Chemoenzymatic Synthesis of a Trisaccharide–Serine Conjugate, Gal(β1-3)Gal(β1-4)Xyl(β1-*O*)–L-Ser, Use of Galactosyl Fluoride as a Donor for Transglycosylation

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The trisaccharide–serine conjugate [Gal(β1-3)Gal(β1-4)Xyl(β)-Ser], constituting the linkage region between the glycosaminoglycan and protein in proteoglycan, was synthesized by using enzymatic transglycosylation with a β-D-galactosidase as a key reaction. The yields of transglycosylation were much improved by using galactosyl fluoride as a donor in comparison with those obtained by a conventional *p*-nitrophenyl (PNP) galactoside. After enzymatic synthesis of Gal(β1-3)Gal(β1-4)Xyl(β)–PNP, cleavage of the PNP group of the fully acetylated trisaccharide, chemical coupling with serine, and final deprotection afforded Gal(β1-3)Gal(β1-4)Xyl(β)–Ser.

Enzymatic synthesis is of increasing importance for synthesis of oligosaccharides, since it allows facile preparation of desired oligosaccharides in short steps.[1,2] Among enzymatic synthesis, transglycosylation catalyzed by glycosidases has apparent advantages in view of the availability of enzymes and rather low specificity for both glycosyl donors and acceptors, allowing the application of this method to a variety of compounds. The major issues in transglycosylation have been how to improve the regioselectivity and the yield which are not always high. In our previous studies, Gal(β1-3)Gal(β1-4)Xyl(β)–Ser (**1**), Gal(β1-3)Gal(β1-4)Xyl(β)–MU [MU = 4-methyl-2-oxo-2*H*-chromen-7-yl (4-methylumbelliferyl)], and GlcA(β1-3)Gal(β1-3)Gal(β1-4)Xyl(β)–MU, corresponding to the linkage region between the glycosaminoglycan (GAG) chain and protein in proteoglycan, were synthesized from Xyl–PNP (PNP = *p*-nitrophenyl) or Xyl–MU by stepwise enzymatic transglycosylation using β-galactosidase (*Escherichia coli*) and β-glucuronidase (bovine liver) as enzymes and the corresponding PNP glycosides as donors.[3,4] Regioselective transglycosylation at the 3′-position of Gal(β1-4)Xyl(β)–PNP or Gal(β1-4)Xyl(β)–MU was achieved by partial protection of the primary hydroxy group of the disaccharide intermediates. The yields of transglycosylation were, however, low, since competitive hydrolysis of a glycosyl donor occurred in preference to the desired transglycosylation.

PNP glycosides have been frequently used as glycosyl donors for transglycosylation, since they are efficiently recognized by glycosidases and their high reactivity enables facile glycosyl transfer to acceptors within short reaction times to suppress hydrolysis of the glycosylated products. Their solubilities in water are, however, not high enough because of the hydrophobicity of the nitrophenyl group. Since the reaction at high concentrations of substrates is advantageous for glycosylation rather than hydrolysis of the donors, glycosyl donors possessing high reactivity and solubility are advantageous for transglycosylation. We therefore examined the use of glycosyl fluorides as glycosyl donors for transglycosylation. Enzymatic glycosylation using glycosyl fluorides as donors was already reported by several authors.[5–10] Shoda et al. extensively studied the enzymatic synthesis of cellulose and their derivatives using cellobiosyl fluoride or its derivatives.[6] In the present study, β-galactosyl fluoride (Gal–F: **2**) was used for β-galactosidase-catalyzed transglycosylation. Application of this method to the improved synthesis of Gal(β1-3)Gal(β1-4)Xyl(β)–Ser (**1**) is described (Fig. 1).

