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Enzymic transfer of 6-modified D-galactosyl residues: synthesis of biantennary penta- and hepta-saccharides having two 6-deoxy-D-galactose residues at the nonreducing end and evaluation of 6-deoxy-D-galactosyl transfer to glycoprotein using bovine β-(1 → 4)-galactosyltransferase and UDP-6-deoxy-D-galactose

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Abstract

UDP-6-Deoxy-D-galactose and UDP-6-deoxy-6-fluoro-D-galactose were synthesized and their transfer to 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) by β-(1 → 4)-galactosyltransferase was examined. The transfer rates of 6-deoxy-D-galactose and 6-deoxy-6-fluoro-D-galactose were 1.3 and 0.2% of that of D-galactosyl transfer, respectively. The 2-acetamido-4-*O*-(6-deoxy-β-D-galactopyranosyl)-2-deoxy-D-glucopyranose (6′-deoxy-*N*-acetyllactosamine) and methyl 2-acetamido-4-*O*-(6-deoxy-6-fluoro-β-D-galactopyranosyl)-2-deoxy-D-glucopyranoside (6′-deoxy-6′-fluoro-*N*-acetyllactosamine) were synthesized enzymatically in 30 and 59% yields, respectively. Further, 6-deoxy-D-galactose could be completely transferred to N-linked type biantennary oligosaccharides having two *N*-acetyl-D-glucosaminyl residues at the nonreducing end to give the corresponding penta- and hepta-saccharides in 55 and 57% yields, respectively. An assay of 6-deoxy-D-galactosyl transfer using asialo agalacto α_1-acid glycoprotein as an acceptor suggested that 6-deoxy-D-galactose was transferred to about 30% of the *N*-acetyl-D-glucosaminyl residues in the N-linked oligosaccharides of the glycoprotein.

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1. Introduction

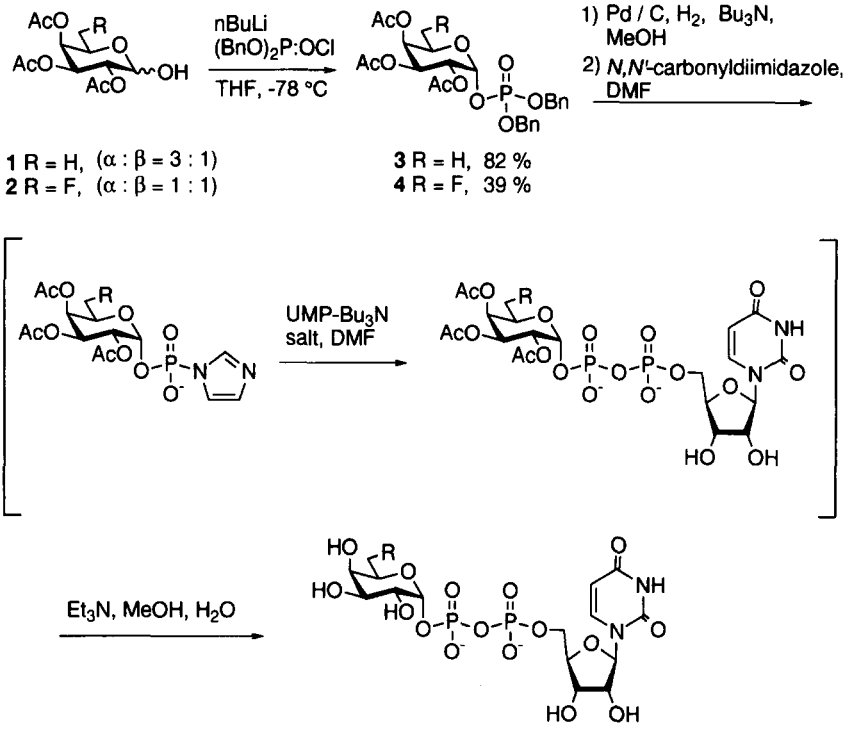
Oligosaccharides in glycoproteins and glycolipids have been shown to be involved in signal transduction in various cellular and molecular recognition events [1–4]. Oligosaccharide analogues that have inhibitory activity towards glycosyltransferases [5–8] or glycosidases [9–11] are useful chemical tools for the investigation of such phenomena. Although syntheses of unnatural glycolipids [12,13] and glycopeptides [14,15] have been reported, a synthetic methodology for glycoprotein analogues has not yet been established. Recent studies of such glycoproteins as erythropoietin [16] and tissue plasminogen activator [17,18], showed that the structure of the carbohydrate moiety may have remarkable effects on their activities. In order to improve the activity of such a glycoprotein or to establish a relationship between the activity and the structure of oligosaccharides on these proteins, it may be of interest to introduce sugar analogues into these oligosaccharides.

Glycosyl transfer using glycosyltransferase and sugar nucleotide analogues is expected to be effective and mild. Since isolation [19], cloning [19] and over-expression using *E. coli* [20] or *Baculovirus* [21] of glycosyltransferases have been established, enzymatic synthesis of oligosaccharides can be done on the millimolar scale, and several in situ regeneration systems of sugar nucleotides [22,23] have also been developed. However, in the enzymatic synthesis of oligosaccharide analogues, the major problem is the low transfer rate of the sugar analogue from its nucleotide, which is often one-hundredth [24] of that of the unmodified sugar nucleotide. Thus it is essential to investigate whether the slow-reacting sugar analogue can actually be transferred to the glycoprotein.

Here we describe the synthesis of UDP-6-deoxy-D-galactose and UDP-6-deoxy-6-fluoro-D-galactose and their transfer to 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetylglucosamine) by commercially available bovine β-(1 → 4)-galactosyltransferase (EC 2. 4. 1. 22) [25]. The transfer of the 6-deoxy-D-galactosyl moiety to N-linked type biantennary oligosaccharides having two 2-acetamido-2-deoxy-β-D-glucopyranoside residues at the nonreducing end and to asialo agalacto α_1-acid glycoprotein is presented.

2. Results and discussion

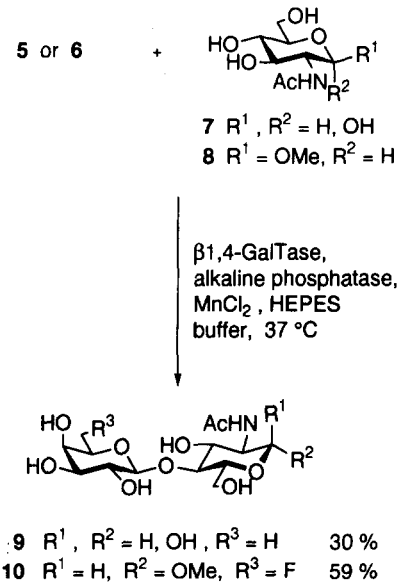
Synthesis of UDP-6-deoxy- and UDP-6-deoxy-6-fluoro-D-galactose.—A synthetic route for UDP-6-deoxy-D-galactose **5** and UDP-6-deoxy-6-fluoro-D-galactose **6** is outlined in Scheme 1. The former was synthesized from peracetylated D-fucose [26], whose selective *O*-deacetylation at the anomeric position with hydrazine acetate led to D-fucose triacetate **1** in 77% yield. Treatment of **1** ($\alpha:\beta = 3:1$) with BuLi and dibenzyl phosphorochloridate [27] gave α-phosphate dibenzyl ester **3** exclusively in 82% yield. Catalytic hydrogenation of **3** in the presence of Pd–C, followed by coupling with



Scheme 1.

N,N'-carbonyldiimidazole, led to the corresponding imidazolide. Condensation of this intermediate with uridine 5'-monophosphate tributylammonium salt [28] in *N,N*-dimethylformamide, and subsequent mild *O*-deacetylation [29] with Et_3N–MeOH–H_2O gave **5** in 19% yield from the phosphotriester **3**. In the same manner 6-fluoro analogue **6** was synthesized in 13% overall yield. On the phosphorylation of **2** an anomeric mixture was obtained and separated by silica gel chromatography to give α-phosphotriester **4** and its β anomer in 39 and 8% yields, respectively.

*Transfer of 6-deoxy- and 6-deoxy-6-fluoro-*D*-galactose by galactosyltransferase.*— Transfer of the 6-modified D-galactosyl residue was monitored by HPLC determination of uridine, which was quantitatively formed in the presence of alkaline phosphatase (EC 3.1.3.1) from the stoichiometrically liberated UDP [30]. This treatment favors the glycosyl transfer, because the transfer reaction is inhibited by UDP. Instead of the frequently used sodium cacodylate and Tris-HCl buffers, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer was used, which was found to accelerate the glycosyl transfer enormously. The assay suggested that both analogues were indeed transferred from the corresponding sugar nucleotides. Although the relative transfer rates of 6-deoxy-D-galactose and 6-deoxy-6-fluoro-D-galactose were 1.3 and 0.2% compared



Scheme 2.

to that of the parent D-galactosyl moiety, these enzymic glycosylations proved to be practical enough for the larger scale preparations as shown in Scheme 2. The glycosylation of GlcNAc **7** (1.4 equiv) with the donor **5** gave *N*-acetyl-6′-deoxylactosamine (**9**) as a sole product in 30% yield based on the donor. Similar reaction of UDP-6-deoxy-6-fluoro-D-galactose (**6**) with equimolar methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**8**) yielded methyl 2-acetamido-4-*O*-(6-deoxy-6-fluoro-β-D-galactopyranosyl)-2-deoxy-D-glucoside (**10**) as a sole product in 59% yield.

Synthesis of biantennary tri- and penta-saccharides as galactosyl acceptors.—As a model reaction for the modification of a carbohydrate chain in a glycoprotein, the enzymatic synthesis of biantennary penta- and hepta-oligosaccharides containing two 6-deoxy-D-galactosyl residues at the nonreducing end was examined.

A trisaccharide **17** and pentasaccharide **25**, which are partial structures of N-linked tetraantennary complex type carbohydrate chains (Fig. 1), were selected as biantennary galactosyl acceptors and synthesized as shown in Schemes 3 and 4.

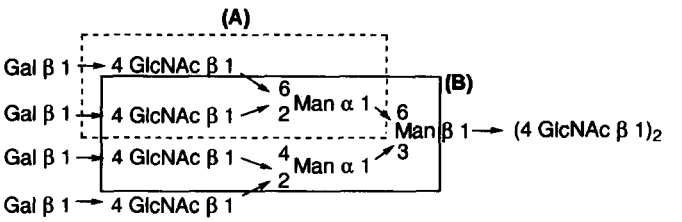
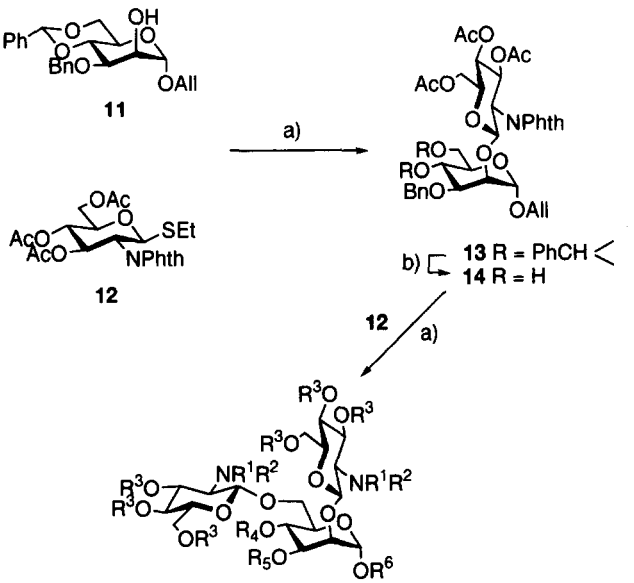


Fig. 1. Partial structures of N-linked tetraantennary complex type carbohydrate chains (**17** and **25**).

