Synthesis of 5-Thio-L-fucose-Containing Disaccharides, as Sequence-Specific Inhibitors, and 2′-Fucosyllactose, as a Substrate of α-L-Fucosidases†

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Four 5-thio-L-fucose-containing disaccharides having α(1→6), α(1→3), α(1→4)GlcNAc, and α(1→2)-Gal linkages (compounds **1**−**4**, respectively) were synthesized as potential α-L-fucosidase inhibitors. The glycosylation reactions using 2,3,4-tri-*O*-acetyl-5-thio-L-fucopyranosyl trichloroacetimidate as a glycosyl donor and BF_3·OEt_2 as a catalyst gave mainly α-linked disaccharides. Only α(1→2)-linked disaccharide **4** showed inhibitory activity (K_i = 0.21 mM) against *Bacillus* α-L-fucosidase which hydrolyzes the Fucα(1→2) linkage specifically. The results suggested that sequence specificity of an enzyme could be estimated from the inhibitory activities of the compounds **1**−**4**. In contrast, every disaccharide showed inhibitory activity (K_i = 30−91 μM) against bovine epididymis α-L-fucosidase.

The glycosidases cleave glycosidic bonds of oligosaccharides in glycoconjugates. Glycosidase inhibitors have been used for studies of *N*-linked oligosaccharide processing in glycoproteins,[1] metabolic disorders such as diabetes,[2] microbial infections,[3] and metastasis.[4] They are also valuable tools for studying enzyme activity[5] and purification of enzymes by affinity chromatography.[6] It has been suggested that α-L-fucosidase (EC 3.2.1.15), which hydrolyzes the terminal α-L-fucopyranosyl linkage, participates in gamete recognition interacting with ascidian[7] and mammal[8] fucose-containing glycoconjugates. Previously, the author synthesized four α-L-fucopyranosyl disaccharides with thioglycosidic linkages to create sequence specific inhibitors.[9] However, their inhibitory activities against two bovine α-L-fucosidases were low and unsatisfactory. Furthermore, the fact that the inhibition mode of three of these disaccharides was of mixed-type suggested the additional binding of these inhibitors to sites different from the substrate binding site of the enzyme. On the other hand, 5-thio-L-fucose, which contains a sulfur atom in the pyranose ring instead of oxygen, was a potent competitive inhibitor of bovine α-L-fucosidase[10] (K_i = 42 μM), and a 5-thio-L-fucose-containing disaccharide with an α(1→2)Gal linkage also showed an excellent competitive inhibition[11] (K_i = 30 μM). We report here the syntheses of four 5-thio-α-L-fucopyranosyl disaccharides, the relationship between their inhibitory activities against two α-L-fucosidases, and the substrate specificity of these enzymes.

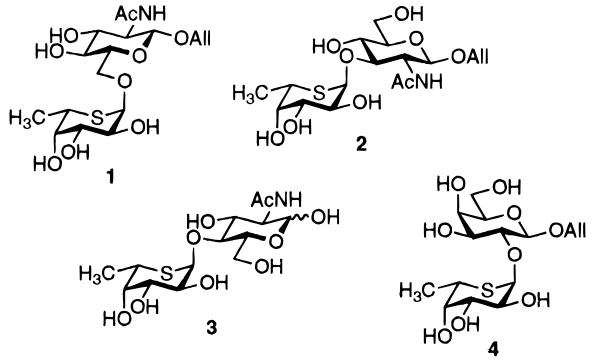

Figure 1.

Results and Discussion

Synthesis. Considering the frequency of L-fucosyl linkages in glycoconjugates, disaccharides having α(1→6), α(1→4), α(1→3)GlcNAc, and α(1→2)Gal linkages (**1**−**4**) were selected as synthetic targets (Figure 1). As the authors communicated previously,[11] 5-thio-α-L-fucopyranosides could be synthesized using 2,3,4-tri-*O*-acetyl-5-thio-L-fucopyranosyl trichloroacetimidate (**6**) as a glycosyl donor and a catalytic amount of BF_3·OEt_2[12] as an activator. The trichloroacetimidate **6** was synthesized from 5-thio-L-fucopyranose tetraacetate **5**[13] in two steps

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† Dedicated to Prof. Hans Paulsen on the occasion of his 75th birthday.

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(1) (a) Fuhrmann, U.; Bause, E.; Ploegh, H. *Biochem. Biophys. Acta* **1985**, *825*, 95. (b) Elbein, A. D. *Annu. Rev. Biochem.* **1987**, *56*, 497. Elbein, A. D. *FASEB J.* **1991**, *5*, 3055.

(2) Truscheit, E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744.

(3) (a) Schwarz, P. M.; Elbein, A. D. *J. Biol. Chem.*, **1985**, *260*, 14452. (b) Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; De Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedena, F.; Ploegh, H. L. *Nature* **1987**, *330*, 74. (c) Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.*, 1**988**, *85*, 9229.

(4) (a) Humphries, M. J.; Matsumoto, K.; White, S. L.; Olden, K. *Cancer Res.* **1986**, *46*, 5215.

(5) Defaye, J.; Gelas, J. In *Studies in Natural Product Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1991, Vol. 8, p 315.

(6) Orgeret, C.; Seillier, E.; Gautier, C.; Defaye, J.; Driguez, H. *Carbohydr. Res.*, **1992**, *224*, 29.

(7) (a) DeSantis, R.; Pinto, M. R. Gamete Interaction in Ascidians: Sperm Binding and Penetration Through the Vitelline Coat. In *Mechanism of Fertilization: Plants to Humans*; Dale, B., Ed.; Springer-Verlag: Berlin, 1990; p 297. (b) Hoshi, M.; DeSantis, R.; Pinto, M. R.; Cotelli, F.; Rosati, F. *Zool. Sci.*, **1985**, *2*, 65.

(8) Huang, T. F., Jr.; Ohzu, E.; Yanagimachi, R. *Gamete Res.*, **1982**, *5*, 355.

(9) Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron: Asymmetry* **1994**, *5*, 2351.

(10) Hashimoto, H.; Fujimori, T.; Yuasa, H. *J. Carbohydr. Chem.*, **1990**, *9*, 683.

(11) Hashimoto, H.; Izumi, M. *Tetrahedron Lett.*, **1993**, *31*, 4949.

(12) Schmidt, R.; R. Kinzy, W. *Adv. Carbohydr. Chem. Biochem.*, **1994**, *50*, 21.

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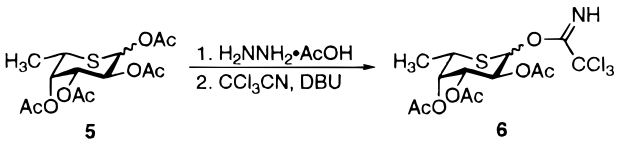
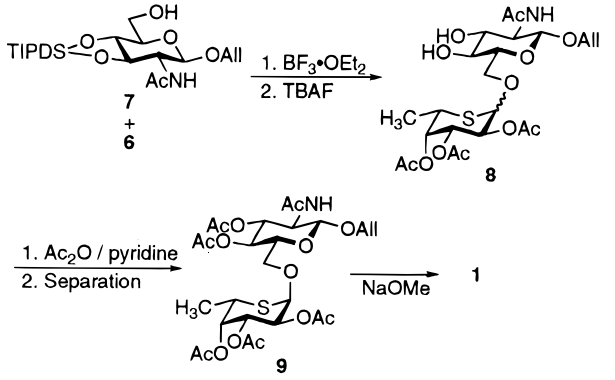
Scheme 1



Scheme 2



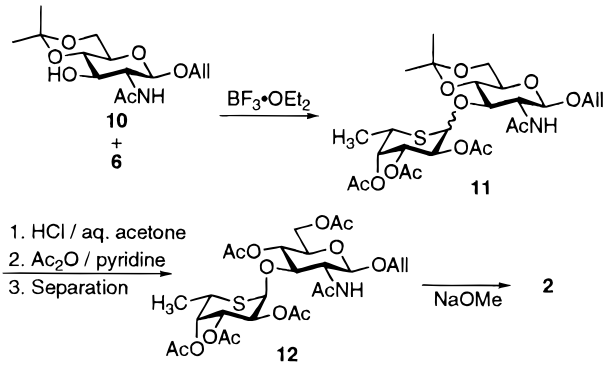
in 95% yield. The anomeric acetyl group of **5** was removed by treatment with hydrazine acetate and the resulting 1-OH derivative was treated with CCl_3CN and DBU[14] to give **6** (Scheme 1).

