Synthesis of Fluorescence-Labeled Galβ1-3Fuc and Galβ1-4Fuc as Probes for the Endogenous Glyco-Epitope Recognized by Galectins in *Caenorhabditis elegans*

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To search for the endogenous glyco-epitope in *Caenorhabditis elegans*, we synthesized labeled Galβ1-3Fuc and Galβ1-4Fuc and examined their binding affinity for *C. elegans* galectin LEC-6 using frontal affinity chromatography analysis. We developed a new strategy for synthesizing the labeled saccharides, in which the labeling unit, the 2-aminopyridine moiety, is coupled with a spacer unit derived from D-mannitol. Our results indicate that Galβ1-4Fuc is the endogenous glyco-epitope present in *C. elegans N*-glycans.

Key words galectin; 2-aminopyridine; frontal affinity chromatography

Galectins are a family of carbohydrate-binding proteins defined by their affinity for β-galactosides-containing glycoconjugates.[1—3] Their binding to saccharides results in various biological phenomena including development, immunity, and tumor metastasis.[4] It is known that a Galβ1-4GlcNAc (*N*-acetyllactosamine) unit is the endogenous glyco-epitope recognized by vertebrate galectins.[5—8] Among galectins from invertebrate species including *Caenorhabditis elegans*, specificity for *N*-acetyllactosamine was reported, although the existence of the glycan containing *N*-acetyllactosamine units has not been confirmed in *C. elegans*.[9—14] This discrepancy raised a question concerning the endogenous glyco-epitope that is recognized by galectins in *C. elegans*. To search for it, Takeuchi *et al.* isolated *N*-type glycoproteins bound by *C. elegans* galectin LEC-6.[15] Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)/TOF analysis in conjunction with glycosidase digestion elucidated that the glycoproteins contain a Gal-Fuc disaccharide unit attached to the innermost GlcNAc (*N*-acetylglucosamine) residue. Among several Gal-Fuc units, in which D-galactose and L-fucose are differentially linked at some positions, we assumed that Galβ1-4Fuc should be the endogenous glyco-epitope because its presence had been confirmed in *C. elegans N*-glycans.

In this study, we synthesized Galβ1-3Fuc and Galβ1-4Fuc derivatives and examined their binding affinity for galectin LEC-6 using frontal affinity chromatography (FAC) analysis.[16] For FAC analysis, saccharides must be labeled with a fluorescent agent. Recently, pyridylamination has been widely used as a labeling method for saccharides because of its high sensitivity.[17—19] This method, however, has a disadvantage in terms of efficiency: the yield is low in the reductive amination for introducing the pyridylamino group at the reducing end of the saccharide. It is even more disadvantageous that this reductive amination requires an aldehyde at the anomeric position of the sugar, which causes the loss of the characteristic nature of the pyranose ring at the reducing end. Therefore we investigated another method for obtaining fluorescence-labeled Gal-Fuc derivatives. In our strategy, the fluorescence unit, the 2-aminopyridine moiety, is coupled with Gal-Fuc *via* a water soluble C6 spacer derived from D-mannitol. Here we describe the synthesis of novel fluorescence-labeled Gal-Fuc derivatives (**1**, **2**) (Fig. 1).

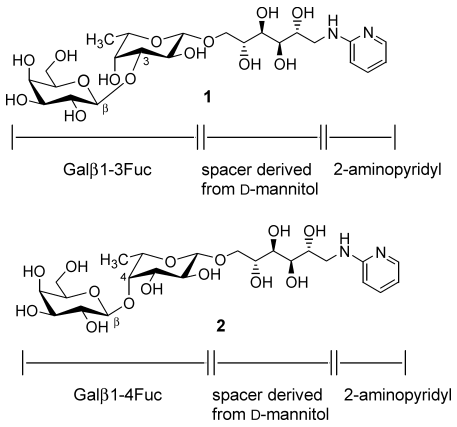

Fig. 1. Structures of Fluorescence-Labeled Gal-Fuc Derivatives (**1**, **2**)

Results and Discussion

Synthesis of Protected Galβ1-3Fuc (14) and Galβ1-4Fuc (17) As shown in Chart 1, allyl α-L-fucopyranoside (**3**)[20] was chosen as the starting material for the synthesis of Galβ1-3Fuc (**14**) and Galβ1-4Fuc (**17**). Using Kamerling conditions,[21] its transformation into cyclic 3,4-*O*-orthoacetate, followed by 2-*O*-acetylation and regioselective orthoester opening by acidic hydrolysis, provided allyl 2,4-*O*-diacetyl-α-L-fucopyranoside (**6**). Similarly, regioselective 4-*O*-acetylation, followed by 2,3-dibenzoylation and deacetylation, provided allyl 2,3-*O*-dibenzoyl-α-L-fucopyranoside (**10**).

In the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf), coupling of the L-fucosyl acceptor **6** with the 2,3,4,5-tetra-*O*-acetylated galactosyl donor **11**, which was expected to be an efficient galactosylating agent because of neighboring group participation,[22] proceeded smoothly to give only the β-linked disaccharide **12** (Chart 2). *O*-Deallylation of **12** with $Pd(PPh_3)_4$, followed by trichloroacetimidation of the hemiacetal formed **13** with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), gave the Galβ1-3Fuc donor **14**.[23]

Similar to the coupling of compounds **11** and **6**, the L-

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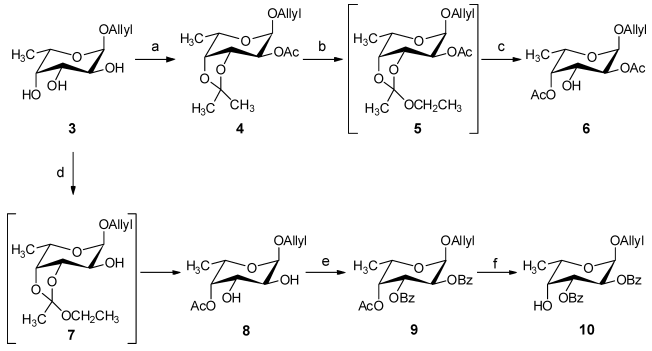
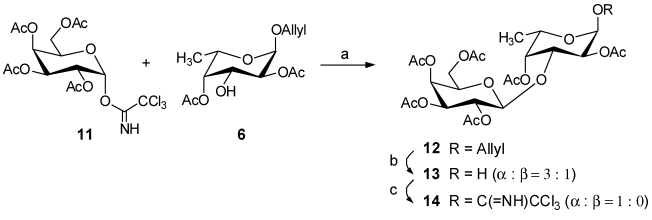


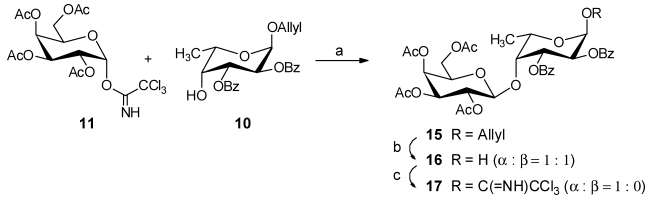
Reagents and conditions: (a) 1) 2,2-dimethoxypropane, *p*-TsOH, DMF, r.t.; 2) Ac_2O, pyridine, r.t., 45% (2 steps); (b) 1) TFA, CH_2Cl_2/H_2O, r.t.; 2) $(EtO)_3CMe$, *p*-TsOH, DMF, r.t.; (c) AcOH, H_2O, r.t., 90% (3 steps); (d) $(EtO)_3CMe$, *p*-TsOH, DMF, r.t.; (e) BzCl, pyridine, r.t. 75% (2 steps); (f) AcCl, MeOH/CH_2Cl_2, r.t., 57%.

