Synthesis of bisubstrate analogues targeting α-1,3-fucosyltransferase and their activities†

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We designed two bisubstrate analogues targeting α-1,3-fucosyltransferases, based on the three dimensional structure of Lewis X, which is the product of a α-1,3-fucosyltransferase reaction. We selected guanosine-5′-diphospho-L-galactose as a donor mimic and 2-hydroxyethyl β-D-galactoside as an acceptor mimic, and tethered these two mimics with a methylene or ethylene linker. For the synthesis, the 6-position of L-galactose and the 6-position of D-galactose were first tethered *via* a methylene or ethylene linker. The L-galactose moiety was then converted to a GDP derivative. Both bisubstrate analogues were moderate inhibitors against α-1,3-fucosyltransferase V and VI. The fact that they were substrates of α-1,3-fucosyltransferase VI suggested that these compounds bound to the donor binding site, but not to the acceptor binding site.

Introduction

Fucose-containing oligosaccharides represent a very important class of biomolecules involved in many important biological processes such as development, differentiation, and cell adhesion during inflammation and tumor metastasis. Fucosyltransferases (FucTs)[1] are the class of enzymes responsible for the biosyntheses of fucose-containing oligosaccharides, and at least 10 FucT genes have been cloned from mammalian cells, including α-1,2-FucT, α-1,3/4-FucT, α-1,3-FucT, α-1,6-FucT, and protein *O*-FucT. All FucTs catalyze the transfer of fucose from the common donor substrate guanosine-5′-diphospho-β-L-fucose (GDP-Fuc). α-1.3-FucTs are involved in the biosynthesis of sialyl Lewis X (sLe^x), which plays a key role in inflammation and tumor metastasis.[2] Therefore, inhibitors of α-1.3-FucTs are potential anti-inflammatory and antitumor agents.[3]

Many FucT inhibitors have been reported, most of which are based on the ground state or transition state structure of GDP-Fuc.[4–6] Several GDP-Fuc mimics demonstrated inhibitory activities in the micromolar range, which is similar to the K_M value of GDP-Fuc.[4] The structure of the most potent FucT inhibitor reported to date consisted of GDP and biphenyl connected through a triazole ring, and exhibited a K_i value of 62 nM (competitive against GDP-Fuc) for α-1,3-FucT VI.[5] The reaction mechanism of α-1,3-FucT V was suggested to follow an ordered-sequential mechanism, meaning that both the donor and acceptor are bound to the enzyme during the transfer reaction (Fig. 1).[7,8] Therefore, the bisubstrate analogue, which contains both donor and acceptor moieties on the same molecule, is an interesting compound for the development of a potent FucT inhibitor. Palcic *et al.* first synthesized a bisubstrate analogue of α-1,2-FucT. They tethered the phosphonate analogue of GDP and the 2-hydroxyl of the acceptor mimic phenyl galactoside with an ethylene linker, and the resulting bisubstrate analogue was a competitive inhibitor against both donor and acceptor (K_i = 16 μM against GDP-Fuc; 2.3 μM against Gal-Ph).[9] Another bisubstrate analogue inhibitor was reported by Wong's group, who synthesized homofuconojirimycin covalently linked to the 3-hydroxyl of the acceptor *N*-acetyllactosamine (LacNAc). The compound itself was a weak inhibitor against α-1,3-FucT V (IC_{50} = 5.7 mM), but demonstrated a potent synergistic inhibition in the presence of GDP (IC_{50} = 31 μM).[10] Bisubstrate analogues consisting of 1-deoxyfuconojirimycin and galactose, and mannose and *N*-acetylgalactosamine have also been reported.[11]

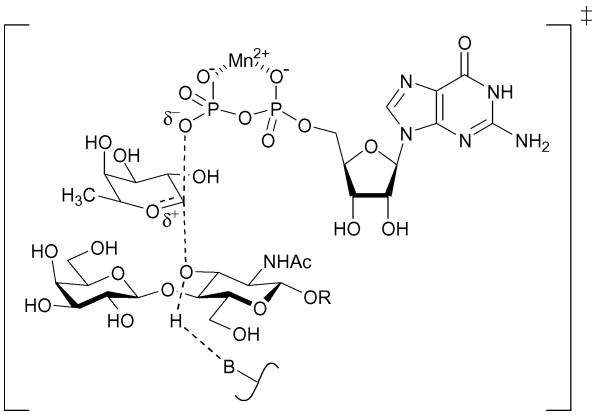

Fig. 1 Proposed transition state for fucosyltransferase V.

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† Electronic supplementary information (ESI) available: NMR spectra of compounds **1**, **2**, **4**, **6–9**, **12–23**, **25–27**, and ESI-MS spectra of FucT VI reaction products using GDP-Fuc, compounds **1**, and **2**. See DOI: 10.1039/b513897c

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We have been studying the inhibitory activities of bisubstrate analogues for various glycosyltransferases to develop potent and selective glycosyltransferase inhibitors. Glycosyltransferases employing a donor sugar-nucleotide usually exhibit different acceptor specificities. Bisubstrate analogues are therefore potential specific inhibitors of glycosyltransferases. We synthesized a bisubstrate analogue containing a donor substrate UDP-galactose and an acceptor substrate methyl *N*-acetylglucosaminide tethered with a methylene linker.[12] This compound exhibited a potent and competitive inhibition against β-1,4-galactosyltransferase (GalT) (K_i = 3.3 μM for UDP-Gal; 1.4 μM for GlcNAc), but a less potent inhibition against β-1,3-GalT and α-1,3-GalT. We also synthesized a bisubstrate analogue for sialyltransferases, although this compound exhibited only a weak inhibition.[13] Herein, we report the design and synthesis of two bisubstrate analogues for α-1,3-FucTs, and the results of their inhibitory activities against α-1,3-FucT V and VI.

Results and discussion

Design of bisubstrate analogues

A straightforward approach for the design of a bisubstrate analogue inhibitor is to utilize the X-ray crystallographic structure of the enzyme with bound substrates. However, no such structure was available for α-1,3-FucTs. We therefore decided to design our bisubstrate analogue based on the three-dimensional structure of the α-1,3-FucT reaction product, Le^x. We speculated that the structure of Le^x would resemble the transition state with regard to the relative position of the donor and the acceptor bound to the enzyme. The resulting designed bisubstrate analogues **1** and **2** are depicted in Fig. 2. Based on the reported[14] three-dimensional structure of Le^x (Fig. 3), 6-position of fucose and the 6-position of galactose are in close proximity, and we decided to connect these positions *via* an alkyl linker. We selected L-Gal as a fucose analogue

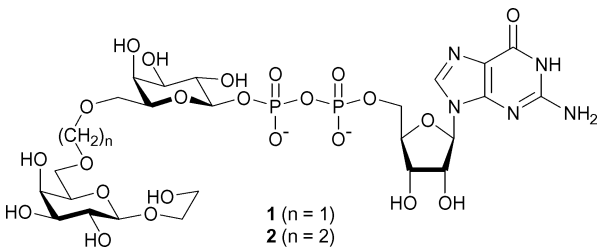


Fig. 2 Designed bisubstrate analogues.

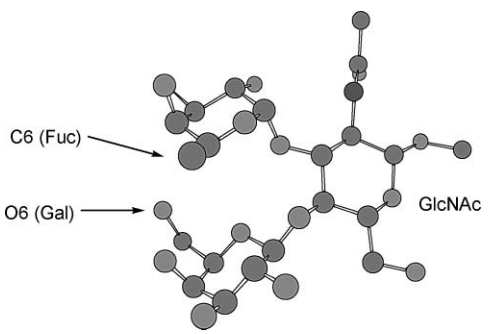
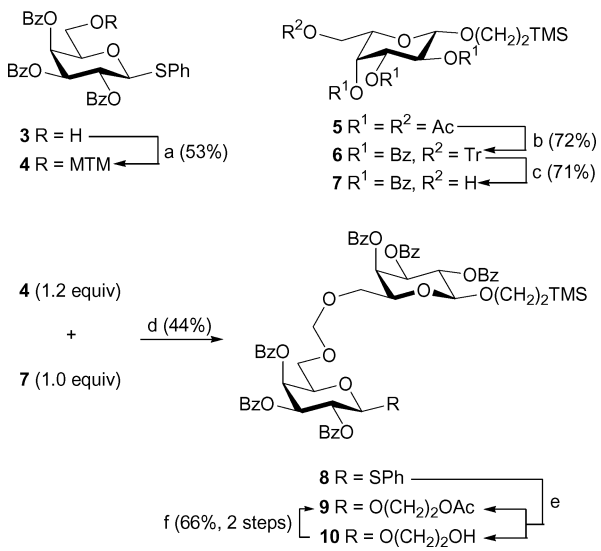


Fig. 3 Three-dimensional structure of Le^x.[14]

so that 6-*O*-alkylation affords a facile tethering. Additionally, we assumed that modifying the 6-position of Fuc would not disturb the binding to the enzyme since α-1,3-FucTs can transfer an L-Gal moiety from GDP-L-Gal.[15] For the acceptor mimic, we decided to employ 2-hydroxyethyl β-D-galactoside instead of LacNAc, to simplify the synthesis. Since the 3-hydroxyl of LacNAc is also considered to be important for binding,[8,14] we inserted a 2-hydroxyethyl aglycon to resemble this hydroxyl group.

