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Novel features of acceptor recognition by β-(1→4)-galactosyltransferase

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Abstract

In order to understand how β-(1→4)-galactosyltransferase recognizes its glycosyl acceptor, substrate specificities were investigated using synthetic 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetylglucosamine) derivatives in which the 1-, 2-, 3-, 4-, and 6-positions were systematically substituted. The hydroxyl groups at the 3-, 4-, and 6-positions were substituted by fluoride, thiol or hydrogen. For modification of the 2- position, the acetamido group was converted to ethylamino-, *N*-methylacetamido- and acetyloxy groups. For the anomeric position, several sugar residues were introduced as the aglycon of *N*-acetylglucosaminide. Galactose transfer assay using synthetic *N*-acetylglucosamine derivatives indicated that both the acetamido group and the 4-hydroxyl group were essential for binding of *N*-acetylglucosamine toward the β-(1→4)-galactosyltransferase. The assay also showed that the *N*-acetylglucosamine having a large substitution at the 6-position can be recognized as an acceptor. It is suggested that in this case the bulky substitutent is positioned away from the catalytic site or out of enzyme. Since the 2-acetamido and the 4-hydroxyl group are essential for recognition, the side composed of the 2, 3, and 4-positions may face the acceptor-binding site.

Keywords: β-(1→4)-galactosyltransferase; Acceptor supecificity; Galactosyl transfer; *N*-acetylglucosamine

1. Introduction

Bovine β-(1→4)-galactosyltransferase (GalTase, EC 2.4.1.90) catalyzes the transfer of a galactose residue from UDP-galactose to the 4-position of 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetylglucosamine). Since the GalTase is available in large quantities bovine milk, it is useful as synthetic catalyst for enzymatic or chemoenzymatic syntheses of galactosyl oligosaccharides [1]. This enzyme has the following unique characteristics. When GalTase forms a complex with lactalbumin, the substrate specificity changes from *N*-

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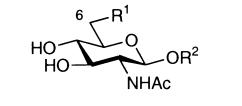
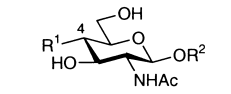
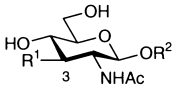
acetylglucosamine to glucose [2,3]. The GalTase is also the most extensively studied enzyme among many kinds of glycosyltransferases. Cloning of GalTase was performed by Narimatsu et al. [4] in 1986, and investigation of the binding site of UDP-galactose was reported by Brew et al. [5] in 1990. In addition, in order to investigate substrate specificity and to design inhibitors, modified *N*-acetylglucosamines [6–8], as well as UDP-modified-galactoses [1,9], have been synthesized. Since modification at the 4-position of *N*-acetylglucosamine interferes with the binding to the GalTase [6,7,10], an acceptor analogue–inhibitor has not been found. Hindsgaul and co-workers reported inhibitory activities of several deoxygenated acceptor–analogue inhibitors toward eight kinds of glycosyltransferases and concluded that the glycosyltransferases were divided into two groups [11]. In the first group the hydroxyl group to be glycosylated in glycosyl acceptor is essential for the binding to the enzyme. In contrast, the enzyme belonging to the second group does not require the hydroxyl group for binding. GalTase belongs to the former group. The 4-hydroxyl group, especially the hydrogen of the 4-hydroxyl group of *N*-acetylglucosamine, is deduced to form a hydrogen bond to the GalTase. However, recognition by the GalTase of *N*-acetylglucosamine has not been investigated in detail. In order to understand how the GalTase recognizes the acceptor moiety, we studied substrate specificities systematically [10] by use of modified *N*-acetylglucosamines of which hydroxyl groups are substituted by fluoride, thiol or hydrogen. For both the fluoro and thiol functions, the nature of the hydrogen-bond formation is expected to be biased to a hydrogen-accepting and a hydrogen-donating role, respectively. In addition, deoxygenation is expected to remove both the hydrogen-accepting and -donating roles of the hydroxyl group. We report here the behaviors of a series of synthetic *N*-acetylglucosamine derivatives toward GalTase.



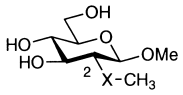
1 R^1 = H, R^2 = Me
2 R^1 = F, R^2 = 3Galβ1-OMe
3 R^1 = SH, R^2 = 6Glcα1-OMe
4 R^1 = $S)_2$, R^2 = 6Glcα1-OMe



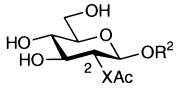
5 R^1 = H, R^2 = 6Glcα1-OMe
6 R^1 = F, R^2 = Me
7 R^1 = SH, R^2 = Me



8 R^1 = H, R^2 = Me
9 R^1 = F, R^2 = 3Galβ1-OMe



10 X = $NHCH_2$
11 X = NMeCO



12 X = O, R^2 = Me
13 X = NH, R^2 = Me
14 X = NH, R^2 = β–3 Gal-(1→ *O*) Me
15 X = NH, R^2 = α–6 Glc-(1→ *O*) Me
16 X = NH, R^2 = α–6 Man-(1→ *O*) Me
17 X = NH, R^2 = α–2 Man-(1→ *O*) Me

2. Results

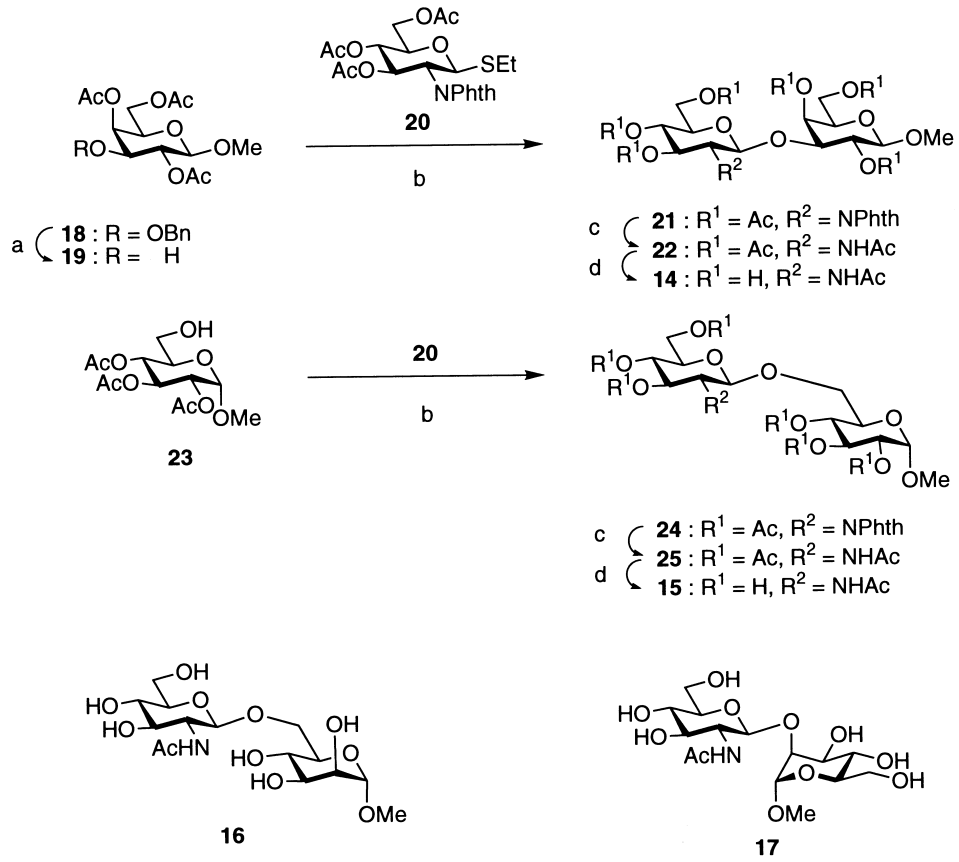
Synthesis of N-*acetylglucosamine analogues*.—In order to investigate substrate specificity, we planned to synthesize several *N*-acetylglucosamine derivatives modified at the 1, 2, 3, 4, and 6-positions as shown in **1**–**17**.

In order to check for specificity toward the anomeric position, we prepared *N*-acetylglucosaminyl disaccharides whose aglycon moieties were varied (**14**–**17**). A disaccharide, β-D-GlcNAc-β-(1→3)-Gal-(1→O)Me **14**, was synthesized [12] by glycosylation of galactoside **19** with **20** [13] and by subsequent deprotection (Scheme 1). The disaccharide **15** was also synthesized in moderate yield in the same manner as in the preparation of **14**. Other disaccharides, **16** [14] and **17** [15] were synthesized according to the reported method.

Deoxygenation at the 6-position of **26** was performed by iodination and subsequent radical reduction. The 3-deoxygenated derivative **8** was derived from benzylidene acetal derivative **28** (Scheme 2) [16]. Thiocarbonylimidazolylation at the 3-position of **28** and subsequent radical reduction and deprotection afforded the 3-deoxygenated derivative **8**. The 4-deoxygenated glycosyl donor **33** was synthesized by radical reduction of the corresponding iodo derivative **32**. Glycosylation of **23** with deoxygenated donor **33** in the presence of MeOTf and subsequent deprotection afforded disaccharide **5**.

Synthetic routes for glycosyl donors having fluoro or thiol groups are shown in Scheme 3. Fluorination at the 6-position of **35** and the 3-position of **39** were performed with diethylaminosulfur trifluoride (DAST) [17]. The 6-thioacetyl derivative **38** was derived from tosyl derivative **37** by the treatment of potassium thioacetate.

Synthesis of the disaccharides having a fluorine atom or a thiol group are shown in Scheme 4. Glycosylation with the fluorinated **36** or **41** or



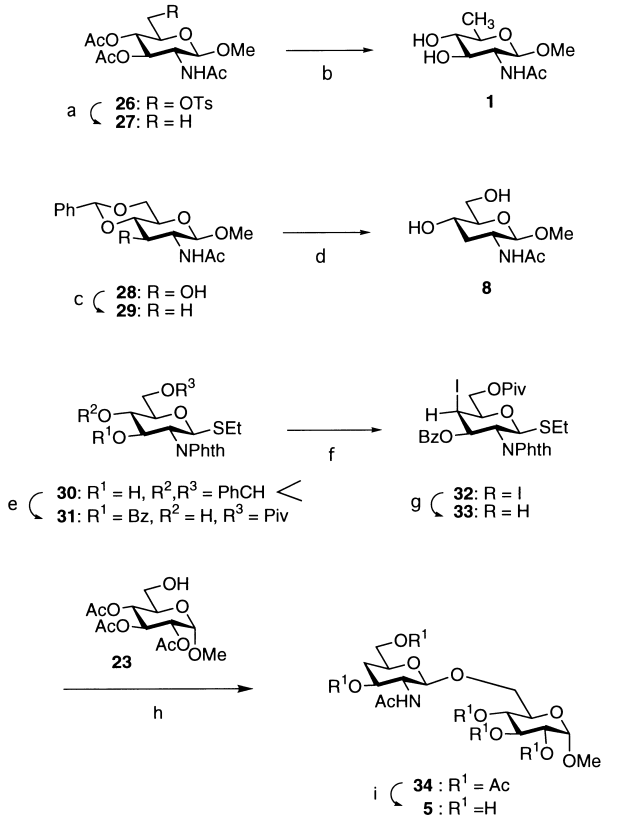
Scheme 1. (a) H_2, $Pd(OH)_2$, MeOH; (b) MeOTf, MS 4A, CH_2Cl_2, **21**: 92%, **24**: 80%; (c) (1) $H_2NNH_2 \cdot H_2O$, EtOH, 90 °C, (2) Ac_2O, pyridine, DMAP, **22**: 90% , **25**: 83%; (d) NaOMe, MeOH, **14**: 90%, **15**: 81%.

thioacetylated donor **38** in the presence of MeOTf and subsequent deprotection afforded desired disaccharides **2**, **9**, and **3**, respectively. S-deacetylation of **47** was carried out in the presence of DL-dithiothreitol in order to avoid disulfide formation. However, the disulfide **4** was also derived from the thiol **3** by spontaneous oxidation [18] in water, and it was then used in the GalTase assay.

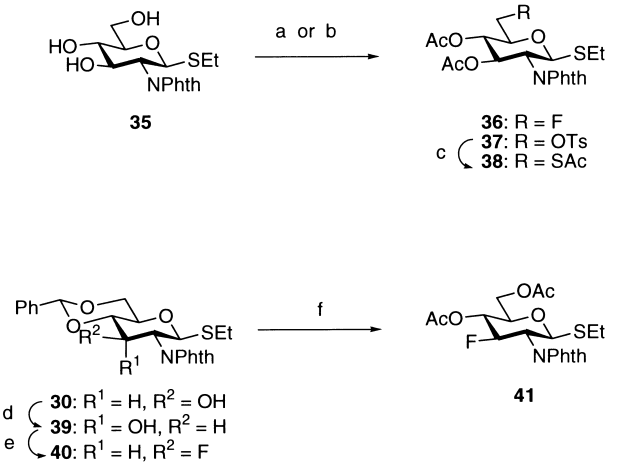
The 4-fluoro **6** and 4-thiol derivatives **7** were synthesized from methyl 2-azido-2-deoxy-β-D-galactopyranoside **48** [19] that was derived from D-galactal by the azidonitration method (Scheme 5). In order to introduce a fluorine atom at the 4-position, the 3- and 6-positions of **48** were selectively acetylated and then treated with DAST to afford the 4-deoxy-4-fluoro compound **50**. After reduction of the azido group by hydrogen sulfide, N-acetylation, followed by O-deacetylation, afforded **6**. Formation of the triflate at the 4-position of **49** and subsequent nucleophilic substitution with potassium thioacetate afforded the 4-thioacetyl derivative **52**. N-Acetylation and O-deacetylation was performed in the same manner as for the preparation of **6**.

