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UDP-*N*-trifluoroacetylglucosamine as an alternative substrate in *N*-acetylglucosaminyltransferase reactions

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Abstract

The synthesis of UDP-*N*-trifluoroacetylglucosamine [uridine 5′-(2-trifluoroacetamido-2-deoxy-α-D-glucopyranosyl diphosphate, UDP-GlcNAc-F_3] is reported. The compound is found to serve as a substrate for the 'core-2' GlcNAc transferase (EC 2.4.1.102) that is involved in the biosynthesis of O-linked glycoproteins and for the GlcNAcT-V transferase (EC 2.4.1.155) that is a key biosynthetic enzyme controlling the branching pattern of cell surface complex Asn-linked oligosaccharides. The trisaccharide β-D-Gal*p*-(1 → 3)-[β-D-Glc*p*NAc-F_3(1 → 6)]-α-D-Gal*p*NAc-OR [R = $(CH_2)_8CO_2Me$] was prepared from β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-OR using the 'core-2' GlcNAc transferase. The tetrasaccharide β-D-Glc*p*NAc-(1 → 2)-[β-D-Glc*p*NAc-F_3-(1 → 6)]-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OR [R = $(CH_2)_7CH_3$] was prepared from β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OR [R = $(CH_2)_7CH_3$] using the GlcNAcT-V transferase. Removal of the trifluoroacetyl group was achieved under mild basic conditions to give the corresponding glucosamine containing tetrasaccharide. These examples demonstrate the feasibility of introducing masked forms of glucosamine residues into oligosaccharides using GlcNAc-specific transferases. The requirement for the trifluoroacetamido group as a specific recognition element was evident in the observation that neither UDP-glucosamine nor UDP-glucose served as a donor substrates for the 'core-2' GlcNAc transferase.

Keywords: *N*-Acetylglucosaminyltransferase; UDP-sugar analog; Oligosaccharide synthesis

1. Introduction

Cellular recognition in nature is often mediated via binding events involving complex carbohydrates on cell surfaces [1]. Compounds that interfere with these recognition processes are candidates as new drug targets in a variety of diseases. For this reason, strategies for the synthesis of oligosaccharides have received a great deal of attention in recent years [2–7]. One promising method involves the use of

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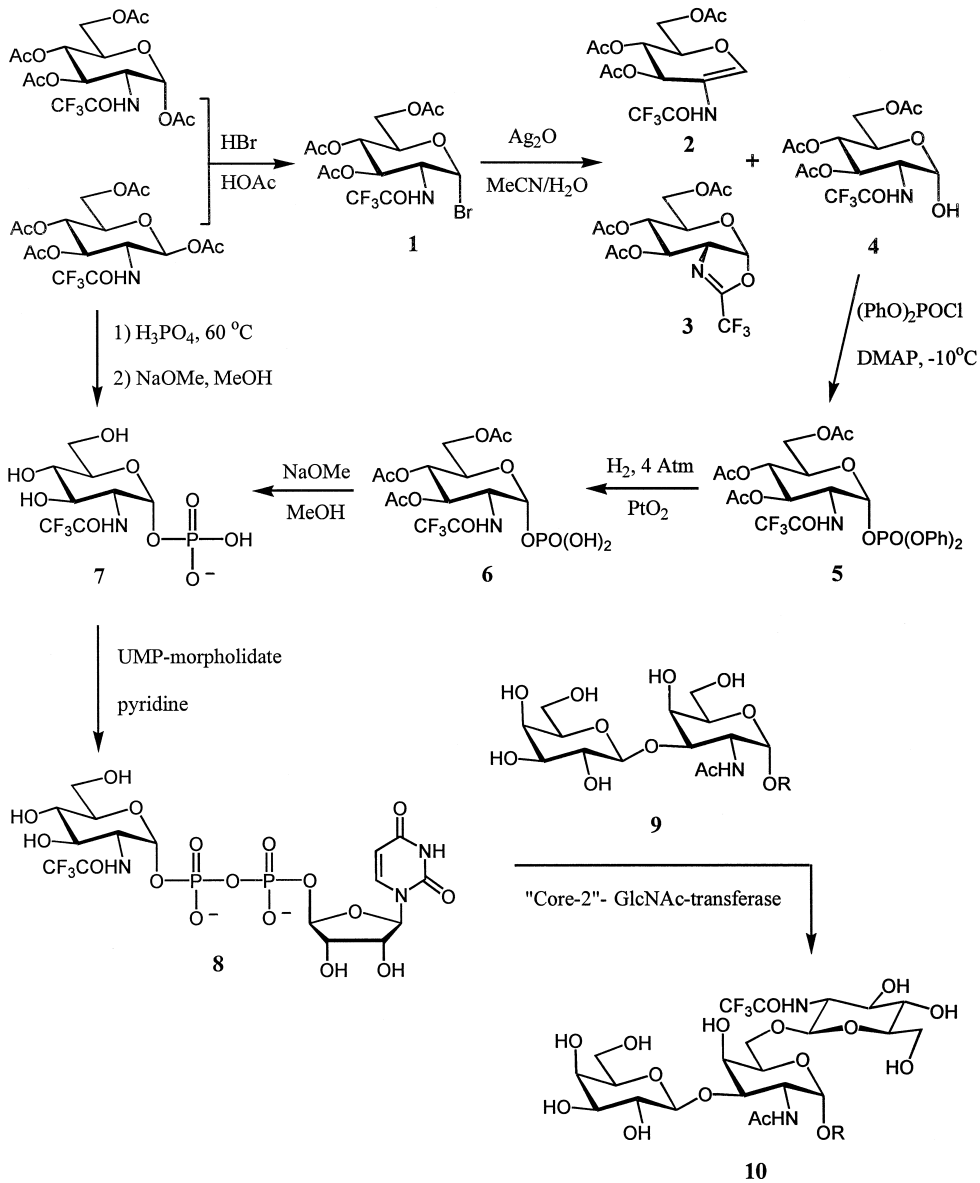
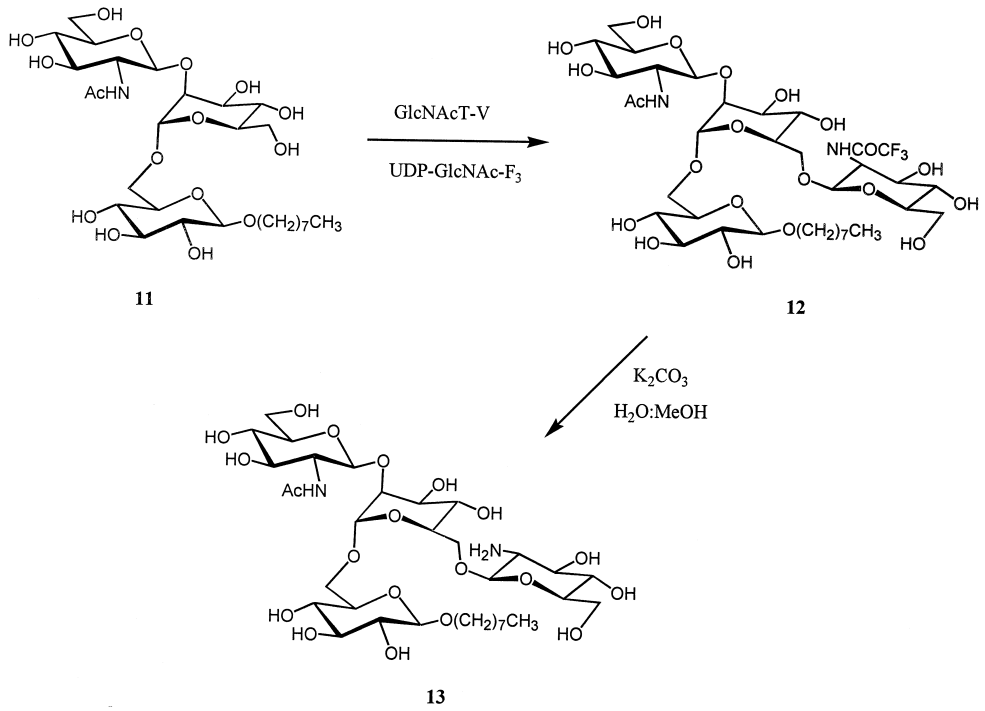
transferase enzymes that employ sugar-nucleotides as the carbohydrate donors [8–10]. The main advantage to this approach is that the enzymes are highly regiospecific and stereospecific with regard to directing the activated donor carbohydrate to the appropriate hydroxyl group of the acceptor, thereby eliminating the need for multiple protection–deprotection steps required in non-enzymatic syntheses. One of the drawbacks of this strategy, is that the specificity of these enzymes precludes the use of alternative donors that differ significantly from the natural donors. This makes the enzymatic synthesis of some unnatural oligosaccharides very difficult.