Synthesis of bisubstrate analogues

The synthesis of methylene-tethered pseudodisaccharide **9** was carried out as shown in Scheme 1. A methylene-tether was designed to form *via* (methylthio)methyl (MTM) ether, which was an effective strategy for the synthesis of the methylene-tethered bisubstrate analogue inhibitor of β-1,4-GalT.[12] To form β-glycosidic linkages in both galactose anomeric centres *via* neighbouring group participation, a benzoyl group was adopted for the protection of the hydroxyl groups. Methylthiomethylation of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**3**)[16] using MTMCl and *N*,*N*-diisopropylethylamine (DIEA) was a very slow reaction and the yield of 6-*O*-MTM ether **4** was only 47% after 2 days at 60 °C. Alternatively, reaction of **3** with DMSO, Ac_2O and AcOH[17] was faster, giving a 54% yield of **4** after 16 h. Trimethylsilylethyl 2,3,4,6-tetra-*O*-acetyl-β-L-galactopyranoside (**5**) was synthesized from L-galactono-1,4-lactone using Thiem's method.[18] Deacetylation of **5**, and regioselective tritylation followed by benzoylation yielded **6**. Methanolysis of 6-*O*-trityl ether **6** yielded 6-OH derivative **7**. The coupling of **4** and **7** was then examined. MeOTf was used as a promoter in order to activate the methylthio group chemoselectively in the presence of the phenylthio group. Using a slight excess (1.2 equiv) of **4**, methylene-tethered pseudodisaccharide **8** was formed at a 47% yield. The structure was confirmed by the appearance of an AB quartet (J = 7.0 Hz) at δ = 4.76 and 4.66, which are peaks of the newly formed methylene acetal. Hydrolysis of **4** was



Scheme 1 *Reagents and conditions*: (a) DMSO, Ac_2O, AcOH; (b) (i) NaOMe, MeOH; (ii) TrCl, pyridine, 50 °C, then BzCl; (c) TsOH, $CHCl_3$, MeOH; (d) MeOTf, MS3A, CH_2Cl_2; (e) $HO(CH_2)_2OAc$, NIS, TfOH, MS4A, CH_2Cl_2, −30 °C; (f) Ac_2O, pyridine.

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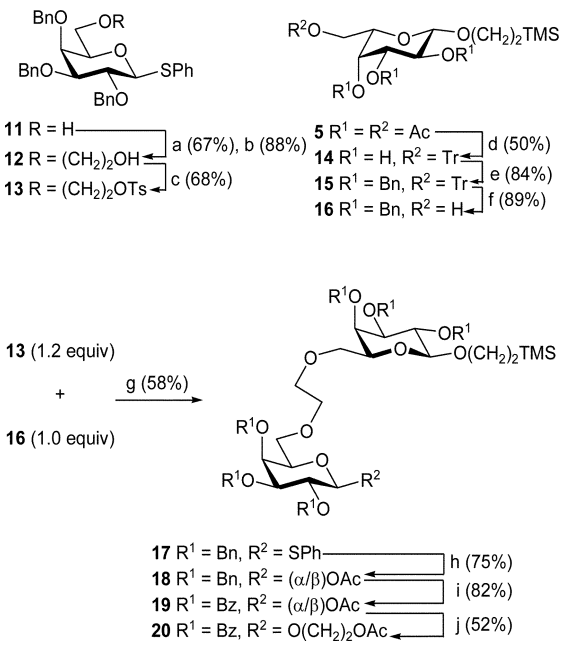
observed as a side reaction yielding **3**, and further condensation of **3** and **4** was also observed. Increasing the amount of **4** to 1.6 equiv did not improve the yield of **8** due to these side reactions. Glycosidation of thioglycoside **8** with commercial grade ethylene glycol monoacetate, which is a mixture of ethylene glycol, its monoacetate, and its diacetate, using NIS and TfOH as activators yielded a mixture of acetoxyethyl glycoside **9** and hydroxyethyl glycoside **10**. The mixture was acetylated to give only **9** at a 66% yield.

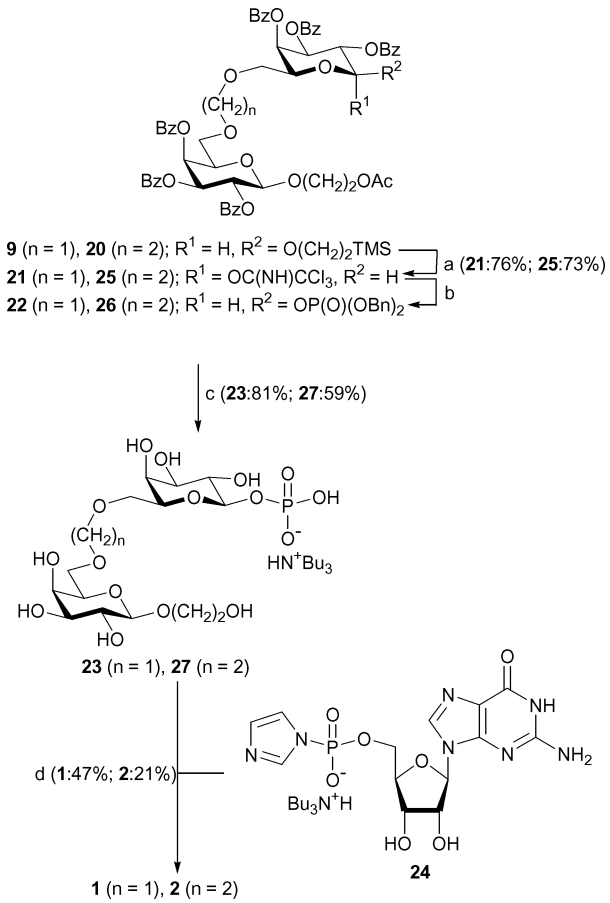
The synthesis of ethylene-tethered pseudodisaccharide is shown in Scheme 2. Ethylene-tether was synthesized using a tetrahydropyranyl (THP) ether of 2-bromoethanol. Introduction of this tether required the activation of hydroxyl groups with NaH, while other hydroxyl groups required protection with a benzyl group. After constructing the ethylene-tethered pseudodisaccharide, the benzyl group was converted to a benzoyl group for the stereoselective formation of β-glycoside as mentioned above. Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**11**)[19] was treated with NaH and 2-(2-bromoethoxy)tetrahydro-2*H*-pyrane, and methanolysis of THP ether gave 6-*O*-hydroxyethyl ether **12** at an 88% yield. Treatment of **12** with TsCl in pyridine gave tosylate **13** at a 68% yield. Trimethylsilylethyl 2,3,4-tri-*O*-benzyl-β-L-galactopyranoside (**16**) was prepared as follows: deacetylation and subsequent selective tritylation of **5** gave 6-*O*-trityl ether **14** at a 50% yield. Benzylation of free hydroxyl groups gave tribenzyl ether **15** at an 84% yield. Methanolysis in the presence of TsOH gave **16** at an 89% yield. Condensation of **16** and 1.2 equiv of tosylate **13** using NaH in DMSO gave ethylene-tethered pseudodisaccharide **17** at a 58% yield after 4 h. The condensation reaction in DMF was much slower (19 h) with comparable yield (52%).



Scheme 2 *Reagents and conditions*: (a) $Br(CH_2)_2OTHP$, NaH, DMF; (b) TsOH, $CHCl_3$–MeOH; (c) TsCl, pyridine; (d) i) NaOMe, MeOH; ii) TrCl, Pyridine, 50 °C; (e) NaH, BnBr, DMF; (f) TsOH, $CHCl_3$, MeOH; (g) NaH, DMSO; (h) (i) NBS, aq. acetone; (ii) Ac_2O, pyridine; (i) (i) H_2, $Pd(OH)_2$/C, EtOAc, MeOH, AcOH; (ii) BzCl, pyridine; (j) (i) H_2NNH_2·AcOH, DMF, 60 °C; (ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2; (iii) $HO(CH_2)_2OAc$, TMSOTf, MS3A, CH_2Cl_2, 0 °C.

Hydrogenation of **17** over Pd/C at atmospheric pressure did not proceed, presumably due to the presence of the sulfur atom. The benzyl groups in **17** could be removed *via* hydrogenation in MeOH at 3.5 atom in the presence of AcOH, but solvolysis of thioglycoside also occurred simultaneously yielding methyl glycoside. Therefore, thioglycoside in **17** was hydrolyzed with NBS in aqueous acetone, and acetylated to give **18** at a 75% yield. Hydrogenation of **18** proceeded smoothly, and **19** was obtained at an 82% yield after benzoylation. Anomeric acetate in **19** was then removed by treatment with H_2NNH_2·AcOH in hot DMF, and converted to acetoxyethyl glycoside **20** *via* trichloroacetimidate at a yield of 52%. Commercial grade ethylene glycol monoacetate was partitioned between $CHCl_3$ and water to remove ethylene glycol from the mixture, so that no formation of hydroxyethyl glycoside was observed in this reaction.