Chart 1. Synthesis of Compounds **6** and **10**



Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS 4 Å, 0 °C, 42%; (b) $Pd(PPh_3)_4$, AcOH, 80 °C, 51%; (c) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 49%.

Chart 2. Synthesis of Compound **14**



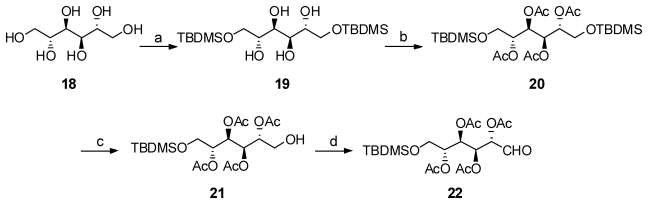
Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS 4 Å, 0 °C, 48%; (b) $Pd(PPh_3)_4$, AcOH, 80 °C, 78%; (c) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 97%.

Chart 3. Synthesis of Compound **17**

fucosyl acceptor **10** reacted with the galactosyl donor **11** to give the β-linked disaccharide **15** (Chart 3). Its transformation into the glycosyl donor **17** was performed in the same manner as described above for the preparation of **14**.

Synthesis of the Spacer Moiety (22) from D-Mannitol Next, we prepared a spacer moiety of the labeled Gal-Fuc derivatives (Chart 4). We chose commercially available D-mannitol as the starting material for this. Primary hydroxyl groups of D-mannitol (**18**) were protected with *t*-butyldimethylsilyl (TBDMS) to give the tetra-ol **19**. Peracetylation of **19**, followed by selective deprotection of TBDMS, afforded the alcohol **21**.[24] To introduce the 2-aminopyridyl group by reductive amination,[25—27] **21** was converted into an aldehyde (**22**) by oxidation.

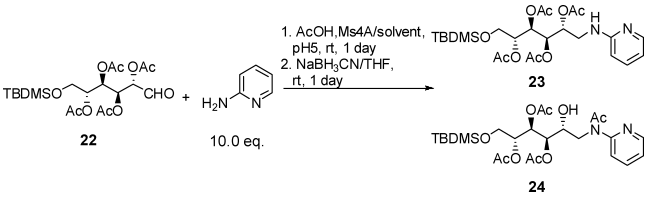
Coupling of the Spacer Moiety with the Aminopyridyl Group by Reductive Amination After preparing the spacer unit **22**, the next step was introducing the aminopyridyl group into it. Unfortunately, reductive amination was a difficult task in this case. As shown in Table 1, an excess amount of 2-aminopyridine was necessary owing to the reduced reactivity of the amino group connected to the aromatic pyridine ring. This is the essential problem in this reaction. Therefore we attempted to develop conditions conductive to the subsequent coupling of the aldehyde **22** and 2-aminopyridine (Table 1). After confirming that the appropriate acidity (pH 5) was necessary for this reaction, it was also found that the solvent and concentration were important. When the alcohols, CH_2Cl_2, and CH_3CN were used as the solvent, satisfactory yields were not obtained (Table 1, entries 1—4), although Et_2O, THF, and DMF appeared promising (entries 5—10). In particular, at a rather high dilution in DMF, the best yield was obtained (entry 9). A detailed examination of the reaction in these solvents allowed us to isolate the minor product **24**, in which the acetyl group migrated to the nucleophilic amino group. It is likely that **24** was formed from **23** during the reaction. We therefore evaluated **24** as a useful intermediate for the synthesis of the labeling unit **26**. Thus a mixture of **23** and **24** was acetylated to give the single compound **25**. The TBDMS group of **25** was deprotonated to form the labeling agent **26** (Chart 5).



Reagents and conditions: (a) TBDMSCl, imidazole, DMF, 0 °C, 79%; (b) Ac_2O, pyridine, r.t., 88%; (c) 1% I_2/MeOH, r.t., 42%; (d) DMSO, $(COCl)_2$, TEA, CH_2Cl_2, −78 °C, 97%.

Chart 4. Synthesis of Compound **22**

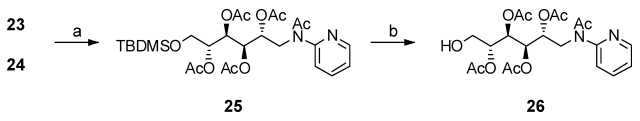
Table 1. Reductive Amination of **22** with 2-Aminopyridine



Entry	Solvent	Concentration (M)	Yield (%)	Ratio **23** : **24**
1	MeOH	0.1	33	1 : 0[a]
2	EtOH	0.1	28	1 : 0[a]
3	CH_2Cl_2	0.1	25	1 : 0[a]
4	CH_3CN	0.1	Trace	
5	Et_2O	0.1	39	1 : 0.3[a]
6	THF	0.1	55	1 : 0.2[a]
7	THF	0.5	25	1 : 0.5[a]
8	DMF	0.1	45	1 : 0.3[b]
9	DMF	0.05	68	1 : 0.3[b]
10	DMF	0.01	51	1 : 0.2[b]

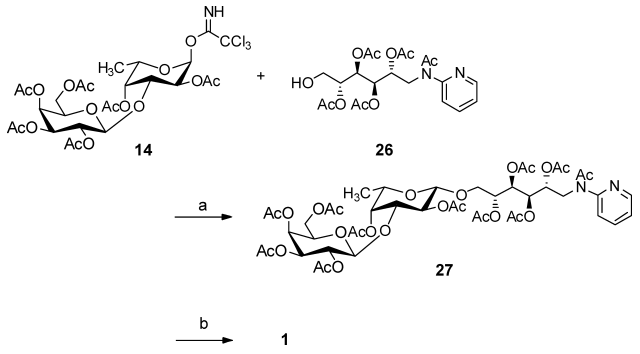
a) Determined by ^{1}H-NMR, *b*) after separation by column chromatography.

Synthesis of Fluorescence-Labeled Gal-Fuc Derivatives (1, 2) For the synthesis of labeled Galβ1-3Fuc and Galβ1-4Fuc, the labeling agent **26** was reacted with Gal-Fuc **14** in the presence of TMSOTf to give **27**. Fortunately, the glycosylation occurred only at the hydroxyl group without occurring at the pyridine-nitrogen, which may be ascribed to a decrease in the nucleophilicity of the nitrogen.[28] Finally, deprotection of acetyl groups gave the target labeled Galβ1-3Fuc **1** (Chart 6). Labeled Galβ1-4Fuc **2** was prepared in a similar manner (Chart 7).



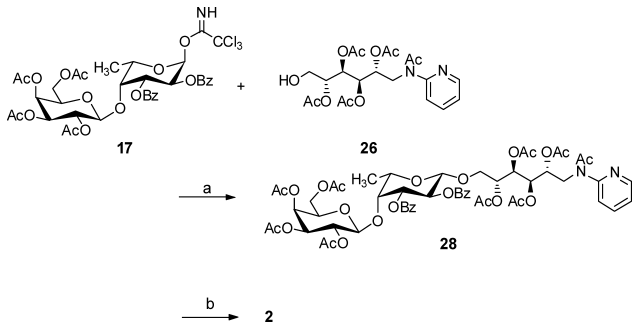
Reagents and conditions: (a) Ac_2O, pyridine, 80 °C, 62%; (b) HF·pyridine, pyridine/THF, r.t., 86%.

