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Chemoenzymatic Synthesis of Uridine Diphosphate-GlcNAc and Uridine Diphosphate-GalNAc Analogs for the Preparation of Unnatural Glycosaminoglycans

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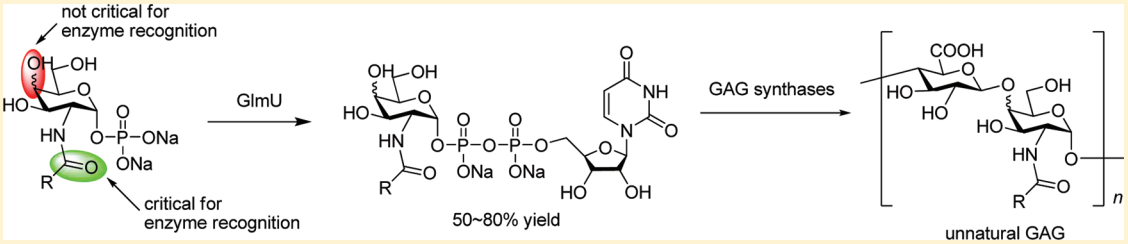
Supporting Information



ABSTRACT: Eight *N*-acetylglucosamine-1-phosphate and *N*-acetylgalactosamine-1-phosphate analogs have been synthesized chemically and were tested for their recognition by the GlmU uridyltransferase enzyme. Among these, only substrates that have an amide linkage to the C-2 nitrogen were transferred by GlmU to afford their corresponding uridine diphosphate(UDP)-sugar nucleotides. Resin-immobilized GlmU showed comparable activity to nonimmobilized GlmU and provides a more facile final step in the synthesis of an unnatural UDP-donor. The synthesized unnatural UDP-donors were tested for their activity as substrates for glycosyltransferases in the preparation of unnatural glycosaminoglycans in vitro. A subset of these analogs was useful as donors, increasing the synthetic repertoire for these medically important polysaccharides.

1. INTRODUCTION

Glycosaminoglycans (GAGs) are a family of polysaccharides that are prevalent both as free glycans and in the core structures of glycans in glycoproteins[1] and glycolipids,[2] affecting cell−cell interactions during various metabolic processes.[3] They are composed of repeating disaccharide units containing hexosamine residues; the other part of the repeat is often a uronic acid residue. *N*-Acetylglucosamine (GlcNAc) is found in heparosan, heparin, heparan sulfate, and hyaluronan. *N*-Acetylgalactosamine (GalNAc) is found in chondroitin sulfate and dermatan sulfate.

Monosaccharides in the form of UDP-nucleotides act as donors being transferred to a glycosyl acceptor by a glycosyltransferase or a synthase in the polysaccharide biosynthetic pathway.[4] Natural and unnatural GAGs can be prepared in the laboratory[5] by using recombinant GAG synthases and chemically prepared UDP-sugar nucleotides. With the advent of recombinant technology, glycosyltransferases and synthases have now become readily available. A major limitation to the enzymatic preparation of GAG polysaccharides and oligosaccharides is the limited availability and cost of sugar nucleotides and of sugar nucleotide analogs and derivatives due to the difficulty of their syntheses.[6] Though they can be prepared in several ways, a purely chemical approach requires a long and tedious synthesis, and yields of UDP-donor are generally low. Enzymatic synthesis offers an alternative to the use of protection and deprotection steps required for chemical synthesis[7] and circumvents the difficulties inherent to the formation of a pyrophosphate bond.

Wang et al. reported the enzymatic synthesis of UDP-GlcNAc/UDP-GalNAc analogs using recombinant *Escherichia coli* *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU).[8] However, they reported low yields, especially for *N*-modified GalNAc-1-phosphate analogs and later turned to recombinant human UDP-GalNAc pyrophosphorylase (AGX1) for the synthesis of UDP-GalNAc analogs.[9] More recently, Chen et al. reported a one-pot three-enzyme synthesis of UDP-

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GlcNAc derivatives using an *N*-acetylhexosamine 1-kinase and GlmU, but only a few derivatives were successfully synthesized.[10] Here we report the chemical synthesis of eight different GlcNAc-1-phosphate and GalNAc-1-phosphate analogs and their recognition by the GlmU enzyme (Figure 1).

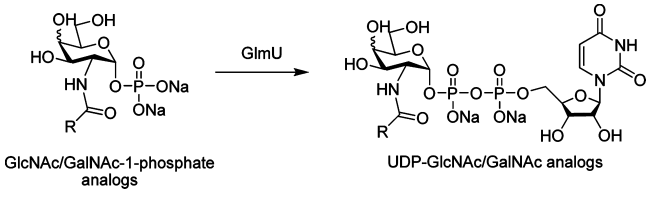


Figure 1. Enzymatic synthesis of UDP-GlcNAc and UDP-GalNAc analogs.

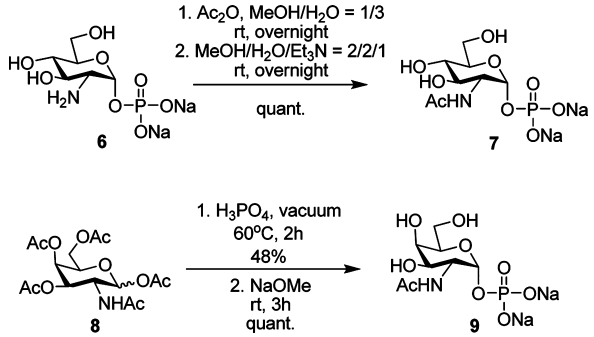
2. RESULTS AND DISCUSSION

2.1. Synthesis of *N*-Acetylglucosamine-1-phosphate and *N*-Acetylgalactosamine-1-phosphate Analogs. Eight natural and unnatural *N*-acetylglucosamine-1-phosphates and *N*-acetylgalactosamine-1-phosphates were chemically synthesized from commercially available monosaccharides. Our first attempt aimed at synthesizing GlcNH$_2$-1-phosphate, because the amine group can be easily functionalized at a later step. As shown in Scheme 1, reactions of glucosamine hydrochloride (**1**) with in situ-generated TfN_3 afforded 2-azido sugar **2** in 89% yield, which was subsequently peracetylated.[11] MacDonald phosphorylation[12] of the protected 2-azido sugar gave monophosphate **4** in a modest 46% yield. Subjecting **4** to Zemplén conditions removed its acetyl groups, affording GlcN$_3$-1-phosphate (**5**). Finally, reduction of **5** by hydrogenation using Lindlar's catalyst[13] afforded GlcNH$_2$-1-phosphate (**6**) in quantitative yield.

Next, GlcNAc-1-phosphate (**7**), the natural substrate of GlmU, was chemically synthesized from **6** through *N*-acetylation using Ac_2O in MeOH/H_2O (1/3) followed by treatment with MeOH/H_2O/Et_3N (2/2/1) to remove any *O*-acetyl groups that had been introduced (Scheme 2). Next, GalNAc-1-phosphate (**9**), an unnatural substrate of GlmU, was synthesized to investigate the impact of the C4 configuration on GlmU recognition. Compound **9**, a C4 epimer of **7**, was prepared from the commercially available galactosamine (**8**).

