Rational Design, Synthesis, and Characterization of Novel Inhibitors for Human β1,4-Galactosyltransferase[||]

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An affinity labeling reagent, uridine 5′-(6-amino-{2-[(7-bromomethyl-2-naphthyl)methoxycarbonylmethoxy]ethoxy}acetyl-6-deoxy-α-D-galactopyranosyl) diphosphate (**1a**), was designed on the basis of 3D docking simulation and synthesized to investigate the functional role of Trp310 residue located in the small loop near the active site of human recombinant galactosyltransferase (βGalT-1). Mass spectrometric analysis revealed that the Trp310 residue of βGalT1 can be selectively modified with the naphthylmethyl group of compound **1a** at the C-3 position of the indole ring. This result motivated us to synthesize novel uridine-5′-diphosphogalactose (UDP-Gal) analogues as candidates for mechanism-based inhibitors for βGalT-1. We found that uridine 5′-(6-O-[10-(2-naphthyl)-3,6,9-trioxadecanyl]-α-D-galactopyranosyl) diphosphate (**2**) is the strongest inhibitor (K_i = 1.86 μM) against UDP-Gal (K_m = 4.91 μM) among compounds reported previously. A cold spray ionization time-of-flight mass spectrometry study demonstrated that the complex of this inhibitor and βGalT-1 cannot interact with an acceptor substrate in the presence of Mn^{2+}.

Introduction

Glycosylation reactions conducted by glycosyltransferases is one of the crucial biological processes for the post-translational modifications of protein(s) and lipid(s) functions that greatly influence various molecular recognition processes including bacterial and viral infections, cell adhesion in inflammation, immune response, cellular differentiation, development, regulation, and many other intercellular communication and signal transductions.[1] Selective inhibitors of glycosyltransferases are therefore of interest because they may lead to the development of novel and potential therapeutic reagents.[2−9] However, it seems that the difficulty of the development of practical glycosyltransferase inhibitors might be due to the complex and dynamic reaction mechanism that involves many components such as a sugar nucleotide as a donor substrate, an acceptor sugar substrate, and a metal ion.

Galactosyltransferases (Gal-T), the most important and ubiquitous superfamily of sugar elongation enzymes in eukaryotes, transfers galactose from uridine-5′-diphosphogalactose (UDP-Gal) to an acceptor substrate. It was known that βGal-T1 undergoes critical conformational changes upon glycosyl donor substrate binding from an open conformation (conformation I) to a closed conformation (conformation II).[10,11] Upon donor substrate binding, tryptophan 314 (Trp314; GenBank accession number X14558) in the small flexible loop plays an important role for the conformational changes in the long flexible loop (residues 345−365) by moving toward the catalytic pocket to interact with the complex of donor substrate and Mn^{2+} ion.[12] This conformational change seems to be an essential event for the subsequent successful binding with an acceptor sugar substrate because the mutation of Trp to Ala drastically reduced both the binding affinity to the sugar nucleotide and catalytic activity of this enzyme.[13] In human βGal-T1 (GenBank accession number X55415), Trp310 located in the consensus sequence (^{309}Gly-Trp-Gly-Gly-Glu-Asp^{314}) is thought to act as a key amino acid for inducing this crucial conformational change (see Supporting Information). It is noteworthy that the movement of Trp314 (Trp310 in human βGal-T1) to the active site of βGal-T1 might be indispensable both for the binding with the donor substrate and for the conformational change in the long flexible loop required for opening the door of the guest room.

Our interest has been focused on the functional role of this Trp310 residue in the dynamic mechanism of the human βGal-T1 activity. We considered that compounds prohibiting the access of the Trp310 existing far from the active site would become a new class of the potent inhibitors for βGal-T1. In the present study, we describe an efficient strategy for the rational design and synthesis of the potent inhibitors of βGal-T1 by utilizing a designated affinity labeling reagent for the Trp310 residue.

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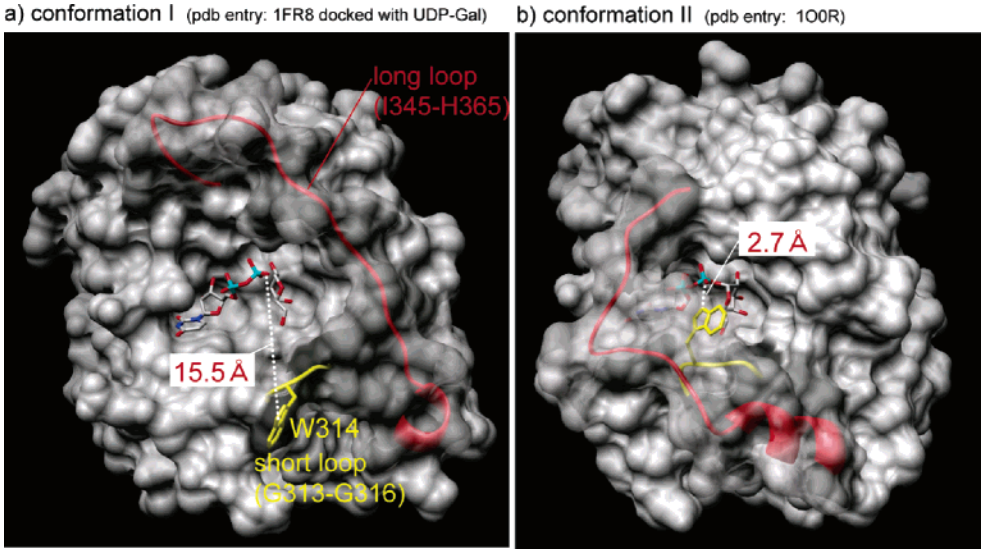


Figure 1. Dynamic conformational change of βGal-T1 along with the binding with UDPGal-Mn^{2+}. Upon a donor substrate binding, Trp314 in the small flexible loop (residues 313−316, yellow) plays a crucial role in the conformational changes of the long flexible loop (residues 345−365, red) by moving toward the catalytic pocket to interact with β-phosphate oxygen of UDP-Gal: (a) open conformation docked with UDP-galactose (conformation I); (b) closed conformation (conformation II). The flipping of Trp314 to the active site of β1,4Gal-T1 is essential both for binding with the donor substrate and for the conformational change in the long flexible loop required for opening the door of the guest room (conformation II). This figure was prepared using UCSF Chimera. UCSF Chimera is a highly extended interactive molecular graphics program. For detail information, see http://www.cgl.ucsf.edu/chimera/.

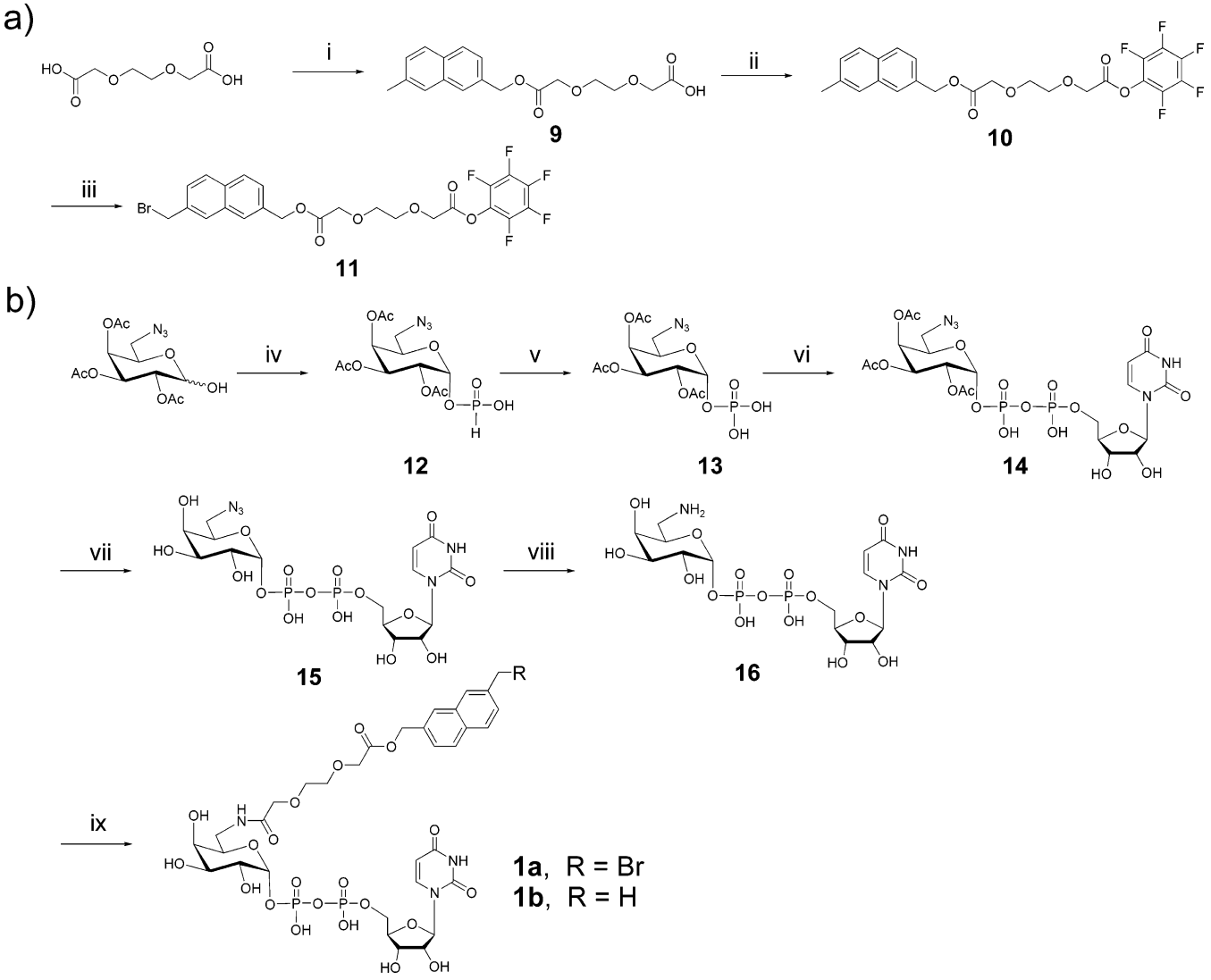
Results and Discussion

A. Affinity Labeling of Trp310 Residue. To estimate the distance between UDP-Gal in the active sites of βGal-T1 and Trp314, UDP-Gal was docked into the binding cavity of conformation I (open conformation, pdb entry: 1FR8). As shown in Figure 1a, it was indicated from X-ray crystallographic data[10] that the distance between Trp314 of the small loop (residues 313−316) and β-phosphate group of the βGal-T1 was found to be 15.5 Å. In addition, that from the 2- or 6-OH group of UDP-Gal was estimated to be 18.8 and 15.7 Å, respectively. In contrast, it was suggested that Trp314 in conformation II (closed conformation, pdb entry: 1O0R) apparently is located near (2.7 Å) the β-phosphate group of the UDP in the binding pocket (Figure 1b).[11] It is likely that the NH (indole ring) group of the Trp314 residue of βGal-T1 may bind with a complex of Mn^{2+}-β-phosphate oxygen atoms of UDP moiety in the donor binding pocket through hydrogen bonding and contribute to hydrophobic interactions with the C5−C6 of galactose of UDP-Gal.[14]

This result prompted us to design and synthesize the site-directed affinity labeling reagent based on the chemical modification at the C-6 position of UDP-Gal as the starting material, since tolerance at the C-6 or C-2 position has been demonstrated in synthetic studies on the donor substrates for βGal-T1.[15−17] As illustrated in Scheme 1, we synthesized the UDP-Gal derivative **1a** having a bromomethyl substituted naphthalene group[18] as an aromatic affinity tag for the indole side chain of the Trp310 residue. The key intermediate **15** derived from a known 2,3,4-tri-*O*-acetyl-6-azide-6-deoxy-galactopyranose[19] was coupled with the active esters **10** and **9** to give the affinity labeling reagent **1a** and **1b** as a chemically stable analogue, respectively. According to the result of the manual docking shown in Figure 1a, the distance between the bromomethyl substituted naphthalene group and the carbon atom at the C-6 position of UDP-Gal was adjusted to be approximately 15 Å by connecting them with 3,6-dioxaoctandioic acid. When a highly reactive bromomethyl naphthalene group of compound **1a** can access the Trp310 in the short loop of human βGal-T1, we expect that nucleophilic attack by a spatially adjacent C-3 position of the indole ring on** this reactive group might afford βGal-T1 covalently modified with the affinity labeling reagent **1a** at Trp310 residue.

