Chemoenzymatic Synthesis of *n*-Hexyl and *O*-β-D-Xylopyranosyl-(1→6)-β-D-glucopyranosides

Masashi KISHIDA,[a,b] Miho NISHIUCHI,[a] Keisuke KATO,[a] and Hiroyuki AKITA*,[a]

[a] *School of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan: and* [b] *Tsukuba Research Institute, Novartis Pharma K.K.; 8 Ohkubo, Tsukuba, Ibaraki 300–2611, Japan.*

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Direct β-glucosidation between 1,6-octanediol (5) and D-glucose (3) using the immobilized β-glucosidase (EC 3.2.1.21) from almonds with the synthetic prepolymer ENTP-4000 gave a mono-β-glucoside (6) in 61.4% yield, which was converted into the *n*-hexyl β-D-glucopyranoside (1) by means of a chemoenzymatic method. The coupling of the *n*-hexyl β-D-glucopyranoside congener (13) and 2,3,4-tri-*O*-acetyl-β-D-xylosyl congener (14), followed by deprotection, afforded the synthetic *n*-hexyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (2), which was identical to the natural 2 with respect to the spectral data and specific rotation.

Key words β-glucosidase; β-glucosidation; lipase; natural product synthesis; *n*-hexyl β-D-glucopyranoside

The Chinese natural medicine "Si Lie Hong Jing Tian" prepared from the underground part of *Rhodiola* (*R.*) *quadrifida* (PALL.) FISCH. *et* MEY., has been prescribed for hemostatic, antibechic, and tonic purposes in traditional Chinese preparations and used as an endermic liniment for burns and contusions. *n*-Hexyl β-D-glucopyranoside (**1**) was isolated as one of chemical constituents of *R. quadrifida* by Yoshikawa *et al.*[1] and reported to increase blood pressure.[2] Meanwhile, *n*-hexyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**) was isolated from the dried roots of *Rehmannia glutinosa* LIBOSH. var. *purpurea* MAKINO.[3] In this paper, we describe the synthesis of *n*-hexyl β-D-glucopyranoside (**1**) and its natural congener (**2**) based on the selective β-glycosidation between the nonprotected D-glucose (**3**) and primary alcohols such as *n*-hexanol (**4**) and 1,6-hexanediol (**5**) catalyzed by the immobilized β-glucosidase (EC 3.2.1.21) from almonds. Retrosynthetically, the synthesis of **2** can be achieved by the coupling reaction of the protected *n*-hexyl β-D-glucopyranoside congener and the protected β-D-xylosyl congener.

Enzymatic β-Glycosidation In case of the direct β-glycosidation between D-glucose (**3**) 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.[4]

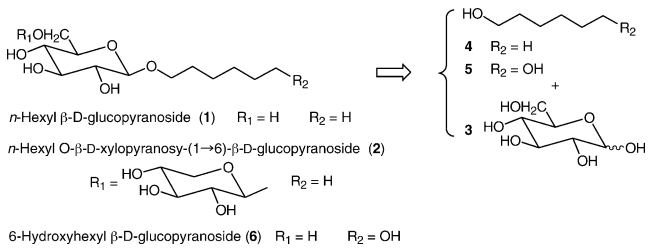


Chart 1

Enzymatic synthesis of *n*-hexyl β-D-glucopyranoside (**1**)[5] and 6-hydroxyhexyl-β-D-glucopyranoside (**6**)[6] using 4-nitrophenyl β-D-glucopyranoside as a glycosyl donor was reported previously by us, while direct β-glycosidation using a large amount of 1,6-hexanediol is reported to give a 6-hydroxyhexyl-β-D-glucopyranoside (**6**) in 61% yield.[4] Those reports are promising for the improved synthesis of *n*-hexyl β-D-glucopyranoside (**1**) and its application to the total synthesis of the natural congener (**2**). 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 (**3**) and 1,8-octanediol (**5**).[7] Then we examined the direct β-glucosidation between D-glucose (**3**) and 1-hexanol (**4**) or/and 1,6-hexanediol (**5**). Immobilization of β-D-glucosidase from almonds on the photocross-linkable resin prepolymer (ENTP-4000) was carried out following the reported procedure.[7]

When a large amount of 1-hexanol (**4**, 23.4 eq) was used as an acceptor for D-glucose (**3**) in the presence of β-glucosidase or the immobilized β-glucosidase, a low yield of *n*-hexyl β-D-glucopyranoside (**1**) (entry 1 in Table 1; 13.5% yield, entry 2 in Table 1; 9.4% yield) was obtained. When the same β-glucosidation was carried out using the recovered immobilized enzyme, the yield of **1** was almost the same as in entry 2 in Table 1 (entry 3 in Table 1). When a large amount of 1,6-hexanediol (**5**; 25.0 eq) was employed for β-glucosidation using the native enzyme and the immobilized enzyme, the yield of **6** was fairly high (entry 4 in Table 1; 67.8% yield, entry 5 in Table 1; 61.4% yield). The recovered enzyme was also found to be effective (entry 6 in Table 1; 47.9% yield).