The two kinds of 2-modified derivatives were synthesized from the known derivative **54** (Scheme 6) [20]. In order to remove the carbonyl group from the acetamido group, compound **54** was reduced with $LiAlH_4$ to afford the 2-ethylamino derivative **10**. N-Methylated derivative **11** was obtained by the treatment of **54** with sodium hydride and methyl iodide, and subsequent O-debenzylation. In this case, the desired derivative **11** was obtained as an E/Z mixture. The 2-*O*-acetyl derivative **12** [21] was prepared from the 4,6-*O*-benzylidene derivative **57** by acid hydrolysis.

Enzyme assays.—Enzyme assays were performed by the reported method of Babad and Hassid [22]. Radioactive galactose was transferred from UDP-[U-^{14}C]-galactose to synthetic *N*-acetylglucosamine derivatives by the action of the GalTase, and then the ^{14}C-labelled 2-acetamido-2-deoxy-lactoside (*N*-acetyllactosaminide) was quantitated by liquid scintillation counting. The kinetic



Scheme 2. (a) (1) NaI, DME, (2) Bu_3SnH, AlBN, toluene, 77% (2 steps); (b) NaOMe, MeOH, 60%; (c) (1) Thiocarbonyldiimidazole, DMAP, CH_2Cl_2; (2) Bu_3SnH, AlBN, toluene, 85% (2 steps); (d) (1) 60% AcOH, (2) Ac_2O, pyridine, (3) NaOMe, MeOH, 77% (3 steps); (e) (1) BzCl, pyridine, (2) 60% AcOH, (3) PivCl, pyridine, 84% (3 steps); (f) (1) Tf_2O, pyridine, CH_2Cl_2, (2) NaI, DME, 99% (2 steps); (g) Bu_3SnH, AlBN, toluene, 87%; (h) (1) MeOTf, MS4A, CH_2Cl_2, (2) $H_2NNH_2{\cdot}H_2O$, EtOH, (3) Ac_2O, pyridine, 66% (3 steps); (i) NaOMe, MeOH, 88%.



Scheme 3. (a) (1) DAST, CH_2Cl_2, (2) Ac_2O, pyridine, 38% (2 steps); (b) (1) TsCl, puridine, (2) Ac_2O, pyridine, 92% (2 steps); (c) KSAc, DMF, 82%; (d) (1) Swern oxidation, (2) $NaBH_4$, EtOH–H_2O, 96% (2 steps), gluco:allo = 1:4, (e) DAST, CH_2Cl_2, 50%, (f) (1) 60% AcOH, (2) Ac_2O, pyridine, 63% (2 steps.

parameters of *N*-acetylglucosamine derivatives obtained for GalTase reactions are summarized in Table 1.

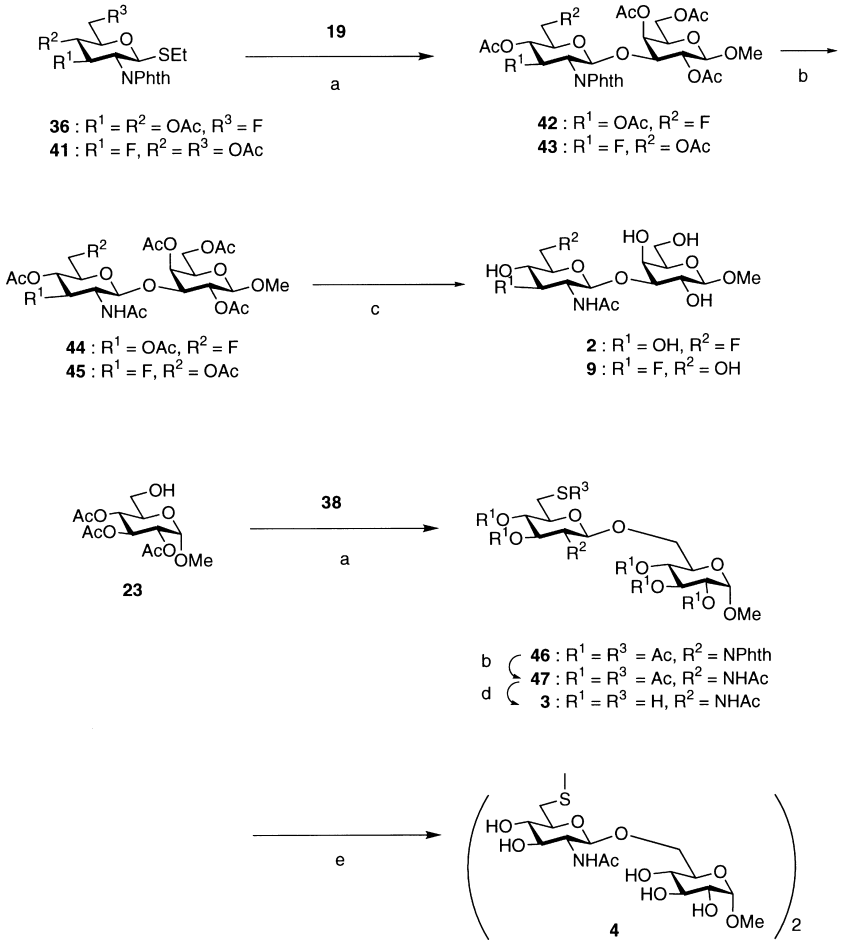
In the case of the 6-modified analogues, the assay indicated that deoxy- **1**, fluoro- **2** and thiol-derivatives **3** were good acceptors. However, in the case of disulfide derivatives **4**, the V_{max} value was far lower than that of native disaccharide **15**.

The 4-modified *N*-acetylglucosamine derivatives, deoxy- **5**, fluoro- **6**, and thiol-derivatives **7** were assayed. However, the assay indicated that these derivatives were neither acceptors nor inhibitors. In addition, even with a 10-fold higher concentration compared to the K_m value of *N*-acetylglucosamine, derivatives **5**, **6**, and **7** did not inhibit the reaction in which *N*-acetyllactosamine was formed by the action of UDP-galactose, *N*-acetylglucosamine, and GalTase. Therefore, this suggests that both hydrogen and oxygen in the 4-hydroxyl group are essential for the binding of *N*-acetylglucosamine to GalTase.

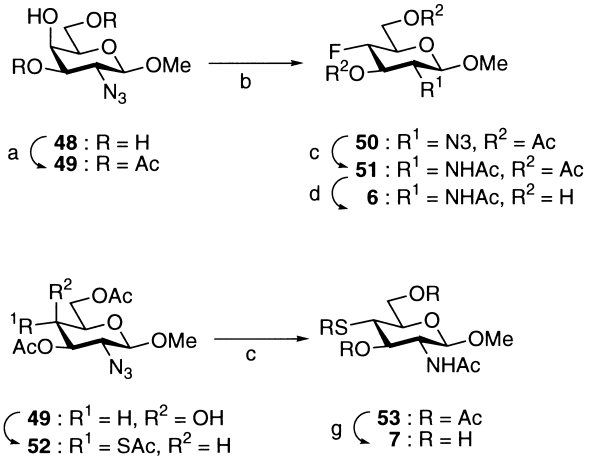
In order to investigate recognition at the 3-position of *N*-acetylglucosamine, 3-deoxygenated **8** and the 3-deoxy-3-fluoro-derivative **9** were used. In the case of the 3-deoxy derivative **8**, the K_m value was elevated by 2.8 fold and the V_{max} value was decreased to 34% compared to those of *N*-acetylglucosamine **13**. On the other hand, in the case of the 3-deoxy-3-fluoro derivative **9**, no transfer activity was observed due to a decrease of V_{max}. However, the affinity was changed only slightly ($K_i = 4.2$ mM). These results suggested that modification at the 3-position affected the reaction velocity rather than the affinity.

It is known that 2-deoxy-2-propionamido-D-glucose is a good acceptor for GalTase [23]. However, GalTase can not recognize 2-ethylamino- **10**, 2-*N*-methylacetamido- **11** and 2-*O*-acetyl-D-glucopyranosides **12**. In addition, even with a 10-fold higher concentration compared to the K_m value of *N*-acetylglucosamine, these derivatives did not act as inhibitors. These results suggested that the whole acetamido group is essential for binding of *N*-acetylglucosamine to the GalTase.

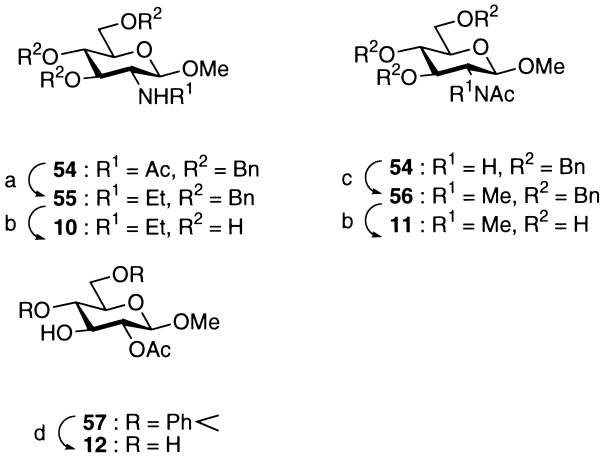
In order to investigate the recognition of the reducing end, or aglycon, of *N*-acetylglucosamine by GalTase, four disaccharides, **14**, **15**, **16**, and **17**, having different aglycons and linkages, were used in the GalTase assay. Since their K_m values were similar, we conclude that GalTase does not recognize the aglycon sugar moiety and linkage to a significant degree.



Scheme 4. (a) MeOTf, MS4A, CH_2Cl_2, **43**: 44%, **46**:75%; (b) (1) $H_2NNH_2{\cdot}O$, EtOH, (2) Ac_2O, pyridine, DMAP, **44**: 44% (3 steps), **45**: 56% (2 steps), **47**: 76%; (c) NaOMe, MeOH, **2**: 82%, **9**: 71%; (d) NH_4OH, DL-dithiothreitol, MeOH, 56%; (e) H_2O (56 mM). 37 °C, 67%.



Scheme 5. (a) Ac_2O, pyridine, CH_2Cl_2, 66%; (b) DAST, CH_2Cl_2, 48%; (c) (1) H_2S, pyridine, triethylamine, H_2O, (2) Ac_2O, pyridine, **51**: 98%, **53**: 84%; (d) NaOMe, MeOH, 91%; (e) (1) Tf_2O, pyridine, CH_2Cl_2, (2) KSAc, DMF, 85% (2 steps); (g) NH_4OH, DL-dithiothreitol, MeOH, 62%.



Scheme 6. (a) $LiAlH_4$, Et_2O, reflux 76%; (b) Pd/C, H_2, MeOH, **10**: 87%, **11**: 85%; (c) NaH, MeI, DMF, 99%; (d) 60% AcOH, 72%.

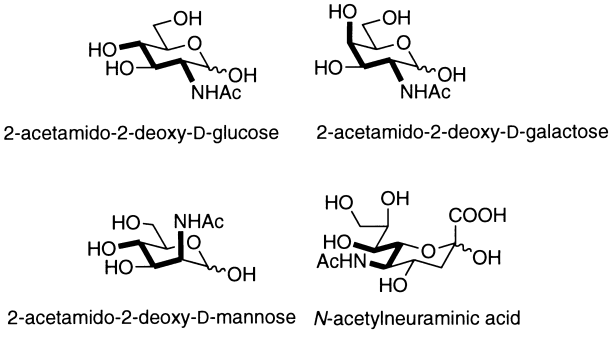
Table 1
Kinetics parameters of GlcNAc-OR derivatives for the galactosylation with bovine β-1,4GalTase

Compd	K_m (nM)	V_{max} (relative
1	0.5	55
2	1.0	94
3	1.2	33
4	0.2	1.7
5	no binding	
6	no binding	
7	no binding	
8	4.2	34
9	2.7[a]	< 1
10	no binding	
11	no binding	
12	no binding	
13	1.5	100
14	1.4	94
15	0.7	66
16	1.0	108
17	2.8	100

[a] K_i value.

3. Discussion

The GalTase assay using the synthetic *N*-acetylglucosamine derivatives suggests that modification at both the 2- and 4-position severely interfered the binding of the *N*-acetylglucosamine to the GalTase. Therefore, GalTase recognized both the acetamido and the 4-hydroxyl groups. In other words, a W-like orientation of the acetamido group and the 4-hydroxyl group is characteristic enough to distinguish *N*-acetylglucosamine from other amino sugars. Since there is no other sugar that has the same W-like orientation among the sugar residues of the glycoconjugates, it may be reasonable that GalTase recognizes the W-like orientation in *N*-acetylglucosamine as a crucial marker, (see structures below).



Nishida et al. reported that the GalTase catalyzes the transfer of a galactose from UDP-galactose to the β-anomeric position of 3-acetamido-3-deoxy-D-glucopyranose [24,25]. This unusual transfer also suggests that the GalTase recognized the W-like orientation as a recognition marker.