As shown in the ESI-TOF mass spectrometry of βGal-T1 in the presence of compound **1a** (Figure 2), advent of the new ion peaks at *m/z* 36 785 and 36 076 clearly suggests that compound **1a** acts as a specific tag for βGal-T. They could be assigned to a product generated by the direct nucleophilic substitution of βGal-T with **1a** (*m/z* 36 785, peak γ) and its deacylated form (*m/z* 36 076, peak β). It was also suggested that the stable analogue **1b** showed a moderate inhibitory effect (K_i = 22.3 μM) on βGal-T activity by a competitive mechanism as indicated in Figure 2d. Further precise analysis of the site labeled by **1a** was carried out by using MALDI-TOF/TOF MS[20] of the tryptic digests of the affinity labeled human recombinant βGal-T1 as shown in Figure 3. An unknown parent ion peak at *m/z* 3273.149 was subjected to the fragmentation experiment to allow for structural characterization of this peptide fragment. It was finally revealed, by using a series of b- and y-ions, that a new precursor ion peak at *m/z* 3273.149 was the ion peak due to the sequence ^{295}QQFLTINGFPNNY-WG*W*GGEDDDIFNR^{320} including the small loop region in which the Trp310 residue was selectively modified with the naphthylmethyl group at the C-3 position of the indole ring (two possible isomers were indicated in Figure 3b). Although careful elucidation of the detectable ion peaks in spectrum 2 of Figure 3a has been carried out by TOF/TOF analysis, no significant candi-

Scheme 1[a]



[a] Reagents and conditions: (i) (1) Cs_2CO_3/MeOH−H_2O, room temp; (2) 2-bromomethyl-7-methylnaphthalene/DMF, 50 °C, 46%; (ii) pentafluorophenyl trifluoroacetate, pyridine/DMF, room temp, 53%; (iii) NBS, AIBN/CCl_4, 80 °C, 27%; (iv) 2-chloro-4*H*-benzodioxaphosphorin 4-one, Et_3N/THF-dioxane, 0 °C; (2) H_3PO_3/CH_3CN, room temp, 47% vol; (1) Dowex 50W-X8 H^+/THF, room temp; (2) *t*BuOOH, I_2/THF, room temp, 65%; (vi) UMP-morpholidate, 1*H*-tetrazole/pyridine, room temp, 62%; (vii) NaOMe/MeOH, −10 °C, 70%; (viii) 10% Pd−C, H_2/H_2O, room temp; (ix) **14** (for **1b**) or **15** (for **1a**), Et_3N/H_2O−dioxane, room temp.

date bearing the naphthylmethyl group except the above-mentioned peptide was obtained, indicating that the Trp310 is a specific amino acid reacting with this affinity labeling reagent **1a**. Therefore, we considered that the aromatic stacking between naphthalene moieties of **1a**,**b** and the indole ring of the Trp310 may be crucial and feasible for designing a new class of potent inhibitors of βGal-T1.

B. Inhibitor Design Based on Affinity Labeling of Trp310. The results of the affinity labeling of Trp310 by **1a** and the potent inhibitory effect by **1b** motivated us to design much more effective inhibitors than **1b** on the basis of the modification study of UDP-Gal. Considering the structural features of compound **1b**, it seemed that an amide bond and an ester bond may reduce the flexibility of the linker moiety that influences the affinity with Trp310 in the flexible small loop. Therefore, we decided to synthesize compounds **2** and **3** bearing a naphthalene group linked by a flexible trioxadecanyl moiety, in which UDP-Gal and naphthalene were combined by ether bonds. Scheme 2 indicates the synthetic route of compound **2** (schemes and experimental details of the synthesis of compounds **3**−**7** are described in the Supporting Information). Thiophenylglycoside **21** was obtained by a key substitution reaction of **18** with an alcohol **20** in 82% yield. Conversion of the thiophenyl group of triol **22** into the phosphate group (five steps) at the amomeric position followed by coupling with UMP-morpholidate afforded compound **2** as an amorphous powder. The K_i values of UDP-Gal derivatives investigated in this paper are listed in Figure 4. It was revealed that compound **2** exhibited the highest inhibitory activity against this enzyme among compounds used in this study. To the best of our knowledge, it was demonstrated that **2** is the strongest competitive inhibitor ($K_i = 1.86\ \mu M$) against UDP-Gal ($K_m = 4.91\ \mu M$) among inhibitors reported (Figure 4a).[4,7] Analogues **4**, **5**, and **7** lost (or reduced) the inhibitory effect on human recombinant βGal-T1, indicating that both the flexible trioxadecanyl [$(CH_2CH_2O)_3$] linker and aromatic (naphthyl) terminal groups as well as two ether bonds are indispensable in achieving the satisfac-

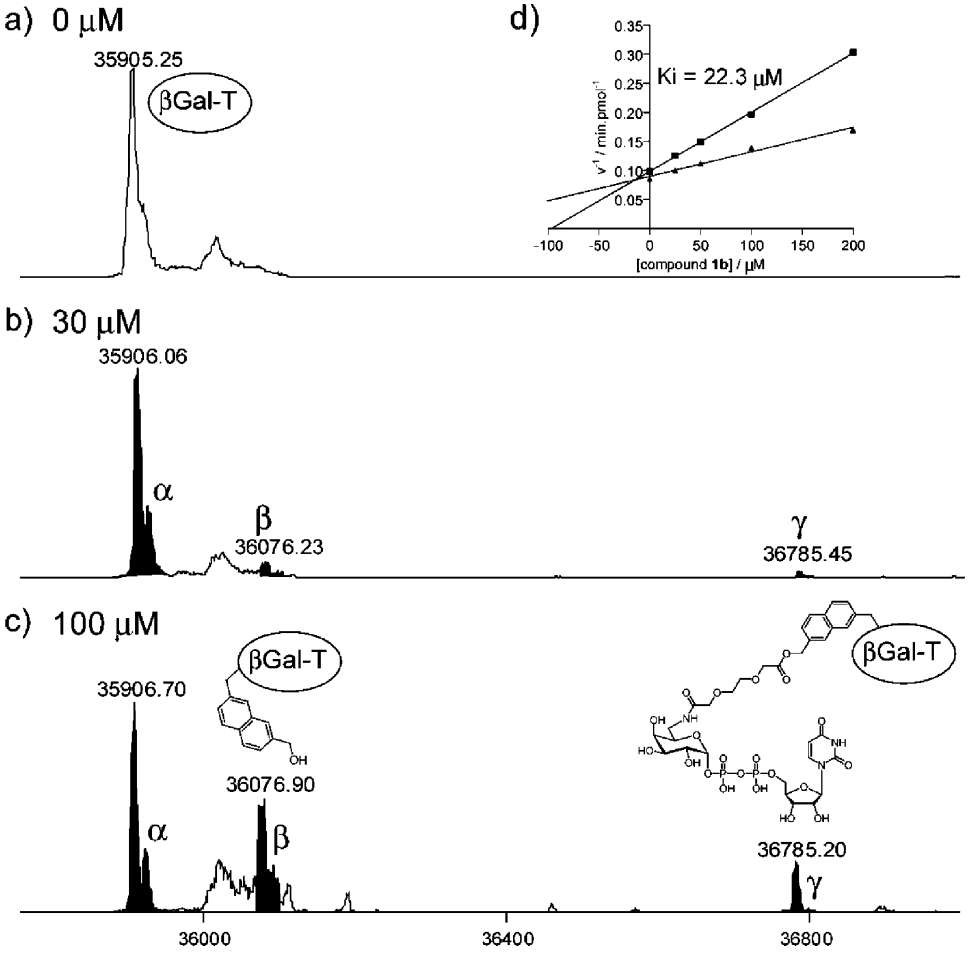


Figure 2. Irreversible binding of **1a** on β-Gal-T1: (a) ESI mass spectrum of β-Gal-T1 in the negative control experiment; (b) in the presence of 30 μM compound **1a**; (c) in the presence of 100 μM compound **1a**; (d) inhibitory effect of compound **1b**. Conditions are the following: LC/MS analysis was conducted in using 2% acetic acid; 100 mM ammonium carbonate (pH 6.8) was used as washing buffer; linear gradient mode was 2%; acetic acid and acetonitrile were the eluents.

tory inhibition. Although compound **3** showed a little improved inhibition in comparison with compound **1b**, modification at the C-6 position seemed to be much more effective than that at the C-2 position of Gal residue. Since we have not synthesized a suicide substrate analogue of compound **3**, the mechanism of the inhibition by **3** is not clear.

Our hypothesis explaining the inhibitory effect observed in the present study is that the synthetic analogues of UDP-Gal, having an aromatic (hydrophobic) group through a flexible linker moiety (**1**−**4**), may disturb the access of the NH (indole ring) group of the Trp310 residue of the human β-Gal-T1 to a complex of Mn^{2+}-β-phosphate oxygen atoms of their own UDP moiety in the donor binding pocket. As a result, it seems that an acceptor substrate cannot bind efficiently with the complex of β-Gal-T1-UDP-Gal analogues because access of Trp310 is thought to be an essential step to induce the change from conformation I to conformation II required for the successful binding of an acceptor substrate.[12,21] If this hypothesis is correct, the complex of β-Gal-T1-**2** does not show binding capacity with an acceptor substrate. A snapshot of the results from electrospray ionization mass spectrometry under nondestructive conditions (CSI-TOF MS)[22] revealed that the complex of human recombinant β-Gal-T1 with **2** and Mn^{2+} ($[M + 12H]^{12+}$, *m/z* 3066.6) exhibited no binding capacity toward an acceptor substrate **8**, while a signal due to the complex of β-Gal-T1 with UDP-Gal and Mn^{2+} (*m/z* 3044.1) shifted to the peak at *m/z* 3030.1 because of the complex of β-Gal-T-UDP-Mn^{2+} and to the peak at *m/z* 3073.1 corresponding to the molecular mass of the complex for β-Gal-T-UDP-Mn^{2+} acceptor substrate **8** under the same conditions (Figure 5). These results are still preliminary but at least justified our hypothesis that these compounds might inhibit the binding of the acceptor substrate with β-Gal-T1 by perturbing a key functional role of the Trp310 in the small loop.

