Supplemental Material

I. Abbreviations

NeuAc: N-acetylneuraminic acid or sialic acid
Gal: Galactose
GlcNAc: N-acetyl-D-glucosamine
GalNAc: N-acetyl-D-galactosamine
Fuc: Fucose
SialylT: sialyltransferase
ST3[Galβ1,3GalNAc]: All enzymes responsible for α2,3sialylation of Galβ1,3GalNAc- (type III structure). This includes cloned ST3Gal-I and -II.
ST3[Galβ1,4GlcNAc]: All enzymes responsible for α2,3sialylation of Galβ1,4GlcNAc- (type II structure). This includes cloned ST3Gal-III, -IV and -VI.
ST6[Galβ1,4GlcNAc]: All enzymes responsible for α2,6sialylation of Galβ1,4GlcNAc- (type II structure). This includes cloned ST6Gal-I.
FT: fucosyltransferase
FT[Sialyl-LacNAc]: Enzyme(s) responsible for α1,3fucosylation of NeuAcα2,3Galβ1,4GlcNAc- (Sialyl-LacNAc) structure
GalT: galactosyltransferase
GalNAcT: N-acetylgalactosaminyl transferase
GlcNAcT: N-acetylglucosaminyl transferase
SulfoT: sulfotransferase
LacNAc: N-acetyllactosamine or Galβ1,4GlcNAc
sLe^X: sialyl Lewis X
F: Fluoro
Me: Methyl
Bn: Benzyl
HU: Hydroxyurea
PI: Propidium iodide
S-N: Sugar-nucleotide

II. Cell cycle analysis by propidium iodide (PI)

For cell cycle analysis, samples containing $0.2\text{-}0.4\times10^6$ cells at different time points in each of the different growth phases, before/after synchronization by HU, were collected, fixed with 80% ethanol in PBS and stored overnight at –20°C. The next day, cells were pelleted by centrifugation and ethanol was discarded. The samples were then equilibrated for 5 min at 37°C in 100µl SSC buffer (150mM sodium chloride, 15mM sodium citrate, pH=7.0). Following this, 100µg/ml DNase-free RNase (Sigma) in SSC buffer was added for 20 min at 37°C, cells were washed twice in SSC, and resuspended in 1ml PBS for 15 min to allow rehydration. These cells were then centrifuged and resuspended in 200µl Tris buffer (100mM Tris, 150mM NaCl, 1mM $CaCl_2$, 0.5mM $MgCl_2$, 0.1% Tween-20, pH 7.4) containing 10µg/ml

PI (propidium iodide) for 20 min in dark at room temperature. Following this, samples were analyzed using a FACSCalibur flow cytometer with CellQuest software. Acquired events were gated on FL2-W versus FL2-A to discriminate singlets from doublets and larger aggregates, and the distribution of singlet cells in various growth phases was quantified using the FL2-A histogram.

III. RNA isolation and real time RT-PCR of glycosyltransferase genes

Total RNA was isolated using the SV total RNA isolation system (Promega, Madison, WI), and cDNA was synthesized from RNA using the iScript reverse transcriptase kit (Bio-Rad, Hercules, CA) according to manufacturer's instructions. Real time PCR reactions, using iQ SYBR-green Supermix (Bio-Rad), were performed on the My iQ Single-Color Real-Time PCR Detection System (Bio-Rad). Primer pairs for each mRNA, corresponding to PSGL-1 and an array of glycosyltransferase genes, were designed based on sequence information deposited in the GenBank (Supplemental Table S1). Relative mRNA levels are derived from ΔCt, the difference of the threshold cycle value of the target mRNA and the Ct value for RPL32, a ribosomal protein mRNA that was used as a reference standard.

IV. Cytometry-bead preparation

Carbodiimide chemistry was used to link anti-PSGL-1 mAb TB5 (Mouse monoclonal IgG1 against protein N-terminus from BioVendor, Candler, NC) to 6μm carboxyl polystyrene beads (Polysciences, Warrington, PA). Briefly, these beads were activated overnight at RT with a mixture of 0.05M EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide·HCl) and 0.05M sulfo-NHS (Pierce, Rockford, IL) in PBS buffer (pH=7.2). The next day, the beads were washed and resuspended in PBS (pH=7.8) containing 20μg TB5 (concentration of 0.2 mg/ml) for 2-3 hours at RT. Unreacted mAb was removed by washing and unreacted active sites were quenched with 40mM ethanolamine for 30 min. Bead surface was then blocked with PBS (pH=7.2) containing 1% BSA prior to use. Polystyrene beads thus obtained are termed “TB5-beads”. Using the same procedure, an isotype matched mAb (anti-CD18/β2-integrin, clone IB4) was conjugated to activated beads to prepare “IB4-beads”.

V. Glycosyltransferase reaction conditions

The following are the conditions for individual enzymatic assays:

A. Sialyltranferase (SialylT): Reaction temperature in all cases was 37°C. α2,3- and α2,6-sialyltransferase (SialylT) assay reactions proceeded for 2h in a mixture containing 100mM sodium cacodylate buffer (pH=6.0), 7.5mM acceptor, 0.2μCi of CMP-[9-^{3}H] NeuAc (specific activity=33.6Ci/mmol) and 5μl cell extract in a total volume of 20μl (1).