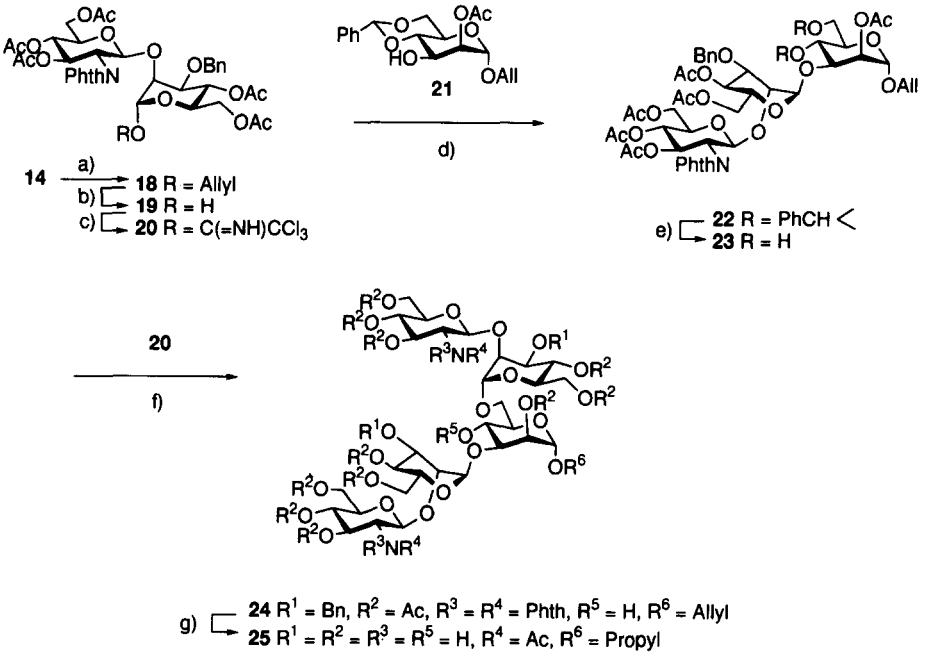


(a) MeOTf / CH_2Cl_2, 6 h, **13**:61%, **15**:71 %; (b) 60% AcOH, 90°C, 30 min, 80 % ; (c) 1) H_2NNH_2-H_2O / EtOH, 100°C, 24 h, 2) Ac_2O, DMAP / Py, 3 h, 40 %, (2 steps); (d) 1) Pd/C, H_2,12 h, 2) NaOMe / MeOH, 40°C, 3 h, 81 % (2 steps).

Scheme 3.

The trisaccharide **17** was derived from allyl 4,6-*O*-benzylidene-α-D-mannopyranoside [31]. Its selective 3-*O*-benzylation [32] with *n*-Bu_2SnO and BnBr led to **11** in 85% yield. Glycosylation of **11** with thioglycoside **12** [33] in the presence of MeOTf furnished disaccharide **13** in 61% yield. Regioselective glycosylation of its *O*-debenzylidenated derivative **14** with **12** led to desired trisaccharide **15** in 71% yield. Deprotection of the *N*-phthaloyl group with hydrazine hydrate and subsequent treatment with acetic anhydride and pyridine afforded the *N*-acetate **16** in 40% yield. *O*-Debenzylation and *O*-deacetylation gave the trisaccharide acceptor **17** in 81% yield.

For the synthesis of the pentasaccharide acceptor **25**, the disaccharide **18** was converted to the corresponding trichloroacetimidate **20**. Isomerization of the allyl group in **18** to an isopropenyl group with Pd–C, followed by hydrolysis with $HgCl_2$ and HgO [34], led to **19** in 81% yield, which was converted to the imidate **20** in 82% yield. Paulsen et al. reported that glycosylation at the 3-position prior to the 6-position of mannoside acceptor is effective for construction of bianntenary oligosaccharide [35]. Therefore, the glycosylation at the 3-position of mannoside derivative **21** was first performed. Coupling of **20** with an acceptor **21**, prepared by selective 2-*O*-acetylation of

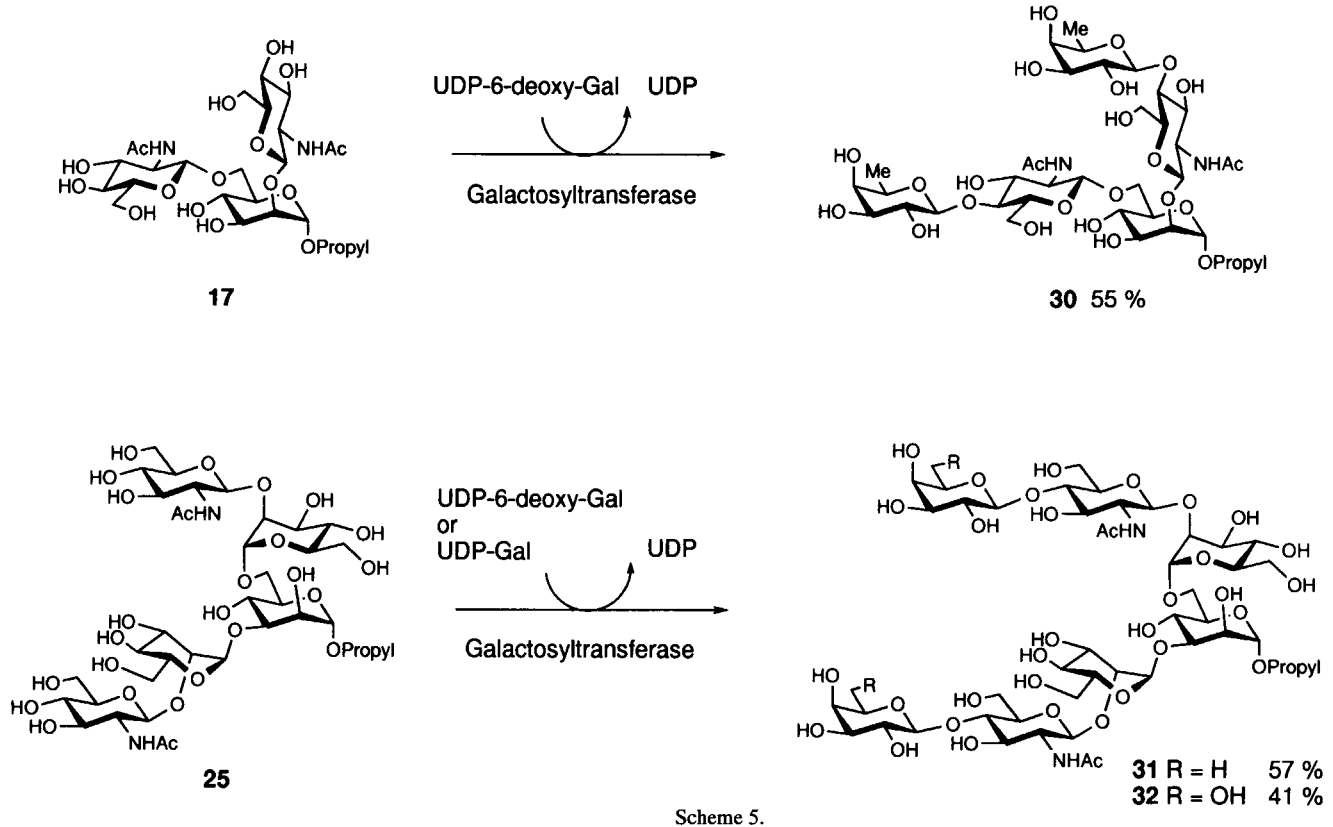


(a) Ac_2O, Py, 90%; (b) 1) Pd/C, H_2, MeOH, 2) $HgCl_2$, HgO, Acetone : H_2O (4 : 1), 81% (2 steps); (c) CCl_3CN, DBU, CH_2Cl_2, 82%; (d) TMSOTf, CH_2Cl_2, MS4A, 56%; (e) 60% AcOH, 90 °C, 71%; (f) BF_3OEt_2, CH_2Cl_2, MS4A, 54%; (g) 1) H_2NNH_2-H_2O, EtOH, 100 °C, 2) Ac_2O, Py, DMAP, 3) NaOMe, MeOH, 4) Pd/C, H_2, MeOH, 37% (4 steps).

Scheme 4.

allyl 4,6-*O*-benzylidene-α-D-mannopyranoside via the orthoester derivative, in the presence of TMSOTf led to trisaccharide **22** in 56% yield. *O*-Debenzylidenation of **22** to give **23** (71%), and subsequent glycosylation with **20** in the presence of boron trifluoride etherate, gave a mixture of pentasaccharide **24** (54%) and its β isomer ($\alpha:\beta = 5:1$). *N*-Dephthaloylation, *N*-acetylation, and *O*-deacetylation of **24** gave **25** in 37% yield.

*Enzymatic synthesis of 6-deoxy-*D*-galactosylated oligosaccharides.*—Glycosylation of the trisaccharide **17** (10 μmol) with 3 equiv of UDP-6-deoxy-D-galactose and 3 units of galactosyltransferase for 48 h, afforded a pentasaccharide **30**, exclusively, in 55% yield (Scheme 5) after successive purification on anion-exchange, reversed-phase, and gel-permeation columns. Reversed-phase TLC analysis of the final mixture suggested that there was no untransferred acceptor **17**, or intermediate monogalactosylated tetrasaccharide. The same procedure was applied to the synthesis of the heptasaccharide **31** from **25**. In this case, however, some intermediate hexasaccharide was detected after 48 h. Therefore, an additional 0.5 units of galactosyltransferase were added, and the reaction was allowed to continue for a further 48 h. The yield of the heptasaccharide **31**, purified in the same manner as described above, was 57%. Newly formed Gal-β-(1 → 4)-GlcNAc linkages were confirmed by the chemical shift of H-4 in the GlcNAc


UDP-6-deoxy-Gal
UDP
Galactosyltransferase
17
30 55 %
UDP-6-deoxy-Gal
or
UDP-Gal
UDP
Galactosyltransferase
25
31 R = H 57 %
32 R = OH 41 %

Scheme 5.

residues of the peracetylated derivatives of **30** and **31**. On the other hand, enzymatic galactosylation of **25** was completed with only 0.75 units of galactosyltransferase, to give a heptasaccharide **32** in 41% yield. Although the transfer rate of 6-modified D-galactose was poor compared to that of D-galactose, these data suggested that enzymatic modification with UDP-(6-modified D-galactose) was useful for a practical synthesis of oligosaccharide analogues.

