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A Search for Pyrophosphate Mimics for the Development of Substrates and Inhibitors of Glycosyltransferases

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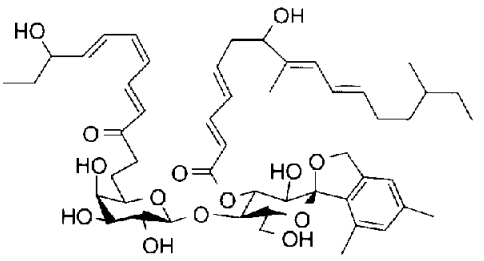
Abstract—The design and synthesis of several β-1,4-galactosyltransferase inhibitors are reported. Mimics of the pyrophosphate–Mn^{2+} complex were the focus of the design. Malonic, tartaric, and monosaccharide moieties were used as replacements of the pyrophosphate moiety, and galactose or azasugars with potent galactosidase inhibitory activity were used as the 'donor' component. Compound **6**, in which glucose was used as the pyrophosphate–Mn^{2+} complex mimic and galactose as the 'donor' component, showed the best inhibitory activity towards the transferase with a K_i of 119.6 μM. © 1997 Elsevier Science Ltd.

Introduction

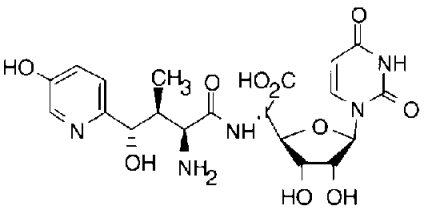
Complex oligosaccharides are synthesized by glycosyltransferases in the endoplasmic reticulum and Golgi complex by the sequential transfer of sugar residues from nucleotide or membrane-bound donors to growing polysaccharide chains.[1] Since carbohydrates are important recognition molecules in biological systems, the profound impact of glycosyltransferases on life processes has made them desirable targets for inhibition. The naturally occurring[2–5] and synthetic[6–19] inhibitors of glycosyltransferases known to date (Figs 1 and 2) include acceptor analogues (halo-sugars and deoxysugars),[10,12,13] unreactive sugar nucleotide analogues,[6,7,14–16] bisubstrate and trisubstrate analogues,[8,9] transition-state analogues,[11,17,18] and the transferases that have been studied for inhibition include galactosyltransferases,[6,7,10] fucosyltransferases,[8,10,11,13] sialyltransferases,[13,15,16] *N*-acetylglucosaminyltransferases,[14] glucose ceramide synthase,[18] and oligosaccharyl transferase.[19] The mechanisms of reactions catalyzed by glycosyltransferases have also been investigated to some extent.[20,21] It is generally postulated that these enzymatic reactions proceed through a half-chair transition state with substantial sp^2 character at the anomeric carbon.

We are particularly interested in the development of glycosyltransferase inhibitors. We have chosen β-1,4-galactosyltransferase (GalT) as a model system. GalT catalyzes the transfer of galactose from UDP-galactose to an acceptor sugar, and this reaction has been proposed to proceed via the transition state illustrated in Figure 3.[2] The pyrophosphate moiety in UDP-galactose is believed to form a six-membered ring in its complex with an essential divalent manganese ion. We have focused our attention on the development of pyrophosphate analogues that are capable of mimicking this pyrophosphate–metal interaction. Since several known GalT inhibitors contain slightly modified pyrophosphate moieties (Fig. 2),[6,7,17] further modification of the pyrophosphate group could lead to more potent inhibitors.

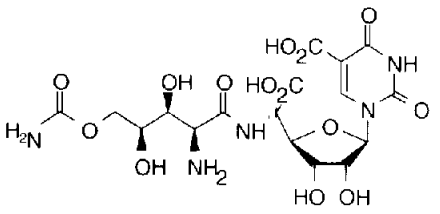
In our study, malonic, tartaric acid, and monosaccharide linkages were chosen as pyrophosphate mimics (Fig. 4). It was hypothesized that both the malonic and tartaric esters could form a complex with Mn^{2+} to mimic the metal–pyrophosphate complex. The naturally occurring GlcNAc phosphotransferase inhibitor tunicamycin apparently utilizes a monosaccharide to mimic the pyrophosphate–Mn^{2+} six-membered ring complex (Fig. 1).[5] Several X-ray crystal structures of ATP or inorganic pyrophosphate and metal ion complexes also show that the six-membered ring complexes that are formed are either chair or boat conformations.[22] Thus, glucose and galactose residues were investigated as other possible pyrophosphate–metal complex mimics. Compounds **1–3** were designed to have a malonic linkage to mimic the pyrophosphate moiety (Fig. 5). The imino-cyclitols in **2–4** were used since they are effective inhibitors of galactosidases,[23] which catalyze the glycosidic cleavage of galactosides through a similar half-chair transition state. Compound **4** employs a tartaric moiety to mimic pyrophosphate. Compounds **5** and **6** were designed to have a monosaccharide to mimic the pyrophosphate–metal complex. Compound **7** was expected to be a potential substrate or inhibitor for the enzyme.



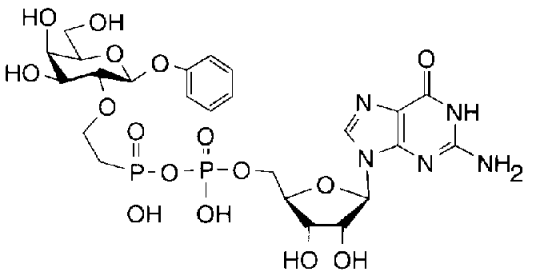
Papulacandin B
glucan synthase inhibitor [2]
$IC_{50} = 1.2 \times 10^{-6}$ M



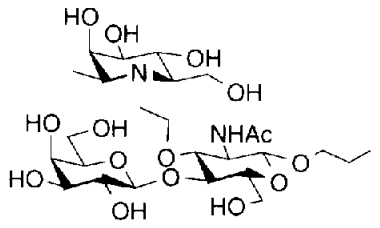
Nikkomycin Z
chitin synthetase inhibitor [2]
$K_i = 1.4 \times 10^{-7}$ M



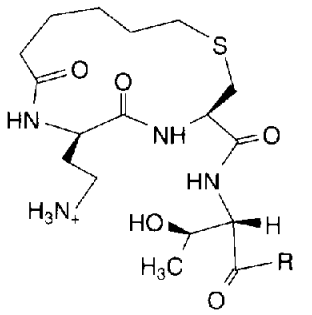
Polyoxin D
chitin synthetase inhibitor [2]
$K_i = 9.0 \times 10^{-7}$ M



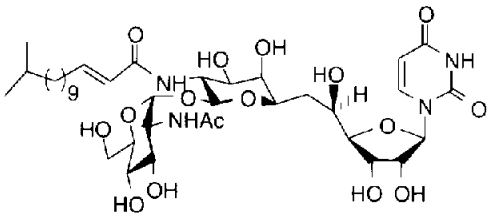
Bisubstrate analogue inhibitor
α 1,2- fucosyltransferase [8]
$K_i = 2.3$-16 μM



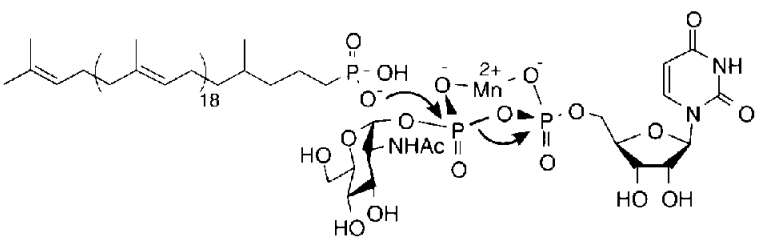
Transition state analog inhibitor
α-1,3-fucosyltransferase [11]
$K_i \sim 31$ μM with 30 μM GDP



Oligosaccharyl transferase inhibitor [19]
$K_i = 37$ nM, R = Val-Thr- *p*-Nitrophe-NH_2



Tunicamycin
inhibitor of GlcNAc phosphotransferase [5f]
$IC_{50} = 7 \times 10^{-9}$ M



GlcNAc phosphotransferase reaction

Figure 1. Examples of glycosyltransferase inhibitors. Tunicamycin appears to mimic the transition state of the GlcNAc phosphotransferase reaction.