N-Acetylglucosamine (GlcNAc) residues are very common components of natural glycoconjugates that are generated by a large class of transferases that utilize UDP-GlcNAc as a donor. The first of such enzymes to be discussed is the UDP-D-Glc*p*NAc:β-D-Gal *p*-(1 → 3)-α-D-Gal*p*NAc (GlcNAc to GalNAc) β-(1 → 6)-GlcNAc transferase that is also referred to as the 'core-2'-GlcNAc transferase (GlcNAcT, EC 2.4.1.102) and is involved in the biosynthesis of O-linked glycoproteins in mammals [11,12]. This enzyme transfers a GlcNAc residue to the O-6 of GalNAc in the disaccharide β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-OR (–OR can be either a hydroxyl residue of a serine or threonine in a protein or a simple aryl or alkyl aglycon). In this paper, we describe the



Scheme 1.



Scheme 2.

synthesis of UDP-*N*-trifluoroacetylglucosamine (UDP-GlcNAc-F_3) and demonstrate that it can serve as an unnatural donor in the GlcNAcT reaction (Scheme 1). We also describe its recognition by the UDP-D-Glc*p*NAc:α-D-Man*p*-(1 → 6)-β-D-Glc*p*NAc transferase V (GlcNAcT-V, EC 2.4.1.155). This enzyme transfers a GlcNAc residue to the O-6 of Man in the trisaccharide β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OR (R = $(CH_2)_7CH_3$) (**11**) (Scheme 2) and controls the branching pattern of asparagine-linked oligosaccharides [13,14].

The ease of removal of the trifluoroacetyl group under relatively mild conditions [15–17] effectively permits the introduction of glucosamine residues into oligosaccharides that normally contain GlcNAc. This could lead to the preparation of a variety of unnatural compounds with potential bioactive properties as well as provide a nucleophilic functionality to which other molecules can be appended using reductive amination chemistry. The direct transfer of UDP-glucosamine was not expected to be feasible because the acetamido functionality of the natural substrate donor was being replaced by a charged ammonium group. Indeed, we have shown that the 'core-2'-GlcNAcT accepts neither UDP-glucose nor UDP-glucosamine as a donor substrate. This indicates the trifluoroacetamido group served as a necessary recognition element in the transferase reaction. In addition, we have demonstrated the feasibility of this method by the enzymatic synthesis of the tetrasaccharide **12** followed by the removal of the trifluoroacetyl group to generate the glucosamine containing tetrasaccharide **13** (Scheme 2).