To construct the (1→6) linkage, allyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside[15] was first examined as an acceptor, but migration of the acetyl group from 4-OH to 6-OH was observed during the glycosylation reaction and the desired disaccharide could not obtained in a pure form. The glycosylation of allyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-glucopyranoside (**7**)[16] with the imidate **6** at room temperature gave a mixture of α- and *β*-linked disaccharide **8** in 70% yield after removal of the TIPDS group. The α-linked disaccharide was formed predominantly in a ratio of 81:19. The disaccharide mixture was acetylated and separated several times on silica gel using $CHCl_3$–MeOH 75:1 as an eluate to give a pure α-linked disaccharide **9**. De-*O*-acetylation of the disaccharide **9** with methanolic sodium methoxide gave α(1→6)-linked disaccharide **1** in 97% yield (Scheme 2).

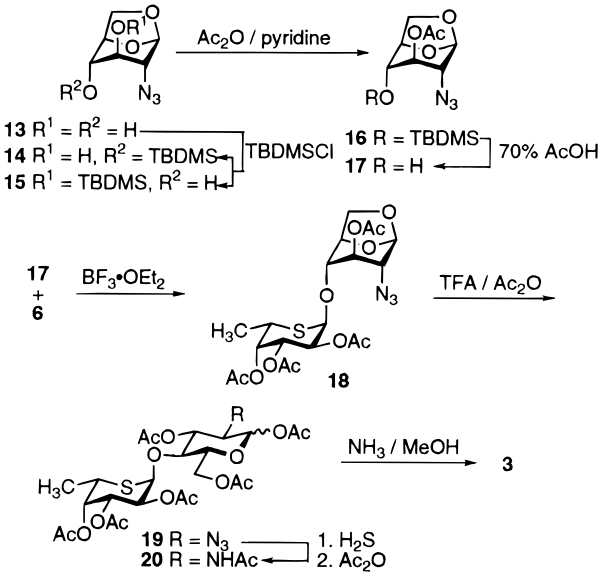
The glycosylation of allyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-*β*-D-glucopyranoside (**10**)[17] with the imidate **6** at 0 °C gave (1→3)-linked disaccharide **11** in 64% yield. The ratio of α- and *β*-glycoside was 82:18. The isopropylidene group was hydrolyzed by treatment with 1 M HCl in acetone[17] and free hydroxyl groups were acetylated to give pentaacetate **12** in 62% yield. The α-linked disaccharide separated in this stage was de-*O*-acetylated with methanolic sodium methoxide to give α-(1→3)-linked disaccharide **2** (Scheme 3).

The synthesis of (1→4)-linked disaccharide **3** required many more steps for preparing the acceptor (Scheme 4). Glycosylation of allyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-*β*-D-glucopyranoside[18] was unsuccessful due to a lack of the reactivity of the 4-OH group. Next the authors

Scheme 3



Scheme 4



examined 1,6-anhydro-2-azido-2-deoxy-*β*-D-glucopyranose derivative **17**[19] as a glycosyl acceptor. The compound **17** was synthesized in three steps from 1,6-anhydro-2-azido-2-deoxy-*β*-D-glucopyranose (**13**) which could be conveniently prepared from D-glucal.[20] Treatment of **13** with 1.2 mol equiv of *tert*-butyldimethylsilyl chloride (TBDMSCl) gave 4-*O*-TBDMS derivative **14**[21] and 3-*O*-TBDMS derivative **15** in 89% yield in a 9:1 ratio. Compound **14** and **15** were separated by MPLC. The 4-*O*-TBDMS derivative **14** was acetylated (94%), and subsequent desilylation in hot aqueous acetic acid gave **17** in 73% yield. Glycosylation of the acceptor **17** proceeded smoothly at −20 °C to give only α-linked disaccharide **18** in 89% yield. The 1,6-anhydro-ring of **18** was acetolyzed under mild conditions, *i.e.* trifluoroacetic acid and acetic anhydride, without a cleavage of the interglycosidic bond. The azido group of **19** was converted to an acetamido group by treatment with H_2S in pyridine and water and subsequent acetylation. *O*-Acetyl groups were removed by treatment with methanolic ammonia to give α(1→4)-linked disaccharide **3** in 67% yield.

For the synthesis of α(1→2)-linked disaccharide, allyl 3,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside (**25**) was used as a glycosyl acceptor (Scheme 5). 1,3,4,6-tetra-*O*-acetyl-

(13) Izumi, M.; Tsuruta, O.; Hashimoto, H. *Carbohydr. Res.*, **1996**, *280*, 287.
(14) Sugimoto, M.; Numata, M.; Koike, K.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.*, **1986**, *156*, c1.
(15) Roy, R.; Jennings, H. J. *Carbohydr. Res.*, **1983**, *112*, 63.
(16) Kosma, P.; Bahnmüller, R.; Schulz, G.; Brade, H. *Carbohydr. Res.*, **1990**, *208*, 37.
(17) Hasegawa, A.; Ozaki, M.; Kiso, M.; Azuma, I. *J. Carbohydr. Chem.*, **1984**, *3*, 331.
(18) Nashed, N. M. *Carbohydr. Res.*, **1979**, *71*, 299–304.
(19) Petitou, M.; Duchaussoy, P. Lederman, I.; Choay, J.; Sinaÿ. P.; Jacquinet, J.-C.; Torri, G. *Carbohydr. Res.*, **1986**, *147*, 221.
(20) Tailler, D.; Jacquinet, J.-C.; Noirot, A.-M.; Beau, J.-M. *J. Chem. Soc., Perkin Trans. 1*, **1992**, 3163.
(21) van Boeckel, C. A. A.; van Aelst, S. F.; Beetz, T. *Recl. Trav. Chim. Pays-Bas*, **1983**, *102*, 415.

Scheme 5

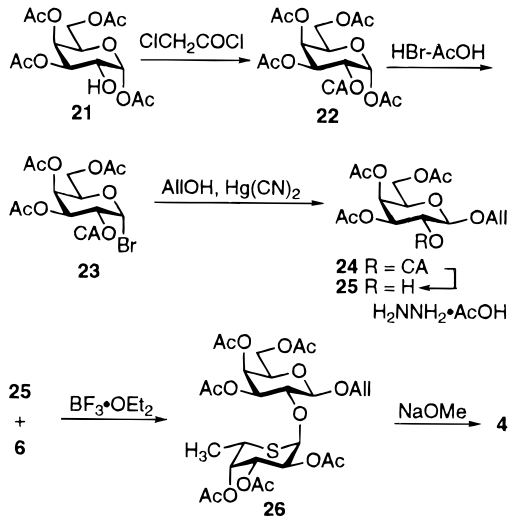


Table 1. Glycosylation Reaction with the Trichloroacetimidate 6

entry	acceptor (equiv)	conditions	% yield[a]	α:β
1	**7** (1.5)	−20 °C, 3 h-rt, overnight	**8**, 70%	81:19
2	**10** (3.0)	0 °C, 7 h	**11**, 76%	82:18
3	**17** (3.0)	−20 °C, 30 min	**18**, 85%	>99:1
4	**25** (1.6)	−20 °C, 15 min	**26**, 86%	98:2

[a] Isolated yield.

α-D-galactopyranose (**21**)[22] was acylated with chloroacetyl chloride and converted to the glycosyl bromide with HBr−acetic acid. The glycosylation of allyl alcohol using mercury(II) cyanide as an activator gave β-glycoside **24** in 81% yield. Dechloroacetylation with hydrazine acetate gave the acceptor **25** in 82% yield. The glycosylation of **25** with the imidate **6** at −20 °C gave α-glycoside **26** in 86% yield. Deacetylation with methanolic sodium methoxide gave target disaccharide **4** in 92% yield.

The results of glycosylation reactions with peracetylated 5-thio-L-fucopyranosyl trichloroacetimidate **6** are summarized in Table 1. The reaction with reactive acceptors (entries 3 and 4) proceeded smoothly at −20 °C with excellent stereoselectivity, and only the desired α-linked disaccharides were obtained. On the other hand, the reaction with less reactive acceptors (entries 1 and 2) required longer reaction times and higher reaction temperatures. As a result of such reaction conditions, the stereoselectivities were decreased to about 4:1. These results suggest that α-glycosides are kinetically favored.