Unnatural UDP-GlcNAc and UDP-GalNAc analogs having a 'clickable'[14] alkyne group were also synthesized. In our initial approach, we attempted to functionalize glucosamine hydrochloride in the first step with propiolic acid under several different coupling conditions, but this led to low yields and separation difficulties. Instead, the stepwise synthesis of GlcN-alkyne-1-phosphate was undertaken as shown in Scheme 3. 2-

Scheme 2. Synthesis of GlcNAc/GalNAc-1-phosphate



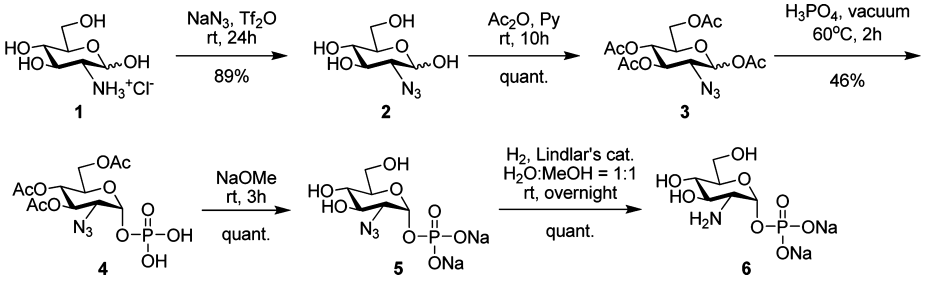
Azido-D-glucose tetraacetate (**3**) was reduced by hydrogenation with Lindlar's catalyst to afford **10**. The amino sugar **10** was then acylated using propiolic anhydride, prepared by treating propiolic acid with DCC in DCM and affording the alkyne derivative in 70% yield. MacDonald phosphorylation[12] followed by de-*O*-acetylation provided GlcN-alkyne-1-phosphate (**13**). GalN-alkyne-1-phosphate (**18**) was similarly synthesized using CBz protection of the amino group (Scheme 4).

Next, a bioisostere of GlcNAc-1-phosphate bearing an *N*-trifluoroacetamide was synthesized (Scheme 5). The *N*-trifluoroacetyl (TFA) group represents an excellent protecting group in the chemoenzymatic synthesis of GAGs,[15] as it can undergo selective deprotection by mild base treatment and then can be readily *N*-sulfonated to afford heparin and heparan sulfate type glycans. First, D-glucosamine hydrochloride (**1**) was protected as an *N*-trifluoroacetyl by treatment of CF_3CO_2Et[16] followed by peracetylation. Phosphorylation[12] followed by Zemplén deprotection afforded GlcN-TFA-1-phosphate (**22**).

A 'thiol-clickable'[17] analog, with an *N*-ene, was next synthesized from commercially available tetra-*O*-acetyl glucosamine (**23**). The per-*O*-acetylated sugar was chosen as the starting material, as the unprotected monosaccharide was prone to polymerization upon treatment with acryloyl chloride. Thus, treatment of the tetraacetate with acryloyl chloride introduced an ene group to afford compound **24**. Phosphorylation proceeding in low yield was followed by deacetylation to give monophosphate **26**.

2.2. Preparation of the UDP-Sugar Nucleotides. After the successful synthesis of a small library of GlcNAc-1-phosphate and GalNAc-1-phosphate analogs, each substrate was tested for its recognition by GlmU from *Escherichia coli*.[15] The enzymatic reaction was performed in Tris buffer with $MgCl_2$, DTT, UTP, and inorganic pyrophosphatase at 30 °C for 3 h (2 days for UDP-GalNAc analogs).[18] The results are shown in Table 1.

Scheme 1. Synthesis of GlcNH$_2$-1-phosphate



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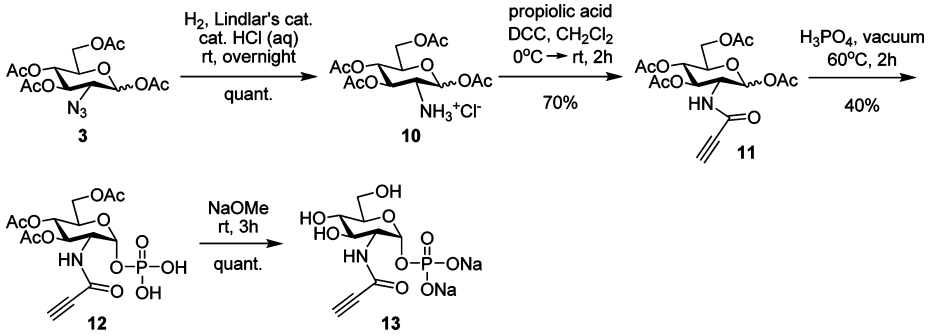
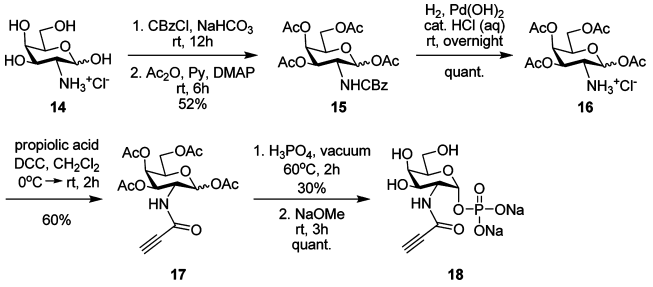
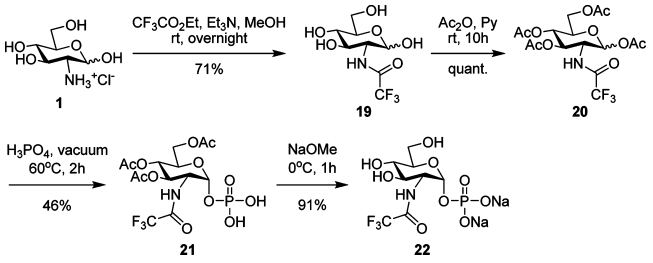
Scheme 3. Synthesis of GlcN-alkyne-1-phosphate



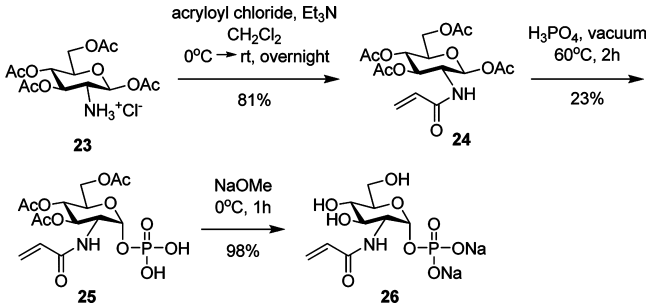
Scheme 4. Synthesis of GalN-alkyne-1-phosphate



Scheme 5. GlcN-TFA-1-phosphate

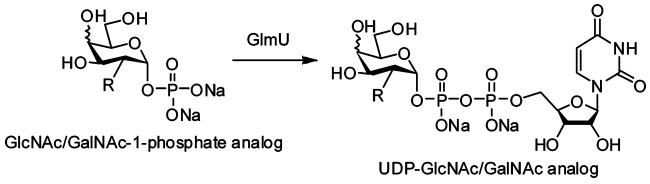


Scheme 6. Synthesis of GlcN-ene-1-phosphate



Out of the eight analogs tested, six were accepted as substrates by GlmU, affording the corresponding UDP-sugar nucleotides in high yields. Neither $GlcN_3$-1-phosphate (**5**) nor $GlcNH_2$-1-phosphate (**6**) was accepted by GlmU. GlcNAc-1-phosphate (**7**) and GlcN-TFA-1-phosphate (**22**) were excellent substrates, yielding more than 70% conversion. Because GalNAc-1-phosphate (**9**) was accepted by GlmU, the 4-OH group appears not to play a critical role in enzyme recognition. Both GlcN-alkyne-1-phosphate (**13**) and GalN-alkyne-1-phosphate (**18**) gave moderate yields of UDP-sugars using GlmU. On the basis of these observations, it appears that the amide bond on the 2-amino functional group is essential for substrate recognition by GlmU but the configuration at C4 is not important.