2. Results and discussion

The synthesis of UDP-*N*-trifluoroacetylglucosamine began with the preparation of the glycosyl bromide **1** from an α/β mixture of 1,3,4,5-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose [18] (Scheme 1). Treatment of **1** with silver oxide in 10:1 acetonitrile:water gave 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucose **4** in 57% yield. The relatively low yield was due to the formation of the peracetylated glucal **2** and oxazoline **3** as by-products in yields of 7 and 12%, respectively. These products have been obtained previously upon treatment of **1** with MeOH under *Koenigs–Knorr* conditions [19]. Compound **4** was phosphorylated by treatment with diphenylchlorophosphate and 4-*N*,*N*-dimethylaminopyridine (DMAP) in CH_2Cl_2 at −10 °C. These conditions are known to produce anomerically enriched α-glycosyl phosphate diphenyl esters for a number of acetylated hexopyranoses [20]. Indeed the α-diphenylphosphate **5** was obtained in 52% yield

Table 1
Selected ^{1}H NMR data[a] for compounds **9** [25] and **10**

Residue	Proton	9	10
GalNAc	H-1	4.88 (3.5)	4.84 (3.8)
	H-2	4.31 (3.5, 11.0)	4.29 (3.8, 11.1)
	H-3	4.02 (3.5, 11.0)	4.00 (3.1, 11.1)
	H-4	4.24 (2.9)	4.21 (3.4)
	NAc	2.02	2.00
βGal	H-1	4.48 (8.0)	4.46 (7.8)
	H-4	3.91 (3.0)	3.91 (3.4)
GlcNAc	H-1		4.63 (8.5)

[a]Numbers in parentheses give coupling constants in Hz.

and no β-anomer was isolated upon purification of the reaction product by conventional chromatography. A by-product ($\sim$15%), whose ^{1}H NMR and mass spectra were consistent with that expected for the corresponding α-glycosyl chloride (3,4,6-tri-*O*-acetyl-2-deoxy 2-triflouroacetamido-α-D-glucopyranosyl chloride), was also obtained. The formation of glycosyl chlorides has been observed during the diphenylphosphorylation of sugars when *n*-butyllithium was used as the base [21]. The phenyl groups in the phosphate triester were removed by hydrogenation over platinum oxide catalyst [22] to give **6**. Low yields ($\sim$30%) were observed, presumably due to acid catalyzed decomposition of the glycosyl phosphates. Cleavage of the *O*-acetyl groups in the presence of the base-sensitive trifluoroacetamido group was accomplished by careful treatment with NaOMe in MeOH to give **7** (68%).

In further work, a more efficient method for the preparation of compound **7** was developed. Treatment of the peracetylated β-*N*-trifluoroacetylglucosamine [18] with neat phosphoric acid at 60 °C (the MacDonald procedure [23]), followed by deacetylation with sodium methoxide in MeOH gave a 27% yield of **7**.

The GlcNAc-F_3 α-phosphate **7** was coupled to UMP using standard Khorana conditions with UMP-morpholidate in dry pyridine [24]. The product UDP-GlcNAc-F_3 **8**, was isolated as its dilithium salt in

Table 2
Selected ^{1}H NMR data[a] for compounds **11** [26], **12** and **13**

Residue	Proton	11	12	13
β-Glc	H-1	4.46 (8.1)	4.45 (8.1)	4.45 (8.1)
	H-2	3.26 (8.0, 9.2)	3.26 (8.5)	3.26 (8.7)
α-Man	H-1	4.90 (1.3)	4.82 (1.5)	4.90
	H-2	4.12 (3.4)	4.08 (3.5)	4.13 (3.5)
β-GlcNAc	H-1	4.57 (8.4)	4.56 (8.4)	4.56 (8.4)
	NAc	2.06	2.04	2.07
β-GlcNAc-F_3	H-1		4.64 (8.4)	
β-GlcNH_2	H-1			4.39 (8.1)
	H-2			2.68 (9.0)

[a]Numbers in parentheses give coupling constants in Hz.

44% yield following purification by anion exchange and size exclusion chromatographies.

'Core-2'-GlcNAc-transferase was obtained from mouse kidney acetone powder by extraction and digestion with trypsin followed by a single step affinity chromatography on UDP-hexanolamine Sepharose (see Section 3). Incubation of β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-OR **9** [25] with a portion of this enzyme extract, in the presence of the donor UDP-GlcNAc-F_3, resulted in the formation of the trisaccharide product **10** in a 62% isolated yield.

The structure of **10** was assigned on the basis of the ^{1}H NMR data showed in Table 1. The new β-glycosidic linkage was established by the H-1 doublet ($J_{1,2}$ 8.5 Hz) at δ 4.63 ppm. This was in agreement with a previous assignment of the *N*-acetylglucosamine analog of this trisaccharide [11]. High resolution electrospray ionization mass spectrometry (HRESIMS) confirmed the composition and size of **10** ($[M + Na]^+ = 833.3173$). The presence of the *N*-trifluoroacetyl group was confirmed by the singlet at δ −76.1 ppm in the ^{19}F NMR.

The trifluoro donor was also incubated with a portion of cloned GlcNAcT-V transferase and acceptor **11**. The resultant tetrasaccharide **12** was obtained in a 56% isolated yield. From the ^{1}H NMR data presented in Table 2, the new β-glycosidic linkage of **12** was confirmed by the H-1 doublet ($J_{1,2}$ 8.4 Hz) at δ 4.64 ppm. The composition of **12** was confirmed by the $[M + Na]^+$ peak at 937.3622 in the HRESIMS spectrum. The presence of the *N*-trifluoroacetyl group was confirmed by the singlet at δ −75.5 in the ^{19}F NMR.

The preparation of **10** and **12** clearly demonstrates the feasibility of using enzymes to introduce a protected form of glucosamine into an oligosaccharide. In order to show that the trifluoroacetamido group was a specific recognition component in the enzymatic reaction, the alternative substrates UDP-glucosamine (bearing a small positively charged group at C-2) and UDP-glucose (bearing a small neutral group at C-2) were tested as substrates. In either case, no detectable transfer was observed after eight days. The presence of an acetamido moiety at C-2 was therefore required for the transfer to occur; as one might expect for these highly specific transferases.