In addition to the above described four potential inhibitors of α-L-fucosidase, 2′-fucosyllactose, which was required for the enzyme assay as a substrate, was newly synthesized in a convenient route as shown in Scheme 6. This synthetic method demonstrated the usefulness of tri-*O*-isopropylidenelactose dimethylacetal **28** which can be easily prepared by treatment of lactose with 2,2-dimethoxypropane in the same manner as reported for *N*-acetyllactosamine.[23] Selective 6-*O*-acetylation followed by glycosylation with per-*O*-*p*-methoxybenzylated 1-thiofucoside **27** in the presence of iodonium di(*sym*-collidine) perchlorate (IDCP) gave α-linked trisaccharide derivative

Scheme 6

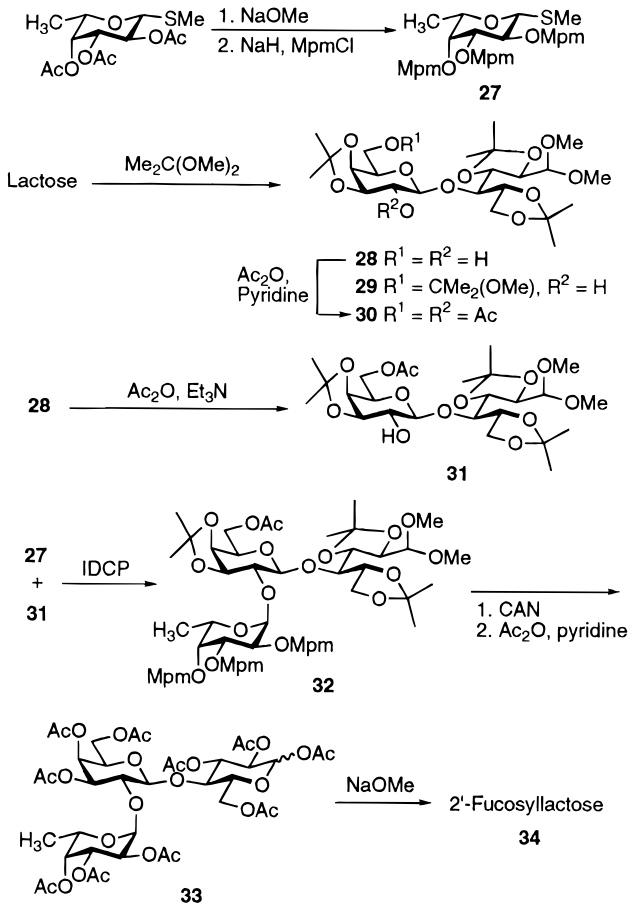


Table 2. K_i Values of 5-Thio-L-fucosyl Disaccharides against α-L-Fucosidases

inhibitor	*Bacillus* sp. K40Tm mM	bovine epididymis, μM
1→6GlcNAc (**1**)	>5	31
1→3GlcNAc (**2**)	>5	91
1→4GlcNAc (**3**)	>5	44
1→2Gal (**4**)	0.21	30

32 exclusively. Simultaneous deprotection of the *O*-*p*-methoxylbenzyl and *O*-isopropylidene groups with CAN followed by acetylation for purification and deacetylation gave 2′-fucosyllactose **34** in 25% yield from **28**.

Enzyme Inhibition. The inhibitory activities of four synthesized disaccharides having different glycosidic linkages were examined against two α-L-fucosidases from different biological sources. One is *Bacillus* sp. K40T α-L-fucosidase which cleaves only α(1→2) linkage, and the other is bovine epididymis α-L-fucosidase whose substrate specificity is not clear. The inhibitory activities of the disaccharides **1**−**4** against *Bacillus* sp. K40T α-L-fucosidase were examined to investigate the relationship between inhibitory activities and sequence specificity of the enzyme. The assay of this enzyme was carried out using 2′-fucosyllactose as a substrate. The K_m value of 2′-fucosyllactose determined from an [S]/*v* vs [S] plot was 0.39 mM. The inhibition mode and the K_i value of these compounds were determined from the Lineweaver−Burk plot and are summarized in Table 2. α(1→2) Linked disaccharide **4** was a potent competitive inhibitor (K_i = 0.21 mM), while the other three disaccharides **1**−**3** had weak or no inhibitory activities up to 5 mM. These results suggest that 5-thio-L-fucose-containing disaccharides were recognized in the same way as natural

(22) Helferich, B.; Zirner, J. *Chem. Ber.*, **1962**, *95*, 2604.
(23) Yoshino, T; Ishido, Y. 17th Japanese Carbohydrate Symposium, July 18−20, 1995, Kyoto: Abstract p 170.

oligosaccharides by Bacillus α-L-fucosidase and the sequence specificity of the enzymes could be confirmed from their inhibitory activities. Next, compounds **1**−**4** were tested as inhibitors of the α-L-fucosidase from bovine epididymis using *p*-nitrophenyl α-L-fucopyranoside as a substrate. Each disaccharide was a competitive inhibitor, which suggested that these disaccharides bind to the catalytic site of the enzyme. Compounds **1**, **3**, and **4** have K_i values that are similar to that of monosaccharide 5-thio-L-fucose. The K_i value of the disaccharide **2**, which carries α(1→3) linkage, was slightly larger than those of the others. However, the $\Delta\Delta G$ between **2** with the largest K_i value and **4** with the smallest K_i value was only 0.66 kcal/mol, which suggests that the difference is not crucial.[24] This result suggested that bovine epididymis α-L-fucosidase has no sequence specificity.

In summary, four 5-thio-L-fucose-containing disaccharide analogs were synthesized using the trichloroacetimidate method. A 5-thio-α-L-fucoside bond was formed predominantly in each case. The fact that only an α-(1→2)-linked disaccharide of these four disaccharides showed inhibitory activity toward *Bacillus* α-1,2-L-fucosidase suggested that these four disaccharides are useful for estimating the sequence specificity of a fucosidase.

Experimental Section

General. NMR spectra were recorded on 270 MHz or 400 MHz instrument. Melting points were uncorrected. Optical rotations were determined with 0.5 dm cell. Column chromatography was performed with Wakogel C-300 (200−300 mesh; Wako Pure Chem.). Medium pressure liquid chromatography (MPLC) was performed with Baker silica gel chromatography packing (40 μm; J. T. Baker). TLC was carried out on plates precoated with silica gel 60 F-254 (E. Merck). α-L-Fucosidase (bovine epididymis), NADP, and L-fucose dehydrogenase (*Pseudomonas* sp.) were purchased from Sigma Chemical Co. α-L-Fucosidase (*Bacillus* sp. K40T) and *p*-nitrophenyl α-L-fucopyranoside were purchased from Seikagaku Kogyo Co.

2,3,4-Tri-*O*-acetyl-5-thio-L-fucopyranosyl Trichloroacetimidate (6). To a solution of 1,2,3,4-tetra-*O*-acetyl-5-thio-L-fucopyranose (**5**) (99.8 mg, 287 μmol) in DMF (2.25 mL) was added H_2NNH_2·AcOH (45.0 mg, 489 μmol). After being stirred at 50 °C for 2.5 h, the solution was cooled to rt, diluted with EtOAc, and washed with brine (3 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (0.33 mL) and CCl_3CN (0.29 mL), and the solution was cooled to 0 °C. To the stirred solution was added DBU (4.2 μL). After being stirred overnight, the reaction mixture was subjected to a column of silica gel and eluted with 2:1 hexane−EtOAc to give **6** as a syrup (0.123 g, 95%, α:β = 86:14): $[\alpha]^{25}_D$ −219 (*c* 0.67, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.64 (brs, 1H), 6.28 (d, 1H, *J* = 3.0 Hz), 5.59 (brs, 1H, *J* = 2.8 Hz), 5.52 (dd, 1H, *J* = 3.0, 10.6 Hz), 5.42 (dd, 1H, *J* = 2.8, 10.6 Hz), 3.68 (q, 1H, *J* = 6.9 Hz), 2.20, 2.01, 2.00 (each s, 3H × 3), 1.18 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.7, 170.1, 169.9, 160.9, 77.4, 72.2, 69.7, 69.3, 35.8, 20.7, 15.8. Anal. Calcd for $C_{14}H_{18}Cl_3NO_7S$: C, 37.30; H, 4.03; N, 3.11; S, 7.11. Found: C, 37.32 ; H, 4.21; N, 3.00; S, 7.5.