Transfer of 6-deoxy-D-galactosyl residue to asialo agalacto α_1-acid glycoprotein.- Since the 6-deoxy-D-galactosyl residue was transferred to the biantennary oligosaccharide acceptors, we investigated its transfer to the asialo agalacto α_1-acid glycoprotein. The number of 2-acetamido-2-deoxy-D-glucopyranosyl residues in this protein subjected to D-galactosylation, was estimated to be ca. 17 [36]. The asialo agalacto α_1-acid glycoprotein was prepared according to the reported procedure [36]. The number of 2-acetamido-2-deoxy-D-glucopyranosyl residue exposed by treatment with the β-galactosidase (*Streptococcus*), was estimated by the number of the [^{14}C]-labeled D-galactose incorporated by treatment with UDP-D-[^{14}C]-galactose and galactosyltransferase. After incubation for 48 h, the assay suggested that the protein had accepted 2.5 equiv of [^{14}C]-labeled D-galactose. However, this value is much less than the expected 17 equiv, probably due to folding of the oligosaccharide chains. Thus, to make the peptide chain looser, a detergent (Nonidet p-40, 0.1%) was added to the incubation mixture. This detergent dramatically elevated the galactosyl transfer rate 48-fold. After incubation for 2 h, 10 equiv of the labeled galactose were incorporated. These conditions were used for the estimation of the 6-deoxy-D-galactosyl transfer, and the assay method is shown in Fig. 2. The asialo agalacto α_1-acid glycoprotein was first incubated with UDP-6-deoxy-D-galactose (3.9 mM) for 18 h in the presence of the galactosyltransferase and alkaline phosphatase, then with UDP-D-[^{14}C]-galactose for 2 h under the above same conditions. The [^{14}C]-labeled D-galactose incorporated into the glycoprotein was estimated to be 5

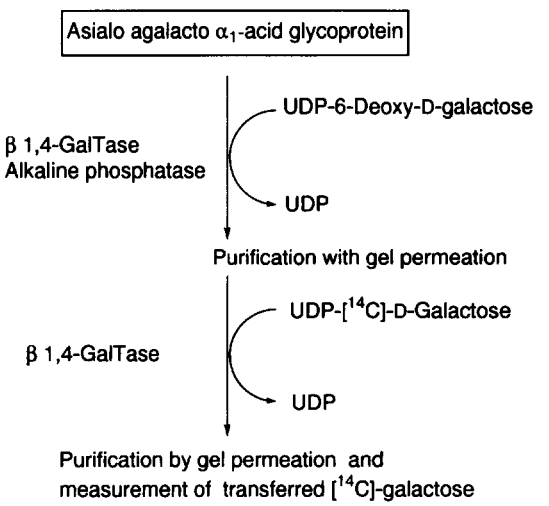


Fig. 2. The assay method used (see text).

equiv. This result indicated that 5 equiv of the 6-deoxy-D-galactoses were incorporated into the protein by the first incubation. The 2-acetamido-2-deoxy-D-glucopyranosyl residue at the nonreducing end of the carbohydrate chain of the glycoprotein may be fully masked with 6-deoxy-D-galactose by use of a larger amount of galactosyltransferase or a higher concentration of UDP-6-deoxy-D-galactose. Thus, 6-modified D-galactose can be transferred by galactosyltransferase not only to an oligosaccharide but also to a glycoprotein.

3. Conclusions

UDP-6-deoxy-D-galactose and UDP-6-deoxy-6-fluoro-D-galactose were shown to be useful donors for modification of carbohydrate chains using galactosyltransferase. The transfer rates of these 6-modified D-galactoses were ca. one-hundredth of that of D-galactose. However, 6-deoxy-D-galactose could be transferred, not only to two kinds of biantennary oligosaccharides having two GlcNAc residues at the nonreducing ends in similar preparative yields to that for D-galactosylation using UDP-D-galactose, but also to those of an asialo agalacto glycoprotein.

4. Experimental

General methods.—^{1}H NMR spectra were recorded with Jeol EX-270 or Bruker AM-500 instruments at 298 K. The chemical shifts are presented in ppm and referenced to tetramethylsilane in $CDCl_3$, or to sodium 3-(trimethylsilyl)propionate in D_2O as the internal or external standard, respectively. ^{13}C NMR spectra were recorded with a Jeol EX-270 instrument at 298 K. The chemical shifts are expressed in ppm and referenced to tetramethylsilane in $CDCl_3$, or to dioxane (67.4 ppm) in D_2O as the internal or external standard, respectively. ^{1}H and ^{13}C NMR data were given only for the clearly assigned and necessary signals for confirmation of structure. ^{31}P NMR spectra were recorded with a Jeol EX-270 instrument at 298 K. The chemical shifts are expressed in ppm and referenced to external H_3PO_4 (0 ppm). Optical rotations were measured with a Jasco DIP-4 instrument. High-resolution mass spectra were recorded on a JMS-SX102A or Shimadzu-Kratos concept-IIH instrument under FAB conditions. All reactions were monitored by TLC (Silica Gel 60 F_{254}, E. Merck) by charring after spraying with 5% H_2SO_4 in MeOH. Wako-Gel C-300 was used for flash column chromatography. Bovine β-(1 → 4)-galactosyltransferase (EC 2.4.1.22), calf intestine alkaline phosphatase (EC 3.1.3.1), and streptococcus 6646K β-galactosidase (EC 3.2.1.23) were purchased from Sigma Chemical Co., Boehringer-Mannheim, and Seikagaku Co., respectively. UDP-α-D-[U-^{14}C]-galactose was purchased from NEN Research Products.

2,3,4-Tri-O-acetyl-6-deoxy-α,β-D-galactopyranose (**1**).—To a solution of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-α,β-D-galactopyranose [26] (950 mg, 2.86 mmol) in DMF (30 mL) was added $H_2NNH_2 \cdot AcOH$ (395 mg, 4.29 mmol). The mixture was stirred at 50°C for 0.5 h, then diluted with EtOAc and washed with brine. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Purification of the residue by flash column

chromatography (1:1 hexane–EtOAc) afforded **1** (622 mg, 77%) as an anomeric mixture ($\alpha:\beta = 3:1$); ^{1}H NMR (270 MHz): δ 5.46 (bs, 0.75 H, α H-1), 5.42 (dd, 0.75 H, $J_{3,4}$ 3.3, $J_{3,2}$ 10.9 Hz, α H-3), 5.31 (dd, 0.75 H, $J_{4,5}$ 1.0 Hz, α H-4), 5.24 (bs, 0.25 H, β H-4), 5.14 (dd, 0.75 H, $J_{2,1}$ 3.6 Hz, α H-2), 5.07–5.05 (m, 0.5 H, β H-2, 3), 4.68 (m, 0.25 H, α H-1), 4.43 (q, 0.75 H, α H-5), 4.13 (b, 0.25 H, β 1-OH), 3.87–3.83 (m, 1 H, β H-5, β 1-OH), 2.17, 2.10, 2.00 (each s, each 3 H, Ac), 1.23 (d, 0.75 H, $J_{6,5}$ 6.6 Hz, β H-6), 1.15 (d, 2.25 H, $J_{6,5}$ 6.6 Hz, α H-6). Anal. Calcd for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.97; H, 6.25.

*2,3,4-Tri-*O*-acetyl-6-deoxy-6-fluoro-α,β-*D*-galactopyranose* (**2**).—To a solution of allyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-β-D-galactopyranoside (57 mg, 0.18 mmol) in MeOH (1 mL) was added Pd–C (10%, 140 mg), and the mixture was stirred at 70°C for 4 h, filtered through a Celite pad, and concentrated in vacuo. To a solution of the residue in 4:1 acetone–H_2O (1.25 mL) were added $HgCl_2$ (58 mg, 0.22 mmol) and HgO (47 mg, 0.22 mmol). The mixture was stirred at room temperature for 10 min and filtered through a Celite pad (washed with $CHCl_3$). The filtrate was washed with brine (×3), dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (3:2 hexane–EtOAc) afforded **2** (42 mg, 84%, $\alpha:\beta = 1:1$); ^{1}H NMR (270 MHz): δ 5.56 (d, 0.5 H, $J_{1,2}$ 3.6 Hz, α H-1), 5.52 (d, 0.5 H, $J_{4,3}$ 3.6 Hz, α H-4), 5.47–5.46 (b, 0.5 H, β H-4), 5.43 (dd, 0.5 H, $J_{3,2}$ 10.9 Hz, α H-3), 5.19 (dd, 0.5 H, α H-2), 5.15–5.05 (m, 1 H, β H-2, β H-3), 4.75–4.72 (m, 0.5 H, β H-1), 4.63–4.32 (m, 2.5 H, β H-6a, β H-6b, α H-5, α H-6a, α H-6b), 4.06–4.01 (m, 0.5 H, β H-5), 2.17, 2.11, 2.01 (each s, each 1.5 H, β Ac), 2.15, 2.11, 2.00 (each s, each 1.5 H, α Ac). Anal. Calcd for $C_{12}H_{17}FO_8$: C, 46.75; H, 5.52. Found: C, 47.24; H, 5.47.

*Allyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-β-*D*-galactopyranoside.*—To a solution of allyl 2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-β-D-galactopyranoside (290 mg, 0.72 mmol) in MeOH (3 mL) was added NaOMe (87 mg, 1.64 mmol) at room temperature and stirring was continued for 2 h. The mixture was neutralized by addition of Dowex 50W-X8 (H^+) and then filtered. The concentrated filtrate was dissolved in acetic anhydride (0.5 mL) and pyridine (0.5 mL) and stirred for 1 h. After concentration of the mixture, purification of the residue by flash column chromatography (5:1 hexane–EtOAc) afforded the title compound (205 mg, 90%); $[\alpha]_D^{24}$ −13.3° (*c* 2.4, $CHCl_3$); ^{1}H NMR (270 MHz): δ 5.93–5.79 (m, 1 H, $CH=CH_2$), 5.44 (dd, 1 H, $J_{4,5}$ 1.0, $J_{4,3}$ 3.3 Hz, H-4), 5.33–5.18 (m, 3 H, H-2, $CH=CH_2$), 5.04 (dd, 1 H, $J_{3,2}$ 10.6 Hz, H-3), 4.55 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.63–4.32 (m, 3 H, $J_{6a,5}$ 6.6, $J_{6b,5}$ 6.5 Hz, H-6a, 6b, $CH_2CH=CH_2$), 4.16–4.10 (m, 1 H, $CH_2CH=CH_2$), 4.09–3.91 (m, 1 H, H-5), 2.15, 2.07, 1.99 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.20, 170.11, 169.43, 133.28, 117.66, 100.09 (C-1), 80.85 (d, $J_{C,F}$ 172.1 Hz, C-6), 71.61 (d, $J_{C,F}$ 24.2 Hz, C-5), 70.89, 70.06, 68.86, 67.14 (d, *J* 6.1 Hz, C-4), 20.75, 20.63, 20.58. Anal. Calcd for $C_{15}H_{20}FO_8$: C, 51.87; H, 5.80. Found: C, 51.55; H, 6.10.