Conclusion

Novel inhibitors for β-Gal-T1 were designed and synthesized on the basis of the affinity labeling of the Trp310 located in the small flexible loop near the active site. Among UDP-Gal analogues studied herein, compound **2** showed the highest inhibitory effect ($K_i = 1.86$ μM). In general, highly conserved Trp residues (G*W*GGE and similar sequences) seem to play an essential role in the enzymatic reactions of many glycosyltransferases, namely, β1,4-galactosyltransferase,[23] β1,3-galactosyltransferase,[24] α1,4-*N*-acetyl-D-hexosaminyltransferase,[25] clostridial cytotoxin glycosyltransferases,[26] and polypeptide *N*-acetyl-D-galactosaminyltransferase.[27] As for other glycosyltransferases, individual 3D structural information of the flexible loop near the active site will become

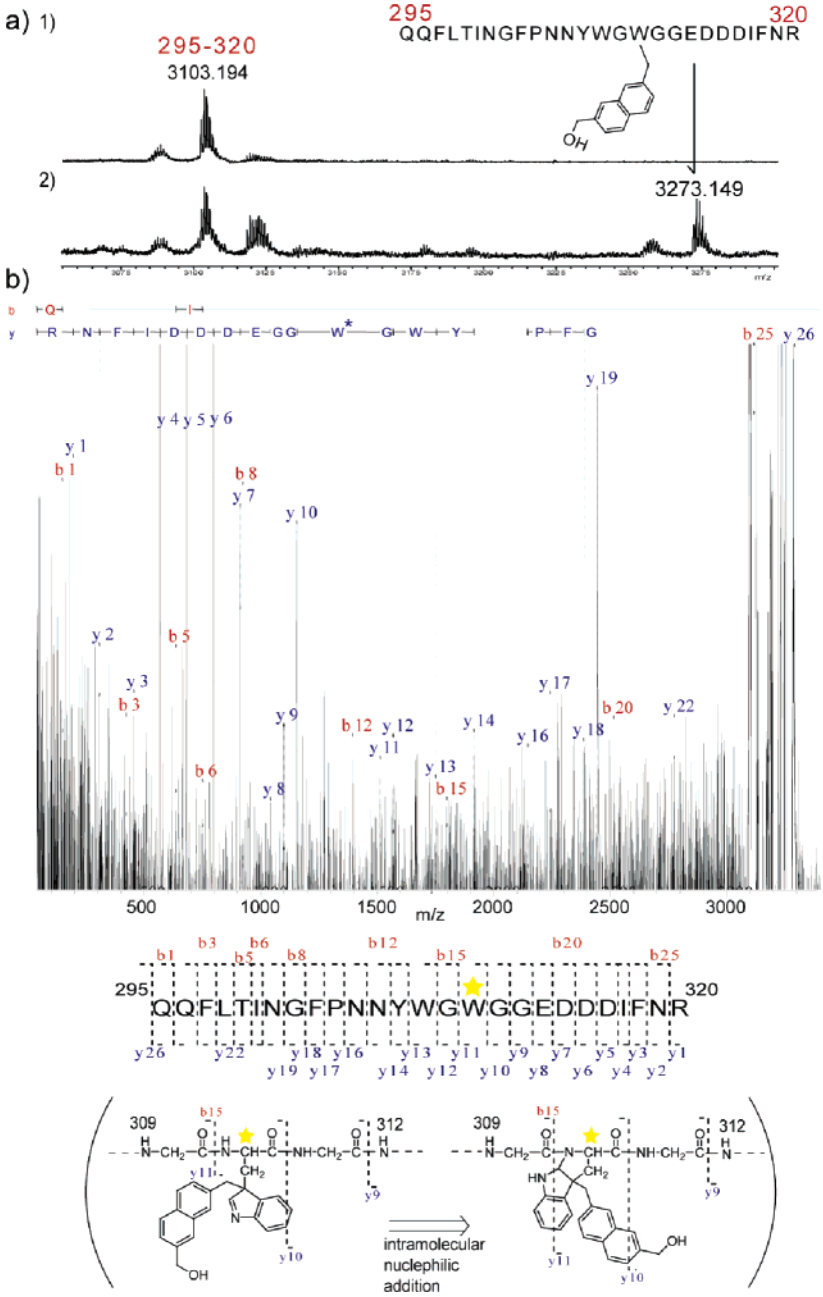


Figure 3. Affinity labeling and proteomics analysis of Trp310 by using a suicide substrate **1a**. A precursor ion peak at *m/z* 3273.149 corresponding to the mass of the peptide fragment Q295-R320 was further employed for precise fragmentation analysis based on a MALDI-TOF/TOF MS method according to the procedure described in our previous report.[18] It was demonstrated that Trp310 can be modified with compound **1a** through a nucleophilic substitution at C-3 position of this indole ring. There is the possibility of a nucleophilic addition reaction by main chain amide nitrogen after the first substitution reaction at C-3 indole, though it is difficult to distinguish these isomers.

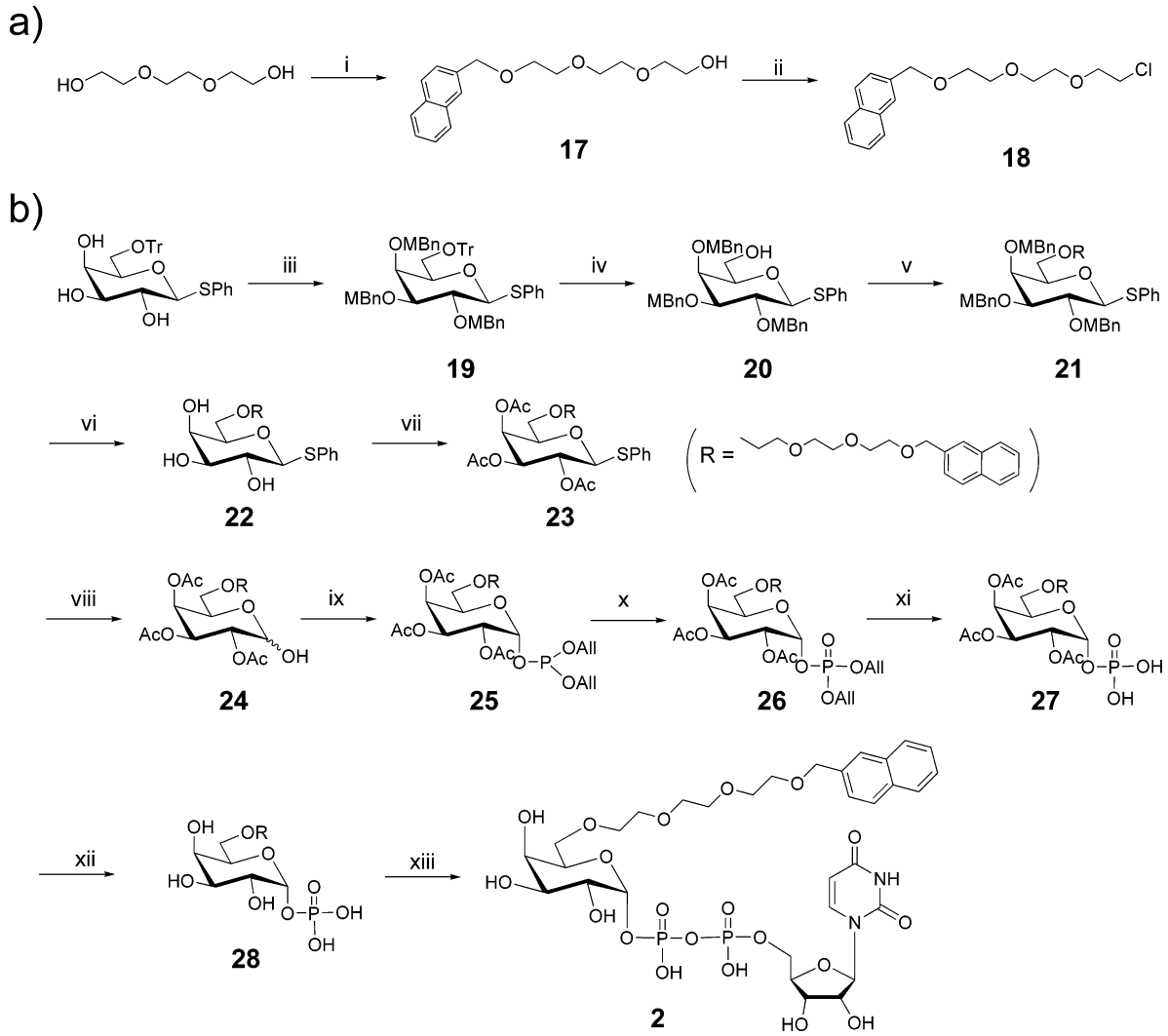
key to the rational design of the dynamic inhibitors. We are currently investigating crystallization of the complex of the human recombinant βGal-T1 with compound **2**, and the results of the structural characterization will be reported as soon as possible.

Experimental Section

Chemicals. Solvents were dried with 4 Å molecular sieves before use. Silica gel column chromatography was performed with Kanto chemical 60N (100−210 μm diameter) and Iatrobead column from Iatron Laboratories, 6RS-8060. All chemical reagents were purchased from Wako Pure Chemical Co. Ltd. Recombinant human β1,4-galactosyltransferase-1 was purchased from Toyobo Co. Ltd. UDP and UDP-galactose were obtained from Yamasa Corporation. MALDI matrixes [2,5-dehydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnaminic acid (CHCA), sinapinic acid (SA)] and MALDI peptide calibration standard mixture containing angiotensin II, bombesin, ACTH (18-39), and somatostatin were purchased from Bruker Daltonics GmbsH.

Structural Characterization. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE 600 and 500 MHz (150 and 125 MHz for ^{13}C, respectively). TMS for organic solvents and TMSP for D_2O were used as the internal standards. Cold spray ionization mass spectrometry (CSI-TOF MS) analyses were performed by using a JMS-T100CS spectrometer (AccuTOF CS, JEOL Co. Ltd., Japan) equipped with an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. MALDI-TOF mass spectra were measured by using an Ultraflex TOF/TOF mass spectrometer equipped with a reflector and controlled by the Flexcontrol 1.2 software package (Brucker

Scheme 2[a]



[a] Reagents and conditions: (i) 2-bromomethylnaphthalene, NaH/DMF, 0 to room temp, 95%; (ii) $SOCl_2$, pyridine/DMF, room temp, 85%; (iii) *p*-methoxybenzyl chloride, NaH/DMF, 0 °C to room temp, 95%; (iv) 1 N HCl/MeOH, CH_2Cl_2, room temp, 92% vol, **18**, NaH/DMF, 0 °C to room temp, 82%; (vi) PhSH, $SnCl_2/CH_2Cl_2$, −78 °C, 61%; (vii) Ac_2O/pyridine, room temp, 96%; (viii) NBS/acetone−H_2O, room temp, 77%; (ix) *N*,*N*-diallyl-diisopropyl phosphoramidite, 1*H*-tetrazole/THF, −40 °C, 49%; (x) *t*BuOOH/THF, −10 °C, 98%; (xi) tetrakis(triphenylphosphine)palladium, Et_2NH/THF, room temp, 91%; (xii) Et_2NH/MeOH, room temp, 88%; (xiii) UMP-morpholidate, 1*H*-tetrazole/pyridine, room temp, 41%.

Daltonics GmbsH, Bremen, Germany). Optical rotation was measured by a Perkin-Elmer polarimeter, model 343. High- and low-resolution mass spectrometry and elemental analysis were carried out at the Center for Instrumental Analysis, Hokkaido University. A high-performance liquid chromatography (HPLC) system was used and was equipped with the following: HITACHI L-7100 pump, HITACHI ODS reverse-phase column (46 mm × 250 mm), and HITACHI F-1050 fluorescence detector.