Results and Discussion

Treatment of 2′,3′-*O*-isopropylidene uridine[34] (**8**) with tosyl chloride in pyridine at room temperature gave tosylate **9** in 80% yield that was subsequently converted to azide **10**.[25] Hydrogenolysis of the azide afforded amine **11**,[25] which was coupled with monomethyl malonic acid to generate **12**. Hydrolysis of **12** gave the UDP analogue **1**, which was found to have no inhibition

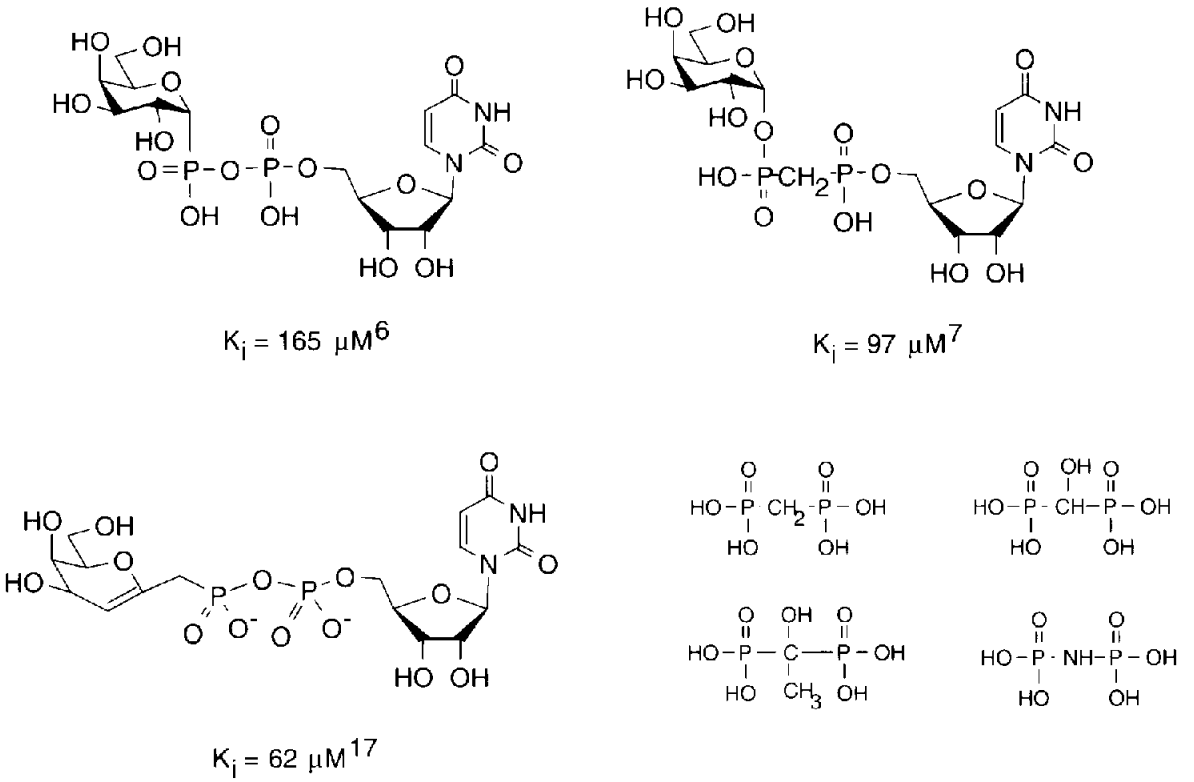


Figure 2. Galactosyltransferase inhibitors containing modified pyrophosphate groups.

at the concentration of 1 mM (Scheme 1). It should be noted that UDP has a K_i of 460 μM against galactosyltransferase,[26] indicating that the malonyl group is a poor mimic of the pyrophosphate–metal complex.

With regards to **2** and **3**, it was expected that both would act as donor sugar transition state analogue inhibitors by mimicking the half-chair conformation and the positive charge developed in the pyranose-ring with the heterocyclic nitrogen protonated at physiological pH. The first problem to be solved was the formation of a UDP analogue that could be produced in good yield and on a large scale to be used for the synthesis of **2**, **3**, and **7**. Several conditions including different solvents and Lewis acids were tested in order to prepare **13** from Meldrum's acid and **8**. Surprisingly, all efforts failed except simply melting the two substrates together

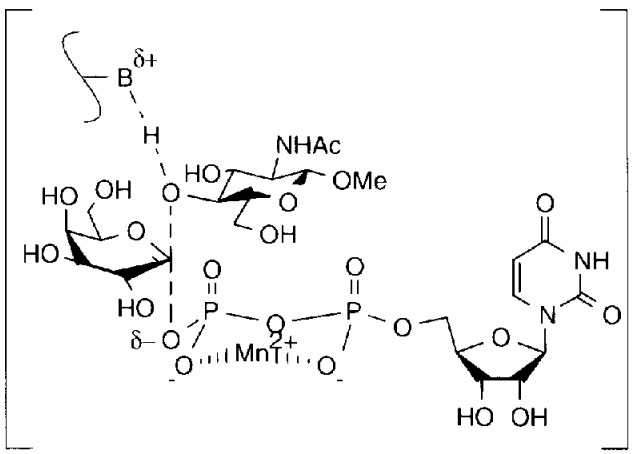


Figure 3. Proposed transition state of the galactosyltransferase reaction.

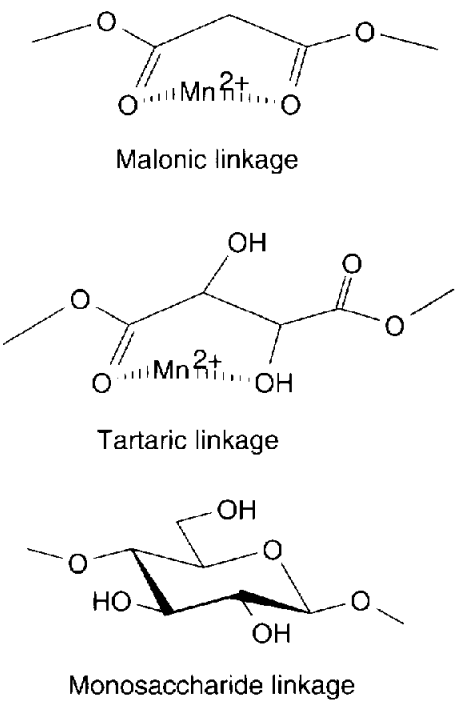


Figure 4. Designed mimics of pyrophosphate–Mn^{2+} complex.

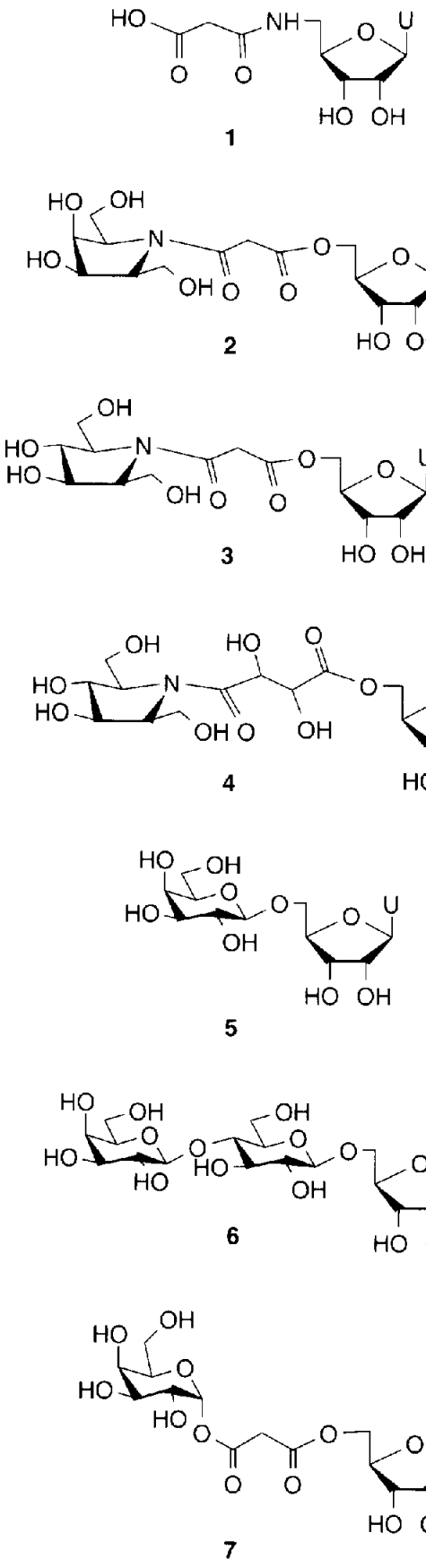
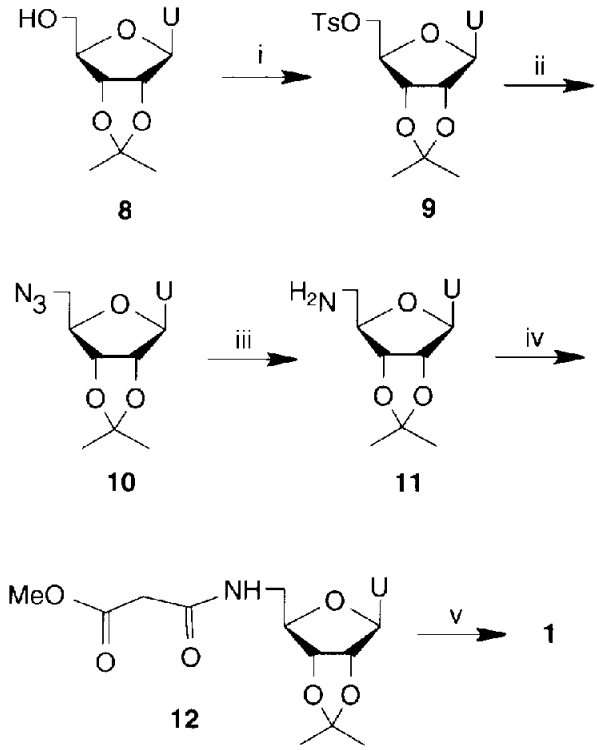
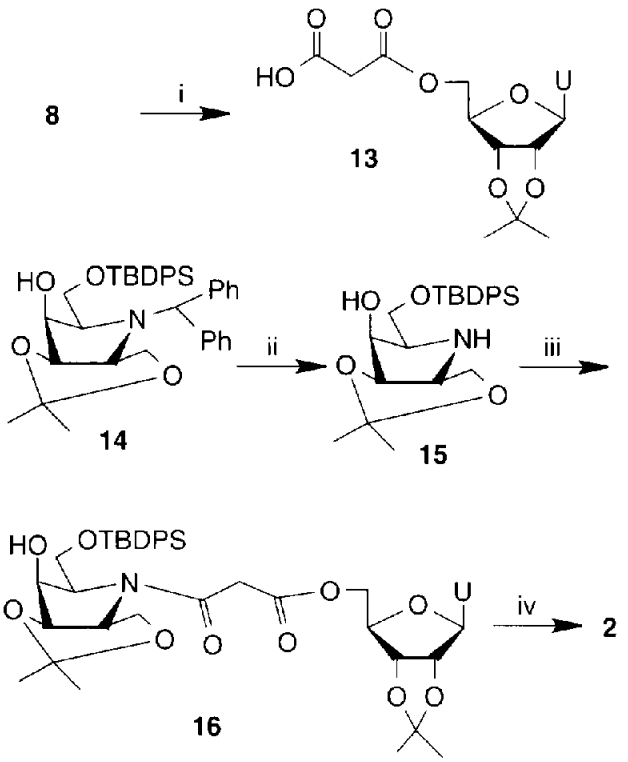