B. N-acetylglucosaminyltransferase (GlcNAcT): β1,3- and β1,2 GlcNAcT assay reactions were carried out for 2h. The reaction mixture in these cases contained 70mM Hepes buffer (pH=7.0), 7mM GlcNAc1,5 lactone (Toronto Research Chemicals, ON, Canada), 0.7mM 2-aceto-amido-1,2-dideoxy nojirimycin (Toronto Research Chemicals), 14mM Mn acetate, 5mM ATP, 2mM NaN_3, 5mM synthetic acceptor or 100μg fetuin-based acceptor, 0.2μCi of UDP-[6-^{3}H]GlcNAc (specific activity=36Ci/mmol) and 10μ1 of cell extract in a total volume of 30μ1.

C. Galactosyltransferase (GalT): β1,4 Galactosyltransferase (GalT) and β1,3GalT assay mixtures contained 100mM Hepes (pH=7.0), 7mM ATP, 20mM Mn acetate, 1mM UDP-Gal, 0.5mM acceptor, 0.05μCi of UDP-[^{14}C]Gal (327mCi/mmol) and 5μl cell extract in a total volume of 20μl. Reaction was carried out for 4h (2).

D. Fucosyltransferase (FT): α1,2-, α1,6-, α1,3- and α1,4-fucosyltransferase (FT) assay reactions were carried out for 2h in a reaction mixture containing 50mM Hepes buffer (pH=7.5), 5mM $MnCl_2$, 7mM ATP, 3mM NaN_3, 3mM synthetic acceptor or 40μg of fetuin-based acceptor, 0.05μCi of GDP-[^{14}C]Fuc (290mCi/mmol) and 5μl cell extract in a total volume of 20μl (3, 4).

E. Sulfotransferase (SulfoT): SulfoT assay reactions were run for 2h with 100mM Tris-maleate (pH=7.2), 5mM Mg acetate, 5mM ATP, 10mM NaF, 10mM BAL, 7.5mM acceptor, 0.5μCi of [35S]PAPS (2.4Ci/mmol) and 10μ1 of cell extract in a volume of 30μ1 (5).

F. ppGalNAc transferase (ppGalNAcT): Reaction mixture (25μl) consists of 100mM Hepes buffer (pH=7.0) with protease inhibitor cocktail III (Calbiochem, La Jolla, CA), 7mM ATP, 20mM Mn acetate,

UDP-[6-^{3}H]GalNAc diluted with cold UDP-GalNAc to a final concentration of 350μM (27,500 cpm/nmol), 10μl cell lysate (13μg/μl) and 50μg (520.4μM) of truncated PSGL-1 N-terminal sequence synthetic peptide (N-term acetylated QATEYEYLDYDFLPETEPPEMKHHHHHHLR, GenScript Corp., Piscataway, NJ). Reaction was carried out at 37ºC for 4h. Separation of His-tagged peptide from the reaction mixture was carried out by addition of 1ml PBS buffer containing 500mM NaCl (binding buffer) to the reaction mixture along with MagneHis^{TM} Ni particles (Promega, Madison, WI) for 30min at RT, followed by extensive (5-times) washing of these particles with binding buffer containing 10mM Imidazole. These particles were then added to 15ml of scintillation fluid and the radioactivity associated with peptide was quantified. Counts from blank control containing everything except the synthetic peptide were less than 10% of the sample signal.

VI. Acceptor specificity

The following text describes the synthetic carbohydrate substrates used in enzymology based assays and potential enzyme activities that they measure. Also provided are a list of potential genes/enzymes that display this activity. Although the substrates have been specifically designed to distinguish between closely related enzymes, it is possible that multiple gene-products/enzymes could catalyze identical reactions on a given substrate. In this case, it is not possible to determine which enzyme is more important in mediating a particular activity. Further, while the text is based on current knowledge of Biochemistry, it is clear that the substrate specificity of glycosyltransferases is not fully defined at the current time.

A. Sialyltransferase (SialylT)

Acceptor specificity of sialyltransferases (SialylTs) is based on our recent paper (1). In this study, we show that while compound **1** (Figure 1A, main manuscript) is acted upon by both cloned α2,6 and α2,3 sialylTs, **2** and **3** can be applied to distinguish between these enzymes. In this regard, α2,3 sialylTs that act at the 3-position of Gal in Galβ1,3GalNAc (abbreviated ST3[Galβ1,3GalNAc]) act on **2** but not **3**. The α2,6 sialyltransferase ST6Gal-I and α2,3 sialylT specific for Galβ1,4GlcNAc (abbreviated ST3[Galβ1,4GlcNAc]) both act on **3** but nor **2**. In order to distinguish between the last two enzymes, we use the acceptors 2-O-MeGalβ1,3GlcNAcβ-O-Bn and 4-O-MeGalβ1,4GlcNAcβ-O-Bn which are sialylated exclusively by ST3[Galβ1,3GlcNAc] and ST3[Galβ1,4GlcNAc] respectively. Our study suggests that these last two acceptors may not be sialylated by either ST6Gal-I or ST3[Galβ1,3GalNAc] (1).