{2-[(7-Methyl-2-naphthyl)methoxycarbonylmethoxy]-ethoxyacetic Acid (9). To a solution of 3,6-dioxaoctandioic acid (500 mg, 2.81 mmol) in a mixed solvent of MeOH (10 mL) and H_2O (10 mL) was added Cs_2CO_3 (914 mg, 2.81 mmol) at room temperature. After being stirred for 20 min at room temperature, the reaction mixture was concentrated in vacuo, the obtained residue was redissolved in a mixed solvent of DMF (20 mL) and H_2O (5 mL), and 2-bromomethyl-7-methylnaphthalene (110 mg, 468 μmol) was added at room temperature. After being stirred for 24 h at 70 °C, the reaction mixture was concentrated and the residue obtained was purified by a Wakogel 50C18 reverse0phase column (water as a eluent) to give compound **9** (82 mg, 46% calculated from 2-bromomethyl-7-methylnaphthalene). ^{1}H NMR (600 MHz, CD_3OD, 27 °C) δ 7.79 (d, 1H, aromatic), 7.72 (m, 2H, aromatic), 7.59 (s, 1H, aromatic), 7.38 (d, 1H, aromatic), 7.31 (d, 1H, aromatic), 5.32 (s, 2H, OCH_2naph), 4.24 (s, 2H, OCH_2C(C=O)Onaphthyl), 3.90 (s, 2H, OCH_2C(=O)OH), 3.63−3.70 (m, 4H, OCH_2CH_2O), 2.47 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3-OD, 27 °C) δ 176.14, 170.99, 135, 92, 133.51, 133.12, 131.54, 128.25, 127.74, 127.20, 126.55, 126.53, 124.66, 70.49, 70.31, 69, 38, 67, 91, 66.50, 20.35; HRMS calcd for $[M + H]^+$ $C_{18}H_{21}O_6$ 333.1338, found 333.1338.

{2-[(7-Methyl-2-naphthyl)methoxycarbonylmethoxy]-ethoxy}acetic Acid Pentafluorophenyl Ester (10). To a solution of **9** (216 mg, 650 μmol) in DMF (10 mL) was added pyridine (155 μL, 1.9 mmol) and pentafluorophenyl trifluoroacetate (218 μL, 1.27 mmol) at room temperature.[28] After being stirred overnight at room temperature, the reaction mixture was concentrated and the obtained residue was purified by silica gel column chromatography (hexane/EtOAc = 2:1) to give **10** (170 mg, 53%). ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.77 (d, 1H, aromatic), 7.71 (m, 2H, aromatic), 7.57 (s, 1H, aromatic), 7.36 (d, 1H, aromatic), 7.32 (d, 1H, aromatic), 5.34 (s, 2H, OCH_2naph), 4.52 (s, 2H, OCH_2C(=O)Opfp), 4.24 (s, 2H, OCH_2C(C=O)O-naphthyl), 3.81−3.86 (m, 4H, OCH_2CH_2O), 2.50 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 170.62, 166.64, 136.22, 133.42, 132.60, 131.52, 128.79, 128.22, 127.51,

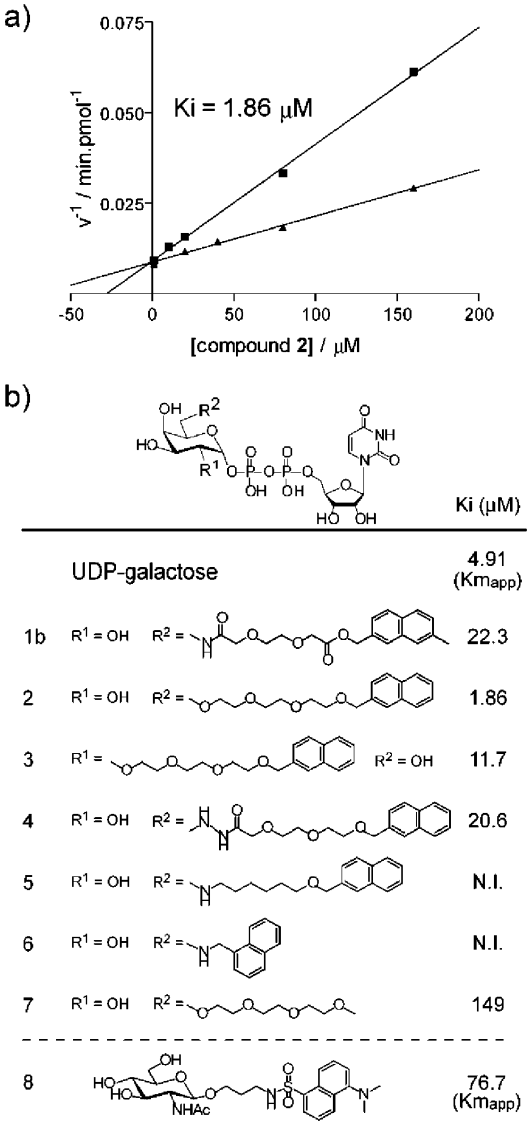


Figure 4. Synthesized inhibitors and their inhibitory activity against βGal-T1. (a) Shown is the effect of compound **2** on βGal-T1 activity in the presence of donor substrate (UDP-Gal) and compound **8** as an acceptor substrate. Conditions are the following: 40 μM (square) and 100 μM (triangle) UDPGal, 0−160 μM compound **8**, 150 μU βGal-T1. (b) Shown are synthetic inhibitors used in this study and their kinetics constants.

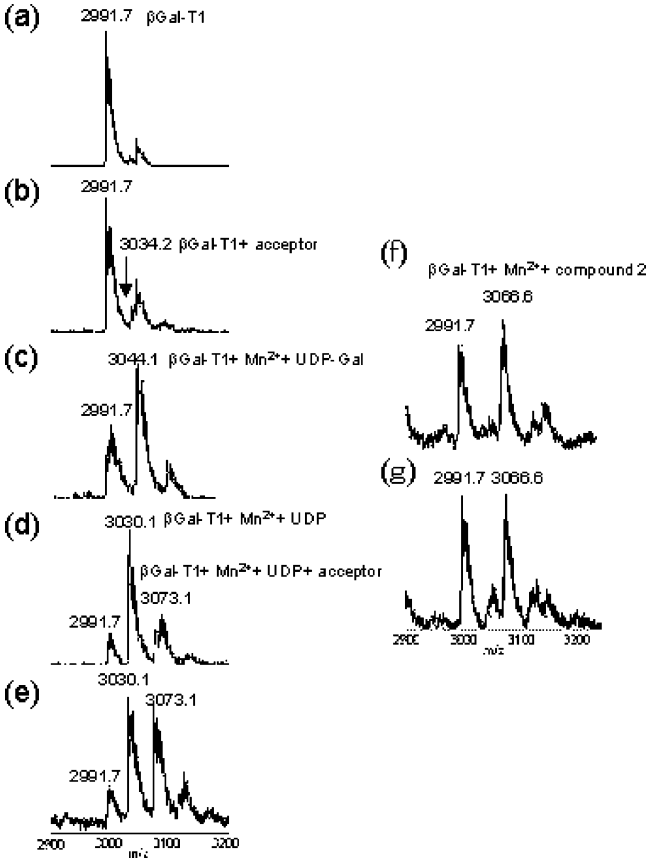


Figure 5. Evidence of competitive inhibition by compound **2** in the dynamic conformational change of βGal-T1 by means of CSI-TOF MS at 37 °C. (a) The ion peak at *m/z* 2991.7 shows $[M + 12H]^{12+}$ of βGal-T1 (95 pmol). (b) Spectrum of βGal-T1 (95 pmol) in the presence of an acceptor **8** (450 pmol). Only a small peak corresponding to the complex was detected at *m/z* 3034.2 in addition to the original signal. (c) Spectrum of βGal-T1 (95 pmol) in the presence of UDP-Gal (400 pmol). A significant signal corresponding to the GalT-Mn^{2+}-UDP-Gal complex was observed at *m/z* 3044.1. (d) A typical spectrum of βGal-T1 (95 pmol) obtained in the presence of both UDP-Gal (400 pmol) and an acceptor substrate **8** (450 pmol). The ion peaks due to βGal-T1-Mn^{2+}-UDP and βGal-T1-Mn^{2+}-UDP-acceptor **8** were observed at *m/z* 3030.1 and *m/z* 3073.1, respectively. (e) A spectrum of βGal-T1 (95 pmol) in the presence of excess amount of acceptor substrate. Compound **8** (450 pmol) was added to the complexes formed in an experiment (d). The signal intensity due to the complex of βGal-T1-Mn^{2+}-UDP-acceptor **8** was drastically enhanced by addition of **8**, indicating that the door for the guest room has been opened by binding with UDP-Gal and the inhibitory effect by UDP was conducted by forming a complex with acceptors using this cavity in conformation II. (f) The spectrum of βGal-T1 (95 pmol) in the presence of compound **2** (400 pmol). A significant signal corresponding to the complex of βGal-T1-Mn^{2+}-**2** was observed at *m/z* 3066.6. (g) The effect of the addition of an acceptor **8** (450 pmol) to the complex of βGal-T1-Mn^{2+}-**2**.

127.20, 126.95, 125.06, 71.29, 71.14, 68.77, 67.84, 67.19, 21.67; HRMS calcd for $[M + H]^+$ $C_{24}H_{20}F_5O_6$ 499.1180, found 498.1198.

{2-[(7-Bromomethyl-2-naphthyl)methoxycarbonyl-methoxy]ethoxy}acetic Acid Pentafluorophenyl Ester (11). To a solution of **10** (10 mg, 20 μmol) in CCl_4 (3 mL) was added *N*-bromosuccinimide (4.3 mg, 24 μmol) and AIBN (330 μg, 2 μmol) at room temperature. After being stirred for 6 h at 80 °C in an argon atmosphere, the reaction mixture was concentrated and the residue obtained was purified by silica gel column chromatography (hexane/EtOAc = 6:1 to 4:1) to give the compound **11** (3 mg, 27%). ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.82−7.84 (m, 4H, aromatic), 7.47−7.54 (m, 2H, aromatic), 5.36 (s, 2H, OCH_2naph), 4.67 (s, 2H, CH_2Br), 4.54 (s, 2H, $OCH_2C(=O)Opfp$), 4.25 (s, 2H, $OCH_2C(C=O)O$-naphthyl), 3.83−3.89 (m, 4H, OCH_2CH_2O); HRMS calcd for $[M + H]^+$ $C_{24}H_{19}BrF_5O_6$ 577.0285, found 577.0287.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl Phosphite Mono-Et_3N Salt (12). To a solution of known 2,3,4-tri-*O*-acetyl-6-azido-6-deoxygalactose (3.0 g, 7.9 mmol) dissolved in a mixed solvent of THF (10 mL) and dioxane (20 mL) was added Et_3N (1.95 mL, 14 mmol) and 2-chloro-4*H*-benzodioxaphosphorin-4-one (1.47 g, 7.3 mmol dissolved in 6 mL of THF) at 0 °C.[29] After being stirred for 20 min at 0 °C, to the reaction mixture was added H_2O (5 mL) and then the mixture was concentrated. The concentrate was redissolved in THF, and the precipitant formed was separated by filtration. The filtrate was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH = 25:1 to 10:1) to give an anomeric mixture of compound **12** (α/β = 9:1) as judged by NMR. To the anomeric mixture compound redissolved in CH_3CN was added H_3PO_3 (3.6 g, 44 mmol), and the mixture was stirred overnight at room temperature in an argon atmosphere.[30] The reaction mixture was neutralized by Et_3N (6.4