Figure 5. Structures of target compounds 1-7.



Scheme 1. Reagents and conditions: (i) TsCl, pyr, 80%; (ii) NaN_3, DMF, 50 °C, 91%; (iii) Pd/C, H_2; (iv) malonyl monomethyl ester, EDAC, HOBT, 34% from 10; (v) LiOH, MeOH:H_2O (3:1); Dowex 50 [H^+], 37%.

without the use of solvent or catalyst. Hydrogenolysis of **14**[23b] gave amine **15** that was then coupled with **13** to generate amide **16** in 62% yield. Deprotection under acidic conditions gave target **2** (Scheme 2). Compound **3** was made starting from *N*-diphenylmethyl 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine.[27] Acetonization followed by hydrogenolysis gave amine **17** in 75% yield, which was then coupled with **13** to generate amide **18**. Deprotection of **18** using TFA afforded target **3** (Scheme 3). The inhibitory activities of **2** and **3** were rather disappointing. Compound **3** was not an inhibitor of the transferase; however, compound **2** did show some inhibition of the enzyme with a K_i of about 1 mM. Even though both **2** and **3** possess a transition state mimic of the donor sugar, they are much less effective inhibitors than UDP, further suggesting that the malonic moiety is a poor pyrophosphate substitute. Perhaps the malonyl–metal ion complex is, if formed, a flat hydrophobic six-membered ring structure while the hydrophilic six-membered ring structure of pyrophosphate–metal ion complex prefers a chair or twist boat form. The reason for **2** being a better inhibitor than **3** is perhaps due to the different stereochemistry at the C-3 position of the azasugar portion of the molecule as the azasugar portion of **2**, 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*S*),4(*R*)-dihydroxypyrrolidine, is a better inhibitor of galactosidase than the azasugar portion of **3**, 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine.[23b] This result also supports the concept that glycosyltransferase reactions

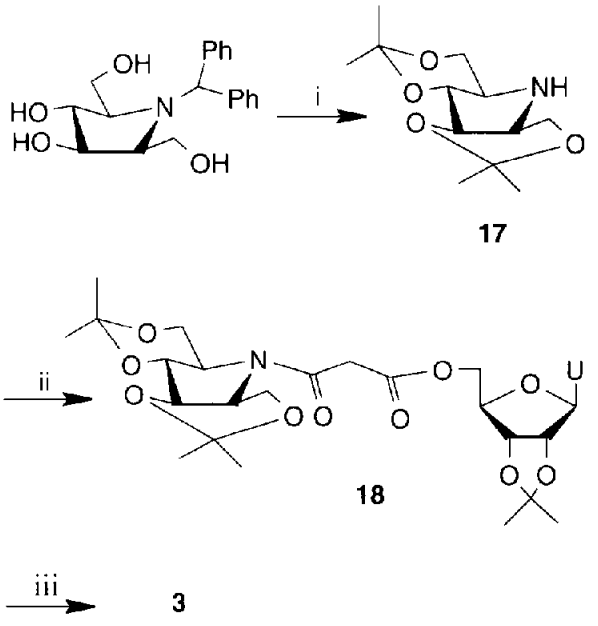


Scheme 2. Reagents and conditions: (i) Meldrum's acid, 66%; (ii) $Pd(OH)_2$, H_2, 50 psi; (iii) **13**, EDAC, HOBT, 62% from **14**; (iv) TFA:H_2O (9:1), 44%.

possess the same donor transition state structure as do the corresponding glycosidase reactions.

A tartaric linkage was incorporated into **4** with the expectation that the increased hydrophilicity (compared to **3**) might increase the inhibitory activity. The preparation is similar to that of compound **3**. Compound **20** was obtained from the coupling of acid **19** and amine **17** using standard amino acid coupling methods. Hydrolysis of methyl ester **20** under basic conditions followed by coupling with amine **11** gave diamide **21** in 41% yield. Deprotection of **21** generated compound **4**, which did not show inhibition towards the enzyme (Scheme 4). This negative result could be due to conformational differences between the tartaric–Mn^{2+} complex and the pyrophosphate–Mn^{2+} complex.

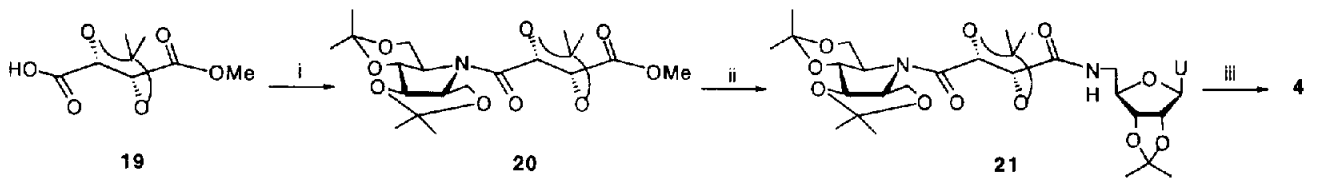
A monosaccharide unit was then used as a surrogate for pyrophosphate to produce the UDP-analogue **5** and the UDP-Gal analogue **6**. Coupling of α-D-galactopyranosyl bromide tetraacetate and **8** at 35 °C gave β-galactoside **22**. Treatment of **22** with sodium methoxide followed by acidic hydrolysis produced analogue **5** (Scheme 5). Kinetic analysis, however, showed that **5** was a very poor



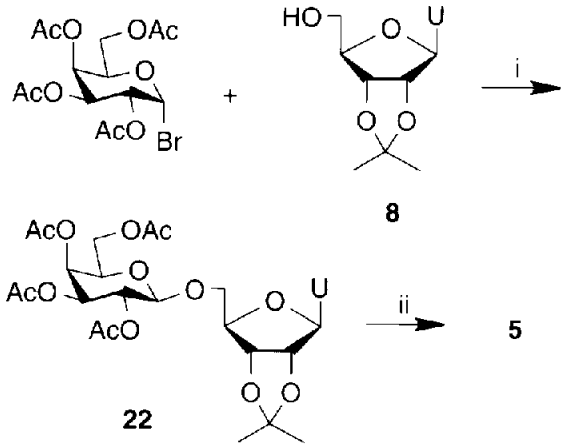
Scheme 3. Reagents and conditions: (i) methoxypropene, CH_2Cl_2-THF, *p*-TsOH; $Pd(OH)_2$, 50 psi, H_2, 75%; (ii) **13**, EDAC, DMAP, CH_2Cl_2, 76%; (iii) TFA:H_2O (9:1), 75%.

inhibitor of the enzyme (K_i > 1 mM). Lactose was converted to peracetate **23** that when treated with hydrogen bromide in acetic acid afforded bromide **24**. Compound **25** was obtained in 60% yield from the glycosylation reaction of **24** and **8**. Following deprotection, compound **6** was obtained in 43% yield (Scheme 6). Compound **6** was found to be a good inhibitor of the enzyme with a K_i = 119.6 μM. This result suggests that the pyrophosphate and metal ion complex may be mimicked by glucose and that this conformation could be crucial for transferase recognition.

Compound **7** was designed to mimic UDP-Gal–Mn^{2+} complex and was expected to be either a substrate or an inhibitor of GalT. The nucleoside-malonic moiety could be a leaving group during the enzymatic reaction though the pKa of the leaving group is relatively higher than that of the phosphate group. The synthesis of **7** started from protected uridine **26** (Scheme 7).[28] Treatment of the latter with benzyl chloromethyl ether in ether afforded the N-3 protected nucleoside **27**. The 5′ hydroxy compound **28** was obtained by treatment of **29** with Bu_4NF. When **28** was melted with Meldrum's acid at 100 °C, **29** was obtained in 64% overall yield from **26**. It was necessary to protect the uridine nitrogen in order to make acid **29** soluble in dichloromethane, as nonpolar solvents gave the best α-selectivity in the following glycosylation reaction. Nucleoside-malonic



Scheme 4. Reagents and conditions: (i) **17**, EDAC, DMAP, 84%; (ii) KOH, MeOH; **11**, EDAC, DMAP, 41%; (iii) TFA:H_2O (9:1), 17%.