Methylene- and ethylene-tethered pseudodisaccharides **9** and **20** were converted to their GDP derivative as shown in Scheme 3. Trimethylsilylethyl glycosides **9** and **20** were hydrolyzed and converted to α-trichloroacetimidates **21** and **25** at yields of 76% and 73%, respectively. Treatment of α-imidates **21** and **25** with recrystallized dibenzyl phosphate according to Schmidt's procedure[20] gave β-phosphates **22** and **26** in good yields. The anomeric configurations of **22** and **26** were determined to be β *via* the anomeric proton of L-Gal (H-1L) in ^1H NMR (**22**: δ 5.69, $J_{1L,2L}$ 7.7 Hz; **26**: δ 5.72, $J_{1L,2L}$ 7.9 Hz). Compound **22** contained a small amount of hydrolyzed product (hemiacetal),



Scheme 3 *Reagents and conditions*: (a) (i) TFA, CH_2Cl_2; (ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2; (b) $(BnO)_2P(O)OH$, CH_2Cl_2; (c) (i) H_2, Pd/C, Et_3N, MeOH; (ii) pyridine, NH_4OH; (d) $MgCl_2$, DMF.

which was difficult to separate because of the instability of the glycosyl phosphate **22**, and used without further purification. Benzyl esters of the phosphate were removed *via* hydrogenation over Pd/C. All benzoyl groups were then removed by treatment with pyridine and NH_4OH, and 1-phosphates **23** and **27** were obtained at yields of 81% and 59% as tri-*n*-butylamine salts, after passing through a column of cation-exchange resin. Condensation of sugar 1-phosphate **23** with poorly soluble GMP-morpholidate even when using a modified procedure[21] was sluggish in our hands. On the other hand, condensation of sugar 1-phosphate **23** with GMP-imidazolidate **24** in the presence of $MgCl_2$ proceeded smoothly and more rapidly than in its absence, as we had reported for the synthesis of GDP-5-thiofucose and GDP-5-thiomannose.[22] After overnight reaction, bisubstrate analogue **1** was isolated with a yield of 47%, following purification *via* anion-exchange chromatography and gel-permeation chromatography. This procedure was also applied to **27**, and bisubstrate analogue **2** was obtained at a yield of 21%. The structure of **1** and **2** were confirmed by ^1H, ^{13}C and ^{31}P NMR spectra as well as HRMS.

Activities of bisubstrate analogues

FucT assay was carried out using [2-(2-pyridylamino)ethyl] β-*N*-acetyllactosaminide (PA-LacNAc)[23] as an acceptor, and the resulting amount of reaction product was quantified *via* HPLC with fluorescence detector. IC_{50} values for bisubstrate analogues **1** and **2** were determined using fixed concentrations of 10 mM PA-LacNAc and 0.1 mM GDP-Fuc for both commercially available FucT V and VI. For FucT V, the IC_{50} value of **1** and **2** were determined to be 0.26 mM and 0.27 mM, respectively. For FucT VI, the IC_{50} values of **1** and **2** were determined to be 0.11 mM and 0.19 mM, respectively (Table 1). Next, we examined whether or not **1** and **2** can serve as a substrate of both FucTs. The enzyme reaction was executed using 0.19 mM of **1** or 0.15 mM of **2**, and 0.14 mM of PA-LacNAc, and the resulting reaction product was analyzed *via* HPLC after 8 and 20 h. Although no new peak was observed for the FucT V reaction, a faster-moving peak was formed for both **1** and **2** in the FucT VI reaction (Fig. 4). Enzyme reaction products

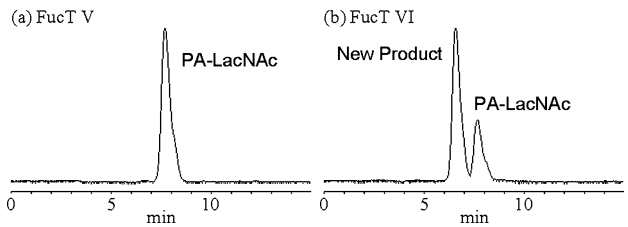


Fig. 4 HPLC profile of the reaction of FucT V or VI with PA-LacNAc and compound **2**.

Table 1 Inhibitory activities of bisubstrate analogues **1** and **2**

Compound	IC_{50}/mM[a]		K_i/μM[b]
1	0.26 (FucT V)	0.11 (FucT VI)	41
2	0.27 (FucT V)	0.19 (FucT VI)	43

[a] The IC_{50} values were determined at the concentrations of 10 mM PA-LacNAc and 0.10 mM GDP-Fuc. [b] The K_i values for FucT V were calculated using the equation, $IC_{50} = K_i(1 + s/K_M)$, in which the reported K_M value for GDP-Fuc (9 μM) was used.[8]

of **1** and **2** were analyzed *via* LC-MS. The *m*/*z* values expected for the Le^x type structures were observed for both products (see the electronic supplementary information, ESI†), suggesting that FucT VI can transfer 6-modified L-Gal moiety to LacNAc. Since **1** and **2** were not substrates of FucT V, the K_i values were calculated to be 41 μM and 43 μM, respectively, assuming that **1** and **2** are competitive inhibitors against GDP-Fuc (Table 1). These values are similar to that of GDP (K_i = 29 μM).[8] Apparently, the D-Gal moiety of **1** and **2** did not bind to the acceptor binding site of FucT VI since **1** and **2** were substrates of FucT VI. We anticipated that the initial binding of GDP-L-Gal moiety would eventually lead to the second binding of the acceptor moiety with the aid of a flexible linker. A molecule having a more rigid structure in the vicinity of the transition state may be more suitable as a bisubstrate analogue inhibitor.

Conclusion

We have successfully synthesized GDP-L-Gal analogues tethered to D-Gal *via* methylene and ethylene linkers. These compounds were found to be moderate inhibitors for FucT V, but substrates of FucT VI. These findings provide new insight regarding the substrate binding site of FucT V and VI. In addition, these findings add new and interesting information, which is useful not only for the development of FucT V specific inhibitors but also for establishing the utility of FucT VI for the modification of fucosylated glycoconjugates.

Experimental

General methods

^1H, ^{13}C and ^{31}P NMR spectra were recorded at 270 MHz or 400 MHz, 67.8 MHz, and 109 MHz, respectively, with JEOL JNM-EX270 or Varian Unity 400 instruments. All chemical shifts are quoted on the δ-scale and were referenced to tetramethylsilane (δ = 0 in $CDCl_3$) or HDO (δ = 4.80 in D_2O) for ^1H NMR, and $CDCl_3$ (δ = 77.0 in $CDCl_3$) or acetone (δ = 29.0 in D_2O) for ^{13}C NMR as an internal standard, and 85% H_3PO_4 (δ = 0) for ^{31}P NMR as an external standard. *J* values are given in Hz. Where indicated, NMR peak assignments were made using COSY; in compounds having D-Gal and L-Gal residues, assignments were indicated with D or L. High-resolution mass spectra were recorded using ESI techniques with TOF detector, with PerSpective Biosystems Mariner Biospectrometry Workstation. Optical rotations were determined using 1.0 dm cell, with Horiba SEPA-200 polarimeter, and $[\alpha]_D$ values are given in $10^{-1}{}^\circ$ cm g^{-1}. Silica gel column chromatography was performed with Kieselgel 60 (70–230 mesh, E. Merck) or Wakogel C300 (200–300 mesh, Wako Chemical). Thin-layer chromatography (TLC) was carried out on Kieselgel 60F254 Art.5715 (E. Merck) glass plates precoated with silica gel with fluorescence indicator. Plates were visualized by irradiation with UV lamp, or dipping in 1% $Ce(SO_4)_2$–1.5% $(NH_4)_6Mo_7O_{24}$–10% H_2SO_4, 5% H_2SO_4 in MeOH, or orcinol in 10% H_2SO_4–EtOH and charred. AG1-X8 was purchased from BioRad. Sephadex G-15 was purchased from Amersham Biosciences. High-performance liquid chromatography (HPLC) was performed using Hitachi L-7100 pump equipped with Waters 470 fluorescence detector and MacIntegrator recorder.

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Effluent was degassed by sonicating under reduced pressure. Inertsil ODS-3 HPLC column (i.d. 4.6 mm × 250 mm) was purchased from GL-Sciences. High-performance liquid chromatography combined with mass spectrometry (LC-MS) was performed using Shimadzu LCMS-2010A system equipped with TOSOH TSK-GEL ODS-100V column (i.d. 2.0 mm × 150 mm). Purity of ethylene glycol monoacetate, purchased from Aldrich, was 50% and contained ethylene glycol and ethylene glycol diacetate as impurities. Commercial ethylene glycol monoacetate was partitioned between $CHCl_3$ and water, and organic layer was concentrated to yield a mixture of ethylene glycol monoacetate and diacetate, and used without further purification. NaH (55% in mineral oil) was washed with hexane prior to use. CH_2Cl_2 was distilled from P_2O_5. α-1,3-Fucosyltransferase V and VI were purchased from Calbiochem.

Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-methylthiomethyl-1-thio-β-D-galactopyranoside (4). To a solution of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**3**, 2.4 g, 4.1 mmol) in DMSO (12.6 cm^3) was added Ac_2O (9.0 cm^3) and AcOH (1.8 cm^3). After being stirred for 1 day at room temperature, cooled saturated aqueous $NaHCO_3$ solution was added to the solution and stirred for additional 1 h. The solution was extracted with EtOAc, and the extract was washed with saturated aqueous $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified on a column of silica gel (hexane–EtOAc 5 : 1) to yield **4** (1.41 g, 53%) as an amorphous solid.; R_f 0.16 (hexane–EtOAc 5 : 1); $[\alpha]_D^{23}$ +110.8 (*c* 1.0 in $CHCl_3$); (Found: C, 64.93; H, 4.94; S, 9.70. $C_{35}H_{32}O_8S_2$ requires C, 65.20; H, 5.00; S, 9.95%); δ_H (270 MHz, $CDCl_3$) 7.98–7.21 (20 H, m), 5.92 (1 H, d, *J* 3.3), 5.70 (1 H, t, *J* 9.9), 5.54 (1 H, dd, *J* 3.3 and 9.9), 5.00 (1 H, d, *J* 9.9), 4.60 (2 H, s), 4.22 (1 H, t, *J* 6.6), 3.77 (1 H, dd, *J* 6.3 and 9.9), 3.71 (1 H, dd, *J* 6.6 and 9.9), 2.07 (3 H, s); δ_C (67.8 MHz, $CDCl_3$) 165.4, 165.2, 164.9, 134.3, 133.3, 133.2, 133.1, 130.7, 129.8, 129.7, 129.7, 129.3, 129.0, 128.8, 128.7, 128.4, 128.4, 128.3, 128.1, 85.4, 76.3, 75.9, 73.3, 68.5, 67.9, 65.9, 14.0.