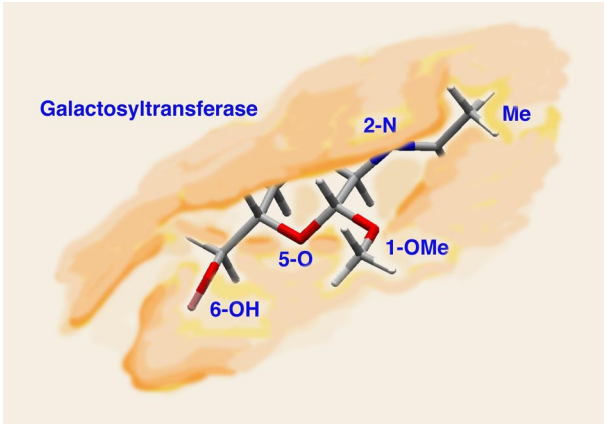


Fig. 1. Schematic representation of the binding of methyl 2-acetamido-2 deoxy-β-D-glucopyranoside to GalTase.

The assay shows that the disulfide disaccharide **4** is an acceptor ($K_m = 0.2$ mM). Hindsgaul and coworkers have also reported the same tendency for the *N*-acetylglucosamine having a large substitution at the 6-position such as a sialic acid methyl ester or fucose to act as galactosyl acceptor [7]. Therefore, the 6-position of *N*-acetylglucosamine is not of importance for its binding to the GalTase. It is reasonable to assume that in this case the bulky substitutent is positioned away from the catalytic site or outside of the enzyme altogether.

Considering the assay results described above, the binding mode of *N*-acetylglucosamine to GalTase can be deduced. Since the 2-acetamido and the 4-hydroxyl groups are essential for recognition, the side that is formed by the 2-, 3-, 4-positions may face the acceptor-binding site. The 6-position, as well as the aglycon at the reducing end, may be positioned outside of the acceptor-binding site. These interrelationships are shown in Fig. 1.

4. Conclusions

In order to investigate the substrate specificity of the β-(1→4)-galactosyltransferase in detail, several *N*-acetylglucosamine analogues, in which the 1-, 2-, 3-, 4-, and 6-positions were individually chemically modified, were synthesized and subjected to enzyme assay. The enzyme assay indicated that the β-(1→4)-galactosyltransferase recognizes the W-like orientation of the 2-acetamido and 4-hydroxyl

groups. Since there is no report of an X-ray crystal structure of the GalTase, derivatization of substrates and their enzyme assays may serve as the best methods to obtain clues for the binding of *N*-acetylglucosamine to the acceptor-binding site of the enzyme. The results obtained here, together with previous ones concerning substrate specificity toward the UDP-galactose [26], enabled us to design and synthesize a potent tricomponent GalTase-inhibitor composed of UDP, galactose, and *N*-acetylglucosamine [27].

5. Experimental section

General.—^{1}H NMR spectra were recorded with JEOL EX-270 or Bruker AM 500 instruments at 298 K. The chemical shifts are presented in ppm and referenced to tetramethylsilane in $CDCl_3$, or sodium 3-(trimethylsilyl)propionate in D_2O as the internal or external standard, respectively. ^{13}C NMR spectra were recorded with a JEOL EX-270 at 298 K. The chemical shifts are expressed in ppm and referenced to tetramethylsilane in $CDCl_3$, or dioxane (67.4 ppm) in D_2O as the internal or external standard, respectively. Optical rotations were measured with a JASCO DIP-4. High-resolution mass spectra were recorded on a JMS-SX102A or Shimazu/Kratos concept-IIH under FAB conditions. All reactions were monitored by TLC (Silica Gel 60-F_{254}, E. Merck) by charring after spraying with 5% H_2SO_4 in methanol. Wako-Gel C-300 was used for flash column chromatography. Bovine β-(1→4)-galactosyltransferase (EC 2.4.1.90) and calf intestinal alkaline phosphatase (EC 3.1.3.1) were purchased from Sigma Chemical Co. and Boehringer Mannheim, respectively. UDP-α-[U-^{14}C]-D-galactose were purchased from NEN Research Products.

Methyl 2,4,6-tri-O-*acetyl-3*-O-*benzyl*-β-D-*galactopyranoside* (**18**). To a solution of methyl β-D-galactopyranoside [28] (5.0 g, 25.7 mmol) in dry MeOH (100 mL) was added di-*n*-butyltin oxide (6.42 g, 25.7 mmol), and the mixture was stirred under reflux for 2 h. The mixture was allowed to cool to room temperature, and it was concentrated in vacuo. The residue was dried by coevaporating with pyridine (twice). To a solution of this residue in dry benzene (200 mL) was added *n*-butylammonium iodide (11.4 g, 30.93 mmol) and benzyl bromide (6 mL, 51.5 mmol), and the mixture was stirred under reflux for 2 h. After quenching the excess benzyl bromide by the addition of the NaOMe (excess), the mixture was concentrated in vacuo. Then saturated aq KF solution 100 mL was added to the residue, which was then stirred at room temperature. The mixture was then diluted with EtOAc, and the organic phase was washed with water and then dried with $MgSO_4$. After concentration of the mixture, the residue was dissolved in acetic anhydride and pyridine (1:1, 10 mL). After stirring for 0.5 h at room temperature, the mixture was concentrated in vacuo. Purification of this residue by the flash column chromatography (3:2 hexane–ethyl acetate) afforded **18** (7.4 g, 70%) : $[\alpha]_D^{23}$ + 40.66 °(*c* 0.9, $CHCl_3$); mp 120–122 °C; ^{1}H NMR: δ 7.4–7.18 (m, 5 H, Ph), 5.52 (d, 1 H, $J_{4,3}$ 2.6 Hz, H-4), 5.12 (dd, 1 H, $J_{2,3}$ 9.9, $J_{2,1}$ 7.9 Hz, H-2), 4.71, 4.39 (d, 1 H, *J* 12.1 Hz, benzyl CH_2), 4.30 (d, 1 H, H-1), 4.19 (d, 2 H, $J_{6,5}$ 6.6 Hz, H-6), 3.83 (dd, 1 H, H-5), 3.55 (dd, 1 H, H-3), 3.48 (s, 3 H, OMe), 2.15, 2.08, 2.03 (s, 3 H, Ac). Anal. Calcd for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found C, 58.35; H, 6.53.

Methyl 2,4,6-tri-O-*acetyl*-β-D-*galactopyranoside* (**19**). To a solution of **18** (3.4 g, 8.27 mmol) in MeOH (70 mL) was added $Pd(OH)_2$ (600 mg, 10%/C), and the mixture was stirred under a hydrogen atmosphere for 12 h. (Caution. Extreme fire hazard!) After stirring for 12 h, the mixture was passed through a Celite pad, and the filtrate was concentrated in vacuo. Purification of the residue by the flash column chromatography (1:2 hexane–ethyl acetate) afforded **19** (2.5 g, 95%): $[\alpha]_D^{23}$ −40.0 ° (*c* 0.4, $CHCl_3$); mp 98–100 °C; ^{1}H NMR: δ 5.33 (dd, 1 H, $J_{4,3}$ 1.0, $J_{4,3}$ 3.6 Hz, H-4), 4.96 (dd, 1 H, $J_{2,3}$ 9.9, $J_{2,1}$ 7.9 Hz, H-2), 4.37 (d, 1 H, H-1), 4.17 (d, 2 H, $J_{6,5}$ 6.6 Hz, H-6), 3.88–3.82 (m, 2 H, H-3, 5), 3.52 (s, 3 H, OMe), 2.41 (d, 1 H, $J_{OH,3}$ 6.3 Hz, OH), 2.18, 2.14, 2.07 (s, 3 H, Ac). Anal. Calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found C, 49.17; H, 6.63.

Methyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranoside* (**21**). To a solution of acceptor **19** (47 mg, 147 μmol), donor **20** (50 mg, 98 μmol) in CH_2Cl_2 (2.5 mL) was added MS 4A (250 mg). After stirring for 1 h at room temperature, MeOTf (55 μL, 490 μmol) was added at 0 °C, and the mixture was stirred at room temperature for 18 h. After addition of triethylamine (136 μL), the mixture was passed through a Celite pad and concentrated in vacuo. Purification of the residue by the flash column chromatography (1:1 hexane–

ethyl acetate) afforded **21** (66 mg, 92%): $[\alpha]_D^{25}$ +7.0° (*c* 0.9, CH_2Cl_2); ^{1}H NMR: δ 7.86–7.72 (m, 4 H, Phth), 5.76 (dd, 1 H, $J_{3',4'}$ 8.9, $J_{3',2'}$ 10.9 Hz, H-3′), 5.43 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.38 (dd, 1 H, $J_{4,5}$ 1.0, $J_{4,3}$ 4.6 Hz, H-4), 5.19 (dd, 1 H, $J_{4',5'}$ 9.9 Hz, H-4′), 4.91 (dd, 1 H, $J_{2,1}$ 7.9, $J_{2,3}$ 9.9 Hz, H-2), 4.40 (dd, 1 H, $J_{6a',5'}$ 2.6, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 3.38 (s, 3 H, OMe), 2.15, 2.10, 2.07, 2.03, 1.84, 1.77 (each s, each 3 H, Ac). Anal. Calcd for $C_{33}H_{39}NO_{18}$: C, 53.73; H, 5.33; N,1.90. Found C, 53.70; H, 5.69; N,1.78.

Methyl (3,4,6-tri-O-*acetyl-2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranoside* (**22**). To a solution of **21** (46 mg, 62.4 mmol) in EtOH (0.5 mL) was added $H_2NNH_2{\cdot}H_2O$ (0.5 mL), and the mixture stirred at 90 °C. After 24 h the mixture was concentrated in vacuo. The residue was dissolved in Ac_2O (0.25 mL) and pyridine (0.25 mL) and stirred for 12 h. After concentration of the mixture in vacuo, purification of the residue by flash column chromatography (ethyl acetate) afforded **22** (36 mg, 90%): $[\alpha]_D^{23}$ +22.95° (*c* 2.9, CH_2Cl_2); ^{1}H NMR: δ 5.66 (d, 1 H, $J_{NH,2'}$ 7.9 Hz, NH), 5.52 (t, 1 H, $J_{3',4'}=J_{3',2'}$ 9.2 Hz, H-3′), 5.38 (d, 1 H, $J_{4,3}$ 3.3 Hz, H-4), 5.15–5.00 (m, 3 H, H-2, 1′, 4′), 4.32 (dd, 1 H, $J_{6'a,5'}$ 2.6, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.31 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 3.70–3.66 (m, 1 H, H-5′), 3.39 (s, 3 H, OMe), 2.12 (s, 3 H, Ac), 2.11 (s, 6 H, Ac×2), 2.08 (s, 3 H, Ac), 2.02 (s, 6 H, Ac×2), 1.91 (s, 3 H, Ac). Anal. Calcd for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found C, 49.69; H, 5.94; N, 2.34.

Methyl (2-acetamido-2-deoxy-β-D-*glucopyranosyl)-(1→3)*-β-D-*galactopyranoside* (**14**). To a solution of **22** (95 mg, 147 μmol) in dry MeOH (2 mL) was added NaOMe (8 mg, 147 μmol), and the mixture was stirred at room temperature. After 20 h the mixture was neutralized by the addition of the resin (Dowex-50W×8, H^+) and passed through a Celite pad. The filtrate was concentrated in vacuo. Purification of the residue over a gel-permeation column (Shephadex G-15, water) afforded **14** [11] (52 mg, 90%): ^{1}H NMR (D_2O, HOD = 4.81): δ 4.70 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.31(d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.14 (d, 1 H, $J_{4,3}$ 3.1 Hz, H-4), 3.90 (bd, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 3.57 (s, 3 H, OMe), 2.04 (s, 3 H, Ac).

Methyl (3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→6)-2,3,4,-tri*-O-*acetyl*-α-D-*glucopyranoside* (**24**). To a solution of acceptor **23** [29] (170 mg, 530 μmol), donor **20** (180 mg, 354 μmol) in CH_2Cl_2 (3 mL) was added MS 4A (1 g). After stirring for 1 h at room temperature, MeOTf (199 μL, 1.76 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 3 h. After addition of triethylamine (490 μL), the mixture was passed through a Celite pad and concentrated in vacuo. Purification of the residue by flash column chromatography (1:2 hexane–ethyl acetate) afforded **24** (220 mg, 80%): $[\alpha]_D^{23}$ +65.2° (*c* 2.6, CH_2Cl_2); ^{1}H NMR: δ 7.87–7.31 (m, 4 H, Phth), 5.78 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{3',2'}$ 10.6 Hz, H-3′), 5.41 (d, 1cH, $J_{1',2'}$ 8.6 Hz, H-1′), 5.34 (dd, 1H, $J_{3,2}=J_{3,4}$ 9.6 Hz, H-3), 5.18 (dt, 1 H, $J_{4',5'}$ 9.6 Hz, H-4′), 4.76 (bt, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.68 (dd, 1 H, $J_{2,1}$ 3.3 Hz, H-2), 4.46 (d, 1 H, H-1), 3.49 (dd, 1 H, $J_{6b,5}$ 7.3, $J_{6b,6a}$ 10.6 Hz, H-6b), 3.02 (s, 3 H, OMe), 2.13, 2.04, 2.01, 1.93, 1.92, 1.87 (each s, each 3 H, Ac). Anal. Calcd for $C_{33}H_{39}NO_{18}$: C, 53.73; H, 5.32; N, 1.90. Found C, 53.91; H, 5.08; N, 1.85.