mL, 46 mmol) and concentrated in vacuo. Purification of the concentrate was conducted by silica gel column chromatography ($CHCl_3$/MeOH = 25:1 to 10:1) to give the pure α anomer compound **12** (1.4 g, 66%) as the mono-Et_3N salt. ^{1}H NMR (500 MHz, CD_3OD, 27 °C) δ 5.86 (dd, 1 H, $J_{1,p}$ = 8.9 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.42−5.45 (m, 2 H, H-3, H-4), 5.21 (m, 1 H, H-2), 4.42 (t, 1 H, H-5), 3.44 (dd, 1 H, $J_{5,6b}$ = 7.6 Hz, $J_{6a,6b}$ = 12.7 Hz, H-6b), 3.20 (dd, 1 H, $J_{5,6a}$ = 5.2 Hz, H-6a), 3.09 (m, 6 H, $(CH_3CH_2)_3N$), 2.16 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 1.98 (s, 3 H, Ac), 1.35 (m, 9 H, $(CH_3CH_2)_3N$); ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C) δ 170.27, 170.25, 169.94, 91.81, 91.78, 68.79, 68.74, 67.69, 67.65, 67.53, 50.66, 45.58, 20.80, 20.66, 8.53; HRMS calcd for $[M + Et_3N + H]^+$ $C_{18}H_{34}N_4O_{10}P$ 497.2013, found 497.2013.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl Phosphate Mono-Et_3N Salt (13). Compound **12** (1.2 g, 2.4 mmol) dissolved in THF (10 mL) was passed though the cation-exchange resin column (Dowex 50W-X8 H^+ form) with additional THF (30 mL) as eluent. To the solution was added *t*BuOOH (580 μL of 5 M solution in decane) and I_2 (4 mg) at room temperature.[31] After being stirred for 24 h at room temperature in an argon atmosphere, the reaction mixture was neutralized by Et_3N (338 μL, 2.4 mmol) and then concentrated. The concentrate was purified by a Wakogel 50C18 reverse-phase column (water as a eluent) to give the compound **13** (810 mg, 65%). ^{1}H NMR (500 MHz, CD_3OD, 27 °C) δ 5.78 (dd, 1 H, $J_{1,p}$ = 7.5 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.47 (dd, 1 H, $J_{4,5}$ = 1.1 Hz, H-4), 5.42 (dd, 1 H, $J_{3,4}$ = 3.4 Hz, H-3), 5.14 (m, 1 H, $J_{2,3}$ = 10.7 Hz, H-2), 4.45 (t, 1 H, $J_{5,6}$ = 6.9 Hz, H-5), 3.50 (dd, 1 H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.27 (dd, 1 H, $J_{5,6b}$ = 7.3 Hz, H-6b), 3.09 (m, 6 H, $(CH_3CH_2)_3N$), 2.16 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 1.98 (s, 3 H, Ac), 1.33 (m, 9 H, $(CH_3CH_2)_3N$); ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C) δ 170.71, 170.28, 169.91, 92.00, 91.96, 68.67, 67.87, 67.82, 67.65, 67.50, 50.32, 45.43, 20.86, 20.65, 8.46; HRMS calcd for $[M + Et_3N + H]^+$ $C_{18}H_{34}N4O_{11}P$ 513.1962, found 513.1954.

Uridine 5′-(2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl) Diphosphate Monoammonium Salt (14). To a solution of **13** (760 mg, 1.48 mmol) in dry pyridine (5 mL) was added UMP-morpholidate (2.0 g, 2.96 mmol) at room temperature. The solution was concentrated in vacuo to complete dryness (removal of water). To the obtained mixture redissolved in dry pyridine (5 mL) was added 1*H*-tetrazole (455 mg, 5.9 mmol) at room temperature, and the mixture was stirred for 24 h in an argon atmosphere.[32] To the reaction solution was added additional UMP-morpholidate (700 mg, 960 μmol), and the mixture was stirred for 2 days at room temperature. The reaction mixture was concentrated in vacuo, and the obtained residue was subjected to purification by ion exchange column chromatography (DEAE-Sephasell, 50 mM NH_4HCO_3 as the eluent). Further purification was conducted by a Wakogel 50C18 reverse-phase column (water as the eluent) to give the compound **14** (650 mg, 62%) as the monoammonium salt. ^{1}H NMR (600 MHz, D_2O, 27 °C) δ 7.98 (d, 1 H, uracil), 5.96−5.98 (m, 2 H, uracil, H′-1), 5.80 (dd, 1 H, $J_{1'',p}$ = 7.7 Hz, $J_{1'',2''}$ = 3.5 Hz, H″-1), 5.52 (d, 1 H, H″-4), 5.38 (dd, 1 H, $J_{2'',3''}$ = 10.7 Hz, $J_{3'',4''}$ = 3.3 Hz, H″-3), 5.22 (m, 1 H, H″-2), 4.51 (t, 1 H, $J_{5'',6''}$ = 6.4 Hz, H″-5), 4.34−4.38 (m, 2 H, H′-2, H′-3), 4.25−4.28 (m, 2 H, H′-4, H′-5 × 1), 4.19 (m, 1 H, H′-5 × 1), 3.56 (dd, 1 H, $J_{5'',6''b}$ = 6.5 Hz, $J_{6''a,6''b}$ = 12.9 Hz, H″-6b), 3.44 (dd, 1 H, $J_{5'',6''a}$ = 6.4 Hz, H″-6a), 2.22 (s, 3 H, Ac), 2.15 (s, 3 H, Ac), 2.02 (s, 3 H, Ac); ^{13}C NMR (150 MHz, D_2O, 27 °C) δ 176.06, 175.78, 175.47, 168.83, 154.49, 144.46, 105.34, 95.31, 95.27, 91.27, 85.88, 85.82, 76.51, 72.29, 71.66, 70.85, 70.80, 70.45, 70.39, 67.61, 67.57, 52.33, 23.00, 22.76, 22.70; HRMS calcd for $[M + NH_3 + H]^+$ $C_{21}H_{33}N_6O_{19}P_2$ 735.1276, found 735.1280.

Uridine 5′-(6-Azido-6-deoxy-α-D-galactopyranosyl) Diphosphate Monoammonium Salt (15). To a solution of **14** (300 mg, 423 μmol) in MeOH (40 mL) was added NaOMe (114 mg, 2.1 mmol in 6 mL of MeOH) at −10 °C. After being stirred for 24 h at −10 °C, to the reaction mixture was added additional NaOMe (10 mg, 184 μmol in 1 mL of MeOH 1 mL) daily for 4 days. The reaction mixture was neutralized by the addition of cation-exchange resin (Dowex 50W-X8 H^+) and filtered by glass filter. The filtrate was concentrated and purified by ion exchange column chromatography (DEAE-Sephasell, 50 mM NH_4HCO_3 as the eluent). Further purification was conducted by gel permeation chromatography (Sephadex G-10, water as the eluent) to give the compound **15** (179 mg, 70%) as the monoammonium salt. ^{1}H NMR (600 MHz, D_2O, 27 °C) δ 7.95 (d, 1 H, uracil), 5.97−5.98 (m, 2 H, uracil, H′-1), 5.61 (dd, 1 H, $J_{1'',p}$ = 7.1 Hz, $J_{1'',2''}$ = 3.5 Hz, H″-1), 4.35−4.38 (m, 2 H, H′-2, H′-3), 4.18−4.28 (m, 4 H, H′-4, H′-5 × 2, H″-5), 3.99 (d, 1 H, H″-4), 3.91 (dd, 1 H, $J_{3'',4''}$ = 3.4 Hz, H″-3), 3.77 (dt, 1 H, $J_{2'',3''}$ = 10.3 Hz, H″-2), 3.56 (dd, 1 H, $J_{5'',6''b}$ = 7.3 Hz, $J_{6''a,6''b}$ = 12.8 Hz, H″-6b), 3.46 (dd, 1 H, $J_{5'',6''a}$ = 6.1 Hz, H″-6a); ^{13}C NMR (150 MHz, D_2O, 27 °C) δ 168.90, 154.42, 144.37, 105.37, 98.47, 98.43, 91.20, 85.97, 85.91, 76.49, 72.84, 72.34, 71.93, 71.90, 71.03, 70.97, 67.65, 67.61, 62.11, 53.02; HRMS calcd for $[M + NH_3 + H]^+$ $C_{15}H_{27}N_6O_{16}P_2$ 609.0959, found 609.0953.

Uridine 5′-(6-Amino-6-deoxy-α-D-galactopyranosyl) Diphosphate Monoammonium Salt (16). To a solution of **15** (4.5 mg, 7.4 mmol) in H_2O (3 mL) was added 10% Pd−C (10 mg), and the mixture was stirred for 30 min at room temperature in a hydrogen atmosphere. The reaction solution was filtered and the filtrate was concentrated to give compound **16** (4.1 mg, 96%). ^{1}H NMR (600 MHz, D_2O, 27 °C) δ 7.92 (d, 1 H, uracil), 5.95−5.98 (m, 2 H, uracil, H′-1), 5.65 (dd, 1 H, $J_{1'',p}$ = 6.5 Hz, $J_{1'',2''}$ = 3.7 Hz, H″-1), 4.35−4.40 (m, 3 H, H′-2, H′-3, H″-5), 4.21−4.29 (m, 3 H, H′-4, H′-5 × 2), 4.03 (d, 1 H, H″-4), 3.95 (dd, 1 H, $J_{3'',4''}$ = 3.3 Hz, H″-3), 3.84 (dt, 1 H, $J_{2'',3''}$ = 10.3 Hz, H″-2), 3.25 (m, 2 H, H″-6a, H″-6b); ^{13}C NMR (150 MHz, D_2O, 27 °C) δ 168.87, 154.48, 144.35, 105.32, 98.38, 98.34, 91.27, 85.85, 85.79, 76.41, 72.34, 72.15, 71.63, 70.69, 70.61, 67.80, 67.76, 62.13, 42.87; HRMS calcd for $[M + H]^+$ $C_{15}H_{26}N_3O_{16}P_2$ 566.0788, found 566.0793.

Uridine 5′-(6-Amino-{2-[(7-bromomethyl-2-naphthyl)-methoxycarbonylmethoxy]ethoxy}acetyl-6-deoxy-α-D-galactopyranosyl) Diphosphate Monoammonium Salt (1a). To a solution of **16** (2.5 mg, 4.3 μmol) in H_2O (2 mL) was added Et_3N (18 μl, 12.9 μmol) and compound **11** (3 mg, 5.3 μmol in 2 mL of THF) at room temperature. After the mixture was stirred for 30 min at room temperature, 5 mL of 20 mM ammonium acetate buffer (pH 6.0) was added and then concentrated. The obtained mixture was used directly for the affinity labeling experiment without purification because 30−40% hydrolysis at the bromomethyl group was confirmed after keeping the temperature at 4 °C for 24 h as judged by TLC and ESI MS (data not shown). ESI MS (negative ion mode) calcd for $[M - H]^-$ $C_{33}H_{41}BrN_3O_{21}P_2$ 956.1, found 956.