Scheme 5. Reagents and conditions: (i) $Hg(CN)_2$, CH_2Cl_2, 35 °C, 66%; (ii) NaOMe, MeOH; AcOH:1 N HCl (3:1), 95%.

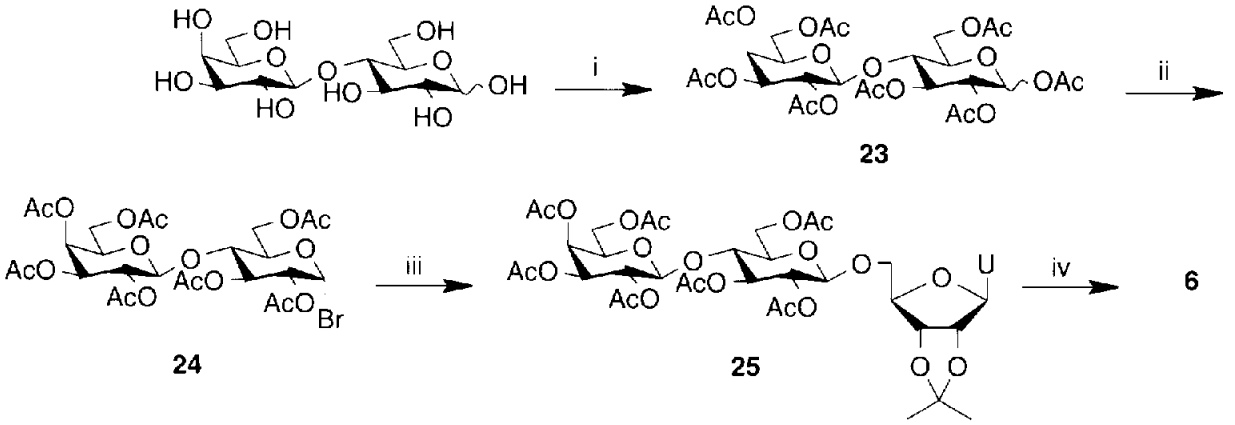
acid **29** was added to a solution of 2,3,4,6-tetrabenzyl galactosyl β-trichloroacetimidate[29] in dichloromethane at −20 °C to provide the protected mimic **30**. Treatment of **30** with $Pd(OH)_2$ in chloroform and ethanol under an atmosphere of hydrogen removed the benzyl and benzyloxy methyl groups while generating enough HCl from reaction with chloroform to hydrolyze the acetonide to afford compound **7**. Enzymatic reactions were attempted at different pH (7.0, 7.5, and 8.0) and with different cations (Mn^{2+}, Mg^{2+}, and Co^{2+}), but no enzymatic product was observed by TLC analysis. Compound **7** was stable for at least 4 days under the room temperature assay conditions stated above. In an inhibition assay, **7** showed no enzyme inhibitory activity under the above conditions. These results seem to reconfirm that malonic ester is not a good pyrophosphate substitute.

In summary, both malonic and tartaric linkages appeared to be ineffective as replacements for pyrophosphate in the development of inhibitors or substrates of GalT. However, the monosaccharide glucose was shown to be a mimic of the pyrophosphate–Mn^{2+} complex, supporting the hypothesis that during the glycosyltransferase reaction, the pyrophosphate and divalent metal ion may form a six-membered complex. Work is in progress to develop more potent glycosyltransferase inhibitors with a monosaccharide spacer between various azasugars and nucleosides.

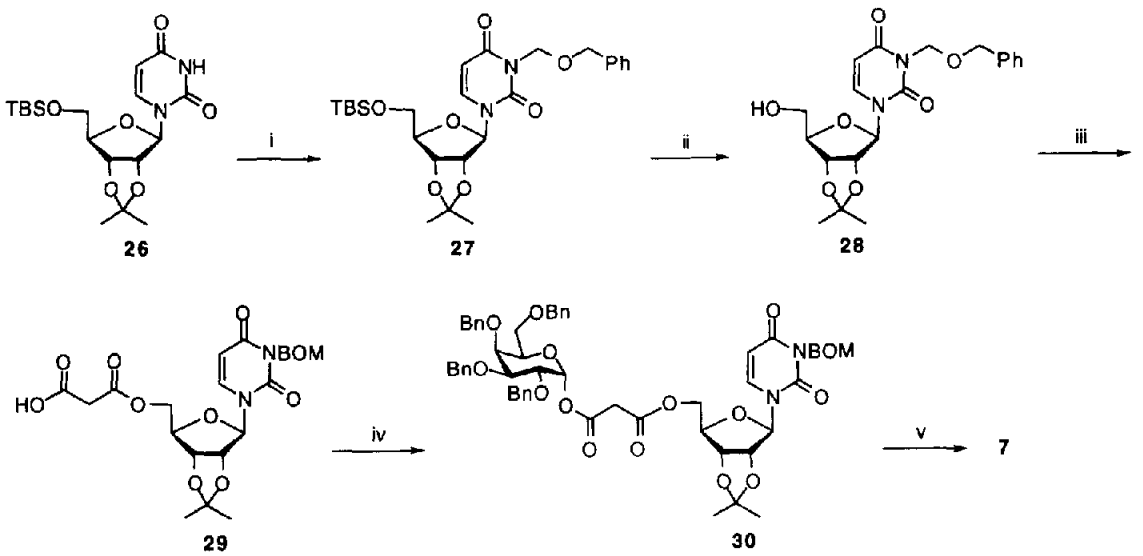
Experimental

2′,3′-*O*-Isopropylidene-5′-*O*-tosyluridine (9). Alcohol **8** (5.4 g, 0.019 mol) was dissolved in pyridine and tosyl chloride (4.3 g, 0.023 mol) was added. The mixture was stirred at room temperature for 3 h and the solvent was evaporated. Water was added to the residue and extracted with ethyl acetate (3 ×). The organic layers were combined, dried over $MgSO_4$ and concentrated. The crude product was purified using a silica gel column (hexane:EtOAc, 1:3–1:8, gradient elution) to afford **9** (6.6 g, 80%) as an oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.55 (s, 3H), 2.45 (s, 3H), 4.26 (m, 2H, ribose-H-5), 4.35 (m, 1H, ribose-H-4), 4.78 (dd, 1H, J = 3.5, 6.5 Hz, ribose-H-3), 4.90 (dd, 1H, J = 2.0, 6.5 Hz, ribose-H-2), 5.66 (d, 1H, J = 2.0 Hz, ribose-H-1), 5.71 (dd, 1H, J = 2.0, 8.0 Hz), 7.23 (d, 1H, J = 8.0 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.77 (d, 2H, J = 8.0 Hz). HRMS calcd for $C_{19}H_{22}N_2O_8SCs$ ($M + Cs^+$) 571.0151, found 571.0152.

2′,3′-*O*-Isopropylidene-5′-azido-5′-deoxyuridine (10). Tosylate **9** (6.6 g, 0.015 mol) was dissolved in DMF (20 mL) and sodium azide (9.8 g, 0.15 mol) was added. The suspension was stirred at 50 °C for 8 h and the solvent evaporated. Water was added and the aqueous solution extracted with ethyl acetate (3 ×). The organic fractions were combined, dried over $MgSO_4$ and concentrated. The residue was purified using a silica gel column (hexane:EtOAc, 1:3) to afford **10** (4.2 g, 91%) as an oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36(s, 3H), 1.57 (s, 3H), 3.63 (d, 2H, J = 5.0 Hz, ribose-H-5), 4.24 (dd, 1H, m, ribose-H-4), 4.83 (dd, 1H, J = 4.0, 6.0 Hz, ribose-H-3), 5.01 (dd, 1H, J = 2.0, 6.5 Hz, ribose-H-2), 5.66 (d, 1H, J = 2.0 Hz, ribose-H-1), 5.78 (d, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.0 Hz), 9.68 (s, 1H, N-H).



Scheme 6. Reagents and conditions: (i) acetic anhydride, pyr, 92%; (ii) hydrogen bromide in acetic acid (30%), CH_2Cl_2; (iii) **8**, $Hg(CN)_2$, CH_2Cl_2, 35 °C, 65%; (iv) NaOMe, MeOH; Dowex 50 [H^+], 43%.



Scheme 7. Reagents and conditions: (i) BOMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 89%; (ii) Bu_4NF, THF, 94%; (iii) Meldrum's acid, 100 °C, 77%; (iv) 2,3,4,6-tetrabenzyl galactosylpyranose β-imidate, CH_2Cl_2, -20 °C, 52%; (v) $Pd(OH)_2$, H_2, EtOH, $CHCl_3$, H_2O, 44%.

HRMS calcd for $C_{12}H_{15}N_3O_8Na$ (M + Na^+) 332.0971, found 332.0969.