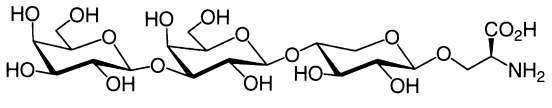

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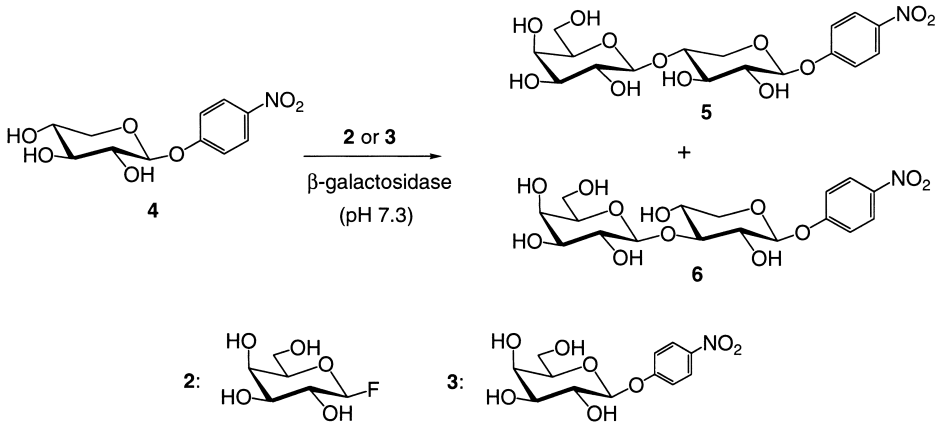
Fig. 1. The structure of trisaccharide–serine conjugate **1**.

Disaccharide, Gal(β1-4)Xyl(β)–PNP (**5**), was prepared by transgalactosylation with Gal–F (**2**) to Xyl–PNP (**4**) (Scheme 1). The higher concentration of the phosphate buffer (200 mM, pH 7.3) was required for the reaction using Gal–F (100 mM) as compared to the concentration (50 mM, pH 7.3) for the reaction with Gal–PNP (**3**). This was important to neutralize HF liberated during the reaction mixture at the optimum pH range of the enzyme. The desired transglycosylated product **5** was obtained in 18.2% yield (Table 1, entry 1), which was similar to that obtained with Gal–PNP (Table 1, entry 4). When the reaction was carried out at a higher concentration of the substrate, i.e., under saturated conditions of Gal–F (**2**) (400 mM) at room temperature, a better yield (27.7%) was obtained as expected (Table 1, entry 2). The concentration of **2** was then increased to 600 mM by warming the solution to 50 °C, where a higher buffer-concentration (1.0 M) was required to maintain the solution at pH 7.3. Although the enzymatic activity decreased at the present high buffer-concentration, the glycosyla-



Scheme 1.

Table 1. The Yields of Disaccharide **5** and Its Regioisomer **6** in Transgalactosylation of Xyl–PNP (**4**) with Gal–F (**2**)[a] or Gal–PNP (**3**)[a] by the Use of β-Galactosidase (*E. coli*)[b]

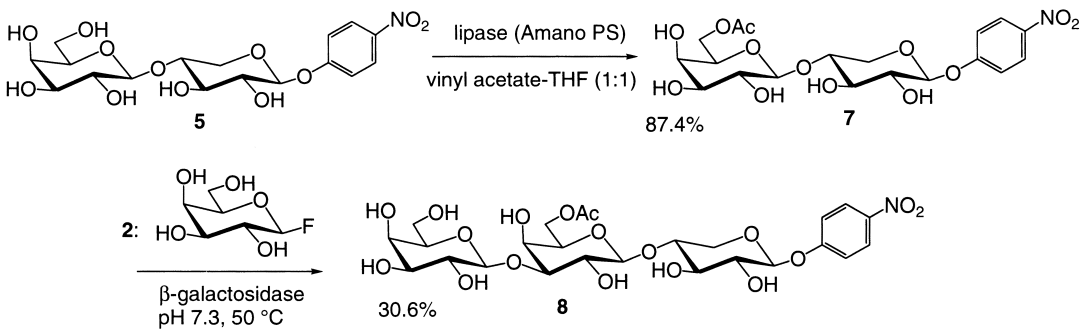
Entry	Donor	Concentration of Donor/mM	Temp/°C	Concentration of Buffer/M	Time/h	Yields[c]/%	
						5	**6**
1	**2**	100	25	0.2	0.5	18.2	2.1
2	**2**	400	25	0.8	2.5	27.7	3.0
3	**2**	600	50	1.0	4.8	34.9	4.1
4	**3**	100	25	0.05	0.6	16.9	2.0

a) All reactions were carried out using 3 molar amounts of the donor to Xyl–PNP. b) 30 mU per 1 μmol of the donor was used. c) Based on HPLC.