2-Trimethylsilylethyl 6-*O*-triphenylmethyl-2,3,4-tri-*O*-benzoyl-β-L-galactopyranoside (6). To a solution of 2-trimethylsilylethyl 2,3,4,6-tetra-*O*-acetyl-β-L-galactopyranoside (**5**, 2.63 g, 5.86 mmol) in MeOH (30 cm^3) was added a catalytic amount of NaOMe. After being stirred for 4 h at room temperature, the solution was neutralized with Dowex 50W-X8 (H^+). The resin was filtered off, and the filtrate was concentrated to give syrup. The syrup was dissolved in pyridine (30 cm^3), and TrCl (2.45 g, 8.79 mmol) was added at room temperature. After being stirred for 16 h at 50 °C, the solution was cooled to 0 °C, and BzCl (3.06 cm^3, 26.3 mmol) was added. After being stirred for 3 h, the reaction was quenched by adding crashed ice. The solution was extracted with $CHCl_3$, and the organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 10 : 1 → 8 : 1) to yield **6** (3.52 g, 72%) as an amorphous solid.; R_f 0.12 (hexane–EtOAc 10 : 1); $[\alpha]_D^{26}$ −63.2 (*c* 1.26 in $CHCl_3$); (Found: C, 73.14; H, 6.25. $C_{51}H_{50}O_9Si$ requires C, 73.36; H, 6.04%); δ_H (270 MHz, $CDCl_3$) 8.11–7.12 (30 H, m), 6.02 (1 H, d, *J* 2.3), 5.65 (1 H, dd, *J* 7.6 and 10.6), 5.57 (1 H, dd, *J* 2.3 and 10.6), 4.74 (1 H, d, *J* 7.6), 4.04–3.98 (2 H, m), 3.61 (1 H, dt, *J* 6.6 and 10.2), 3.48 (1 H, dd, *J* 5.9 and 10.2), 3.27 (1 H, br t, *J* 8.4), 1.13 (2 H, m), −0.08 (9 H, s); δ_C (67.8 MHz, $CDCl_3$) 165.5, 165.2, 165.0, 143.2, 132.98, 132.96, 132.9, 130.0, 129.9, 129.7, 129.6, 129.5, 129.3, 128.9, 128.4, 128.3, 128.2, 128.14, 128.08, 127.7, 126.9, 100.9, 86.9, 72.6, 72.1, 70.1, 68.2, 67.7, 61.1, 18.1, −1.4.

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzoyl-β-L-galactopyranoside (7). To a solution of **6** (1.6 g, 1.9 mmol) in $CHCl_3$–MeOH (2 : 1 v/v, 19 cm^3) was added $TsOH{\cdot}H_2O$ (164 mg, 0.952 mmol) at 0 °C. After being stirred for 3 h at room temperature, the solution was diluted with $CHCl_3$, and washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 3 : 1 → 1 : 1) to yield **7** (0.80 g, 71%) as a colourless syrup.; R_f 0.19 (hexane–EtOAc 3 : 1); $[\alpha]_D^{30}$ −174 (*c* 0.84 in $CHCl_3$); (Found: C, 64.68; H, 5.96. $C_{32}H_{36}O_9Si$ requires C, 64.85; H, 6.12%); δ_H (270 MHz, $CDCl_3$) 8.13–7.21 (15 H, m), 5.84 (1 H, dd, *J* 10.4 and 7.9), 5.80 (1 H, m), 5.57 (1 H, dd, *J* 3.3 and 10.4), 4.82 (1 H, d, *J* 7.9), 4.11–4.00 (2 H, m), 3.89–3.80 (1 H, m), 3.69–3.80 (2 H, m), 0.92 (2 H, m), −0.06 (9 H, s); δ_C (67.8 MHz, $CDCl_3$) 166.7, 165.4, 165.1, 133.7, 133.2, 133.0, 130.0, 129.61, 129.59, 129.35, 128.61, 128.56, 128.49, 128.20, 128.18, 101.0, 73.9, 71.9, 70.0, 69.0, 67.9, 60.5, 18.1, −1.4.

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranosid-6-yloxymethyl)-β-L-galactopyranoside (8). To a solution of **7** (290 mg, 0.489 mmol) and **4** (380 mg, 0.589 mmol) in CH_2Cl_2 (8.0 cm^3) was added MS3A (300 mg) under Ar atmosphere. After being stirred for 1 h at room temperature, MeOTf (67 mm^3, 0.59 mmol) was added. Another portion of MeOTf (67 mm^3, 0.59 mmol) was added after 2.5 h and 4.5 h, and the suspension was stirred for an additional 1 h. The suspension was neutralized with Et_3N, and filtered through a pad of celite. The filtrate was concentrated, and the residue was purified on a column of silica gel (toluene–EtOAc 25 : 1 → 20 : 1) to yield crude **8**. This was again purified on a column of silica gel (hexane–EtOAc 5 : 2) to yield pure **8** (254 mg, 44%) as a colourless syrup.; R_f 0.37 (hexane–EtOAc 2 : 1); $[\alpha]_D^{30}$ −6.5 (*c* 1.07, $CHCl_3$); δ_H (400 MHz, $CDCl_3$, COSY) 8.07–7.12 (35 H, m, Ar), 5.89 (1 H, br d, $J_{4L,3L}$ 3.7, H-4L), 5.87 (1 H, br d, $J_{4D,3D}$ 2.7, H-4D), 5.74 (1 H, dd, $J_{2L,3L}$ 10.5, $J_{2L,1L}$ 7.9, H-2L), 5.67 (1 H, t, $J_{2D,1D} = J_{2D,3D}$ 9.9, H-2D), 5.50 (1 H, dd, H-3L), 5.48 (1 H, dd, H-3D), 4.93 (1 H, d, H-1D), 4.76 (1 H, d, H-1L), 4.66 (1 H, d, *J* 7.0, OCH_2O), 4.56 (1 H, d, *J* 7.0, OCH_2O), 4.13–4.03 (3 H, m, H-5L, 5D, OCH_2CH_2Si), 3.78–3.59 (5 H, m, H-6aL, 6bL, 6aD, 6bD, OCH_2CH_2Si), 0.90 (2 H, m, OCH_2CH_2Si), − 0.08 (9 H, s, $SiMe_3$); δ_C (67.8 MHz, $CDCl_3$) 165.4, 165.3, 165.1, 165.0, 164.9, 134.25, 133.28, 133.1, 133.0, 130.6, 129.9, 129.8, 129.63, 129.59, 129.5, 129.3, 129.1, 128.9, 128.8, 128.72, 128.68, 128.44, 128.41, 128.32, 128.27, 128.14, 128.09, 127.8, 100.9, 96.2, 85.1, 76.2, 73.2, 72.4, 72.1, 69.9, 68.4, 67.8, 67.7, 66.5, 66.3, 18.1, −1.4; *m/z* (ESI) 1211.3625 (M^+ + Na. $C_{66}H_{64}O_{17}SSiNa$ requires 1211.3531).

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2-acetoxyethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosid-6-yloxymethyl)-β-L-galactopyranoside (9). To a solution of **8** (99.6 mg, 83.7 μmol) and ethylene glycol monoacetate* (38 mm^3, 0.21 mmol) in CH_2Cl_2 (1.0 cm^3) was added MS4A (50 mg) under Ar atmosphere. After being stirred for 1 h at room temperature, the suspension was

* In this case, commercial grade ethylene glycol monoacetate was used without any purification.