*Allyl 2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-β-*D*-galactopyranoside.*—Allyl 4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranoside [37] (580 mg, 1.12 mmol) was dissolved in 60% AcOH (35 mL). The mixture was stirred at 100°C for 1.5 h and concentrated in vacuo. Purification of the residue by flash column chromatography (1:2 hexane–EtOAc) afforded allyl 2,3-di-*O*-benzoyl-β-D-galactopyranoside (440 mg). To a solution of this diol in CH_2Cl_2 (1.5 mL) was added with stirring at −78°C dieth-

ylaminosulfur trifluoride (146 mL, 1.19 mmol), then the mixture was allowed to warm to room temperature and stirred for 48 h. The temperature was elevated to 50°C and kept for 5 h. After cooling, MeOH (300 μL) was added to the mixture and the stirring was continued for 1 h at room temperature. The residue obtained by concentration of the mixture was purified by flash column chromatography (3:1 hexane–EtOAc) to afford the title compound (204 mg, 45%); $[\alpha]_D^{23}$ +74.9° (*c* 4.1, CH_2Cl_2); ^{1}H NMR (270 MHz): δ 8.01–7.32 (m, 10 H, Ph), 5.87–5.73 (m, 1 H, C*H* = CH_2), 5.77 (dd, 1 H, $J_{2,1}$ 7.9, $J_{2,3}$ 10.2 Hz, H-2), 5.33 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.29–5.10 (m, 2 H, CH = CH_2), 4.78 (d, 1 H, H-1), 4.70 (dd, 2 H, $J_{6,5}$ 4.7, $J_{6,F}$ 46.7 Hz, H-6), 4.43–4.34 (m, 2 H, H-4, CH_2CH = CH_2), 4.21–4.13 (m, 1 H, CH_2CH = CH_2), 4.00 (dq, 1 H, $J_{5,F}$ 5.9 Hz, H-5), 2.48 (d, 1 H, $J_{4,OH}$ 5.6 Hz, 4-OH); ^{13}C NMR: δ 165.84, 165.37, 133.55, 133.42, 133.17, 129.88, 129.74, 129.50, 128.89, 128.50, 128.35, 117.75, 100.03 (C-1), 81.77 (d, $J_{C,F}$ 169.6 Hz, C-6), 74.21, 73.04 (d, $J_{C,F}$ 23.1 Hz, C-5), 69.92, 69.52, 67.16 (d, $J_{C,F}$ 6.1 Hz, C-4). Anal. Calcd for $C_{23}H_{23}FO_7$: C, 64.18; H, 5.39. Found: C, 63.84; H, 5.05.

2,3,4-Tri-O-*acetyl-6-deoxy-α*-D-*galactopyranosyl 1-phosphate, dibenzyl ester* (**3**).—To a solution of **1** (300 mg, 1.07 mmol, α:β = 3:1) in THF (6 mL) was added a hexane solution of *n*-BuLi (0.66 mL, 1.07 mmol) at −78°C, and after 2 min a solution (THF, 2 mL) of dibenzyl phosphorochloridate (3.21 mmol). The temperature was allowed to warm to −60°C, and after 15 min the mixture was diluted with ether. The diluted solution was washed with aq $NaHCO_3$, dried with $MgSO_4$, and concentrated. The residue obtained was purified by flash column chromatography (3:2 hexane–EtOAc) to afford **3** (473 mg, 82%); $[\alpha]_D^{24}$ +78.2° (*c* 1.3, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.41–7.26 (m, 10 H, Ph), 5.91 (dd, 1 H, $J_{1,2}$ 3.3, $J_{1,P}$ 6.3 Hz, H-1), 5.33 (dd, 1 H, $J_{3,4}$ 3.3, $J_{3,2}$ 10.9 Hz, H-3), 5.27 (d, 1 H, H-4), 5.20 (ddd, 1 H, $J_{2,P}$ 3.0 Hz, H-2), 5.10–5.05 (m, 4 H, CH_2), 4.16 (q, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 2.16, 1.99, 1.91 (each s, each 3 H, Ac), 1.05 (d, 3 H, Me); ^{13}C NMR: δ 170.46, 170.13, 169.95, (each C = O), 135.52, 135.42, 128.73, 128.68, 128.03, 127.92, 94.94 (d, $J_{C,P}$ 6.1 Hz, C-1), 70.53 (C-4), 69.58 (CH_2 × 2), 67.38 (C-3), 67.08 (d, $J_{C,P}$ 7.3 Hz, C-2), 66.83 (C-5), 20.66, 20.59, 20.52 (CO*C*H_3), 15.72 (C-6); ^{31}P NMR δ −1.78. Anal. Calcd for $C_{26}H_{31}O_{11}P$: C, 56.73; H, 5.64. Found: C, 56.70; H, 6.10.

2,3,4-Tri-O-*acetyl-6-deoxy-6-fluoro-α*-D-*galactopyranosyl 1-phosphate, dibenzyl ester* (**4**).—To a stirred solution of **2** (96 mg, 0.35 mmol, α:β = 1:1) in THF (2 mL) was added a hexane solution of *n*-BuLi (0.215 mL, 0.35 mmol) at −78°C. After 5 min, a solution of the dibenzylphosphorochloridate (1.03 mmol) in THF (3 mL) was added to the mixture, which was allowed to warm to −60°C. The work up as described in the preparation of **3** and purification by flash column chromatography (4:1 diethyl ether–hexane) afforded **4** (72 mg, 39%) and its β anomer (14 mg, 8%).

Physical data for **4**: $[\alpha]_D^{24}$ +69.1° (*c* 1.1, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.41–7.31 (m, 10 H, Ph), 5.97 (dd, 1 H, $J_{1,2}$ 3.3, $J_{1,P}$ 6.9 Hz, H-1), 5.49 (bd, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 5.33 (dd, 1 H, $J_{3,2}$ 10.9 Hz, H-3), 5.23 (ddd, 1 H, $J_{2,P}$ 2.6 Hz, H-2), 5.11–5.02 (m, 4 H, CH_2), 4.32 (dd, 2 H, $J_{6,5}$ 5.8, $J_{6,F}$ 42.6 Hz, H-6), 4.26 (dq, 1 H, $J_{5,F}$ 16.2 Hz, H-5), 2.14, 2.00, 1.91 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.03, 169.95, 169.81 (each C = O), 135.40, 135.31, 135.27, 128.75, 128.66, 128.60, 128.54, 128.05, 128.01, 94.43 (d, $J_{C,P}$ 4.9 Hz, C-1), 80.64 (d, $J_{C,F}$ 172.1 Hz, C-6), 20.57, 20.52, 20.43 (each

$COCH_3$); ^{31}P NMR δ −1.89. Anal. Calcd for $C_{26}H_{30}FO_{11}P$: C, 54.92; H, 5.28. Found: C, 55.37; H, 5.87. β Anomer: $[\alpha]_D^{24}$ +9.4° (*c* 1.0, $CHCl_3$); ^1H NMR: δ 7.40–7.27 (m, 10 H, Ph), 5.47 (bd, 1 H, $J_{4,3}$ 3.3 Hz, H-4), 5.39–5.30 (m, 2 H, H-2, 3), 5.11–5.02 (m, 4 H, CH_2), 4.58–4.30 (m, 2 H, $J_{6a,5} = J_{6b,5} = 5.6$, $J_{6a,F} = J_{6b,F} = 46.52$ Hz, H-6a,6b), 4.06 (ddd, 1 H, $J_{5,F}$ 11.9 Hz, H-5), 2.17, 1.99, 1.93 (each s, each 3 H, Ac); ^{13}C NMR: δ 96.71 (d, $J_{C,P}$ 4.9 Hz, C-1), 80.29 (d, $J_{C,P}$ 173.3 Hz, C-6), 20.57 ($COCH_3$), 20.52 ($COCH_3 \times 2$); ^{31}P NMR δ −2.48.

Uridine 5′-(6-deoxy-α-D-galactopyranosyl) diphosphate, diammonium salt (**5**).—Hydrogen was introduced to a mixture of **3** (460 mg, 0.85 mmol), Bu_3N (205 mg, 1.11 mmol), and Pd–C (10%, 100 mg) in MeOH (20 mL). After 2 h, the mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo to afford 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-galactopyranosyl 1-phosphate, tributylammonium salt; ^1H NMR (270 MHz, CD_3OD): δ 5.70 (dd, 1 H, $J_{1,2}$ 3.3, $J_{1,P}$ 7.6 Hz, H-1), 5.41 (dd, 1 H, $J_{3,4}$ 3.3, $J_{3,2}$ 10.9 Hz, H-3), 5.30 (d, 1 H, H-4), 5.08 (bd, 1 H, H-2), 4.46 (q, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 2.16, 2.06, 1.91 (each s, each 3 H, Ac); ^{31}P NMR (CD_3OD): δ 0.16.

To a solution of this glycosyl phosphate in DMF (5 mL) was added *N,N′*-carbonyldiimidazole (220 mg, 1.36 mmol), and the mixture was stirred at room temperature for 12 h. After stopping the reaction by adding MeOH (16 mg, 0.51 mmol), the mixture was stirred for 0.5 h, concentrated in vacuo, and coevaporated with dry pyridine (×3). To a solution of this residue in DMF (5 mL) was added uridine 5′-monophosphate, tributylammonium salt (1.02 mmol). After stirring for 12 h at room temperature under Ar, the mixture was concentrated in vacuo at 35°C, and the residue was dissolved in a mixed solution of triethylamine (1.5 mL), MeOH (10 mL), and water (4.6 mL), and the solution was stirred for 24 h. The residual solution obtained by concentration of the mixture at room temperature was diluted with water and then loaded onto an anion-exchange column (Dowex 1-X8, formate, 1.5 × 15 cm). The UDP-6-deoxy-D-galactose was eluted with aq NH_4CO_3 (50 mM–1.0 M). The eluates with 300–500 mM NH_4CO_3 were combined and lyophilized. Further purification (twice) of the residue by gel-permeation chromatography (Sephadex G-15, 2.5 × 60 cm, water) afforded **5** (85 mg, 19%); $[\alpha]_D^{25}$ +49.3° (*c* 0.4, H_2O); ^1H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 7.98 (d, 1 H, $J_{6,5}$ 8.3 Hz, H-6), 6.00 (d, 1 H, H-5), 5.98 (d, 1 H, $J_{1',2'}$ 5.3 Hz, H-1′), 5.57 (dd, 1 H, $J_{1'',2''}$ 3.6, $J_{1'',P}$ 6.6 Hz, H-1″), 3.93 (dd, 1 H, $J_{3'',4''}$ 3.3, $J_{3'',2''}$ 10.2 Hz, H-3″), 3.83 (d, 1 H, H-4″), 3.76 (ddd, 1 H, $J_{2'',P}$ 3.0 Hz, H-2″), 1.23 (d, 3 H, $J_{6'',5''}$ 6.6 Hz, H-6″); ^{31}P NMR: δ −10.55, −12.05 (each d, *J* 19.8 Hz); HRFABMS (negative-ion) Calcd for $C_{15}H_{23}N_2O_{16}P_2$: 549.0523. Found: 549.0527.