Table 1. GlcNAc/GalNAc-1-phosphate Analog Acceptance by GlmU[a]



	Substrate	R	Yield (%)	Product
GlcNAc & analogs	**5**	N_3	n.r.	-
	6	NH_2	n.r.	-
	7*		85	UDP-GlcNAc, **27**
	13		50	UDP-GlcN-alkyne, **28**
	22		78	UDP-GlcN-TFA, **29**
	26		65	UDP-GlcN-ene, **30**
GalNAc & analogs	**9***		70	UDP-GalNAc, **31**
	18		60	UDP-GalN-alkyne, **32**

[a] * = natural precursor.

Immobilization of $(His)_6$-tagged enzymes on Ni beads has been useful in carbohydrate synthesis.[19,20] Because the reactions above using the soluble enzyme showed promising yields, we examined the immobilization of GlmU for future applications in large-scale synthesis. Taking advantage of the C-terminal $(His)_6$-tag, GlmU was immobilized on Ni Sepharose beads that were then added to the reaction mixture using **22** as the substrate. Immobilized GlmU gave high yields of UDP-GlcN-TFA, comparable to that of the solution-based reaction. The same beads could be recycled and reused for a second reaction and retained high enzymatic activity. Recycling the enzyme through its immobilization avoids the requirement of repeated enzyme expression and can allow for continuous processes and eases the purification of UDP-sugar nucleotide product.

Although UDP-$GlcN_3$ and UDP-$GlcNH_2$ could not be synthesized using GlmU, they were chemically synthesized from the aforementioned $GlcN_3$-1-phosphate (**5**) (Scheme 7). Compound **5** was converted to its pyridinium salt and then stirred with UMP-morpholidate[21] in pyridine for 5 days to

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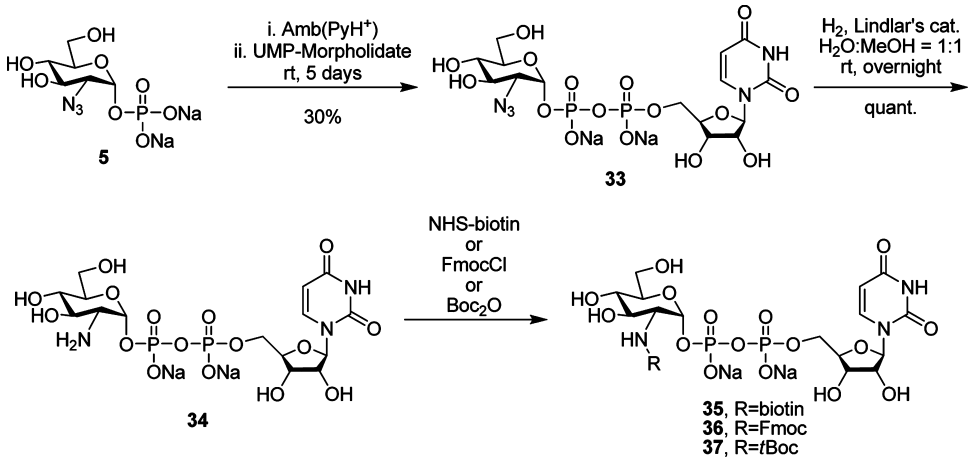
Scheme 7. Chemical Synthesis of UDP-GlcN Derivatives



afford UDP-GlcN$_3$ (**33**). The azide group was then reduced to an amine by hydrogenation using Lindlar's catalyst, affording UDP-GlcNH$_2$ (**34**). This free amino group was then functionalized to afford the following unnatural UDP-GlcNAc analogs: UDP-GlcN-biotin (**35**), UDP-GlcN-Fmoc (**36**), and UDP-GlcN-*t*Boc (**37**).

These ten UDP-sugar nucleotides were then tested for incorporation by various GAG synthases in vitro. PmHAS and PmCS produce hyaluronan and chondroitin polysaccharides, respectively.[22] PmHS1 and PmHS2 are two homologous isozymes that both can polymerize heparosan, the precursor of heparan sulfate and heparin.[5] The hexosamine analogs were tested in a chain polymerization assay using radioactively labeled UDP-[^{3}H]GlcUA; a radioactive signal results only if the hexosamine is copolymerized into the disaccharide repeats of a GAG product.[5,23]

Table 2. Analog Usage by GAG Synthases As Monitored by Copolymerization Assay[5,23]

	[^{3}H]GlcUA incorporation (disintegrations/min ×10^{-3})			
UDP-donor	PmHAS	PmCS	PmHS1	PmHS2
27[a]	70−120	na[b]	25−80	25−70
28	1	na	12	16
29	34	na	NS[c]	12
30	7	na	12	14
33	NS[d]	na	NS[d]	NS[d]
35	NS[d]	na	NS[d]	NS[d]
36	NS	na	NS	1.2
37	6	na	13	13
31[a]	na	110[d]	na	na
32	na	82[d]	na	na

[a]Natural precursor positive control (PmCS only utilizes UDP-GalNAc derivatives while the remainder of the synthases only employ UDP-GlcNAc derivatives). [b]na, not applicable. [c]NS, not significant (≤5-fold over background of the negative control without any UDP-hexosamine present). [d]Values for overnight reaction time.

The results shown in Table 2 demonstrate that some analogs function well with one or more synthases, but a few analogs did not incorporate into polymer under our conditions. In addition to increasing the chemical functionality of synthetic GAGs, the substrate specificity information yields insights into the synthases' recognition of their donor precursors.

3. CONCLUSIONS

We synthesized a small library of GlcNAc/GalNAc-1-phosphate analogs for their recognition by the GlmU uridyltransferase to synthesize UDP-GlcNAc/GalNAc analogues. Only analogs with an amide linkage at the C-2 nitrogen were successfully accepted by GlmU and gave the product in moderate to high yields. Analogs without an amide linkage were converted to UDP-sugar donors by chemical synthesis. The UDP-sugars were then tested for incorporation by GAG synthases using a radioactive assay, which showed that natural and unnatural UDP-sugars were incorporated into the polysaccharide. These results demonstrate that unnatural GAGs with a desired functionality can be synthesized in vitro and have promising therapeutic applications.

4. EXPERIMENTAL SECTION

General. ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, or 600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, or 800 MHz (800 MHz for ^{1}H NMR, 200 MHz for ^{13}C NMR) with Topsin 2.1 software. Mass data were acquired by high-resolution ESI-MS. Thin-layer chromatography (TLC) was carried out using plates of silica gel 60 with fluorescent indicator and revealed with UV light (254 nm) when possible and Von's reagent or ninhydrin solution in ethanol. Flash chromatography was performed using silica gel 230−400 mesh. Yields are given after purification, unless otherwise noted. When reactions were performed under anhydrous conditions, the mixtures were maintained under argon. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom (version 2.1) software for systematic names in organic chemistry.

Expression of GlmU Enzyme. The procedures for the expression and purification of GlmU were described previously.[24] Briefly, GlmU was cloned into a PET 21b vector (Novagen) to form a C-terminal (His)$_6$-tagged fusion protein. The expression was carried out in BL21 star cells (Invitrogen). The protein was purified by a Ni-Sepharose 6 Fast Flow column (GE Health) following a standard procedure.

General Procedure for the MacDonald Phosphorylation. Crystalline phosphoric acid (8.1 mmol) was dried in vacuo over phosphorus pentoxide for 12 h. The 1-*O*-acetyl sugar derivative (1 mmol) was added, and the mixture was heated at 60 °C in vacuo. After 2 h, heating was ceased and the resulting dark black mixture was dissolved in anhydrous THF (5 mL). The solution was cooled to 0 °C, and concentrated ammonium hydroxide (0.5 mL) was added until pH ~7. The precipitate of ammonium phosphate was filtered off and washed with THF (20 mL). The combined filtrate and washings were evaporated to give a syrupy residue that was purified by flash column chromatography (EtOAc 100% to EtOAc/MeOH 50/50).

