Synthesis of *N*-Acetyllactosamine Derivatives with Variation in the Aglycon Moiety for the Study of Inhibition of Sialyl Lewis x Expression

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*Herein we describe an inhibition study of the sialyl Lewis x (sLe^x) expression on a human monocytic cell line (U937), using a series of peracetylated N-Acetyllactosamine (LacNAc) analogues with variation at the aglycon moiety. It was found that the extent of inhibition was related to the hydrophobicity and structure of the aglycon. In general, peracetylated LacNAc analogues with a naphthyl or biphenyl aglycon (**3**, **4**, **6**, and **7**) were better in suppression of sLe^x expression than a benzyl derivative (**2**). Steady-state kinetic experiments with human α-1,3-fucosyltransferases IV and VI (FucT IV and VI, EC 2.4.1.65) revealed that the deacetylated LacNAc-aglycons with naphthyl (**18**, **19**, and **20**) or biphenyl (**17**) moieties exhibited higher affinity to the fucosyltransferases than aglycon moieties with smaller hydrophobic groups (**14**, **15**, and **16**). These results are in agreement with the findings of the U937 cell-based experiments, and suggest that the higher enzyme affinity LacNAc-aglycons make better acceptor decoys and, hence, the observed differences in LacNAc-aglycon inhihitory effects on sLe^x expression.*

KEYWORDS:

carbohydrates · enzymes · glycoproteins · glycosyltransferases · inhibitors

Introduction

The key step in inflammatory response is the recruitment of vascular leukocytes from the flowing blood stream to the scene of inflamed tissue, which is mediated by a cascade of molecular interactions between the leukocyte and blood vessel endothelial cells.[1] Such interactions are initiated by the binding of the cell surface glycoprotein ligands containing sialyl Lewis x (sLe^x) to selectin receptors displayed on the surface of leukocytes and the blood vessel.[2, 3] Circulating tumor cells also take advantage of similar ligand – receptor interaction during hematogenous metastasis.[4] Therefore, agents that abrogate the ligand – receptor binding may prove useful for treating deleterious inflammation and tumor cell metastasis. This can be achieved either by saturating the binding capacity of selectin receptors or by down-regulating the expression of sLe^x ligand. For the former strategy, exogenous sLe^x oligosaccharides,[5] sLe^x glycomimetics,[6] and multivalent sLe^x ligands[7] have been developed, while the latter approach has been attempted with glycosyltransferase inhibitors[8, 9] or competing substrates,[10–14] which either inhibit or divert the enzymatic assembly of sLe^x ligand in the Golgi bodies. However, most small molecules that inhibit the glycosyltransferases are not cell-permeable and can not reach the Golgi bodies.[13]

One tactic is to use an exogenous decoy acceptor (i.e., a competing acceptor) which in principle resembles the acceptor in the four-partner transition state complex that characterizes fucosyltransferase reactions (a sugar donor, a nucleotide, a divalent cation, and an acceptor). The decoy acceptor can act as a substrate for the fucosyltransferase and interrupt the utilization of endogenous acceptors to form the active natural ligand (Scheme 1). The concept of a decoy acceptor was first realized in 1973 by Okayama et al.,[10] who reported that β-D-xyloside would act as a substrate for the assembly of glycosaminoglycans and thereby inhibit the biosynthesis of glycosaminoglycan chains on endogenous proteoglycan core proteins. Benzyl-α-D-GalNAc was later discovered to suppress the biosynthesis of *O*-linked glycoproteins in HL-60 leukemia cells.[11] A more recent demonstration of this concept was reported by Esko et al., who prepared peracetylated forms of Galβ1,3GlcNAc, Galβ1,4GlcNAc, GlcNAcβ1,3Gal, and Galβ1,3GalNAc with naphthyl or naphthyl-