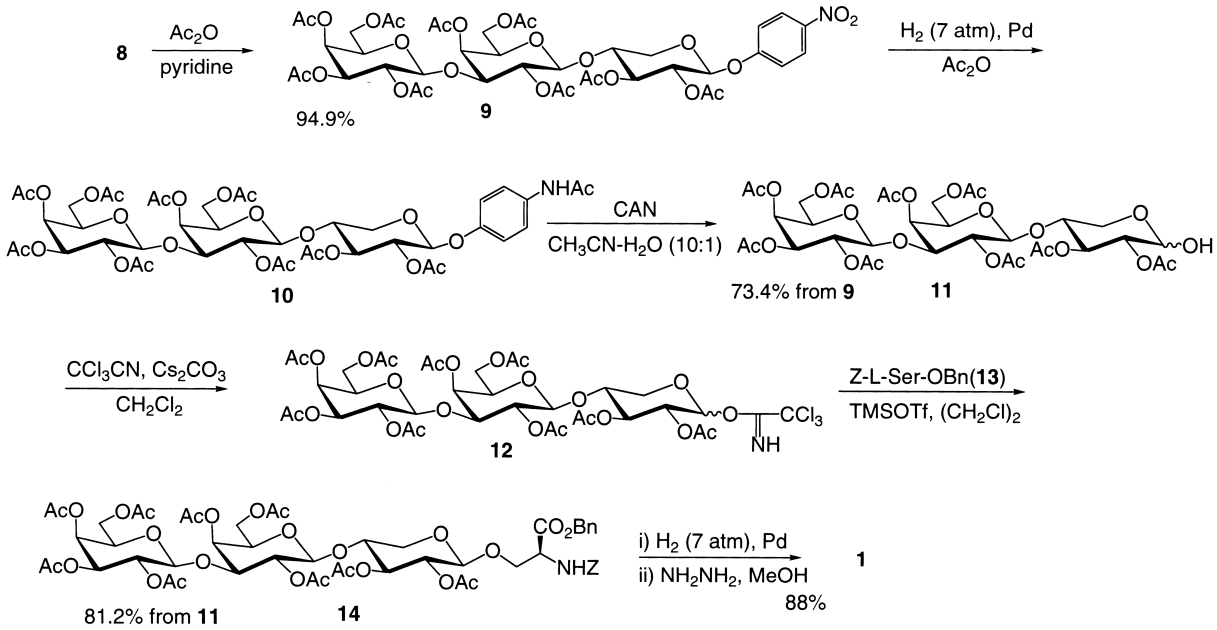
tion proceeded effectively to give the desired disaccharide **5** in a yield as high as 34.9% (entry 3). In a preparative scale synthesis where the reaction was carried out under the same conditions, the desired **5** was obtained in 32.9% yield after HPLC purification. The structures of both **5** and its regioisomer **6** were confirmed by NMR spectrum (^{1}H–^{1}H COSY, HMQC, and HMBC).

Enzymatic galactosylation of the hydroxy group of the 3′-position of the disaccharide **5** was then investigated to form the trisaccharide, Gal(β1-3)Gal(β1-4)Xyl(β)–PNP (Scheme 2). Since the acceptor **5** itself in this reaction is a good substrate of galactosidase, facile enzymatic hydrolysis was expected when direct glycosylation to **5** would be attempted. Furthermore, glycosylation at the more reactive 6′-position rather than the 3′-position would occur preferentially.[2t] In our previous studies, we already overcame these problems by selective protection of the primary hydroxy group of its Gal residue with an acetyl group.[3,4] As in our previous works, the regioselective introduction of an acetyl group to the 6′-position of **5** was readily carried out by the use of lipase (celite-immobilized Amano PS) in vinyl acetate and THF (1:1) to give the 6′-*O*-monoacetylated disaccharide **7** in 87.4% yield. Regioselective transglycosylation to 3′-position of **7** was then effected by the use of 5 mol amt. of Gal–F (**2**) in a phosphate buffer at 50 °C to give the desired trisaccharide **8** in 30.6% yield with 60.5% recovery of **7**. The yield was much improved in comparison with that of Gal–PNP (**3**) (23.1%).[3]

The trisaccharide **8** was then subjected to chemical glycosylation with L-serine derivative **13** as described in our preliminary communication (Scheme 3).[3] After complete *O*-acetylation, the PNP group of the resultant **9** was removed as follows.[11] The PNP group was changed into *p*-acetamidophenyl (AAP) group by catalytic hydrogenolysis in Ac_2O. The AAP group was then removed by ammonium cerium(IV) ni-



Scheme 2.