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General Procedure for Zemplén Deacetylation. Sodium methoxide (3 mmol) in MeOH (0.5 M) was added to the peracetylated phosphate (1 mmol) in MeOH (7 mL) at 0 °C. The reaction was stirred at 0 °C for 1 h and quenched with Amberlite IR-120 (H^+). Amberlite was removed through filtration, and the filtrate was concentrated in vacuo to afford the fully deprotected phosphate in quantitative yield.

General Procedure for the Enzymatic Preparation of the UDP-Sugar Nucleotides. A reaction mixture containing the monophosphate, glucosamine-1-phosphate acetyltransferase/N-acetyl glucosamine-1-phosphate uridyltransgerase (GlmU), Tris-HCl (46 mM, pH 7.0), $MgCl_2$ (5 mM), dithiothreitol (200 μM), UTP (2.5 mM), and inorganic pyrophosphatase (0.012 units/μL), from Sigma-Aldrich, was incubated at 30 °C for 3 h. For GalNAc substrates, a second portion of GlmU was added to the reaction mixture on the second day. The completion of the reaction was monitored by TLC (*n*-butanol/formic acid/methanol = 4/8/1) or through MS. Then three reaction volumes of ethanol was added to quench the reaction and stored overnight at −20 °C. Centrifugation at 3000 rcf for 30 min afforded the crude product in the supernatant which was then concentrated and further purified on a BioGel (Bio-Rad) P2 column (2.5 × 65 cm) and eluted with H_2O. Fractions were collected, and those containing the product as determined by UV absorbance at 262 nm were combined and freeze-dried to afford the pure product.

2-Azido-2-deoxy-D-glucopyranose-3,4,6-triacetate-1-(dihydrogen phosphate) (4). Brown oil; ^{1}H NMR (500 MHz, MeOD): δ (ppm) 5.73 (s, 1H), 5.49 (t, J = 9.5 Hz, 1H), 5.12 (t, J = 9.74 Hz, 1H), 4.36 (d, J = 10 Hz, 2H), 4.14 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 2.08 (s, 3H), 2.04 (s, 6H); ^{13}C NMR (125 MHz, MeOD): δ (ppm) 171.1, 170.2, 170.0, 93.4, 70.7, 68.4, 68.0, 61.4, 61.2, 61.1; HRMS-FAB: [M − H] calcd for $C_{12}H_{17}N_3O_{11}P^-$ 410.0606; found: 410.0601.

2-Azido-2-deoxy-D-glucopyranose-1-(dihydrogen phosphate) (5). Light brown amorphous solid; ^{1}H NMR (500 MHz, D_2O): δ (ppm) 5.54 (dd, 1H, J = 7.4 Hz, 3.1 Hz), 3.88−3.72 (m, 4H), 3.48 (t, 1H, J = 9.5 Hz), 3.29 (d, 1H, J = 2.5 Hz); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 94.0, 72.8, 70.7, 69.5, 62.8, 60.3; HRMS-FAB: [M − H] calcd for $C_6H_{11}N_3O_8P^-$ 284.0289; found: 284.0289.

2-Amino-2-deoxy-D-glucopyranose-1-(dihydrogen phosphate) (6). Lindlar's catalyst (20 mg) was added to a stirred solution of **5** (75 mg, 0.228 mmol) in MeOH/H_2O, 1/1 (10 mL). A hydrogen balloon was attached to the reaction flask, and the reaction was stirred overnight at room temperature. The reaction mixture was filtered through Celite and rinsed with water. The filtrate and washings were combined and concentrated to afford **6** in quantitative yield. ^{1}H NMR (800 MHz, D_2O): δ (ppm) 5.59 (1H, dd, J = 7.4 Hz, 3.1 Hz), 3.90−3.84 (3H, m), 3.73 (1H, m), 3.45 (1H, m), 3.26 (1H, m); ^{13}C NMR (200 MHz, D_2O): δ (ppm) 90.8, 72.1, 69.6, 69.4, 60.1, 54.4; HRMS-FAB: [M − H] calcd for $C_6H_{13}NO_8P^-$ 258.0384; found: 258.0391.

2-Deoxy-2-[(1-oxo-2-propyn-1-yl)amino]-D-glucopyranose-1,3,4,6-tetraacetate (11). To a stirred solution of DCC (740 mg, 3.59 mmol) in dry CH_2Cl_2 (19.5 mL) was slowly added propiolic acid (0.18 mL, 2.92 mmol) at 0 °C. After 1 h, **10** (1.267 g, 2.924 mmol), dissolved in dry CH_2Cl_2 (7 mL), and DIPEA were added dropwise. The reaction mixture was stirred for 1 h at 0 °C, warmed to room temperature, and then stirred for another 2 h before the solvents were removed in vacuo. The crude product was purified by silica gel chromatography (MeOH/CH_2Cl_2, 1/20) to yield **11** as a colorless amorphous solid (1.20 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.01 (d, 1 H, J = 9.4 Hz), 5.69 (d, 1 H, J = 8.7 Hz), 5.14 (m, 2 H), 4.34 (m, 1 H), 4.27 (dd, 1 H, J = 4.2, 12.5 Hz), 4.12 (d, 1 H, J = 12.5 Hz), 3.81 (m, 1 H), 2.13−2.04 (4s, 12 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.2, 170.7, 169.5, 169.4, 152.1, 92.0, 76.5, 74.7, 72.5, 72.2, 68.0, 61.6, 52.6, 20.8−20.5; HRMS-FAB: [M + Na] calcd for $C_{17}H_{21}NNaO_{10}^+$ 422.1063; found: 422.1065.

2-Deoxy-2-[(1-oxo-2-propyn-1-yl)amino]-D-glucopyranose-3,4,6-triacetate-1-(dihydrogen phosphate) (12). Brown oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.01 (d, J = 9.4 Hz, 1 H), 5.69 (d, J = 8.7 Hz, 1 H), 5.14 (m, 2 H), 4.34 (m, 1 H), 4.27 (dd, J = 4.2, 12.5 Hz, 1 H), 4.12 (d, J = 12.5 Hz, 1 H), 3.81 (m, 1 H), 2.13−2.04 (4s, 12 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.2, 170.7, 169.5, 169.4, 152.1, 92.0, 76.5, 74.7, 72.5, 72.2, 68.0, 61.6, 52.6, 20.8−20.5; HRMS-FAB: [M + Na] calcd for $C_{17}H_{21}NNaO_{10}^+$ 422.1063; found: 422.1065.

2-Deoxy-2-[(1-oxo-2-propyn-1-yl)amino]-D-glucopyranose-1-(dihydrogen phosphate) (13). Light brown amorphous solid; ^{1}H NMR (500 MHz, D_2O): δ (ppm) 5.32 (dd, J = 3.3, 7.0 Hz,1 H), 3.87 (dt, J = 2.6, 10.5 Hz, 1 H), 3.72 (dd, J = 2.4, 4.2 Hz, 1 H), 3.70−3.64 (m, 3 H) 3.38 (dd, J = 9.1, 10.0 Hz, 1 H), 3.35 (s, 1 H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 152.5, 95.5, 77.8, 75.0, 73.2, 63.9, 62.9, 56.7, 56.6; HRMS-FAB: [M − H] calcd for $C_9H_{13}NO_9P^-$ 310.0328; found: 310.0323.

