td, 1H, *J* = 6.0, 9.6 Hz), 2.04 (td, 2H, *J* = 6.3, 6.3 Hz), 1.85 (dq, 2H, *J* = 7.3, 7.6 Hz), 0.83 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.7, 165.6, 165.5, 165.3, 165.1, 165.0, 133.6, 133.43, 133.35, 133.32, 133.1, 133.0, 129.85, 129.82, 129.78, 129.7, 129.43, 129.35, 129.3, 129.1, 128.8, 128.7, 128.44, 128.38, 128.21, 124.3, 101.0, 100.8, 73.9, 72.9, 71.9, 70.3, 69.8, 69.7, 69.6, 68.3, 68.2, 62.3, 27.3, 20.5, 14.1 ; *Anal*. Calcd for $C_{59}H_{54}O_{16}$: C, 69.36; H, 5.34. Found: C, 69.54; H, 5.34.

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

Compound (**12**) (0.30 g, 0.29 mmol) was dissolved in 4 mL of methanol/THF (1:1) and 25% NaOMe in MeOH (0.072 g, 0.35 mmol) was added. The solution was stirred for 1 h. A small amount of ion exchange resin (Amberlyst 15, H^+-form) was added to remove sodium ions. The reaction mixture was diluted with methanol and resin was filtered off and washed thoroughly. The filtrate was evaporated with silica gel (1.0 g) to dryness and the residue was purified by flash column chromatography on silica gel (8 g, CH_2Cl_2/MeOH (5:1)) to afford **3** (0.096 g, 83 %) as a colorless amorphous solid. **3**: mp 69.0-71.0 °C; $[\alpha]_D^{23}$ –31.0° (c= 0.74, MeOH); IR (KBr) 3415, 2878, 1370, 1257, 1080, 781, 644 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz) δ: 5.49-5.35 (m, 2H), 4.31 (d, *J* = 6.8 Hz, 1H), 4.26 (d, 1H, *J* = 7.8 Hz), 4.08 (dd, 1H, *J* = 2.3, 11.4 Hz), 3.88-3.83 (m, 2H), 3.81-3.78 (m, 1H), 3.73 (dd, 1H, *J* =

5.6, 11.4 Hz), 3.59 (dd, 1H, *J* = 6.8, 8.8 Hz), 3.55-3.50 (m, 3H), 3.45-3.41 (m, 1H), 3.36-3.30 (m, 2H), 3.19-3.15 (m, 1H) 2.37 (td, 2H, *J* = 7.1, 7.1 Hz), 2.07 (dq, 2H, *J* = 7.6, 7.6 Hz), 0.97 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR (methanol-d_4, 100 MHz) δ: 134.5 (C-4), 125.9 (C-3), 105.1 (Ara-C1), 104.4 (Glc-C1), 77.9 (Glc-C3), 76.9 (Glc-C5), 75.0 (Glc-C2), 74.2 (Ara-C3), 72.4 (Ara-C2), 71.6 (Glc-C4), 70.6 (C-1), 69.5 (Glc-C6 and Ara-C4), 66.7 (Ara-C5), 28.8 (C-2), 21.6 (C-5), 14.6 (C-6); HR FAB-MS *m/z*: Calcd for $C_{17}H_{30}O_{10}$: 395.1917 $(M+1)^+$. Found: 395.1912.

(*Z*)-3-Hexenyl 2,3,4,2',3',4'-*O*-hexabenzoyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (13)

A 50-mL three-necked round-bottom flask covered with aluminum foil, was charged with **7** (0.57 g, 1.0 mmol), 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide[9] (**10**) (1.08 g, 2.0 mmol), tetramethylurea (0.35 g, 3.0 mmol) and CH_2Cl_2 (4.0 mL). To this solution was added AgOTf (0.51 g, 2.0 mmol) at 0 °C under argon atmosphere and the mixture was stirred for 12 h at rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel (80 g, *n*-hexane/AcOEt (3:1-2:1)) to afford **13** (0.90 g, 87%), which was recrystallized from acetone/*n*-hexane to give a pure **13** (0.58 g, 56%) as a colorless solid. **13**: mp 175.0-176.0 °C; $[\alpha]_D^{27}$ +54.8° (c= 0.59, $CHCl_3$); IR (KBr) 1732, 1263, 1100, 1028, 708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.08-8.05 (m, 2H), 7.99-7.93 (m, 6H), 7.85-7.82 (m, 2H), 7.82-7.79 (m, 2H), 7.62-7.58 (m, 1H), 7.54-7.35 (m, 13H), 7.31-7.23 (m, 4H), 5.90 (t, 1H, *J* = 9.6 Hz), 5.77 (dd, 1H, *J* = 3.3, 10.1 Hz), 5.70 (dd, 1H, *J* = 1.8, 3.3 Hz), 5.63 (t, 1H, *J* = 10.1 Hz), 5.50 (dd, 1H, *J* = 7.8, 9.8 Hz), 5.45 (t, 1H, *J* = 9.8 Hz), 5.19-5.16 (m, 3H), 4.87 (d, 1H, *J* = 7.8 Hz), 4.16-4.10 (m, 2H), 3.98 (td, 1H, *J* = 6.6, 9.4 Hz), 3.94-3.91 (m, 2H), 3.57 (td, 1H, *J* = 7.1, 9.4 Hz), 2.26 (td, 2H, *J* = 6.8, 6.8 Hz), 1.91-1.83 (m, 2H), 1.27 (d, 3H, *J* = 6.3 Hz), 0.81 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.8, 165.7, 165.40, 165.37, 165.1, 133.7, 133.5, 133.4, 133.3, 133.2, 133.1, 133.0, 129.9, 129.8, 129.71, 129.66, 129.5, 129.4, 129.3, 129.2, 128.9, 128.7, 128.53, 128.47, 128.4, 128.3, 128.2, 124.2, 101.2, 98.3, 74.5, 72.9, 71.9, 71.8, 70.5, 70.1, 69.8, 67.2, 66.9, 27.5, 20.5, 17.6, 14.1; *Anal*. Calcd for $C_{60}H_{56}O_{16}$: 68.76; H, 5.46. Found: C, 68.71; H, 5.51.