Chart 5. Synthesis of Compound **26**



Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS 4 Å, 0 °C, 55%; (b) NaOMe, MeOH, r.t., 96%.

Chart 6. Synthesis of Compound **1**



Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS 4 Å, 0 °C, 34%; (b) NaOMe, MeOH, r.t., 96%.

Chart 7. Synthesis of Compound **2**

The binding affinity of the labeled Galβ1-3Fuc **1** and Galβ1-4Fuc **2** for galectin LEC-6 was examined in FAC analysis. Using an immobilized LEC-6, retardation of the labeled Galβ1-4Fuc **2** was significantly greater than that of the labeled Galβ1-3Fuc **1**, the details of which have been reported in a separate paper.[29] The results support our assumption that Galβ1-4Fuc is the endogenous glyco-epitope present in *C. elegans N*-glycans.

Conclusion

We synthesized labeled Galβ1-3Fuc and Galβ1-4Fuc and examined their binding affinity for galectin LEC-6 using FAC analysis. To improve the pyridylamination method, we developed a new strategy, in which the labeling unit, the 2-aminopyridine moiety, is coupled with a spacer unit derived from D-mannitol. Our results indicate that Galβ1-4Fuc is the endogenous glyco-epitope present in *C. elegans N*-glycans.[29]

Experimental

General Procedures All reactions sensitive to air or moisture were conducted under an argon atmosphere. Materials were obtained from commercial suppliers. All anhydrous solvents were purified according to standard methods. The NMR spectra (^{1}H, ^{13}C) were determined on a JEOL 600 MHz (ECP-600) or 400 MHz (AL-400) spectrometer, using $CDCl_3$ (with TMS for ^{1}H-NMR and chloroform-*d* for ^{13}C-NMR as the internal reference) solution, unless the otherwise noted. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane as an internal standard and coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), broad (br). High resolution mass spectra (HR-MS) were obtained on LCMS-IT-TOF (Shimadzu, Kyoto, Japan) for electrospray ionization (ESI). Sodium trifluoroacetate (TFA-Na) was used as internal standard for high resolution MS. Optical rotations were determined using a DIP-370 (Shimadzu, Kyoto, Japan) digital polarimeter in 100-mm cells and the sodium D line (589 nm) at room temperature in the solvent and concentration indicated. Infrared spectra (IR) were recorded on a JASCO FT/IR-410 spectrometer using sodium chloride plates or potassium bromide pellets. Absorbance frequencies are recorded in reciprocal centimeters (cm^{-1}). Analytical thin layer chromatography was carried out using Merck silica gel 60 F_{254}. Column chromatography was used silica gel Wakogel C-300 (45—60 μm), NH silicagel (Chromatorex® FujiSilysia Chemical, 100—200 mesh). Reverse phase Column chromatography was used ODS-SS10200T.

Allyl 2-*O*-Acetyl-3,4-*O*-isopropylidene-α-L-fucopyranoside (4) To a solution of **3** (200 mg, 0.98 mmol) in DMF (1 ml) were added 2,2-dimethoxypropane (0.6 ml, 4.9 mmol) and *p*-toluenesulfonic acid (*p*-TsOH) (30 mg). The mixture was stirred at room temperature for 12 h, yielding a new product (*Rf*=0.42) as indicated by TLC (EtOAc/hexane, 1 : 1). Then, the solution was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 and concentrated. A solution of the residue in pyridine/acetic anhydride (2 ml, 3 : 1) was stirred for 12 h, when TLC (EtOAc/hexane, 1 : 1) showed the formation of **4** (*Rf*=0.72). The mixture was concentrated using toluene as an azeotropic solvent, and column chromatography (EtOAc/hexane, 1 : 3) of the residue afforded **4** (127 mg, 45%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.33 (3H, d, *J*=6.8 Hz), 1.32 (3H, s), 1.50 (3H, s), 2.09 (3H, s), 3.96 (1H, m), 4.06 (1H, dd, *J*=2.4, 5.2 Hz), 4.13 (2H, m), 4.30 (1H, dd, *J*=5.2, 8.0 Hz), 4.87 (1H, dd, *J*=3.6, 8.0 Hz), 4.92 (1H, d, *J*=3.6 Hz), 5.17 (1H, m), 5.26 (1H, m), 5.84 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16.3, 21.1, 26.4, 28.1, 63.2, 68.3, 72.0, 73.4, 76.1, 95.1, 109.3, 117.4, 133.5, 170.5. IR (neat) cm^{-1}: 2988, 1741, 1373, 1238, 1130, 1080, 1059, 758. HR-MS (ESI) *m/z*: 309.1314 (M+Na), Calcd for 309.1309, $C_{14}H_{22}O_6Na$ (M+Na). $[\alpha]_D^{24}$ −191.5 (*c*=0.6, $CHCl_3$).

Allyl 2,4-Di-*O*-acetyl-α-L-fucopyranoside (6) To a solution of **4** (80 mg, 0.28 mmol) in CH_2Cl_2 (2 ml) and water (0.2 ml), trifluoroacetic acid (0.21 ml, 2.8 mmol) was added. The mixture was stirred for 45 min at room temperature (removal of the isopropylidene group (*Rf*=0.07) was observed on TLC). The solution was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried and concentrated. To a solution of the residue in dry DMF (1 ml) and trimethyl orthoacetate (77 μl, 0.42 mmol), *p*-TsOH (5.7 mg, 0.03 mmol) was added. After 1 h, 80% acetic acid (2 ml) was added and stirred for 40 min, the mixture was co-concentrated with toluene and a solution of the residue in CH_2Cl_2 was washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. Then column chromatography (EtOAc/hexane, 1 : 2 to 1 : 1) of the residue afforded **6** (73 mg, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.13 (3H, d, *J*=6.4 Hz), 2.12 (3H, s), 2.17 (3H, s), 3.99 (1H, m), 4.12 (2H, m), 4.23 (1H, dd, *J*=3.6, 10.4 Hz), 4.96 (1H, dd, *J*=4.0, 10.4 Hz), 5.03 (1H, d, *J*=4.0 Hz), 5.19 (1H, m), 5.23 (1H, m), 5.30 (1H, m), 5.86 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16.2, 20.9, 21.1, 64.9, 67.1, 68.6, 71.5, 73.7, 95.4, 117.5, 133.5, 171.1, 171.2. IR (neat) cm^{-1}: 3462, 2986, 1741, 1433, 1373, 1236, 1132, 1099, 1059, 756. HR-MS (ESI) *m/z*: 311.1106 (M+Na), Calcd for 311.1101, $C_{13}H_{20}O_7Na$ (M+Na). $[\alpha]_D^{24}$ −178.9 (*c*=1.0, $CHCl_3$).

Allyl 4-*O*-Acetyl-2,3-di-*O*-benzoyl-α-L-fucopyranoside (9) To a stirred solution of allyl fucoside **3** (500 mg, 2.5 mmol) in DMF (7 ml) was added triethyl orthoacetate (540 ml, 3.0 mmol) and *p*-TsOH (33 mg, 0.17 mmol). After 30 min (the starting material disappeared by TLC monitoring), the solution was poured into H_2O, and extracted with Et_2O. The combined organic layer was dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 1) to give **8**. To a stirred solution of **8** in pyridine (10 ml) was added BzCl (0.71 ml, 6.1 mmol). The reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was poured into H_2O, and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 3 : 2) to give **9** (838 mg, 75%, 2 steps).