With regard to the relationship between our designation of ST3[Galβ1,3GalNAc] and previously cloned sialyltransferases, two enzymes ST3Gal-I and ST3Gal-II predominantly act to sialylate Galβ1,3GalNAc (Type III) type oligosaccharides and have low activity towards Galβ1,4GlcNAc (Type II) or Galβ1,3GlcNAc (Type I) chains (6).

Three different sialyltransferases namely, ST3Gal-III, ST3Gal-IV and ST3Gal-VI act on Galβ1,4GlcNAc (Type II), consistent with their involvement in the formation of sLe^X (7, 8). Of these ST3Gal-III shows strong preference towards Galβ1,3GlcNAc type (Type I) while ST3Gal-IV exclusive acts on Galβ1,4GlcNAc (Type II). The later enzyme is a major enzyme contributing to leukocyte selectin function in knockout mice models (9). ST3Gal-III expression has also been correlated with the formation of sLe^X in lung carcinomas (10). In our studies, we attribute the activity on acceptor 2-O-MeGalβ1,3GlcNAcβ-O-Bn by ST3[Galβ1,3GlcNAc] to ST3Gal-III, while activity towards 4-O-MeGalβ1,4GlcNAcβ-O-Bn by ST3[Galβ1,4GlcNAc] is likely due to both ST3Gal-IV and -VI.

B. Galactosyltransferase (GalT)

Acceptor specificity of GalT is based on our earlier studies (2). GlcNAcβ1,6GalNAcα-O-Bn was used as an acceptor for both the attachment of Gal to the 4-position of GlcNAc and for the attachment of Gal to the 3-position of GalNAc. 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn was used to examine the activity of only the former enzyme in the absence of the later. Similarly 4-F-GlcNAcβ1,6GalNAcα-O-Bn and GalNAcα-O-Bn were used to study GalT activity at the 3-position of GalNAc (core 1 β1,3GalT-I). A combination of two cloned β1,4GalTs, β1,4GalT-I and -IV, likely act on 3-O-MeGalβ1,3(GlcNAcβ1,6) GalNAcα-O-Bn in our assay. Of these, GalT-IV is a major enzyme involved in the formation of polyLacNAc structures in O-glycans, while GalT-I has similar function in N-glycans (11).

C. N-acetylglucosaminyl transferase (GlcNAcT)

The primary goal of our studies of GlcNAcT is to compare the attachment of GlcNAc to the 3-position of Gal in either the Galβ1,4GlcNAc (Type II) or Galβ1,3GalNAc (Type III) residues. In this regard, Compound **3** and the tetrasaccharide Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-O-Bn quantify the extension of lactosamine (LacNAc) type structures (referred to as LacNAc extension β1,3GlcNAcT activity). Compound **2** on the other hand measures the rate of Core-1 extension (referred to as Core-1

extension β1,3GlcNAcT activity). Data for Globo-H (Galβ1,3GalNAcβ1,3Galα-O-Me) and fetuin triantennary acceptor are also provided.

D. Fucosyltransferase (FT)

Previous studies with FTs guide the choice of acceptor specificities (3, 12). Different FTs (FT-IV/-V/-VI/-IX) can fucosylate the LacNAc chain in the core-2 structures. Among these, the myeloid α1,3FT-IV is abundant in HL-60 cells while α1,3 FT-IX is the main enzyme involved in CD15/Le^X epitope formation in neutrophils. Both these enzymes act more efficiently on Galβ1,4GlcNAcβ (LacNAc) compared to sialyl-LacNAc (NeuAcα2,3Galβ1,4GlcNAcβ) (12-14). α1,3FT-VII, on the other hand, exclusively transfers Fuc to sialyl-LacNAc. Among the FTs, only α1,3/4FT-III acts on Galβ1,3GlcNAcβ- to give Le^a (Lewis-a) type structures. GlcNAcβ1,4GlcNAcβ-O-Bn is used as a specific acceptor for FT-VI. While most FTs attach fucose to the GlcNAcβ residue, only α1,2FT attaches fucose to Galβ in the formation of blood group type antigens. Thus, Galβ-O-Bn is used to measure α1,2FT activity. Further, acceptors where the 2-position of Gal is blocked with a methyl substituent, 2-O-MeGalβ1,4GlcNAcβ-O-Bn and 2-O-MeGalβ1,3GlcNAcβ-O-Bn, were used for the measurement of α1,3FT-IV/FT-IX and α1,3/4 FT-III exclusively in the absence of α1,2FT activity.

E. Sulfotransferase (sulfoT)

Core-2 based acceptors were also used to examine sulfotransferase activity. Previously, we have shown that Compound **1** is acted upon by Gal:3-O-sulfotransferases (clones Gal3ST-2, -3 and -4) (5). Among the Gal:3-O-sulfotransferases, while compound **2** was specific for Gal3ST-4 compound **3** was acted upon by Gal3ST-2 and -3. The trisaccharide 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn was acted upon by GlcNAc:6-O-sulfotransferase but not the Gal:3-O-sulfotransferases. Finally, all compounds (**1**, **2** and **3**) can serve as a substrate for GlcNAc:6-O-sulfotransferases.