cooled to −30 °C. To the suspension was added NIS (36 mg, 0.16 mmol) and TfOH (0.3 M in Et_2O, 83 mm^3, 25 μmol), subsequently. After being stirred for 30 min, the suspension was diluted with $CHCl_3$ and filtered through a pad of celite. The filtrate was washed with 10% aqueous $Na_2S_2O_3$ solution and saturated aqueous $NaHCO_3$ solution, subsequently. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 2 : 1 → 1 : 1) to yield a mixture of **9** and hydroxyethyl glycoside **10**. The mixture was acetylated with Ac_2O (0.5 cm^3) and pyridine (0.5 cm^3) and purified by silica gel column chromatography (hexane–EtOAc 3 : 2) to yield **9** (65.2 mg, 66%) as a colourless syrup; R_f 0.16 (hexane–EtOAc 2 : 1); $[\alpha]_D^{30}$ + 4.8 (*c* 1.27, $CHCl_3$); δ_H (400 MHz, $CDCl_3$, COSY) 8.07–7.13 (30 H, m, Ar), 5.88–5.87 (2 H, m, H-4L, 4D), 5.75 (1 H, dd, $J_{2D,3D}$ 10.5, $J_{2D,1D}$ 8.1, H-2D), 5.74 (1 H, dd, $J_{2L,3L}$ 10.4, $J_{2L,1L}$ 7.9, H-2L), 5.52 (1 H, dd, $J_{3D,4D}$ 3.4, H-3D), 5.49 (1 H, dd, $J_{3L,4L}$ 3.4, H-3L), 4.79 (1 H, d, H-1D), 4.76 (1 H, d, H-1L), 4.67 (1 H, d, *J* 6.9, OC*H*$_2$O), 4.57 (1 H, d, *J* 6.9, OC*H*$_2$O), 4.18–4.03 (6 H, m, H-5L, 5D, OC*H*$_2$CH$_2$Si,OC*H*$_2$C*H*$_2$O), 3.83–3.61 (6 H, m, H-6aL, 6bL, 6aD, 6bD, OC*H*$_2$CH$_2$Si, OC*H*$_2$CH$_2$O), 1.69 (3 H, s, Ac), 0.91 (2 H, m, OCH$_2$C*H*$_2$Si), −0.07 (9 H, s, SiMe$_3$); δ_C (67.8 MHz, $CDCl_3$) 170.6, 165.4, 165.3, 165.0, 164.9, 133.4, 133.3, 133.07, 133.02, 132.9, 129.9, 129.6, 129.59, 129.46, 129.3, 129.1, 129.0, 128.8, 128.7, 128.48, 128.47, 128.2, 128.16, 128.11, 101.3, 100.9, 96.1, 72.7, 72.4, 72.1, 71.8, 69.9, 69.6, 68.4, 67.8, 67.5, 66.3, 63.0, 20.4, 18.1, −1.4; *m/z* (ESI) 1205.3908 (M^+ + Na. $C_{64}H_{66}O_{20}SiNa$ requires 1205.3814).

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-hydroxyethyl)-1-thio-β-D-galactopyranoside (12). To a solution of phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**11**, 2.47 g, 4.55 mmol) in DMF (30 cm^3) was added NaH (55% in oil, 800 mg, 18.3 mmol) at 0 °C. After being stirred for 1 h at 0 °C, 2-(2-bromoethoxy)-tetrahydro-2*H*-pyrane (2.7 cm^3, 18 mmol) was added, and stirred overnight at room temperature. MeOH was added to the solution to destroy excess reagents, and diluted with EtOAc. The solution was washed with brine, and the organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 3 : 1 → 1 : 1) to yield THP–ether (2.04 g, 67%) as a colourless syrup.

The syrup was dissolved in $CHCl_3$–MeOH (2 : 1 v/v, 30 cm^3) and TsOH·H_2O (262 mg, 1.52 mmol) was added at 0 °C. After being stirred for 1 h at room temperature, the solution was diluted with $CHCl_3$, and washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 2 : 1 → 3 : 2 → 1 : 1) to yield **12** (1.56 g, 88%) as an amorphous solid.; R_f 0.19 (hexane–EtOAc 2 : 1); $[\alpha]_D^{26}$ + 1.7 (*c* 1.1, $CHCl_3$); (Found: C, 71.45; H, 6.37; S, 5.74. $C_{35}H_{38}O_6S$ requires C, 71.65; H, 6.53; S, 5.47%); δ_H (270 MHz, $CDCl_3$) 7.58–7.19 (20 H, m), 4.98 (1 H, d, *J* 10.5), 4.81–4.72 (4 H, m), 4.65 (1 H, d, *J* 9.6), 4.62 (1 H, d, *J* 11.9), 3.94 (1 H, t, *J* 9.6 Hz), 3.90 (1 H, d, *J* 2.6 Hz), 3.65–3.41 (8 H, m); δ_C (67.8 MHz, $CDCl_3$) 138.5, 138.12, 138.06, 133.9, 131.5, 128.7, 128.4, 128.2, 128.1, 127.9, 127.7, 127.6, 127.49, 127.48, 127.1, 87.8, 84.2, 77.41, 77.37, 77.2, 75.7, 74.3, 73.5, 72.9, 72.5, 69.9, 61.8.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-{2-(*p*-toluenesulfonyloxy)ethyl}-1-thio-β-D-galactopyranoside (13). To a solution of **12** (1.37 g, 2.33 mmol) in pyridine (20 cm^3) was added TsCl (1.36 g, 6.98 mmol) at room temperature. After being stirred for 3 h, the solution was diluted with $CHCl_3$, and washed with saturated aqueous $NaHCO_3$, 1 M HCl and water, subsequently. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 4 : 1 → 3 : 1 → 2 : 1) to yield **13** (1.16 g, 68%) as a colourless syrup; R_f 0.14 (hexane–EtOAc 4 : 1); $[\alpha]_D^{26}$ + 0.9 (*c* 1.21, $CHCl_3$); (Found: C,67.77; H, 5.95; S, 8.73. $C_{42}H_{44}O_8S_2$ requires C, 68.08; H, 5.99; S, 8.66%); δ_H (270 MHz, $CDCl_3$) 7.76–7.18 (24 H, m), 4.95 (1 H, d, *J* 11.9), 4.81–4.71 (4 H, m), 4.61 (1 H, d, *J* 9.9), 4.60 (1 H, d, *J* 11.5), 4.05 (2 H, m), 3.95–3.88 (2 H, m), 3.59 (1 H, dd, *J* 2.6 and 9.2), 3.54–3.44 (5 H, m), 2.39 (3 H, s); δ_C (67.8 MHz, $CDCl_3$) 144.8, 138.7, 138.3, 138.2, 134.0, 133.1, 131.5, 129.8, 128.7, 128.4, 128.3, 128.2, 127.8, 127.7, 127.5, 127.4, 127.1, 87.6, 84.1, 77.3, 77.1, 75.6, 74.3, 73.4, 72.7, 69.7, 68.9, 68.8, 21.6.

2-Trimethylsilylethyl 6-*O*-triphenylmethyl-β-L-galactopyranoside (14). To a solution of **5** (3.35 g, 7.47 mmol) in MeOH (80 cm^3) was added catalytic amount of NaOMe. After being stirred for 1 h, the solution was neutralized with Dowex 50W-X8 (H^+). The resin was filtered off, and the filtrate was concentrated. The residue was dissolved in pyridine (50 cm^3), and TrCl (3.12 g, 11.2 mmol) was added at room temperature. After being stirred overnight at 50 °C, MeOH was added to the solution, and the resulting solution was concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 2 : 3) to yield **14** (1.98 g, 50%) as a colourless syrup; R_f 0.20 (hexane–EtOAc 2 : 3); $[\alpha]_D^{30}$ + 15.3 (*c* 1.09, $CHCl_3$); (Found: C, 68.70; H, 7.24. $C_{30}H_{38}O_6Si$ requires C, 68.93; H, 7.33%); δ_H (270 MHz, $CDCl_3$) 7.47–7.23 (15 H, m), 4.23 (1 H, d, *J* 7.6), 4.07–3.97 (2 H, m), 3.65–3.54 (4 H, m), 3.46 (1 H, dd, *J* 5.6 and 9.4), 3.36 (1 H, dd, *J* 6.4 and 9.4), 1.02 (2 H, m), 0.01 (9 H, s); δ_C (67.8 MHz, $CDCl_3$) 143.5, 128.5, 127.8, 127.1, 102.3, 89.0, 73.6, 73.5, 72.3, 69.1, 67.3, 62.6, 18.4, −1.3;

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-β-L-galactopyranoside (15). To a solution of **14** (214 mg, 0.410 mmol) in DMF (4.0 cm^3) was added NaH (55% in oil, 161 mg, 3.69 mmol) at 0 °C. After being stirred for 1 h at 0 °C, BnBr (0.3 cm^3, 2.5 mmol) was added, and the solution was stirred for 30 min at room temperature. MeOH was added to the solution, and the resulting solution was diluted with EtOAc, and washed with brine. Organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 20 : 1) to yield **15** (274 mg, 84%) as a colourless syrup.; R_f 0.16 (hexane–EtOAc 20 : 1); $[\alpha]_D^{25}$ + 2.6 (*c* 0.9, $CHCl_3$); (Found: C, 76.91; H, 7.03. $C_{51}H_{56}O_6Si$ requires C, 77.24; H, 7.12%); δ_H (270 MHz, $CDCl_3$) 7.41–7.11 (30 H, m), 4.92 (1 H, d, *J* 10.9), 4.83–4.46 (5 H, m), 4.32 (1 H, d, *J* 7.9), 4.00 (1 H, dt, *J* 7.6 and 10.2), 3.85 (1 H, d, *J* 2.6) 3.76 (1 H, dd, *J* 7.9 and 9.6) 3.62–3.44 (3 H, m), 3.36 (1 H, t *J* 6.3) 3.20 (1 H, dd, *J* 6.3 and 8.9) 1.03 (2 H, m), 0.01 (9 H, s); δ_C (67.8 MHz, $CDCl_3$) 143.8, 138.6, 128.6, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.1, 126.9, 103.4, 86.8, 82.2, 79.7, 75.1, 74.2, 74.1, 73.6, 73.1, 67.3, 62.8, 18.6, −1.3.