Deprotection of tetrasaccharide **12** was performed using a previously published procedure [17] which employed potassium carbonate in aqueous MeOH. These mild conditions allowed the isolation of the desired tetrasaccharide **13** after purification by reversed-phase chromatography.

3. Experimental

General methods.—Melting points were recorded on a Reichert melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter. TLC was performed on Silica Gel 60F_{254} (E. Merck, Darmstadt) with detection by charring after spraying with 5% H_2SO_4 or by spraying with a solution containing H_2SO_4 (31 mL) ammonium molybdate (21 g), and cerium sulfate (1 g) in water (500 mL) and then heating (110 °C for 5 min). Flash column chromatography was performed using Silica Gel 60 (230–400 mesh). All reagents were commercially available unless otherwise stated. The disaccharide β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-OR [R = $(CH_2)_8CO_2Me$] (**9**) and the trisaccharide β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OR [R = $(CH_2)_7CH_3$] (**11**) were respectively synthesized as previously described [25,27]. UDP-GlcNAc was obtained from Sigma (St. Louis, MO). UDP-[6-^{3}H(N)]GlcNAc (specific activity, 60 Ci/mmol) was from American Radiolabeled Chemicals (St. Louis, MO). Liquid scintillation counting was performed with a Beckman LS-1801 instrument using Ecolite (+) cocktail from ICN Radiochemicals (Costa Mesa, CA). Iatrobeads refers to a beaded Silica Gel (No. 6RS-8060) manufactured by Iatron Laboratories (Tokyo). Ion exchange and gel chromatographic supports were from BioRad Laboratories (Missisauga, ON). Pyridine, acetonitrile, CH_2Cl_2, and Et_3N were distilled under N_2 from calcium hydride. Methanol was distilled under N_2 from magnesium methoxide. All reactions performed in organic solvents were carried out under argon. ^{1}H NMR spectra were recorded at 400 MHz (Bruker WH-400) or at 600 MHz (Varian spectrometer) in solutions in deuterium oxide with acetone as the external standard (δ 2.23 ppm) and at 30 °C (for compounds **9** and **13**). ^{13}C NMR spectra were recorded at 75.43 MHz (Varian XL-300), ^{31}P NMR spectra were recorded at 81.0 MHz and ^{19}F NMR at 188.0 MHz with proton decoupling (Bruker AC-200). The ^{31}P chemical shifts are expressed relative to external phosphoric acid (0.00 ppm). The ^{19}F chemical shifts are expressed relative to $CFCl_3$ for solutions in $CDCl_3$ (0.00 ppm) or AcOH for solutions in deuterium oxide (−76.53 ppm). Signals upfield of $CFCl_3$ were assigned negative values. Desorption chemical ionization (DCI) mass spectra were recorded on a Delsi Nermag R10-10C mass spectrometer using ammonia as the chemical ionization gas. High resolution liquid secondary ion (HRLSI) mass spectra were

recorded on a Kratos Concept II HQ mass spectrometer. High resolution electrospray ionization (HRESI) mass spectrometry of **10** was obtained using a Micromass ZabSpec Hybrid Sector-TOF spectrometer using a 1% solution of ArOH in water:MeOH (1:1) as liquid carrier. Millex-GV (0.22 mm) filters units were from Millipore, and C_{18} Sep-Pak sample-preparation cartridges were from Waters Associates. Microanalysis were carried out by Mr. Peter Borda in the microanalytical laboratory at UBC.

3,4,6-Tri-O-*acetyl-2-deoxy-2-trifluoroacetamido-α*-D-*glucose* (**4**).—3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide **1** (1.08 g, 2.3 mmol) was dissolved in 10 mL of dry acetonitrile containing silver oxide (0.50 g), water (1 mL) was added, and the mixture was stirred for 2 h in the dark. The resulting suspension was filtered, concentrated, and separated on a silica gel column using 1:3 EtOAc–hexanes. Fractions of higher mobility were segregated for further purification. Later fractions were pooled and evaporated to give **4** as a white solid. (0.53 g, 57%): mp 174 °C, lit. 173–174 °C [18]; $[\alpha]_D^{22}$ +20.0° (*c* 0.5, $CHCl_3$), lit. +25.0° (*c* 1, $CHCl_3$) [18]; R_f 0.16 (1:1 EtOAc–hexanes); ^{1}H NMR ($CDCl_3$): δ 6.64 (br d, 1 H, $J_{2,NH}$ 9.1 Hz, NH), 5.34 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.6 Hz, H-3), 5.33 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.13 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.29 (ddd, H-2) 4.25–4.08 (m, 3 H, H-5, H-6, H-6′) 2.08, 2.03, 2.00 (3s, each 3 H, 3Ac); ^{13}C NMR ($CDCl_3$): δ 171.40, 171.05, 169.48 (*C*OCH_3, 3Ac), 157.30, (q, *C*OCF_3, $J_{C,F}$ 37.7 Hz), 115.53 (q, CF_3, $J_{C,F}$ 287.7 Hz), 90.87 (C-1), 70.46 (C-5), 67.82 (C-3), 67.71 (C-4), 61.89 (C-6), 52.76 (C-2), 20.77, 20.60, 20.48 (CH_3, 3Ac); ^{19}F NMR ($CDCl_3$): δ −76.6; DCIMS *m/z* 419 ($[M + NH_4]^+$, 100%). Anal. Calcd. for $C_{14}H_{18}O_9NF_3$: C, 41.90; H, 4.52; N, 3.49. Found: C, 42.15; H, 4.32; N, 3.29.