(*Z*)-3-Hexenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (4)

Compound (**13**) (0.30 g, 0.29 mmol) was dissolved in 4 mL of methanol/THF (1:1) and 25% NaOMe in MeOH (0.072 g, 0.35 mmol) was added. The solution was stirred for 1 h. A small amount of ion exchange resin (Amberlyst 15, H^+-form) was added to remove sodium ions. The reaction mixture was diluted with methanol and resin was filtered off and washed thoroughly. The filtrate was evaporated with silica gel (1.0 g) to dryness and the residue was purified by flash column chromatography on silica gel (8 g, CH_2Cl_2/MeOH (5:1)) to afford **4** (0.114 g, 96 %) as a colorless amorphous solid. **4**: mp

74.5-76.0 °C; $[\alpha]_D^{25}$ –53.9° (c= 0.63, MeOH){lit. $[\alpha]_D^{20}$ –48.26° (c= 0.50, MeOH) [1f] }; IR (KBr) 3373, 2931, 1372, 1049, 614 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz) δ: 5.48-5.35 (m, 2H), 4.74 (d, 1H, *J* = 1.3 Hz), 4.25 (d, 1H, *J* = 7.8 Hz), 3.97 (dd, 1H, *J* = 1.8, 11.1 Hz), 3.84-3.78 (m, 2H), 3.70-3.63 (m, 2H), 3.61 (dd, 1H, *J* = 6.1, 11.4 Hz), 3.53 (td, 1H, *J* = 7.6, 9.4 Hz), 3.41-3.29 (m, 3H), 3.27 (t, 1H, *J* = 9.1 Hz), 2.38 (td, 2H, *J* = 6.8, 6.8 Hz), 2.08 (qd, 2H, *J* = 7.3, 7.3 Hz), 1.26 (d, 3H, *J* = 6.1 Hz), 0.97 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (methanol-d_4, 100 MHz) δ: 134.5 (C-4), 125.9 (C-3), 104.4 (Glc-C1), 102.3 (Rham-C1), 78.1 (Glc-C3), 76.8 (Glc-C5), 75.1 (Glc-C2), 74.0 (Rham-C4), 72.4 (Rham-C3), 72.2 (Rham-C2), 71.6 (Glc-C4), 70.6 (C-1), 69.8 (Rham-C5), 68.1 (Glc-C6), 28.8 (C-2), 21.5 (C-5), 18.0 (Rham-C6), 14.7 (C-6); HR FAB-MS *m/z*: Calcd for $C_{18}H_{32}O_{10}$: 409.2074 $(M+1)^+$. Found: 409.2050.

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3. a) According to Dr. T. Uchiyama's private communication, isolation and structural elucidation of natural product (**3**) from *Hippophae rhamnoides* were reported as a poster presentation at the 123th Annual Meeting of Pharmaceutical Society of Japan (N. Machida, M. Makino, T. Uchiyama, S. Kitanaka, and Y. Fujimoto, Abstract Paper (II), p. 152 (2003)). Low value of specific rotation of **3**

was presumably contributed to the contamination of a small amount of impurity, but structural elucidation was carried out by spectrospecific analysis and chemical degradation procedure. b) Spectral data of natural product (**3**): Yellow amorphous powder; $[\alpha]_D$ –3.5° (c=1, MeOH); ^{1}H NMR (methanol-d_4, 300 MHz) δ: 5.40 (m, 2H), 4.30 (d, J = 6.7 Hz, 1H), 4.26 (d, 1H, J = 7.9 Hz), 4.08 (dd, 1H, J = 2.3, 11.1 Hz), 3.83 (m, 2H), 3.72 (dd, 1H, J = 5.3, 10.6 Hz), 3.55 (m, 4H), 2.37 (br quart, 2H, J = 7.0 Hz), 2.07 (br quin, 2H, J = 8.0 Hz), 0.97 (t, 3H, J = 8.0 Hz); ^{13}C NMR (methanol-d_4, 100 MHz) δ: 133.2 (C-4), 124.6 (C-3), 103.9 (Ara-C1), 103.2 (Glc-C1), 76.8 (Glc-C3), 75.7 (Glc-C5), 73.9 (Glc-C2), 73.1 (Ara-C3), 71.2 (Ara-C2), 70.5 (Glc-C4), 69.5 (C-1), 68.4 (Glc-C6 and Ara-C4), 65.6 (Ara-C5), 27.8 (C-2), 20.6 (C-5), 13.7 (C-6); HR FAB-MS *m/z*: Calcd for $C_{17}H_{30}O_{10}$: 393.17607 $(M-H)^-$. Found: 393.17602.

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