2-Deoxy-2-[[(phenylmethoxy)carbonyl]amino]-D-galactopyranose-1,3,4,6-tetraacetate (15). Galactosamine·HCl (5.0 g, 23 mmol) was dissolved in 130 mL of 0.5 M NaOMe in MeOH and stirred for 35 min. The reaction mixture was cooled to 0 °C, 3.23 mL (23.2 mmol) of Et_3N and 5.0 g (23 mmol) of benzyl chloroformate were added, and the mixture was stirred for 5 h. The thick suspension was concentrated and dissolved in 25 mL of Ac_2O and 50 mL of pyridine. A catalytic amount of *N*,*N*-(dimethylamino)pyridine (DMAP) was added, and the solution was stirred overnight. It was concentrated and coevaporated with toluene (6 × 25 mL). The resulting syrup was dissolved in 40 mL of $CHCl_3$ and washed with saturated $CuSO_4$ (2 × 20 mL), saturated $NaHCO_3$ (2 × 25 mL), and brine (1 × 25 mL). The combined aqueous layers were extracted with 40 mL of $CHCl_3$, and the combined organic layers were dried over Na_2SO_4. The crude product was concentrated and purified by silica gel chromatography, eluting with a gradient of 200/1 to 20/1, $CHCl_3$/MeOH, to yield 6.3 g (52%) of a yellow syrup comprising a mixture of anomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (m, 5 H), 6.22 (dd, 1 H, J = 3.65 Hz), 5.39 (m, 1 H), 5.12−4.99 (m, 3 H), 4.43 (m, 1 H), 4.12−3.98 (m, 3 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 1.98 (s, 3 H), 1.89 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.3, 170.3, 168.8, 155.4, 136.7, 128.5, 91.4, 77.3, 68.4, 68.0, 67.1, 66.7, 61.2, 48.6, 20.8, 20.5, 20.4. HRMS-FAB: [M + Na] calcd for $C_{22}H_{25}NNaO_{10}^+$ 486.1376; found: 486.1379.

2-Amino-2-deoxy-D-galactopyranose-1,3,4,6-tetraacetate Hydrochloride (16). ^{1}H NMR (500 MHz, CD_3OD): δ 6.37 (d, 1 H, J = 3.6 Hz), 5.50 (dd, 1 H, J = 1.3, 3.2 Hz), 5.36 (dd, 1 H, J = 3.2, 8.3 Hz), 4.43 (m, 1 H), 4.12 (d, 1 H, J = 6.6 Hz), 4.09 (d, 1 H, J = 6.6 Hz), 3.96 (dd, 1 H, J = 3.6, 11.4 Hz), 2.21 (s, 3 H), 2.14 (s, 3 H), 2.06 (s, 3 H), 1.89 (s, 3 H); δ 169.8, 169.4, 168.8, 168.5, 91.6, 67.6, 65.6, 61.2, 55.4, 42.4, 19.1, 17.3, 15.8, 11.2. HRMS-FAB: [M + Na] calcd for $C_{14}H_{21}NNaO_9^+$ 370.1114; found: 370.1117.

2-Deoxy-2-[(1-oxo-2-propyn-1-yl)amino]-D-galactopyranose-1,3,4,6-tetraacetate (17). Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.10 (d, 1 H, J = 3.6 Hz), 5.32 (m, 1 H), 5.09 (dd, 1 H, J = 3.2, 11.6 Hz), 4.56 (dd, 1 H, J = 3.2, 11.6 Hz), 4.16 (t, 1 H, J = 6.6 Hz), 3.97 (m, 2 H), 3.57 (m, 1 H), 2.93 (s, 1 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.90 (s, 3 H), 1.89 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 170.2, 169.4, 168.8, 152.3, 92.5, 77.2, 74.4, 71.5, 70.1, 66.2, 61.3, 49.4, 24.8−22.0; HRMS-FAB: [M + Na] calcd for $C_{17}H_{21}NNaO_{10}^+$ 422.1063; found: 422.1068.

2-Deoxy-2-[(1-oxo-2-propyn-1-yl)amino]-α-D-galactopyranose-1-(dihydrogen phosphate) (18). Light brown amorphous solid; ^{1}H NMR (500 MHz, CD_3OD): δ 5.32 (dd, 1 H, J = 3.4, 7.2 Hz), 4.17 (dt, 1 H, J = 2.6, 10.8 Hz), 4.00 (dd, 1 H, J = 5.7, 6.3 Hz), 3.86 (m, 1 H), 3.80 (dd, 1 H, J = 3.1, 10.0 Hz), 3.59 (m, 1 H), 3.34 (s, 1 H); ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) 152.6, 91.4, 68.0, 67.9, 67.5, 67.2, 66.0, 61.9, 61.3; HRMS-FAB: [M − H] calcd for $C_9H_{13}NO_9P^-$ 310.0328; found: 310.0321.

2-Deoxy-2-[(trifluoroacetyl)amino]-D-glucopyranose (19). Et_3N (3.50 mL, 25 mmol) and CF_3CO_2Et (3.8 mL, 32 mmol) were added to D-glucosamine hydrochloride (5.375 g, 25 mmol) in MeOH (15 mL). The reaction mixture was stirred at room temperature overnight. The solution was concentrated in vacuo and recrystallized in MeOH to afford N-trifluoroacetylated **19** (α/β = 1:0.8) (5.26 g, 19.2 mmol, 71%). ^{1}H NMR (500 MHz, D_2O): δ (ppm) 8.28 (d, J = 0.76 Hz, 1H), 5.11 (d, J = 3.49 Hz, 1H), 4.63 (d, J = 12.5 Hz, 1H), 3.84 (dd, J = 10.73, 3.53 Hz, 1H), 3.77 (d, J = 1.44 Hz, 1H), 3.75−3.73 (m, 1H), 3.73−3.71 (m, 1H), 3.71−3.69 (m, 1H), 3.68 (d, J = 2.31

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Hz, 1H), 3.64 (d, J = 5.20 Hz, 1H), 3.63 (d, J = 2.43 Hz, 1H), 3.62−3.60 (m, 1H), 3.59 (dd, J = 4.87, 2.14 Hz, 1H), 3.49−3.45 (m, 1H), 3.34 (ddd, J = 10.06, 2H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 94.8, 90.5, 76.7, 73.8, 71.7, 70.9, 70.7, 70.4, 61.4, 61.2, 57.7, 55.1; HRMS-FAB: [M − H] calcd for $C_8H_{11}F_3NO_6^-$ 274.0544; found: 274.0541.

2-Deoxy-2-[(trifluoroacetyl)amino]-D-glucopyranose-1,3,4,6-tetraacetate (20). Pyridine (56.3 mL) and Ac_2O (32.5 mL) were added to **19** (5.26 g, 19.2 mmol). The reaction mixture was stirred for 3 h at room temperature. The solution was concentrated in vacuo and coevaporated with toluene (10 mL) three times. The resulting residue was recrystallized in Et_2O (15 mL) and petroleum ether (5 mL) to afford peracetylated **20** (α:β = 1:0.33, 8.51 g, 19.2 mmol, quant). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.60 (d, J = 9.28 Hz, 1H), 6.51 (d, J = 8.50 Hz, 1H), 6.27 (d, J = 3.66 Hz, 1H), 5.76 (d, J = 8.71 Hz, 1H), 5.35−5.28 (m, 1H), 5.25 (t, J = 9.74, 1H), 5.22−5.16 (m, 1H), 4.45 (ddd, J = 10.84, 8.69, 3.66 Hz, 1H), 4.33−4.27 (m, 2H), 4.15 (dd, J = 12.56, 2.28 Hz, 1H), 4.09 (dd, J = 12.50, 2.33 Hz, 1H), 4.04 (ddd, J = 9.95, 3.97, 2.32 Hz, 1H), 3.85 (ddd, J = 9.50, 4.50, 2.18 Hz, 1H), 2.23 (s, 3H), 2.13 (s, 6H), 2.11 (s, 6H), 2.08 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ (ppm) 171.7, 170.7, 169.2, 168.6, 157.5, 157.2, 116.5, 114.2, 91.7, 89.6, 72.8, 71.9, 70.1, 69.7, 67.9, 67.2, 61.6, 61.3, 53.1, 51.7; HRMS-FAB: [M − H] calcd for $C_{16}H_{19}F_3NO_{10}^-$ 442.0967; found: 442.0961.