3, 4, 6 - Tri - O - *acetyl - 5 - anhydro - 2 - deoxy - 2 - trifluoroacetamido*-D-*arabino-hex-1-enitol* (**2**).—Obtained in 7% yield as a by-product of the chromatographic purification of **4**. Further purification by flash chromatography using 9:1 benzene–ether was required in order to separate this compound from the oxazoline **3**. Clear oil, $[\alpha]_D^{22}$ −46.5° (*c* 1, $CHCl_3$), lit. −45° (*c* 1, $CHCl_3$) [18]; R_f 0.25 (7:3 benzene–ether); ^{1}H NMR ($CDCl_3$): δ 8.19 (br s, 1 H, NH), 7.69 (s, 1 H, H-1), 5.22 (dd, 1 H, $J_{3,4} = J_{4,5}$ 3.6 Hz, H-4), 5.15 (dd, 1 H, $J_{3,5}$ 1.1 Hz, H-3), 4.45–4.40 (ddd, 1 H, $J_{5,6}$ 7.9, $J_{5,6'}$ 4.1 Hz, H-5), 4.35 (dd, 1 H, J_{gem} 11.9 Hz, H-6), 4.22 (dd, 1 H, H-6′) 2.12, 2.10, 2.08 (3s, each 3 H, 3Ac); ^{13}C NMR ($CDCl_3$): δ 172.66, 170.48, 169.54 (*C*OCH_3, 3Ac), 154.73 (q, $J_{C,F}$ 37.5 Hz, *C*OCF_3), 115.53 (q, $J_{C,F}$ 286.4 Hz, CF_3), 141.07 (C-1) 128.34 (C-2) 73.19, 66.40, 66.32, 60.92 (C-3, C-4, C-5, C-6) 20.82, 20.79, 20.75 (CH_3, 3Ac); ^{19}F NMR ($CDCl_3$): δ −76.4; DCIMS *m/z* 401 ($[M + NH_4]^+$, 100%). Anal. Calcd. for $C_{14}H_{16}O_8NF_3$: C, 43.87; H, 4.21; N, 3.65. Found: C, 43.58; H, 4.28; N, 3.90.

2-Trifluoromethyl-(3,4,6-tri-O-*acetyl-1,2-dideoxy-α*-D-*glucopyrano)[2,1*-D*]-Δ²-oxazo line* (**3**).—Obtained as a clear oil as described above; $[\alpha]_D^{22}$ +3.6° (*c* 2.2, $CHCl_3$), lit. +5.5° (*c* 2, $CHCl_3$) [18]; R_f 0.32 (7:3 benzene–ether); ^{1}H NMR ($CDCl_3$): δ 6.27 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 5.31 (dd, 1 H, $J_{2,3} = J_{3,4}$ 2.4 Hz, H-3), 4.93 (ddd, 1 H, $J_{2,4}$ 1.3, $J_{4,5}$ 8.2 Hz, H-4), 4.36 (m, 1 H, H-2) 4.28–4.15 (m, 2 H, H-6, H-6′), 3.63 (m, 1 H, H-5), 2.10, 2.08, 2.06 (3s, each 3, 3Ac); ^{13}C NMR ($CDCl_3$): δ 170.60, 169.33, 169.02 (*C*OCH_3, 3Ac), 156.22 (q, $J_{C,F}$ 41.0 Hz, *C*NCF_3), 115.90 (q, $J_{C,F}$ 273.0 Hz, CF_3), 102.22 (C-1), 68.77, 68.60, 67.51, 63.97, 63.20 (C-2, C-3, C-4, C-5, C-6) 20.77, 20.63, 20.62 (CH_3, 3Ac); ^{19}F NMR ($CDCl_3$): δ −71.3; DCIMS *m/z* 401 ($[M + NH_4]^+$, 100%). Anal. Calcd. for $C_{14}H_{16}O_8NF_3$: C, 43.87; H, 4.21; N, 3.65. Found: C, 44.11; H, 4.19; N, 3.57.

Diphenyl (3, 4, 6 - tri - O - *acetyl - 2 - deoxy - 2 - trifluoroacetamido - α* - D - *glucopyranosyl) phosphate* (**5**).—A mixture of **4** (1.24 g, 3.1 mmol) and DMAP (0.87 g, 7.1 mmol) in 25 mL of dry CH_2Cl_2 was stirred at room temperature for 10 min and then cooled to −10 °C. Diphenylchlorophosphate (1.4 mL, 7.1 mmol) was added dropwise and the solution was stirred at this temperature for 2 h. The mixture was then diluted with CH_2Cl_2 (30 mL) and washed with ice-cold water, ice-cold 0.5 M HCl and saturated $NaHCO_3$. After drying the organic phase over $MgSO_4$, it was concentrated to a small residue which was purified by column chromatography using 2:3 EtOAc–hexanes to afford the title compound as white crystals (1.02 g, 52%): mp 65 °C; $[\alpha]_D^{20}$ +60.0° (*c* 1, $CHCl_3$); R_f 0.27 (2:3 EtOAc–hexanes); ^{1}H NMR ($CDCl_3$): δ 7.40–7.08 (m, 10 H, 2Ph), 6.88 (br d, 1 H, $J_{2,NH}$ 8.7 Hz, NH), 6.01 (dd, 1 H, $J_{H,P}$ 6.0 Hz, $J_{1,2}$ 3.2 Hz, H-1), 5.32 (dd, 1 H, $J_{3,4}$ 9.9, $J_{2,3}$ 11.3 Hz, H-3), 5.20 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.41 (ddd, 1 H, H-2), 4.20–3.88 (m, 3 H, H-5, H-6, H-6′), 2.03, 2.01, 1.99 (3s, each 3 H, 3Ac); ^{13}C NMR ($CDCl_3$): δ 170.92, 170.54, 169.21 (*C*OCH_3, 3Ac), 157.77 (q, $J_{C,F}$ 38.4 Hz, *C*OCF_3), 129.96, 129.89, 125.85, 125.80, 119.91, 119.85, 119.82, 119.76 (2Ph), 115.45 (q, $J_{C,F}$ 287.6 Hz, CF_3), 96.12 (d, $J_{C,P}$ 7 Hz, C-1), 70.03 (C-5), 69.39 (C-3), 67.20 (C-4), 60.91 (C-6), 52.48 (d, $^2J_{C,P}$ 8.4 Hz, C-2), 20.53, 20.45, 20.24