Allyl *O*-(2,3,4-Tri-*O*-acetyl-5-thio-α-L-fucopyranosyl)-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (9). To a solution of **6** (74.9 mg, 0.166 mmol) and allyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranoside (**7**) (126 mg, 0.250 mmol) in CH_2Cl_2 (5.6 mL) was added powdered MS4A (370 mg), and the mixture was stirred for 1 h under Ar. To the solution cooled to −20 °C was added dropwise a solution of BF_3·OEt_2 (6.7 μL, 54 μmol) in CH_2Cl_2 (1.4 mL). The solution was gradually warmed to rt and stirred overnight. The solution was neutralized with Et_3N (30 μL, 0.215 mmol), diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was dissolved in THF (49.5 mL). This solution was mixed with a solution of 1 M Bu_4NF in THF (0.5 mL). After being stirred for 45 min, the solvent was evaporated, and the residue was dissolved in $CHCl_3$. The solution was washed twice with brine, and the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified on a column of silica gel (8:1 $CHCl_3$−MeOH) to give **8** as a syrup (64.1 mg, 70%, α:β = 81:19). The compound **8** was dissolved in pyridine (1 mL) and acetic anhydride (0.5 mL) and stirred overnight. Methanol was added to the solution, and the solvents were evaporated. The residue was chromatographed several times on a column of silica gel (75:1 $CHCl_3$−MeOH) to isolate α-linked disaccharide **9**: mp 181−182 °C (from ether−petroleum ether); $[\alpha]^{20}_D$ −148 (*c* 0.63, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.94−5.80 (m, 1H), 5.50 (bs, 1H), 5.46 (d, 1H, *J* = 8.6 Hz, NH), 5.41−5.33 (m, 2H), 5.26 (dd, 1H, *J* = 7.6, 9.9 Hz), 5.29−5.19 (m, 2H), 5.00 (t, 1H, *J* = 9.9 Hz), 4.80 (d, 1H, *J* = 2.0 Hz), 4.69 (d, 1H, *J* = 8.3 Hz), 4.34−4.27 (m, 1H), 4.12−4.04 (m, 1H), 3.89 (dd, 1H, *J* = 2.3, 10.9 Hz), 3.83 (ddd, 1H, *J* = 7.6, 8.3, 8.6 Hz), 3.65 (ddd, 1H, *J* = 2.3, 5.6, 9.9 Hz), 3.52 (dd, 1H, *J* = 5.6, 10.9 Hz), 3.48 (q, 1H, *J* = 6.9 Hz), 2.18, 2.08, 2.04, 2.03, 1.98, 1.96 (each s, 3H × 6), 1.14 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.9, 170.8, 170.8, 170.2, 169.9, 169.5, 133.5, 117.8, 99.5, 80.9, 73.4, 72.6, 72.4, 71.1, 69.6, 69.4, 69.0, 62.1, 54.7, 34.3, 20.9, 20.8, 20.7, 15.8. Anal. Calcd for $C_{27}H_{39}NO_{14}S$: C, 51.18; H, 6.20; N, 2.21. Found: C, 50.98; H, 6.16; N, 2.14.

Allyl *O*-(5-Thio-α-L-fucopyranosyl)-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (1). To a solution of **9** (25.8 mg, 41 μmol) in MeOH (1.7 mL) was added a solution of 0.5 M NaOMe in MeOH (0.25 mL). After being stirred for 1 h, the solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated in vacuo, and the residue was chromatographed on a column of Bio-Gel P-2 (water) and lyophilized to give **1** as a white powder (16.7 mg, 97%): $[\alpha]^{23}_D$ −254 (*c* 0.21, H_2O); ^{1}H NMR (270 MHz, D_2O) δ 5.98−5.84 (m, 1H), 5.34−5.25 (m, 2H), 4.71 (d, 1H, *J* = 3.0 Hz), 4.58 (d, 1H, *J* = 8.2 Hz), 4.36−4.29 (m, 1H), 4.20−4.13 (m, 2H), 4.03 (bs, 1H), 4.00 (dd, 1H, *J* = 3.0, 10.2 Hz) , 3.88 (dd, 1H, *J* = 3.0, 10.2 Hz), 3.77−3.71 (m, 2H), 3.66 (m, 1H), 3.58−3.51 (m, 2H), 3.42 (q, 1H, *J* = 7.3 Hz), 2.04 (s, 3H), 1.21 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR (67.8 MHz, D_2O) δ 175.4, 134.2, 119.0, 101.0, 84.1, 75.8, 75.0, 74.6, 71.9, 71.4, 71.3, 70.8, 68.3, 56.3, 37.0, 22.9, 16.4. Anal. Calcd for $C_{17}H_{29}NO_9S$: C, 48.22; H, 6.90; N, 3.31. Found: C, 47.97; H, 6.50; N, 3.55.

Allyl *O*-(2,3,4-Tri-*O*-acetyl-5-thio-α/β-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (11). To a solution of **6** (80.5 mg, 0.179 mmol) and allyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**10**) (164 mg, 0.545 mmol) in CH_2Cl_2 (5.6 mL) was added powdered MS4A (400 mg), and the mixture was stirred for 1 h under Ar. After being cooled to 0 °C, a solution of BF_3·OEt_2 (7.2 μL, 58 μmol) in CH_2Cl_2 (1.5 mL) was added dropwise. After being stirred for 7 h, the reaction mixture was neutralized with Et_3N and diluted with $CHCl_3$. The solution was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified on a column of silica gel (1:1 hexane−EtOAc) to give **11** as a crystal (80.6 mg, 76%, α:β = 82:18): mp 125−127 °C (from Et_2O−petroleum ether); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.85−5.79 (m, 1H), 5.65 (d, 1H, *J* = 8.6 Hz), 5.48 (bs, 1H), 5.40 (dd, 1H, *J* = 10.6, 3.0 Hz), 5.27 (dd, 1H, *J* = 2.6, 10.6 Hz), 5.23−5.17 (m, 2H), 5.07 (d, 1H, *J* = 2.6 Hz), 4.78 (d, 1H, *J* = 8.2 Hz), 4.34−4.01 (m, 2H), 4.31 (bt, 1H), 3.93 (dd, 1H, *J* = 5.3, 10.9 Hz), 3.83−3.61 (m, 4H), 3.35 (dt, 1H, *J* = 5.3, 9.9 Hz), 2.17, 2.12, 1.98, 1.95 (each s, 3H × 4), 1.52, 1.37 (each s, 3H × 2), 1.10 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.2, 170.10, 170.09, 170.0, 133.7, 117.7, 99.9, 99.5, 86.1, 75.2, 73.3, 72.9, 71.7, 69.9, 68.8, 67.1, 62.2, 57.1, 34.2, 29.1, 23.4, 21.1, 20.8, 20.7, 19.1, 15.8. Anal. Calcd for $C_{26}H_{39}NO_{12}S$: C, 52.96; H, 6.67; N, 2.38. Found: C, 53.29; H, 6.93; N, 2.38.

Allyl *O*-(2,3,4-Tri-*O*-acetyl-5-thio-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopy-

(24) Sphor, U.; Paszkiwicz-Hnatiw, E.; Morishima, N.; Lemieux, R. U. *Can J. Chem.*, **1992**, *70*, 254 and references therein.

ranoside (12). To a solution of **11** (38.9 mg, 66.0 μmol) in acetone (4.3 mL) was added 1 M HCl (0.29 mL). After being boiled under reflux for 2 h, the solution was neutralized with barium oxide, and the solid was filtered off. The filtrate was concentrated in vacuo, and the residue was acetylated with acetic anhydride and pyridine and a catalytic amount of 4-(dimethylamino)pyridine. The mixture was purified by silica gel column chromatography (2:3 hexane−EtOAc) to give **12** as a syrup (25.8 mg, 62%, α:β = 85:15). α-Linked disaccharide was separated by silica-gel column chromatography (50:1 $CHCl_3$−MeOH): $[\alpha]^{17}_D$ −153 (*c* 0.63, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.88−5.82 (m, 1H), 5.66 (d, 1H, *J* = 7.6 Hz), 5.46 (bs, 1H), 5.39 (m, 2H), 5.24−5.19 (m, 2H), 5.04 (t, 1H, *J* = 8.9 Hz), 5.03 (d, 1H, *J* = 2.3 Hz), 4.99 (d, 1H, *J* = 7.9 Hz), 4.49 (t, 1H, *J* = 8.9 Hz), 4.36−4.29 (m, 1H), 4.23 (dd, 1H, *J* = 5.3, 12.2 Hz), 4.12 (dd, 1H, *J* = 3.0, 12.2 Hz), 4.10−4.03 (m, 1H), 3.70 (ddd, 1H, *J* = 3.0, 5.3, 8.9 Hz), 3.43−3.32 (m, 2H), 2.16, 2.11 (each s, 3H × 2), 2.10 (s, 6H), 1.97, 1.95 (each s, 3H × 2), 1.12 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 170.8, 170.7, 169.8, 169.5, 133.5, 118.0, 98.2, 80.6, 76.2, 72.6, 71.8, 71.4, 70.5, 70.1, 68.6, 62.6, 57.5, 34.8, 29.4, 23.5, 21.1, 20.8, 20.7, 20.6, 16.0. Anal. Calcd for $C_{27}H_{39}NO_{14}S$: C, 51.18; H, 6.20; N, 2.21. Found: C, 50.93; H, 6.47; N, 2.20.