Synthesis of *n*-Hexyl β-D-Glucopyranoside (1) and *n*-Hexyl *O*-β-D-Xylopyranosyl-(1→6)-β-D-glucopyranoside (2) As the yield of *n*-hexyl β-D-glucopyranoside (**1**) from

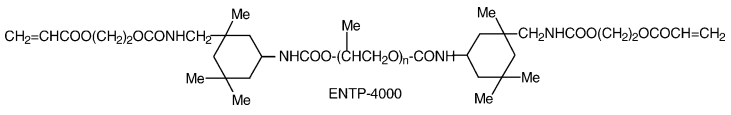
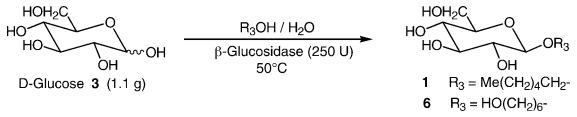


Fig. 1

* To whom correspondence should be addressed. e-mail: akita@phar.toho-u.ac.jp

Table 1



Entry	R_3OH (g)	β-Glucosidase	Time (d)	Product (**1** or **6**), yield (%)
1	$Me(CH_2)_5OH$ **4** (14.6)	Native	4	**1** (13.5)
2	$Me(CH_2)_5OH$ **4** (14.6)	A	4	**1** (9.4)
3[a]	$Me(CH_2)_5OH$ **4** (14.6)	Recovered A	4	**1** (9.0)
4	$HO(CH_2)_6OH$ **5** (18.0)	Native	6	**6** (67.8)
5	$HO(CH_2)_6OH$ **5** (18.0)	A	6	**6** (61.4)
6[b]	$HO(CH_2)_6OH$ **5** (18.0)	Recovered A	6	**6** (47.9)

A immobilized β-glucosidase with ENTP-4000. *a*) The same immobilized enzyme in entry 2 was employed again after filtration. *b*) The same immobilized enzyme in entry 5 was employed again after filtration.

direct β-glucosidation was low, conversion of **6** into the desired β-glucoside (**1**) was carried out in five steps, including the chemoenzymatic method. Acetylation of **6** gave quantitatively a pentaacetate (**7**), which was treated with the lipase Amano P from *Pseudomonas* sp. to provide a monoalcohol (**8**) in 80% yield along with the starting material (**7**). In this enzymatic hydrolysis, the terminal acetyl group in the side chain was selectively hydrolyzed and other acetyl groups in the sugar moiety were found to be intact. Treatment of **8** with iodine (I_2) in the presence of triphenylphosphine (Ph_3P) gave quantitatively the corresponding iodide (**9**), which was subjected to reduction with $NaBH_4$ to give a tetraacetate (**10**) in 88% yield. Finally, treatment of **10** with K_2CO_3 in MeOH provided the desired β-glucoside (**1**) in 87% yield. Consequently, the overall yield (41.6% yield) of **1** from D-glucose (**3**) *via* six steps is considerably improved in comparison to that (8.8—13.5% yield) with the direct β-glucosidation of 1-hexanol (**4**).

Tritylation of **1** gave a trityl ether (**11**; 79% yield) along with the starting material (**1**; 20% recovery). Benzoylation of **11** afforded a benzoate (**12**) in 97% yield, which was subjected to hydrogenolysis in the presence of 20% $Pd(OH)_2$-C to provide the desired **13** in 97% yield. On the other hand, methylthio 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**14**) was synthesized by applying the reported method[8] based on the reaction of (methylthio)trimethylsilane and tetra-*O*-acetyl-β-D-xylopyranoside obtained by acetylation of D-xylose. By following the reported procedure,[9] the coupling reaction of *n*-hexyl β-D-glucopyranoside congener (**13**) and methylthio 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**14**) in the presence of silver triflate (AgOTf) and phenylselenochloride (PhSeCl) gave the coupled product (**15**) in 69% yield. Finally, treatment of **15** with K_2CO_3 in MeOH provided quantitatively the synthetic *n*-hexyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**). The spectral data (^{1}H-, ^{13}C-NMR) and specific rotation ($[\alpha]_D$ −50.0 (MeOH)) of the synthetic (**2**) were identical with those (^{1}H-, ^{13}C-NMR and $[\alpha]_D$ −48.0 (MeOH)) of the natural product **2**.[3]