Methyl (3,4,6-tri-O-*acetyl-2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1→_6)-2,3,4-tri*-O-*acetyl*-α-D-*glucopyranoside* (**25**). To a solution of **24** (100 mg, 1.28 mmol) in EtOH (1.0 mL) was added $H_2NNH_2{\cdot}H_2O$ (1.0 mL), and the mixture was stirred at 90 °C. After 24 h, the mixture was concentrated in vacuo. The residue was dissolved in acetic anhydride (1.0 mL) and pyridine (1.0 mL) and stirred for 12 h. After concentration of the mixture in vacuo, purification of the residue by flash column chromatography (1:2 hexane–ethyl acetate) afforded **25** (73 mg, 83%): $[\alpha]_D^{23}$ +7.9° (*c* 4.3, CH_2Cl_2); mp 220–223 °C; ^{1}H NMR: δ 5.97 (d, 1 H, $J_{NH,2'}$ 8.6 Hz, NH), 5.48 (t, 1 H, $J_{3,2}=J_{3,4}$ 9.2 Hz, H-3), 5.21 (t, 1 H, $J_{3',2'}=J_{3',4'}$ 9.2 Hz, H-3′), 5.14–5.05 (m, 2 H, H-4, 4′), 4.94 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.85 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 4.50 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.24 (dd, 1 H, $J_{6'a,5'}$ 4.6, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 3.39 (s, 3 H, OMe), 2.08, 2.07, 2.06, 2.04, 2.02, 2.00, 1.97 (s, 3 H, Ac). Anal. Calcd for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found C, 49.97; H, 5.62; N, 2.05.

Methyl (2-acetamido-2-deoxy-β-D-*glucopyranosyl)-(1→6)*-α-D-*glucopyranoside* (**15**). To a solution of **25** (52 mg, 80 μmol) in dry MeOH (1 mL) was added NaOMe (2 mg, 40 μmol), and the mixture was stirred at room temperature. After 20 h, the mixture was neutralized by the addition of the resin (Dowex-50W×8, H^+) and then passed through a Celite pad. The filtrate was concentrated in vacuo. Purification of the residue over a gel-permeation column (Shephadex G-15, water)

afforded **15** (26 mg, 81%): $[\alpha]_D^{23}$ +51.7° (*c* 0.7, H_2O); ^1H NMR (D_2O, HOD = 4.81): δ 4.56 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1′), 3.69 (dd, 1 H, $J_{3,4} = J_{3,2}$ 9.2 Hz, H-3), 3.57 (dd, 1 H, $J_{2,1}$ 3.6 Hz, H-2), 3.44 (s, 3 H, OMe), 2.08 (s, 3 H, Ac); *m*/*z* Calcd for $C_{15}H_{27}NO_{11}Na$: 420.1482 (M + Na); Found 420.1502.

Methyl 3,4-di-O-*acetyl-2-acetamido-2-deoxy-6*-O-p-*toluenesulfonyl*-β-D-*glucopyranoside* (**26**). To a solution of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**13**) [30] (400 mg, 1.74 mmol) in pyridine (5 mL) was added *p*-toluenesulfonyl chloride (398 mg, 2.08 mmol) at 0 °C, and the mixture was stirred for 2 h at 0 °C. The mixture was diluted with ethyl acetate and washed with aq $NaHCO_3$. The organic phase was dried with $MgSO_4$ and concentrated in vacuo. The residue was dissolved in acetic anhydride and pyridine (0.5 mL:0.5 mL), and the mixture was stirred at room temperature. Concentration of this mixture gave crystalline **26** that was recrystallized from ethanol (390 mg, 48%): $[\alpha]_D^{23}$ +19.7° (*c* 0.6, CH_2Cl_2); mp 192 °C (dec); ^1H NMR: δ 7.78, 7.35 (d, each 2 H, *J* 8.6 Hz, Ar), 5.42 (d, 1 H, $J_{NH,2}$ 9.2 Hz, NH), 5.22 (dd, 1 H, $J_{3,4}$ 9.2, $J_{3,2}$ 10.6 Hz, H-3), 4.90 (bdd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.51 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1), 4.13 (dd, 1 H, $J_{6a,5}$ 3.3, $J_{6a,6b}$ 10.9 Hz, H-6a), 4.07 (dd, 1 H, $J_{6b,5}$ 5.6 Hz, H-6b), 3.85–3.71 (m, 2 H, H-2, 5), 3.43 (s, 3 H, OMe), 2.46 (s, 3 H, Ts-Me), 2.01, 2.00, 1.94 (s, 3 H, Ac). Anal. Calcd for $C_{20}H_{27}NO_{10}S$: C, 50.73; H, 5.75; N, 2.96. Found C,50.26; H, 5.72; N, 3.14.

Methyl 3,4-di-O-*acetyl-2-acetamido-2,6-dideoxy*-β-D-*glucopyranoside* (**27**). To a solution of methyl 3,4-di-*O*-acetyl-2-acetamido-2-deoxy-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**26**) (200 mg, 0.422 mmol) in dimethoxyethane (3 mL) was added NaI (126 mg, 0.846 mmol), and the mixture was stirred at 80 °C. After 4 h the mixture was diluted with ethyl acetate and washed with brine (×2). The organic phase was dried and then concentrated in vacuo. After drying this residue by vacuum pump, a solution of this residue in dry toluene (20 mL) was treated with AIBN (10 mg) and tri-*n*-butyltin hydride (227 μL, 0.846 mmol), and the mixture was stirred under reflux. After 2 h the mixture was concentrated in vacuo and ethyl acetate and saturated aq KF (5 mL each) were added to the residue, and the mixture was stirred for 1 h. The organic phase was washed with brine (×3), dried with $MgSO_4$ and concentrated in vacuo. Purification of this residue by flash column chromatography (ethyl acetate) afforded **27** (98 mg, 77%): $[\alpha]_D^{23}$ −38.0° (*c* 0.3, DMSO); ^1H NMR: δ 5.44 (d, 1 H, $J_{NH,2}$ 8.3 Hz, NH), 5.19 (dd, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 9.2 Hz, H-3), 4.82 (t, ^1H, $J_{4,5}$ 9.6 Hz, H-4), 4.50 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 3.89 (dt, 1 H, H-2), 3.59–3.51 (m, 3 H, H-5, 6a, 6b), 3.49 (s, 3 H, OMe), 2.04, 2.03, 1.95 (s, 3 H, Ac), 1.25 (d, 3 H, $J_{6,5}$ 6.3 Hz, H-6). Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found C,51.90; H, 6.80; N, 4.38.

Methyl 2-acetamido-2,6-dideoxy-β-D-*glucopyranoside* (**1**). To a solution of methyl 3,4-di-*O*-acetyl-2-acetamido-2,6-dideoxy-β-D-glucopyranoside (**27**) (41 mg, 0.135 mmol) in dry MeOH (0.5 mL) was added a catalytic amount of NaOMe, and the mixture was stirred at room temperature. After 5 h, the mixture was neutralized by the addition of the resin (Dowex-50W×8, H^+) and filtered. Purification of the concentrated filtrate over a gel-permeation column (Shephadex G-15, water) afforded **1** (18 mg, 60%): $[\alpha]_D^{23}$ −46.3° (*c* 2.0, H_2O); ^1H NMR (D_2O, HOD = 4.81): δ 4.21 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 3.47 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.30–3.23 (m, 2 H, H-3, 5), 3.27 (s, 3 H, OMe), 2.99 (t, 1 H, $J_{4,3}$ 9.2 Hz, H-4), 1.82 (s, 3 H, Ac), 1.11 (d, 3 H, $J_{6,5}$ 6.3 Hz, H-6); *m*/*z* Calcd for $C_9H_{17}NO_5Na$: 242.1005 (M + Na); Found 242.1032.

Methyl 2-acetamido-4,6-O-*benzylidene-2,3-dideoxy*-β-D-*glucopyranoside* (**29**). To a solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**28**) [16] (250 mg, 771 μmol) and 4-dimethylaminopyridine (47 mg, 385 μmol) in CH_2Cl_2 (12 mL) was added 1,1-thiocarbonyldiimidazole (165 mg, 925 μmol), and the mixture was stirred under reflux for 1 h. The mixture was allowed to cool to room temperature, and then it was diluted with $CHCl_3$. The organic phase was washed with brine and then dried with $MgSO_4$. After concentration of the organic phase in vacuo, a solution of this residue in toluene (30 mL) was treated with AIBN (10 mg) and tri-*n*-butyltin hydride (415 μL, 1.54 mmol) and stirred under reflux. After 2 h, the mixture was allowed to cool to room temperature and *n*-hexane (20 mL) was added. Crystalline **29** was isolated by filtration (203 mg, 85%): $[\alpha]_D^{25}$ −86.4° (*c* 0.5, DMSO); mp 270 °C (dec); ^1H NMR (DMSO-d_6): δ 7.94 (d, 1 H, $J_{NH,2}$ 8.6 Hz, NH), 7.43–7.33 (m, 5 H, Ph), 5.59 (s, 1 H, PhCH), 4.37 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.20 (dd, 1 H, $J_{6a,5}$ 5.0, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.73 (t, 1 H, $J_{6b,5}$ 10.2 Hz, H-6b), 2.13 (ddd, 1 H, $J_{3e,4} = J_{3e,2}$

4.6, $J_{3e,3a}$ 7.6 Hz, H-3e), 1.82 (s, 3 H, Ac), 1.58 (ddd, 1 H, $J_{3a,4} = J_{3a,2}$ 11.9 Hz, H-3a). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found C, 62.12; H, 6.66; N, 4.77.

Methyl 2-acetamido-2,3-dideoxy-β-D-glucopyranoside (**8**). The 3-deoxy derivative **29** (101 mg, 328 μmol) was dissolved in 60% acetic acid and stirred at 100 °C. After 6 h the mixture was concentrated in vacuo. In order to purify the crude product, this residue was dissolved in acetic anhydride and pyridine (0.25 mL:0.25 mL). After 1 h, the mixture was concentrated in vacuo, and purification of this residue by flash column chromatography (3:97 MeOH–ethyl acetate) afforded per acetylated **8**. To a solution of the acetate derivative in dry MeOH (1 mL) was added NaOMe (catalytic amount), and the mixture was stirred at room temperature. The mixture was neutralized by the addition of the resin (Dowex-50W×8, H^+) and passed through a Celite pad. Purification of the concentrated filtrate over a gel-permeation column (Shephadex G-15, water) afforded **8** (56 mg, 77%): $[\alpha]_D^{23}$ −34.8° (*c* 1.2, H_2O); ^{1}H NMR (D_2O, HOD = 4.81): *d* 4.46 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 3.95 (dd, 1 H, $J_{6a,5}$ 2.3, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.74 (dd, 1 H, $J_{6b,5}$ 6.3, H-6b), 3.54 (s, 3 H, OMe), 3.52–3.46 (m, 1 H, H-5), 2.32 (ddd, 1 H, $J_{3e,4} = J_{3e,2}$ 4.9, $J_{3e,3a}$ 12.5 Hz, H-3e), 2.02 (s, 3 H, Ac), 1.58 (ddd, 1 H, $J_{3a,4} = J_{3a,2}$ 12.2 Hz, H-3a); *m/z* Calcd for $C_9H_{17}NO_5Na$: 242.1005 (M + Na); Found 242.1003.

Ethyl 3-O-benzoyl-2-deoxy-2-phthalimido-6-O-pivaloyl-1-thio-β-D-glucopyranoside (**31**). To a solution of ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **30** [13] (7.3 g, 16.5 mmol) in pyridine (5 mL) was added benzoyl chloride (2.5 g, 18.2 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with $CHCl_3$ and washed with 1% HCl solution and then water. After drying with $MgSO_4$, the organic phase was concentrated in vacuo. The residue was dissolved in 60% acetic acid, and the mixture was stirred at 100 °C. After 1 h, the mixture was concentrated in vacuo. To a solution of this residue in pyridine (20 mL) was slowly added pivaloyl chloride (2.19 g, 18.1 mmol) at 0 °C, and the mixture was stirred for 2 h. The mixture was then diluted with ethyl acetate and washed with aq $NaHCO_3$, and concentrated. Purification of these residue by flash column chromatography (3:2 hexane–ethyl acetate) afforded **31** (7.55 g, 84%): $[\alpha]_D^{23}$ +45.9° (*c* 2.2, CH_2Cl_2); ^{1}H NMR: *d* 8.11–7.33(m, 9 H, Ar), 5.93 (dd, 1 H, $J_{3,2}$ 10.2, $J_{3,4}$ 8.6 Hz, H-3), 5.54 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 4.50 (t, 1 H, H-2), 4.50 (dd, 1 H, $J_{6a,5}$ 2.6, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.43 (dd, 1 H, $J_{6b,5}$ 4.3 Hz, H-6b), 3.88–3.83 (m, 1 H, H-5), 3.75 (t, 1 H, $J_{4,5}$ 8.6 Hz, H-4), 2.78–2.61 (m, 2 H, SCH_2), 1.25 (m, 12 H, *t*-Bu, SCH_2CH_3). Anal. Calcd for $C_{28}H_{31}NO_8S$: C, 62.09; H, 5.77; N, 2.59. Found C, 62.38; H, 5.35; N, 2.53.