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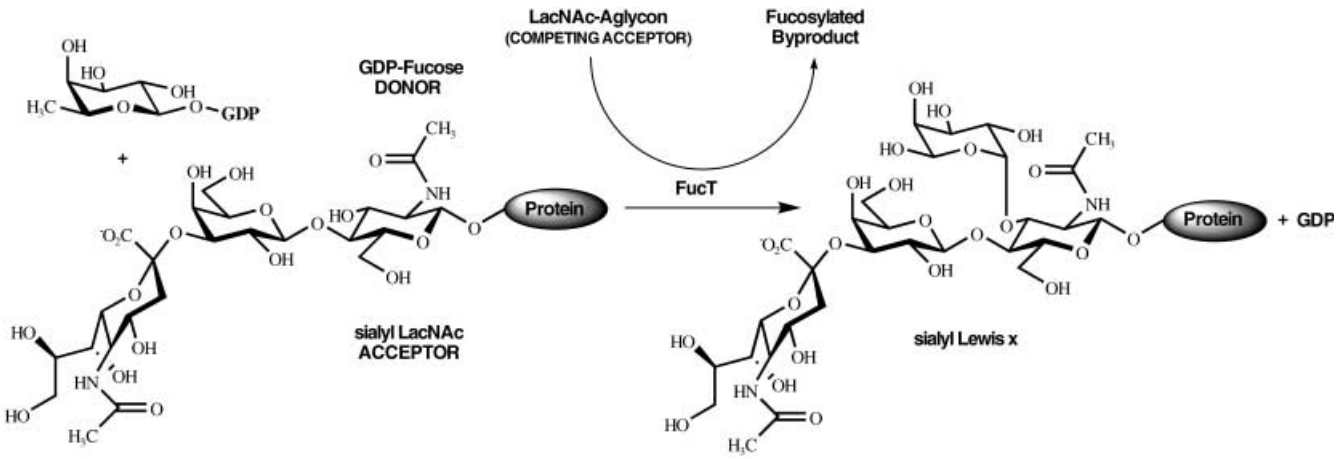


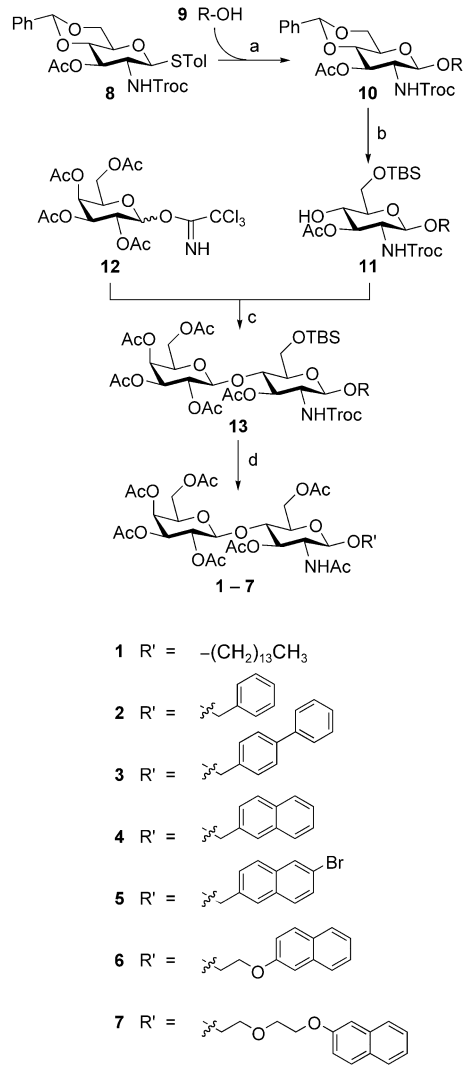
Scheme 1. Fucosyltransferase (FucT) catalyzes the transfer of the fucose moiety from GDP-fucose to LacNAc or sialyl LacNAc glycoconjugates of proteins or lipids. The physiological fucose transfer reaction can be inhibited by introduction of a high affinity substrate, LacNAc-aglycon, which then diverts the fucose moiety from endogenous glycoconjugates.