Scheme 3.

trate [$NH_4Ce(NO_3)_6$, (CAN)] oxidation to give the trisaccharide **11** in 73.4% yield from **9**. The reaction of **11** with CCl_3CN in the presence of Cs_2CO_3 gave glycosyl trichloroacetimidate **12**,[12] which was then coupled with Z–L-Ser–OBn (**13**) by the use of trimethylsilyl triflate (TMSOTf) as a catalyst in 1,2-dichloroethane to give the protected serine conjugate **14**. Catalytic hydrogenolysis of **14** and subsequent hydrazinolysis[13] afforded the desired Gal(β1-3)Gal(β1-4)Xyl(β1-*O*)–L-Ser (**1**) in 88% yield. The structure of **1** was confirmed by the NMR spectra (^{1}H–^{1}H COSY, HMQC, and HMBC) and the matrix-assisted laser desorption/ionization (MALDI) mass spectrum.

The combination of chemical and enzymatic methods described above provides a facile overall procedure for synthesis of trisaccharide derivatives with a minimum use of protecting groups. The use of *p*-nitrophenyl glycoside as a temporary protection of reducing-end sugar moiety enables not only facile purification of the products by reversed phase HPLC owing to its strong UV absorption and appropriate lipophilicity but also easy transformation from a simple sugar chain to a complex glycoconjugate. Transglycosylation by using galactosyl fluoride at high concentrations proved to be effective to improve the yields of glycosylated product.

Experimental

β-Galactosidase (*E. coli*, EC 3.2.1.23) was purchased from Sigma Chemical Co. Lipase (EC 3.1.1.3), Amano PS was a generous gift from Amano Pharmaceutical Co., Ltd. (Aichi, Japan). Xyl–PNP (**3**) was purchased from Sigma Chemical Co. Gal–F (**2**) was prepared according to the literature.[14,15] Kieselgel 60 F_{254} (E. Merck), 0.5 mm, was used for silica-gel preparative TLC. HPLC was performed using Shimadzu LC-6AD liquid chromatograph fitted with a YMC-GEL ODS-M 120 S-5 column, 20 × 250 mm (designated column A), or a Cosmosil 5C18AR column, 20 × 250 mm (column B). The yields and regioselectivities of the products were determined from the peak areas in the analytical HPLC chromatograms. FAB-MS spectra were obtained using a JEOL SX-102 mass spectrometer. Positive-ion MALDI-MS were performed using a Voyager Elite XL time-of-flight mass spectrometer equipped with a delayed-extraction system (PerSeptive Biosystem).[16] α-Cyano-4-hydroxycinnamic acid was used as a matrix. NMR spectra were recorded on a JEOL JNM-GSX 270 (270 MHz), VARIAN-UNITY 600 NMR (600 MHz), or JEOL LA 500 (500 MHz) spectrometer, using D_2O or $CDCl_3$ as a solvent. The chemical shifts of the protons are given in δ values as determined either with TMS in $CDCl_3$ or HDO (δ 4.65) in D_2O as the internal standard. The galactose residue connected to the xylose in the compound **1**, **8**, **9**, **11**, or **14** is designated Gal_A and the other galactose is designated Gal_B, respectively, in the NMR assignment.