Compound **8**: ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.11 (3H, d, *J*=6.6 Hz), 2.14 (3H, s), 3.76 (1H, dd, *J*=3.6, 9.6 Hz), 3.95 (1H, dd, *J*=3.6, 9.6 Hz), 4.03 (2H, m), 4.18 (1H, m), 4.92 (1H, d, *J*=3.6 Hz), 5.18 (1H, dd, *J*=1.8, 3.6 Hz), 5.20 (1H, m), 5.28 (1H, m), 5.52 (1H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.1, 20.8, 65.2, 68.8, 69.4, 69.8, 73.1, 97.6, 117.9, 133.5, 171.3.

IR (neat) cm^{-1}: 3649, 3422, 2988, 1734, 1421, 1375, 1246, 1132, 1082, 1039, 756. HR-MS (ESI) *m/z*: 269.0981 (M+Na), Calcd for 269.0996, $C_{11}H_{18}O_6Na$ (M+Na). $[\alpha]_D^{20}$ −171.1 (*c*=0.3, $CHCl_3$).

Compound **9**: ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.22 (3H, d, *J*=6.6 Hz), 2.17 (3H, s), 4.05 (1H, m), 4.24 (1H, m), 4.34 (1H, m), 5.16 (1H, m), 5.28 (1H, d, *J*=3.6 Hz), 5.31 (1H, m), 5.54 (1H, dd, *J*=1.2, 3.6 Hz), 5.59 (1H, dd, *J*=3.6, 10.8 Hz), 5.85 (2H, m), 7.37 (4H, m), 7.51 (2H, m), 7.90 (2H, m), 7.99 (2H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.9, 20.6, 64.7, 68.7, 68.8, 68.9, 71.4, 95.7, 117.5, 128.3 (×2), 128.4 (×2), 129.5 (×2), 133.1 (×2), 133.3 (×2), 133.5, 134.5 (×2), 165.6, 166.1, 170.4. IR (neat) cm^{-1}: 2986, 2939, 1747, 1726, 1421, 1373, 1280, 1228, 1130, 1072, 1032, 758, 711. HR-MS (ESI) *m/z*: 477.1532 (M+Na), Calcd for 477.1520, $C_{25}H_{26}O_8Na$ (M+Na). $[\alpha]_D^{20}$ −160.8 (*c*=0.5, $CHCl_3$).

Allyl 2,3-Di-*O*-benzoyl-α-L-fucopyranoside (10) To a stirred solution of **9** (118 mg, 0.3 mmol) in CH_2Cl_2 (0.5 ml) were added AcCl (0.3 ml) and MeOH (2.2 ml). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was poured into H_2O, and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 4) to give **10** (61 mg, 57%) and recovery of **9** (20 mg, 17%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.35 (3H, d, *J*=6.6 Hz), 4.05 (1H, m), 4.16 (1H, br), 4.22 (1H, m), 4.26 (1H, m), 5.15 (1H, m), 5.26 (1H, d, *J*=3.6 Hz), 5.30 (1H, m), 5.64 (1H, dd, *J*=3.6, 10.8 Hz), 5.73 (1H, dd, *J*=3.0, 10.8 Hz), 5.85 (1H, m), 7.38 (4H, m), 7.51 (2H, m), 7.99 (4H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.0, 65.5, 68.6, 68.7, 70.8, 71.7, 95.7, 117.3, 128.4 (×2), 128.5 (×2), 129.7 (×2), 129.8 (×2), 133.2, 133.3 (×2), 133.6 (×2), 165.8, 166.1. IR (neat) cm^{-1}: 3649, 3067, 2984, 1718, 1280, 1165, 1111, 1070, 1030, 758, 711. HR-MS (ESI) *m/z*: 435.1427 (M+Na), Calcd for 435.1414, $C_{23}H_{24}O_7Na$ (M+Na). $[\alpha]_D^{20}$ −152.6 (*c*=1.0, $CHCl_3$).

Allyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-fucopyranoside (12) To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-2,2,2-trichloroacetimidate **11** (1.82 g, 3.7 mmol), **6** (710 mg, 2.5 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (24 ml) was added TMSOTf (223 μl, 1.23 mmol) at 0 °C under Ar. The mixture was stirred for 1.5 h at 0 °C, and then neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic phase was dried, filtered, and concentrated. The residue was purified by silica gel chromatography (EtOAc/toluene, 1 : 2) to yield **12** (645 mg, 42%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.12 (3H, d, *J*=6.6 Hz), 1.97 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 2.14 (3H, s), 3.91 (1H, m), 4.01 (1H, m), 4.07 (2H, m), 4.15 (1H, m), 4.23 (2H, m), 4.56 (1H, d, *J*=7.8 Hz), 4.98 (1H, dd, *J*=3.6, 10.2 Hz), 5.04 (1H, d, *J*=3.6 Hz), 5.08 (1H, dd, *J*=7.8, 10.2 Hz), 5.12 (1H, dd, *J*=3.6, 10.2 Hz), 5.21 (1H, m), 5.25 (1H, m), 5.30 (1H, m), 5.36 (1H, dd, *J*=1.2, 3.6 Hz), 5.87 (1H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.9, 20.4, 20.5, 20.5, 20.7, 20.7, 60.9, 64.8, 66.7, 68.4, 68.5, 68.8, 70.3, 70.9, 71.1, 74.0, 95.6, 99.9, 117.5, 133.5, 169.3, 169.9 (×2), 170.0, 170.3, 170.5. IR (neat) cm^{-1}: 3024, 2986, 2941, 1747, 1433, 1371, 1232, 1167, 1132, 1079, 756. HR-MS (ESI) *m/z*: 641.2065 (M+Na), Calcd for 641.2052, $C_{27}H_{38}O_{16}Na$ (M+Na). $[\alpha]_D^{24}$ −70.4 (*c*=0.7, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-acetyl-L-fucopyranose (13) To a stirred solution of **12** (645 mg, 1.0 mmol) in HOAc (7.0 ml) was added $Pd(PPh_3)_4$ (361 mg, 0.31 mmol). The reaction mixture was stirred at 80 °C for 1.5 h and cooled to room temperature, to which was added toluene and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 2 : 3) to give **13** (305 mg, 51%, $\alpha:\beta$=3 : 1).