VII. Deriving *in vivo* estimates of rate constants (k_{vivo}) from *in vitro* experimental data[1]

An explicit relation between the apparent first order rate constant (k) reported for our *in vitro* assay (Table 2, main manuscript) and that which is expected *in situ* or *in vivo* (k_{vivo}) is derived. This derivation is based on the concept that glycosyltransferase reactions proceed via a bi-bi reaction mechanism (Eq. S1). The exact mechanism followed depends on the individual glycosyltransferase, the relative concentrations of the acceptor (A) and activated monosaccharide/sugar-nucleotide (M) in the reaction mixture (15, 16). Depending on the circumstance, either enzyme-acceptor (EA) complex or enzyme-monosaccharide (EM) complex formation can precede enzyme-monosaccharide-acceptor (EMA) complex formation. In the final step, the nucleotide (N) and glycan product (P) are formed.

$$\left.\begin{array}{l} \mathrm{E}+A \xleftarrow{K_1} \mathrm{EA}+M \\ \mathrm{E}+M \xleftarrow{K_{1'}} \mathrm{EM}+A \end{array}\right\} \xleftarrow{K_2 \text{ or } K_{2'}} \mathrm{EMA} \xrightarrow{k_{cat}} \mathrm{E}+\mathrm{N}+\mathrm{P} \qquad \text{(S1)}$$

Based on the above scheme, the following are reaction velocity expressions under our *in vitro* experimental and *in vivo* conditions:

In vitro estimates for Sialyl-, Fucosyl- and GlcNAc- transferase activity. Under our *in vitro* assay conditions, [EA] formation likely precedes [EMA] since $[A]_{vitro} >> [M]_{vitro}$ and $[A]_{vitro} > K_{M,\,acc}$ (Table S2). Thus, the reaction is limited by monosaccharide rather than acceptor concentration (upper reaction in Eq. 1). Using the rapid equilibrium assumption[2] , an expression for the velocity of product formation (V_{vitro}) can be derived:

$$V_{vitro} = k_{cat} \cdot \frac{[E_0]_{vitro}}{K_2\left(\frac{K_1}{[A]_{vitro}} + 1\right) + [M]_{vitro}} \cdot [M]_{vitro} = k_{cat} \cdot \frac{[E_0]_{vitro}[M]_{vitro}}{K_{M,mono} + [M]_{vitro}} \qquad \text{(S2)}$$

[1] Many of the mathematical expressions have either *vitro* or *vivo* in subscript, for example $[M]_{vitro}$ and k_{vivo}. The subscript *vitro* is used for measurements/parameters associated with the *in vitro* enzymology studies. The subscript *vivo* is used to describe the same parameter *in situ/in vivo* in the cellular Golgi.

[2] Rapid equilibrium assumption involves solving the following equations simultaneously: $K_1 = [E][A]/[EA]$; $K_2 = [EA][M]/[EMA]$; $[E_0] = [E] + [EA] + [EMA]$; V_{vitro} ($= k_{cat}[EMA]$)

Here, terms in square brackets ([]) denote concentration of individual species and the Michaelis-Menten constant ($K_{M,\,mono} = K_2\,(K_1/[\mathrm{A}] + 1)$) is calculated with respect to the monosaccharide since this is the rate limiting species. Further, since $K_{M,mono} > [M]_{vitro}$, the above expression simplifies to:

$$V_{vitro} = k_{cat} \cdot \frac{[E_0]_{vitro}[M]_{vitro}}{K_{M,mono}} = k[M]_{vitro} \tag{S3}$$

where k is the apparent first order rate constant that is quantified in our assays in Table 2.

In situ/vivo enzyme kinetics. *In situ,* $[\mathrm{M}]_{vivo} >> [\mathrm{A}]_{vivo}$ and $[\mathrm{M}]_{vivo} >> K_{M,\,mono}$. Thus, the reaction velocity *in vivo* (V_{vivo}) derived similar to above is:

$$V_{vivo} = k_{cat} \cdot \frac{[E_0]_{vivo}[A]_{vivo}}{K_{M,acc} + [A]_{vivo}} \tag{S4}$$

Similar to above, $K_{M,\,acc} = K_{1'}\,(K_{2'}/[\mathrm{M}] + 1)$. Further simplification is possible since $K_{M,\,acc} >> [A]_{vivo}$. Thus, the above expression can be simplified to a first order reaction with respect to acceptor concentration, $[A]_{vivo}$.

$$V_{vivo} = \left(k_{cat} \cdot \frac{[E_0]_{vivo}}{K_{M,acc}} \right) \cdot [A]_{vivo} = k_{vivo}[A]_{vivo} \tag{S5}$$

where, $k_{vivo} = k_{cat} \cdot \frac{[E_0]_{vivo}}{K_{M,acc}}$. Assuming that k_{cat} is the same both *in vivo* and *in vitro*, it follows from Eq. S3 and S5 that:

$$k_{vivo} = k \cdot \frac{[E_0]_{vivo}}{[E_0]_{vitro}} \cdot \frac{K_{M,mono}}{K_{M,acc}} \tag{S6}$$

In the above expression since $[E_0]_{vivo}/[E_0]_{vitro}$ is the same for all enzymes (it is the ratio of enzyme concentration in Golgi with respect to that in *in vitro* enzyme assays). Thus, it follows that k_{vivo} is proportional to $k \cdot (K_{M,\,mono}/K_{M,\,acc})$.