Compound 12. Azide **10** (309 mg, 1 mmol) was dissolved in MeOH (5 mL) and Pd/C (10%, 20 mg) was added to the solution. The suspension was stirred under hydrogen (1 atm) for 1 h. The catalyst was filtered through Celite and the solvent evaporated to afford amine **11** which was used for the next step without purification. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36(s, 3H), 1.58 (s, 3H), 2.98 (dd, 1H, J = 6.0, 13.0 Hz, ribose-H-5), 3.06 (dd, 1H, J = 4.5, 13.0 Hz, ribose-H-5), 4.10 (m, 1H, ribose-H-4), 4.78 (dd, 1H, J = 4.5, 6.5 Hz, ribose-H-3), 4.95 (dd, 1H, J = 2.5, 6.5 Hz, ribose-H-2), 5.69 (d, 1H, J = 2.5 Hz, ribose-H-1), 5.73 (d, 1H, J = 8.0 Hz), 7.38 (d, 1H, J = 8.0 Hz). HRMS calcd for $C_{12}H_{18}N_2O_5$ (M + H^+) 284.1246, found 284.1244.

Malonyl monomethyl ester (132 mg, 1 mmol) was dissolved in dry dichloromethane and EDAC (191.7 mg, 1 mmol) and HOBT (135.1 mg, 1 mmol) were added. The mixture was stirred for 5 min and amine **11** was added. The mixture was stirred at room temperature for 3 h, diluted with dichloromethane, and washed with water. The organic layer was dried over $MgSO_4$, concentrated, and the crude product was purified by silica gel chromatography (hexane:EtOAc, 1:3–1:5, gradient elution) to obtain **12** (129 mg, 34%) as an oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.56 (s, 3H), 3.37 (s, 2H), 3.54 (ddd, 1H, J = 4.0, 8.0, 14.5 Hz, ribose-H-5), 3.75 (s, 3H), 3.80 (m, 1H, ribose-H-5), 4.25 (m, 1H, ribose-H-4), 4.79 (dd, 1H, J = 4.0, 6.5 Hz, ribose-H-3), 5.08 (dd, 1H, J = 2.5, 6.5 Hz, ribose-H-2), 5.47 (d, 1H, J = 2.5 Hz, ribose-H-1), 5.76 (d, 1H, J = 8.0 Hz), 7.27 (d, 1H , J = 8.0 Hz).

Compound 1. Ester **12** (129 mg, 0.34 mmol) was dissolved in MeOH:H_2O (3:1, 3 mL) and LiOH 1. The mixture was stirred at 50 °C for 12 h, cooled to room temperature and neutralized to pH 7 with satd $NaHCO_3$. The aqueous solution was concentrated, the residue applied to a Bio Gel P2 column and eluted with water to obtain **1** (39 mg, 37%) as a colorless foam. ^1H NMR (500 MHz, CD_3OD) δ 3.14 (m, 2H), 3.52 (dd, 1H, J = 4.5, 14.0 Hz, ribose-H-5), 3.63 (dd, 1H, J = 5.5, 14.0 Hz, ribose-H-5), 4.00 (m, 1H, ribose-H-4), 4.07 (dd, 1H, J = 5.0, 5.5 Hz, ribose-H-3), 4.21 (dd, 1H, J = 5.0, 5.5 Hz, ribose-H-2), 5.76 (d, 1H, J = 8.0 Hz), 5.82 (d, 1H, J = 5.0 Hz, ribose-H-1), 7.74 (d, 1H, J = 8.0 Hz). ^{13}C NMR (125 MHz, D_2O) δ 40.82, 70.68, 73.67, 82.41, 90.62, 102.81, 142.58, 142.64, 151.98, 166.63, 170.02, 172.41. HRMS calcd for $C_{12}H_{16}N_3O_8$ (M + H^+) 330.0937, found 330.0945.

Compound 13. A mixture of **8** (1.0 g, 3.52 mmol) and Meldrum's acid (507 mg, 3.52 mmol) was melted at 100 °C for 1 h with magnetic stirring, followed by 30 min under aspirator vacuum. The crude product was purified by silica gel chromatography to afford **13** ($CHCl_3$:MeOH:H_2O, 8:2:0.2, 860 mg, 66%) as a colorless glass. ^1H NMR (400 MHz, CD_3OD) δ 1.26 (s, 3H), 1.45 (s, 3H), 4.28 (br, s, 3H), 4.92 (dd, J = 2.3, 6.4 Hz, 1H), 5.65 (d, J = 8.0 Hz, 1H), 5.71 (d, J = 2.3 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 25.52, 27.44, 82.08, 85.48, 85.77, 94.70, 102.88, 115.28, 144.01, 151.75, 166.06, 169.87, 172.75. HRMS calcd for $C_{15}H_{19}N_2O_6$ (M + H^+) 371.1091, found 371.1090.

Compound 16. Compound **14** was dissolved in MeOH and $Pd(OH)_2$ was added. The suspension was shaken under a hydrogen atmosphere (55 psi) overnight. The catalyst was filtered and the solvent evaporated and dried under vacuum to obtain crude **15**, which was used without further purification. Acid **13** (90.0 mg, 0.24 mmol), EDAC (53.8 mg, 0.28 mmol), and HOBT (37.9 mg, 0.28 mmol) were dissolved in dry dichloromethane and the mixture stirred for 5 min followed by the addition of amine **15** (82.5 mg, 0.187 mmol). The mixture was stirred for 3 h and diluted with dichloromethane. The organic layer was washed with water,

dried over $MgSO_4$, and concentrated. Silica gel chromatography (EtOAc:hexane, 2:1–6:1, gradient elution) gave compound **16** (92 mg, 62%) as an oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.06 (s, 9H), 1.35 (s, 3H), 1.37 (s, 3H), 1.57 (s, 3H), 1.58 (s, 3H), 3.58 (dd, 1H, J = 4.0, 11.0 Hz), 3.94 (dd, 1H, J = 9.0, 11.0 Hz), 4.13 (m, 2H), 4.25 (dd, 1H, J = 4.5, 9.0 Hz), 4.38 (m, 3H), 4.83 (m, 1H, ribose-H-4), 5.00 (m, 4H, ribose-H-2,3,5,5), 5.65 (d, 1H, J = 2.5 Hz, ribose-H-1), 5.69 (d, 1H, J = 8.0 Hz), 7.31 (d, 1H, J = 8.0 Hz), 7.37–7.42 (m, 6H), 7.65–7.66 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.17, 19.04, 21.44, 25.25, 25.91, 26.83, 27.10, 41.99, 54.52, 60.82, 62.12, 64.28, 68.61, 72.18, 80.71, 84.17, 84.79, 94.42, 102.73, 114.69, 114.71, 139.98, 130.01, 135.49, 135.63, 142.32, 149.84, 162.67, 166.88, 167.31. HRMS calcd for $C_{20}H_{51}N_3O_{12}SiCs$ ($M + Cs^+$) 926.2296, found 926.2270.

Compound 2. Ester **16** (25 mg, 0.032 mmol) was dissolved in TFA:H_2O (9:1, 2 mL) and the mixture stirred at room temperature for 5 min. Water (2 mL) was added and the solvent evaporated. Purification by silica gel chromatography ($CHCl_3$:MeOH, 3:1) and Bio Gel P2 column chromatography (water) afforded compound **2** (6.6 mg, 44%) as a colorless foam. Both ^1H NMR and ^{13}C NMR spectra showed the existence of two rotamers at room temperature. ^1H NMR (500 MHz, CD_3OD) δ 3.84–3.89 (m, 5H), 4.07–4.40 (m, 9H), 5.76 (2d, 1H, J = 8.0 Hz), 5.85 (2d, 1H, J = 4.5 Hz), 7.72 (2d, 1H, J = 8.0 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 61.4, 61.7, 61.9, 62.3, 63.9, 64.4, 65.3, 71.1, 71.2, 71.4, 72.0, 72.2, 75.0, 75.7, 83.1, 83.1, 86.4, 90.6, 91.0, 91.2, 102.7, 103.0, 103.1, 142.6, 142.7, 142.7, 163.2, 163.4, 166.2. HRMS calcd for $C_{18}H_{25}N_3O_{12}Na$ ($M + Na^+$) 498.1336, found 498.1350.

Compound 17. To a solution of *N*-diphenylmethyl 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (150 mg, 0.45 mmol) and methoxypropene (0.44 mL, 4.5 mmol) in CH_2Cl_2:THF (5 mL) was added *p*-toluenesulfonic acid (100 mg) at room temperature. After 12 h, saturated aqueous $NaHCO_3$ was added and the resulting mixture extracted with dichloromethane. The organic layer was washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated. A solution of this residue in MeOH (10 mL) was hydrogenated with $Pd(OH)_2$ (40 mg) under 50 psi of hydrogen for 4 h. The catalyst was filtered and the solvent removed under vacuum. The residue was purified by silica gel column chromatography (EtOAc) to give **17** (82 mg, 75%) as an oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (s, 3H), 1.38 (s, 3H), 1.48 (s, 3H), 1.55 (s, 3H), 2.78 (dt, 1H, J = 4.5, 10.3 Hz), 3.50 (dd, 1H, J = 2.3, 7.8 Hz), 3.55 (t, 1H, J = 10.3 Hz), 3.72 (m, 1H), 3.83 (dd, 1H, J = 4.5, 10.3 Hz), 3.85 (t, 1H, J = 10.3 Hz), 4.04 (dd, 1H, J = 4.5, 10.3 Hz), 4.21 (dd, 1H, J = 6.6, 7.8 Hz).