(CH_3, 3Ac); ^{19}F NMR ($CDCl_3$): δ −76.1; ^{31}P NMR ($CDCl_3$): δ −14.39; DCIMS *m/z* 651 ($[M + NH_4]^+$, 100%). Anal. Calcd. for $C_{26}H_{27}O_{12}NPF_3$: C, 49.30; H, 4.30; N, 2.21. Found: C, 49.08; H, 4.41; N, 2.22.

*Monotriethylammonium salt of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-*D*-glucopyranosyl phosphate* (**6**).—A solution of **5** (250 mg, 0.4 mmol) in 1:1 EtOAc–MeOH (10 mL) was hydrogenated (4 atm) in the presence of platinum oxide catalyst (25 mg). After 4 h, the catalyst was removed by filtration and Et_3N (2 mL) was added to the filtrate. Evaporation of the solvent afforded a syrup that was purified by preparative thin layer chromatography using 10:10:1 $CHCl_3$–MeOH–water to give the free acid form of **6** as a white hygroscopic solid (60 mg, 32%), this was converted to the monotriethylammonium salt by passing through a small column (3 cm) of Dowex 50W-X8 (triethylammonium form): R_f 0.6 (10:10:1 $CHCl_3$–MeOH–water); ^{1}H NMR (deuterium oxide): δ 5.53 (dd, 1 H, $J_{1,P}$ 7.1 Hz, $J_{1,2}$ 3.1 Hz, H-1), 5.42 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.8 Hz, H-3), 5.13 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.50–4.14 (m, 4 H, H-2, H-5, H-6, H-6′) 2.14, 2.10, 2.02 (3s, each 3 H, 3Ac); ^{13}C NMR (deuterium oxide): δ 174.07, 173.43, 173.14 (*C*OCH_3, 3Ac), 159.64 (q, $J_{C,F}$ 38.4 Hz, *C*OCF_3), 115.86 (q, $J_{C,F}$ 284.5 Hz, CF_3), 92.79 (d, $J_{C,P}$ 5.5 Hz, C-1), 71.17 (C-5), 68.63 (C-3), 68.31 (C-4), 62.05 (C-6), 52.57 (d, $^2J_{C,P}$ 8.4 Hz, C-2), 20.41, 20.37, 20.20 (CH_3, 3Ac); ^{19}F NMR (deuterium oxide): δ −75.4; ^{31}P NMR (deuterium oxide): δ −1.07; HRLSIMS *m/z*: 480.0522 (calcd. for $C_{14}H_{19}O_{12}NPF_3$, 480.0519).

*Monopyridinium salt of 2-deoxy-2-trifluoroacetamido-α-*D*-glucopyranosyl phosphate* (**7**).—Compound **6** (180 mg, 0.4 mmol) was dissolved in 3 mL of dry MeOH. The solution was cooled to 0 °C and 0.50 mL of NaOMe in MeOH (1 M) was added. After stirring for 20 min, the reaction was quenched using Dowex 50W-X8 resin (pyridinium form, previously washed with MeOH). The resin was filtered off and the methanolic solution was evaporated. The residue was dissolved in a small amount of water, passed through a small column (3 cm) of Dowex 50W-X8 (pyridinium form) resin and lyophilized to give **7** as the monopyridinium salt (120 mg, 68%): ^{1}H NMR (deuterium oxide): δ 5.48 (dd, 1 H, $J_{1,P}$ 7.2, $J_{1,2}$ 3.2 Hz, H-1), 4.04 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 3.93–3.72 (m, 4 H, H-3, H-5, H-6, H-6′), 3.53 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4); ^{13}C NMR (deuterium oxide): δ 159.61 (q, $J_{C,F}$ 37.6 Hz, *C*OCF_3), 115.96 (q, $J_{C,F}$ 284.7 Hz, CF_3), 93.12 (d, $J_{C,P}$ 5.8 Hz, C-1), 73.04 (C-5), 70.20 (C-3), 69.80 (C-4), 60.50 (C-6), 54.65 (d, $^2J_{C,P}$ 8.0 Hz, C-2); ^{19}F NMR (deuterium oxide): δ −75.7; ^{31}P NMR (deuterium oxide): δ −1.33; HRLSIMS *m/z* 354.0189 (calcd. for $C_8H_{12}O_9NPF_3$: 354.0202).

Preparation of **7** *by the MacDonald procedure*.—Crystalline phosphoric acid (500 mg, 5.1 mmol) was dried in vacuo over phosphorus pentoxide for 12 h. Solid 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranose (280 mg, 0.63 mmol) was added and the mixture was heated at 60 °C in vacuo. The formation of a melt and the evolution of CH_3COOH vapors was observed. 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. 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 applied to two plates of silica gel (2 mm thickness) and eluted with 10:10:1 $CHCl_3$–MeOH–water. Isolation of the corresponding zone gave crude **6** as a light brown solid. This material was *O*-deacetylated and purified as described above to give **7** (73 mg, 27%).