Uridine 5′-(6-deoxy-6-fluoro-α-D-galactopyranosyl) diphosphate, diammonium salt (**6**).—In the same manner as described in the preparation of **5**, the glycosyl dibenzyl phosphate **4** (96 mg, 0.178 mmol) was debenzylated to give 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-α-D-galactopyranosyl phosphate, tributylammonium salt; ^1H NMR (270 MHz): δ 5.80 (dd, 1 H, $J_{1,2}$ 3.3, $J_{1,P}$ 7.3 Hz, H-1), 5.54 (bs, 1 H, H-4), 5.43 (dd, 1 H, $J_{3,4}$ 3.3, $J_{3,2}$ 10.9 Hz, H-3), 5.17 (bddd, 1 H, H-2), 2.17, 2.13, 2.09 (each s, each 3 H, Ac); ^{31}P NMR (CD_3OD): δ 0.10, and then converted to **6** in 13% total yield (12 mg); $[\alpha]_D^{25}$ +50.5° (*c* 0.2, H_2O); ^1H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 7.98 (d, 1 H, $J_{6,5}$ 8.3 Hz, H-6), 6.00 (d, 1 H, H-5), 5.98 (d, 1 H, $J_{1',2'}$ 4.6 Hz, H-1′), 5.66 (dd, 1 H, $J_{1'',2''}$ 3.6, $J_{1'',P}$ 6.6 Hz, H-1″), 4.65 (bd, 2 H, $J_{6'',F}$ 45.9 Hz, H-6″); ^{31}P NMR: δ −10.44,

−12.10 (each d, J 19.8 Hz); HRFABMS (negative-ion) Calcd for $C_{15}H_{22}FN_2O_{16}P_2$: 567.0429. Found: 567.0440.

O-(6-Deoxy-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose (**9**).—A solution containing the donor **5** (82 mg, 138 μmol), the acceptor **7** (43 mg, 194 μmol), bovine serum albumin (5 mg), alkaline phosphatase (100 units), β-(1 → 4)-galactosyltransferase (4 units), and $MnCl_2$ (100 mM) in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (0.1 M, pH 7.0, total volume 585 μL) was incubated at 37°C for 5 days. The mixture was directly loaded onto a column of anion-exchange resin (1 × 5 cm, Cl^- form), and the disaccharide was eluted with water. This fraction was then loaded onto a charcoal column (2 × 13 cm). The inorganic compounds were eluted with water, and the disaccharide and uridine were eluted with an aq EtOH (20 → 50% gradient). Further purification of the disaccharide fraction by gel-permeation chromatography (Sephadex G-15, 1.0 × 80 cm, water) afforded **9** (15 mg, 30%); ^{1}H NMR (500 MHz, D_2O, 303 K): δ 4.42 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 2.04 (s, 3 H, Ac), 1.25 (d, 3 H, $J_{6',5'}$ 6.5 Hz, 6'-Me); HRFABMS (positive-ion): Calcd for $C_{14}H_{25}NO_{10}Na$: 390.1377. Found: 390.1375.

Methyl O-(6-deoxy-6-fluoro-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**10**).—A solution containing the donor **6** (5 mg, 8.3 μmol), the acceptor **8** (2 mg, 8.2 μmol), bovine serum albumin (2 mg), alkaline phosphatase (100 units), β-(1 → 4)-galactosyltransferase (2 units), and $MnCl_2$ (40 mM) in HEPES buffer (0.1 M, pH 7.0, total volume 200 μL) was incubated at 37°C for 48 h. The mixture was directly loaded onto a column of anion-exchange resin [1 × 5 cm, Dowex (Cl^- form)]. The disaccharide was eluted with water and then purified on an reversed-phase (C_{18}) column (2 × 10 cm, H_2O → 9:1 H_2O–MeCN). Further purification of the disaccharide by gel-permeation chromatography (Sephadex G-15, 1.0 × 80 cm, water) afforded **10** (1.9 mg, 59%); ^{1}H NMR (500 MHz, D_2O, 303 K): δ 4.57 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.50 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.05 (d, 1 H, $J_{4',3'}$ 3.5 Hz, H-4'), 3.99 (dd, 1 H, $J_{6a,5}$ 2.0, $J_{6a,6b}$ 13.0 Hz, H-6a), 3.89 (dd, 1 H, $J_{6b,5}$ 5.2 Hz, H-6b), 3.82–3.78 (m, 2 H, H-2,3), 3.74 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.74 (dd, 1 H, $J_{3',2'}$ 9.3 Hz, H-3'), 3.68–3.65 (m, 1 H, H-5), 3.62 (dd, 1 H, H-2'), 3.56 (s, 3 H, OMe), 2.09 (s, 3 H, Ac); HRFABMS (positive-ion): Calcd for $C_{15}H_{26}FNO_{10}$: 400.1619. Found: 400.1607.

Allyl 3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (**11**).—To a solution of allyl 4,6-*O*-benzylidene-α-D-mannopyranoside [31] (5.03 g, 16.2 mmol) in dry MeOH (90 mL) was added *n*-Bu_2SnO (4.85 g, 19.5 mmol), and the mixture was stirred at 90°C for 2 h, allowed to cool to room temperature and concentrated in vacuo. The residue was dried by coevaporating with dry benzene and dissolved in dry benzene (180 mL). To this solution was added BnBr (3.85 mL, 32.5 mmol) and *n*-Bu_4NI (7.19 g, 19.5 mmol), and the mixture was stirred at reflux for 2 h and concentrated in vacuo. The residue was triturated with aq KF at room temperature, and the product was extracted with EtOAc. The extract was dried with $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography (3:2 hexane–EtOAc) afforded **11** (5.54 g, 85%); $[\alpha]_D^{24}$ +41° (*c* 5.5, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.50–7.17 (m, 10 H, Ar), 5.93–5.78 (m, 1 H, C*H* = CH_2), 5.57 (s, 1 H, PhC*H*), 5.29–5.15 (m, 2 H, CH = CH_2), 4.83 (bs, 1 H, H-1), 4.81, 4.66 (each d, each 1 H, $J_{a,b}$ 11.9 Hz, PhCH_2), 4.25–4.21 (m, 1 H, H-5), 4.16–4.07 (m, 2 H, CH_2CH = CH_2), 3.13 (d, 1 H, $J_{OH,2}$ 1.7 Hz, OH); ^{13}C

NMR: δ 138.00, 137.55, 133.49, 129.74, 128.96, 128.89, 128.44, 128.19, 127.89, 127.83, 126.04, 117.81, 101.56, 99.23, 78.88, 75.72, 73.08, 69.95, 68.82, 68.16, 63.43. Anal. Calcd for $C_{23}H_{26}O_6$: C, 69.33; H, 6.58. Found: C, 69.58; H, 6.34.

Allyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**13**).—To a stirred mixture of glycosyl donor **12** (189 mg, 0.37 mmol), acceptor **11** (222 mg, 0.56 mmol), and 4A molecular sieves (1 g) in CH_2Cl_2 (7 mL) was added MeOTf (0.21 mL, 1.85 mmol) at 0°C, and the mixture was allowed to warm to room temperature. After 15 h, triethylamine (0.52 mL, 3.7 mmol) was added to the mixture which was then filtered through a Celite pad. Concentration of the filtrate in vacuo and purification of the residue by flash column chromatography (3:2 hexane–EtOAc) afforded **13** (72 mg, 61%); $[\alpha]_D^{24}$ +8.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.89–7.71, 7.45–7.23 (m, 14 H, Ar), 5.89 (dd, 1 H, $J_{3',2'}$ 10.9, $J_{3',4'}$ 8.9 Hz, H-3′), 5.83–5.69 (m, 1 H, C*H* = CH2), 5.46 (s, 1 H, PhC*H*), 5.44 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 5.19 (dd, 1 H, $J_{4',5'}$ 10.2 Hz, H-4′), 5.19–5.10 (m, 2 H, CH = CH_2), 4.72, 4.66 (each d, each 1 H, $J_{a,b}$ 12.2 Hz, PhCH_2), 4.53 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.49 (dd, 1 H, H-2′), 4.32 (dd, 1 H, $J_{6'a,5'}$ 4.6, $J_{6'a,6'b}$ 12.0 Hz, H-6′a), 4.22 (dd, 1 H, $J_{6'b,5'}$ 2.3 Hz, H-6′b), 4.16–4.08 (m, 1 H, H-2), 4.00–3.84 (m, 4 H, H-3,4,5′, CH_2CH = CH_2), 3.79–3.71 (m, 2 H, H-6a, CH_2CH = CH_2), 3.57–3.49 (m, 1 H, H-5), 3.17 (dd, 1 H, $J_{6b,5} = J_{6b,6a}$ = 10.2 Hz, H-6b), 2.06, 2.05, 1.89 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.74, 170.20, 169.47, 138.43, 137.54, 134.23, 133.03, 131.62, 128.80, 128.19, 128.14, 127.56, 127.46, 126.02, 123.39, 118.02, 101.45, 97.03, 96.87, 78.17, 77.25, 75.58, 73.93, 72.13, 71.72, 70.40, 69.13, 68.39, 68.19, 63.92, 62.25, 54.52, 20.75, 20.66, 20.54. Anal. Calcd for $C_{43}H_{45}NO_{15}$: C, 63.31; H, 5.56; N, 1.72. Found: C, 63.02; H, 5.58; N, 1.83.

Allyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3-*O*-benzyl-α-*D*-mannopyranoside* (**14**).—The disaccharide **13** (424 mg, 0.50 mmol) was dissolved in 60% AcOH (10 mL), and the solution was stirred at 90°C for 0.5 h and concentrated in vacuo. Purification of the residue by flash column chromatography (1:3 hexane–EtOAc) afforded **14** (304 mg, 80%); $[\alpha]_D^{24}$ +0.3° (*c* 2.7, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.88–7.75, 7.39–7.27 (m, 9 H, Ar), 5.90 (dd, 1 H, $J_{3',2'}$ 10.9, $J_{3',4'}$ 8.9 Hz, H-3′), 5.84–5.71 (m, 1 H, C*H* = CH_2), 5.38 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.21–5.11 (m, 3 H, H-4′, CH = CH_2), 4.76, 4.39 (each d, each 1 H, $J_{a,b}$ 10.9 Hz, PhCH_2), 4.54 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.42 (dd, 1 H, H-2′), 4.29 (dd, 1 H, $J_{6'a,5'}$ 4.6, $J_{6'a,6'b}$ 12.1 Hz, H-6′a), 4.20 (dd, 1 H, $J_{6'b,5'}$ 2.3 Hz, H-6′b), 4.13 (bs, 1 H, H-2), 3.99–3.86 (m, 2 H, H-5′, CH_2CH = CH_2), 3.78–3.66 (m, 3 H, H-3,4, CH_2CH = CH_2), 3.46–3.36 (m, 2 H, H-5,6a), 3.17 (dd, 1 H, $J_{6b,5}$ 4.6, $J_{6b,6a}$ 10.2 Hz, H-6b), 2.04, 2.01, 1.89 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.65, 170.19, 169.49, 137.70, 134.43, 133.13, 131.52,128.44, 128.30, 127.99, 123.47, 117.93, 96.69, 96.30, 77.25, 72.94, 72.06, 71.77, 70.55, 70.26, 69.15, 68.25, 66.97, 62.98, 62.25, 54.55, 20.65, 20.54. Anal. Calcd for $C_{36}H_{41}NO_{15}$: C, 59.42; H, 5.68; N, 1.92. Found: C, 59.20; H, 5.75; N, 1.89.

*Allyl 3-*O*-benzyl-2,6-bis-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranoside* (**15**).—To a mixture of acceptor **14** (40 mg, 53 μmol), donor **12** (33 mg, 64 μmol), and 4A molecular sieves (100 mg) in dry CH_2Cl_2 (1 mL) was added MeOTf (15 μL, 130 μmol). After 12 h, triethylamine (74 μL, 530 μmol) was added to the mixture, which was then filtered through a Celite pad.