Conclusion

In conclusion, direct β-glucosidation between 1,6-hexanediol (**5**) and D-glucose (**3**) using the immobilized β-glucosidase from almonds with the synthetic prepolymer ENTP-4000 gave a mono-β-glucoside (**6**) in 61.4% yield, which was converted into the *n*-hexyl β-D-glucopyranoside (**1**) using the chemoenzymatic method. The coupling of the *n*-hexyl β-D-glucopyranoside congener (**13**) and methylthio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**14**), followed by deprotection, afforded the synthetic *n*-hexyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**2**), which was consistent with the natural **2** with respect to the spectral data and specific rotation.

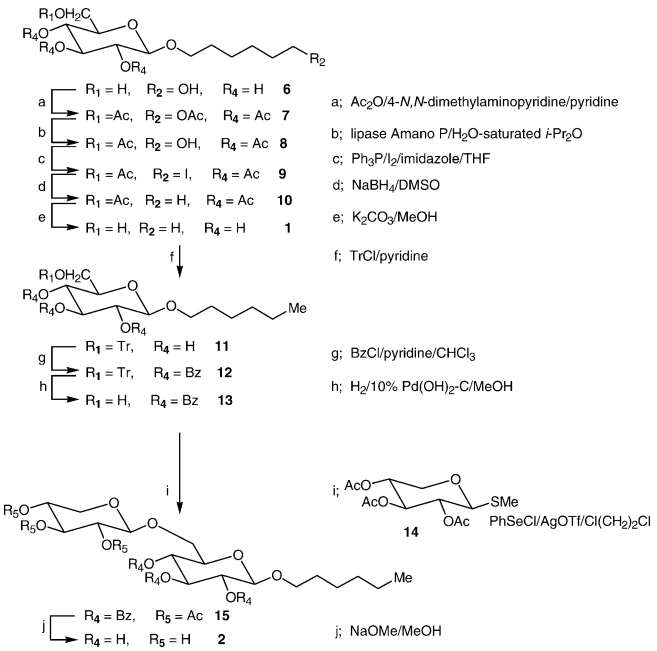


Chart 2

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 mass spectra were obtained with a JEOL JMS-AX 500 (matrix; glycerol) 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 Transglycosylation. Synthesis of *n*-Hexyl β-D-Glucopyranoside (1) 1) Entry 1: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1-hexanol (14.6 g, 143.1 mmol), water (2 ml) and β-glucosidase 100 mg (250 units) was incubated for 4 d at 50 °C. The reaction mixture was directly chromatographed on silica gel (35 g) to give 1-hexanol (12.0 g, 82.1% recovery) from the $CHCl_3$ eluent and β-glucoside (**1**, 218.7 mg, 13.5% yield) as colorless crystals from the $CHCl_3$/MeOH=10 : 1 eluent. **1**: mp 88—89 °C; $[\alpha]_D^{27}$ −34.9° (*c*=0.5, H_2O); IR (KBr): 3410, 2927, 1075, 1030 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 4.93 (1H, d, *J*=4.9 Hz), 4.90 (2H, dd, *J*=4.9, 12.7 Hz), 4.46 (1H, t, *J*=5.9 Hz), 4.09 (1H, d, *J*=7.8 Hz), 3.75 (1H, sixtet, *J*=6.8 Hz), 3.66 (1H, dd, *J*=5.9, 11.7 Hz), 0.86 (3H, t, *J*=6.8 Hz); ^{13}C-NMR (MeOH-d_4): δ 104.4, 78.1, 77.9, 75.1, 71.7, 70.9, 62.8, 32.9, 30.8, 26.8, 23.7, 14.4; FAB-MS *m/z*: 265 $(M+1)^+$; *Anal.* Found: C, 54.28; H, 9.25. Calcd for $C_{12}H_{24}O_6$: C, 54.53; H, 9.15%.

2) Entry 2: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1-hexanol (14.6 g, 143.1 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 1-hexanol (10.4 g, 71.2% recovery) from the $CHCl_3$ eluent and β-glucoside (**1**, 152 mg, 9.4% yield) as colorless crystals from the HCl_3/MeOH=10 : 1 eluent.

3) Entry 3: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1-hexanol (14.6 g, 143.1 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 1-hexanol (11.0 g, 75.3% recovery) from the $CHCl_3$ eluent and β-glucoside (**1**, 145.7 mg, 9.0% yield) as colorless crystals from the $CHCl_3$/MeOH=10 : 1 eluent.