Table S1: Quantitative RT-PCR primers used to assay glycosyltransferase transcripts

Enzyme/Protein	Gene Name	GenBank Accession #	Forward primer (5'→3')	Reverse primer (5'→3')
PSGL-1*	SELPLG	NM_003006	GCAGATGAAGCCGAGAAAGC	CAGTCAGAGGAGTGGTGTCAG
ST6Gal-I*	ST6GAL1	NM_173216	GGACCCATCTGTATACCACTC	ATGTCCCATAGCTCCCAAGG
ST3Gal-VI*	ST3GAL6	NM_006100	GGCACCTGTGGAAATGAAAC	AACTTATCGCTACCATACAAGG
ST3Gal-IV*	ST3GAL4	NM_006278	CCCATCTTCCTGCGGCTTG	CCTGAGGCTCTGGATGTTCTTG
ST3Gal-III	ST3GAL3	NM_174963	TGATCGGTTGGGCTTCCTC	GGTGCTGGCTTGGAGAAC
ST3Gal-II	ST3GAL2	NM_006927	CTTCAAGTATATCCACGACAG	CAGTAGTGGTGCCAGTTG
ST3Gal-I	ST3GAL1	NM_003033	GCAAAGATCAGAGTGAAACAG	CACCTCATCGCAGACATG
α1,3FT-VII	FUT7	NM_004479	CTCCGAGGCATCTTCAAC	CTCCTGGAAGTTGCTGAC
α1,3FT-IV	FUT4	NM_002033	GTGTTGGACTACGAGGAG	CCAGTTGAAGAGGTTACTTG
Core 2 β1,6GlcNAcT-I*	C2GNT / GCNT1	NM_001097634	AAGGTGGAAGAAGCGGTATGAG	CTGACCACGAAGTAGGCACTG
Core 1 β1,3GalT-I	C1GALT1	NM_020156	AGCAGGAGATTCCAGAGATACC	TCAGAGCAGCAACCAGGAC
β1,4GalT-IV*	B4GALT4	AB024436	GGCGAAGACGATGACCTCAG	ATCCGTTCTGCGTTCACCTC
β1,4GalT-I	B4GALT1	NM_001497	TCATCATTCCATTCCGCAACC	ATTGAGGAGCTTAGCACGATTG
β1,3GlcNAcT-3	B3GNT3	NM_014256	GACTTCCACGACTCCTTCTTC	CGTCATCATCCCCGTTGAG
β1,3GlcNAcT-2*	B3GNT2	NM_006577	TGGAACCGAGAGCAAGAG	TCAGATGGCTTATATTGGAGAG
β1,3GlcNAcT-1	B3GNT1	NM_006876	GGCACCAATGTCTCCTAC	CACTGGTTGCTCTGATCC
RPL32	RPL32	NM_000994	AGCGTAACTGGCGGAAAC	CGTTGTGGACCAGGAACTTC

* PCR products for these genes were purified from agarose gel and sequenced to confirm validity of the RT-PCR assay. These are the primary genes products from which important conclusions are drawn in this manuscript.

Table S2: Enzymology experiment parameters: Studies with HL-60 cells [a]

1. Enzyme	2. Acceptor used[b]	3. Sugar-nucleotide (S-N)	4. Acceptor ($[A]_{vitro}$, μM)	5. Reaction time (hr)	6. Cell lysate[c] (μg)	7. Radioactive S-N (μM)	8. Unlabeled S-N[d] (μM)	9. S-N in assay from cell source[e] (μM)	10. Consumption of radio-sugar[a] (%)	11. K_M for S-N ($K_{M,\ mono}$, μM) (Reference)[f]	12. K_M for acceptor ($K_{M,\ acc}$, μM) (Reference)[f]
Sialyltransferase											
ST3[Galβ1,3GalNAc]	Galβ1,3(3-O-MeGalβ1,4GlcNAcβ1,6)GalNAcα-O-Bn **(#2)**	CMP-NeuAc	7500	2	36.5	0.2976	-	0.4928	0.6	23 (17)	400 (18)
ST3[Galβ1,4GlcNAc]	4-O-Me Galβ1,4 GlcNAcβ-O-Bn	CMP-NeuAc	7500	2	36.5	0.2976	-	0.4928	0.6	74.1 (19)	300 (20, 21)
ST3[Galβ1,4GlcNAc] and ST6[Galβ1,4GlcNAc]	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	CMP-NeuAc	7500	2	36.5	0.2976	-	0.4928	0.6	74.1 (19)	
Galactosyltransferase											
β1,4GalT	3-O-Me Galβ1,3 (GlcNAcβ1,6) GalNAcα-O-Bn	UDP-Gal	500	4	36.5	7.6453	1000	1.3724	9.9	31 (22)	290 (11, 23)
β1,3GalT	4-Fluoro GlcNAcβ1,6 GalNAcα-O-Bn	UDP-Gal	500	4	36.5	7.6453	1000	1.3724	2.6	630 (24)	
Fucosyltransferase											
α1,3FT	2-O-Me Galβ1,4 GlcNAcβ-O-Bn	GDP-Fuc	3000	2	36.5	8.6207	-	0.3289	12.7	10 (15)	1500 (16)
α1,3FT[Sialyl-LacNAc]	NeuAcα2,3Galβ1,4 GlcNAcβ-O-Bn	GDP-Fuc	3000	2	36.5	8.6207	-	0.3289	3.4	16.4 (25)	3080 (25)
GlcNAc transferase											
LacNAc extension β1,3GlcNAcT	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	UDP-GlcNAc	5000	2	73	0.1852	-	3.4115	5.1	200 (26)	710 (11)
GalNAc transferase											
ppGalNAcT	Truncated PSGL-1 N-terminal sequence peptide	UDP-GalNAc	520.4	4	130	350 (27500cpm/nmol)		Not known	0.32[g]	10-62 (27)	20 (27)