Uridine 5′-(6-Amino-{2-[(7-methyl-2-naphthyl)methoxycarbonylmethoxy]ethoxy}acetyl-6-deoxy-α-D-galactopyranosyl) Diphosphate Monoammonium Salt (1b). To a solution of **16** (10 mg, 17 μmol) in H_2O (2 mL) was added Et_3N (4.8 μL, 34 μmol) and compound **10** (25 mg, 51 μmol in dioxane 4 mL) at room temperature. After being stirred for 30 min at room temperature, the reaction mixture was concentrated and the residue obtained was purified by a Wakogel 50C18 reverse-phase column (60% MeOH as a eluent). The obtained compound was passed through a cation-exchange resin column (Dowex 50W-X8, Na^+ form) to give compound **1b** (18 mg, 84%). ^{1}H NMR (600 MHz, D_2O, 27 °C) δ 7.66−7.87 (m, 5 H, uracil, aromatic × 4), 7.41−7.45 (m, 2 H, aromatic × 2), 5.81−5.83 (m, 2 H, H′-1, uracil), 5.60 (dd, 1 H, $J_{1'',P}$ = 7.1 Hz, $J_{1'',2''}$ = 3.6 Hz, H″-1), 5.36 (s, 2 H, OCH_2naph), 4.15−4.31 (m, 7 H, H′-2, H′-3, H′-4, H′-5a, H′-5b, $OCH_2C{=}O$ × 2), 4.13 (t, 1 H, H″-5, $J_{4'',5''}$ = 6.7 Hz), 4.00 (m, 2H, $OCH_2C{=}O$ × 1, H″-4), 3.86−3.88 (m, 2H, $OCH_2C{=}O$ × 1, H″-3), 3.76−3.78 (m, 3H, OCH_2CH_2O, H″-2), 3.70−3.72 (m, 2H, OCH_2CH_2O), 3.48 (dd, 1 H, $J_{6a'',6b''}$ = 13.8 Hz, $J_{5'',6a''}$ = 5.9 Hz, H″-6a), 3.31 (dd, 1H, $J_{5'',6b''}$= 7.4 Hz, H″-6b), 2.48 (s, 3H, CH_3naph); ^{13}C NMR (150 MHz, D_2O, 27 °C) δ 175.36, 174.96, 168.59, 144.07, 139.64, 135.79, 135.58, 133.78, 131.68, 130.91, 130.28, 129.41, 129.36, 127.76, 105.13, 98.48, 98.43, 91.31, 85.77, 85.71, 76.49, 73.07, 72.31, 72.18, 72.01, 71.91, 71.06, 71.00,

70.82, 70.12, 67.55, 67.51, 57.05, 41.88, 23.47, 21.77, 17.67; HRMS calcd for $[M + H]^+$ $C_{33}H_{44}N_3O_{21}P_2$ 880.1943, found 880.1924.

10-(2-Naphthyl)-3,6,9-trioxadecanol (17). A solution of triethyleneglycol (399 μL, 3 mmol) in DMF (8 mL) was cooled to 0 °C, and sodium hydride (54 mg, 1.5 mmol) was added. After the mixture was stirred for 30 min at 0 °C, 2-bromomethylnaphthalene (276 mg, 1.2 mmol) was added to the solution, and the mixture was stirred for 1 h at 0 °C and was allowed to warm to room temperature for further stirring. After the mixture was stirred for 2 h at room temperature, the reaction was stopped by the addition of MeOH and concentrated. The residue obtained was purified by silica gel column chromatography (toluene/EtOAc = 3:1 to 1:1) to give **17** (202 mg, 70%). ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.79−7.83 (dd, 4 H, naphthalene), 7.46−7.49 (m, 3 H, naphthalene), 4.73 (s, 2 H, OCH_2-naphthalene), 3.70 (m, 10 H, $[OCH_2CH_2]$, OCH_2CH_2OH), 3.61 (m, 2 H, $[OCH_2CH_2]$); ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C) δ 136.00, 133.62, 133.35, 128.52, 128.21, 128.04, 126.89, 126.43, 126.20, 126.16, 73.73, 72.87, 71.03, 71.00, 70.75, 69.74, 62.13; HRMS calcd for $[M + H]^+$ $C_{17}H_{23}O_4$ 291.1596, found 291.1588.

1-Chloro-10-(2-naphthyl)-3,6,9-trioxadecane (18). To a solution of **17** (200 mg, 688 μmol) in CH_2Cl_2 (5 mL) was added pyridine (100 μL) and thionyl chloride (200 μL, 2.75 mmol) at room temperature. After being stirred for 24 h, the reaction solution was concentrated and the residue obtained was purified by silica gel column chromatography (toluene/EtOAc = 2:1) to give **18** (190 mg, 89%). ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.79−7.83 (m, 4 H, naphthalene), 7.46−7.48 (m, 3 H, naphthalene), 4.73 (s, 2 H, OCH_2-naphthalene), 3.74 (t, 2 H, OCH_2CH_2−Cl), 3.67−3.72 (m, 8 H, $[OCH_2CH_2]$), 3.61 (t, 2 H, $[OCH_2CH_2]$); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 135.74, 133.25, 132.97, 128.14, 127.84, 127.67, 126.46, 126.07, 125.83, 125.78, 73.34, 71.36, 70.74, 70.68, 70.65, 69.43, 42.72; HRMS calcd for $[M]^+$ $C_{17}H_{21}O_3Cl$ 308.1179, found 308.1170.

Phenyl 2,3,4-*p*-Methoxybenzyl-1-thio-6-*O*-triphenylmethyl-β-D-galactopyranoside (19). To a solution of phenyl 1-thio-6-*O*-triphenylmethyl-β-D-galactopyranoside[33] (5.66 g, 11 mmol) in dry DMF (50 mL) was added sodium hydride (1.8 g, 49.5 mmol) at 0 °C. After the mixture was stirred for 30 min, *p*-methoxybenzyl chloride (4.9 mL, 36.3 mmol) was added to the solution and further stirred overnight at room temperature. The reaction was stopped by addition of MeOH, and the mixture was concentrated in vacuo. The residue obtained was dissolved in $CHCl_3$, and the organic layer was washed with brine, dried by $MgSO_4$, filtered, and concentrated. The residual syrup was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to give **19** (9.4 g, 98%) as a syrup; $[\alpha]^{20}_D$ +20.9° (*c* 0.85, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.53 (m, 2 H, aromatic), 7.37−7.40 (m, 6 H, aromatic), 7.21−7.31 (m, 17 H, aromatic), 7.14 (dd, 3 H, aromatic), 7.05 (d, 2 H, aromatic), 6.84−6.87 (dd, 4 H, aromatic), 6.74 (d, 2 H, aromatic), 4.74 (d, 1 H, OCHH-phenyl), 4.63−4.70 (m, 4 H, OCH_2-phenyl × 2), 4.57 (d, 1 H, H-4), 4.44 (d, 1 H, OCHH-phenyl), 3.83 (t, 1 H, $J_{3,4}$ = 9.1 Hz, H-3), 3.77−3.82 (m, 10 H, H-1, OCH_3 × 3), 3.53−3.56 (dd, 1 H, $J_{5,6b}$ = 6.3 Hz, $J_{6a,b}$ = 9.8 Hz, H-6b), 3.46 (dd, 1 H, $J_{2,3}$ = 9.2 Hz, H-2), 3.28 (t, 1 H, H-5), 3.14 (dd, 1 H, $J_{5,6a}$ = 6.3 Hz, H-6a); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 159.32, 159.25, 158.98, 143.95, 134.73, 131.51, 130.94, 130.86, 130.64, 130.55, 130.37, 130.03, 129.46, 129.32, 129.23, 128.78, 128.69, 128.53, 127.96, 127.87, 127.29, 127.05, 126.76, 113.84, 113.79, 113.49, 87.79, 86.96, 83.95, 77.77, 75.29, 73.72, 72.58, 63.25, 55.32; HRMS calcd for $[M + Na]^+$ $C_{55}H_{54}O_8NaS$ 897.3437, found 897.3456.

Phenyl 2,3,4-*p*-Methoxybenzyl-1-thio-β-D-galactopyranoside (20). To a solution of **19** (9.1 g, 10.4 mmol) in a mixed solvent ($CHCl_3$/MeOH = 2:1, 75 mL) was added 1 N HCl/MeOH (20.8 mL, 20.8 mmol) at room temperature. After being stirred for 90 min, the mixture was neutralized by addition of Et_3N (3.49 mL, 25.0 mmol) and concentrated. The residue obtained was purified by silica gel column chromatography (hexane/EtOAc = 2:1) to give **20** (6.08 g, 92%) as a crystal (from EtOH and hexane); $[\alpha]^{20}_D$ −4.9° (*c* 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.53 (d, 2 H, aromatic), 7.20−7.33 (m, 9 H, aromatic), 6.85−6.90 (m, 6 H, aromatic), 4.89 (d, 1 H, OCHH-phenyl), 4.75 (d, 1 H, OCHH-phenyl), 4.66−4.71 (m, 3 H, OCHH-phenyl × 3), 4.62 (d, 1 H, H-4), 4.56 (d, 1 H, OCHH-phenyl), 3.90 (t, 1 H, $J_{3,4}$ = 9.5 Hz, H-3), 3.76−3.84 (m, 11 H, H-1, H-5, OCH_3 × 3), 3.56 (dd, 1 H, $J_{2,3}$ = 9.2 Hz, H-2), 3.45 (dd, 1 H, $J_{6a,b}$ = 11.3 Hz, H-6b), 3.39 (dd, 1 H, H-6a); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 159.32, 134.14, 131.46, 130.53, 130.43, 130.35, 129.95, 129.89, 129.27, 128.84, 127.12, 113.91, 113.81, 113.78, 87.84, 83.99, 78.77, 77.33, 75.32, 73.70, 72.83, 72.77, 62.32, 55.29; HRMS calcd for $[M + H]^+$ $C_{36}H_{41}O_8S$ 633.2522, found 633.2532.

Phenyl 2,3,4-*p*-Methoxybenzyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-1-thio-β-D-galactopyranoside (21). To a solution of **20** (2.3 g, 3.6 mmol) in dry DMF (8 mL) was added sodium hydride (262 mg, 7.2 mmol) at 0 °C.[34] After the mixture was stirred for 30 min, compound **18** (2.22 g, 7.2 mmol) in dry DMF (8 mL) was added to the reaction mixture at 0 °C. After the mixture was stirred for 12 h, the reaction was stopped by addition of MeOH and the solution was concentrated. The residue obtained was dissolved in $CHCl_3$, washed with brine, dried by $MgSO_4$, filtered, and concentrated. The residual syrup was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100:1) to give **21** (2.8 g, 82%) as a syrup; $[\alpha]^{20}_D$ +13.8° (*c* 0.9, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.77−7.82 (m, 4 H, aromatic), 7.54 (m, 2 H, aromatic), 7.45−7.47 (m, 3 H, aromatic), 7.19−7.31 (m, 9 H, aromatic), 6.86 (m, 6 H, aromatic), 4.87 (d, 1 H, OCHH-phenyl), 4.56−4.73 (m, 9 H, H-4, OCH_2-naphthalene, OCH_2-phenyl × 3), 3.88 (m, 2 H, H-3, OCH_2CHH-Gal), 3.76−3.83 (m, 9 H, OCH_3 × 3), 3.63−3.67 (m, 9 H, H-1, $[OCH_2CH_2]$), 3.54−3.57 (m, 6 H, H-2, H-5, H-6, $[OCH_2CH_2]$, OCH_2CHH-Gal), 3.48 (m, 1 H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 159.20, 159.10, 135.77, 134.42, 133.27, 132.98, 131.35, 131.00, 130.63, 130.49, 129.95, 129.57, 129.15, 128.73, 128.13, 127.85, 127.68, 126.91, 126.44, 126.07, 125.82, 125.77, 113.82, 113.74, 113.58, 87.80, 83.91, 77.35, 75.24, 73.96, 73.32, 73.16, 72.37, 70.74, 70.69, 70.66, 70.58, 70.42, 69.72, 69.46, 60.38, 55.27; HRMS calcd for $[M + Na]^+$ $C_{57}H_{60}O_8NaS$ 927.3754, found 927.3741.