Purification of the concentrated filtrate by flash column chromatography (1:2 hexane–EtOAc) afforded **15** (46 mg, 71%); $[\alpha]_D^{24}$ −5.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.88–7.64, 7.36–7.18 (m, 13 H, Ar), 5.80 (dd, 1 H, $J_{3',2'}$ 11.0, $J_{3',4'}$ 9.0 Hz, H-3′), 5.71 (dd, 1 H, $J_{3'',2''}$ 10.7, $J_{3'',4''}$ 9.0 Hz, H-3″), 5.64–5.57 (m, 1 H, C*H* = CH2), 5.36 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 5.15 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4′), 5.09–4.99 (m, 2 H, CH = CH_2), 5.08 (dd, 1 H, $J_{4'',5''}$ 10.0 Hz, H-4″), 5.03 (d, 1 H, $J_{1'',2''}$ 8.3 Hz, H-1″), 4.69, 4.30 (each d, each 1 H, $J_{a,b}$ 10.7 Hz, PhCH_2), 4.39 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.33 (dd, 1 H, H-2′), 4.25 (dd, 1 H, $J_{6''a,5''}$ 5.1, $J_{6''a,6''b}$ 12.1 Hz, H-6″a), 4.23 (dd, 1 H, $J_{6'a,5'}$ 4.9, $J_{6'a,6'b}$ 12.1 Hz, H-6′a), 4.17 (dd, 1 H, H-2″), 4.16 (dd, 1 H, $J_{6'a,5'}$ 2.4 Hz, H-6′b), 4.11 (dd, 1 H, $J_{6''b,5''}$ 2.4 Hz, H-6″b), 4.05 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.82 (ddd, 1 H, H-5′), 3.75 (dd, 1 H, $J_{6a,5}$ 2.0, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.74 (ddd, 1 H, H-5″), 3.70–3.66 (m, 1 H, CH_2CH = CH_2), 3.51 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.45 (ddd, 1 H, $J_{5,4}$ 9.8, $J_{5,6b}$ 7.6 Hz, H-5), 3.37–3.33 (m, 1 H, CH_2CH = CH_2), 3.32 (dd, 1 H, H-4), 2.85 (dd, 1 H, H-6b), 2.05, 2.04, 2.03, 1.99, 1.87, 1.85 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.74, 170.62, 170.15, 169.52, 169.49, 167.35, 167.29, 137.81, 134.28, 133.03, 131.46, 128.39, 128.21, 127.90, 124.02, 123.66, 123.54, 117.75, 99.12, 96.44, 95.70, 77.23, 77.18, 72.45, 72.04, 71.66, 71.21, 70.98, 70.64, 70.48, 70.35, 69.11, 67.40, 66.65, 62.07, 54.54, 54.41, 34.93, 29.70, 20.74, 20.65, 20.50, 20.47. Anal. Calcd for $C_{56}H_{60}N_2O_{24}$: C, 58.73; H, 5.28; N, 2.45. Found: C, 58.21; H, 5.47; N, 2.33.

*Propyl 2,6-bis-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranoside* (**16**).—Trisaccharide **15** (119 mg, 98 μmol) was dissolved in 1:1 EtOH–$H_2NNH_2 \cdot H_2O$ (1.4 mL), and the solution was stirred at 100°C for 12 h and concentrated in vacuo. The residue was dissolved in acetic anhydride (0.5 mL) and pyridine (0.5 mL) containing a catalytic amount of 4-dimethylaminopyridine, and the solution was stirred at room temperature for 12 h, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc) afforded **16** (45 mg, 40%); $[\alpha]_D^{24}$ −5.0° (*c* 0.6, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.36–7.18 (m, 5 H, Ph), 6.63 (d, 1 H, $J_{NH,2}$ 10.0 Hz, NH), 5.98 (dd, 1 H, $J_{3',2'} = J_{3',4'} = 9.8$ Hz, H-3′), 5.53 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.28 (t, 1 H, $J_{4,3} = J_{4,5} = 10.0$ Hz, H-4), 5.09–5.01 (m, 2 H, H-3″,4″), 4.97 (t, 1 H, $J_{4',5'}$ 9.8 Hz, H-4′), 4.86 (bs, 1 H, H-1), 4.74, 4.31 (each d, each 1 H, $J_{a,b}$ 11.5 Hz, PhCH_2), 4.21 (dd, 1 H, $J_{6'a,5'}$ 4.9, $J_{6'a,6'b}$ 12.5 Hz, H-6′a), 4.19–4.11 (m, 4 H, H-1″,2,3,2′), 3.83 (dd, 1 H, $J_{6''a,5''}$ 3.7, $J_{6''a,6''b}$ 10.0 Hz, H-6″a), 3.82–3.78 (m, 1 H, H-5′), 3.66–3.62 (m, 1 H, H-5″), 3.41–3.37 (m, 1 H, H-5), 2.07 (s, 9 H, Ac × 3), 2.06, 2.04, 2.02, 2.01, 1.96, 1.93 (each s, each 3 H, Ac), 1.59–1.55 (m, 2 H, Pr), 0.90 (t, *J* 7.3 Hz, Pr); ^{13}C NMR: δ 172.85, 171.14, 170.58, 170.51, 169.95, 169.81, 169.40, 138.43, 128.12, 127.40, 103.04, 96.84, 96.48, 74.18, 72.90, 71.75, 71.68, 71.61, 70.67, 70.06, 69.68, 69.20, 68.82, 68.66, 67.74, 62.44, 62.10, 60.32, 56.89, 53.42, 31.55, 23.50, 23.36, 22.77, 22.62, 21.19, 21.01, 20.84, 20.68, 20.58, 14.20, 14.11, 10.58. Anal. Calcd for $C_{46}H_{64}N_2O_{23}$: C, 54.54; H, 6.37; N, 2.77. Found: C, 54.64; H, 6.52; N, 2.54.

*Propyl 2,6-bis-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranoside* (**17**).—To a solution of **16** (38 mg, 34 μmol) in MeOH (1 mL) was added a catalytic amount of Pd-C (10%) and then the mixture was stirred under H_2. After 12 h, the mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. To a methanolic solution of the residue, thoroughly dried with a vacuum pump, was added a catalytic amount of NaOMe and the mixture was stirred at 40°C. After 3 h, the mixture

was allowed to cool to room temperature, neutralized with Dowex 50w-x8 (H^+), and filtered through a Celite pad. The filtrate was concentrated in vacuo, and purification of the residue on a charcoal column (1.5 × 5 cm, water → EtOH) afforded **17** (16 mg, 81%); $[\alpha]_D^{25}$ −12.0° (*c* 0.8, H_2O); ^{1}H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 4.86 (d, 1 H, *J* 1.7 Hz, Man H-1), 4.59 (d, 1 H, *J* 7.6 Hz, GlcNAc H-1), 4.56 (d, 1 H, *J* 7.9 Hz, GlcNAc H-1), 2.09, 2.06 (s, 6 H, Ac), 1.69–1.61 (m, 2 H, CH_2), 0.96 (t, 3 H, *J* 7.6 Hz, CH_3); ^{13}C NMR (D_2O): δ 175.36, 175.00, 102.30, 100.52, 97.46, 77.52, 76.62, 74.53, 74.01, 72.41, 70.78, 70.69, 70.46, 70.17, 68.30, 61.58, 61.40, 56.31, 56.26, 47.45; HRFABMS (positive-ion): Calcd for $C_{25}H_{44}N_2O_{16}Na$: 651.2589. Found: 651.2623.

Allyl O-*(3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1 → 2)-4,6-di*-O-*acetyl-3*-O-*benzyl-α*-D-*mannopyranoside* (**18**).—The disaccharide **14** (351 mg, 0.482 mmol) was dissolved in pyridine (1.5 mL) and acetic anhydride (1.5 mL), and the solution was stirred at room temperature for 2 h, and concentrated in vacuo. Purification of the residue by flash column chromatography (2:3 hexane–EtOAc) afforded **18** (395 mg, 90%); $[\alpha]_D^{25}$ −19° (*c* 0.5, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.83–7.69, 7.34–7.23 (m, 9 H, Ar), 5.87–5.72 (m, 1 H, C*H* = CH_2), 5.81 (dd, 1 H, $J_{3',2'}$ 10.6, $J_{3',4'}$ 9.2 Hz, H-3′), 5.50 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 5.21–5.12 (m, 3 H, H-4′, CH = CH_2), 5.04 (t, 1 H, $J_{4,3} = J_{4,5} = 9.2$ Hz, H-4), 4.71, 4.45 (each d, each 1 H, $J_{a,b}$ 11.9 Hz, PhCH_2), 4.47 (dd, 1 H, H-2′), 2.04, 2.03, 1.97, 1.95, 1.87, (each s, each 3 H, Ac); ^{13}C NMR: δ 170.69, 170.24, 169.41, 169.31, 138.04, 134.12, 133.17, 131.53, 129.04, 128.26, 127.62, 125.30, 123.41, 117.93, 96.91, 96.37, 77.91, 77.27, 74.91, 73.87, 72.06, 70.96, 70.60, 69.02, 68.79, 68.44, 67.76, 62.98, 62.98, 62.16, 54.39, 21.46, 20.79, 20.72, 20.61, 20.49. Anal Calcd for $C_{40}H_{45}NO_{17}$: C, 59.18; H, 5.59; N, 1.73. Found: C, 59.29; H, 5.42; N, 1.69.

O-*(3,4,6-Tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1 → 2)-4,6-di*-O-*acetyl-3*-O-*benzyl-α*-D-*mannopyranose* (**19**).—To a solution of **18** (352 mg, 0.433 mmol) in MeOH (4 mL) was added Pd–C (10%, 450 mg), and the mixture was stirred at 70°C for 7 h. The mixture was diluted with $CHCl_3$, filtered, and the filtrate was concentrated in vacuo. To a solution of the residue in 4:1 acetone–H_2O (7.5 mL) was added $HgCl_2$ (235 mg, 0.866 mmol) and HgO (188 mg, 0.866 mmol), and the mixture was stirred at room temperature for 20 min. After removal of undissolved materials, the filtrate was diluted with $CHCl_3$, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (1:2 hexane–EtOAc) afforded **19** (270 mg, 81%); $[\alpha]_D^{24}$ −31.0° (*c* 1.2, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.78–7.67, 7.34–7.28 (m, 9 H, Ar), 5.85 (dd, 1 H, $J_{3',2'}$ 10.6, $J_{3',4'}$ 9.2 Hz, H-3′), 5.74 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 5.22 (dd, 1 H, $J_{4,3} = J_{4,5} = 9.6$ Hz, H-4), 4.96 (dd, 1 H, $J_{4',5'}$ 9.2 Hz, H-4′), 4.71, 4.54 (each d, each 1 H, *J* 12.2 Hz, PhCH_2), 4.27 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 2.04, 2.02, 1.98, 1.89, 1.88 (s, 3 H, Ac); ^{13}C NMR: δ 170.80, 170.46, 170.19, 169.49, 169.41, 138.02, 134.21, 131.39, 128.30, 127.83, 127.69, 123.48, 96.05, 91.32, 73.91, 73.57, 72.02, 70.98, 70.62, 69.07, 68.67, 67.71, 63.20, 62.08, 60.43, 54.39, 20.81, 20.72, 20.61, 20.49, 14.20. Anal. Calcd for $C_{37}H_{41}NO_{17}$: C, 57.59; H, 5.35; N, 1.81. Found: C, 57.16; H, 5.53; N, 1.91.