[a] acceptor + [^{3}H/^{14}C]sugar-nucleotide donor + cell lysate (containing glycosyltransferase)→ [^{3}H/^{14}C] product. Radioactive product is isolated using Dowex/C-18 chromatography or Nickel beads, and measured using scintillation counter. Based on this measurement, the amount of consumed radioactive-sugar nucleotide is quantified.

[b] All acceptor compounds were chemically synthesized. Composition was validated using NMR and Mass Spectrometry (see references (28-32)).

[c] Cell lysate amount used in each assay. This lysate contains the glycosyltransferase.

[d] In GalT assays only, 1mM unlabeled sugar nucleotide was added to the reaction mixture in addition to the radio-labeled sugar-nucleotide.

[e] Estimated based on amount of S-N reported in mammalian cells by Tomiya *et. al.*(33) and amount of cell lysate used in each assay (see footnote c).

[f] As seen, acceptor is in large excess compared to sugar-nucleotide in all cases except for GalT where 1mM exogenous UDP-Gal is added. K_M values are tabulated from various sources in literature under conditions where either sugar-nucleotide is limiting (K_M for S-N, $K_{M,\ mono}$) or acceptor is limiting (K_M for acceptor, $K_{M,\ acc}$).

[g] Estimated value based on transfer of GalNAc to the synthetic peptide.

Table S3: Glycosyltransferase analysis with cell cycle

Enzyme	Acceptor	% glycosyltransferase activity at specified times [a] (1mM hydroxyurea (HU) added at 0h and removed at 13h)					
		0h [a]	13h	20h	24h	31h	38h
Sialyltransferase							
ST3[Galβ1,3GalNAc]	Galβ1,3(3-O-MeGalβ1,4GlcNAcβ1,6)GalNAcα-O-Bn **(#2)**	100.0	96.1	100.8	107.2	111.5	141.5
ST3[Galβ1,3GlcNAc]	2-O-MeGalβ1,3GlcNAcβ-O-Bn	100.0	78.5	76.3	101.1	108.6	92.5
ST3[Galβ1,4GlcNAc] & ST6[Galβ1,4GlcNAc]	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	100.0	48.0	49.9	54.4	64.1	81.6
Galactosyltransferase							
β1,4GalT & β1,3GalT	GlcNAcβ1,6GalNAcα-O-Bn	100.0	91.6	94.5	99.5	97.7	103.5
β1,3GalT	4-F-GlcNAcβ1,6GalNAcα-O-Bn	100.0	120.9	115.2	125.5	118.7	112.5
β1,4GalT	Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Al	100.0	86.3	91.0	96.8	94.9	100.7
β1,4GalT	3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	100.0	74.9	78.8	83.3	81.2	88.1
GlcNAc transferase							
β1,3GlcNAcT	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	100.0	54.4	60.5	65.6	60.9	95.8
Fucosyltransferase							
α1,3FT	2-O-MeGalβ1,4GlcNAcβ-O-Bn	100.0	106.2	106.5	115.1	109.0	108.9
α1,3FT	GalNAcβ1,4GlcNAcβ-O-Bn	100.0	109.0	106.8	115.0	114.2	109.5
α1,3FT[Sialyl-LacNAc]	NeuAcα2,3Galβ1,4GlcNAcβ-O-Bn	100.0	101.1	101.4	119.7	114.1	99.4
α1,6FT	Fetuin triantennary asialo agalacto glycopeptide	100.0	70.2	65.9	83.7	82.7	76.1

[a] CPM data are presented as % with respect to HL-60 cells cultured in the absence of hydroxyurea.