methene aglycons. These derivatives efficiently crossed the hydrophobic barrier of the cell and Golgi membranes and down-regulated the expression of sLe^x ligands, eventually leading to suppression of intercellular adhesion.[12–14]

Herein we describe the synthesis of a series of peracetyl LacNAc acceptor decoys and the effect of the various aglycons (**1**–**7**) on the inhibition of sLe^x expression. Feeding of the compounds to U937 cells revealed differential inhibition of sLe^x expression. Both the hydrophobicity and the structure of the appended aglycon can modulate the extent of inhibition of sLe^x expression. For comparison, these peracetylated LacNAc-aglycons were deacetylated (**16** to **20**), and the kinetic constants of these compounds were measured with FucT IV and VI. Steady-state kinetic experiments with FucT IV and VI also indicated that the LacNAc-aglycon affinity to the enzyme is dependent on the hydrophobicity of the aglycon moiety. Overall, the kinetic constants acquired from the enzyme kinetic study confirm the cell-based results acquired with U937 cells.

Results and Discussion

The synthesis of peracetyl LacNAc acceptor decoys has been accomplished by a chemical approach,[14] but this had poor or moderate yields, probably because of the poor C-4 hydroxy acceptor site on the glucosaminyl unit. A more convenient synthetic route for these peracetyl acceptors was therefore developed. In the new synthetic route three building blocks were used: a galactosyl donor, a glucosaminyl donor-acceptor, and different aglycon alcohols (Scheme 2). The design of the glucosaminyl donor-acceptor is crucial, since it acts as a donor for the aglycon alcohol and as an acceptor for the galactosyl unit. The C-3 and C-6 hydroxy groups must be selectively protected with appropriate protecting groups such that the nucleophilicity of the free C-4 hydroxy function is preserved, and the protecting groups have to survive the subsequent reaction conditions. From our previous experience in the choice of hydroxy- and amino-protecting groups,[15] the thio-D-glucopyranoside **8** was



Scheme 2. Synthesis of peracetyl LacNAc acceptor decoys by using the three building blocks galactosyl donor, glucosaminyl donor-acceptor, and different aglycon alcohols. Reagents and conditions: a) NIS, TMSOTf, CH_2Cl_2, −23 °C; b) (1) 80% aq. acetic acid, 80 °C; (2) TBDMSCl, Im, THF, RT c) TMSOTf, CH_2Cl_2, −23 °C; d) (1) HF·py, THF, RT; (2) Zn, DMAP, Ac_2O/CH_2Cl_2.

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prepared from glucosamine hydrogen chloride according to the literature procedure,[16] and was then coupled with an aglycon alcohol **9**, in the presence of *N*-iodosuccinimide (NIS) and triflic acid (TfOH), to give the *O*-glycosides **10**.[17] Compounds **10** were treated with 80% acetic acid at 80 °C to remove the benzylidene acetal, and subsequent selective C6-silylation with *tert*-butyldimethylsilyl chloride (TBSCl) furnished the glucosaminyl acceptors **11**. These were glycosylated with the peracetyl galactosyl trichloroacetimidate **12**[18] in the presence of the trimethylsilyl triflate (TMSOTf) to give the disaccharides **13**. The glycosylation yields of **11** and **12** varied from 40% to 80% depending on the chemical structure of the aglycon on **12**. Poor yields were observed with glucosaminyl acceptor **13** bearing an ethylene glycol tether. The oxygen atom on the ethylene glycol spacer can probably associate with the terminal hydroxy function and hence decrease its nucleophilicity, leading to a poorer glycosylation yield. Conversion of disaccharides **13** into the corresponding peracetyl LacNAc decoys was accomplished by removal of the C-6 TBS silyl ether with pyridinium hydrogen fluoride (HF · py), followed by removal of the C-2 trichloroethoxycarbonyl (Troc) protection group and the acetylation of both the C-2 amino and the C-6 hydroxy group with Zn dust reduction in acetic anhydride.[19, 20] With different aglycons, peracetyl LacNAc decoys **1** – **7** were obtained.