Synthesis of 6-Hydroxyhexyl β-D-Glucopyranoside (6) 4) Entry 4: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1,6-hexanediol (18.0 g, 152.5 mmol), and β-glucosidase 100 mg (250 unit) was incubated for 6 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (150 g) to give 1,6-octanediol (17.0 g, 94.4% recovery) from the $CHCl_3$/MeOH=20 : 1 eluent and β-glucoside (**6**, 1.16 g, 67.8% yield) as colorless crystals from the $CHCl_3$/MeOH=9 : 1 eluent. **6**: mp 109—111 °C; $[\alpha]_D^{28}$ −32.5° (*c*=0.46, MeOH); IR (KBr): 3374, 2934, 2864, 1079, 1024 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 4.92 (1H, d, *J*=4.9 Hz), 4.88 (2H, dd, *J*=4.9, 11.7 Hz), 4.45 (1H, t, *J*=5.9 Hz), 4.33 (1H, t, *J*=4.6 Hz), 4.09 (1H, d, *J*=7.8 Hz), 3.75 (1H, q, *J*=6.8 Hz), 3.66 (1H, dq, *J*=1.6, 5.9 Hz); ^{13}C-NMR (D_2O, acetone): δ 103.0, 76.7, 76.7, 74.0, 71.4, 70.5, 62.6, 61.6, 32.0, 29.5, 25.7, 25.6; FAB-MS *m/z*: 281 $(M+1)^+$; *Anal.* Found: C, 51.04; H, 9.01. Calcd for $C_{12}H_{24}O_7$: C, 51.42; H, 8.63%.

5) Entry 5: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1,6-hexanediol (18.0 g, 152.5 mmol), water (2 ml), and the immobilized β-glucosidase was incubated for 6 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give 1,6-hexadiol (17.0 g, 94.4% recovery) from the $CHCl_3$/MeOH=20 : 1 eluent and β-glucoside (**6**, 1.05 g, 61.4% yield) as colorless crystals from the $CHCl_3$/MeOH=9 : 1 eluent.

6) Entry 6: A mixture of D-glucose **3** (1.1 g, 6.1 mmol), 1,6-hexanediol (18.0 g, 152.51 mmol), water (2 ml), and the recovered immobilized β-glucosidase was incubated for 6 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly chromatographed on silica gel (35 g) to give 1,6-hexadiol (17.1 g, 95.0% recovery) from the $CHCl_3$/MeOH=20 : 1 eluent and β-glucoside (**6**, 820 mg, 47.9% yield) as colorless crystals from the $CHCl_3$/MeOH=9 : 1 eluent.

Conversion of 6 into *n*-Hexyl β-D-Glucopyranoside (1) 1) A mixture of **6** (300 mg, 1.07 mmol), Ac_2O (874 mg, 8.5 mmol), and 4-*N*,*N*-dimethylaminopyridine (DMAP; 10 mg, 0.08 mmol) in pyridine (1 ml, 12.4 mmol) was stirred for 1 h at room temperature. The reaction mixture was 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 (15 g, *n*-hexane/AcOEt (2 : 1)) to afford **7** (525 mg, quantitative yield) as a colorless syrup. **7**: $[\alpha]_D^{23}$ −16.6. (*c*=0.62, $CHCl_3$); IR (KBr): 1753 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 5.21 (1H, t, *J*=9.4 Hz), 5.09 (1H, t, *J*=9.4 Hz), 4.98 (1H, dd, *J*=8.0, 9.4 Hz), 4.50 (1H, d, *J*=8 Hz), 4.27 (1H, dd, *J*=4.8, 12.2 Hz), 4.14 (1H, dd, *J*=2.4, 12.2 Hz), 4.05 (2H, t, *J*=6.8 Hz), 3.87 (1H, dt, *J*=6.4, 9.8 Hz), 3.71—3.67 (1H, m), 3.48 (1H, dt, *J*=6.4, 9.8 Hz), 2.09 (3H, s), 2.04 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 2.01 (3H, s), 1.65—1.55 (4H, m), 1.40—1.30 (4H, m); ^{13}C-NMR ($CDCl_3$): δ 171.2 (s), 170.7 (s), 170.4 (s), 169.5 (s), 169.3 (s), 100.8 (d), 72.9 (d), 71.8 (d), 71.4 (d), 70.0 (t), 68.5 (d), 64.4 (t), 62.0 (t), 29.3 (t), 28.6 (t), 25.6 (t), 25.5 (t), 21.0 (q), 20.8 (q), 20.7 (q), 20.6 (q), 20.6 (q); FAB-MS *m/z*: 513 $(M+Na)^+$, 491 $(M+H)^+$.