Phenyl 6-*O*-(10-[2-Naphthyl]-3,6,9-trioxadecanyl)-1-thio-β-D-galactopyranoside (22). To a solution of **21** (2.45 g, 2.7 mmol) in CH_2Cl_2 (25 mL) was added thiophenol (994 μL, 9.72 mmol), and the mixture was cooled to −78 °C. A solution of $SnCl_4$ (1 M solution of $SnCl_4/CH_2Cl_2$, 8.50 mmol) was added to the reaction mixture and stirred for 20 min at −78 °C.[35] The reaction was stopped by addition of saturated $NaHCO_3$ and $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and brine and then concentrated. The residue obtained was purified by silica gel column chromatography ($CHCl_3$/MeOH = 40:1) to give **22** (900 mg, 61%) as a syrup; $[\alpha]^{20}_D$ +18.7° (*c* 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD, 27 °C) δ 7.83−7.86 (m, 4 H, naphthalene), 7.45−7.54 (m, 5 H, naphthalene, phenyl), 7.20−7.30 (m, 3 H, phenyl), 4.73 (s, 2 H, OCH_2-naphthalene), 4.58 (d, 1 H, $J_{1,2}$ = 9.7 Hz, H-1), 3.86 (d, 1 H, H-4), 3.59−3.76 (m, 16 H, H-2, H-5, H-6a, H-6b, $[OCH_2CH_2]$), 3.48 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 135.55, 133.25, 133.00, 132.07, 128.89, 128.20, 127.88, 127.70, 127.64, 126.61, 126.11, 125.89, 125.86, 88.53, 74.76, 73.31, 70.78, 70.56, 70.49, 70.36, 70.13, 69.75, 69.33, 68.61; HRMS calcd for $[M + H]^+$ $C_{29}H_{37}O_8S$ 545.2209, found 545.2204.

Phenyl 2,3,4-Tri-*O*-acetyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-1-thio-β-D-galactopyranoside (23). Compound **22** (1.06 g, 1.94 mmol) was dissolved in pyridine (10 mL), and to the solution was added acetic anhydride (6 mL) at room temperature. After being stirred for 12 h, the reaction mixture was concentrated and the residual syrup was purified by silica gel column chromatography (hexane/EtOAc = 4:1 to 2:1) to give **23** (1.3 g, 99%) as a syrup. $[\alpha]^{20}_D$ −0.9° (*c* 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.77−7.83 (m, 4 H, naphthalene), 7.45−7.50 (m, 5 H, naphthalene, phenyl), 7.26−7.32 (m, 3 H, phenyl), 5.44 (d, 1 H, H-4), 5.23 (t, 1 H, $J_{2,3}$ = 9.9 Hz, H-2), 5.03 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3), 4.73 (s, 2 H, OCH_2-naphthalene), 4.70 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1),

3.86 (t, 1 H, $J_{5,6}$ = 6.2 Hz, H-5), 3.62−3.70 (m, 8 H, [OCH_2-CH_2]), 3.48−3.61 (m, 6 H, H-6a, H-6b, [OCH_2CH_2]), 2.10 (s, 6 H, Ac × 2), 2.00 (s, 3 H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 170.12, 169.99, 169.50, 135.82, 133.29, 132.99, 132.08, 128.90, 128.14, 127.88, 127.69, 126.47, 126.07, 125.81, 86.62, 76.02, 73.33, 72.21, 71.13, 70.69, 70.66, 70.52, 69.48, 69.20, 67.81, 67.56, 20.85, 20.67, 20.61; HRMS calcd for $[M + H]^+$ $C_{35}H_{43}O_{11}S$ 671.2526, found 671.2548.

2,3,4-Tri-*O*-acetyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-D-galactopyranose (24). To a solution of **23** (500 mg, 745 μmol) in mixed solvent (acetone/H_2O = 20:1, 10 mL) was added *N*-bromosuccinimide (398 mg, 2.23 mmol) at room temperature. After being stirred for 90 min, the mixture was concentrated and dissolved in $CHCl_3$. The solution was washed with saturated $NaHCO_3$ and brine, and the organic layer was dried by $MgSO_4$, filtered, and concentrated. The residue obtained was purified by silica gel column chromatography (hexane/EtOAc = 3:1 to 1:2) to give **24** (333 mg, 77%) as a syrup. ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.83 (m, 4 H, naphthalene), 7.45−7.48 (m, 3 H, naphthalene), 5.49 (m, 0.8 H, H-3α), 5.41−5.43 (m, 1 H, H-1α, H-4β), 5.16 (dd, 0.8 H, H-2α), 5.09 (dd, 0.2 H, H-2β), 5.05 (dd, 0.2 H, H-3β), 4.74 (s, 2 H, OCH_2-naphthalene), 4.67 (t, 0.2 H, H-1β), 4.48 (dd, 0.8 H, H-5α), 4.24 (s, 0.8 H, H-4α), 3.86 (t, 0.2 H, H-5β), 3.57−3.72 (m, 12 H, [OCH_2CH_2] × 3), 3.43−3.55 (m, 2 H, H-6a, H-6b), 1.92−2.13 (m, 9 H, Ac × 3); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 170.75, 170.70, 170.53, 170.45, 170.25, 169.84, 135.95, 135.90, 133.51, 133.23, 128.40, 128.11, 127.93, 126.74, 126.32, 126.10, 126.04, 95.88, 90.73, 73.52, 72.39, 71.26, 71.21, 71.15, 71.05, 70.90, 70.87, 70.82, 70.73, 70.67, 70.62, 70.14, 70.05, 69.62, 69.34, 68.89, 67.94, 67.32, 21.05, 20.92, 20.89, 20.83; HRMS calcd for $[M + Na]^+$ $C_{29}H_{38}O_{12}Na$ 601.2261, found 601.2237.

Diallyl 2,3,4-Tri-*O*-acetyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-α-D-galactopyranosyl Phosphite (25). To a solution of **24** (106 mg, 183 μmol) in CH_3CN (2 mL) was added 1*H*-tetrazole (23 mg, 329 μmol), and the mixture was cooled to −40 °C followed by the addition of *N*,*N*-diallyl-diisopropyl phosphoramidite (73 μL, 274 μmol).[36] After the mixture was stirred for 7 h at −40 °C, EtOAc was added and the mixture was washed with saturated $NaHCO_3$ and brine. The organic layer was dried by $MgSO_4$, filtered, and concentrated. The residue obtained was purified by silica gel column chromatography (hexane/EtOAc = 4:1 to 3:1, containing 0.5 vol % Et_3N) to give **25** (65 mg, 49%). $[\alpha]^{20}_D$ +17.0° (*c* 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.78−7.83 (m, 4 H, naphthalene), 7.26−7.48 (m, 3 H, naphthalene), 5.88−5.96 (m, 2 H, CH_2=$CHCH_2$− × 2), 5.74 (dd, 1 H, $J_{1,P}$ = 8.4 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.50 (d, 1 H, H-4), 5.36 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.17−5.33 (m, 5 H, CH_2=$CHCH_2$− × 2, H-2), 4.73 (s, 2 H, OCH_2-naphthalene), 4.34−4.41 (m, 5 H, CH_2=$CHCH_2$− × 2, H-5), 3.54−3.71 (m, 12 H, [OCH_2CH_2]), 3.44−3.54 (m, 2 H, $J_{6a,b}$ = 11.0 Hz, H-6a, H-6b), 2.12 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 1.98 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C) δ 170.25, 170.12, 170.00, 135.81, 134.37, 134.35, 133.29, 132.99, 128.14, 127.87, 127.69, 126.47, 126.06, 125.82, 116.82, 116.75, 90.93, 90.80, 73.34, 71.01, 70.68, 70.64, 70.46, 69.47, 69.19, 68.48, 68.26, 67.61,63.47, 63.40, 63.32, 63.26, 20.77, 20.71, 20.66; HRMS calcd for $[M]^+$ $C_{35}H_{47}O_{14}P$ 722.2703, found 722.2692.

Diallyl 2,3,4-Tri-*O*-acetyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-α-D-galactopyranosyl Phosphate (26). To a solution of **25** (59 mg, 82 μmol) in THF (2 mL) was added Et_3N (114 μL, 820 μmol) and cooled to −10 °C followed by the addition of *tert*-butyl hydroperoxide (49 μL, 246 μmol). After being stirred for 4 h at −10 °C, the reaction mixture was concentrated and the residue obtained was dissolved in $CHCl_3$. The solution was washed with saturated $NaHCO_3$ and brine. The organic layer was dried by $MgSO_4$, filtered, and concentrated to give **26** (60 mg, 98%). $[\alpha]^{20}_D$ +47.8° (*c* 1.00, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, 27 °C) δ 7.78−7.82 (m, 4 H, naphthalene), 7.45−7.48 (m, 3 H, naphthalene), 5.91−5.95 (m, 3 H, H-1, CH_2=$CHCH_2$− × 2), 5.52 (d, 1 H, H-4), 5.33−5.40 (m, 3 H, CH_2=$CHCH_2$−, H-3), 5.23−5.28 (m, 3 H, CH_2=$CHCH_2$−, H-2), 4.72 (s, 2 H, OCH_2-naphthalene), 4.55−4.60 (m, 4 H, CH_2=$CHCH_2$− × 2), 4.35−4.37 (t, 1 H, $J_{5,6a}$ = 6.3 Hz, H-5), 3.56−3.69 (m, 13 H, [OCH_2CH_2], H-6b), 3.46−49 (dd, 1 H, $J_{6a,b}$ = 10.2 Hz, H-6a), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 1.99 (s, 3 H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 170.03, 169.94, 169.87, 135.82, 133.28, 132.98, 132.24, 132.19, 132.13, 128.11, 127.85, 127.67, 126.43, 126.05, 125.80, 125.79, 118.66, 118.53, 94.56, 73.30, 71.02, 70.66, 70.44, 69.57, 69.47, 68.93, 68.54, 68.51, 68.45, 68.41, 68.02, 67.27, 67.22, 67.17, 20.69, 20.63, 20.58; HRMS calcd for $[M + H]^+$ $C_{35}H_{48}O_{15}P$ 739.2731, found 739.2701.

2,3,4-Tri-*O*-acetyl-6-*O*-(10-[2-naphthyl]-3,6,9-trioxadecanyl)-α-D-galactopyranosyl Phosphate Mono-Et_3N Salt (27). To a solution of **26** (60 mg, 83 μmol) in THF (2 mL) was added Et_2NH (172 μL, 1.66 mmol) and tetrakis(triphenylphosphine)palladium (19 mg, 16.6 μmol) at room temperature.[37] After being stirred for 2 h at room temperature, the solution was concentrated and the residue obtained was purified by Iatrobeads column chromatography ($CHCl_3$/MeOH = 2:1, containing 0.5 vol % Et_3N) to give **27** (57 mg, 91%). $[\alpha]^{20}_D$ +40.9° (*c* 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 7.82−7.86 (m, 4 H, naphthalene), 7.31−7.52 (m, 3 H, naphthalene), 5.79 (dd, 1 H, $J_{1,P}$ = 7.7 Hz, $J_{1,2}$ = 3.3 Hz, H-1), 5.53 (d, 1 H, H-4), 5.44 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3), 5.16 (dt, 1 H, $J_{2,3}$ = 10.7 Hz, H-2), 4.76 (s, 2 H, OCH_2-naphthalene), 4.49 (t, 1 H, $J_{5,6a}$ = 6.6 Hz, H-5), 3.53−3.73 (m, 13 H, [OCH_2CH_2], H-6a), 3.48 (dd, 1 H, $J_{5,6b}$ = 7.8 Hz, $J_{6a,b}$ = 9.5 Hz, H-6b), 3.03 (dd, 6 H, CH_3CH_2N × 3), 2.15 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 1.99 (s, 3 H, Ac), 1.30 (t, 9 H, CH_3CH_2N × 3); ^{13}C NMR (150 MHz, $CDCl_3$, 27 °C) δ 170.79, 170.20, 169.96, 135.86, 133.29, 132.99, 128.13, 127.87, 127.68, 126.45, 126.07, 125.83, 92.04, 91.99, 73.30, 70.77, 70.62, 70.59, 70.54, 70.34, 69.50, 68.88, 68.65, 68.20, 68.16, 67.81, 67.50, 45.44, 41.44, 20.95, 20.72, 11.17, 8.95; HRMS calcd for $[M + Na]^+$ $C_{29}H_{39}O_{15}PNa$ 681.1924, found 681.1937.