Ethyl 3-O-benzoyl-2,4-dideoxy-4-iodo-2-phthalimido-6-O-pivaloyl-1-thio-β-D-galactopyranoside (**32**). To a solution of pyridine (1.5 g, 19.2 mmol) in CH_2Cl_2 (50 mL) was added trifluoromethanesufonic anhydride (2.7 g, 9.6 mol) at 0 °C. After 15 min, thioglycoside **31** (2.6 g, 4.8 mmol) in CH_2Cl_2 (5 mL) was added, and the mixture was stirred at room temperature for 30 min. The mixture was diluted with $CHCl_3$ and washed with aq 1% HCl, water and $NaHCO_3$. The organic phase was dried with $MgSO_4$ and concentrated in vacuo. The residue was used directly for the next step without purification. The residue was dissolved in dry dimethoxyethane (50 mL) and treated with NaI (3.5 g, 24 mol). After stirring at room temperature for 12 h, the mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography (3:2 hexane–ethyl acetate) afforded **32** (2.55 g, 99%): $[\alpha]_D^{23}$ +63.8° (*c* 4.4, CH_2Cl_2); ^{1}H NMR: *d* 7.97–7.36 (m, 9 H, Ar), 5.54 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.35 (dd, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 4.3 Hz, H-3), 4.97 (dd, 1 H, $J_{4,5}$ 1.0 Hz, H-4), 4.91 (t, 1 H, H-2), 4.39 (dd, 1 H, $J_{6a,5}$ 6.6, $J_{6a,6b}$ 11.6 Hz, H-6a), 4.14 (dd, 1 H, $J_{6b,5}$ 5.9 Hz, H-6b), 3.48 (m, 1 H, H-5), 2.82–2.68 (m, 2 H, SCH_2), 1.23 (m, 12 H, *t*-Bu, SCH_2CH_3). Anal. Calcd for $C_{28}H_{30}INO_7S$: C, 51.56; H, 4.60; N, 2.15. Found C, 51.29; H, 4.54; N, 2.01.

Ethyl 3-O-benzoyl-2,4-dideoxy-2-phthalimido-6-O-pivaloyl-1-thio-β-D-glucopyranoside (**33**). To a solution of **32** (740 mg, 1.38 mmol) in toluene (13 mL) was added AIBN (10 mg) and tri-*n*-butyltin hydride (0.74 mL, 2.80 mmol), and the mixture was stirred under reflux. After 2.5 h the mixture was allowed to cool to room temperature, and the mixture was diluted with a solution of ether and saturated aq KF (50 mL:50 mL). The mixture was stirred for 2.5 h, and then the organic phase was washed with brine (×3) and dried with $MgSO_4$. After concentration in vacuo, purification of the residue by flash column chromatography

(hexane→ 3:2 hexane–ethyl acetate) afforded **33** (492 mg, 87%): $[\alpha]_D^{23}$ +59.9° (*c* 6.0, EtOH); ^{1}H NMR: δ 7.90–7.33 (m, 9 H, Ar), 5.92 (ddd, 1 H, $J_{3,2}=J_{3,4a}$ 10.6, $J_{3,4e}$ 5.3 Hz, H-3), 5.53 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 4.45 (t, 1 H, H-2), 4.27–4.04 (m, 3 H, H-5, 6, 6′), 2.80–2.60 (m, 2 H, SCH_2), 2.47 (dd, 1 H, $J_{4e,4a}$ 11.6 Hz, H-4e), 1.77 (dt, 1 H, $J_{4a,5}$ 11.6 Hz, H-4a), 1.40–1.17 (m, 12 H, *t*-Bu, SCH_2CH_3). Anal. Calcd for $C_{28}H_{31}NO_7S$: C, 63.98; H, 5.95; N, 2.67. Found C, 64.32; H, 6.10; N, 2.56.

Methyl (3,6-di-O-*acetyl-2-acetamido-2,4-dideoxy*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl*-α-D-*glucopyranoside* (**34**). The preparation of disaccharide **34** with donor **33** (111 mg, 211 μmol) and acceptor **23** (130 mg, 406 μmol) was performed with the same manner in the preparation of **22**. Purification of this disaccharide by flash column chromatography (ethyl acetate) afforded **34** (105 mg, 66% 2 steps): $[\alpha]_D^{23}$ +50.0° (*c* 1.9, CH_2Cl_2); mp 172–174 °C; ^{1}H NMR: δ 5.93 (d, 1 H, $J_{NH,2}$ 8.6 Hz, NH), 5.48 (dd, 1 H, $J_{3,2}=J_{3,4}$ 9.6 Hz, H-3), 5.12 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 5.92 (ddd, 1 H, $J_{3',2'}=J_{3',4'a}$ 11.2, $J_{3',4'e}$ 5.0 Hz, H-3′), 4.94 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.86 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 4.32 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.22–4.05 (m, 3 H, H-6′a, 6′b, 6a), 3.93–3.83 (m, 2 H, H-2′, 5), 3.76–3.70 (m,1 H, H-5′), 3.39 (s, 3 H, OMe), 3.36 (dd, 1 H, $J_{6b,5}$ 4.0, $J_{6b,6a}$ 11.6 Hz, H-6b), 2.08 (s, 3 H, Ac), 2.06 (s, 6 H, Ac×2), 2.06, 1.99 (s, 3 H, Ac), 1.63 (ddd, 1 H, $J_{4'a,5'}=J_{4'a,3'}=J_{4'a,4'e}$ 11.9 Hz, H-4′a). Anal. Calcd for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found C, 50.48; H, 6.20; N, 2.32.

Methyl (2-acetamido-2,4-dideoxy-β-D-*glucopyranosyl)-(1→6)*-α-D-*glucopyranoside* (**5**). O-Deacetylation was performed in the same manner as for the preparation of **14**. Purification with the gel-permeation column (Sephadex G-15, water) afforded **5** (88%): $[\alpha]_D^{25}$ +35.6° (*c* 1.0, H_2O); ^{1}H NMR (D_2O, HOD = 4.81): δ 4.47 (d, 1 H, $J_{1',2'}$ 12.9 Hz, H-1′), 3.43 (s, 3 H, OMe), 2.08 (s, 3 H, Ac), 2.06 (ddd, 1 H, $J_{4'e,5'}=J_{4'a,3'}$ 4.6, $J_{4'a,4'e}$ 11.5 Hz, H-4′e), 1.48 (ddd, 1 H, $J_{4'a,5'}=J_{4'a,3'}$ 11.5 Hz, H-4′a); *m/z* Calcd for $C_{15}H_{27}NO_{10}Na$: 404.1533; (M + Na); Found 404.1539.

Ethyl 3,4-di-O-*acetyl-2,6-dideoxy-6-fluoro-2-phthalimido-1-thio*-β-D-*glucopyranoside* (**36**). To a solution of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**35**) [13] (310 mg, 0.87 mmol) in CH_2Cl_2 (6.4 mL) was added diethylaminosulfur trifluoride (DAST, 634 μL, 5.20 mmol) at −40 °C, and the mixture was allowed to warm to room temperature. After 1 h, MeOH (2 mL) was added at −10 °C, and the mixture was concentrated in vacuo. Acetic anhydride and pyridine were added to the residue with stirring at room temperature. After concentration of the mixture, purification of the residue by flash column chromatography (3:1 hexane–ethyl acetate) afforded **36** (145 mg, 38%): $[\alpha]_D^{23}$ +43.0° (*c* 0.4, $CHCl_3$); mp 127–129 °C; ^{1}H NMR: δ 7.88–7.33 (m, 4 H, Phth), 5.86 (dd, 1 H, $J_{3,2}=J_{3,4}$ 9.9 Hz, H-3), 5.50 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.14 (bdd, 1 H, $J_{4,5}$ 10.2 Hz, H-4), 4.52 (dd, 2 H, $J_{6,5}$ 4.3, $J_{6,F}$ 47.18 Hz, H-6), 4.39 (bt, 1 H, H-2), 3.99–3.85 (m, 1 H, H-5), 2.76–2.61 (m, 2 H, SCH_2), 2.06, 1.87 (s, 3 H, Ac), 1.22 (t, 3 H, *J* 7.6 Hz, SCH_2CH_3). Anal. Calcd for $C_{20}H_{22}FNO_7S$: C, 54.67; H, 5.01; N, 3.18. Found C, 54.55; H, 5.06; N, 3.14.

Ethyl 3,4-di-O-*acetyl-2-deoxy-2-phthalimido-6-O*-p-*toluenesulfonyl-1-thio*-β-D-*glucopyranoside* (**37**). To a solution of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**35**) (4.26 g, 12.1 mmol) in pyridine (30 mL) was added *p*-toluenesulfonyl chloride (2.76 g, 14.5 mmol) at 0 °C. After 1 h the mixture was poured into aq $NaHCO_3$ and extracted with $CHCl_3$. The organic phase was washed with 1% aq HCl and water. After drying with $MgSO_4$, the mixture was concentrated in vacuo. The residue was dissolved in acetic anhydride and pyridine and stirred for 2 h. After concentration of the mixture in vacuo, purification of the concentrated mixture by flash column chromatography (3:1 hexane–ethyl acetate) afforded **37** (6.57 g, 92%): $[\alpha]_D^{23}$ +46.7° (*c* 2.4, CH_2Cl_2); ^{1}H NMR: δ 7.86–7.75 (m, 6 H, Ar), 7.36 (d, 2 H, *J* 7.9 Hz, Ar), 5.79 (dd, 1 H, $J_{3,4}$ 9.2, $J_{3,2}$ 10.2 Hz, H-3), 5.42 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.02 (dd, 1 H, $J_{4,5}$ 10.2 Hz, H-4), 4.31 (dd, 1 H, H-2), 4.18 (dd, 1 H, $J_{6a,5}$ 3.3, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.10 (dd, 1 H, $J_{6b,5}$ 3.3 Hz, H-6b), 3.95–3.88 (m, 1 H, H-5), 2.69–2.54 (m, 2 H, SCH_2), 2.46 (s, 3 H, Ts), 2.00, 1.84 (s, 3 H, Ac), 1.18 (t, 3 H, *J* 7.3 Hz, SCH_2CH_3). Anal. Calcd for $C_{27}H_{29}NO_{10}S_2$: C, 54.81; H, 4.94; N, 2.37. Found C, 54.94; H, 4.57; N, 2.25.

Ethyl 3,4-di-O-*acetyl-2-deoxy-2-phthalimido-6-thioacetyl-1-thio*-β-D-*glucopyranoside* (**38**). To a solution of **37** (5.57 g, 9.45 mmol) in *N*,*N*-dimethylformamide (30 mL) was added potassium thioacetate (5.39 g, 47.2 mmol), and the mixture was stirred at 80 °C under an argon atmosphere. After 3 h the mixture was concentrated in vacuo. Purification of the residue by flash column chromatography (2:1 hexane–ethyl acetate) afforded **38**

(4.08 g, 82%): $[\alpha]_D^{23}$ +47.2° (*c* 0.5, $CHCl_3$); mp 148–150 °C; ^{1}H NMR: δ 7.92–7.67 (m, 4 H, Phth), 5.80 (dd, 1 H, $J_{3,2}$ 9.0, $J_{3,4}$ 10.11 Hz, H-3), 5.45 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.06 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.36 (dd, 1 H, H-2), 3.82 (ddd, 1 H, $J_{5,6a}$ 4.0, $J_{5,6b}$ 8.0 Hz, H-5), 3.35 (dd, 1 H, $J_{6a,6b}$ 16.0 Hz, H-6a), 3.07 (dd, 1 H, H-6b), 2.81–2.56 (m, 2 H, SCH_2), 2.36, 2.09, 1.85 (s, 3 H, Ac), 1.23 (t, 3H, *J* 7.6 Hz, SCH_2CH_3). Anal. Calcd for $C_{22}H_{25}NO_8S_2$: C, 53.32; H, 5.09; N, 2.83. Found C, 53.25; H, 5.09; N, 2.85.

*Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-*D*-allopyranoside* (**39**). To a cold (−78 °C) stirred solution of oxalyl chloride (11.5 g, 90.7 mmol) in CH_2Cl_2 (300 mL) was slowly added DMSO (8.84 g, 113 mmol). After the mixture was stirred at −78 °C for 10 min, ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **30** (10.0 g, 22.7 mmol) in CH_2Cl_2 (50 mL) was added, and the mixture was stirred at that temperature for 20 min. Triethylamine (ca. 25 mL) was slowly added, and then the mixture was allowed to warm to room temperature. The mixture was diluted with $CHCl_3$ and washed with aq $NaHCO_3$. After drying with $MgSO_4$, the mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate and H_2O (300 mL, 3:1), and to this mixture was added $NaBH_4$ (4.2 g) at 0 °C. After 30 min the mixture was diluted with ethyl acetate and washed with water. After drying with $MgSO_4$, the organic phase was concentrated in vacuo. Purification of the residue by flash column chromatography (3:1 hexane–ethyl acetate) afforded a diastereomeric mixture (9.6 g, 96%; gluco:allo = 1:4). The allopyranoside **39** was recrystallized from hexane–ethanol: $[\alpha]_D^{23}$ −80.5° (*c* 1.3, $CHCl_3$); mp 160–162 °C; ^{1}H NMR: δ 7.90–7.33 (m, 9 H, Ar), 6.02 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 5.62 (s, 1 H, PhCH), 4.47–4.41 (m, 3 H, H-2, 3, 4), 4.27 (ddd, 1 H, *J* 5.3, 9.6 Hz, H-5), 3.82 (bt, 2 H, H-6a, 6b), 3.14 (s, 1 H, OH), (dd, 1 H, H-6b), 2.78–2.65 (m, 2 H, SCH_2), 1.24 (t, 3 H, *J* 7.6 Hz, SCH_2CH_3). Anal. Calcd for $C_{23}H_{23}NO_6S$: C, 62.57; H, 5.25; N, 3.17. Found C, 62.54; H, 5.21; N, 3.15.