Compound **13α**: ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.13 (3H, d, *J*=10.2 Hz), 1.97 (3H, s), 2.06 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 2.12 (3H, s), 2.14 (3H, s), 3.91 (1H, m), 4.09 (1H, m), 4.21 (1H, m), 4.27 (1H, dd, *J*=4.8, 15.6 Hz), 4.30 (1H, m), 4.57 (1H, d, *J*=11.4 Hz), 4.98 (1H, dd, *J*=5.4, 15.6 Hz), 5.09 (1H, dd, *J*=11.4, 15.6 Hz), 5.15 (1H, dd, *J*=5.4, 15.6 Hz), 5.27 (1H, m), 5.37 (1H, d, *J*=5.4 Hz), 5.43 (1H, t, *J*=5.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.9, 20.5, 20.6, 20.6, 20.7, 20.8, 20.9, 60.9 65.0, 66.8, 68.9, 68.9, 70.4, 70.9, 71.2, 73.6, 91.0, 100.1, 169.4, 170.1, 170.1, 170.3, 170.4, 170.6. IR (neat) cm^{-1}: 3462, 2988, 1749, 1373, 1236, 1078, 760. HR-MS (ESI) *m/z*: 601.1736 (M+Na), Calcd for 601.1739, $C_{24}H_{34}O_{16}Na$ (M+Na). $[\alpha]_D^{24}$ −47.2 (*c*=0.6, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-fucopyranosyl Trichloroacetimidate (14) To a stirred solution of **13** (80 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (1.4 ml) was added trichloroacetonitrile (111 μl, 1.1 mmol) and DBU (4.5 μl, 0.03 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C. The solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 1) to give **14** (49 mg, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.17 (3H, d, *J*=6.0 Hz), 1.97 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.14 (3H, s), 2.15 (3H, s), 3.92 (1H, m), 4.11 (2H, m), 4.26 (1H, m), 4.33 (1H, dd, *J*=3.6, 10.4 Hz), 4.62 (1H, d, *J*=7.6 Hz), 4.99 (1H, dd, *J*=3.6, 10.8 Hz), 5.10 (1H, dd, *J*=7.6, 10.8 Hz), 5.32 (1H, dd, *J*=3.6, 10.4 Hz), 5.37 (2H, m), 6.51 (1H, d, *J*=3.6 Hz), 8.61 (1H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16.2, 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 61.2, 66.9, 67.6, 68.0, 68.8, 70.2, 70.6, 71.1, 73.3, 91.1, 94.4, 95.6, 160.8, 169.3, 169.9, 170.0, 170.0, 170.2, 170.4. IR (neat) cm^{-1}: 3026, 1747, 1674, 1371, 1234, 1078, 760. HR-MS (ESI) *m/z*: 744.0836 (M+Na), Calcd for 744.0835, $C_{26}H_{34}O_{16}Cl_3Na$ (M+Na). $[\alpha]_D^{20}$ −75.0 (*c*=0.6, $CHCl_3$).

Allyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (15) To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-2,2,2-trichloroacetimidate **11** (3 g, 6.3 mmol), **10** (1.7 g, 4.2 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (40 ml) was added TMSOTf (377 μl, 2.1 mmol) at 0 °C under Ar. The mixture was stirred for 1 h at 0 °C, and then neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic phase was dried, filtered, and concentrated. The residue was purified by silica gel chromatography (EtOAc/hexane, 1 : 1) to yield **15** (1.5 g, 48%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.28 (3H, d, *J*=6.6 Hz), 1.91 (3H, s), 1.94 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 3.16 (2H, m), 3.60 (1H, m), 3.98 (1H, m), 4.16 (2H, m), 4.24 (1H, d, *J*=3.0 Hz), 4.41 (1H, d, *J*=7.8 Hz), 4.91 (1H, dd, *J*=3.0, 10.2 Hz), 5.10 (1H, m), 5.14 (1H, d, *J*=3.0 Hz), 5.20 (1H, d, *J*=3.6 Hz), 5.25 (2H, m), 5.55 (2H, m), 5.49 (1H, m), 7.34 (4H, m), 7.47 (2H, m), 7.96 (2H, m), 8.03 (2H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.9, 20.5, 20.5, 20.6, 20.8, 60.4, 65.3, 66.7, 68.6 (×2), 69.3, 69.9, 70.4, 70.8, 76.5, 95.7, 101.7, 117.2, 128.1 (×2), 128.3, 129.7 (×4), 130.0 (×2), 132.9 (×2), 133.0, 133.7, 165.8, 166.1, 169.2, 169.9, 170.0, 170.2. IR (neat) cm^{-1}: 3024, 2984, 1751, 1369, 1282, 1222, 1111, 1072, 756, 713. HR-MS (ESI) *m/z*: 765.2375 (M+Na), Calcd for 765.2365, $C_{37}H_{42}O_{16}Na$ (M+Na). $[\alpha]_D^{20}$ −98.8 (*c*=1.2, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-L-fucopyranose (16) To a stirred solution of **15** (1.1 g, 1.5 mmol) in AcOH (10 ml) was added $Pd(PPh_3)_4$ (504 mg, 0.44 mmol). The reaction mixture was stirred at 80 °C for 1 h and cooled to room temperature, to which was added toluene and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 2 : 3) to give **16** (802 mg, 78%, $\alpha:\beta$=1 : 1).

Compound **16** ($\alpha:\beta$=1 : 1 mixture): ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.24 (3H, d, *J*=6.6 Hz), 1.31 (3H, d, *J*=6.6 Hz), 1.85 (3H, s), 1.86 (3H, s), 1.90 (3H, s), 1.91 (3H, s), 2.00 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.06 (3H, s), 3.12 (4H, m), 3.56 (2H, m), 3.79 (1H, m), 4.14 (1H, d, *J*=3.0 Hz), 4.21 (2H, m), 4.37 (3H, m), 4.70 (1H, m), 4.88 (2H, m), 5.12 (3H, m), 5.21 (2H, dd, *J*=7.8, 10.2 Hz), 5.43 (1H, m), 5.52 (2H, m), 7.28-7.98 (20H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.0, 16.2, 20.5, 20.5, 20.5, 20.6, 60.3, 60.4, 65.3, 66.7, 66.8, 68.9, 69.3, 69.4, 69.5, 70.2, 70.4, 70.4, 70.5, 70.8, 71.8, 72.6, 75.5, 76.5, 90.8, 96.0, 101.7, 128.1, 128.1, 128.3, 129.3, 129.5, 129.6, 129.7, 129.8, 129.9, 130.0, 133.1, 133.2, 133.3, 133.4, 165.8, 166.0, 166.1, 166.7, 169.2, 169.9, 170.0, 170.0, 170.1, 170.1, 170.2. IR (neat) cm^{-1}: 3447, 3022, 1749, 1369, 1219, 1176, 1111, 1072, 758, 711. HR-MS (ESI) *m/z*: 725.2041 (M+Na), Calcd for 725.2052, $C_{34}H_{38}O_{16}Na$ (M+Na). $[\alpha]_D^{20}$ −141.6 (*c*=0.9, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl Trichloroacetimidate (17) To a stirred solution of **16** (429 mg, 0.61 mmol) in anhydrous CH_2Cl_2 (6 ml) were added trichloroacetonitrile (490 μl, 4.9 mmol) and DBU (18 μl, 0.12 mmol) at 0 °C. After the mixture was stirred for 30 min at 0 °C, the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 2 to 1 : 1) to give **17** (504 mg, 97%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.34 (3H, d, *J*=6.6 Hz), 1.92 (3H, s), 1.95 (3H, s), 2.05 (3H, s), 2.09 (3H, s), 3.11 (1H, dd, *J*=6.6, 11.4 Hz), 3.19 (1H, dd, *J*=7.2, 11.4 Hz), 3.62 (1H, m), 4.36 (2H, m), 4.44 (1H, d, *J*=8.4 Hz), 4.93 (1H, dd, *J*=3.6, 10.8 Hz), 5.16 (1H, m), 5.28 (1H, dd, *J*=8.4, 10.8 Hz), 5.57 (1H, dd, *J*=3.6, 10.8 Hz), 5.83 (1H, dd, *J*=3.6, 10.8 Hz), 6.65 (1H, d, *J*=3.6 Hz), 7.32 (2H, m), 7.37 (2H, m), 7.48 (2H, m), 7.94 (2H, m), 8.03 (2H, m), 8.53 (1H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.0, 20.5, 20.5, 20.6, 20.8, 60.4, 66.7, 67.2, 68.3, 69.2, 69.8, 70.6, 70.7, 75.7, 91.0, 94.3, 101.7, 128.1, 128.3 (×2), 129.2, 129.7 (×3), 129.8, 130.0 (×2), 133.2, 133.3, 160.8, 165.4, 166.1, 169.1 (×2), 170.0, 170.2. IR (neat) cm^{-1}: 3024, 1751, 1371, 1221, 1118, 1070, 758, 711. HR-MS (ESI) *m/z*: 868.1144 (M+Na), Calcd for 868.1148, $C_{36}H_{38}O_{16}Na$ (M+Na). $[\alpha]_D^{20}$ −117.4 (*c*=0.8, $CHCl_3$).