O-*(3,4,6-Tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1 → 2)-3*-O-*benzyl-α,β*-D-*mannopyranosyl trichloroacetimidate* (**20**).—To a solution of **19** (264 mg,

0.342 mmol) in CH_2Cl_2 (4 mL) was added CCl_3CN (74 mg, 0.51 mmol) and DBU (16 mg, 0.10 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was loaded onto a column directly and eluted with 2:3 hexane–EtOAc to afford **20** (256 mg, 82%); $[\alpha]_D^{24}$ +3.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz): δ 8.58 (s, 1 H, NH), 7.83–7.69, 7.31–7.25 (m, 9 H, Ar), 6.07 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 5.81 (dd, 1 H, $J_{3',2'}$ 10.9, $J_{3',4'}$ 9.2 Hz, H-3'), 5.65 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1'), 5.25–5.13 (m, 2 H, H-4,4'), 4.70, 4.52 (each d, each 1 H, *J* 12.2 Hz, PhCH_2), 4.50 (dd, 1 H, H-2'), 2.05, 2.04, 1.99, 1.94, 1.88 (s, 3 H, Ac); ^{13}C NMR: δ 170.65, 170.20, 169.40, 169.20, 159.82, 137.45, 134.21, 131.41, 128.37, 127.92, 127.85, 123.57, 96.85, 95.09, 90.58, 77.27, 73.78, 72.16, 71.93, 71.28, 70.91, 70.66, 68.91, 66.74, 62.37, 62.01, 54.34, 22.64, 20.75, 20.72, 20.63, 20.50, 20.47, 14.20, 14.12. Anal. Calcd for $C_{39}H_{41}Cl_3N_2O_{17}$: C, 51.13; H, 4.51; N, 3.06. Found: C, 51.53; H, 4.78; N, 2.98.

*Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**21**).—To a solution of the allyl 4,6-*O*-benzylidene-α-D-mannopyranoside [31] (2.18 g, 7.03 mmol) in CH_2Cl_2 (10 mL) was added $CH_3C(OMe)_3$ (5.91 g, 49.2 mmol) and pyridinum *p*-toluenesulfonate (530 mg, 2.11 mmol), and the mixture was stirred at room temperature for 10 min. The mixture was diluted with $CHCl_3$, washed with aq $NaHCO_3$, dried with $MgSO_4$, and concentrated in vacuo. The residue was dissolved in 80% AcOH (35 mL) and stirred at room temperature for 15 min. The mixture was diluted with $CHCl_3$, washed with aq $NaHCO_3$, dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (1:1 hexane–EtOAc) afforded **21** (2.26 g, 91%); $[\alpha]_D^{24}$ +34.0° (*c* 0.9, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.52–7.34 (m, 5 H, Ar), 5.98–5.83 (m, 1 H, C*H* = CH_2), 5.61 (s, 1 H, PhC*H*), 5.36–5.21 (m, 2 H, CH_2), 5.25 (dd, 1 H, $J_{2,1}$ 1.6, $J_{2,3}$ 3.6 Hz, H-2), 4.85 (d, 1 H, H-1), 2.18 (s, 3 H, Ac); ^{13}C NMR: δ 170.56, 137.12, 133.17, 129.27, 128.35, 126.25, 118.00, 102.21, 97.66, 79.05, 72.18, 68.70, 68.48, 67.21, 63.47, 20.99. Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.33. Found: C, 61.47; H,6.28.

*Allyl 2-*O*-acetyl-3-*O*-[4,6-di-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranosyl]-4,6-*O*-benzylidene-α-*D*-manno-pyranoside* (**22**).—To a solution of donor **20** (75 mg, 82 μmol) and acceptor **21** (86 mg, 245 μmol) in CH_2Cl_2 was added 4A molecular sieves (130 mg), and the mixture was stirred at room temperature for 30 min. Then TMSOTf (8 μL, 41 μmol) was added to the mixture at 0°C, and stirring was continued for 30 min. After removal of undissolved materials, the filtrate was diluted with $CHCl_3$, washed with aq $NaHCO_3$, dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (1:1 hexane–EtOAc) afforded **22** (50 mg, 56%); $[\alpha]_D^{23}$ −16.0° (*c* 3.2, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.85–6.95 (m, 14 H, Ar), 5.95–5.85 (m, 1 H, C*H* = CH_2), 5.58 (dd, 1 H, $J_{3'',2''}$ 11.0, $J_{3'',4''}$ 9.3 Hz, H-3''), 5.34–5.26 (m, 2 H, CH_2), 5.16 (dd, 1 H, $J_{2,1}$ 1.3, $J_{2,3}$ 3.7 Hz, H-2), 5.07 (d, 1 H, $J_{1'',2''}$ 8.6 Hz, H-1''), 5.05 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-1'), 5.03 (dd, 1 H, $J_{4'',5''}$ 10.0 Hz, H-4''), 4.87 (dd, 1 H, $J_{4',3'} = J_{4',5'} =$ 9.3 Hz, H-4'), 4.77 (d, 1 H, H-1), 4.60, 4.39 (each d, each 1 H, *J* 12.5 Hz, PhCH_2), 4.32 (dd, 1 H, H-2''), 4.03 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.83 (dd, 1 H, $J_{4,3} = J_{4,5} = 10.0$ Hz, H-4), 2.06, 2.00, 1.98, 1.97, 1.88, 1.87 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.71, 170.53, 170.22, 169.74, 169.49, 169.16, 138.20, 137.32, 134.12, 133.08, 131.55, 130.08, 128.77, 128.32, 127.76, 127.44, 126.79, 123.56, 118.51, 102.53, 97.82, 97.61, 95.51,

79.01, 77.25, 73.85, 72.33, 71.95, 71.41, 71.21, 70.40, 70.08, 68.86, 68.68, 68.55, 67.26, 63.58, 63.05, 61.08, 54.12, 20.84, 20.74, 20.66, 20.50, 15.27. Anal. Calcd for $C_{55}H_{61}NO_{23}$: C, 59.83; H, 5.57; N, 1.27. Found: C, 59.80; H,5.67; N,1.33.

*Allyl 2-*O*-acetyl-3-*O*-[4,6-di-*O*-acetyl-2-*O*-(3,4,6 -tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**23**).—The trisaccharide **22** (511 mg, 0.463 mmol) was dissolved in 60% AcOH (12 mL), and the solution was stirred at 90°C for 1 h, then concentrated in vacuo. Purification of the residue by flash column chromatography (1:3 hexane–EtOAc) afforded **23** (335 mg, 71%); $[\alpha]_D^{24}$ +12.0° (*c* 0.7, $CHCl_3$); ^{1}H NMR (270 MHz): δ 7.82–7.69, 7.32–7.17 (m, 9 H, Ar), 5.92–5.78 (m, 1 H, $CH=CH_2$), 5.78 (dd, 1 H, $J_{3'',2''}$ 10.9, $J_{3'',4''}$ 9.2 Hz, H-3″), 5.58 (d, 1 H, $J_{1'',2''}$ 8.6 Hz, H-1″), 5.60–5.20 (m, 2 H, CH_2), 5.17 (dd, 1 H, $J_{4'',5''}$ 10.0 Hz, H-4″), 5.00–4.99 (m, 2 H, H-1′,2), 4.95 (dd, 1 H, $J_{4',3'} = J_{4',5'} = 8.6$ Hz, H-4′), 4.78 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.64, 4.53 (each d, each 1 H, *J* 12.2 Hz, CH_2), 4.42 (dd, 1 H, H-2″), 2.06, 2.04, 2.02, 2.00, 1.98, 1.97, 1.88, 1.87 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.81, 170.70, 170.24, 169.97, 169.52, 169.45, 137.99, 134.32, 133.26, 131.35, 128.26, 127.62, 127.55, 123.61, 117.91, 98.85, 96.75, 96.58, 77.29, 76.62, 76.14, 74.91, 74.27, 72.18, 72.04, 71.77, 71.30, 70.57, 69.34, 69.09, 68.25, 68.00, 67.62, 63.00, 62.39, 62.10, 54.43, 20.84, 20.74, 20.63, 20.58, 20.47. Anal. Calcd for $C_{48}H_{57}NO_{23}$: C, 56.75; H, 5.65; N, 1.38. Found: C, 56.47; H,5.64; N,1.37.

*Allyl 2-*O*-acetyl-3,6-bis-*O*-[4,6-di-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**24**).—To a solution of donor **20** (295 mg, 322 μmol) and acceptor **23** (218 mg, 215 μmol) in CH_2Cl_2 was added 4A molecular sieves (435 mg), and the mixture was stirred at room temperature for 30 min. Then $BF_3 \cdot OEt_2$ (13 μL, 110 μmol) was added to the mixture at 0°C, and stirring was continued for 3 h. The mixture was diluted with $CHCl_3$, washed with aq $NaHCO_3$, dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (1:2 hexane–EtOAc) afforded **24** (205 mg, 54%); $[\alpha]_D^{23}$ −7.0° (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CHCl_3$ 7.26 ppm): δ 7.86–7.70, 7.35–7.21 (m, 18 H, Ar), 5.78 (dd, 1 H, *J* 8.9, 10.9 Hz, GlcNAc H-3), 5.77 (dd, 1 H, *J* 9.4, 10.7 Hz, GlcNAc H-3), 5.57 (d, 1 H, *J* 8.5 Hz, GlcNAc H-1), 4.76 (bs, 1 H, Man H-1), 4.68 (d, 1 H, *J* 2.1 Hz, Man H-1), 2.06–1.86 (m, Ac); ^{13}C NMR: δ 170.85, 170.73, 170.69, 170.65, 170.60, 170.26, 170.22, 170.15, 169.77, 169.45, 169.38, 138.02, 137.97, 137.68, 134.30, 133.69, 133.24, 131.50, 131.37, 128.37, 128.32, 128.26, 127.89, 127.81, 127.76, 127.67, 127.61, 127.53, 123.54, 118.17, 99.28, 97.09, 96.71, 96.28, 77.30, 75.04, 74.61, 74.16, 73.39, 72.15, 72.09, 71.77, 71.63, 71.45, 71.30, 70.83, 70.74, 70.60, 70.57, 69.63, 69.56, 69.05, 69.00, 68.71, 68.14, 68.05, 67.96, 67.12, 66.68, 65.84, 62.91, 62.10, 61.99, 54.45, 54.38, 36.66, 29.70, 29.34, 29.20, 29.13, 27.21, 24.71, 22.70, 20.84, 20.79, 20.72, 20.65, 20.59, 20.49, 15.27, 14.12. Anal. Calcd for $C_{85}H_{96}N_2O_{39}$: C, 57.69; H, 5.47; N, 1.58. Found: C, 57.26; H,5.90; N,1.52.