*Ethyl 4,6-*O*-benzylidene-2,3-dideoxy-3-fluoro-2-phthalimido-1-thio-*β*-*D*-glucopyranoside* (**40**). To a solution of allopyranoside **39** (850 mg, 1.93 mmol) in CH_2Cl_2 (20 mL) was added DAST (932 mg, 5.78 mmol) at −40 °C. The mixture was allowed to slowly warm to room temperature and stirred for 2 h. The mixture was again cooled to −10 °C, and MeOH (6 mL) was added. Purification of the concentrated mixture by flash column chromatography (3:1 hexane–ethyl acetate) afforded **40** (431 mg, 50%): $[\alpha]_D^{23}$ +7.1° (*c* 2.2, $CHCl_3$); mp 149–151 °C; ^{1}H NMR: δ 7.98–7.20 (m, 9 H, Ar), 5.59 (s, 1 H, PhCH), 5.50 (ddd, 1 H, $J_{3,2} = J_{3,4}$ 8.6, $J_{3,F}$ 44.4 Hz, H-3), 5.40 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 2.68 (q, 2 H, *J* 7.3 Hz, SCH_2), 1.17 (t, 3 H, SCH_2CH_3). Anal. Calcd for $C_{23}H_{22}FNO_5S$: C, 62.29; H, 5.00; N, 3.16. Found C, 61.82; H, 4.95; N, 3.27.

*Ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-fluoro-2-phthalimido-1-thio-*β*-*D*-glucopyranoside* (**41**). The fluoro sugar **40** (2.5 g, 5.63 mmol) was dissolved in 70% acetic acid (50 mL) and the mixture was stirred at 60 °C. After 3 h the mixture was concentrated in vacuo, and the residue was treated with acetic anhydride and pyridine (10 mL:10 mL) for 30 min. Purification of the concentrated mixture by flash column chromatography (3:1 hexane–ethyl acetate) afforded **41** (1.57 g, 63%): $[\alpha]_D^{23}$ +22.9° (*c* 2.5, $CHCl_3$); ^{1}H NMR: δ 7.88–7.76 (m, 4 H, Phth), 5.48–5.24 (m, 2 H, H-3,4), 5.33 (d, 1 H, $J_{1,2}$ 10.9 Hz, H-1), 4.51 (ddd, 1 H, $J_{2,3} = J_{2,F}$ 9.9 Hz, H-2), 4.32 (dd, 1 H, $J_{6a,5}$ 4.9, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.20 (bd, 1 H, H-6b), 3.85 (m, 1 H, H-5), 2.77–2.62 (m, 2 H, SCH_2), 2.12 (s, 6 H, Ac), 1.22 (t, 3 H, *J* 7.6 Hz, SCH_2CH_3). ^{13}C NMR: δ 170.51, 169.15, 167.92, 166.86, 89.18 (d, $J_{3,F}$ 190.4 Hz, C-3), 80.73 (d, $J_{1,F}$ 6.1 Hz, C-1), 75.20 (d, $J_{5,F}$ 7.4 Hz, C-5), 68.92 (d, $J_{4,F}$ 18.3 Hz, C-4), 61.98 (C-6), 53.69 (d, $J_{2,F}$ 18.3 Hz, C-2), 24.10 (SCH_2), 20.60, 20.52 (each Ac), 14.68 ($SCCH_3$). Anal. Calcd for $C_{20}H_{22}FNO_7S$: C, 54.67; H, 5.01; N, 3.18. Found C, 54.64; H, 5.17; N, 3.10.

*Methyl(3,4-di-*O*-acetyl-2-acetamido-2,6-dideoxy-6-fluoro-*β*-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside* (**44**). Preparation of disaccharide **42** with donor **36** (200 mg, 420 μmol) and acceptor **19** (270 mg, 850 μmol) was performed in the same manner as in the preparation of **21**. Purification of crude disaccharide by flash column chromatography (1:5 hexane–ethyl acetate) afforded disaccharide **42** (176 mg). This disaccharide was dissolved in $EtOH:H_2NNH_2{\cdot}H_2O$ (1.4 mL: 1.4 mL) and the mixture was stirred at 90 °C. After 24 h the mixture was concentrated in vacuo. The residue was treated with acetic anhydride (0.5 mL), pyridine (0.5 mL) and DMAP at room temperature. After 6 h the mixture was concentrated in vacuo and purification of this residue by flash column chromatography (1:5 hexane–ethyl acetate) afforded **44** (113 mg, 44%): $[\alpha]_D^{23}$ +43.0° (*c* 2.6,

CH_2Cl_2); ^{1}H NMR: δ 5.72 (d, 1 H, $J_{NH,2'}$ 7.6 Hz, NH), 5.53 (dd, 1 H, $J_{3',2'}$ 10.6, $J_{3',4'}$ 9.6 Hz, H-3′), 5.41 (bd, 1 H, $J_{4,3}$ 3.3 Hz, H-4), 5.13 (dd, 1 H, $J_{2,3}$ 9.9, $J_{2,1}$ 8.3 Hz, H-2), 5.09 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.92 (bdd, 1 H, $J_{4',5'}$ 9.9 Hz, H-4′), 4.45 (dd, 2 H, $J_{6',5'}$ 3.3, $J_{6',F}$ 46.5 Hz, H-6′), 4.31 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.17–4.08 (m, 2 H, H-6a.b), 3.49 (s, 3 H, OMe), 3.37 (dt, 1 H, H-2′), 2.13, 2.11, 2.07, 2.03, 2.02, 1.92 (s, 3H, Ac); ^{13}C NMR: δ 170.60, 170.44, 170.10, 169.75, 169.7, 169.56 (each carbonyl), 101.94 (C-1), 99.68 (C-1′), 81.57 (d, $J_{6',F}$ 174.6 Hz, C-6′), 76.26, 72.66 (d, $J_{5',F}$ 20.8 Hz, C-5′), 71.19 (d, $J_{3',F}$ 4.9 Hz, C-3′), 70.78, 69.20, 68.59 (d, $J_{4',F}$ 7.3, C-4′), 62.16 (C-6), 56.84 (OMe), 55.99 (C-2′), 23.29, 21.10, 20.77, 20.74, 20.66, 20.63 (each Ac-Me); Anal. Calcd for $C_{25}H_{36}FNO_{15}$: C, 49.26; H, 5.95; N, 2.30. Found C, 49.35; H, 5.84; N, 2.91.

Methyl (2-acetamido-2,6-dideoxy-6-fluoro-β-D-*glucopyranosyl)-(1→3)*-β-D-*galactopyranoside* (**2**). O-Deacetylation of **44** (162 mg, 266 μmol) by the same manner in the preparation of **14** afforded **2** (87 mg, 82%): $[\alpha]_D^{23}$ −8.0° (*c* 0.4, H_2O); ^{1}H NMR (D_2O, HOD = 4.81): *d* 4.76 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.73 (bd, 2 H, $J_{6',F}$ 47.1 Hz, H-6′), 4.12 (bd, 1 H, $J_{4,3}$ 2.9 Hz, H-4), 3.57 (s, 3 H, OMe), 2.04 (s, 3 H, Ac); ^{13}C NMR (TPS = 0.00 ppm): δ 177.69, 106.60 (C-1), 105.45 (C-1′), 84.79 (d, $J_{6',F}$ 167.9 Hz, C-6′), 84.84 (C-3), 77.44 (C-5), 76.96 (d, $J_{5',F}$ 17.7 Hz, C-5′), 76.09, 72.54, 71.31 (d, $J_{4',F}$ 6.23 Hz, C-4′), 71.13 (C-4), 63.63 (C-6), 59.90 (OMe), 58.37 (C-2′), 24.88 (Me); *m*/*z* Calcd for $C_{15}H_{26}FNO_{10}Na$: 422.1439 (M + Na); Found 422.1438.

Methyl (4,6-di-O-*acetyl-2,3-dideoxy-3-fluoro-2-phthalimido*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranoside* (**43**). Glycosylation of **19** (434 mg, 1.36 mmol) with donor **41** (400 mg, 0.905 mmol) in the same manner in the preparation of **21** afforded crude disaccharide. Purification of crude disaccharide by flash column chromatography (1:1 hexane–ethyl acetate) afforded **43** (280 mg, 44%): $[\alpha]_D^{23}$ −1.4° (*c* 2.0, EtOH); ^{1}H NMR: δ 7.87–7.74 (m, 4 H, Phth), 5.38 (bd, 1 H, $J_{4,3}$ 3.6 Hz, H-4), 5.24 (dt, 1 H, $J_{3',4'} = J_{3',2'}$ 8.9, $J_{3',F}$ 48.2 Hz, H-3′), 5.25 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.92 (dd, 1 H, $J_{2,1}$ 7.9, $J_{2,3}$ 9.9 Hz, H-2), 4.41 (bd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.36 4.24 (m, 1 H, H-2′), 4.21 (d, 1 H, H-1), 4.19–4.04 (m, 3 H, H-6′b, 6a, 6b), 3.84–3.70 (m, 3 H, H-3. 5. 5′), 3.38 (s, 3 H, OMe), 2.16, 2.12, 2.11, 2.07, 1.78 (each s, each 3 H, Ac); ^{13}C NMR: δ 170.83, 170.64, 170.10, 169.92, 169.14, 169.96 (each carbonyl), 134.36, 131.55, 123.56, 123.36, 101.69 (C-1), 97.58 (d, $J_{1',F}$ 11.0 Hz, C-1′), 88.12 (d, $J_{3',F}$ 190.4 Hz, C-3′), 75.96 (C-3), 71.12 (d, $J_{5',F}$ 4.9 Hz, C-5′), 71.09, 70.39, 68.97 (d, $J_{4',F}$ 18.3 Hz, C-4′), 68.89, 62.14, 60.99, 56.51 (OMe), 54.90 (d, $J_{2',F}$ 19.0 Hz, C-2′), 20.81, 20.74, 20.70, 20.61, 20.52 (each Me). Anal. Calcd for $C_{31}H_{34}FNO_{16}$: C, 53.53; H, 4.93; N, 2.01. Found C, 53.81; H, 5.35; N, 2.06.

Methyl (4,6-di-O-*acetyl-2-acetamido-2,3-dideoxy-3-fluoro*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranoside* (**45**). *N*-Dephthalimidation and acetylation of **43** (100 mg, 0.14 mmol) by the same manner in the preparation of **44** afforded crude disaccharide. Purification of this crude disaccharide by flash column chromatography (ethyl acetate) afforded **45** (49 mg, 56%): $[\alpha]_D^{23}$ −8.0° (*c* 0.3, $CHCl_3$); mp 189–191 °C; ^{1}H NMR: δ 5.88 (d, 1 H, $J_{NH,2'}$ 6.6 Hz, NH), 5.38 (d, 1 H, $J_{4,3}$ 3.3 Hz, H-4), 5.31 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.36 (bd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.31 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.65–3.61 (m, 1 H, H-5′), 3.50 (s, 3 H, OMe), 2.99–2.91 (m, 1 H, H-2′), 2.12 (s, 3 H, Ac), 2.11(s, 6 H, Ac×2), 2.10, 2.08, 1.99 (each s, each 3 H, Ac); ^{13}C NMR: δ 171.16, 170.74, 170.64, 169.77, 169.70, 169.43 (each carbonyl), 102.03 (C-1), 98.34 (d, $J_{1',F}$ 11.0 Hz, C-1′), 88.70 (d, $J_{3',F}$ 185.6 Hz, C-3′), 76.57, 71.21, 70.67 (d, $J_{5',F}$ 9.8 Hz, C-5′), 70.48 (C-2), 69.43 (C-4), 69.14 (d, $J_{4',F}$ 18.3 Hz, C-4′), 62.03 (C-6), 61.17 (C-6′), 58.40 (d, $J_{2',F}$ 17.1 Hz, C-2′), 56.87 (OMe), 23.58, 21.04, 20.84, 20.74 (Ac-Me). Anal. Calcd for $C_{25}H_{36}FNO_{15}$: C, 49.26; H, 5.95; N, 2.30. Found C, 49.03; H, 6.02; N, 2.31.

Methyl (2-acetamido-2,3-dideoxy-3-fluoro-β-D-*glucopyranosyl)-(1→3)*-β-D-*galactopyranoside* (**9**). O-Deacetylation of **45** (84 mg, 138 μmol) in the same manner in the preparation of **2** afforded **9** (39 mg, 71%): $[\alpha]_D^{23}$ −17.5° (*c* 0.8, H_2O); ^{1}H NMR (D_2O, DSS = 0.00 ppm): δ 4.76 (d, ^{1}H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.50 (ddd, 1 H, $J_{3',2'}$ 8.6, 9.8, $J_{3',F}$ 52.2 Hz, H-3′), 4.29 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.12 (bd, 1 H, $J_{4,3}$ 3.4 Hz, H-4), 3.55 (s, 3 H, OMe), 2.03 (s, 3 H, Ac); *m*/*z* Calcd for $C_{15}H_{26}FNO_{10}Na$: 422.1439 (M + Na); Found 422.1470.