Allyl *O*-(5-Thio-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-*β*-D-glucopyranoside (2). To a solution of **12** (28.1 mg, 44 μmol) in MeOH (1.7 mL) was added a solution of 0.5 M NaOMe in MeOH (0.25 mL). After being stirred for 2 h, the solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated in vacuo, and the residue was chromatographed on a column of Bio-Gel P-2 (water) and lyophilized to give **2** as a white powder (11.9 mg, 63%): $[\alpha]^{23}_D$ −213 (*c* 0.20, H_2O); ^{1}H NMR (270 MHz, D_2O) δ 5.97−5.83 (m, 1H), 5.33−5.24 (m, 2H), 4.83 (bs, 1H), 4.57 (d, 1H, *J* = 7.9 Hz), 4.37−4.30 (m, 1 H), 4.19−4.12 (m, 1 H), 4.02 (bs, 1H), 3.98−3.84 (m, 4 H), 3.82 (dd, 1H, *J* = 11.5, 2.6 Hz), 3.75 (dd, 1H, *J* = 3.3, 11.9 Hz), 3.53 (q, 1H), 3.50−3.49 (m, 2H), 2.01 (s, 3H), 1.16 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, D_2O) δ 175.2, 134.1, 119.0, 100.8, 84.2, 78.5, 76.6, 75.0, 71.6, 71.3, 69.3, 61.6, 56.0, 37.0, 23.0, 16.2. Anal. Calcd for $C_{17}H_{29}NO_9S$: C, 48.22; H, 6.90; N, 3.31. Found: C, 47.87; H, 6.59; N, 3.44.

3-*O*-Acetyl-1,6-anhydro-2-azido-4-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-*β*-D-glucopyranose (16). To a solution of 1,6-anhydro-2-azido-2-deoxy-*β*-D-glucopyranose (**13**) (570 mg, 3.04 mmol) in DMF (3.7 mL) were added imidazole (470 mg, 6.90 mmol) and *tert*-butyldimethylsilyl chloride (600 mg, 3.98 mmol). After being stirred overnight, the solvent was evaporated in vacuo, and the residue was dissolved in $CHCl_3$. The solution was washed subsequently with 1 M HCl and water. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified on a column of silica gel (3:1 hexane−EtOAc) to give 1,6-anhydro-2-azido-4-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-*β*-D-glucopyranose (**14**) and 1,6-anhydro-2-azido-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-*β*-D-glucopyranose (**15**) as a syrup (813 mg, 89%, **14**:**15** = 9:1). The compound **14** was isolated by MPLC (3:1 hexane−EtOAc). **14**: $[\alpha]^{25}_D$ +3.8 (*c* 1.1, CH_2Cl_2); lit.:[20] $[\alpha]^{25}_D$ +5.7 (*c* 1, CH_2Cl_2).

The compound **14** (298 mg, 0.988 mmol) was acetylated with pyridine (2 mL) and acetic anhydride (1 mL) to give crystalline **16** (320 mg, 94%): mp 68−70 °C ($CHCl_3$); $[\alpha]^{20}_D$ +47 (*c* 1.0 $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.50 (brs, 1H), 4.84 (m, 1H), 4.46 (brd, 1H, *J* = 5.6 Hz), 4.00 (dd, 1H, *J* = 1.0, 7.6 Hz), 3.76 (dd, 1H, *J* = 5.6, 7.6 Hz), 3.59 (brs, 1H), 3.05 (s, 1H), 2.11 (s, 3H), 0.94 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 169.4, 100.3, 76.9, 72.9, 69.5, 65.0, 58.8, 25.7, 21.0, 18.1, −4.9, −5.0. Anal. Calcd for $C_{14}H_{25}N_3O_5Si$: C, 48.96; H, 7.34; N, 12.23. Found: C, 48.87; H, 7.39; N, 12.20.

3-*O*-Acetyl-1,6-anhydro-2-azido-2-deoxy-*β*-D-glucopyranose (17). A solution of **16** (0.317 g, 0.922 mmol) in 70% aqueous AcOH (20 mL) was heated at 80 °C for 21 h. Most of the solvents were evaporated in vacuo, and the remaining solvents were coevaporated three times with toluene. The residue was purified on a column of silica gel to give **17** as a syrup (0.155 g, 73%): $[\alpha]^{27}_D$ −6.8 (*c* 1.9, $CHCl_3$), lit.:[18] $[\alpha]^{23}_D$ −6.5 (*c* 1, $CHCl_3$).

***O*-(2,3,4-Tri-*O*-acetyl-5-thio-α-L-fucopyranosyl)-(1→4)-3-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-*β*-D-glucopyranose (18).** To a solution of **6** (99.8 mg, 0.221 mmol) and **17** (155 mg, 0.675 mmol) in CH_2Cl_2 (6.8 mL) was added powdered MS4A (500 mg), and the mixture was stirred for 1 h under Ar. To the solution cooled to −20 °C was added dropwise a solution of BF_3·OEt_2 (8.9 μL, 71 μmol) in CH_2Cl_2 (1.8 mL). After being stirred for 30 min, the solution was neutralized with Et_3N. The solution was diluted with $CHCl_3$ and filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by MPLC (1:1 hexane−EtOAc) to give **18** as a syrup (102 mg, 89%): $[\alpha]^{17}_D$ −122 (*c* 0.85, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.55 (bs, 1H), 5.53 (s, 1H), 5.45 (dd, 1H, *J* = 3.0, 10.6 Hz), 5.30 (dd, 1H, *J* = 3.3, 10.6 Hz), 5.09 (d, 1H, *J* = 3.0 Hz) 5.02 (s, 1H), 4.53 (bd, 1H, *J* = 5.6 Hz), 3.97 (bd, 1H, *J* = 7.3 Hz), 3.82 (dd, 1H, *J* = 5.6, 7.3 Hz), 3.71 (s, 1H), 3.67 (q, 1H, *J* = 6.9 Hz), 3.09 (s, 1H), 2.18, 2.12, 2.08, 1.99 (each s, 3H × 4), 1.17 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.0, 170.1, 169.7, 169.4, 100.6, 78.1, 73.0, 72.5, 71.7, 71.2, 71.1, 69.1, 64.9, 58.4, 34.9, 21.0, 20.9, 20.7, 20.7, 15.9. Anal. Calcd for $C_{20}H_{27}N_3O_{11}S$: C, 46.42; H, 5.26; N, 8.12. Found: C, 46.48; H, 5.35; N, 7.95.

***O*-(2,3,4-Tri-*O*-acetyl-5-thio-α-L-fucopyranosyl)-(1→4)-1,3,6-tri-*O*-acetyl-2-azido-2-deoxy-α/*β*-D-glucopyranose (19).** To a solution of **18** (42.3 mg, 81.7 μmol) in acetic anhydride (0.5 mL) was added dropwise a solution of trifluoroacetic acid−acetic anhydride (1:5, 0.6 mL) at 0 °C, and the mixture was stirred overnight at rt. The solvents were evaporated in vacuo, and the residue was purified on a column of silica gel (3:2 hexane−EtOAc) to give **19** as a syrup (45.4 mg, 90%, α:β = 3:1): ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.26 (d, 0.75H, *J* = 3.6 Hz), 5.56 (d, 0.25H, *J* = 8.6 Hz), 5.54−5.46 (m, 2.75H), 5.30 (dd, 0.75H, *J* = 3.0, 11.2 Hz), 5.23 (dd, 0.25H, *J* = 3.0, 11.2 Hz), 5.15 (dd, 0.25H, *J* = 8.6, 9.9 Hz), 4.71 (d, 0.75 H, *J* = 2.6 Hz), 4.68 (d, 0.25 H, *J* = 2.6 Hz), 4.51−4.42 (m, 1H), 4.35−4.25 (m, 1.25H), 4.07−4.02 (m, 0.75H), 3.81−3.74 (m, 1H), 3.55 (dd, 0.25H, *J* = 8.6, 9.9 Hz), 3.54 (m, 1H), 3.81−3.43 (m, 1H), 3.42 (dd, 0.75H, *J* = 3.6, 10.2 Hz), 2.24, 2.20, 2.19, 2.18, 2.17, 2.16, 2.08, 2.07, 2.06, 1.96, 1.95 (each s), 1.14 (d, 2.25H, *J* = 6.9 Hz), 1.12 (d, 0.75H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.7, 170.4, 170.1, 170.0, 169.9, 168.7, 90.0, 83.5, 77.6, 72.6, 71.4, 71.1, 70.7, 68.6, 61.9, 60.9, 35.4, 21.6−20.6, 15.9. Anal. Calcd for $C_{24}H_{33}N_3O_{14}S$: C, 46.52; H, 5.37; N, 6.78; S, 5.17. Found: C, 46.78; H, 5.38; N, 6.41; S, 4.83.