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzyl-β-L-galactopyranoside (16). To a solution of **15** (2.47 g, 3.11 mmol) in $CHCl_3$–MeOH (2:1 v/v, 40 cm^3) was added TsOH·H_2O (267 mg, 1.55 mmol) at 0 °C. After being stirred for 2 h at room temperature, the solution was diluted with $CHCl_3$, and washed with brine. Aqueous layer was extracted with $CHCl_3$, and combined organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column

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of silica gel (hexane–EtOAc 5 : 1 → 1 : 1) to yield **16** (1.53 g, 89%) as a colourless syrup.; R_f 0.19 (hexane–EtOAc 3 : 1); $[\alpha]_D^{30}$+ 24.6 (*c* 0.71, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.35–7.22 (15 H, m), 4.97–4.63 (6 H, m), 4.37 (1 H, d, *J* 7.9), 4.00 (1 H, dt, *J* 7.6 and 9.2 Hz), 3.85–3.73 (3 H, m), 3.63–3.48 (3 H, m), 3.36 (1 H, t, *J* 5.6), 1.03 (2 H, m), 0.01 (9 H, s); δ_C (67.8 MHz, $CDCl_3$) 138.8, 138.4, 138.3, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.6, 127.5, 127.4, 103.6, 82.3, 79.7, 75.1, 74.5, 74.1, 73.3, 73.0, 67.4, 62.0, 18.5, −1.5; *m/z* (ESI) 573.2648 (M^+ + Na. $C_{32}H_{42}O_6SiNa$ requires 573.2591).

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzyl-6-*O*-(phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranosid-6-yloxyethyl)-β-L-galactopyranoside (17). To a solution of **16** (359 mg, 0.652 mmol) in DMSO (5.0 cm^3) was added NaH (55% in oil, 136 mg, 3.13 mmol) at 0 °C. After being stirred for 1 h, **13** (602 mg, 0.812 mmol) was added to the solution. After being stirred for 4 h, the reaction was quenched with MeOH, and the solution was diluted with EtOAc. The solution was washed with brine, and the aqueous layer was extracted with EtOAc. Combined organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 5 : 1 → 7 : 2) to yield **17** (426 mg, 58%) as a colourless syrup.; R_f 0.41 (hexane–EtOAc 3 : 1); $[\alpha]_D^{30}$+ 12.0 (*c* 0.87, $CHCl_3$); δ_H (400 MHz, $CDCl_3$, COSY) 7.57–7.18 (35 H, m, Ar), 4.97–4.60 (12 H, m, PhCH_2 × 6), 4.62 (1 H, d, $J_{1D,2D}$ 9.6, H-1D), 4.34 (1 H, d, $J_{1L,2L}$ 8.0, H-1L), 3.98 (1 H, dt, *J* 7.8, 9.6, OC$H_2$$CH_2$Si), 3.93 (1 H, br d, $J_{4D,3D}$ 2.7, H-4D), 3.92 (1 H, t, $J_{2D,3D}$ 9.6, H-2D), 3.84 (1 H, br d, $J_{4L,3L}$ 2.1, H-4L), 3.79 (1 H, dd, $J_{2L,3L}$ 9.8, H-2L), 3.61–3.35 (13 H, m, H-3D, 5D, 6aD, 6bD, 3L, 5L, 6aL, 6bL, OC$H_2$$CH_2Si, OCH_2CH_2$O), 1.02 (2 H, m, OCH_2CH_2Si), −0.01 (9 H, s, $SiMe_3$); δ_C (100 MHz, $CDCl_3$) 138.7, 138.5, 138.3, 138.2, 134.1, 131.5, 128.8, 128.4, 128.3, 128.2, 128.1, 127.9, 127.73, 127.68, 127.50, 127.46, 127.0, 103.4, 87.7, 82.2, 79.7, 75.6, 75.1, 74.3, 73.4, 73.3, 73.2, 72.9, 72.7, 70.5, 69.6, 67.3, 18.4, 1.5; *m/z* (ESI) 1141.5036 (M^+ + Na. $C_{67}H_{78}O_{11}SSiNa$ requires 1141.4932).

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzyl-6-*O*-(1-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranos-6-yloxyethyl)-β-L-galactopyranoside (18). To a solution of **17** (420 mg, 0.375 mmol) in acetone–water (9 : 1 v/v, 10 cm^3) was added NBS (200 mg, 1.12 mmol) at room temperature. After being stirred for 20 min, another portion of NBS (30 mg, 0.169 mmol) was added. After being stirred for 20 min, TLC analysis revealed that all **17** was consumed. The solution was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$, and water, respectively. The organic layer was dried ($MgSO_4$) and concentrated to yield crude hemiacetal (433 mg) as a syrup. The syrup was acetylated with pyridine (4.0 cm^3) and Ac_2O (3.0 cm^3) and purified by silica gel column chromatography (hexane–EtOAc 4 : 1 → 3 : 1 → 2 : 1) to yield **18** (302 mg, 75%, α : β = 6 : 4) as a colourless syrup; δ_H (270 MHz, $CDCl_3$) 7.34–7.23 (30 H, m), 6.35 (0.6 H, d, *J* 3.6), 5.53 (0.4 H, d, *J* 8.2), 4.96–4.57 (12 H, m), 4.33 (1 H, d, *J* 7.9), 4.17–3.33 (18 H. m), 2.07 (1.8 H, s), 2.02 (1.2 H, s), 1.02 (2 H, m), 0.00 (9 H, s); *m/z* (ESI) 1091.4861 (M^+ + Na. $C_{63}H_{76}O_{13}SiNa$ requires 1091.4953).

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α/β-D-galactopyranos-6-yloxyethyl)-β-L-galactopyranoside (19). To a solution of **18** (113 mg, 0.105 mmol) in EtOAc–MeOH–AcOH (2/2/1 v/v/v, 5.0 cm^3) was added $Pd(OH)_2$/C (20%, 35 mg). The suspension was stirred for 20 h under H_2 gas at atmospheric pressure. The suspension was filtered through a pad of celite, and the filtrate was concentrated to give hexaol (56.2 mg) as a syrup. The syrup was dried by concentrating from pyridine three times. The residue was dissolved in pyridine (2.0 cm^3), and BzCl (0.15 cm^3, 1.3 mmol) was added to the solution. After being stirred for 17 h at room temperature, the reaction was quenched with crashed ice, and extracted twice with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 5 : 2 → 3 : 2) to yield **19** (99.4 mg, 82%, α : β = 6 : 4) as an amorphous solid.; δ_H (270 MHz, $CDCl_3$) 8.08–7.20 (30 H. m), 6.65 (0.6 H, d, *J* 3.3), 6.07 (0.4 H, d, *J* 8.2), 5.97–5.47 (6 H, m), 4.79 (0.4 H, d, *J* 8.2), 4.76 (0.6 H, d, *J* 8.2), 4.51 (0.6 H, m), 4.25 (0.4 H, m), 4.11–3.44 (10 H, m), 2.17 (1.8 H, s), 2.09 (1.2 H, s), 0.92 (2 H, m), −0.06 (9 H, s).

2-Trimethylsilylethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2-acetoxyethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosid-6-yloxyethyl)-β-L-galactopyranoside (20). To a solution of **19** (94.2 mg, 82 μmol) in DMF (2.0 cm^3) was added H_2NNH_2·AcOH (7.9 mg, 86 μmol). After being stirred for 10 min at 60 °C, the solution was diluted with $CHCl_3$, and washed with brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (hexane–EtOAc 3 : 2 → 5 : 4) to yield hemiacetal (70.4 mg) as a colourless syrup.

CCl_3CN (126 mm^3, 1.26 mmol) and Cs_2CO_3 (41 mg, 0.13 mmol) was added to a solution of the hemiacetal (70.4 mg, 63 μmol) in CH_2Cl_2 (3.0 cm^3) at room temperature under Ar atmosphere. After being stirred overnight, precipitate was filtered off, and the filtrate was concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 2 : 1) to yield trichloroacetimidate (72 mg) as a colourless syrup.

MS3A (100 mg) was added to a solution of the imidate (72 mg, 57 μmol) and ethylene glycol monoacetate (46 mg, 140 μmol) in CH_2Cl_2 (2.0 cm^3) under Ar atmosphere. After being stirred for 1 h, the suspension was cooled to 0 °C, and a solution of TMSOTf (2.0 mm^3, 11 μmol) in CH_2Cl_2 (0.1 cm^3) was added. After being stirred for 20 min at 0 °C, the reaction was neutralized with Et_3N, and the solid was filtered off. The filtrate was concentrated, and the residue was treated with pyridine (1.0 cm^3) and Ac_2O (1.0 cm^3) to facilitate the purification of **20**. Purification by silica gel column chromatography (hexane–EtOAc 2 : 1 → 3 : 2) yielded **20** (51 mg, 52%) with a small contamination of ethylene glycol diacetate.; R_f 0.30 (hexane–EtOAc 3 : 2); δ_H (400 MHz, $CDCl_3$, COSY) 8.08–7.22 (30 H, m, Ar), 5.88–5.86 (2 H, m, H-4L, 4D), 5.75 (1 H, dd, $J_{2D,3D}$ 10.4, $J_{2D,1D}$ 8.2, H-2D), 5.73 (1 H, dd, $J_{2L,3L}$ 10.4, $J_{2L,1L}$ 8.1, H-2L), 5.53 (1 H, dd, $J_{3D,4D}$ 3.4, H-3D), 5.51 (1 H, dd, $J_{3L,4L}$ 3.4, H-3L), 4.81 (1 H, d, H-1D), 4.77 (1 H, d, H-1L), 4.20–4.04 (6 H, m, H-5L, 5D, OC$H_2$$CH_2Si, OCH_2CH_2$OAc), 3.84 (1 H, ddd, *J* 4.1, 6.2 and 11.1, OC$H_2$$CH_2$OAc), 3.69–3.61 (5 H, m, H-6aL, 6bL, 6aD,6bD, OC$H_2$$CH_2$Si), 3.59–3.54 (2 H, m, OCH_2CH_2O), 3.50–3.45 (2 H, m, OCH_2CH_2O), 1.71 (3 H, s, Ac), 0.93 (2 H, m, OCH_2CH_2Si), −0.06 (9 H, s, $SiMe_3$); δ_C (67.8 MHz, $CDCl_3$) 170.6, 165.4, 165.31, 165.28, 165.2, 165.0, 164.9, 133.32, 133.26, 133.02, 132.97, 132.9, 129.8, 129.6, 129.4, 129.3, 129.2, 129.1, 128.81, 128.78, 128.5, 128.2, 128.13, 128.1, 101.3, 100.9, 72.8, 72.5, 72.0, 71.7, 70.8, 70.0, 69.7, 69.2, 69.1, 68.5, 67.7, 67.6, 63.1, 29.7, 20.4, 18.1, −1.4.