β-D-Galactopyranosyl Fluoride[15] **(2).** To the solution of galactose pentaacetate (2.15 g, 5.51 mmol) in CH_2Cl_2 (20 mL) was added HBr–AcOH (6.0 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at r.t. for 2 h and then concentrated in vacuo. To the solution of the residue in anhydrous CH_3CN (150 mL) was added AgF (0.801 g, 6.31 mmol) and the mixture was stirred at r.t. for 12 h. The insoluble materials were removed by filtration through celite. The filtrate was concentrated in vacuo and the residue was purified by silica-gel column chromatography [toluene–acetone (7:1)] to give the fully acetylated galactosyl fluoride (1.40 g, 72.5%, m.p.: 98 °C). To the solution of this compound in anhydrous MeOH (20 mL) was added NaOMe (0.1 mL, 1 M MeOH solution) and the mixture was stirred at 0 °C for 10 h. The reaction was quenched with Dowex 5W-X8 (H^+ form). After the resin was removed, the solution was concentrated in vacuo to give β-galactosyl fluoride (**2**) as a solid: Yield 0.712 g (97.8%); $[\alpha]_D^{25}$ −32.6 (*c* 1.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 6.33 (1H, d, J = 7.2 Hz, H-1: Gal), 4.11 (1H, dd, J = 12, 5.2 Hz, H-5: Gal), 3.77 (1H, d, J = 3.1 Hz, H-4: Gal), 3.64–3.43 (2H, m), 3.38–3.21 (2H, m, H-6: Gal); FAB-MS [positive, matrix: glycerol (Gro)] *m/z* 183.2 [$(M + H)^+$].

***p*-Nitrophenyl *O*-β-D-Galactopyranosyl-(1 → 4)–β-D-xylopyranoside (5) and *p*-Nitrophenyl *O*-β-D-Galactopyranosyl-(1 → 3)-β-D-xylopyranoside (6).** To a solution of Gal–F (**2**)

(141.0 mg, 0.774 mmol) and Xyl–PNP (**4**) (70.0 mg, 0.258 mmol) in a phosphate buffer (1.0 M, pH 7.3, 1.30 mL) was added β-galactosidase (50 U[17]) at 50 °C. After the mixture was allowed to stand at 50 °C for 2 h, the reaction was stopped by heating the mixture at 80 °C for 1 min. The mixture was filtered and the filtrate concentrated in vacuo. The residue was purified by HPLC [column A, H_2O–CH_3CN (18%), flow rate: 8 mL min^{-1}, detection: UV at 320 nm, retention time: 16.9 min (**5**) and 27.1 min (**6**)] to give Gal(β1-4)Xyl(β)–PNP (**5**) (36.8 mg, 32.9%) and Gal(β1-3)Xyl(β)–PNP (**6**) (3.6 mg, 3.2%) as colorless powder after lyophilization with recovery of **4** (38.6 mg, 55.1%). **5**: $[\alpha]_D^{25}$ −64.9 (*c* 4.95, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 8.14 (2H, d, *J* = 9.2 Hz, H-3: PNP), 7.12 (2H, d, *J* = 9.4 Hz, H-2: PNP), 5.13 (1H, d, *J* = 7.6 Hz, H-1: Xyl), 4.37 (1H, d, *J* = 7.8 Hz, H-1: Gal), 4.07 (1H, dd, *J* = 12, 5.2 Hz, H-5ex: Xyl), 3.84 (1H, m, H-4: Xyl), 3.79 (1H, d, *J* = 3.2 Hz, H-4: Gal), 3.64–3.55 (6H, m), 3.51 (1H, dd, *J* = 7.2, 11.0 Hz, H-2: Gal); ^{13}C NMR (125 MHz, D_2O) δ 160.346 (C-4: PNP), 141.439 (C-1: PNP), 124.872 (C-3: PNP), 116.265 (C-2: PNP), 100.980 (C-1: Gal), 98.633 (C-1: Xyl), 75.000 (C-4: Xyl), 74.120 , 72.385, 71.200, 69.432 (C-2: Gal), 67.400 (C-4: Gal), 61.988 (C-5: Xyl), 59.890 (C-6: Gal); FAB-MS [positive, Matrix: Glycerol (Gro)] *m/z* 434.2 [$(M + H)^+$]. Found: C, 47.11; H, 5.31; N, 3.29%. Calcd for $C_{17}H_{23}NO_{12}$: C, 47.12; H, 5.35; N, 3.23%. **6**: ^{1}H NMR (600 MHz, D_2O) δ 7.71 (1H, d, *J* = 8.8 Hz, H-3: PNP), 7.21 (2H, d, *J* = 8.8 Hz, H-2: PNP), 5.21 (1H, d, *J* = 7.4 Hz, H-1: Xyl), 4.33 (1H, d, *J* = 7.7 Hz, H-1: Gal), 4.15 (1H, dd, *J* = 4.9, 10.9 Hz, H-5eq: Xyl), 3.62 (1H, d, *J* = 2.9 Hz, H-4: Gal), 3.80–3.73 (2H, m, H-4: Xyl; H-2; Xyl), 3.70 (1H, dd, *J* = 3.4, H-3: Xyl) 3.61–3.55 (6H, m); ^{13}C NMR (125 MHz, D_2O) δ 162.447 (C-4: PNP), 143.627 (C-1: PNP), 127.019 (C-3: PNP), 117.436 (C-2: PNP), 104.136 (C-1: Gal), 100.730 (C-1: Xyl), 84.485 (C-3: Xyl), 76.251 , 73.512, 73.092, 72.154, 69.530, 68.659, 65.788 (C-5: Xyl), 62.004 (C-6: Gal); FAB-MS (positive, Matrix: Gro) *m/z* 434.2 [$(M + H)^+$].