1,6-Di-*O*-(*t*-butyldimethylsilyl)-D-mannitol (19) To a stirred solution of D-mannitol (**18**) (45 g, 0.25 mol) in DMF (165 ml) were added TBDMSCl (74 g, 0.54 mol) and imidazole (55 g, 0.54 mol) at 0 °C. The mixture was stirred for 1 h at 0 °C. Then H_2O was added to the mixture and the suspen-

tion was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 3) to give **19** (80 g, 79%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.00 (6H×2, s), 0.81 (9H×2, s), 3.12 (1H×2, d, J=4.8 Hz), 3.52 (1H×2, d, J=6.0 Hz), 3.70 (4H×2, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: −5.4 (×4), 18.3 (×2), 25.9 (×6), 64.3 (×2), 70.9 (×2), 72.1 (×2). IR (neat) cm^{-1}: 3422, 2955, 2932, 2858, 1471, 1257, 1217, 1070, 839, 760. HR-MS (ESI) m/z: 411.2602 (M+H), Calcd for 411.2593, $C_{18}H_{43}O_6Si_2$ (M+H). $[\alpha]_D^{20}$ −1.26 (c=0.5, $CHCl_3$).

2,3,4,5-Tetra-*O*-acetyl-1,6-di-*O*-(*t*-butyldimethylsilyl)-D-mannitol (20) To a stirred solution of **19** (6 g, 0.015 mol) in pyridine (15 ml) was added Ac_2O (7 ml, 0.073 mol) at 0 °C. The mixture was stirred for 12 h at room temperature. Then H_2O was added to the mixture and the suspention was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 5) to give **20** (7.7 g, 88%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: −0.01 (3H×2, s), 0.00 (3H×2, s), 0.85 (9H×2, s), 2.02 (3H×2, s), 2.05 (3H×2, s), 3.59 (1H×2, dd, J=7.8, 16.8 Hz), 3.71 (1H×2, dd, J=5.4, 16.8 Hz), 4.94 (1H×2, m), 5.45 (1H×2, d, J=12.0 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: −5.54 (×4), 18.1 (×2), 20.9 (×2), 21.0 (×2), 25.6 (×6), 61.4 (×2), 67.8 (×2), 70.8 (×2), 169.5 (×2), 169.7 (×2). IR (neat) cm^{-1}: 2955, 2932, 2858, 1749, 1371, 1253, 1222, 1118, 1064, 1035, 758. HR-MS (ESI) m/z: 579.3015 (M+H), Calcd for 579.3015, $C_{26}H_{51}O_{10}Si_2$ (M+H). $[\alpha]_D^{20}$ +18.9 (c=0.5, $CHCl_3$).

2,3,4,5-Tetra-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-D-mannitol (21) The compound **20** (6.4 g, 0.011 mol) was added to the 1% I_2/methanol solution (40 ml) and stirred for 18 h at room temperature. Then 3% $Na_2S_2O_3$ was added to the mixture and the suspension was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 2 : 3) to give **21** (2.1 g, 42%) and recovery of **20** (3.2 g, 52%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.00 (3H, s), 0.06 (3H, s), 0.86 (9H, s), 2.03 (3H, s), 2.06 (3H, s), 2.08 (3H, s), 2.13 (3H, s), 2.55 (1H, m), 3.50 (1H, m), 3.59 (1H, dd, J=4.8, 11.4 Hz), 3.66 (1H, dd, J=3.0, 11.4 Hz), 3.72 (1H, m), 4.77 (1H, m), 5.05 (1H, m), 5.35 (1H, dd, J=1.8, 10.2 Hz), 5.48 (1H, dd, J=1.8, 9.0 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: −5.5 (×2), 18.1, 20.6, 20.8, 20.9, 21.0, 25.6 (×3), 60.5, 61.8, 67.6, 67.8, 70.0, 70.4, 169.4, 169.7, 170.3, 171.9. IR (neat) cm^{-1}: 3504, 2932, 2858, 1749, 1471, 1371, 1226, 1035, 839, 777. HR-MS (ESI) m/z: 487.1975 (M+Na), Calcd for 487.1970, $C_{20}H_{36}O_{10}SiNa$ (M+Na). $[\alpha]_D^{20}$ +14.4 (c=0.5, $CHCl_3$).

2,3,4,5-Tetra-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-D-mannose (22) To a cold solution of DMSO (141 μl, 1.99 mmol) in dry CH_2Cl_2 (10 ml) at −78 °C, $(COCl)_2$ (84 μl, 0.99 mmol) was added slowly and the mixture was stirred for 15 min. Then the mixture was added to the solution of **21** (308 mg, 0.66 mmol) in dry CH_2Cl_2 (10 ml). After being stirred for 2 h at −78 °C, the mixture was warmed to r.t., treated with triethylamine (370 μl, 2.65 mmol) and then washed with saturated aqueous NH_4Cl solution. The reaction products were extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 2 : 1) to give **22** (300 mg, 97%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.00 (3H, s), 0.01 (3H, s), 0.85 (9H, s), 2.03 (3H, s), 2.06 (3H, s), 2.09 (3H, s), 2.16 (3H, s), 3.62 (1H, dd, J=6.6, 16.8 Hz), 3.71 (1H, dd, J=4.8, 16.8 Hz), 5.01 (2H, m), 5.51 (2H, m), 9.42 (1H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: −5.6 (×2), 18.2, 20.5, 20.6, 20.7, 21.0, 25.8 (×3), 61.8, 67.9, 68.2, 70.7, 74.8, 170.4, 170.7, 170.9, 170.9, 196.6. HR-MS (ESI) m/z: 485.1831 (M+H), Calcd for 485.1813, $C_{20}H_{35}O_{10}Si$ (M+H).

2,3,4,5-Tetra-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-1-deoxy-1-(2-pyridinylamino)-D-mannitol (23) and 3,4,5-Tri-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-1-deoxy-1-(*N*-acetyl-*N*-2-pyridinylamino)-D-mannitol (24) To a solution of aldehyde **22** (50 mg, 0.11 mmol) and 4 Å molecular sieves in anhydrous DMF (2 ml) were added 2-aminopyridine (102 mg, 1.1 mmol) and AcOH (840 μl), and the mixture was stirred for 23 h at room temperature. Then $NaBH_3CN$ in THF solution (0.22 mmol, 216 μl) was added to the mixture. After 24 h, the mixture was neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic phase was dried, filtered, and concentrated. The residue was purified by silica gel chromatography (EtOAc/toluene, 3 : 2) to yield **23** (30 mg, 50%) and **24** (10 mg, 18%).