Methyl (3,4-di-O-*acetyl-2-deoxy-2-phthalimido-6-thioacetyl*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl*-α-D-*glucopyranoside* (**46**). Glycosylation of acceptor **23** (170 mg, 0.53 mmol) with donor **38** (185 mg, 0.35 mmol) in the same manner as in the preparation of **42** afforded crude disaccharide. Purification of this disaccharide by flash column chromatography (1:2 hexane–ethyl acetate) afforded

46 (211 mg, 75%): $[\alpha]_D^{23}$ +67.7° (*c* 4.2, CH_2Cl_2); ^1H NMR: δ 7.85–7.72 (m, 4 H, Phth), 5.74 (dd, 1 H, $J_{3',4'}$ 8.9, $J_{3',2'}$ 10.6 Hz, H-3′), 5.36 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 5.35 (dd, 1 H, $J_{3,2}=J_{3,4}$ 9.9 Hz, H-3), 5.05 (dt, 1 H, $J_{4',5'}$ 9.9 Hz, H-4′), 4.76 (dd, 1 H, $J_{4,5}$ 10.2 Hz, H-4), 4.70 (dd, 1 H, $J_{2,1}$ 3.3 Hz, H-2), 4.53 (d, 1 H, H-1), 4.29 (dd, 1 H, H-2′), 3.90–3.77 (m, 3 H, H-5, 5′, 6a), 3.48 (dd, 1 H, $J_{6b,5}$ 7.3, $J_{6b,6a}$ 10.9 Hz, H-6b), 3.30 (dd, 1 H, $J_{6'a,5'}$ 3.0, $J_{6'a,6'b}$ 14.2 Hz, H-6′a), 3.12 (dd, 1 H, $J_{6'b,5'}$ 14.2 Hz, H-6′a), 3.06 (s, 3 H, OMe), 2.37, 2.09, 2.01, 1.93, 1.92, 1.85 (each s, each 3 H, Ac). Anal. Calcd for $C_{33}H_{39}NO_{17}S$: C, 52.59; H, 5.22; N,1.86. Found C, 53.05; H, 4.70; N, 1.64.

Methyl (3,4-di-O-*acetyl-2-acetamido-2-deoxy-6-thioacetyl-*β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl-*α-D-*glucopyranoside* (**47**). N-Dephthalation and acetylation of **46** (230 mg, 0.288 mmol) in the same manner as in the preparation of **44** afforded crude disaccharide. Purification of this disaccharide by flash column chromatography (1:3 hexane–ethyl acetate) afforded **47** (156 mg, 76%): $[\alpha]_D^{23}$ +56.6° (*c* 2.2, CH_2Cl_2); ^1H NMR: δ 5.91 (d, 1 H, $J_{NH,2'}$ 8.6 Hz, NH), 5.48 (dd, 1 H, $J_{3,2}=J_{3,4}$ 9.6 Hz, H-3), 5.17 (dd, 1 H, $J_{3',2'}=J_{3',4'}$ 9.6 Hz, H-3′), 5.10 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.95 (dd, 1 H, $J_{4',5'}$ 9.6 Hz, H-4′), 4.95 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.86 (dd, 1 H, H-2), 4.43 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.08–3.87 (m, 3 H, H-2′, 5, 6a), 3.57 (t, 1 H, H-5′), 3.40 (s, 3 H, OMe), 3.38 (dd, 1 H, $J_{6b,5}$ 3.6, $J_{6b,6a}$ 12.2 Hz, H-6b), 3.27 (dd, 1 H, $J_{6'a,5}$ 3.0, $J_{6'a,6'b}$ 14.5 Hz, H-6′a), 3.00 (dd, 1 H, $J_{6'b,5'}$ 7.3 Hz, H-6′b), 2.34, 2.08 (each s, each 3 H, Ac), 2.07 (s, 6 H, Ac×2), 2.03, 2.00, 1.97 (s, 3 H, Ac). Anal. Calcd for $C_{27}H_{39}NO_{16}S$: C, 48.72; H, 5.91; N, 2.10. Found C, 48.51; H, 5.85; N, 2.06.

*Methyl (2-acetamido-2-deoxy-6-thio-*β-D-*glucopyranosyl)-(1→6)-*α-D-*glucopyranoside* (**3**). To a solution of disaccharide **47** (95 mg, 133 μmol) and DL-dithiothreitol (204 mg, 1.33 mol) in degassed MeOH (0.5 mL) was added NH_4OH (28%, 3 mL), and the mixture was stirred at room temperature. After 12 h the mixture was concentrated in vacuo at 35 °C. Purification of residue over a gel-permeation column (Sephadex G-15, H_2O) afforded **3** (31 mg, 56%): $[\alpha]_D^{23}$ +37.9° (*c* 2.9, H_2O); ^1H NMR: (D_2O, HOD = 4.81): δ 4.58 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 3.44 (s, 3H, OMe), 3.06 (bd, 1 H, $J_{6'a,6'b}$ 13.5 Hz, H-6′a), 2.80 (dd, 1 H, $J_{6'b,5'}$ 6.9 Hz, H-6′b), 2.08 (s, 3H, Ac); negative-ion FAB: *m*/*z* Calcd for $C_{15}H_{26}NO_{10}S$: 412.1278 (M - H); Found 412.1286.

*Methyl (2-acetamido-2-deoxy-6-thio-*β-D-*glucopyranosyl)-(1→6)-*α-D-*glucopyranoside-6′-yl disulfide* (**4**). The disaccharide **3** (6 mg, 14 μmol) was dissolved in H_2O (250 mL), and this solution was stirred at 37 °C. After 48 h the mixture was concentrated in vacuo. Purification of this residue over a gel-permeation column (Sephadex G-15, H_2O) afforded disulfide tetrasaccharide **4** (4 mg, 67%): $[\alpha]_D^{23}$ +128.8° (*c* 0.2, H_2O); ^1H NMR (HOD = 4.81): δ 4.53 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 3.64 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2′), 3.64 (dd, 1 H, $J_{4',3'}=J_{4',5'}$ 9.7 Hz, H-4′), 3.52 (dd, 1 H, $J_{2,1}$ 3.6, $J_{2,3}$ 10.0 Hz, H-2), 3.38 (s, 3 H, OMe), 3.35–3.29 (m,1 H, H-6′a), 2.93 (dd, 1 H, $J_{6'b,5'}$ 8.4, $J_{6'b,6'a}$ 13.6 Hz, H-6′b), 2.03 (s, 3 H, Ac). *m*/*z* Calcd for $C_{30}H_{52}N_2O_{20}S_2Na$: 847.2453 (M + Na); Found 847.2472.

Methyl 3,6-di-O-*acetyl-2-azido-2-deoxy-*β-D-*galactopyranoside* (**49**). To a solution of methyl 2-azido-2-deoxy-β-D-galactopyranoside (**48**) [19] (341 mg, 1.56 mmol) and pyridine (541 mg) in CH_2Cl_2 (10 mL) stirring at 0 °C was added acetic anhydride (349 mg, 3.43 mmol) in CH_2Cl_2 (7 mL) over 15 min. After 1 h the mixture was allowed to warm to room temperature and stirred for 3 h. The reaction was quenched by addition of MeOH (1 mL), and the mixture was concentrated in vacuo. Purification of the residue by flash column chromatography (1:1 hexane–ethyl acetate) afforded **49** (310 mg, 66%): $[\alpha]_D^{23}$ +1.4° (*c* 1.1, CH_2Cl_2); mp 145–147 °C; ^1H NMR: δ 4.72 (dd, 1 H, $J_{3,4}$ 3.0, $J_{3,2}$ 10.6 Hz, H-3), 4.36 (dd, 1 H, $J_{6a,5}$ 6.6, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.27 (dd, 1 H, $J_{6b,5}$ 5.9 Hz, H-6b), 4.37 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.98 (dd, 1 H, $J_{4,OH}$ 5.3 Hz, H-4), 3.73 (dd, 1 H, H-2), 3.68 (bt, 1 H, H-5), 3.59 (s, 3 H, OMe), 2.33 (d, 1 H, 4-OH), 2.18, 2.09 (s, 3 H, Ac). Anal. Calcd for $C_{11}H_{17}N_3O_7$: C, 43.57; H, 5.65; N, 13.86. Found C, 43.40; H, 5.71; N, 13.42.

Methyl 3,6-di-O-*acetyl-2-azido-2,4-dideoxy-4-fluoro-*β-D-*glucopyranoside* (**50**). To a solution of methyl 3,6-di-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (**49**) (80 mg, 0.27 mmol) in CH_2Cl_2 (2 mL) was added diethylaminosulfur trifluoride (97 μL, 0.80 mmol) at −40 °C under argon atmosphere. After 4 h the mixture was allowed to warm to −10 °C, and excess diethylaminosulfur trifluoride was quenched by the addition of MeOH (0.3 mL). The mixture was directly concentrated in vacuo. Purification of the residue by flash column chromatography (1:3 hexane–ethyl acetate) afforded **50** (38 mg, 48%): $[\alpha]_D^{25}$ −32.7° (*c* 3.3, CH_2Cl_2);

^{1}H NMR: δ 5.13 (ddd, 1 H, $J_{3,2}$ 10.2, $J_{3,4}$ 8.9, $J_{3,F}$ 13.9 Hz, H-3), 4.42 (ddd, 1 H, $J_{6a,5}=J_{6a,F}$ 2.0, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.41 (ddd, 1 H, $J_{4,5}$ 8.9, $J_{4,F}$ 50.8 Hz, H-4), 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.26 (ddd, 1 H, $J_{6b,5}$ 5.0, $J_{6b,F}$ 1.7 Hz, H-6b), 3.76–3.65 (m, 1 H, H-5), 3.59 (s, 3 H, OMe), 3.42 (bdd, 1 H, $J_{2,F}$ 0.5 Hz, H-2), 2.17, 2.11 (s, 3 H, Ac); ^{13}C NMR: δ 170.75, 169.83, 103.16 (C-1), 87.15 (d, $J_{4,F}$ 188.0 Hz, C-4), 72.11 (d, $J_{3,F}$ 19.5 Hz, C-3), 71.46 (d, $J_{5,F}$ 23.2 Hz, C-5), 63.78 (d, $J_{2,F}$ 7.3 Hz, C-2), 62.23 (C-6), 57.77 (OMe), 21.13, 20.99 (each Ac).Anal. Calcd for $C_{11}H_{16}FN_3O_6$: C, 43.28; H, 5.28; N, 13.77. Found C, 43.71; H, 5.35; N, 13.69.

Methyl 3,6-di-O-*acetyl-2-acetamido-2,4-dideoxy-4-fluoro*-β-D-*glucopyranoside* (**51**). To a solution of methyl 3,6-di-*O*-acetyl-2-azido-2,4-dideoxy-4-fluoro-β-D-glucopyranoside (**50**) (25 mg, 83 μmol) in triethylamine–pyridine–H_2O (0.1 mL:0.5 mL: 0.25 mL) was introduced H_2S gas, and the mixture was stirred at room temperature. After 30 min the mixture was concentrated in vacuo. The residue was treated with acetic anhydride and pyridine. After being stirred for 30 min at room temperature, the mixture was concentrated in vacuo. Purification of the residue by flash column chromatography (ethyl acetate) afforded **51** (26 mg, 98%): $[\alpha]_D^{25}$ −32.9° (*c* 1.73, CH_2Cl_2); mp 180–182 °C; ^{1}H NMR: δ 5.80 (d, 1 H, $J_{NH,2}$ 9.2 Hz, NH), 5.29 (ddd, 1 H, $J_{3,2}$ 9.2, $J_{3,4}$ 9.2, $J_{3,F}$ 14.5 Hz, H-3), 4.50 (ddd, 1 H, $J_{4,5}$ 9.2, $J_{4,F}$ 50.8 Hz, H-4), 4.49 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.25 (dd, 1 H, $J_{6b,5}$ 4.3, $J_{6b,6a}$ 12.0 Hz, H-6b), 3.97 (ddd, 1 H, H-2), 3.76–3.71 (m, 1 H, H-5), 3.49 (s, 3 H, OMe), 2.11 (s, 6 H, Ac×2), 1.97 (s, 3 H, Ac); ^{13}C NMR: δ 1701.01, 170.64, 170.35 (each carbonyl), 101.85 (C-1), 86.87 (d, $J_{4,F}$ 186.8 Hz, C-4), 72.46 (d, $J_{3,F}$ 18.3 Hz, C-3), 71.25 (d, $J_{5,F}$ 24.4 Hz, C-5), 62.14 (C-6), 56.87 (OMe), 53.93 (d, $J_{2,F}$ 6.1 Hz, C-2), 23.32 (NAc), 20.77 (Ac×2). Anal. Calcd for $C_{13}H_{20}FNO_7$: C, 48.60; H, 6.30; N, 4.36. Found C, 48.60; H, 6.42; N, 4.34.

Methyl 2-acetamido-2,4-dideoxy-4-fluoro-β-D-*glucopyranoside* (**6**). O-Deacetylation of **51** (63 mg, 0.20 mmol) in the same manner as in the preparation of **14** afforded **6** (42 mg, 91%): $[\alpha]_D^{23}$ −22.7° (*c* 2.5, H_2O); ^{1}H NMR (D_2O, HOD = 4.81): δ 4.54 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 4.42 (bdt, 1 H, $J_{4,3}=J_{4,5}$ = 8.9, $J_{4,F}$ = 51.5 Hz, H-4), 3.55 (s, 3 H, OMe), 2.08 (s,3 H, Ac); *m*/*z* Calcd for $C_9H_{16}FNO_5Na$: 260.0911 (M + Na); Found 260.0925.