***O*-(2,3,4-Tri-*O*-acetyl-5-thio-α-L-fucopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α/*β*-D-glucopyranose (20).** A solution of **19** (21.6 mg, 34.9 mmol) in pyridine−water (1:1, 1 mL) was saturated with H_2S at rt. After being stirred overnight, the reaction mixture was concentrated in vacuo. The residue was dissolved in pyridine (1 mL) and acetic anhydride (0.5 mL) and stirred overnight. To the solution was added MeOH, and the solvents were evaporated. The residue was purified by silica gel chromatography (1:4 hexane−EtOAc) to give **20** as a syrup (21.2 mg, 100%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.09 (d, 0.75H, *J* = 3.3 Hz), 5.68−5.65 (m, 0.5H), 5.58 (d, 0.75H, *J* = 8.9 Hz), 5.51−5.44 (m, 2H), 5.31−5.24 (m, 1.75H), 5.12 (dd, 0.25H, *J* = 7.6, 9.9 Hz), 4.79 (d, 1H, *J* = 2.6 Hz), 4.55 (bd, 0.25H), 4.51 (bd, 0.75H), 4.38 (ddd, 0.75H, *J* = 3.3, 8.9, 10.2 Hz), 4.29−4.19 (m, 1.25H), 3.99−3.96 (m, 0.75 H), 3.89−3.80 (m, 1.25H), 3.50−3.45 (m, 1H), 2.23, 2.17, 2.13, 2.12, 2.10, 2.09, 2.08, 1.97, 1.94 (each s), 1.13 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.8−168.8, 90.5, 82.8, 77.2, 72.5, 72.4, 71.1, 70.5, 68.7, 62.2, 51.4, 35.2, 23.1−20.6, 15.9. Anal. Calcd for $C_{26}H_{37}NO_{15}S$: C, 49.13; H, 5.87; N, 2.20. Found: C, 48.91; H, 6.15; N, 1.99.

***O*-(5-Thio-α-L-fucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (3).** A solution of **20** (32 mg, 52 μmol) in MeOH (2 mL) was saturated with NH_3 and stirred overnight. The reaction mixture was concentrated, and the residue was purified on a column of silica gel (6:2:1 EtOAc−MeOH−water) and Sephadex G-15 (water) and lyophilized to give **3** as a white powder (13.5 mg, 67%, α:β = 3:2): $[\alpha]^{23}_D$ −237 (5 min) −241 (70 h) (*c* 0.50, H_2O); ^{1}H NMR (270 MHz, D_2O, at 60 °C) δ 5.20 (d, 0.6H, *J* = 3.6 Hz), 4.79 (d, 0.6H, *J* = 3.3 Hz), 4.78 (d, 0.4H, *J* = 3.3 Hz), 4.71 (d, 0.4H, *J* = 7.6 Hz), 4.03−3.56 (m, 9H), 2.04 (s, 3H), 1.17 (d, 3H, *J* = 7.3 Hz); ^{13}C

NMR (67.8 MHz, D_2O) δ 175.3, 95.8, 91.5, 83.9, 76.22, 76.15, 75.8, 75.1, 73.4, 71.5, 71.3, 70.2, 60.8, 58.0, 55.3, 37.2, 23.0, 22.7, 16.3. Anal. Calcd for $C_{14}H_{25}NO_9S$: C, 43.86; H, 6.57; N, 3.65. Found: C, 43.60; H, 6.49; N, 3.92.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2-chloroacetyl)-α-D-galactopyranose (22). To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**21**) (2.01 g, 5.77 mmol) in $(CH_2Cl)_2$ (17 mL) at 0 °C was added a solution of chloroacetyl chloride (0.93 mL, 11.7 mmol) in $(CH_2Cl)_2$ (5.6 mL). After being stirred at 0 °C for 1 h, ice was added to the reaction mixture and it was diluted with $CHCl_3$. The organic layer was washed subsequently with 1 M HCl, saturated $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated in vacuo. The residue was crystallized from EtOH−Et_2O to give **22** (1.91 g, 78%): mp 115.5−117 °C; $[\alpha]^{26}_D$ +89 (*c* 1.1, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.40 (d, 1H, *J* = 2.3 Hz), 5.52 (brs, 1H), 5.40−5.39 (m, 2H), 4.37 (brt, 1H, *J* = 6.6 Hz), 4.12−4.09 (m, 2H), 4.01 (s, 2H), 2.17 (s, 6H), 2.05, 2.01 (each s, 3H × 2). Anal. Calcd for $C_{16}H_{21}ClO_{11}$: C, 45.24; H, 4.98. Found: C, 44.91: H, 5.03.

Allyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2-chloroacetyl)-β-D-galactopyranoside (24). To a solution of **22** (0.559 g, 1.32 mmol) in CH_2Cl_2 (7.5 mL) at 0 °C was added 25% HBr−AcOH (13.5 mL). After being stirred for 3 h at 0 °C, the solution was diluted with CH_2Cl_2 and washed subsequently twice with ice−water and saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated to give 3,4,6-tri-*O*-acetyl-2-*O*-(2-chloroacetyl)-α-D-galactopyranosyl bromide (**23**) as a syrup (0.570 g, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.70 (d, 1H, *J* = 4.0 Hz), 5.53 (dd, 1H, *J* = 1.3, 3.3 Hz), 5.45 (dd, 1H, *J* = 3.3, 10.6 Hz), 5.13 (dd, 1H, *J* = 4.0, 10.6 Hz), 4.50 (dt, 1H, *J* = 1.3, 6.6 Hz), 4.20 (dd, 1H, *J* = 6.6, 11.4 Hz), 4.12 (dd, 1H, *J* = 6.6, 11.4 Hz), 4.11 (s, 2H), 2.17, 2.07, 2.02 (each s, 3H × 3).

To a mixture of AllOH (1.8 mL, 26 mmol), $Hg(CN)_2$ (1.33 g, 5.27 mmol), and powdered MS3A (0.855 g) in toluene−$MeNO_2$ (1:1, 26 mL) was added dropwise a solution of **23** (0.570 g, 1.28 mmol) in toluene−$MeNO_2$ (1:1, 3.9 mL), and the mixture was stirred for 3 days and filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2. The solution was washed twice with water, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified on a column of silica gel (3:2 hexane−EtOAc) to give **24** as a syrup (0.438 g, 81%): $[\alpha]^{25}_D$ −1.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.93−5.79 (m, 1H), 5.41 (dd, 1H, *J* = 1.0 Hz), 5.29 (dd, 1H, *J* = 7.9, 10.6 Hz), 5.32−5.19 (m, 2H), 5.07 (dd, 1H, *J* = 3.3, 10.6 Hz), 4.57 (d, 1H, *J* = 7.9 Hz), 4.40−4.32 (m, 1H), 4.15−4.07 (m, 1H), 4.20 (dd, 1H, *J* = 6.6, 11.2 Hz), 4.13 (dd, 1H, *J* = 6.6, 11.2 Hz), 4.04 (s, 2H), 3.92 (dt, 1H, *J* = 1.0, 6.6 Hz), 2.16, 2.06, 1.99 (each s, 3H × 3); ^{13}C NMR (67.8 Hz, $CDCl_3$) δ 170.4, 170.2, 170.1, 166.0, 133.1, 118.0, 99.6, 70.7, 70.7, 70.6, 70.2, 67.0, 61.2, 40.5, 20.6, 20.5. Anal. Calcd for $C_{17}H_{23}ClO_{10}$: C, 48.29; H, 5.48. Found: C, 48.16 ; H, 5.46.

Allyl 3,4,6-Tri-*O*-acetyl-β-D-galactopyranoside (25). To a solution of **24** (0.438 g, 1.04 mmol) in EtOAc−MeOH (1:1, 16 mL) was added H_2NNH_2·AcOH (0.292 g, 3.17 mmol). After being stirred for 5 h, the solvents were evaporated and the residue was dissolved in EtOAc. The solution was washed with brine, and the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified on a column of silica gel (1:1 → 1:2 hexane−EtOAc) to give **25** as a syrup (0.295 g, 82%): $[\alpha]^{25}_D$ +2.6 (*c* 1.8, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.03−5.88 (m, 1H), 5.39 (dd, 1H, *J* = 1.0, 3.6 Hz), 5.37−5.23 (m, 2H), 4.94 (dd, 1H, *J* = 3.6, 10.2 Hz), 4.45−4.38 (m, 1H), 4.20−4.13 (m, 1H), 4.42 (d, 1H, *J* = 7.6 Hz), 4.19 (dd, 1H, *J* = 6.6, 11.2 Hz), 4.12 (dd, 1H, *J* = 6.6, 11.2 Hz), 3.90 (dt, 1H, *J* = 1.0, 6.6 Hz), 3.84 (dd, 1H, *J* = 7.6, 10.2 Hz), 2.49 (brs, 1H), 2.13 (s, 3H), 2.05 (s, 6H); ^{13}C NMR (67.8 Hz, $CDCl_3$) δ 170.4, 170.3, 170.2, 133.3, 118.5, 102.0, 72.5, 70.7, 70.5, 69.1, 67.1, 61.3, 20.7, 20.6, 20.6. Anal. Calcd for $C_{15}H_{22}O_9$: C, 52.02; H, 6.40. Found: C, 51.81; H, 6.26.