2-Deoxy-2-[(trifluoroacetyl)amino]-D-glucopyranose-3,4,6-triacetate-1-(dihydrogen phosphate) (21). ^{1}H NMR (500 MHz, D_2O): δ (ppm) 5.70−5.55 (m, 1H), 5.43 (s, 1H), 5.14 (s, 1H), 4.36 (s, 2H), 4.24−4.09 (m, 1H), 3.34 (d, J = 19.11 Hz, 1H), 2.08 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 171.3, 170.4, 169.8, 157.2, 114.7, 92.7, 71.2, 68.2, 68.1, 61.4, 52.8; HRMS-FAB: [M − H] calcd for $C_{14}H_{18}F_3NO_{12}P^-$ 480.0524; found: 480.0524.

2-Deoxy-2-[(trifluoroacetyl)amino]-D-glucopyranose-1-(dihydrogen phosphate) (22). ^{1}H NMR (500 MHz, D_2O): δ (ppm) 5.44 (dd, J = 7.32, 3.23 Hz, 1H), 4.03 (ddd, J = 10.57, 3.17, 1.84 Hz, 1H), 3.94 (ddd, J = 10.12, 4.89, 2.22 Hz, 1H), 3.77 (dd, J = 12.38, 4.94 Hz, 1H), 3.87 (ddd, J = 12.31, 9.99, 5.66 Hz, 1H), 3.51 (dd, J = 10.06, 9.14 Hz, 1H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 159.4, 116.7, 92.6, 72.6, 69.9, 69.5, 60.2, 54.3; HRMS-FAB: [M − H] calcd for $C_8H_{12}F_3NO_9P^-$ 354.0207; found: 354.0206.

2-Deoxy-2-[(1-oxo-2-ethen-1-yl)amino]-β-D-glucopyranose-1,3,4,6-tetraacetate (24). Et_3N (145 μL, 1.04 mmol) was added to a stirred solution of 1,3,4,6-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose·HCl (200 mg, 0.521 mmol). The solution was cooled to 0 °C, and acryloyl chloride (50 μL, 0.625 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature. The solution was concentrated in vacuo and purified on silica gel chromatography (hexanes 100% to hexanes/EtOAc = 1/1) to afford **24** as a clear syrup (170 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.96 (d, J = 9.59 Hz, 1H), 6.20 (d, J = 17.1 Hz, 1H), 6.00 (dd, J = 17.1, 10.4 Hz, 1H), 5.68 (d, J = 8.8 Hz, 1H), 5.61 (d, J = 9 Hz, 1H), 5.17 (t, J = 11 Hz, 1H), 5.07 (t, J = 9.65 Hz, 1H), 4. 34 (dd, J = 19.42, 9.61 Hz, 1H), 4.21 (td, J = 9.10, 4.57 Hz, 1H), 4.08 (dd, J = 12.44, 2.15 Hz, 1H), 3.81 (ddd, J = 9.85, 4.55, 2.19 Hz, 1H), 2.04−1.95 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.2, 170.9, 169.7, 169.4, 166.0, 130.1, 127.4, 92.4, 72.6, 72.6, 68.0, 61.7, 61.7, 52.5, 50.3; HRMS-FAB: [M + H] calcd for $C_{17}H_{24}NO_{10}^+$ 402.1400; found: 402.1395.

2-Deoxy-2-[(1-oxo-2-ethen-1-yl)amino]-α-D-glucopyranose-3,4,6-triacetate-1-(dihydrogen phosphate) (25). Brown oil; ^{1}H NMR (500 MHz, MeOD): δ (ppm) 6.28 (d, J = 9.92 Hz, 17 Hz, 1H), 6.22 (dd, J = 17.5, 2.05 Hz,1H), 5.66 (dd, J = 9.93, 2.05 Hz, 1H), 5.52 (dd, J = 6.63, 3.26 Hz, 1H), 5.36 (dd, J = 10.49, 9.59 Hz, 1H), 5.11 (t, J = 9.75, 9.75 Hz, 1H), 4.39−4.34 (m, 1H), 4.31 (dd, J = 9.20, 6.05 Hz, 2H), 4.29−4.26 (m, 1H), 4.17−4.11 (m, 1H), 2.00 (s, 6H), 1.94 (s, 3H); ^{13}C NMR (125 MHz, MeOD): δ (ppm) 171.1, 170.5, 169.9, 166.7, 130.3, 126.1, 93.7, 71.4, 68.3, 68.2, 61.5, 51.9, 50.3 ; HRMS-FAB: [M − H] calcd for $C_{15}H_{21}NO_{12}P^-$ 438.0807; found: 438.0800.

2-Deoxy-2-[(1-oxo-2-ethen-1-yl)amino]-α-D-glucopyranose-3,4,6-triacetate-1-(dihydrogen phosphate) (26). Light brown amorphous solid; ^{1}H NMR (500 MHz, D_2O): δ (ppm) 6.14 (dd, J = 17.14, 10.20 Hz, 1H), 6.04 (dd, J = 17.14, 1.35 Hz, 1H), 5.60 (dd, J = 10.23, 1.34 Hz, 1H), 5.28 (dd, J = 6.63, 3.26 Hz, 1H), 3.88−3.83 (m, 1H), 3.68 (dd, J = 6.66, 3.67 Hz, 1H), 3.65−3.62 (m, 3H), 3.59 (m, 1H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 165.8, 129.4, 128.0, 93.5, 72.6, 70.4, 69.4, 60.1, 53.7; HRMS-FAB: [M − H] calcd for $C_9H_{15}NO_9P^-$ 312.0490; found: 312.0486.

***P*′-[2-(Acetylamino)-2-deoxy-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate), (UDP-GlcNAc, 27).** ^{1}H NMR (500 MHz, D_2O): δ (ppm) 7.87 (dd, J = 8.08, 2.41 Hz, 1H), 5.93−5.86 (m, 2H), 5.44 (d, J = 3.28 Hz, 1H), 4.32−4.25 (m, 2H), 4.24−4.08 (m, 3H), 3.94−3.88 (m, 1H), 3.86 (ddd, J = 8.03, 5.20, 2.66 Hz, 1H), 3.80 (dd, J = 17.63, 9.17 Hz, 1H), 3.73 (ddd, J = 18.49, 9.53, 5.39 Hz, 2H), 3.47 (ddd, J = 9.52, 5.51, 3.38 Hz, 1H), 2.02−1.95 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 174.7, 166.7, 152.1, 141.4, 102.6, 94.3, 88.4, 83.0, 73.7, 72.9, 70.8, 69.5, 69.4, 64.9, 60.2, 53.5, 22.0; HRMS-FAB: [M − H] calcd for $C_{17}H_{26}N_3O_{17}P_2^-$ 606.0743; found: 606.0734.