ACCEPTOR + [^{3}H/^{14}C]sugar-nucleotide donor + cell lysate (containing glycosyltransferase)

↓

[^{3}H/^{14}C] PRODUCT

↓

Purify [^{3}H/^{14}C] PRODUCT using Dowex/C-18 chromatography and confirm unique product using TLC

Product /lane	Enzyme assayed	Acceptor	Donor sugar-nucleotide
	Sialyltransferase		
I	ST3[Galβ1,3GalNAc]	Galβ1,3(3-O-MeGalβ1,4GlcNAcβ1,6)GalNAcα-O-Bn **(#2)**	CMP-[9-^{3}H]NeuAc
II	ST3[Galβ1,4GlcNAc] & ST6[Galβ1,4GlcNAc]	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	CMP-[9-^{3}H]NeuAc
	Fucosyltransferase		
III	α1,3FT	2-O-MeGalβ1,4GlcNAcβ-O-Bn	GDP-[^{14}C]Fuc
IV	α1,3FT	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-O-Bn	GDP-[^{14}C]Fuc
	Galactosyltransferase		
V	β1,4GalT	3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	UDP-[^{14}C]Gal
VI	β1,3GalT	4-F-GlcNAcβ1,6GalNAcα-O-Bn	UDP-[^{14}C]Gal
	GlcNActransferase		
VII	LacNAc extension β1,3GlcNAcT	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)	UDP-[^{14}C]GlcNAc
VIII	LacNAc extension β1,3GlcNAcT	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-O-Bn	UDP-[^{14}C]GlcNAc

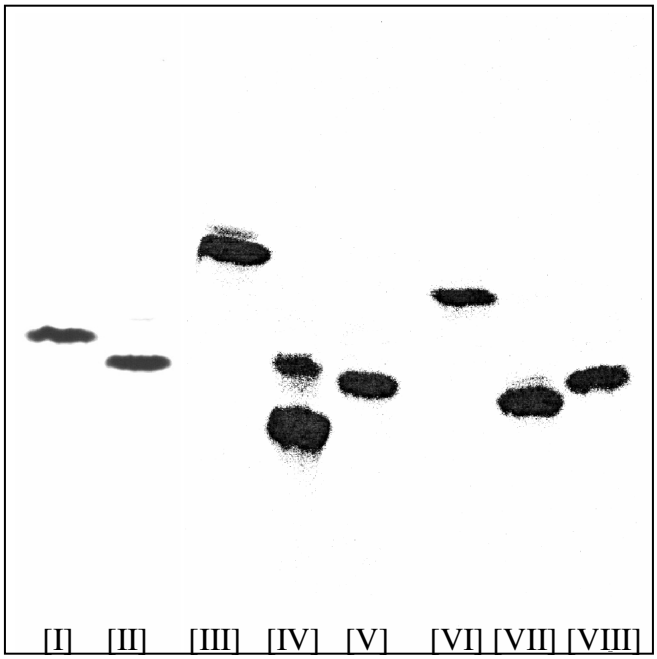

Figure S1. Specific/unique radioactive products are formed in our assays: The radioactive product(s) of eight typical enzymatic assays (acceptor and sugar-nucleotides in individual lanes are listed in Table above) was separated from unreacted sugar-nucleotide donors using chromatography techniques described in Methods. Products were separated using silica gel GHLF thin layer chromatography plates (Analtech) with 1-Propanol/NH_4OH/H_2O (12/2/5, v/v) as the mobile phase. Radioactive products were visualized either by fluorography at -70^{0}C using Biomax Light film (Eastman Kodak) after spraying the TLC plates with Enhance (Dupont) in the case of ^{3}H-labeled products (lanes I, II), or by using the Cyclone phosphorimager system with SR screen (PerkinElmer) in the case of ^{14}C-labeled products (III-VIII). TLC shows that unique products are formed for each of the eight reactions except lane IV. This is expected since the acceptor used in lane IV (Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-O-Bn) has two sites of fucosylation. Thus, as seen both mono- and di-fucosylated products are formed here. Besides TLC analysis of products, mass spectrometry analysis of selected reaction products has been performed in our earlier publications (1, 34).

Figure S2
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Enzyme	Donor	Acceptor
β1,4GalT	UDP-[^{14}C]Gal	3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn
β1,3GalT	UDP-[^{14}C]Gal	GalNAcα-O-Bn
β1,3GlcNAcT	UDP-[^{14}C]GlcNAc	3-O-MeGalβ1,3(Galβ1,4GlcNAcβ1,6)GalNAcα-O-Bn (**#3**)
ST3[Galβ1,3GalNAc]	CMP-[^{14}C]NeuAc	Galβ1,3GalNAcα-O-Bn
α1,3FucT	GDP-[^{14}C]Fuc	GalNAcβ1,4GlcNAcβ-O-Bn