Allyl *O*-(2,3,4-Tri-*O*-acetyl-5-thio-L-fucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranoside (26). To a solution of **6** (20.2 mg, 44.8 μmol) and **25** (25.0 mg, 72.2 μmol) in $(CH_2Cl)_2$ (1.04 mL) was added powdered MS4A (152 mg), and the mixture was stirred for 1 h under Ar. To the solution cooled to −20 °C was added dropwise a solution of BF_3·OEt_2 (1.9 μL, 15 μmol) in $(CH_2Cl)_2$ (0.40 mL). After being stirred for 15 min at −20 °C, the solution was neutralized with Et_3N (8 mL), diluted with EtOAc, and filtered through a pad of Celite. The filtrate was evaporated, and the residue was purified on a column of silica gel (3:2 → 8:7 → 1:1 hexane−EtOAc) to give **26** as a syrup (24.6 mg, 86%). (α:β = 98:2); α anomer: mp 167−169 °C; $[\alpha]^{25}_D$ −191 (*c* 0.78, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.01−5.87 (m, 1H), 5.45 (dd, 1H, *J* = 1.3, 2.6 Hz), 5.37−5.24 (m, 2H), 5.35 (dd, 1H, *J* = 2.6, 10.6 Hz), 5.32 (d, 1H, *J* = 3.3 Hz), 5.23 (dd, 1H, *J* = 2.6, 10.6 Hz), 5.09 (d, 1H, *J* = 2.6 Hz), 5.08 (dd, 1H, *J* = 3.3, 9.9 Hz), 4.53 (d, 1 H, *J* = 7.6 Hz), 4.45−4.39 (m, 1H), 4.14−4.07 (m, 1H), 4.18 (dd, 1H, *J* = 6.6, 10.9 Hz), 4.14 (dd, 1H, *J* = 7.6, 9.9 Hz), 4.11 (dd, 1H, *J* = 6.6, 10.9 Hz), 3.88 (d, 1H, *J* = 6.6 Hz), 3.73 (dq, 1H, *J* = 1.3, 7.3 Hz), 2.17, 2.14, 2.05, 2.00, 1.984, 1.981 (each s, 3H × 6), 1.08 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.8, 170.6, 170.4, 170.3, 170.1, 169.9, 133.1, 118.7, 100.8, 80.5, 73.6, 72.7, 72.0, 71.5, 70.7, 70.5, 68.7, 67.3, 61.2, 34.0, 20.7, 20.6, 20.5, 15.6. Anal. Calcd for $C_{27}H_{38}O_{15}S$: C, 51.10; H, 6.04; S, 5.05. Found: C, 51.06; H, 5.90; S, 4.73.

Allyl *O*-(5-Thio-α-L-fucopyranosyl)-(1→2)-β-D-galactopyranoside (4). To a solution of **26** (81.1 mg, 0.128 mmol) in MeOH (3 mL) and CH_2Cl_2 (0.9 mL) was added a solution of 0.5 M NaOMe in MeOH (0.5 mL). After 75 min, the solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated in vacuo, and the residue was chromatographed on a column of Sephadex G-15 (water) and lyophilized to give **4** as a white powder (45.0 mg, 92%): $[\alpha]^{23}_D$ −269 (*c* 0.29, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 6.08−5.93 (m, 1H), 5.41−5.28 (m, 2H), 5.09 (d, 1 H, *J* = 3.3 Hz), 4.53 (d, 1H, *J* = 7.3 Hz), 4.44−4.38 (m, 1H), 4.25−4.18 (m, 1H), 4.02 (d, 1H, *J* = 3.0 Hz), 4.00 (dd, 1 H, *J* = 3.3, 10.2 Hz), 3.90−3.83 (m, 3H), 3.84 (dd, 1H, *J* = 3.0, 10.2 Hz), 3.82−3.72 (m, 2H), 3.68 (dd, 1H, *J* = 4.6, 7.9 Hz), 3.52 (q, 1H, *J* = 7.3 Hz), 1.17 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (67.8 MHz, D_2O) δ 134.1, 119.9, 101.8, 84.5 76.9, 75.8, 75.0, 74.6, 72.0, 71.4, 69.8, 61.7, 36.9, 16.4. Anal. Calcd for $C_{15}H_{26}O_9S$: C, 47.11; H, 6.85. Found: C, 46.89; H, 6.63.

Methyl 2,3,4-Tri-*O*-(*p*-methoxybenzyl)-1-thio-β-L-fucopyranoside (27). To a solution of methyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (1.564 g, 4.88 mmol) in MeOH (19 mL) was added a catalytic amount of 0.5 M NaOMe, and the mixture was stirred for 1 h at rt. The solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated, and the residual syrup was dried in high vacuum. To a solution of the residue in DMF (11.5 mL) was added NaH (1.02 g, 55%) at 0 °C, and the mixture was stirred for 30 min. To the solution was added MpmCl (3.2 mL) and stirred overnight at rt. Methanol was added to the solution, and the mixture was diluted with EtOAc. The solution was washed four times with brine, and the organic layer was dried and concentrated. The residue was purified on a column of silica gel (3:1 → 12:5 hexane−EtOAc) to give **27** as a crystal (1.965 g, 73%): $[\alpha]^{26}_D$ −8.1 (*c* 1.2, $CHCl_3$); mp 73−75 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.34−7.26 (m, 6H), 6.90−6.83 (m, 6H), 4.92−4.59 (m, 6H), 4.26 (d, 1H, *J* = 9.6 Hz), 3.816 (s, 3H), 3.804 (s, 3H), 3.796 (s, 3H), 3.78 (dd, 1H, *J* = 9.2, 9.6 Hz), 3.56 (d, 1H, *J* = 3.0 Hz), 3.52 (dd, 1H, *J* = 3.0, 9.2 Hz), 3.46 (q, 1H, *J* = 6.3 Hz), 2.19 (s, 3H), 1.16 (d, 3H, *J* = 6.3 Hz). Anal. Calcd for $C_{31}H_{38}O_7S$: C, 67.12; H, 6.90. Found: C, 67.03; H, 7.35.

***O*-(3,4-*O*-Isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3;5,6-di-*O*-isopropylidene-D-glucose Dimethyl Acetal (28).** To a solution of CSA (13 mg) in $Me_2C(OMe)_2$ (6.16 mL) was added lactose (1.00 g, 2.78 mmol), and the mixture was stirred at 80 °C until it became clear. The solution was cooled to rt and neutralized with Et_3N. The solution was concentrated in vacuo, and the residue was purified by MPLC (2:3 → 1:3 hexane−EtOAc) to give **28** (0.594 g, 42%) and its 6′-*O*-(2-methoxy-2-propyl) isomer **29** (0.500 g, 31%). The structure of **28** was determined as its diacetate **30**. **28**: $[\alpha]^{23}_D$ +35.7 (*c* 2.0, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.60 (dd, 1H, *J* = 6.9, 7.9 Hz), 4.43 (d, 1H, *J* = 8.3 Hz), 4.37 (d, 1H, *J* = 6.9 Hz), 3.50 (s, 3H), 3.49 (s, 3H), 1.50 (s, 6H), 1.39 (s, 6H), 1.32 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 110.0, 109.4, 107.8, 106.6, 102.9, 78.9, 77.7, 77.1, 75.3, 74.9, 74.2, 73.6, 73.1, 64.0, 61.9, 57.1, 53.9 27.6−23.4. Anal. Calcd for $C_{23}H_{40}O_{12}$:

C, 54.32; H, 7.93. Found: C, 54.62; H, 8.17. **29**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.44 (s, 3H), 3.43 (s, 3H), 3.21 (s, 3H), 1.52 (s, 3H), 1.50 (s, 3H), 1.38 (s, 6H), 1.34 (s, 9H), 1.33 (s, 3H). **30**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.02 (dd, 1H, J = 6.9, 7.9 Hz), 4.79 (d, 1H, J = 7.9 Hz), 4.46 (t, 1H, J = 6.3 Hz), 4.39 (dd, 1H, J = 5.0, 11.9 Hz), 4.36 (d, 1H, J = 6.3 Hz), 4.31 (dt, 1H, J = 2.3, 6.3 Hz), 4.24 (dd, 1H, J = 7.3, 11.9 Hz), 4.16−4.10 (m, 2H), 4.08 (dd, 1H, J = 1.7, 2.3 Hz), 3.98−3.93 (m, 4H), 3.42 (s, 6H), 2.13 (s, 3H), 2.09 (s, 3H), 1.56 (s, 3H), 1.48 (s, 3H), 1.37 (s, 3H), 1.33 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.8, 169.6, 110.9, 110.7, 108.0, 105.0, 100.2, 78.2, 78.1, 77.3, 75.1, 73.8, 73.7, 72.6, 70.9, 64.6, 63.4, 55.5, 53.0, 27.7, 27.6, 26.4, 26.3, 26.1, 24.7, 20.9, 20.8.