***P*′-[2-(1-Oxo-2-propyn-1-yl)amino-2-deoxy-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-alkyne, 28).** White fluffy powder; ^{1}H NMR (600 MHz, D_2O): δ (ppm) 7.89 (d, J = 8.1 Hz, 1 H), 5.89 (d, J = 4.6 Hz, 1 H), 5.88 (d, J = 8.9 Hz, 1 H), 5.47 (dd, J = 3.2, 6.9 Hz, 1 H), 4.28 (m, 2 H), 4.20 (m, 1 H), 4.18−4.16 (m, 1 H), 4.12−4.09 (m, 1 H), 3.99 (dt, J = 3.1, 10.5 Hz, 1 H), 3.87−8.83 (m, 1 H), 3.79−3.77 (m, 1 H), 3.76−3.70 (m, 2 H), 3.49−3.44 (m, 2 H); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 166.2, 154.8, 151.8, 141.6, 102.6, 94.1, 88.4, 88.3, 83.2, 73.8, 72.0, 69.6, 68.3, 67.4, 64.9, 60.2, 50.3, 50.2; HRMS-FAB: [M] calcd for $C_{18}H_{25}N_3O_{17}P_2^+$ 617.0659; found: 617.0656.

***P*′-[2-Deoxy-2-[(2,2,2-trifluoroacetyl)amino]-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-TFA, 29).** White fluffy powder; ^{1}H NMR (600 MHz, D_2O): δ (ppm) 7.88 (d, J = 8.11 Hz, 1H), 5.90 (m, 2H), 5.58−5.51 (dd, 1H, J = 7.0 Hz, 3.2 Hz), 4.29 (m, 2H), 4.21 (s, 1H), 4.20−4.14 (m, 1H), 4.11 (ddd, J = 11.68, 5.39, 3.12 Hz, 1H), 4.04 (d, J = 10.57 Hz, 1H), 3.88 (dd, J = 20.10, 9.82 Hz, 2H), 3.81 (d, J = 11.83 Hz, 1H), 3.78−3.73 (m, 1H), 3.52 (dd, J = 9.53, 5.39 Hz, 1H); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 166.4, 160.0, 151.9, 141.8, 117.0, 102.7, 93.9, 88.5, 83.2, 73.9, 73.1, 70.3, 69.7, 69.5, 64.9, 60.2, 54.4; HRMS-FAB: [M − H] calcd for $C_{17}H_{23}F_3N_3O_{17}P_2^-$ 660.0460; found: 660.0463.

***P*′-[2-Deoxy-2-[(1-oxo-2-ethen-1-yl)amino]-2-deoxy-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-ene, 30).** White fluffy powder; ^{1}H NMR (800 MHz, D_2O): δ (ppm) 7.88 (d, J = 8.09 Hz, 1H), 6.35−6.29 (dd, J = 10.4, 18.4 Hz, 1H), 6.19−6.13(d, J = 16.8 Hz, 1H), 5.92−5.86 (m, 2H), 5.74−5.68 (d, J = 10.4 Hz, 1H), 5.46−5.42 (m, 1H), 4.31−4.25 (m, 2H), 4.21−4.18 (m, 1H), 4.18−4.08 (m, 2H), 3.97−3.93 (m, 1H), 3.88−3.84 (m, 1H), 3.78−3.73 (m, 3H), 3.50−3.46 (m, 1H); ^{13}C NMR (200 MHz, D_2O): δ (ppm) 168.7, 166.1, 151.7, 141.4, 129.6, 127.9, 102.5, 94.4, 88.3, 83.1, 73.7, 72.9, 70.9, 69.5, 69.3, 64.8, 60.1, 53.6; HRMS-FAB: [M − H] calcd for $C_{18}H_{26}N_3O_{17}P_2^-$618.0743; found: 618.0741.

***P*′-[2-(Acetylamino)-2-deoxy-α-D-galactopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate), (UDP-GalNAc, 31).** ^{1}H NMR (500 MHz, D_2O): δ (ppm) 7.79 (d, J = 8.2 Hz, 1 H), 5.81 (br d, J = 4.2 Hz, 1 H); 5.79 (br d, J = 8.3, 1 H); 5.39 (dd, J = 6.9, 3.3 Hz, 1 H); 4.22−4.19 (m, 2 H), 4.13−4.08 (m, 3 H), 4.05−4.01 (m, 2 H); 3.88 (d, J = 3.0 Hz, 1 H), 3.78 (dd, J = 11.8, 3.2 Hz, 1 H); 3.73 (m, 1 H), 3.62 (m, 1H), 1.92 (s, 3 H); HRMS-FAB: [M] calcd for $C_{17}H_{27}N_3O_{17}P_2^+$ 607.0816; found: 607.0818.

***P*′-[2-(1-Oxo-2-propyn-1-yl)amino-2-deoxy-α-D-galactopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GalN-alkyne, 32).** White fluffy powder; ^{1}H NMR (600 MHz, D_2O): δ (ppm) 7.87 (d, J = 8.1 Hz, 1 H), 5.89 (d, J = 4.8 Hz, 1 H), 5.86 (d, J = 8.2 Hz, 1 H), 5.50 (dd, J = 3.4, 6.9 Hz, 1 H), 4.28 (m, 2 H), 4.22 (dt, J = 3.1, 10.6 Hz, 1 H), 4.20 (m, 1 H), 4.17 (m, 1 H), 4.11−4.09 (m, 2 H), 3.97 (br d, J = 3.0 Hz, 1 H), 3.91 (dd, J = 3.1, 10.9 Hz, 1 H), 3.70−3.64 (m, 3 H); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 166.2, 155.0, 151.8, 141.6, 102.6, 94.2, 88.4, 83.2, 83.1, 73.7, 72.0, 69.7, 68.3, 67.5, 65.0, 60.9, 50.3, 50.2; HRMS-FAB: [M] calcd for $C_{18}H_{25}N_3O_{17}P_2^+$ 617.0659; found: 617.0661.

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***P′*-[2-Azido-2-deoxy-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN$_3$, 33).** UMP-morpholidate (211 mg, 0.303 mmol) was dried by dissolving in anhydrous pyridine (5 mL) and concentrating in vacuo three times. Fully deprotected phosphate **5** (77 mg, 0.217 mmol) was converted to its PyH^+ form by passing through an Amberlite (PyH^+) column. UMP-morpholidate was dissolved in pyridine (5 mL) and added to phosphate **5** (PyH^+). The reaction mixture was stirred for 5 days at room temperature. The solvent was removed by concentration, and the resulting residue was dissolved in H_2O (10 mL). Insoluble material was removed through filtration, and the soluble filtrate was concentrated in vacuo. The residue was loaded onto a BioGel P2 column (2.5 × 65 cm) and eluted with H_2O. Fractions were collected, and those containing the product as determined by TLC (*n*BuOH/HCO_2H/H_2O = 4/8/1) were combined and freeze-dried to afford a white powder (50 mg, 0.0756 mmol, 35%). ^{1}H NMR (500 MHz, D_2O): δ (ppm) 7.90 (d, *J* = 8.12 Hz, 1H), 5.91 (m, 2H), 5.61 (dd, *J* = 7.48, 3.32 Hz, 1H), 4.30 (m, 2H), 4.23−4.18 (m, 1H), 4.15 (m, 2H), 3.89−3.83 (m, 2H), 3.78 (dd, *J* = 12.47, 2.34 Hz, 1H), 3.71 (dd, *J* = 12.51, 4.28 Hz, 1H), 3.46 (dd, *J* = 10.12, 9.16 Hz, 1H), 3.30 (m, 1H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 166.6, 152.2, 141.9, 102.9, 94.6, 88.5, 83.5, 74.0, 73.0, 70.9, 69.9, 69.6, 63.1, 63.0, 60.3; HRMS-FAB: [M − H] calcd for $C_{15}H_{22}N_5O_{16}P_2^-$ 590.0542; found: 590.0539.