Compound (**23**): ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.00 (3H, s), 0.06 (3H, s), 0.85 (9H, s), 1.94 (3H, s), 2.04 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 3.20 (1H, m), 3.59 (1H, dd, J=6.0, 11.4 Hz), 3.69 (1H, dd, J=3.6, 11.4 Hz), 3.97 (1H, m), 4.96 (1H, m), 5.03 (1H, m), 5.42 (1H, dd, J=2.4, 9.0 Hz), 5.49 (1H, dd, J=2.4, 9.0 Hz), 6.38 (1H, d, J=8.4 Hz), 6.53 (1H, m), 7.36 (1H, m), 8.02 (1H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: −5.6 (×2), 18.1, 20.7, 20.8, 20.9, 21.0, 25.7 (×3), 40.6, 61.7, 67.9, 68.7, 69.4, 70.6, 107.7, 113.0, 137.3, 147.6, 158.3, 169.4, 170.0, 170.4, 170.7. IR (neat) cm^{-1}: 3020, 2957, 2932, 2853, 1743, 1371, 1217, 1057, 839, 761. HR-MS (ESI) m/z: 541.2558 (M+Na), Calcd for 541.2576, $C_{25}H_{40}O_9N_2Si_2Na$ (M+Na). $[\alpha]_D^{20}$ +75.9 (c=0.1, $CHCl_3$).

Compound (**24**): ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.00 (3H, s), 0.01 (3H, s), 0.85 (9H, s), 2.01 (3H, s), 2.06 (3H, s), 2.08 (3H, s), 2.16 (3H, s), 3.36 (1H, m), 3.61 (1H, dd, J=5.4, 11.4 Hz), 3.71 (2H, m), 5.02 (2H, m), 5.45 (2H, m), 6.67 (2H, m), 7.67 (1H, m), 8.13 (1H, dd, J=1.2, 6.0 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: −5.6 (×2), 18.1, 20.6, 20.7, 20.8, 20.9, 25.7 (×3), 42.5, 61.6, 67.7, 68.0, 68.8, 70.3, 108.1, 112.6, 141.4, 145.7, 155.2, 169.5, 170.2, 170.6, 171.0.

2,3,4,5-Tetra-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-1-deoxy-1-(*N*-acetyl-*N*-2-pyridinylamino)-D-mannitol (25) To a stirred solution of **23** (286 mg, 0.53 mol) in pyridine (1 ml) was added Ac_2O (0.5 ml) at room temperature. The mixture was stirred for 12 h at 80 °C. Then H_2O was added to the mixture and the suspension was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/toluene, 1 : 1) to give **25** (191 mg, 62%) and recovery of **23** (42 mg, 15%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.03 (6H, d, J=0.3 Hz), 0.85 (9H, s), 1.78 (3H, s), 1.98 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.11 (3H, s), 3.57 (1H, dd, J=5.6, 11.6 Hz), 3.69 (1H, dd, J=4.0, 11.6 Hz), 4.07 (1H, dd, J=8.8, 14.4 Hz), 4.18 (1H, d, J=13.6 Hz), 4.97 (1H, m), 5.23 (1H, m), 5.33 (1H, dd, J=2.4, 8.0 Hz), 5.40 (1H, dd, J=2.4, 8.4 Hz), 7.20 (2H, dd, J=4.8, 7.2 Hz), 7.74 (1H, m), 8.49 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: −5.5 (×2), 18.2, 20.8, 20.8, 20.9, 21.0, 22.9, 25.7 (×3), 47.9, 61.5, 67.9, 69.2, 69.3, 70.8, 121.5, 127.3, 130.4, 138.4, 149.0, 169.5, 169.7, 169.8, 170.1, 170.4. IR (neat) cm^{-1}: 2957, 2932, 2858, 1749, 1471, 1437, 1371, 1221, 1122, 1062, 754. HR-MS (ESI) m/z: 583.2687 (M+H), Calcd for 583.2681, $C_{27}H_{43}O_{10}N_2Si$ (M+H). $[\alpha]_D^{20}$ +53.3 (c=0.5, $CHCl_3$).

2,3,4,5-Tetra-*O*-acetyl-1-deoxy-1-(*N*-acetyl-*N*-2-pyridinylamino)-D-mannitol (26) To the solution of **25** (60 mg, 0.10 mmol) in pyridine (200 μl), THF (100 μl), and HF-pyridine (343 μl) were added and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution, and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/toluene, 4 : 1) to give **26** (42 mg, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.70 (3H, s), 1.90 (3H, s), 2.01 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 3.43 (1H, m), 3.64 (1H, m), 3.93 (1H, m), 4.15 (1H, d, J=14.4 Hz), 4.74 (1H, m), 5.26 (3H, m), 7.10 (1H, m), 7.15 (1H, m), 7.69 (1H, m), 8.43 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.7, 20.7, 20.8, 21.0, 22.9, 48.2, 60.4, 67.7, 68.9, 69.2, 70.1, 121.4, 128.2, 129.0, 138.2, 149.2, 169.9, 170.1, 170.2, 170.6, 171.9. IR (neat) cm^{-1}: 3464, 3016, 1747, 1664, 1437, 1371, 1224, 1047, 756. HR-MS (ESI) m/z: 491.1658 (M+Na), Calcd for 491.1636, $C_{21}H_{28}O_{10}N_2Na$ (M+Na). $[\alpha]_D^{25}$ +26.8 (c=0.2, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-acetyl-β-L-fucopyranosyl-(1→6)-2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(*N*-acetyl-*N*-2-pyridinylamino)-D-mannitol (27) To the solution of **14** (49 mg, 0.07 mmol) and **26** (48 mg, 0.10 mmol) in dry CH_2Cl_2 (1 ml), containing activated molecular sieves (4 Å, 25 mg), was stirred at 0 °C. TMSOTf (12 μl, 0.07 mmol) was added, and the mixture was stirred for 30 min, when TLC (CH_2Cl_2/MeOH, 10 : 1) showed the formation of a new spot. The mixture was neutralized with pyridine, then filtered and washed with aqueous $NaHCO_3$ solution, dried, filtered, and concentrated. Column chromatography (NH silicagel, EtOAc/toluene, 10 : 1) of the residue afford **27** (36 mg, 55%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.17 (3H, d, J=6.6 Hz), 1.79 (3H, s), 1.96 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.06 (3H, s), 2.11 (3H, s), 2.12 (3H, s), 2.14 (3H, s), 3.68 (1H, m), 3.71 (1H, dd, J=3.6, 12.0 Hz), 3.80 (1H, dd, J=6.6, 12.0 Hz), 3.87 (1H, m), 3.91 (1H, dd, J=3.6, 10.2 Hz), 4.06 (1H, m), 4.07 (1H, dd, J=7.2, 10.8 Hz), 4.16 (1H, dd, J=6.0, 10.8 Hz), 4.19 (1H, m), 4.45 (1H, d, J=8.4 Hz), 4.53 (1H, d, J=7.8 Hz), 4.96 (1H, dd, J=3.6, 10.8 Hz), 4.99 (1H, m), 5.04 (1H, dd, J=7.8, 10.8 Hz), 5.12 (1H, m), 5.18 (1H, d, J=3.6 Hz), 5.25 (1H, m), 5.30 (1H, m), 5.34 (1H, m), 5.35 (1H, dd, J=1.8, 3.6 Hz), 7.22 (2H, m), 7.76 (1H, m), 8.49 (1H, dd, J=1.8, 5.4 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.2, 20.5, 20.6, 20.7, 20.7, 20.7, 20.7, 20.8, 20.8, 20.8, 20.9, 22.9, 47.8, 60.9, 66.1, 66.7, 68.2, 68.8, 68.8, 69.2, 69.3, 69.3, 69.4, 69.5, 69.8, 70.5, 71.1, 99.5, 100.1, 121.4, 122.0, 129.1, 138.1, 149.1, 163.3, 169.3, 169.4, 169.6, 169.9, 170.0, 170.1, 170.2, 170.2, 170.3, 170.6. IR (neat) cm^{-1}: 3020, 1749, 1371, 1217, 1132, 1076, 756, 669. HR-MS (ESI) m/z: 1029.3557 (M+H), Calcd for 1029.3558, $C_{45}H_{61}O_{25}N_2$ (M+H). $[\alpha]_D^{25}$ +7.16 (c=0.3, $CHCl_3$).