Previously, Sarkar et al. had shown that cells take up per-*O*-acetylated disaccharides similar to those described here and rapidly deacetylate them.[12–14] These compounds act as substrates, leading to the formation of sialylated and fucosylated oligosaccharides such as sLe^x. Priming of oligosaccharides in this way diverts their synthesis from endogenous glycoproteins. To test how the aglycon affected the activity of LacNAc as an inhibitor, the per-*O*-acetylated analogues **1** – **7** were fed to U937 cells. A monoclonal antibody (CSLEX-1) was used to quantitate the expression of sLe^x on the surface of the cells, and the degree of inhibition was compared to the control disaccharide **4**.[13] When fed to U937 cells at 10 – 50 μM, compounds **1** and **5** were toxic, causing acute cell lysis. In the case of **1**, this was most probably a result of the aglycon's detergent-like properties after the removal of the acetyl protecting groups inside the cell. In contrast, treatment of U937 cells with **2** – **4**, **6**, and **7** inhibited the expression of sLe^x to varying degrees, usually in a dose-dependent manner (Figure 1). Interestingly, **2**, with a small benzyl aglycon structure, was less effective than **3**, **4**, **6**, and **7**, which may be related to the hydrophobicity of the aglycon. The enhanced efficacies of **3**, **4**, **6**, and **7** could reflect enhanced uptake or a preference of target enzymes for a more complex aglycone. Similar results have been observed for β-D-xylosides.[21, 22] To explain the observed phenomenon, selected peracetyl LacNAc decoys **2** – **4**, **6**, and **7** were deprotected to give the LacNAc decoys **16**,[23] and **17** – **20** (Scheme 3), and their enzyme-binding kinetics were examined with a commercially available human α-1,3-fucosyltransferase VI.

Table 1 illustrates the kinetic constants for the deacetylated LacNAc-aglycons (**16** – **20**), also including **14** and **15**,[24] together with the corresponding K_m values for GDP-fucose. Arranged in the order of decreasing K_m values, it is evident that there is a correlation between the hydrophobicity of the aglycon group

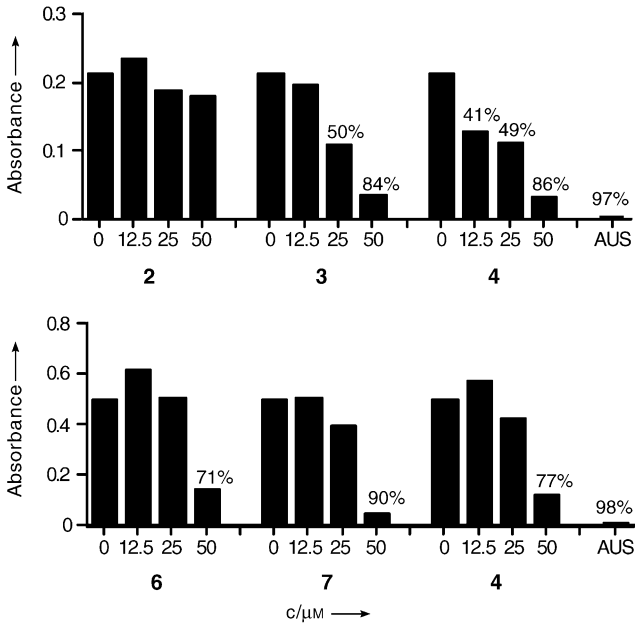
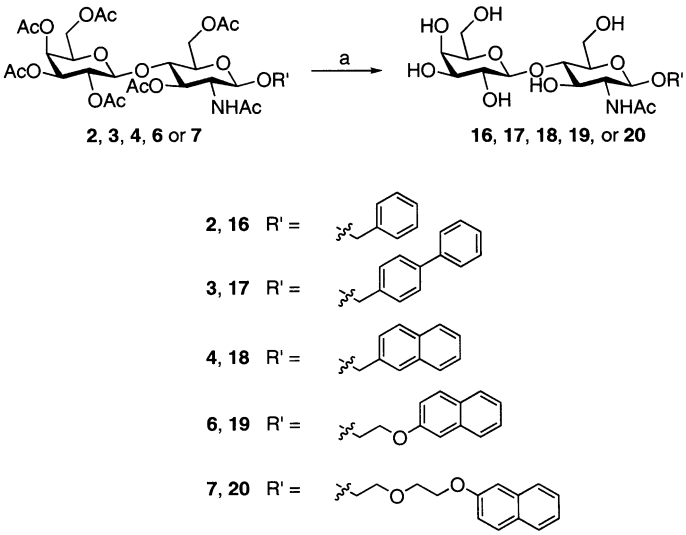