Compound 18. A mixture of **17** (24.3 mg, 0.1 mmol), acid **13** (37.0 mg, 0.1 mmol), EDAC (210.9 mg, 0.11 mmol), and DMAP (12.2 mg, 0.1 mmol) in dichloromethane (4 mL) was stirred at room temperature for one day. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to afford **18** ($CHCl_3$:MeOH, 20:1, 45.2 mg, 76%) as an oil. ^1H NMR (DMSO-d_6, 400 MHz, at 343 K) δ 1.30 (s, 3H), 1.32 (s, 3H), 1.33 (s, 3H), 1.35 (s, 3H), 1.46 (s, 3H), 1.50 (s, 3H), 3.43 (m, 1H), 3.56 (m, 1H), 4.05 (m, 2H), 4.09 (t, 1H, J = 10.2 Hz), 4.28 (m, 2H), 4.79 (dd, 1H, J = 3.6, 6.4 Hz), 4.99 (dd, 1H, J = 2.4, 6.4 Hz), 5.59 (d, 1H, J = 8.0 Hz), 5.80 (d, 1H, J = 2.4 Hz), 7.62 (d, 1H, J = 8.0 Hz). HRMS calcd for $C_{27}H_{37}N_3O_{12}Cs$ ($M + Cs^+$) 728.1432, found 728.1450.

Compound 3. A solution of **18** (30 mg, 0.050 mmol) in 90% trifluoroacetic acid (2 mL) was stirred for 10 min at room temperature. Water (2 mL) was added and the reaction mixture concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH:10% NH_4OH, 6:4:1) to give **3** (18.0 mg, 75%) as a colorless foam. ^1H NMR (D_2O, 400 MHz) δ 3.68 (dd, 1H, J = 7.0, 12.3 Hz), 3.71–3.88 (m, 4H), 3.97 (dd, 1H, J = 4.3, 11.8 Hz), 4.30 (m, 2H), 4.24–4.38 (m, 3H), 4.48 (m, 2H), 5.86 (d, 1H, J = 4.3 Hz), 5.89 (d, 1H, J = 8.1 Hz), 7.72 (d, 1H, J = 8.1 Hz). HRMS calcd for $C_{18}H_{26}N_3O_{12}$ ($M + H^+$) 476.1516, found 476.1510.

Compound 20. A mixture of azasugar **17** (50 mg, 0.21 mmol), tartaric acid monoester **19** (46 mg, 0.23 mmol), EDAC (43 mg, 0.23 mmol), and DMAP (25 mg, 0.21 mmol) in dichloromethane (5 mL) was stirred at room temperature for 13 h. The solvent was removed under reduced and the residue was purified by silica gel column chromatography to afford **20** (hexane:EtOAc, 2:1, 74 mg, 84%) as an oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 6H), 1.43 (s, 3H), 1.47 (s, 3H), 1.52 (s, 3H), 1.58 (s, 3H), 3.27 (m, 1H), 3.67 (t, 1H, J = 9.3 Hz), 3.81 (s, 3H), 4.09–4.15 (m, 2H), 2.17 (dd, 1H, J = 8.3, 10.6 Hz), 4.27–4.38 (m, 3H), 4.63 (m, 2H), 5.33 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.26, 24.98, 25.86, 26.10, 27.56, 28.96, 52.71, 53.66, 55.92, 60.30, 65.64, 70.11, 75.21, 76.89, 77.02, 99.97, 100.53, 113.20. HRMS calcd for $C_{20}H_{32}NO_9$ ($M + H^+$) 430.2077, found 430.2069.

Compound 21. To a solution of **20** (70 mg, 0.16 mmol) in MeOH (1 mL) was added a KOH (14.0 mg, 0.24 mmol)/MeOH solution (1 mL) at room temperature and the reaction mixture stirred for 7 h. The solvent was removed under reduced pressure and the residue dissolved in water (10 mL) and the pH of the solution was adjusted to 3.5. The aqueous solution was saturated with brine and extracted with ether (3 ×). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated. A mixture of this residue, amine **11** (22 mg, 0.0776 mmol), EDAC (16.3 mg 0.0854 mmol), and DMAP (19.5 mg, 0.0776 mmol) in dichloromethane (2 mL) was stirred at room temperature for 11 h. The solvent was removed under reduced pressure and the residue purified by silica gel column chromatography to afford **21** ($CHCl_3$:MeOH, 20:1, 21.6 mg, 41%) as an oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.34 (s, 3H), 1.37 (s, 3H), 1.40 (s, 3H), 1.43 (s, 3H), 1.50 (s, 3H), 1.55 (s, 3H), 1.59 (s, 3H), 3.25 (m, 1H), 3.65 (m,

1H), 3.73 (dd, 1H, J = 5.1, 11.5 Hz), 3.82 (dd, 1H, J = 5.5, 11.5 Hz), 3.84 (m, 2H), 4.12 (m, 2H), 4.17 (d, 1H, J = 8.3, 10.5 Hz), 4.27 (m, 1H), 4.33 (m, 1H), 4.38 (dd, 1H, J = 5.1, 9.0 Hz), 4.90 (dd, 1H, J = 3.7, 6.5 Hz), 4.99 (dd, 1H, J = 2.3, 6.5 Hz), 5.09 (d, 1H, J = 6.5 Hz), 5.70 (d, 1H, J = 2.3 Hz), 5.76 (d, 1H, J = 8.0 Hz), 7.34 (d, 1H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.26, 25.01, 25.18, 26.21, 27.03, 27.62, 28.97, 44.20, 53.69, 55.89, 60.29, 60.66, 65.62, 67.07, 70.15, 82.00, 84.46, 94.58, 96.08, 100.52, 102.67, 102.78, 114.74, 142.18, 143.58, 149.79, 162.53, 163.05. HRMS calcd for $C_{31}H_{44}N_4O_{13}$ (M^+) 680.2905, found 680.2884.

Compound 4. The solution of **21** (12 mg, 0.0176 mmol) in 90% TFA (1 mL) was stirred for 30 min at room temperature. Water (10 mL) was added and the mixture concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH:10% NH_4OH, 6:4:1) to give **4** (1.6 mg, 17%) as a colorless foam. ^{1}H NMR (D_2O, 400 MHz) δ 3.62 (d, 2H, J = 4.4 Hz), 3.66 (dd, 1H, J = 7.4, 12.1 Hz), 3.73 (m, 1H), 3.78 (m, 1H), 3.80 (m, 1H), 3.88 (dd, 1H, J = 3.6, 12.0 Hz), 4.00 (dd, 1H, J = 4.4, 12.0 Hz), 4.11–4.21 (m, 4H), 4.37 (d, 1H, J = 4.7 Hz), 4.40 (d, 1H, J = 4.7 Hz), 4.89 (d, 1H, J = 4.7 Hz), 5.80 (d, 1H, J = 4.3 Hz), 5.89 (d, 1H, J = 8.1 Hz), 7.68 (d, 1H, J = 8.1 Hz). HRMS calcd for $C_{19}H_{28}N_4O_{13}Cs$ ($M + Cs^+$) 653.0707, found 653.0702.

2′,3′-*O*-Isopropylidene-5′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactoyranosyl) uridine (22). A suspension of **8** (1.0 g, 3.52 mmol) in anhydrous dichloromethane (20 mL) was heated to 35 °C and mercury cyanide (1.38, 5.46 mmol) was added. α-D-Galactopyranosyl bromide tetraacetate (2.17 g, 5.28 mmol) in anhydrous dichloromethane (20 mL) was added within 2 h. After stirring at 35 °C for 6 h, more bromide (723 mg, 1.76 mmol) was added within 0.5 h. The reaction was stirred for an additional 12 h before the catalyst was filtered. The filtrate was concentrated and purified by silica gel chromatography (hexane:EtOAc, 1:4) to give **22** (1.43 g, 66%, R_f = 0.4, hexane:EtOAc, 1:8) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.57 (s, 3H), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.21 (s, 3H, OAc), 3.66 (dd, 1H, J = 3.0, 10.5 Hz, ribose-H-5), 3.92 (m, 1H, Gal-H-5), 4.10 (dd, 1H, J = 4.0, 11.0 Hz, Gal-H-6), 4.18 (dd, 1H, J = 6.5, 11.0 Hz, Gal-H-6), 4.21 (dd, 1H, J = 2.0, 10.5 Hz, ribose-H-5), 4.45 (m, 1H, ribose-H-4), 4.47 (d, 1H, J = 7.5 Hz, Gal-H-1), 4.66 (dd, 1H, J = 2.0, 6.0 Hz, ribose-H-3), 4.76 (dd, 1H, J = 2.5, 6.0 Hz, ribose-H-2), 5.02 (dd, 1H, J = 3.5, 10.5 Hz, Gal-H-3), 5.09 (dd, 1H, J = 7.5, 10.5 Hz, Gal-H-2), 5.40 (dd, 1H, J = 3.5, 3.5 Hz, Gal-H-4), 5.75 (d, 1H, J = 8.0 Hz), 5.95 (d, 1H, J = 2.5 Hz, ribose-H-1), 7.61 (d, 1H, J = 8.0 Hz), 8.89 (s, 1H, N-H). HRMS calcd for $C_{26}H_{34}N_2O_{15}Cs$ ($M + Cs^+$) 747.1014, found 747.1010.