2) A suspension of **7** (504 mg, 1.03 mmol), lipase Amano P (504 mg) in water-saturated *i*-Pr_2O (50 ml) was incubated for 47 h at 33 °C. The reaction mixture was filtered with the aid of celite and the filtrate was evaporated to give a residue. It was chromatographed on silica gel (20 g) to afford **7** (96 mg, 19% recovery) from the *n*-hexane/AcOEt (2 : 1) eluent and **8** (369 mg, 80% yield) as a colorless syrup from the *n*-hexane/AcOEt (2 : 1) eluent. **8**: $[\alpha]_D^{25}$ −18.1° (*c*=0.43, $CHCl_3$); IR (KBr): 3561, 1753 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 5.20 (1H, t, *J*=9.5 Hz), 5.09 (1H, t, *J*=9.5 Hz), 4.98 (1H, dd, *J*=7.8, 9.5 Hz), 4.49 (1H, d, *J*=7.8 Hz), 4.26 (1H, dd, *J*=4.8, 12.3 Hz), 4.14 (1H, dd, *J*=2.3, 12.3 Hz), 3.87 (1H, dt, *J*=6.6, 9.5 Hz), 3.69 (1H, ddd, *J*=2.3, 4.8, 12.3 Hz), 3.63 (2H, t, *J*=6.6 Hz), 3.49 (1H, dt, *J*=6.6, 9.5 Hz), 2.09 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 2.00 (3H, s), 1.59—1.54 (4H, m), 1.38—1.35 (4H, m); ^{13}C-NMR ($CDCl_3$): δ 170.7 (s), 170.4 (s), 169.4 (s), 169.3 (s), 100.8 (d), 72.9 (d), 71.8 (d), 71.4 (d), 70.0 (t), 68.5 (d), 62.7 (t), 62.0 (t), 32.6 (t), 29.3 (t), 25.6 (t), 25.4 (t), 20.7 (q), 20.6 (q), 20.6 (q), 20.6 (q); FAB-MS *m/z*: 449 $(M+1)^+$. *Anal.* Found: C, 53.07; H, 7.27. Calcd for $C_{20}H_{32}O_{11}$: C, 53.56; H, 7.19%.

3) To a solution of **8** (540 mg, 1.21 mmol), Ph_3P (948 mg, 3.62 mmol), and imidazole (246 mg, 3.62 mmol) in THF (10 ml) was added a solution of I_2 (810 mg, 3.19 mmol) in THF (2 ml). The whole mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a residue. It was chromatographed on silica gel (15 g, *n*-hexane/AcOEt (3 : 1)) to afford **9** (599 mg, 89% yield) as a colorless amorphous solid. **9**: mp 96—98 °C; $[\alpha]_D^{26}$ −16.3° (*c*=0.41, $CHCl_3$); IR (KBr): 1752 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 5.20 (1H, t, *J*=9.6 Hz), 5.09 (1H, t, *J*=9.6 Hz), 4.98 (1H, dd, *J*=8.0, 9.6 Hz), 4.49 (1H, d, *J*=8.0 Hz), 4.27 (1H, dd, *J*=4.4, 12.4 Hz), 4.15 (1H, dd, *J*=2.4, 12.4 Hz), 3.87 (1H, dt, *J*=6.5, 9.8 Hz), 3.68 (1H, ddd, *J*=2.4, 4.4, 12.4 Hz), 3.49 (1H, dt, *J*=6.7, 9.8 Hz), 3.18 (2H, t, *J*=6.8 Hz), 2.09 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 1.85—1.78 (2H, m), 1.61—1.56 (2H, m), 1.38—1.35 (4H, m); ^{13}C-NMR ($CDCl_3$): δ 170.5 (s), 170.2 (s), 169.3 (s), 169.1 (s), 100.8 (d), 72.9 (d), 71.8 (d), 71.3 (d), 69.9 (t), 68.5 (d), 62.0 (t), 33.4 (t), 30.1 (t), 29.2 (t), 24.8 (t), 20.8 (q), 20.7 (q), 20.7 (q), 20.6 (w), 20.6 (q), 6.9 (t); FAB-MS *m/z*: 597 $(M+K)^+$.