***p*-Nitrophenyl *O*-(6-*O*-Acetyl-β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside (7).** To a suspension of disaccharide **5** (200 mg, 0.462 mmol) in THF (50 mL) and vinyl acetate (50 mL) was added immobilized lipase[18] (Amano PS) (2.1 g). The mixture was stirred at r.t. for 23 h and then filtered. After the filtrate was concentrated in vacuo, the residue was purified by HPLC [column B, solvent: H_2O–CH_3CN, flow rate: 8 mL min^{-1}, gradient: 20–50% (0–30 min), detection: UV at 320 nm, retention time: 17.8 min] to give colorless powder after lyophilization: Yield 192 mg (87.4%); $[\alpha]_D^{25}$ −58.9 (*c* 1.92, DMF); ^{1}H NMR (500 MHz, D_2O) δ 8.13 (2H, d, *J* = 9.3 Hz, H-3: PNP), 7.11 (2H, d, *J* = 9.6 Hz, H-2: PNP), 5.13 (1H, d, *J* = 7.5 Hz, H-1: Xyl), 4.38 (1H, d, *J* = 8.8 Hz, H-1: Gal), 4.17 (2H, d, *J* = 5.0 Hz, H-6: Gal), 4.05 (1H, dd, *J* = 5.3, 13 Hz, H-5eq: Xyl), 3.83 (1H, d, *J* = 3.5 Hz, H-4: Gal), 3.81–3.78 (1H, m, H-4: Xyl), 3.61 (1H, dd, *J* = 9.1, 9.1 Hz), 3.56–3.48 (3H, m), 3.41 (1H, dd, *J* = 7.5, 10.0 Hz, H-5ax: Xyl), 2.07 (3H, s, Ac); FAB-MS (positive, Matrix: Gro) *m/z* 476.2 [$(M + H)^+$]. Found: C, 48.09; H, 5.32; N, 3.00%. Calcd for $C_{19}H_{25}NO_{13}$: C, 48.00; H, 5.30; N, 2.95%.