5′-*O*-β-D-Galactopyranosyl uridine (5). β-Galactoside **22** (600 mg, 0.98 mmol) was dissolved in anhydrous methanol (10 mL) and NaOMe (300 mg, 5.55 mmol) was added. The mixture was stirred at room temperature for 0.5 h. The solid was filtered, the solvent evaporated, and the residue redissolved in AcOH:1 N HCl (3:1, 5 mL) and the mixture stirred at room temperature for 3 h. The solution was then neutralized to pH 7 by the addition of 5 N NaOH solution and the mixture concentrated. The residue was purified using a Bio Gel P2 column to give compound **5** (380 mg, 95%, R_f = 0.3, $CHCl_3$:MeOH:H_2O, 3:2:0.25) as a colorless foam. ^{1}H NMR (500 MHz, D_2O) δ 3.49 (dd, 1H, J = 8.0, 10.0 Hz, Gal-H-2), 3.59 (dd, 1H, J = 3.5, 10.0 Hz, Gal-H-3), 3.68 (dd, 1H, J = 4.0, 11.5 Hz, Gal-H-6), 3.72 (dd, 1H, J = 8.5, 11.5 Hz, Gal-H-6), 3.82 (m, 1H, Gal-H-5), 3.86 (m, 1H, Gal-H-4), 4.18 (m, 1H, ribose-H-5), 4.20 (m, 2H, ribose-H-4, ribose-H-5), 4.26 (dd, 1H, J = 5.0, 5.5 Hz, ribose-H-3), 4.32 (dd, 1H, J = 4.5, 5.5 Hz, ribose-H-2), 4.40 (d, 1H, J = 8.0 Hz, Gal-1-H), 5.85 (d, 1H, J = 8.0 Hz), 5.87 (d, 1H, J = 4.5 Hz, ribose-H-1), 7.90 (d, 1H, J = 8.0 Hz). ^{13}C NMR (125 MHz, D_2O) δ 61.54, 68.97, 69.04, 70.11, 71.31, 73.17, 74.19, 75.65, 83.52, 89.69, 102.67, 103.36, 142.50, 152.20 166.86. HRMS calcd for $C_{15}H_{22}N_2O_{11}Na$ ($M + Na^+$) 429.1121, found 429.1116.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactoyranosyl)-D-glucopyranose (23). A mixture of lactose (6.84 g, 0.02 mol) and acetic anhydride (40.8 g, 0.4 mol) in pyridine was stirred at room temperature overnight. After evaporation of the solvent, satd $NaHCO_3$ was added to the residue and the solution extracted with ethyl acetate (3×). The organic fractions were combined, dried over $MgSO_4$ and concentrated. Purification by silica gel chromatography (EtOAc:hexane, 1:1) afforded **23** (12.5 g, 92%, α:β, 1:1.37.) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) α form δ 1.97–2.19 (8xs, 8x3H, 8xOAc), 3.84 (dd, 1H, J = 9.5, 9.5 Hz, H-4), 4.01 (m, 1H, H-5), 4.16 (m, 1H, H-6), 4.47 (m, 1H, H-6), 4.49 (m, 1H, H-6), 4.95 (m, 2H, H-3′ and H-5′), 5.01 (dd, 1H, J = 3.5, 10.5 Hz, H-2), 5.12 (m, 3H, H-2′, 2xH-6′), 5.36 (dd, 1H, J = 4.0, 4.0 Hz, H-4′), 5.47 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 6.26 (d, 1H, J = 3.5 Hz, H-1). β form δ 1.97–2.19 (8xs, 8x3H, 8xOAc), 3.77 (m, 1H, H-5), 3.85 (dd, 1H, J = 9.0, 9.5 Hz, H-4), 4.16 (m, 1H, H-6), 4.47 (m, 1H, H-6), 4.49 (d, 1H, J = 8.0 Hz, H-1′), 4.95 (m, 2H, H-3′ and H-5′), 5.12 (m, 3H, H-2′ and 2xH-6′), 5.05 (dd, 1H, J = 8.0, 9.5 Hz, H-3), 5.25 (dd, 1H, J = 9.5, 9.5 Hz, H-3), 5.36 (dd, 1H, J = 4.0, 4.0 Hz, H-4′), 5.68 (d, 1H, J = 8.0 Hz, H-1). HRMS calcd for $C_{28}H_{38}O_{19}Cs$ ($M + Cs^+$) 811.1062, found 811.1070.

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-α-D-glucopyranosyl bromide (24). Peracetate **23** (2.0 g, 2.95 mmol) was dissolved in dichloromethane (50 mL) and hydrogen bromide in acetic acid (30%, 20 mL) was added at 0 °C. The mixture was warmed up to room temperature and stirred for 2 h. Ice cold water was added to the mixture with stirring followed by extraction of the aqueous layer with dichloromethane (3×). The combined organic layers were dried over $MgSO_4$ and concentrated. Bromide **24** (α form) was used for glycosylation without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.10 (7xs, 7x3H, 7xOAc), 3.88 (m, 2H), 4.08 (dd, 1H, J = 7.5,

11.0 Hz, H-6), 4.16 (dd, 1H, J = 6.0, 11.0 Hz, H-6), 4.20 (m, 2H), 4.50 (m, 1H), 4.52 (d, 1H, J = 8.0 Hz, H-1′) 4.77 (dd, 1H, J = 4.0, 10.0 Hz, H-2), 4.96 (dd, 1H, J = 3.5, 10.5 Hz, H-3′), 5.13 (dd, 1H, J = 8.0, 10.5 Hz, H-2′), 5.36 (dd, 1H, J = 3.5, 3.5 Hz, H-4′), 5.56 (dd, 1H, J = 9.5, 9.5 Hz), 6.53 (d, 1H, J = 4.0 Hz, H-1).

2′,3′-*O*-Isopropylidene-5′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl) uridine (25). Alcohol **8** (284 mg, 1.00 mmol) and mercury cyanide (505 mg, 2.00 mmol) were suspended in dry dichloromethane (5 mL) and **24** (800 mg, 1.14 mmol) in dry dichloromethane was added within 1 h and the mixture stirred at 35 °C for 12 h. The reaction mixture was filtered, poured into water, and extracted with dichloromethane (3×). The organic layers were combined, dried over $MgSO_4$, and concentrated. The crude residue was purified by silica gel chromatography (EtOAc:hexane, 4:1–8:1, gradient elution) to afford **25** (585 mg, 65%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.58 (s, 3H), 1.89–2.16 (7xs, 7x3H, 7xOAc), 3.64 (m, 2H, ribose-H-5, Glc-H-5), 3.80 (dd, 1H, J = 9.5, 9.5 Hz, Glc-H-4), 3.92 (m, 1H, Gal-H-5), 4.14 (m, 5H, ribose-H-5, 2xGlc-H-6, 2xGlc-H-6), 4.42 (m, 1H, ribose-H-4), 4.49 (d, 2H, J = 8.0 Hz, Glc-H-1, Gal-H-1), 4.67 (dd, 1H, J = 3.0, 6.5 Hz, ribose-H-3), 4.74 (dd, 1H, J = 2.5, 6.5 Hz, ribose-H-2), 4.81 (dd, 1H, J = 8.0, 10.0 Hz, Glc-H-2), 4.97 (dd, 1H, J = 3.5, 10.5 Hz, Gal-H-3), 5.11 (dd, 1H, J = 8.0, 10.5 Hz, Gal-H-2), 5.22 (dd, 1H, J = 10.0, 10.0 Hz, Glc-H-3), 5.36 (dd, 1H, J = 1.0, 3.5 Hz, Gal-H-4), 5.75 (d, 1H, J = 8.5 Hz), 5.89 (d, 1H, J = 2.5 Hz, ribose-H-1), 7.54 (d, 1H, J = 8.5 Hz), 8.84 (b, 1H, N-H). HRMS calcd for $C_{38}H_{50}N_2O_{23}Cs$ (M + Cs^+) 1035.1859, found 1035.1899.