*Propyl 3,6-bis-*O*-[2-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**25**).—The pentasaccharide **24** (80 mg, 45 μmol) was dissolved in 1:1 $H_2NNH_2 \cdot H_2O$–EtOH (2 mL), and the solution was stirred at 100°C for 12 h, then concentrated in vacuo. The residue was dissolved in 1:1 acetic anhydride–pyridine (2 mL) containing a catalytic amount of DMAP. After stirring for

44 h, the mixture was concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc), gave an *N*-acetylated pentasaccharide, to a solution of which in dry MeOH (1 mL) was added NaOMe (8 mg), and the mixture was stirred at 40°C for 18 h. Undissolved materials were filtered off, and the filtrate was concentrated in vacuo. To a solution of the residue in MeOH (1 mL) was added 20% Pd–C (50 mg) and the mixture was stirred at 60°C for 2 h under H_2. The mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. Purification of the residue by gel permeation chromatography (Sephadex G-15, 1.0 × 80 cm, water) afforded **25** (16 mg, 37%); $[\alpha]_D^{26}$ +16.0° (*c* 0.5, H_2O); ^{1}H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 5.16, 4.95, 4.87 (bs, 1 H, Man H-1), 4.60 (bd, 2 H, *J* 8.6 Hz, GlcNAc H-1 × 2), 2.09, 2.08 (each s, each 3 H, Ac), 1.67–1.63 (m, 2 H, CH_2), 0.96 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C NMR (D_2O): δ 175.59, 134.87, 100.66, 100.45, 100.37, 100.23, 97.57, 79.42, 77.37, 77.14, 76.62, 74.23, 74.09, 73.65, 72.00, 70.65, 70.44, 70.20, 68.05, 66.43, 66.30, 62.39, 61.42, 56.13, 23.11, 22.75, 20.43, 10.73; HRFABMS (positive-ion): Calcd for $C_{37}H_{64}N_2NaO_{26}$: 975.3645. Found: 975.3698.

Enzymatic preparation of pentasaccharide **30** *with two 6-deoxy-*D*-galactosyl residues.*—A solution containing the donor **5** (17.6 mg, 30 μmol), acceptor **17** (6 mg, 10 μmol), bovine serum albumin (2 mg), alkaline phosphatase (100 units), β-(1 → 4)-galactosyltransferase (3 units), and $MnCl_2$ (40 mM) in HEPES buffer (0.1 M, pH 7.0, total volume 400 μL) was incubated at 37°C for 48 h. Workup and purification of the product in the manner as described for the preparation of **10** afforded the pentasaccharide **30** (4.9 mg, 55%); $[\alpha]_D^{26}$ −28.0° (*c* 0.3, H_2O); ^{1}H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 4.57, 4.53 (each d, each 1 H, *J* 7.8 Hz, GlcNAc H-1 × 2), 4.43–4.41 (bd, 2 H, Gal H-1 × 2), 4.21 (d, 1 H, *J* 10.7 Hz, Man H-6), 4.05 (bs, 1 H, Man H-2), 2.06, 2.03 (each s, each 3 H, Ac), 1.65–1.60 (bq, 2 H, OCH_2–), 1.26 (d, 6 H, *J* 6.3 Hz, Gal-6Me × 2), 0.94 (t, 3 H, *J* 7.6 Hz, CH_3); ^{13}C NMR (D_2O): δ 175.40, 174.93, 103.77, 102.19, 100.34, 97.43, 80.09, 79.91, 77.52, 76.64, 75.38, 74.03, 73.46, 73.19, 72.70, 72.40, 71.93, 71.84, 71.43, 70.92, 70.71, 70.46, 70.17, 68.32, 60.93, 56.26, 55.74, 23.21, 23.02, 22.71, 16.08, 10.85; HRFABMS (positive-ion): Calcd for $C_{37}H_{65}N_2O_{24}$: 921.3928. Found: 921.3940.

^{1}H NMR data for the GlcNAc residues of the peracetylated derivative of **30** (500 MHz, $CDCl_3$): δ 7.13 (d, 1 H, *J* 6.9 Hz, GlcNAc NH_a), 6.49 (d, 1 H, *J* 9.9 Hz, GlcNAc NH_b), 5.83 (dd, 1 H, *J* 10.7, 8.7 Hz, GlcNAc H-3_a), 5.32 (d, 1 H, *J* 8.2 Hz, GlcNAc H-1_a), 5.10–5.03 (m, GlcNAc H-3_b), 4.48 (bd, 1 H, GlcNAc H-6_a), 4.29 (bd, 1 H, GlcNAc H-6_a), 4.20 (dd, 1 H, *J* 6.3, 11.6 Hz, GlcNAc H-6_b), 4.16–4.05 (m, GlcNAc H-2_b), 3.78–3.70 (m, GlcNAc H-4_b), 3.68–3.49 (m, GlcNAc H-4_a,5_a,5_b), 2.87 (ddd, 1 H, GlcNAc H-2_a).

Enzymatic preparation of heptasaccharide **31** *with two 6-deoxy-*D*-galactosyl residues.*—Enzymic 6-deoxy-D-galactosylation of pentasaccharide acceptor **25** (10 mg, 10.5 μmol) in the manner as described in the preparation of **30** afforded the heptasaccharide **31** (7.5 mg, 57%); $[\alpha]_D^{24}$ +13.0° (*c* 0.7, H_2O); ^{1}H NMR (500 MHz, D_2O, 303 K): δ 5.12 (bs, 1 H, Man H-1), 4.91 (d, 1 H, *J* 1.4 Hz, Man H-1), 4.84 (d, 1 H, *J* 1.5 Hz, Man H-1), 4.59 (d, 1 H, *J* 7.6 Hz, GlcNAc H-1), 4.58 (d, 1 H, *J* 6.8 Hz, GlcNAc H-1), 4.42 (d, 2 H, *J* 7.8 Hz, Gal H-1 × 2), 4.18 (dd, 1 H, *J* 3.4 Hz, Man H-2), 4.11 (dd, 1 H, *J* 3.4 Hz, Man H-2), 4.09 (bd, 1 H, Man H-2), 2.05 (s, 6 H, Ac), 1.62 (m, 2

H, CH_2), 1.25 (d, 6 H, *J* 6.3 Hz, Gal H-6 × 2), 0.92 (t, 3 H, *J* 7.4 Hz, CH_3); ^{13}C NMR (D_2O): δ 175.47, 103.75, 100.64, 100.16, 79.92, 79.39, 77.34, 77.12, 75.38, 73.65, 73.46, 72.77, 72.02, 71.91, 71.82, 71.43, 70.63, 70.44, 68.05, 66.47, 66.29, 62.39, 60.79, 55.59, 31.00, 23.09, 22.73, 16.06, 10.69; HRFABMS (positive-ion): Calcd for $C_{49}H_{84}N_2NaO_{34}$: 1267.4804. Found: 1267.4879.

^1H NMR data for the GlcNAc residues of the peracetylated derivative of **31** (500 MHz, $CDCl_3$): δ 5.82 (d, 1 H, *J* 7.6 Hz, GlcNAc NH_a), 5.69 (d, 1 H, *J* 8.8 Hz, GlcNAc NH_b), 5.29 (dd, 1 H, *J* 8.5 Hz, GlcNAc H-3_a), 5.16–5.09 (m, GlcNAc H-3_b), 4.73 (d, 1 H, *J* 6.9 Hz, GlcNAc H-1_a), 4.59 (d, 1 H, *J* 7.5 Hz, GlcNAc H-1_b), 3.70–3.62 (m, GlcNAc H-4_a, 4_b).

Enzymatic preparation of a heptasaccharide **32** *with two* D-*galactosyl residues.*— Enzymic galactosylation of pentasaccharide acceptor **25** (4.1 mg, 4.30 μmol) using one-fourth the amount of β-(1 → 4)-galactosyltransferase in a similar manner to the preparation of **30** afforded the heptasaccharide **32** (2.2 mg, 41%); $[\alpha]_D^{24}$ +22.0° (*c* 0.2, H_2O); ^1H NMR (270 MHz, D_2O, HOD 4.81 ppm): δ 5.18 (bs, 1 H, Man H-1), 4.97 (bs, 1 H, Man H-1), 4.89 (bs, 1 H, Man H-1), 4.63 (bd, 2 H, GlcNAc H-1 × 2), 4.52 (d, 2H, *J* 7.6 Hz, Gal H-1 × 2), 2.10 (s, 6 H, Ac × 2), 1.72–1.64 (m, 2 H, CH_2), 0.97 (t, 3 H, *J* 7.3 Hz, CH_3); HRFABMS (positive-ion): Calcd for $C_{49}H_{84}N_2NaO_{36}$: 1299.4702. Found: 1299.4771.

Transfer assay of 6-modified galactosyl residues.—The transfer reaction was carried out in 60 mM HEPES buffer (pH 7.0) of 100 μL final volume containing GlcNAc **7** (20 mM), donor (150 mM) **5** or **6**, $MnCl_2$ (10 mM), alkaline phosphatase (25 U), and β-(1 → 4)-galactosyltransferase (7 mU). On the contrary, only 0.07 mU of β-(1 → 4)-galactosyltransferase was used for the galactosyl transfer from UDP-Gal. The mixture was incubated at 37°C until 15% of the donor was consumed. The liberated uridine was quantitated by HPLC (Asahipak GS 320 column with 200 mM phosphate buffer, pH 3.0). The relative transfer rates of UDP-6-deoxy- and 6-deoxy-6-fluoro-D-galactoses were 1.3 and 0.2% of D-galactosyl transfer [25].

Assay of D-*[^{14}C]-galactose transfer to asialo agalacto* α_1*-acid glycoprotein.*—The transfer reaction was performed in HEPES buffer (39 mM, pH 7.0, 51 μL final volume) containing UDP-D-[^{14}C]-galactose (392 μM, 6.1×10^4 cpm), asialo agalacto α_1-acid glycoprotein (52 μM), $MnCl_2$ (4 mM), KCl (78 mM), β-(1 → 4)-galactosyltransferase (1 mU), and Nonidet p-40 (0.1%). The mixture was incubated at 37°C for 60 min, and the reaction was stopped by heating the mixture at 55°C. The mixture was passed through a gel-permeation column (1 cm × 25 cm, Sephadex G-50, 0.1 M CH_3COONH_4), and then the isotope counts of the void fractions (2.5–4.5 mL) were corrected by measuring the absorbance of OD_{280} and measured with a liquid scintillation counter. For the negative control, the mixture without β-(1 → 4)-galactosyltransferase was used. The isotope counts after 0.5, 1, and 2 h incubations were measured with 3 data points for each time, and the transferred D-[^{14}C]-galactose was estimated to be 5.2, 8.6, and 10 equiv/protein, respectively.

Assay of 6-deoxygalactosyl transfer to asialo agalacto α_1*-acid glycoprotein.*—The transfer reaction was performed in HEPES buffer (39 mM, pH 7.0, 1030 μL final volume) containing UDP-6-deoxy-D-galactose (3.9 mM), asialo agalacto α_1-acid glycoprotein (52 μM), $MnCl_2$ (4 mM), KCl (78 mM), β-(1 → 4)-galactosyltransferase (200

mU), alakaline phosphatase (200 U), and Nonidet p-40 (0.1%). The mixture was incubated at 37°C for 18 h. After incubation, the mixture was passed through the same gel-permeation column as above, and the void fractions were corrected and lyophilized. Thus was obtained the asialo α_1-acid glycoprotein containing 6-deoxygalactose, which was then used as a D-[^{14}C]-galactosyl acceptor. The second incubation, i.e., the D-[^{14}C]-galactosyl transfer, was performed under identical conditions as that for the assay of D-[^{14}C]-galactosyl transfer to asialo agalacto α_1-acid glycoprotein. The isotope count of the purified glycoprotein was measured by liquid scintillation to be 5 equiv/protein. Thus the equivalents of 6-deoxy-D-alactose transferred to the protein was estimated to be 5.

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