*Dilithium uridine 5′-(2-deoxy-2-trifluoroacetamido-α-*D*-glucopyranosyl diphosphate)* (**8**).—A solution of 4-morpholine *N,N′*-dicyclohexylcarboxamidinium uridine 5′-phosphomorpholidate (210 mg, 0.31 mmol) in anhydrous pyridine (5–7 mL) was concentrated to dryness in vacuo. The process of dissolution in fresh dry pyridine and concentration was repeated twice, dry air being admitted into the flask after each concentration. Separately, an aqueous solution of the monopyridinium salt of sugar phosphate **7** (100 mg, 0.22 mmol) was treated similarly. A solution of the residue in pyridine was added to the dry phosphomorpholidate described above. This solution was concentrated in vacuo and redissolved in ~5 mL of dry pyridine. The resulting solution was sealed, kept at room temperature for 5 days and then concentrated. The residual syrup was redissolved in 10 mL of water, applied onto a column of Dowex 1X4-200 (chloride form, strongly basic), and eluted with a gradient of lithium chloride (0.1–1 M) at pH 3.5. The sugar nucleotide containing fractions were pooled, concentrated to a small volume, and desalted by passing through a column of Bio-Gel P-2 column (2.5 × 45 cm) eluted with water. The appropriate fractions were pooled and lyophilized to give 67 mg (44%) of **8** as its dilithium salt (dihydrate): ^{1}H NMR (deuterium oxide): δ 7.92 (d, 1 H, $J_{5,6}$ 8.1 Hz, H-6),

5.93 (d, 1 H, $J_{1',2'}$ 4.9 Hz, H-1′), 5.91 (d, 1 H, H-5), 5.57 (dd, 1 H, $J_{1'',P}$ 7.1, $J_{1'',2''}$ 3.3 Hz, H-1″), 4.34–4.28 (m, 2 H, H-2′, H-3′), 4.25–4.12 (m, 3 H, H-4″, H-5′a, H-5′b), 3.95–3.72 (m, 4 H, H-2″, H-5″, H-6″a, H-6″b, H-3″), 3.54 (dd, 1 H, $J_{3'',4''} = J_{4'',5''}$ 9.6 Hz, H-4″); ^{13}C NMR (deuterium oxide): δ 160.4 (q, $J_{C,F}$ 37.7 Hz, *C*OCF$_3$), 167.2 (C-4), 152.7 (C-2), 142.5 (C-6), 116.6 (q, $J_{C,F}$ 284.4 Hz, CF$_3$), 103.5 (C-5), 94.6 (d, $J_{C,P}$ 5.9 Hz, C-1″), 89.3 (C-1′), 84.1 (d, $J_{C,P}$ 9.2 Hz, C-4′), 74.7 (C-3′), 73.9 (C-5″), 71.2 (C-2′), 70.5, 70.3 (C-3″, C-4″), 65.8 (d, $J_{C,P}$ 5.5 Hz, C-5′), 61.1 (C-6″), 55.3 (d, $^2J_{C,P}$ 8.8 Hz, C-2″); ^{19}F NMR (deuterium oxide): δ −74.9; ^{31}P NMR (deuterium oxide): δ −11.01 (d, *J* 20.3 Hz, P*β*), −12.90 (d, *J* 20.3 Hz, P*α*); HRLSIMS *m/z* 674.07928 (calcd. for $C_{17}H_{23}O_{17}N_3P_2F_3Li_2$, 674.0775). Anal. Calcd. for $C_{17}H_{22}N_3O_{17}P_2F_3Li_2 \cdot 2H_2O$: C, 28.79; H, 3.70; N, 5.92. Found: C, 28.68; H, 3.79; N, 5.71.

*Preparation of the glycosyltransferase 'core-2'-GlcNAcT (UDP-*D*-GlcpNAc:β-*D*-Gal-(1 → 3)-α-*D*-GalpNAc - β - (1 → 6) - GlcNAc transferase (EC 2.4.1.102)).*—Mouse kidney acetone powder (1 g, Sigma) was suspended in 25 mL NaOAc buffer (A: 0.1 M NaOAc, pH 6.0; 0.2 M NaCl, 0.01 M EDTA) containing 12 mg trypsin and stirred vigorously overnight at 4 °C. The slurry was centrifuged at 6000 × *g* for 20 min at 4 °C. Soybean trypsin inhibitor (5 mg) was added to the supernatant which was stored at 4 °C. The pellet was resuspended in 25 mL of buffer A containing 12 mg of trypsin and stirred vigorously overnight at 4 °C. This preparation was centrifuged at 6000 × *g* for 20 min at 4 °C. The addition of 5 mg of soybean trypsin inhibitor to the combined supernatants was followed by the overnight dialysis verses 1 L of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (B: 0.05 M HEPES, pH 6.5; 0.005 M manganese(II) chloride, 0.1% 2-mercaptoethanol). Dialysis tubing with a molecular weight cutoff of 12 000–14 000 Da was used. The dialysate was centrifuged at 100 000 × *g* for 30 min at 4 °C and the supernatant was then assayed for GlcNAcT activity (~170 mU). This crude extract was then loaded, at a flow rate of 0.15 mL/min, onto a 20 mL UDP-hexanolamine Sepharose 4B gel (3.4 μmol/mL) column which had previously been equilibrated with buffer B. The column was then washed with buffer B (40 mL) and subsequently eluted with 30 mL of 0.5 M NaCl buffer (C: 0.05 HEPES, pH 6.5; 0.5 M NaCl, 0.1% 2-mercaptoethanol). Fractions (1 mL) were collected in plastic tubes and assayed for 'core-2' activity. The fractions containing the highest transferase activity (~119 mU/6 mL) were used for preparative glycosylations.