5′-*O*-β-D-Galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl uridine (6). Compound **25** (585 mg, 0.65 mmol) was dissolved in anhydrous MeOH and NaOMe (100 mg, 1.85 mmol) was added and the mixture stirred at room temperature for 1 h. Dowex 50 (H^+) (~1 g) and H_2O (2 mL) were added, and the mixture was stirred at 50 °C for 12 h. The resin was then filtered and the solution neutralized to pH 7 using satd $NaHCO_3$. After concentration, the crude residue was applied to a Bio Gel P2 column and eluted with water to obtain **6** (245 mg, 67 %) as a colorless foam. ^{1}H NMR (500 MHz, D_2O) δ 3.30 (dd, 1H, J = 8.0, 9.5 Hz), 3.48 (dd, 1H, J = 8.0, 10.0 Hz), 3.55 (m, 1H, H-5), 3.60 (m, 3H), 3.72 (m, 4H), 3.85 (m, 2H), 3.93 (dd, 1H, J = 2.0, 12.0 Hz), 4.17 (m, 1H), 4.22 (m, 1H), 4.27 (dd, 1H, J = 5.0, 5.5 Hz, ribose-H-3), 4.32 (dd, 1H, J = 4.5, 5.0 Hz, ribose-H-2), 4.39 (d, 1H, J = 8.0 Hz), 4.50 (d, 1H, J = 8.0 Hz), 5.82 (d, 1H, J = 8.0 Hz), 5.86 (d, 1H, J = 4.5 Hz, ribose-H-1), 7.86 (d, 1H, J = 8.0 Hz). ^{13}C NMR (125 MHz, D_2O) δ 60.53, 61.48, 68.99, 70.08, 71.39, 72.94, 73.42, 74.19, 74.84, 75.25, 75.81, 78.84, 83.41, 89.82, 102.61, 103.43, 142.42, 152.33, 166.89. HRMS calcd for $C_{21}H_{32}N_2O_{16}Cs$ (M + Cs^+) 701.0806, found 701.0810.

1-(5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3-(benzyloxymethyl)uracil (27). To a 0 °C solution of 1-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil **(26)** (2.2 g, 5.4 mmol) and *i*-Pr_2NEt (4.7 mL, 27.0 mmol) in dichloromethane (50 mL) was added benzyl chloromethyl ether (60%, 1.9 mL, 8.1 mmol) in a dropwise manner. The reaction mixture was stirred at room temperature for 4 h then MeOH was added (2 mL) and the reaction stirred for 30 min. The reaction mixture was poured into saturated $NaHCO_3$ (100 mL) and extracted with chloroform (5×). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent evaporated. Purification of the residue by silica gel column chromatography (EtOAc:hexanes, 1:3) afforded **27** (2.5 g, 89%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.04 (s, 3H), 0.85 (s, 9H), 1.32 (s, 3H), 1.55 (s, 3H), 3.73 (dd, 1H, J = 11.5, 3.1 Hz), 3.87 (dd, 1H, J = 11.5, 2.4 Hz), 4.31 (dd, 1H, J = 5.4, 2.8 Hz), 4.62 (dd, 1H, J = 6.1, 2.6 Hz), 4.66 (s, 2H), 4.70 (dd, 1H, J = 6.1, 2.8 Hz), 5.42 (d, 1H, J = 9.7 Hz), 5.46 (d, 1H, J = 9.7 Hz), 5.65 (d, 1H, J = 8.2 Hz), 5.88 (d, 1H, J = 2.6 Hz), 7.34–7.17 (m, 5H), 7.61 (d, 1H, J = 8.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.69, −5.66, 18.09, 25.17, 25.58, 27.09, 63.15, 70.08, 72.02, 80.15, 85.46, 86.78, 92.86, 101.34, 113.67, 127.38, 128.10, 137.78, 138.96, 150.72, 162.45. HRMS: calcd for $C_{26}H_{38}O_7N_2Si$ (M + Na^+) 541.2346, found 541.2369.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-3-(benzyloxymethyl)uracil (28). To a solution of **27** (822 mg, 1.5 mmol) in tetrahydrofuran (20 mL) was added a 1 M solution of Bu_4NF (1.74 mL). The reaction mixture was stirred for 3 h, poured into ethyl acetate (100 mL) and washed twice with H_2O. The organic layer was dried over $MgSO_4$, filtered, and the solvent evaporated. The residue was purified by silica gel column chromatography (EtOAc:hexanes, 1:1) to afford **28** (597 mg, 94%) as an oil. $[\alpha]^{25}_D$ −13.0 (*c* 0.6; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.55 (s, 3H), 3.76–3.66 (m, 1H), 3.87–3.78 (m, 1H), 7.50 (d, 1H, J = 8.1 Hz), 4.26 (dd, 1H, J = 5.9, 3.0 Hz), 4.66 (s, 2H), 4.81 (dd, 1H, J = 6.3, 2.6 Hz), 4.84 (dd, 1H, J = 6.3, 3.0 Hz), 5.40 (d, 1H, J = 9.7 Hz), 5.44 (d, 1H, J = 9.8 Hz), 5.67 (d, 1H, J = 8.2 Hz), 5.68 (d, 1H, J = 2.1 Hz), 7.34–7.20 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.02, 26.96, 62.10, 70.14, 72.12, 80.20, 84.22, 86.72, 94.80, 101.38, 113.87, 127.38, 127.52, 128.09, 137.46, 140.78, 150.75, 162.64. HRMS: calcd for $C_{20}H_{24}O_7N_2$ (M + Na^+) 427.1481, found 427.1475. Anal. calcd for $C_{20}H_{24}O_7N_2$: C, 59.39; H, 5.98; N, 6.93. Found: C, 59.08; H, 6.09; N, 7.01.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-3-(benzyloxymethyl)uracil-5′-malonic acid (29). Alcohol **28** (597 mg, 1.48 mmol) and Meldrum's acid (212 mg, 1.48 mmol) were melted together at 100 °C for 1 h with magnetic stirring, followed by 30 min under aspirator vacuum. The reaction mixture was cooled and the residue purified by silica gel column chromatography (MeOH:$CHCl_3$, 1:4) to afford **29** (559 mg, 77%) as a clear glass. $[\alpha]^{25}_D$ +11.2 (*c* 0.4; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 3H), 1.54 (s, 3H), 3.37 (br s, 2H), 4.40-4.31 (m, 4H), 4.66 (s, 2H), 4.79 (dd, 1H, J = 6.3, 2.8 Hz), 4.89 (dd, 1H, J = 6.5, 1.9 Hz), 5.40 (d, 1H, J = 9.8 Hz), 5.44 (d, 1H, J = 9.8 Hz), 5.64 (d, 1H, J = 1.9

Hz), 5.77 (d, 1H, J = 8.1 Hz), 7.33–7.20 (m, 6H), 8.70 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.17, 27.02, 41.16, 64.74, 70.30, 72.29, 80.54, 84.58, 84.82, 94.91, 101.78, 114.42, 127.60, 127.69, 128.26, 137.58, 140.72, 150.62, 163.09, 166.92, 169.77. HRMS: calcd for $C_{23}H_{26}O_{10}N_2$ (M + H^+) 491.1666, found 491.1674.

Compound 30. To a 20 °C solution of 2,3,4,6-tetrabenzyl galactosyl β-trichloroacetimidate (210 mg, 0.31 mmol) in CH_2Cl_2 (15 mL) was added **29** (285 mg, 0.58 mmol) dissolved in dichloromethane (3 mL) in a dropwise manner. The reaction mixture was allowed to warm to room temperature, the solvent evaporated and the residue purified by silica gel column chromatography (EtOAc:hexanes, 1:1) to afford **30** (161 mg, 52%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.56 (s, 3H), 3.50–3.47 (m, 2H), 3.87 (dd, 1H, J = 10.1, 2.6 Hz), 4.07–4.01 (m, 2H), 4.18 (dd, 1H, J = 10.0, 3.64 Hz), 4.99–4.30 (m, 17 H), 5.42 (d, 1H, J = 9.8 Hz), 5.47 (d, 1H, J = 9.8 Hz), 5.61 (d, 1H, J = 1.9 Hz), 5.74 (d, 1H, J = 5.74 Hz), 6.38 (d, 1H, J = 3.7 Hz), 7.15 (d, 1H, J = 8.1 Hz), 7.40–7.25 (m, 26H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.18, 27.02, 41.20, 64.61, 68.13, 70.20, 72.01, 72.16, 72.86, 73.35, 73.38, 74.17, 74.79, 74.93, 78.43, 80.61, 84.33, 84.89, 92.15, 94.98, 102.13, 114.45, 127.27, 127.48, 127.52, 127.57, 127.67, 127.71, 127.84, 128.04, 128.17, 128.20, 128.28, 128.31, 137.62, 137.78, 138.23, 138.34, 140.57, 150.62, 162.34, 164.93, 165.48. HRMS: calcd for $C_{57}H_{60}O_{15}N_2$ (M + Cs^+) 1145.3048, found 1145.2980.

Compound 7. $Pd(OH)_2$ (100 mg) was suspended in a solution of **30** (148 mg, 0.146 mmol) dissolved in EtOH (20 mL), $CHCl_3$ (5 mL), and H_2O (1 mL). After purging with argon, H_2 was bubbled through the reaction mixture. After 6 h, the reaction mixture was filtered through Celite and the solvent evaporated. The residue was purified through silica gel using water as eluent to give a white foam (112 mg). A portion of the solid (64 mg, 57%) was applied to a Bio Gel P-2 (fine, 71 g, H_2O) column to afford **7** (18 mg, 44%) as a white foam. ^{13}C NMR (100 MHz, D_2O) δ 45.82, 63.40, 70.75, 71.15, 71.57, 71.71, 72.89, 74.27, 75.19, 77.57, 91.52, 94.69, 98.86, 104.89, 143.86, 154.08, 162.54, 168.62, 173.02. HRMS: calcd for $C_{18}H_{24}N_2O_{14}$ (M + H^+) 493.1306, found 493.1297.

Inhibitor Analysis

The galactosyltransferase assays were carried out as previously described.[6,7]

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