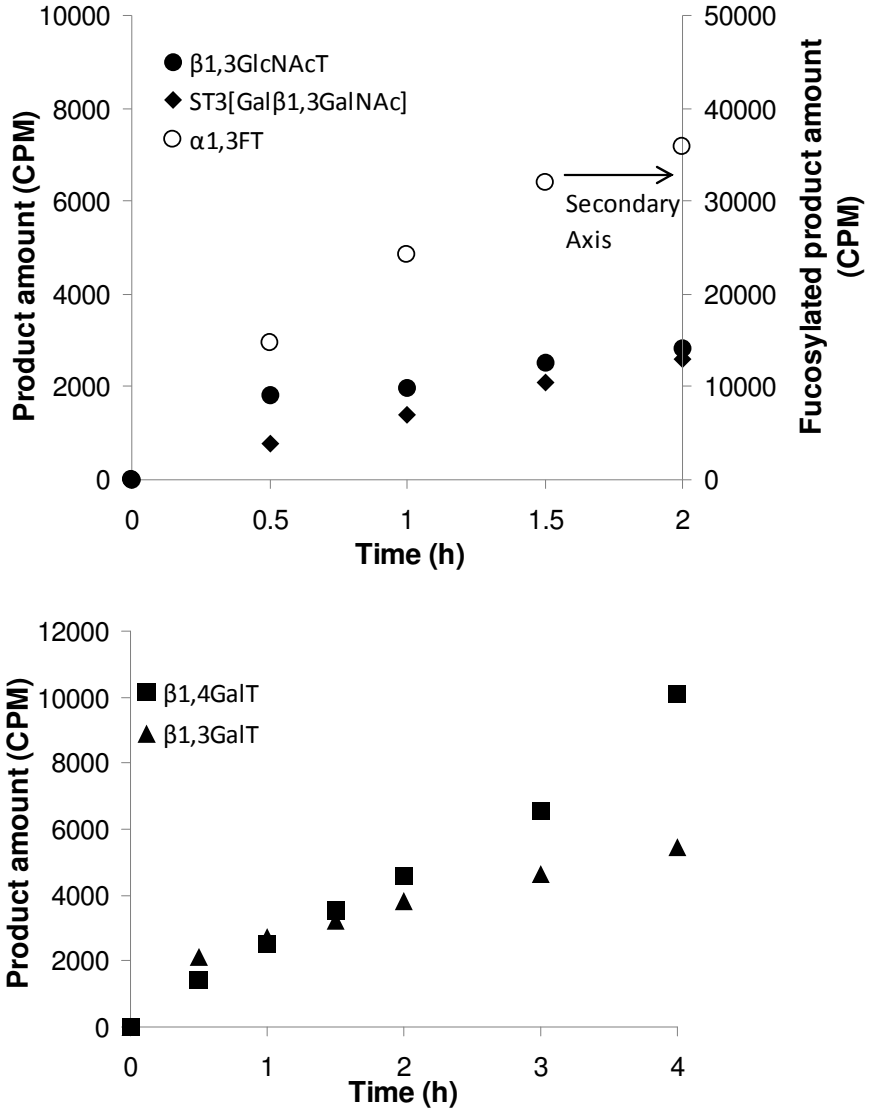

Figure S2. Time course data: Activity assays were performed for selected enzymes using corresponding acceptors mentioned in Table above, under conditions outlined in Methods. Extent of reaction was measured, following chromatographic separation of product, at different time points using scintillation counter. Data suggest that product formation varies approximately linearly during the time course of our assays: 2h for sialylT, FT and GlcNAcT, and 4h for GalT.

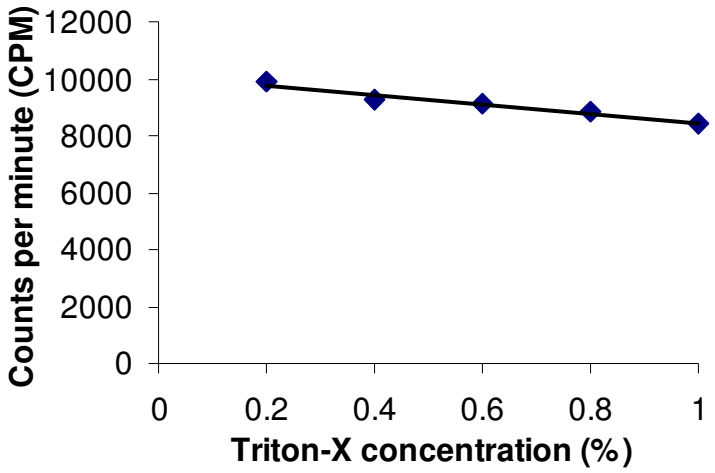

Figure S3. Effect of detergent: Transfer of sialic acid from CMP-[^{14}C]NeuAc (donor) to core-2 acceptor (Compound **2**) was measured using HL-60 cell extract as a source of sialyltransferase (ST3[Galβ1,3GalNAc]). Each 20µl reaction volume contained 7.5mM acceptor, 2µl of HL-60 cell extract and CMP-[^{14}C]NeuAc. Triton-X concentration was varied from 0.2-1.0% while holding all other parameters at the same level in all runs. Radioactive product separated using Sep-Pak column was quantified using scintillation counter for each Triton-X concentration. CPM values quantify the extent of reaction in 2h. Concentration of detergent in assays reported in this paper is 0.5% Triton-X for GalT, FT and sialylT, and 0.66% for GlcNAcT. Varying detergent concentration has less than 10% effect on rate constants reported in this paper.

Supplemental Material References

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