6-*O*-(10-[2-Naphthyl]-3,6,9-trioxadecanyl)-α-D-galactopyranosyl Phosphate Mono-Et_3N Salt (28). To a solution of **27** (57 mg, 75 μmol) in MeOH (2 mL) was added Et_2NH (500 μL), and the mixture was stirred for 3 days at room temperature. The reaction mixture was concentrated, and the residue obtained was purified by ion exchange column chromatography (DEAE Sephasell, 10−50 mM NH_4HCO_3 as a eluent). The obtained residue redissolved in H_2O was passed through a cation-exchange resin column (Dowex 50W-X8, H^+ form followed by Et_3NH^+ form) to give **28** (44 mg, 88%). $[\alpha]^{20}_D$ +41.6° (*c* 1.00, D_2O). ^{1}H NMR (500 MHz, D_2O, 27 °C) δ 7.87−7.95 (m, 4 H, naphthalene), 7.52−7.59 (m, 3H, naphthalene), 5.52 (dd, 1H, $J_{1,P}$ = 7.3 Hz, $J_{1,2}$ = 3.6 Hz, H-1), 4.72 (s, 2 H, OCH_2-naphthalene), 4.20 (t, 1 H, $J_{5,6}$ = 6.0 Hz, H-5), 3.93 (d, 1 H, H-4), 3.88 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3), 3.80 (dt, 1 H, $J_{2,3}$ = 10.3 Hz, H-2), 3.61−3.71 (m, 14 H, H-6a, H-6b, [OCH_2CH_2]), 3.18 (dd, 8 H, CH_3CH_2N × 4), 1.27 (t, 12 H, CH_3CH_2N × 4); ^{13}C NMR (125 MHz, D_2O, 27 °C) δ 137.70, 135.65, 135.52, 131.05, 130.65, 130.43, 129.97, 129.33, 129.24, 129.14, 97.67, 97.62, 75.52, 72.60, 72.34, 72.27, 72.20, 72.16, 72.00, 71.73, 71.42, 71.06, 71.00, 49.36, 10.93; HRMS calcd for $[M + H + Et_3N]^+$ $C_{29}H_{49}NO_{12}P$ 634.2992, found 634.3003.

Uridine 5′-(6-*O*-[10-{2-Naphthyl}-3,6,9-trioxadecanyl]-α-D-galactopyranosyl) Diphosphate Disodium Salt (2). Compound **28** (26 mg, 39 μmol) was dissolved in dry pyridine (1 mL) and concentrated in vacuo three times to remove water. To the residual syrup **28** in dry pyridine (1 mL) was added 1*H*-tetrazole (11 mg, 156 μmol) and UMP-morpholidate (54 mg, 78 μmol, 1 mL in pyridine solution) at room temperature. After being stirred for 2 days, the mixture was evaporated and the residue obtained was subjected to purification by ion exchange column chromatography (DEAE-Sephasell, 10−150 mM NH_4HCO_3 as the eluent). Further purification by gel permeation column chromatography (Sephadex G-15, water as an eluent) and cation-exchange resin column (Dowex 50W-X8, Na^+ form) afforded compound **2** (14 mg, 41%). The compound was lyophilized to give an amorphous powder. ^{1}H NMR (500 MHz, D_2O, 27 °C) δ 7.87−7.97 (m, 6 H, naphthalene, H-5), 7.55−7.61 (m, 3 H, naphthalene), 5.90 (d, 1 H, $J_{1',2'}$ = 4.8 Hz, H′-1),

5.88 (d, 1 H, H-6), 5.64 (dd, 1 H, $J_{1'',P} = 7.4$ Hz, $J_{1'',2''} = 3.5$ Hz, H″-1), 4.77 (s, 2 H, OCH_2-naphthalene), 4.19−4.34 (6 H, H′-2, H′-3, H′-4, H′-5a, H′-5b, H″-5), 3.94 (d, 1 H, H″-4), 3.89 (dd, 1 H, $J_{2'',3''} = 10.2$ Hz, $J_{3'',4''} = 3.2$ Hz, H″-3), 3.61−3.80 (m, 15 H, H″-2, H″-6a, H″-6b, [OCH_2CH_2]); ^{13}C NMR (150 MHz, D_2O, 27 °C) δ 165.85, 151.38, 141.26, 134.77, 132.69, 132.56, 128.11, 127.71, 127.49, 127.07, 126.39, 126.30, 126.23, 102.33, 95.63, 95.59, 88.27, 82.93, 82.87, 73.58, 72.64, 69.69, 69.51, 69.44, 69.37, 69.31, 69.18, 68.97, 68.87, 68.54, 68.24, 68.19, 64.63, 64.66; HRMS calcd for $[M + Na]^+$ $C_{32}H_{44}N_2O_{20}NaP_2$ 861.1860, found 861.1843.

Construction of the Complex Model of UDP-Galactose and β1,4-Galactosyltransferase (Conformation I). Since the crystal structure of β1,4GalT (PDB entry 1FR8), which is in conformation I, includes UDP in the active site, UDP-galactose extracted from conformation II (PDB ently 1O0R) was superimposed onto the UDP in 1FR8 so that the root-mean-square deviation (rmsd) between the UDP part of UDP-galactose and UDP in 1FR8 becomes the smallest. Construction of the complex model was carried out by using the SYBYL 6.9 program (Tripos, Inc.).

Affinity Labeling of βGal-T1. Recombinant human β1,4-galactosyltransferase-1 (50 μg, NP_001488 from NCBInr database, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) was incubated in the presence or absence of the bromomethyl substrate **1a** (final concentration of 0, 30, 100 μM for the calculation of labeling efficiency and 500 μM for the MS/MS analysis) in 100 μL of 20 mM Tris-maleate buffer (pH 6.5) containing 50 mM NaCl and 2 mM $MnCl_2$ at 37 °C for 10 h. The reaction mixture was directly reduced and alkylated (10 mM DTT for 1 h at 40 °C and then 50 mM iodoacetamide for 2 h at room temperature), and then the protein was obtained with acetone precipitation. The protein was digested with 0.5 μg of trypsin in 100 mM ammonium bicarbonate (pH 8.3). MALDI-TOF MS and LIFT TOF/TOF spectra were measured after desalting by means of 10 μL of C-18 ZipTips (Millipore) according to the manufacturer's instructions.

Detection of the Affinity Labeled βGal-T1 by ESI-MS. The LC/MS analysis was conducted by JMS-T100CS (AccuTOF CS, JEOL Co. Ltd., Japan) equipped with Inertsil C8 (GL Sciences Inc., Japan). The HPLC system was equilibrated with 2% acetic acid at 50 μL/min flow speed before sample injection. After the sample injection (1 μL, corresponding to about 14 pmol of protein), sample washing was conducted with 2% acetic acid for 5 min and then with 100 mM ammonium carbonate buffer (pH 6.8) for 10 min. The protein was eluted by the linear gradient mode of 2% acetic acid and acetonitrile (acetonitrile from 0% to 100% in 10 min).

Preparation of Matrix Crystals for MALDI. The matrix solutions were prepared as follows: DHB (10 mg) was dissolved in water (1 mL), and CHCA was prepared as a saturated solution in 3:1 (v/v) of acetonitrile/water. An amount of 0.5 μL of matrix solution was applied to the target spot of the Anchorchip plate (Bruker Daltonics), which was equipped with 384 hydrophilic anchors on an otherwise hydrophobic surface, and 1 μL of the sample solution was added and then dried at room temperature. We employed these samples (about 1−10 pmol) with TOF/TOF modes using the above preparation procedure.

MALDI-TOF MS. MALDI-TOF mass spectra were measured by using an Ultraflex TOF/TOF mass spectrometer equipped with a reflector and controlled by the Flexcontrol 1.2 software package (Brucker Daltonics GmbsH, Bremen, Germany). In the MALDI-TOF MS reflector mode, ions generated by a pulsed UV laser beam (nitrogen laser, $\lambda = 337$ nm, 5 Hz) were accelerated to a kinetic energy of 23.5 kV. Metastable ions generated by laser-induced decomposition of the selected precursor ions were analyzed without any additional collision gas.

In the MALDI-TOF/TOF mode, precursor ions were accelerated to 8 kV and selected in a timed ion gate. The fragments were further accelerated by 19 kV in the LIFT cell (LIFT means "lifting" the potential energy for the second acceleration of ion source), and their masses were analyzed after the ion reflector passage. Masses were automatically annotated by using the FlexAnalysis 2.0 software package. External calibration of MALDI mass spectra was carried out using singly charged monoisotopic peaks of a mixture of human angiotensin II (*m/z* 1046.542), bombesin (*m/z* 1619.823), ACTH (18-39) (*m/z* 2465.199), and somatostatin 28 (*m/z* 3147.472). The mixture of these peptides was measured at the central spot of a 3 × 3 square by using external calibration. To achieve mass accuracy better than 60 ppm, internal calibration was carried out by doping the matrix solution with a mixture of the calibration peptides. Calibration of these mass spectra was performed automatically by utilizing a customized macro command of the Flex Control 2.1 software package. The macro command was used for the calibration of the monoisotopic singly charged peaks of the above-mentioned peptides. TOF/TOF spectra were annotated with the BioTools 2.1 software package.

Determination of Inhibitory Constants. The inhibitory activity of the inhibitors **1b**−**7** for human βGal-T1 was measured by the following procedure. UDP-galactose (40 or 100 μM), inhibitors **1b**−**7** (0−200 μM), and the dansyl fluorescently labeled *N*-acetylglucosamine **8** (50 μM) were dissolved in the reaction buffer (50 mM Hepes, pH 6.5, 10 mM $MnCl_2$, 0.25 mg/mL bovine serum albumin, total volume of 100 μL). The enzymatic reaction was started by the addition of βGal-T1 (40−150 μU) and incubated at 37 °C for 10 min. Inactivation was quickly done by heating the reaction solution at 100 °C for 3 min. The reaction mixture was centrifuged for 5 min, and the supernatant (20 μL) was injected into the HPLC system to detect the product (Galβ1,4GlcNAc-dansyl) formation by measuring fluorescence at 525 nm under excitation at 340 nm. The obtained data were plotted and analyzed by employing Dixon and Webb's method.

Cold Spray Ionization Time-of-Flight Mass Spectrometry (CSI-TOF MS). Commercially available recombinant human βGal-T1 was further purified by dialysis with 50 mM ammonium acetate buffer (pH 6.0) to remove salts and detergent before CSI-TOF MS measurements. A solution of purified enzyme was concentrated by ultrafiltration with microcon YM-10 (Millipore). The contents and concentration of sample solutions for CSI-TOFMS analysis are as follows: total volume (20 μL), buffer [25 mM ammonium acetate (pH 6.0)], βGal-T1 (95 pmol), $MnCl_2$ (2 nmol), UDP (400 pmol), and acceptor substrate **8** (450 pmol). All CSI-TOF mass spectra were measured and recorded at 37 °C according to the conditions described in our previous paper.[19]

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Supporting Information Available: Synthesis procedures and experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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