***p*-Nitrophenyl *O*-β-D-Galactopyranosyl-(1 → 3)-*O*-(6-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside (8).** To a solution of disaccharide **7** (20.0 mg, 0.0421 mmol) and Gal–F (**2**) (37.9 mg, 0.208 mmol) in a phosphate buffer (1.0 M, pH 7.3, 0.35 mL) was added β-galactosidase (5 U) at 50 °C. After the solution was allowed to stand at 50 °C for 1.5 h, the reaction was stopped by addition of AcOH (0.1 mL). The mixture was filtered and the filtrate concentrated in vacuo. The residue was purified by HPLC [column B, solvent: 0.1% aqueous AcOH–CH_3CN, flow rate: 8 mL min^{-1}, gradient: 20–38% (0–30 min), detection: UV at 300 nm, retention time: 17.1 min] to give **8** (8.2 mg, 30.6%) as colorless powder after lyophilization with recovery of **7** (12.1 mg, 60.5%). **8**: $[\alpha]_D^{25}$ −49.0 (*c* 1.51, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 8.16 (2H, d, *J* = 11 Hz, H-3: PNP), 7.14 (2H, d, *J* = 9 Hz, H-2: PNP), 5.15 (1H, d, *J* = 7.5 Hz, H-1: Xyl), 4.49–4.46 (2H, m, H-1: Gal_A; H-1: Gal_B), 4.24–4.16 (2H, m), 4.14 (1H, d, *J* = 3.0 Hz, H-4: Gal_A), 4.09 (1H, dd, *J* = 5.1, 12.1 Hz, H-5eq: Xyl), 3.86–3.80 (2H, m, H-5: Gal; H-4: Xyl), 3.82 (1H, d, *J* = 3.0 Hz, H-4: Gal_B), 3.72 (1H, dd, *J* = 3.5, 9.5 Hz, H-3: Gal_A), 3.68–3.48 (9H, m), 2.04 (3H, s, Ac); FAB-MS (positive, Matrix: Gro) *m/z* 638.1 [$(M + H)^+$]. Found: C, 46.98; H, 5.50; N, 2.15%. Calcd for $C_{25}H_{35}NO_{18}$: C, 47.10; H, 5.53; N, 2.20%.

***p*-Nitrophenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-β-D-xylopyranoside (9).** To a solution of trisaccharide **8** (40.2 mg, 0.0631 mmol) in pyridine (2 mL) was added Ac_2O (1.5 mL) at 0 °C. After the mixture was stirred at r.t. for 12 h, the mixture was concentrated in vacuo. The residue was purified by silica-gel preparative TLC [$CHCl_3$-acetone (10:1)] to give colorless powder after lyophilization: Yield 58.3 mg (94.9%); $[\alpha]_D^{25}$ +16.3 (*c* 4.92, $CHCl_3$); FAB-MS [positive, Matrix: *p*-nitrobenzyl amine (NBA)] *m/z* 974.4 [$(M + H)^+$]. Found: C, 50.62; H, 5.20; N, 1.40%. Calcd for $C_{41}H_{51}NO_{26}$: C, 50.57; H, 5.28; N, 1.44%.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-D-xylopyranose (11).** Compound **9** (52.1 mg, 0.0535 mmol) was hydrogenated with Pd (30 mg, 0.28 mmol) in Ac_2O (4 mL) under 7 kg cm^{-2} of hydrogen at r.t. for 2 days. The catalyst was removed by filtration and the filtrate concentrated in vacuo. To the solution of the resulting **10** in CH_3CN–H_2O (10:1) (2 mL) was added CAN (358 mg, 0.53 mmol) at 0 °C. After being stirred for 20 min, the mixture was diluted with EtOAc (10 mL) and washed with water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel preparative TLC [$CHCl_3$-acetone (4:1)] to give colorless powder after lyophilization: Yield 33.5 mg (73.4%); $[\alpha]_D^{25}$ −3.64 (*c* 3.50, $CHCl_3$); FAB-MS (positive, Matrix: NBA) *m/z* 853.3 [$(M + H)^+$]. Found: C, 49.01; H, 5.60%. Calcd for $C_{35}H_{48}O_{24}$: C, 49.30; H, 5.67%.

***N*-Benzyloxycarbonyl-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3-di-*O*-acetyl-β-D-xylopyranosyl)-(1 → 3)-L-serine Benzyl Ester (14).** To a solution of compound **11** (37.4 mg, 0.0439 mmol) and Cs_2CO_3 (7.0 mg, 0.21 mmol) in dry CH_2Cl_2 (1 mL) was added CCl_3CN (57 μL, 0.57 mmol) at r.t.. After being stirred for 1.5 h, the mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in benzene and lyophilized to give the trichloroacetimidate **12** as colorless powder. To a mixture of **12**, *N*-benzyloxycarbonyl-L-serine benzyl ester (**13**) (17.3 mg, 0.0525 mmol), and molecular sieves 4A (200 mg) in dry 1,2-dichloroethane (1 mL) was added a solution of TMSOTf (3.5 μL, 0.023 mmol) in 1,2-dichloroethane (0.2 mL) under argon atmosphere at −20 °C. After being stirred for 3 h, the mixture was diluted with CH_2Cl_2, filtered, and washed with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel preparative TLC [$CHCl_3$–acetone (7:1)] to give colorless powder after lyophilization: Yield 41.5 mg (81.2%); $[\alpha]_D^{25}$ −9.87 (*c* 3.14, $CHCl_3$); ^{1}H