Radiochemical assay for GlcNAcT.—GlcNAcT activity was measured using a modified version of a previously published procedure [11]. Each assay tube contained: 1.6 μL of 500 mM GlcNAc solution, 1.0 μL of 10 mM UDP-GlcNAc, 1.5 μL of a 3.6 mM solution of acceptor **1**, 0.5 μL of UDP-6-[^{3}H]GlcNAc (~40 000 dpm), and 7.5 μL of HEPES buffer (0.2 mM HEPES, pH 6.5; 2% Triton X-100). To this total of 12.1 μL was added 7.9 μL of crude or diluted enzyme sample. The mixture was incubated for 90 min at 37 °C, diluted with approximately 3 mL of water and loaded onto a C_{18} Sep-Pak cartridge which had been pre-equilibrated with water. The cartridge was washed with 50 mL of water and the product eluted into a scintillation vial with 4 mL of MeOH. The radioactivity of the eluants were quantitated by liquid scintillation counting using 10 mL of scintillation cocktail.

*Enzymatic conversion of β-Gal-(1 → 3)-α-GalNAc-OR (***9***) into β-Gal-(1 → 3)-[β-GlcpNAc-F$_3$ (1 → 6)]-α - GalpNAc - OR* (**10**).—UDP-*N*-trifluoroacetylglucosamine (0.46 mg, 0.64 μmol), **9** (0.8 mg, 1.45 μmol), bovine serum albumin (25 μL of a 100 mg/mL aqueous solution) and alkaline phosphatase from calf intestine (1 U), were added to a 1.5 mL microcentrifuge tube. This was followed by the addition of 490 μL of GlcNAcT (14 mU) and 474 μL of 50 mM HEPES buffer, pH 6.5 containing 0.1% 2-mercaptoethanol. The reaction mixture was incubated at room temperature. Additional donor (0.38 mg, 0.53 μmol) was added after 48 h. After 96 h donor (0.3 mg, 0.42 μmol) and alkaline phosphatase (0.5 U) were added to the reaction. After 8 days, the reaction was worked up by loading it onto two pre-washed C_{18} Sep-Pak cartridges. The cartridges were washed with 30 mL of water and the crude product eluted with 30 mL of MeOH. The MeOH eluant was evaporated to dryness and the residue chromatographed on an Iatrobead-column with 30:20:1 CH_2Cl_2–MeOH–water. The product fractions were pooled, concentrated, dissolved in water and filtered through a 0.22 μm filter before lyophilization. Trisaccharide **10** was obtained in 62% (0.73 mg) isolated yield. Partial ^{1}H NMR assignments are reported in Table 1. HRESIMS *m/z* 833.3173 (calcd. for $C_{32}H_{53}N_2O_{18}F_3Na$: 833.3143).

UDP-glucose and UDP-glucosamine were used as donors in the above reaction but no transfer was observed after 8 days.

Glucosyltransferase GlcNAcT - V [UDP - D *-*

GlcpNAc:α-D-Manp-(1→6)-β-D-GlcpNAc-V transferase (GlcNAcT-V, EC 2.4.1.155)].—The 97 kDa GlcNAcT-V transferase was cloned from rat kidney [27] and was a generous gift from Dr. M. Pierce, Department of Biochemistry, University of Georgia.

Radiochemical assay for GlcNAcT-V.—Enzyme activity was monitored using a modified version of a previously published procedure [28]. Each assay tube contained: 8.0 μL of 1.38 mM UDP-GlcNAc, 1.0 μL of a 0.8 mM solution of acceptor **11**, 1.0 μL of UDP-6-[^{3}H]GlcNAc (~10^5 dpm), and 5.0 μL of sodium cacodylate buffer (100 mM sodium cacodylate, pH 6.5; 20 mM EDTA; 40% glycerol; 2 mg/mL bovine serum albumin). The work-up of the incubated samples was performed in the same manner as given previously for the radiochemical assay for GlcNAcT.

Enzymatic conversion of β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Glcp-OR (R=(CH$_2$)$_7$CH$_3$) (**11**) into β-D-*GlcpNAc-(1→2)-[β-D-GlcpNAc-F$_3$-(1→6)]-α-D-Manp-(1→6)-β-D-Glcp-OR (R=(CH$_2$)$_7$CH$_3$)* (**12**).—UDP-*N*-trifluoroacetylglucosamine (2.4 mg, 3.34 μmol), **11** (4.2 mg, 6.39 μmol), 500 μL of 50 mM sodium cacodylate buffer, pH 6.5 (containing 10 mM EDTA, 20% glycerol and 1 mg/mL bovine serum albumin) and 50 μL of GlcNAcT-V (23 mU) were added to a 1.5 mL microcentrifuge tube. The reaction mixture was kept at room temperature. Additional donor (1.9 mg, 2.64 μmol) was added after 72 h. After 2 weeks, the reaction was worked up using the same C_{18} Sep-Pak procedure that was employed previously for the GlcNAcT-reaction. The dried MeOH residue was chromatographed on an Iatrobead-column eluted with 65:35:3 $CHCl_3$–MeOH–water. The pooled product fractions were concentrated, dissolved in water and filtered through a 0.22 μm filter before lyophilization. Tetrasaccharide **12** was obtained in a 56% (3.26 mg) isolated yield. Partial ^{1}H NMR assignments are reported in Table 2. HRESIMS *m/z* 937.3622 (calcd. for $C_{36}H_{61}N_2O_{21}F_3Na$: 937.3617).

Deprotection of β-D-GlcpNAc-(1→2)-[β-D-GlcpNAc-F$_3$-(1→6)]-α-D-Manp-(1→6)-β-D-Glcp-OR (R=(CH$_2$)$_7$CH$_3$) (**12**).—Deprotection of tetrasaccharide **12** (0.5 mg, 0.55 μmol) was successfully accomplished using potassium carbonate in water–MeOH (1:15). The reaction was complete after 1 week at 40 °C. A C_{18} Sep-Pak work-up was used to obtain the deprotected tetrasaccharide **13**. Partial ^{1}H NMR assignments are reported in Table 2. HRESIMS *m/z* 819.3984 (calcd. for $C_{34}H_{63}N_2O_{20}$: 819.3974).

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