***O*-β-D-Galactopyranosyl-(1→3)-*O*-β-L-fucopyranosyl-(1→6)-1-deoxy-**

1-(2-pyridinylamino)-D-mannitol (1) To the solution of **27** (17 mg, 0.017 mmol) and NaOMe (5.4 mg, 0.099 mmol) in MeOH (1 ml) was stirred at room temperature for 5 h. Then the mixture was concentrated, and the residue was purified by reverse phase silica gel chromatography (H_2O/MeOH, 1 : 1 to 1 : 4) to yield **1** (9 mg, 96%). ^{1}H-NMR (600 MHz, CD_3OD) δ: 1.29 (3H, d, J=6.6 Hz), 3.50 (1H, dd, J=3.6, 10.2 Hz), 3.55 (2H, m), 3.60 (2H, m), 3.64 (1H, m), 3.68 (3H, m), 3.77 (3H, m), 3.81 (4H, m), 3.90 (1H, d, J=9.0 Hz), 4.07 (1H, dd, J=4.8, 10.2 Hz), 4.31 (1H, d, J=7.8 Hz), 4.40 (1H, d, J=7.8 Hz), 6.55 (1H, m), 6.65 (1H, d, J=9.0 Hz), 7.44 (1H, m), 7.88 (1H, dd, J=1.2, 4.8 Hz). ^{13}C-NMR (150 MHz, CD_3OD) δ: 16.7, 46.5, 62.6, 70.3, 70.3, 70.5, 70.7, 71.2, 71.4, 71.7, 72.0, 72.3, 72.6, 74.7, 77.2, 81.6, 102.7, 104.6, 110.5, 113.4, 139.0, 147.1, 160.7. HR-MS (ESI) m/z: 567.2396 (M+H), Calcd for $C_{23}H_{38}O_{14}N_2$, 567.2396. $[\alpha]_D^{25}$ −22.1 (c=0.2, EtOH).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-L-fucopyranosyl-(1→6)-2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(*N*-acetyl-*N*-2-pyridinylamino)-D-mannitol (28) To the solution of **17** (88 mg, 0.10 mmol) and **26** (58 mg, 0.12 mmol) in dry CH_2Cl_2 (2 ml), containing activated molecular sieves (4 Å, 40 mg), was stirred at 0 °C. TMSOTf (10 μl, 0.05 mmol) was added, and the mixture was stirred for 1 h, when TLC (CH_2Cl_2/MeOH, 10 : 1) showed the formation of a new spot. The mixture was neutralized with pyridine, then filtered and washed with aqueous $NaHCO_3$ solution, dried, filtered, and concentrated. Column chromatography (NH silicagel, EtOAc/toluene, 10 : 1) of the residue afforded **28** (41 mg, 34%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.30 (3H, d, J=6.6 Hz), 1.66 (3H, s), 1.86 (3H, s), 1.86 (3H, s), 1.90 (3H, s), 1.96 (3H, s), 1.99 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 2.06 (3H, s), 3.09 (1H, m), 3.14 (1H, m), 3.54 (1H, m), 3.67 (1H, dd, J=6.0, 12.0 Hz), 3.75 (2H, m), 3.95 (1H, m), 4.08 (1H, m), 4.35 (1H, d, J=8.4 Hz), 4.58 (1H, d, J=7.8 Hz), 4.86 (1H, dd, J=3.6, 10.8 Hz), 5.03 (2H, m), 5.08 (1H, m), 5.21 (3H, m), 5.31 (1H, m), 5.53 (2H, m), 7.13 (2H, m), 7.29 (4H, m), 7.43 (2H, m), 7.66 (1H, m), 7.89 (2H, m), 7.96 (2H, m), 8.41 (1H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.0, 20.4, 20.5, 20.6, 20.7, 20.7, 20.7, 20.8, 20.9, 22.9, 47.8, 60.3, 65.3, 65.7, 66.7, 68.4, 68.5, 68.6, 69.2, 69.2, 69.4, 70.1, 70.5, 70.8, 73.1, 100.2, 101.7, 121.4, 128.1, 128.1, 128.2, 128.2, 128.3, 129.0, 129.6, 129.7, 129.9, 129.9, 130.0, 130.1, 133.0, 133.1, 138.1, 149.0. 163.3, 165.0, 166.0, 169.6, 169.9, 170.0, 170.1, 170.1, 170.1, 170.2, 170.2. IR (neat) cm^{-1}: 2986, 1747, 1371, 1226, 1178, 1111, 1070, 756, 713. HR-MS (ESI) m/z: 1153.3853 (M+H), Calcd for 1153.3871, $C_{55}H_{65}O_{25}N_2$ (M+H). $[\alpha]_D^{25}$ −71.8 (c=0.5, $CHCl_3$).

***O*-β-D-Galactopyranosyl-(1→4)-*O*-β-L-fucopyranosyl-(1→6)-1-deoxy-1-(2-pyrydinylamino)-D-mannitol (2)** To the solution of **28** (20 mg, 0.017 mmol) and NaOMe (6 mg, 0.10 mmol) in MeOH (1 ml) was stirred at room temperature for 12 h. Then the mixture was concentrated, and the residue was purified by reverse phase silica gel chromatography (H_2O/MeOH, 1 : 1) to yield **2** (9 mg, 96%). ^{1}H-NMR (600 MHz, CD_3OD) δ: 1.36 (3H, d, J=6.6 Hz), 3.48 (3H, m), 3.55 (2H, m), 3.61 (2H, m), 3.68 (1H, m), 3.74 (4H, m), 3.82 (4H, m), 3.87 (1H, m), 4.05 (1H, dd, J=4.8, 10.2 Hz), 4.28 (1H, d, J=7.8 Hz), 4.30 (1H, d, J=7.8 Hz), 6.56 (1H, m), 6.63 (1H, d, J=9.0 Hz), 7.45 (1H, m), 7.88 (1H, dd, J=1.2, 5.4 Hz). ^{13}C-NMR (150 MHz, CD_3OD) δ: 16.6, 62.5, 70.3, 70.5, 71.2, 71.3, 72.0, 72.2, 72.8, 72.9, 73.0, 73.3, 74.0, 74.5, 77.1, 81.0, 105.0, 105.8, 110.7, 113.4, 139.1, 147.9, 160.6. HR-MS (ESI) m/z: 567.2373 (M+H), Calcd for 567.2396, $C_{23}H_{39}O_{14}N_2$ (M+H). $[\alpha]_D^{25}$ −40.6 (c=0.3, EtOH).

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