NMR (500 MHz, $CDCl_3$) δ 7.45 (10H, m, $PhCH_2$), 5.71 (1H, NH), 5.56 (1H, d, J = 8.5 H), 5.37 (1H, dd, J = 0.7, 3.2 Hz, H-4: Gal), 5.35 (1H, dd, J = 0.9, 3.4 Hz, H-4: Gal_B), 5.21–5.01 (9H, m), 4.93 (1H, dd, J = 3.4, 10.5 Hz, H-3: Gal_B), 4.75 (1H, dd, J = 6.2, 7.6 Hz, H-2: Gal_A), 4.54 (2H, m, H-1: Xyl; H-2: Ser), 4.42 (1H, d, J = 6.0 Hz, H-1: Gal_B), 4.37 (1H, d, J = 7.9 Hz, H-1: Gal_A), 4.24–4.06 (4H, m), 4.00 (1H, dd, J = 7.1, 11.5 Hz, H-5ex: Xyl), 3.86–3.79 (6H, m), 3.66 (1H, m, H-5: Gal), 3.20 (1H, dd, J = 8.0, 12.1 Hz, H-5: Xyl), 2.41–1.98 (27H, Ac); FAB-MS (positive, Matrix: NBA) m/z 1164.5 [$(M + H)^+$]. Found: C, 54.39; H, 5.60; N, 1.18%. Calcd for $C_{53}H_{65}NO_{28}$: C, 54.68; H, 5.63; N, 1.20%.

***O*-β-D-Galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 3)-L-serine (1).** Compound **14** (14.7 mg, 0.0126 mmol) was hydrogenated with Pd (20 mg, 0.19 mmol) in MeOH (1 mL) under 7 kg cm^{-2} of hydrogen at r.t. for 1h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To the solution of the residue in MeOH (4 mL) was added hydrazine monohydrate (1 mL) at 0 °C. After 5 h, the reaction was stopped by addition of acetone and the mixture was concentrated in vacuo. The residue was purified by gel permeation chromatography [LH-20 (Pharmacia Biotech), H_2O] and HPLC [column B, solvent: H_2O, flow rate: 8 mL min^{-1}, detection: UV at 220 nm, retention time: 7.1 min] to give colorless powder after lyophilization: Yield 6.2 mg (88%); $[\alpha]_D^{25}$ +3.71 (c 5.21, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.49 (1H, d, J = 7.2 Hz, H-1: Xyl), 4.41 (1H, d, J = 7.4 Hz, Gal_B), 4.35 (1H, d, J = 7.3 Hz, H-1: Gal_A), 4.15 (1H, dd, J = 3.8, 10.1 Hz, H-3: Ser), 4.07 (1H, d, J = 3.2 H, H-4, Gal_B), 4.00 (1H, dd, J = 3.1, 8.2 Hz, H-5ex: Xyl), 3.93–3.89 (2H, m, H-2: Ser; H-3: Ser), 3.80 (1H, d, J = 3.4 Hz, H-4: Gal), 3.75–3.46 (13H, m), 3.31–3.23 (2H, m, H-5ax: Xyl; H-2: Gal_A). ^{13}C NMR (125 MHz, D_2O) δ 172.293 (COOH), 105.172 (C-1: Xyl), 103.461 (C-1: Gal_B), 102.285 (C-1: Gal_A), 82.922 (C-3: Gal_A), 77.295 (C-4: Xyl), 75.963, 75.831, 74.606, 73.495 (C-2: Gal_A), 73.413, 71.932 (C-2: Xyl), 70.723, 69.473, 68.626 (C-3: Ser), 68.838 (H-5: Xyl), 61.946 (H-6: Gal), 61.848 (H-6: Gal), 55.391 (C-2: Ser). MALDI-MS Found: m/z 584.1114. Calcd for $C_{20}H_{35}NO_{17}Na$ (M + Na): 584.1803.

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