***O*-(6-*O*-Acetyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1→4)-2,3:5,6-di-*O*-isopropylidene-D-glucose Dimethyl Acetal (31).** To a solution of **28** (3.356 g, 6.59 mmol) in CH_2Cl_2 (33.7 mL) was added Ac_2O (1.18 mL) and Et_3N (2.67 mL). After being stirred for 18 h at rt, MeOH was added and the solution was concentrated in vacuo. The residue was purified by MPLC (3:2 → 1:1 hexane−EtOAc) to give **31** (2.434 g, 67%) and diacetate **30** (0.188 g, 5%) and recovered **28** (0.963 g, 28%): $[\alpha]^{25}_D$ +32.1 (*c* 2.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45 (dd, 1H, J = 6.2, 7.6 Hz), 4.42 (d, 1H, J = 8.2 Hz), 4.37 (d, 1H, J = 6.2 Hz), 4.35 (dd, 1H, J = 4.7, 11.6 Hz), 4.30 (dd, 1H, J = 7.2, 11.6 Hz), 4.28 (dt, 1H, J = 6.5, 2.6 Hz), 4.16 (dd, 1H, J = 6.5, 8.7 Hz), 4.11 (dd, 1H, J = 2.3, 5.7 Hz), 4.09 (d, 1H, J = 1.5, 2.6 Hz), 4.07 (t, 1H, J = 5.7, 7.6 Hz), 4.02 (dd, 1H, J = 2.6, 8.7 Hz), 3.95 (ddd, 1H, J = 2.3, 4.7, 7.2 Hz), 3.90 (dd, 1H, J = 1.5, 7.6 Hz), 3.56 (dd, 1H, J = 7.6, 8.2 Hz), 3.47 (bs, 1H), 3.44 (s, 3H), 3.44 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.7, 110.4, 110.4, 108.3, 105.1, 103.7, 79.0, 78.0, 77.8, 76.4, 75.0, 74.1, 73.3, 71.4, 64.6, 63.5, 56.1, 53.2, 28.1, 27.2, 26.3, 26.2, 25.6, 23.8, 20.8. Anal. Calcd for $C_{25}H_{42}O_{13}$: C, 54.54; H, 7.69. Found: C, 54.71; H, 7.95.

***O*-(2,3,4-Tri-*O*-(*p*-methoxybenzyl)-α-L-fucopyranosyl)-(1→2)-*O*-(6-*O*-acetyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1→4)-2,3:5,6-di-*O*-isopropylidene-D-glucose Dimethyl Acetal (32).** A mixture of **31** (1.172 g, 2.12 mmol) and IDCP (0.719 g, 1.52 mmol) in $(CH_2Cl)_2$−Et_2O (1:5) (12.3 mL) and MS4A (1.03 g) was stirred for 1 h at 0 °C under Ar atmosphere. To the stirred solution was added **27** (0.567 g, 1.02 mmol), and the mixture was stirred for 1 h at rt. The solution was diluted with $CHCl_3$, and the solid was filtered off. The filtrate was washed with 2 M aqueous $Na_2S_2O_3$, and the organic layer was dried and concentrated in vacuo. The residue was purified by MPLC (3:2 hexane−EtOAc) to give **32** as a syrup (0.701 g, 65%): $[\alpha]^{25}_D$ −34.0 (*c* 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.23 (m, 6H), 6.90−6.82 (m, 6H), 5.53 (d, 1H, J = 3.6 Hz), 4.90−4.63 (m, 6H), 4.61 (d, 1H, J = 6.6 Hz), 4.49 (dd, 1H, J = 5.9, 6.3 Hz), 4.36 (d, 1H, J = 6.3 Hz), 4.33 (dd, 1H, J = 4.0, 11.5 Hz), 4.25−4.21 (m, 2H), 4.18 (t, 1H, J = 5.9 Hz), 4.08−3.87 (m, 9H), 3.81 (s, 3H), 3.802 (s, 3H), 3.797 (s, 3H), 3.69 (t, 1H, J = 6.9 Hz), 3.42 (s, 6H), 2.09 (s, 3H), 1.46 (s, 3H), 1.43 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H), 1.32 (s, 3H), 1.29 (s, 3H), 1.05 (d, 3H, J = 6.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.7, 159.1, 158.9, 131.5, 131.2, 131.0, 129.9, 129.2, 128.9, 113.7, 113.52, 113.47, 110.2, 110.0, 108.6, 105.2, 101.2, 95.2, 80.1, 79.0, 77.6, 77.5, 77.4, 76.1, 75.2, 75.0, 74.3, 74.2, 73.6, 72.8, 72.3, 70.8, 66.4, 65.2, 63.3, 55.9, 55.2, 53.1, 27.8, 27.3, 26.84, 26.76, 26.5, 25.2, 20.8, 16.8. Anal. Calcd for $C_{55}H_{76}O_{20}$: C, 54.54; H, 7.69. Found: C, 54.71; H, 7.95.

***O*-(2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α/*β*-D-glucopyranose (33).** A solution of **32** (0.219 g, 0.206 mmol) and CAN (0.68 g, 1.24 mmol) in 9:1 (v/v) MeCN−H_2O (3.1 mL) was stirred at rt. After TLC indicated completion of the reaction, pyridine (22 mL) and Ac_2O (11 mL) and a catalytic amount of DMAP was added and stirred overnight. To the reaction mixture was added MeOH. The mixture was then diluted with $CHCl_3$. The solution was washed subsequently with water and saturated aqueous $NaHCO_3$. The organic layer was dried and concentrated in vacuo. The residue was purified on a column of silica gel (3:2 hexane−EtOAc) to give **33** as a syrup (0.128 g, 72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (d, 0.25H, J = 4.0 Hz), 5.69 (d, 0.75H, J = 8.3 Hz), 5.37 (d, 1H, J = 3.6 Hz), 4.41 (d, 1H, J = 7.6 Hz), 1.22 (d, 3H, J = 7.6 Hz); ^{13}C NMR for α anomer (67.8 MHz, $CDCl_3$) δ 170.6−168.6, 100.0, 95.6, 88.8, 73.5, 73.2, 71.6, 71.0, 70.7, 70.6, 69.02, 68.95, 67.9, 67.4, 66.9, 64.7, 60.9, 60.7, 20.9−20.4, 15.3. Anal. Calcd for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.53; H, 5.98.

***O*-α-L-Fucopyranosyl-(1→2)-*O*-*β*-D-galactopyranosyl-(1→4)-D-glucopyranose (34).** To a solution of **33** (0.102 g, 0.118 mmol) in MeOH (16.8 mL) was added a catalytic amount of NaOMe, and the mixture was stirred overnight at rt. The solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was evaporated, and the residue was chromatographed on a column of Sephadex G-15 (water) and lyophilized to give **34** as a white solid (47 mg, 81%): ^{1}H NMR (400 MHz, D_2O) δ 5.37 (d, J = 2.7 Hz), 5.28 (d, J = 3.7 Hz), 4.70 (d, J = 8.1 Hz), 4.58 (d, J = 7.8 Hz), 4.31 (q, J = 6.5 Hz), 4.28 (q, J = 6.5 Hz), 1.28 (d, J = 6.5 Hz).

Enzyme Inhibition Assays. A reaction solution which contained α-L-fucosidase (*Bacillus* sp. K40T) and 2′-fucosyllactose (0.37, 0.53, 1.0, 1.7 mM) and inhibitors (three different concentrations in the range 0.14−0.56 mM and 0 mM) and bovine serum albumin in 56 mM phosphate buffer (pH 6.5, 150 μL) was incubated at 37 °C for 20 or 30 min. The enzyme reaction was quenched at 100 °C for 5 min and the released L-fucose was analyzed using fucose dehydrogenase−NADP system.[25,26]

A reaction solution containing *p*-nitrophenyl α-L-fucopyranoside (0.067, 0.10, 0.17, 0.33 mM), bovine epididymis α-L-fucosidase, and inhibitors (three different concentrations in the range of 0.018−0.21 mM) in 20 mM sodium citrate buffer (pH 5.8, 300 μL) was incubated at 25 °C for 30 or 40 min. The reaction was quenched with cooling in an ice bath and by adding glycine buffer (pH 10.0, 500 μL), and the amount of *p*-nitrophenol was measured by the absorbance at 400 nm.

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(25) Tsay, G. C.; Daeson, G. *Anal. Biochem.*, **1977**, *78*, 423.
(26) Compound **1** showed 7% inhibition at 5 mM.