Bis(ammonium) guanosine 5′-{6-*O*-(2-hydroxyethyl β-D-galacto-pyranosid-6-yloxymethyl)-β-L-galactopyranosyl diphosphate} (1). To a solution of **9** (69.5 mg, 58.7 μmol) in CH_2Cl_2 (0.6 cm^3) was added TFA (1.2 cm^3). After being stirred for 30 min at room temperature, the solution was diluted with toluene and concentrated. The remaining TFA was coevaporated several times with toluene, and the residue was purified on a column of silica gel (hexane–EtOAc 3 : 2 → 1 : 1) to yield hemiacetal (62.7 mg) as a colourless syrup.

CCl_3CN (120 mm^3, 1.2 mmol) and Cs_2CO_3 (38 mg, 0.12 mmol) was added to a solution of the hemiacetal (62.7 mg) in CH_2Cl_2 (2.5 cm^3) at room temperature under Ar atmosphere. After being stirred overnight, precipitate was filtered off, and the filtrate was concentrated. The residue was purified on a column of silica gel (hexane–EtOAc 2 : 1 → 3 : 2) to yield trichloroacetimidate **21** (54.8 mg, 76% in two steps) as an amorphous solid.; R_f 0.51 (hexane–EtOAc 1 : 1); δ_H (270 MHz, $CDCl_3$) 8.74 (1 H, s), 8.08–7.20 (30 H, m), 6.84 (1 H, d, *J* 3.3), 6.04–5.83 (4 H, m), 5.76 (1 H, dd, *J* 7.7 and 10.5), 5.56 (1 H, dd, *J* 2.3 and 10.5), 4.81 (1 H, d, *J* 7.7), 4.62–4.54 (3 H, m), 4.17–4.08 (4 H, m), 3.84–3.70 (5 H, m), 1.70 (3 H, s).

Compound **21** was dried by concentrating three times from dry toluene prior to use. A solution of recrystallized dibenzyl phosphate (15 mg, 54 μmol) in CH_2Cl_2 (0.2 cm^3) was added dropwise to a solution of **21** (54.6 mg, 44.5 μmol) in CH_2Cl_2 (2.0 cm^3) at room temperature under Ar atmosphere. After being stirred for 1 h, the solution was concentrated. The residue was purified on a column of silica gel (toluene–EtOAc 3 : 1 → 2 : 1 containing 1% Et_3N) to yield β-phosphate **22** (60.7 mg) which contained a small amount of corresponding hemiacetal. Compound **22** was used in the next reaction without further purification.; R_f 0.37 (toluene–EtOAc 2 : 1); δ_H (400 MHz, $CDCl_3$) 8.08–7.00 (40 H, m), 5.93 (1 H, br d, *J* 2.9), 5.89 (1 H, dd, *J* 7.7 and 10.2), 5.86 (1 H, m), 5.74 (1 H, dd, *J* 8.0 and 10.4), 5.69 (1 H, t, *J* 7.7), 5.57 (1 H, dd, *J* 2.9 and 10.2), 5.53 (1 H, dd, *J* 3.3 and 10.4), 5.16, 5.09 (1 H × 2, each dd, *J* 7.0 and 11.9), 4.86, 4.76 (1 H × 2, each dd, *J* 7.0 and 11.9), 4.78 (1 H, d, *J* 8.0), 4.58, 4.50 (1 H × 2, each d, *J* 7.1), 4.27–4.05 (4 H, m), 4.03 (1 H, br t, *J* 6.6), 3.82–3.63 (5 H, m), 1.69 (3 H, s); δ_P (109 MHz, $CDCl_3$) − 2.32; *m/z* (ESI) 1365.3611 (M^+ + Na. $C_{73}H_{67}O_{23}PNa$ requires 1365.3709).

Pd/C (10%, 50 mg) was added to a solution of **22** and Et_3N (7.3 mm^3, 52 μmol) in MeOH (3.0 cm^3). The suspension was stirred under H_2 gas at atmospheric pressure for 12 h, and TLC analysis showed only one product. The suspension was filtered through a pad of celite, and the filtrate was concentrated. The residue was dissolved in NH_4OH–pyridine (4 : 1 v/v, 2.0 cm^3) and stirred for 20 h at room temperature. The solution was concentrated, and the residue was dissolved in water and passed through ODS silica gel column. Void fractions were collected and passed through a column of Dowex 50W-X8 (*n*-Bu_3NH^+), and lyophilized to yield deprotected phosphate **23** (24.7 mg, 81% in three steps).; R_f 0.31 (^{i}PrOH–water–NH_4OH 5 : 3 : 1); δ_H (270 MHz, D_2O) 4.90 (1 H, t, *J* 7.6), 4.48 (1 H, d, *J* 7.6), 4.00–3.54 (17 H, m), 3.18–3.13 (6 H, m), 1.75–1.64 (6 H, m), 1.47–1.33 (6 H, m), 1.01–0.93 (9 H, m); δ_P (109 MHz, D_2O) −2.32.

n-Bu_3N (13 mm^3, 52 μmol) was added to a solution of GMP (17.3 mg, 47.6 μmol) in DMF (0.8 cm^3). The solution was concentrated to give GMP tri-*n*-butylammonium salt. The salt was dissolved in DMF (0.8 cm^3) and *N*,*N*′-carbonyldiimidazole (10.8 mg, 66.7 μmol) was added under Ar atmosphere. After being stirred overnight, MeOH was added to the solution and stirred for 30 min to destroy all excess reagents. The solution was concentrated to yield GMP–imidazolidate **24**. Compound **23** (24.6 mg, 36.1 μmol) and **24** were dried by concentrating three times from dry DMF, separately, then combined and dissolved in DMF (1.5 cm^3). $MgCl_2$ was dried by concentrating three times from dry DMF. A solution of $MgCl_2$ (17.5 mg, 18.47 μmol) in DMF (0.5 cm^3) was added to the solution of **23** and **24**. After being stirred overnight, the reaction mixture was concentrated, and the residue was poured onto a column of AG1-X8 (HCO_2^-) and eluted with a linear gradient of NH_4HCO_3 (0–1 M). Fractions containing **1** were collected and lyophilized. The residue was purified by gel permeation chromatography (Sephadex G-15, water) to yield **1** (14.1 mg, 47%) as a white solid after lyophilization.; R_f 0.41 (^{i}PrOH–H_2O–NH_4OH 5 : 3 : 1); δ_H (400 MHz, D_2O, 40 °C, COSY)†† 8.08 (1 H, s, H-8), 5.92 (1 H, d, $J_{1',2'}$ 6.3, H-1′), 4.96 (1 H, dd, $J_{1L,P}$ 8.5, $J_{1L,2L}$ 7.6, H-1L), 4.79–4.74 (3 H, m, H-2′, OCH_2O), 4.52 (1 H, dd, $J_{3',2'}$ 5.3, $J_{3',4'}$ 3.3, H-3′), 4.36 (1 H, d, $J_{1D,2D}$ 7.8, H-1D), 4.35–4.32 (1 H, m, H-4′), 4.20 (2 H, m, H-5′a, 5′b), 4.00–3.95 (1 H, m, OCH_2CH_2OH), 3.91 (1 H, br d, $J_{4D,3D}$ 3.5, H-4D), 3.90 (1 H, br d, $J_{4L,3L}$ 3.4, H-4L), 3.86–3.71 (9 H, m, H-5L, 6aL, 6bL, 5D, 6aD, 6bD, OCH_2CH_2OH), 3.67 (1 H, dd, $J_{3L,2L}$ 10.1, H-3L), 3.66 (1 H, dd, $J_{3D,2D}$ 9.9, H-3D), 3.61 (1 H, dd, H-2L), 3.53 (1 H, dd, H-2D); δ_C (67.8 MHz, D_2O) 160.3, 155.0, 152.3, 137.9, 116.7, 103.4, 99.05, 98.96, 95.77, 87.2, 84.4, 84.3, 74.5, 74.2, 74.1, 73.1, 72.7, 71.9, 71.8, 71.7, 71.3, 71.1, 69.3, 69.1, 68.2, 67.4, 66.0, 65.9, 61.4; δ_P (109 MHz, D_2O) −10.43 (d, *J* 20.8), −12.44 (d, *J* 20.8); *m/z* (ESI) 864.1579 (M^+ + Na. $C_{25}H_{41}N_5O_{23}P_2Na$ requires 864.1565).