4) A mixture of **9** (750 mg, 1.34 mmol) and $NaBH_4$ (102 mg, 2.68 mmol) in DMSO (15 ml) was stirred for 40 min at room temperature. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a residue. It was chromatographed on silica gel (10 g, *n*-hexane/AcOEt (1 : 1)) to afford **10** (574 mg, 99% yield) as colorless needles. **10**: mp 51—53 °C (*n*-hexane/AcOEt); $[\alpha]_D^{27}$ −20.2° (*c*=0.41, $CHCl_3$); IR (KBr): 1746 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 5.20 (1H, t, *J*=9.6 Hz), 5.08 (1H, t, *J*=9.6 Hz), 4.98 (1H, dd, *J*=8.0, 9.6 Hz), 4.49 (1H, d, *J*=8.0 Hz), 4.26 (1H, dd, *J*=4.4, 12.2 Hz), 4.14 (1H, dd, *J*=2.4, 12.2 Hz), 3.87 (1H, dt, *J*=6.4, 9.6 Hz), 3.69 (1H, ddd, *J*=2.4, 4.4, 12.2 Hz), 3.47 (1H, dt, *J*=6.8, 9.6 Hz), 2.08 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 2.00 (3H, s), 1.60—1.52 (2H, m), 1.34—1.24 (6H, m), 0.88 (3H, t, *J*=6.8 Hz); ^{13}C-NMR ($CDCl_3$): δ 170.7 (s), 170.3 (s), 169.4 (s), 169.3 (s), 100.9 (d), 72.9 (d), 71.8 (d), 71.4 (d), 70.2 (t), 68.5 (d), 62.1 (t), 31.5 (t), 29.4 (t), 25.5 (t), 22.6 (t), 20.7 (q), 20.6 (q), 20.6 (q), 20.6 (d), 14.0 (q); FAB-MS *m/z*: 471 $(M+K)^+$. *Anal.* Found: C, 54.94; H, 7.47. Calcd for $C_{20}H_{32}O_{10}\cdot 1/3H_2O$: C, 54.73; H, 7.51%.

5) A mixture of **10** (400 mg, 0.93 mmol) and K_2CO_3 (128 mg, 0.93 mmol) in MeOH (10 ml) was stirred for 25 min at room temperature. The reaction mixture was evaporated to give a residue, which was chromatographed on silica gel (10 g, $CHCl_3$/MeOH (9 : 1)) to afford **1** (213 mg, 87% yield) as a colorless amorphous crystals. The ^{1}H- and ^{13}C-NMR spectra of the present **1** were identical to those of enzymatic β-glucoside **2**.

Conversion of 2 into the *n*-Hexyl β-D-Glucopyranoside Congener (13) 1) A mixture of **1** (1.02 g, 3.86 mmol) and TrCl (1.6 g, 5.73 mmol) in pyridine (3 ml, 37.2 mmol) was stirred for 12 h at room temperature. The reaction mixture was diluted with toluene (100 ml) and evaporated under reduced pressure to give a residue, which was chromatographed on silica gel (20 g) to afford **11** (1.55 g, 79% yield) as a colorless amorphous solid from the $CHCl_3$/MeOH (20 : 1) eluent and starting material **1** (204 mg, 20% recovery) from the $CHCl_3$/MeOH (5 : 1) eluent. **11**: mp 51—53 °C; $[\alpha]_D^{24}$ −46.5° (*c*=0.56, $CHCl_3$); IR (KBr): 3236 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 7.46—7.43 (6H, m), 7.31—7.21 (9H, m), 4.26 (1H, d, *J*=7.6 Hz), 3.88 (1H, dt, *J*=6,8, 9.4 Hz), 3.56—3.50 (3H, m), 3.45—3.42 (4H, m), 3.15 (3H, br s), 1.66—1.60 (2H, m), 1.37—1.26 (6H, m), 0.87 (3H, t, *J*=6.8 Hz); ^{13}C-NMR

($CDCl_3$): δ 143.7 (s, Ph-4°), 128.3 (d, Ph-3°), 127.6 (d, Ph-3°), 126.91 (d, Ph-3°), 102.3 (d), 86.9 (s), 76.8 (d), 73.7 (d), 73.5 (d), 72.0 (d), 70.0 (t), 64.3 (t), 31.5 (t), 29.6 (t), 25,6 (t), 22.5 (t),14.0 (q); FAB-MS *m/z*: 529 $(M+K)^+$. *Anal.* Found: C, 72.29; H, 7.51. Calcd for $C_{31}H_{38}O_6 \cdot 1/2H_2O$: C, 72.21; H, 7.62%.