Methyl 3,6-di-O-*acetyl-2-azido-2-deoxy-4-thioacetyl*-β-D-*glucopyranoside* (**52**). To a solution of pyridine (158 mg, 1.99 mmol) in CH_2Cl_2 (5 mL) stirring at 0 °C was added trifluoromethanesulfonic anhydride (167 μL, 1.0 mmol). After 10 min **49** (150 mg, 0.50 mmol in CH_2Cl_2 (5 mL) was added to this mixture at 0 °C. After 30 min the mixture was diluted with $CHCl_3$ and washed with 1% aq HCl, H_2O and aq $NaHCO_3$. The organic phase was dried with $MgSO_4$ and was concentrated in vacuo. The residue was further dried by use of a vacuum pomp and then dissolved in *N*,*N*-dimethylformamide (2 mL). To this solution was added potassium thioacetate (280 mg, 2.49 mmol) at 0 °C. After being stirred for 30 min, the mixture was directly applied to flash column chromatography (3:1 hexane–ethyl acetate) to give **52** (151 mg, 85%): $[\alpha]_D^{25}$ −16.4° (*c* 0.6, CH_2Cl_2); ^{1}H NMR: δ 5.04 (dd, 1 H, $J_{3,4}$ 9.9, $J_{3,2}$ 10.9 Hz, H-3), 4.37 (dd, 1 H, $J_{6a,5}$ 5.3, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.28 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.22 (dd, 1 H, $J_{6b,5}$ 2.0 Hz, H-6b), 3.79 (m, 1 H, H-5), 3.61(dd, 1 H, $J_{4,5}$ 10.9 Hz, H-4), 3.59 (s, 3 H, OMe), 3.45 (dd, 1 H, H-2), 2.33, 2.10, 2.09 (s, 3 H, Ac). Anal. Calcd for $C_{13}H_{19}N_3O_7S$: C, 43.21; H, 5.30; N, 11.63. Found C, 43.66; H, 5.33; N, 12.22.

Methyl 3,6-di-O-*acetyl-2-acetamido-2-deoxy-4-thioacetyl*-β-D-*glucopyranoside* (**53**). Reduction of azido **52** (218 mg, 0.61 mmol) and acetamidation in the same manner as in the preparation of **51** afforded crude **53**. Purification of this crude product by flash column chromatography (9:1 ethyl acetate–MeOH) afforded **53** (190 mg, 84%): $[\alpha]_D^{25}$ −18.5° (*c* 4.0, CH_2Cl_2); mp 149–151 °C;^{1}H NMR: δ 5.73 (d, 1 H, $J_{NH,2}$ 8.9 Hz, NH), 5.25 (dd, 1 H, $J_{3,4}=J_{3,2}$ 10.6 Hz, H-3), 4.53 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.37 (dd, 1 H, $J_{6a,5}$ 4.6, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.26 (dd, 1 H, $J_{6b,5}$ 2.3 Hz, H-6b), 3.97–3.83 (m, 2 H, H-2, 5), 3.65(dd, 1 H, $J_{4,5}$ 10.6 Hz, H-4), 3.49 (s, 3 H, OMe), 2.33, 2.09, 2.04, 1.95 (s, 3 H, Ac). Anal. Calcd for $C_{15}H_{23}NO_8S$: C, 47.74; H, 6.14; N, 3.71. Found C, 47.88; H, 5.62; N, 3.77.

Methyl 2-acetamido-2-deoxy-4-thio-β-D-*glucopyranoside* (**7**). 4-Thioacetyl derivative **53** (20 mg, 54 μmol) and DL-dithiothreitol (83 mg, 0.54 mmol) were dissolved in MeOH–NH_4OH (0.5 mL:3 mL). After stirring for 12 h at room temperature, the mixture was concentrated in vacuo. Purification of the residue by flash column chromatography (7:3 ethyl acetate–MeOH) and subsequent gel-permeation chromatography (Sephadex G-15, H_2O) afforded **7** (8 mg, 62%):

$[\alpha]_D^{23}$ −60.2° (*c* 0.7, H_2O); ^1H NMR (D_2O, HOD = 4.81): δ 4.47 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.10 (dd, 1 H, $J_{6a,5}$ 2.0, $J_{6a,6b}$ 12.5 Hz, H-6a), 3.91 (dd, 1 H, $J_{6b,5}$ 5.3, H-6b), 3.68 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 3.59–3.50 (m, 1 H, H-5), 3.54 (s, 3 H, OMe), 3.47 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 2.77 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 2.07 (s, 3 H, Ac). *m/z* Calcd for $C_9H_{18}NO_5S$: 252.0906 (M + H); Found 252.0911.

Methyl 2-aminoethyl-3,4,6-tri-O-*benzyl-2-deoxy*-β-D-*glucopyranoside* (**55**). To a solution of methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**54**) [20] (300 mg, 0.59 mmol) in THF (3 mL) was added $LiAlH_4$ (113 mg, 2.97 mmol), and the mixture was stirred under reflux. After 2 days, the mixture was poured into water and extracted with ethyl acetate. The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification of the crude product by flash column chromatography (ethyl acetate) afforded **55** (203 mg, 70%): $[\alpha]_D^{25}$ 16.6° (*c* 0.7, $CHCl_3$); ^1H NMR (300 MHz): δ 7.35∼7.19 (m, 15 H, Ph), 4,95 (d, 1 H, *J* 11.5 Hz, benzyl CH_2), 4.79 (d, 1 H, *J* 10.8 Hz, benzyl CH_2), 4.70∼4.54 (m, 4 H, benzyl CH_2), 4.19 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.66 (s, 3 H, OMe), 2.88∼2.86, 2.69∼2.63 (each m, each 1 H, CH_2), 2.57 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 0.97 (t, 1 H, *J* 7.2 Hz, CH_3); Anal. Calcd for $C_{30}H_{37}NO_5$: C, 73.29; H, 7.58; N, 2.85. Found C, 73.08; H, 7.82; N, 2.83.

Methyl 2-aminoethyl-2-deoxy-β-D-*glucopyranoside* (**10**). To a solution of tri-*O*-benzyl derivative **55** (200 mg, 0.41 mmol) and Pd/C (cat) in MeOH (2 mL) (Caution: Extreme fire hazard!) was introduced hydrogen gas. After stirring for 5 h at room temperature, the mixture was filtered and the filtrate was concentrated in vacuo. Purification of the crude product over a gel-permeation column (Sephadex G-15, H_2O) afforded **10** (78 mg, 87%): $[\alpha]_D^{25}$ −23.9° (*c* 0.4, H_2O); ^1H NMR (D_2O, HOD = 4.81): δ 4.45 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.92 (dd, 1 H, $J_{6a,5}$ 1.9, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.72 (dd, 1 H, $J_{6b,5}$ 5.5 Hz, H-6b), 3.56 (s, 3 H, OMe), 3.00∼2.74 (m, 1 H, CH_2), 2.53 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2), 1.11 (t, 3 H, *J* 7.2 Hz, CH_3); *m/z* Calcd for $C_9H_{19}NO_5Na$: 244.1161 (M + Na); Found 244.1174.

Methyl 2-methylacetamido-3,4,6-tri-O-*benzyl-2-deoxy*-β-D-*glucopyranoside* (**56**). To a solution of tri-*O*-benzyl derivative **54** (400 mg, 0.79 mmol) in DMF (2 mL) was added NaH (60% in oil, 63 mg, 1.58 mmol), and the mixture was stirred at room temperature. After 30 min, MeI (222 mg, 1.58 mmol) was added to this mixture, and the mixture was stirred for another 30 min. The mixture was diluted with ethyl acetate and washed with brine (twice). The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification of the crude product by flash column chromatography (3:2 hexane–ethyl acetate) afforded **56** (406 mg, 99%): $[\alpha]_D^{25}$ 25.3° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.35∼7.20 (m, 15 H, Ph), 4.35 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1) 3.48 (s, 3 H, OMe), 2.72 (s, 3 H, NMe), 2.16 (s, 3 H, Ac); Anal. Calcd for $C_{31}H_{37}NO_6$: C, 71.65; H, 7.17; N, 2.69. Found C, 71.16; H, 7.10; N, 2.61.

Methyl 2-methylacetamido-2-deoxy-β-D-*glucopyranoside* (**11**). O-Debenzylation of **56** (400 mg, 0.77 mmol) in the same manner as in the preparation of **10** afforded **11** as an E, Z mixture (ca. 1:1, 163 mg, 85%): ^1H NMR (D_2O, HOD = 4.81, 323 K): δ 4.99 (d, 0.5 H, $J_{1,2}$ 8.0 Hz, H-1), 4.22∼4.18 (m, 1 H, H-6a), 4.04∼4.00 (m, 1 H, H-6b), 3.77 (s, 3 H, OMe), 3.30, 3.14 (each s, each 1.5 H, Me), 2.43 (s, 3 H, Ac); *m/z* Calcd for $C_{10}H_{19}NO_6Na$: 272.1110 (M + Na); Found 272.1124.

Methyl 2-O-*acetyl-4,6*-O-*benzylidene*-β-D-*glucopyranoside* (**57**). To a solution of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside [21] (140 mg, 0.49 mmol) in CH_2Cl_2 (14 mL) were added acetic anhydride (60 mg, 0.59 mmol), pyridine (58 mg, 0.74 mmol) and DMAP (40 mg) in CH_2Cl_2 (1 mL) at 0 °C. After stirring 1 h at that temperature, MeOH (2 mL) was added and the mixture was concentrated in vacuo. Purification of the crude product by flash column chromatography (1:1 hexane–ethyl acetate) afforded 2-*O*-acetyl derivative **57** [21] (46 mg, 29%), 3-*O*-acetyl derivative (51 mg) and 2,3-di-*O*-acetyl derivative (23 mg) $[\alpha]_D^{25}$ −69.6° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.51∼7.25 (m, 15 H, Ph), 5.53 (s, 1 H, CH), 4.92 (dd, $J_{2,1}$ 7.9, $J_{2,3}$ 9.2 Hz, H-2), 4.42 (d, 1 H, H-1) 4.36 (dd, 1 H, $J_{6a,5}$ 5.0, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.86 (ddd, 1 H, $J_{3,OH}$ 3.6, $J_{3,4} = J_{3,2}$ 9.2 Hz, H-3), 3.79 (dd, 1 H, $J_{6b,5}$ 10.2 Hz, H-6b), 3.57 (dd, $J_{4,5}$ 9.3 Hz, H-4), 3.50 (s, 3 H, OMe), 2.74 (d, 1 H, OH), 2.13 (s, 3 H, Ac); *m/z* Calcd for $C_{16}H_{20}O_7Na$: 347.1107 (M + Na); Found 347.1112.

Methyl 2-O-*acetyl*-β-D-*glucopyranoside* (**12**). 2-*O*-Acetyl derivative **57** (24 mg, 74 μmol) was dissolved in 60% acetic acid (2.0 mL). The mixture was stirred 1 h at 100 °C. After concentration in vacuo, the crude mixture was purified on a gel-permeation column (Sephadex G-15, H_2O) to

afford **12** [20] (13 mg, 72%): ^{1}H NMR (300 MHz, D_2O, HOD = 4.81): δ 4.68 (dd, $J_{2,1} = J_{2,3} = 9.4$ Hz, H-2), 4.57 (d, 1 H, H-1), 3.97 (dd, 1 H, $J_{6a,5}$ 1.6, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.522 (s, 3 H, OMe), 3.49∼3.47 (m, 2 H, H-4,5), 2.16 (s, 3 H, Ac); *m/z* Calcd for $C_9H_{16}O_7Na$: 259.0794 (M + Na); Found 259.0821.

Enzyme assay of synthetic N-*acetylglucosaminide analogues*. Apparent kinetics parameters of β-(1→4)-galactosyltransferase toward synthetic *N*-acetylglucosaminide analogues was examined under the following conditions. An assay solution containing UDP-[U-^{14}C]-galactose (9.92 GBq/mmol, 90 pmol), the various concentrations of *N*-acetylglucosaminide analogue, bovine serum albumin (0.1 μg) $MnCl_2$ (10 mM) and β,-(1→4)-galactosyltransferase in TRIS buffer (20 mM, pH 7.5, total volume 30 μL) was incubated 37 °C. The reaction was followed up to 15% consumption of UDP-galactose. The ^{14}C-labeled *N*-acetyllactosaminide analogue was isolated by passage of the mixture diluted with 1 mL of water through a Pasteur pipette column of Dowex- 1×8 (Cl^-), and further eluted by 1 mL of water. The ^{14}C-labeled *N*-acetyllactosaminide analogue was measured by scintillation counting. The assay was performed in duplicate, and K_m and V_{max} were determined using Lineweaver–Burk plot, and the results are summarized in Table 1.

The inhibition assay with respect to methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was performed with a solution containing a UDP-[U-^{14}C]-D-galactose (9.92 GBq/mmol, 90 pmol), methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (0.5–3 mM), synthetic *N*-acetylglucosamine analogue (0–3 mM), bovine serum albumin (0.1 μg), $MnCl_2$ (10 mM) and β-(1→4)-galactosyltransferase in TRIS buffer (20 mM, pH 7.5, total volume 30 μL). The [^{14}C]-labeled *N*-acetyllactosamine was quantitated by the same manner as above, and the K_i value was obtained from the Lineweaver–Burk plot.

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