Bis(ammonium) guanosine 5′-{6-*O*-(2-hydroxyethyl β-D-galacto-pyranosid-6-yloxyethyl)-β-L-galactopyranosyl diphosphate} (2). Compound **20** (57.7 mg, 48.2 μmol) was treated with CH_2Cl_2–TFA (1 : 2 v/v, 1.0 cm^3) as mentioned for **9**. Purification by silica gel column chromatography (hexane–EtOAc 1 : 1 → 2 : 3) yielded hemiacetal (50.8 mg).

A solution of the hemiacetal (50.8 mg, 46.3 μmol) in CH_2Cl_2 (2.0 cm^3) was treated with CCl_3CN (90 μL, 0.90 mmol) and Cs_2CO_3 (31.5 mg, 96.7 μmol) as mentioned for **21**. Purification by silica gel column chromatography (hexane–EtOAc 3 : 2 → 5 : 4) yielded trichloroacetimidate **25** (41.8 mg, 73% in two steps) as an amorphous solid.; R_f 0.48 (hexane–EtOAc 1 : 1); δ_H (270 MHz, $CDCl_3$) 8.62 (1 H, s), 8.09–7.20 (30 H, m), 6.85 (1 H, d, *J* 3.6), 6.04–6.00 (2 H, m), 5.89 (1 H, dd, *J* 3.6 and 10.3), 5.82 (1 H, d, *J* 3.0), 5.74 (1 H, dd, *J* 7.9 and 10.6), 5.51 (1 H, dd, *J* 3.0 and 10.6), 4.79 (1 H, d, *J* 7.9), 4.64 (1 H, m), 4.18–3.43 (13 H, m), 1.70 (3 H, s).

A solution of **25** (40.7 mg, 32.8 μmol) in CH_2Cl_2 (1.5 cm^3) was treated with a solution of recrystallized dibenzyl phosphate (11 mg, 54 μmol) in CH_2Cl_2 (0.1 cm^3) as mentioned for **22**. Purification by silica gel column chromatography (toluene–EtOAc 2 : 1 → 3 : 2) yielded β-phosphate **26** (34.2 mg).; R_f 0.47 (toluene–EtOAc 1 : 1); δ_H (400 MHz, $CDCl_3$, COSY) 8.10–7.01 (40 H, m, Ar), 5.94 (1 H, dd, $J_{4L,3L}$ 3.4, $J_{4L,5L}$ 0.8, H-4L), 5.89 (1 H, dd, $J_{2L,3L}$ 9.4, $J_{2L,1L}$ 7.9, H-2L), 5.86 (1 H, dd, $J_{4D,3D}$ 3.5, $J_{4D,5D}$ 0.8, H-4D),

†† ^{1}H NMR spectra were recorded at 40 °C to avoid the overlap of HDO. The δ values of H-8 peaks recorded at 25 °C (**1**: 8.079 ppm, **2**: 8.081 ppm) were used as internal standards.

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5.74 (1 H, dd, $J_{2D,3D}$ 10.4, $J_{2D,1D}$ 8.1, H-2D), 5.72 (1 H, t, $J_{1L,P}$ 7.9, H-1L), 5.60 (1 H, dd, H-3L), 5.55 (1 H, dd, H-3D), 5.13, 5.10, 4.88 (1 H × 3, each dd, $J_{CH2,P}$ 7.2, J 11.9, PhCH_2), 4.82 (1 H, d, H-1D), 4.78 (1 H, dd, $J_{CH2,P}$ 7.2, J 11.9, PhCH_2), 4.25 (1 H, br t, J 6.3, H-5L), 4.12–4.08 (3 H, m, OCH_2CH_2OAc), 4.05 (1 H, br t, J 6.3, H-5D), 3.85–3.80 (1 H, m, OCH_2CH$_2$OAc), 3.64–3.37 (8 H, m, H-6aL, 6bL, 6aD, 6bD, OCH$_2$CH$_2$O), 1.70 (3 H, s, Ac); δ_P (109 MHz, $CDCl_3$) − 2.24.

A solution of **26** (17.0 mg, 13 μmol) in MeOH–EtOAc (10 : 1 v/v, 2.0 cm^3) was hydrogenated in the presence of Et_3N (2.0 μL, 14 μmol) and Pd/C (10%, 10 mg). Then, deacylation using pyridine–NH_4OH (4 : 1 v/v, 2.0 cm^3) and subsequent workup was carried out as mentioned for the synthesis of **23** yielded deprotected phosphate **27** (6.6 mg, 59% in three steps). ^{1}H and ^{31}P NMR spectra suggested that **27** was obtained as a mixture of tri-*n*-butylammonium salt and pyridinium salt. Compound **27** was used without further purification to avoid decomposition during purification; R_f 0.17 (^{i}PrOH–water–NH_4OH 7 : 3 : 1); δ_H (270 MHz, D_2O) 4.86 (1 H, t, J 7.7), 4.42 (1 H, d, J 7.6), 4.00–3.50 (20 H, m), 3.15–3.09 (6 H, m), 1.66–1.63 (6 H, m), 1.37–1.32 (6 H, m), 0.95–0.89 (9 H, m); δ_P (109 MHz, D_2O) − 0.68.

Condensation of **24**, derived from GMP (4.0 mg, 11 μmol), and **27** (6.6 mg, 9.5 μmol) was carried out as mentioned for the synthesis of **1**. The same purification procedures for **1** yielded **2** (1.7 mg, 21%).; R_f 0.23 (^{i}PrOH–water–NH_4OH 7 : 3 : 1); δ_H (400 MHz, D_2O, 40 °C, COSY)†† 8.08 (1 H, s, H-8), 5.91 (1 H, d, $J_{1',2'}$ 6.3, H-1′), 4.95 (1 H, t, $J_{1L,P} = J_{1L,2L}$ 7.8, H-1L), 4.79 (1 H, br t, J 7.8, H-2′), 4.53 (1 H, dd, $J_{3',2'}$ 5.2, $J_{3',4'}$ 2.9, H-3′), 4.41 (1 H, d, $J_{1D,2D}$ 7.9, H-1D), 4.33 (1 H, m, H-4′), 4.20 (2 H, m, H-5′a, 5′b), 3.98 (1 H, m, OCH_2CH$_2$OH), 3.92–3.90 (2 H, m, H-4L, 4D), 3.85–3.64 (15 H, m, H-3L, 5L, 6aL, 6bL, 3D, 5D, 6aD, 6bD, OCH_2CH_2O, OCH_2CH_2OH), 3.60 (1 H, dd, $J_{2D,3D}$ 10.1, H-2L), 3.53 (1 H, dd, $J_{2L,3L}$ 9.9, H-2D); δ_P (109 MHz, D_2O) −10.48 (d, J 20.8), −12.37 (d, J 20.8); m/z (ESI) 878.1784 (M^+ + Na. $C_{26}H_{43}N_5O_{23}P_2Na$ requires 878.1722).

HPLC analysis of fucosyltransferase reaction

HPLC was performed by a reverse phase column (Inertsil ODS-3; i.d. 4.6 mm × 250 mm). The column effluent was monitored by fluorescence detector with excitation at 320 nm and emission at 400 nm. The column was eluted isocratically with 0.1 M NH_4OAc–MeCN (97 : 3) at the flow rate of 1.0 cm^3 min^{-1}. Retention time was 5.4 min for PA-LacNAc and 4.9 min for the enzyme reaction product.

Fucosyltransferase assay

A solution of 100 mM cacodylate buffer (pH 6.02) containing 0.1 mM GDP-Fuc, 10 mM PA-LacNAc, 10 mM $MnCl_2$, 0.16 mU FucT V and appropriate amount of inhibitor was adjusted to 10 mm^3. The solution was incubated for 10 min at 37 °C, and the reaction was stopped by heating for 1 min in boiling water. The cooled solution was diluted with 50 mm^3 of water, and an aliquot (20 mm^3) was subjected to the HPLC analysis.

FucT VI assay was carried out under the same conditions mentioned for FucT V but using 0.08 mU of FucT VI instead, and the reaction time was 30 min.

Analysis of fucosyltransferase VI reaction products

A solution of 100 mM cacodylate buffer (pH 6.02) containing 10 mM PA-LacNAc, 10 mM $MnCl_2$, 1.4 mU FucT VI, 0.3 U calf intestine alkaline phosphatase and 10 mM of GDP-Fuc, or **1**, or **2** was adjusted to 20 mm^3. The solution was incubated for 24 h at 25 °C. An aliquot (1 mm^3) of the reaction solution was diluted with 19 mm^3 of water, and analyzed *via* LC-MS. HPLC was performed by a reverse phase column (TSK-GEL ODS-100V; i.d. 2.0 mm × 150 mm). The column effluent was monitored by UV–vis detector at 320 nm, and ESI-MS detector. The column was eluted isocratically with MeOH–0.1% formic acid (6 : 94) at the flow rate of 0.18 cm^3 min^{-1}; For GDP-Fuc: m/z (ESI) 650.5 (M^+ + H, 100), 694.5 (M^- + HCO_2, 100), 648.5 (M^- − H, 21).; For **1**: m/z (ESI) 902.6 (M^+ + H, 100), 900.7 (M^- − H, 100), 946.6 (M^- + HCO_2, 16).; For **2**: m/z (ESI) 916.7 (M^+ + H, 100), 914.8 (M^- −H, 100), 960.6 (M^- + HCO_2, 18).

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