2) A mixture of **11** (1.0 g, 1.92 mmol), BzCl (1.332 g, 9.45 mmol), and pyridine (2 ml, 25.3 mmol) in $CHCl_3$ (5 ml) was stirred for 45 min at 0 °C. The reaction mixture was diluted with $CHCl_3$ (5 ml) and 10% aqueous H_2SO_4 (20 ml) and extracted with AcOEt. The organic layer was washed with 7% aqueous $NaHCO_3$ and 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 (10 : 1)) to afford **12** (1.57 g, 97% yield) as a colorless syrup. **12**: $[\alpha]_D^{31}$ −18.6° (c=0.14, $CHCl_3$); IR (KBr): 1731 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 7.96 (2H, dd, J=1.2, 8.4 Hz), 7.82 (2H, dd, J=1.2, 8.4 Hz), 7.70 (2H, dd, J=1.2, 8.4 Hz), 7.52—7.35 (11H, m), 7.32—7.25 (4H, m), 7.18—7.08 (9H, m), 5.78 (1H, t, J=9.8 Hz), 5.68 (1H, t, J=9.8 Hz), 5.53 (1H, dd, J=7.8, 9.8 Hz), 4.79 (1H, d, J=7.8 Hz), 3.99 (1H, dt, J=6.5, 9.8 Hz), 3.85 (1H, ddd, J=2.5, 4.8, 10.5 Hz), 3.61 (1H, dt, J=6.8, 9.8 Hz), 3.34 (1H, dd, J=2.5, 10.5 Hz), 3.27 (1H, dd, J=4.8, 10.5 Hz), 1.65—1.54 (2H, m), 1.27—1.11 (6H, m), 0.76 (3H, t, J=7.0 Hz); ^{13}C-NMR ($CDCl_3$): δ 165.7, 164.9, 164.6, 143.5 (3C), 132.9 (3C), 129.7, 129.6, 129.5, 129.4, 129.1, 128.9, 128.5 (9C), 128.1, 128.1, 128.0, 127.6 (9C), 126.7 (3C), 101.2, 86.7, 73.9, 73.4, 72.2, 70.0, 69.7, 62.6, 31.6, 29.6, 25.7, 22.6, 14.1; FAB-MS *m/z*: 841 $(M+Na)^+$. *Anal.* Found: C, 76.37; H, 6.11. Calcd for $C_{52}H_{50}O_9$: C, 76.26; H, 6.15%.

3) A mixture of **12** (1.0 g, 1.22 mmol) and 20% $Pd(OH)_2$–C (500 mg) in MeOH (100 ml) was subjected to catalytic hydrogenolysis at ambient temperature 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 (20 g, *n*-hexane/AcOEt (5 : 1)) to afford **13** (688 mg, 97% yield) as a colorless syrup. **13**: IR (KBr): 3461, 1739 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.95 (4H, t, J=7.6 Hz), 7.84 (2H, d, J=7.6 Hz), 7.51 (2H, q, J=7.6, 13.6 Hz), 7.43—7.35 (5H, m), 5.93 (1H, t, J=9.4 Hz), 5.49 (1H, t, J=9.4 Hz), 4.82 (1H, d, J=8.0 Hz), 3.94 (1H, dt, J=6.8, 9.6 Hz), 3.87—3.74 (4H, m), 3.54 (1H, dd, J=6.8, 16.0 Hz), 2.58 (1H, s), 1.58—1.45 (2H, m), 1.27—1.20 (6H, m), 0.74 (3H, t, J=6.8 Hz); ^{13}C-NMR ($CDCl_3$): δ 165.8, 165.6, 164.8, 133.5, 133.0 (2C), 129.8, 129.6 (3C), 129.3 (2C), 128.7, 128.5, 128.3, 128.2 (2C), 128.1 (2C), 101.2, 74.6, 72.8, 71.9, 70.3, 69.6, 61.4, 31.5, 29.5, 25.5, 22.5, 14.0. High (FAB)-MS (matrix; *m*-nitrobenzyl alcohol) *m/z*: 577.2446. Calcd for $C_{33}H_{36}O_9$: 577.2438.

***n*-Hexyl *O*-β-D-Xylopyranosyl-(1→6)-β-D-Glucopyranoside (1)** 1) To a solution of tetra-*O*-acetyl-β-D-xylopyranoside (407 mg, 1.28 mmol) in CH_2Cl_2 (5 ml) under an argon atmosphere was added (methylthio)trimethylsilane (Me_3SiSMe; 382 mg, 3.2 mmol) and 45% $BF_3 \cdot Et_2O$ (200 μl, 0.63 ml) and the whole mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with 7% aqueous $NaHCO_3$ (10 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 (8 g, *n*-hexane/AcOEt (20 : 1)) to afford **14** (245 mg, 62% yield) as colorless needles. **14**: $[\alpha]_D^{31}$ −74.9° (c=0.9, $CHCl_3$); IR (KBr): 1747 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 5.20 (1H, t, J=8.8 Hz), 5.00 (1H, t, J=9.3 Hz), 4.97 (1H, dt, J=5.2, 9.3 Hz), 4.40 (1H, d, J=8.8 Hz), 4.21 (1H, dd, J=5.2, 11.6 Hz), 3.39 (1H, dd, J=9.3, 11.6 Hz), 2.16 (3H, s), 2.08 (3H, s), 2.05 (6H, s); ^{13}C-NMR ($CDCl_3$): δ 169.7, 169.6, 169.3, 83.3, 72.5, 69.0, 68.8, 65.9, 20.8 (3C), 11.6; FAB-MS *m/z*: 307 $(M+1)^+$. *Anal.* Found: C, 47.08; H, 5.95. Calcd for $C_{12}H_{18}O_7S$: C, 47.05; H, 5.92%.