***P′*-[2-Amino-2-deoxy-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcNH$_2$, 34).** 1H NMR (800 MHz, D_2O): δ (ppm) 7.87 (d, *J* = 8.09 Hz, 1H), 5.93−5.85 (m, 2H), 5.64−5.56 (m, 1H), 4.29 (d, *J* = 2.66 Hz, 2H), 4.23−4.19 (m, 1H), 4.19−4.09 (m, 2H), 3.85−3.81 (m, 1H), 3.80−3.72 (m, 2H), 3.72−3.66 (m, 1H), 3.45−3.39 (m, 1H), 3.01−2.91 (m, 1H); ^{13}C NMR (200 MHz, D_2O): δ (ppm) 166.6, 152.2, 141.9, 102.7, 94.7, 88.5, 83.1, 73.8, 73.2, 71.8, 69.6, 69.1, 65.0, 60.1, 54.7; HRMS-FAB: [M − H] calcd for $C_{15}H_{24}N_3O_{16}P_2^-$ 564.0637; found: 564.0638.

***P′*-[2-Deoxy-2-[[5-[(3a*S*,4*S*,6a*R*)-hexahydro-2-oxo-1*H*-thieno-[3,4-*d*]imidazol-4-yl]-1-oxopentyl]amino]-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-biotin, 35).** To a stirred solution of **34** (5 mg, 8.2 μmol) in HEPES buffer (2.5 mL, pH 8) was added 2.5 mL of a 10 mM biotin-NHS solution in DMF at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then concentrated. The crude mixture was purified on a BioGel P2 column (2.5 × 65 cm) and eluted with H_2O. Fractions were collected, and those containing the product as determined by TLC (*n*BuOH/HCO_2H/H_2O = 4/8/1) were combined and freeze-dried to afford **35** as a white powder. ^{1}H NMR (600 MHz, D_2O): δ (ppm) 7.92−7.87 (d, *J* = 7.8 Hz, 1H), 5.92−5.83 (m, 2H), 5.44−5.39 (m, 1H), 4.54−4.49 (m, 1H), 4.37−4.33 (m, 1H), 4.31−4.24 (m, 2H), 4.21−4.18 (m, 1H), 4.18−4.08 (m, 2H), 3.95−3.90 (m, 1H), 3.88−3.83 (m, 1H), 3.82−3.77 (m, 1H), 3.76−3.69 (m, 2H), 3.49−3.43 (m, 1H), 2.95−2.88 (m, 1H), 2.72−2.65 (m, 1H), 2.32−2.25 (m, 1H), 1.70−1.29 (m, 8H); ^{13}C NMR (150 MHz, D_2O): δ 177.5, 166.2, 158.1, 153.3, 141.7, 102.6, 94.6, 84.5, 83.1, 73.8, 73.0, 70.9, 69.6, 64.9, 62.0, 60.2, 55.1, 48.1, 39.7, 35.4, 27.9, 27.5, 25.0; HRMS-FAB: [M − H] calcd for $C_{25}H_{38}N_5O_{18}P_2S^-$ 790.1413; found: 790.1401.

***P′*-[2-Deoxy-2-[[fluorenylmethoxycarbonyl]amino]-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-Fmoc, 36).** To a stirred solution of **34** (5 mg, 8.2 μmol) in 1,4-dioxane/H_2O, 1/1 (0.8 mL), were added $NaHCO_3$ (6.9 mg, 8.2 μmol) and FmocCl (5.3 mg, 20.5 μmol). The reaction was stirred overnight at room temperature and then concentrated. The crude mixture was purified on a BioGel P2 column (2.5 × 65 cm) and eluted with H_2O. Fractions were collected, and those containing the product as determined by TLC (*n*BuOH/HCO_2H/H_2O, 4/8/1) were combined and freeze-dried to afford **36** as a white powder. ^{1}H NMR (600 MHz, D_2O): δ (ppm) 7.92−7.87 (d, *J* = 7.8 Hz, 1H), 5.92−5.83 (m, 2H), 5.44−5.39 (m, 1H), 4.54−4.49 (m, 1H), 4.37−4.33 (m, 1H), 4.31−4.24 (m, 2H), 4.21−4.18 (m, 1H), 4.18−4.08 (m, 2H), 3.95−3.90 (m, 1H), 3.88−3.83 (m, 1H), 3.82−3.77 (m, 1H), 3.76−3.69 (m, 2H), 3.49−3.43 (m, 1H), 2.95−2.88 (m, 1H), 2.72−2.65 (m, 1H), 2.32−2.25 (m, 1H), 1.70−1.29 (m, 8H); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 163.1, 162.9, 143.5, 141.0, 140.8, 128.0, 127.5, 125.5, 125.4, 120.1, 117.3, 102.3, 94.9, 88.5, 82.8, 73.6, 73.0, 71.7, 71.2, 64.9, 60.4, 46.8; HRMS-FAB: [M − H] calcd for $C_{30}H_{34}N_3O_{18}P_2^-$ 786.1318; found: 786.1318.

***P′*-[2-Deoxy-2-[[(1,1-dimethylethoxy)carbonyl]amino]-α-D-glucopyranosyl] Ester Uridine 5′-(Trihydrogen diphosphate) (UDP-GlcN-*t*Boc, 37).** Boc_2O (4.53 μL, 0.0197 mmol) was added to a stirred solution of **34** (8 mg, 0.0131 mmol) in 1,4-dioxane/H_2O, 1/1 (1 mL). The pH of the solution was adjusted to pH 10 using 10% NaOH (aq), and the reaction was stirred overnight at room temperature. A second portion of Boc_2O (4.53 μL, 0.0197 mmol) was added, and the reaction was again stirred overnight at room temperature. Ethyl acetate (1 mL) was added to the reaction mixture, and the organic phase was removed from the resulting biphasic mixture. The aqueous phase was loaded onto a BioGel P2 column (2.5 × 65 cm) and eluted with H_2O. Fractions were collected, and those containing the product as determined by TLC (*n*BuOH/HCO_2H/H_2O = 4/8/1) were combined and freeze-dried to afford **37** as a white powder (5.0 mg, 0.007 mmol, 30%). ^{1}H NMR (500 MHz, D_2O): δ (ppm) 7.96 (d, *J* = 8.00 Hz, 1H), 6.00 (d, *J* = 4.00 Hz, 1H), 5.98−5.95 (m, 2H), 5.54−5.49 (m, 1H), 4.37 (t, *J* = 4.33 Hz, 2H), 4.29 (s, 1H), 4.24 (s, 1H), 4.20 (dd, *J* = 6.04, 2.82 Hz, 1H), 3.93 (dd, *J* = 10.00, 3.16 Hz, 1H), 3.86−3.82 (m, 1H), 3.72 (d, *J* = 5.77 Hz, 1H), 3.68 (s, 1H), 3.54 (d, *J* = 9.43 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, D_2O): δ (ppm) 178.2, 167. 4, 157.9, 141.5, 102.7, 95.0, 88.0, 85.2, 81.3, 73.8, 72.9, 69.6, 69.5, 65.9, 64.8, 60.2, 55.0, 27.6; HRMS-FAB: [M − H] calcd for $C_{20}H_{32}N_3O_{18}P_2^-$ 664.1162; found: 664.1166.

Radioactive Assay. A given UDP-hexosamine analogue (1−4 mM) and radioactive ^{3}H-UDP-GlcUA (0.6−1 mM, 0.1 μCi) were coincubated in 20−50 μL reactions containing 50 mM Tris pH 7.2 and 1 mM $MnCl_2$ (as well as 1 M ethylene glycol for PmHAS and PmCS) with 0.5−45 μg of purified recombinant enzyme at 30 °C for 3−16 h. The reaction mixture was then quenched with detergent and analyzed by descending paper chromatography.[23] As a negative control for assay background, a reaction with no UDP-hexosamine was tested in parallel (chain polymerization can only occur when UDP-GlcUA and a functional UDP-hexosamine are present simultaneously).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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