2) To a mixture of PhSeCl (161 mg, 0.83 mmol) and 4A molecular sieves (1 g) in 1,2-dichloroethane (3 ml) was added silver triflate (AgOTf; 216 mg, 0.83 mmol) with stirring at 0 °C under an argon atmosphere. To the above-mentioned reaction mixture was added a solution of **13** (338 mg, 0.58 mmol) and **14** (350 mg, 1.14 mmol) in 1,2-dichloroethane (2 ml) and the whole mixture was stirred for 2 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 (15 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 (4 : 1)) to afford **15** (339 mg, 69% yield) as a colorless amorphous solid. **15**: $[\alpha]_D^{31}$ −27.81° (c=0.41, $CHCl_3$); IR (KBr): 1741 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 7.95 (2H, t, J=7.6 Hz), 7.92 (2H, d, J=7.6 Hz), 7.81 (2H, d, J=7.6 Hz), 7.53—7.48 (2H, m), 7.42—7.35 (5H, m), 7.28—7.24 (2H, m), 5.86 (1H, t, J=9.6 Hz), 5.46 (1H, t, J=8.8 Hz), 5.42 (1H, t, J=9.2 Hz), 5.14 (1H, t, J=8.8 Hz), 4.94—4.87 (2H, m), 4.77 (1H, d, J=8.0 Hz), 4.60 (1H, d, J=6.8 Hz), 4.08 (1H, dd, J=5.0, 11.6 Hz), 4.00—3.91 (3H, m), 3.77 (1H, dd, J=7.4, 11.6 Hz), 3.53 (1H, dt, J=6.8, 9.4 Hz), 3.33 (1H, dd, J=8.8, 11.6 Hz), 2.07 (3H, s), 2.05 (3H, s), 2.03 (3H, s), 1.60—1.46 (2H, m), 1.27—1.05 (6H, m), 0.74 (3H, t, J=6.8 Hz); ^{13}C-NMR ($CDCl_3$): δ 169.6, 169.4, 168.9, 165.4, 164.9, 164.7, 133.2, 132.9, 132.8, 129.5 (2C), 129.4 (2C), 129.1, 128.6, 128.5, 128.1 (2C), 128.0 (2C), 100.8, 100.4, 73.7, 72.8, 71.7, 71.1, 70.4, 70.0, 69.6, 68.6, 67.8, 61.8, 31.3, 29.3, 25.4, 22.4, 20.7 (3C), 13.9; FAB-MS *m/z*: 857 $(M+Na)^+$. *Anal.* Found: C, 61.91; H, 6.17. Calcd for $C_{44}H_{50}O_{16} \cdot H_2O$: C, 61.96; H, 6.15%.

3) A mixture of **15** (88 mg, 0.10 mmol) and NaOMe (11 mg, 0.20 mmol) in MeOH (3 ml) was stirred for 1 h at room temperature. The reaction mixture was evaporated to give a residue, which was chromatographed on silica gel (8 g, $CHCl_3$/MeOH (7 : 1)) to afford **2** (41 mg, 99% yield) as a colorless amorphous solid. **2**: $[\alpha]_D^{22}$ −50.0° (c=0.3, MeOH); IR (KBr): 3366, 1040 cm^{-1}, ^{1}H-NMR (MeOH-d_4): δ 4.32 (1H, d, J=7.6 Hz), 4.25 (1H, d, J=8.0 Hz), 4.08 (1H, dd, J=2.0, 11.6 Hz), 3.90—3.72 (2H, m), 3.74 (1H, dd, J=5.6, 11.6 Hz), 3.57—3.40 (3H, m), 3.35 (2H, t (like)), 3.31—3.29 (2H, m), 3.23—3.16 (2H, m), 1.42—1.28 (8H, m), 0.91 (3H, t, J=6.8 Hz); ^{13}C-NMR (MeOH-d_4): δ 105.3, 104.2, 77.9, 77.6, 76.8, 74.9, 74.7, 71.3, 71.1, 71.0, 69.6, 66.8, 32.9, 30.8, 26.8, 23.7, 14.5; FAB-MS *m/z*: 419 $(M+Na)^+$.

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