Figure 1. *Inhibition of sLe^x biosynthesis by LacNAc-alycones. U937 cells were treated with various per-O-acetylated LacNAc-aglycons for 4 days at the indicated concentrations. Binding of mAb CSLEX-1 was measured by ELISA (see Experimental Section). The two experiments shown in the top and bottom panels were performed on different days. The values represent the average of triplicate experiments, which varied by $\leq 10\%$.*



Scheme 3. Synthesis of deactetyl LacNAc decoys. *Reagents and conditions: a) NaOMe, MeOH/CH_2Cl_2, RT.*

and the K_m value of the acceptor molecule. The K_m for GDP-fucose follows the same trend. The k_{cat} values are for the most part very similar for all LacNAc-aglycons (**14** – **20**): from 2 to 4 sec^{-1}.

The aglycon contribution to LacNAc affinity is a phenomenon previously observed for FucT III, V, and VI.[24, 25, 26] The hydrophobicity in the aglycon group in LacNAc affinity is also

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Table 1. LacNAc-aglycon specificity of human α-1,3-fucosyltransferase VI.

	LacNAc-R	$K_{LacNAc-R}$ [mM]	$K_{GDP-Fuc}$ [mM]	k_{cat} [sec^{-1}]	$\frac{k_{cat}}{K_{GDP-Fuc}}$ [M^{-1} sec^{-1}]	$\frac{k_{cat}}{K_{LacNAc-R}}$ [M^{-1} sec^{-1}]
14	**R** = H (α,β-mixture)	4.6 ± 0.2	5.5 ± 0.6	3.7 ± 0.1	670 000 ± 70 000	1 100 ± 50
15		0.39 ± 0.02	5.5 ± 1.4	3.3 ± 0.2	600 000 ± 100 000	12 000 ± 1 000
16		0.41 ± 0.03	5.0 ± 0.5	2.2 ± 0.05	440 000 ± 40 000	6 300 ± 500
17		0.28 ± 0.02	5.3 ± 0.4	2.2 ± 0.04	410 000 ± 30 000	12 000 ± 900
18		0.12 ± 0.02	4.8 ± 0.5	1.8 ± 0.03	370 000 ± 40 000	21 000 ± 3 000
19		0.064 ± 0.008	4.5 ± 0.3	2.8 ± 0.05	620 000 ± 40 000	61 000 ± 8,000
20		0.076 ± 0.009	4.7 ± 0.8	1.6 ± 0.07	340 000 ± 60 000	34,000 ± 4,000

[a] Values obtained from fits to Michaelis – Menten Eq. (1). All LacNAc-aglycons are of the form Galβ1,4GlcNAcβ-aglycon, except for compound **14**.

important for FucT VI, as shown by the results in Table 1. It is not clear if this aglycon binding site is a normal component for substrate specificity of FucT VI, since no fucosyltransferase X-ray crystal structure is available, or if the aglycon group simply binds to a "secondary" binding site that enhances affinity. Replacement of the hydrogen atom on the anomeric hydroxy group of **14** with an alkyl ester (**15**) resulted in a reduction in the K_m value of greater than 11-fold for the acceptor molecule. Another sixfold reduction in K_m value is observed if the alkyl ester is replaced with a suitably positioned naphthylene group as in **19**.

Hydrophobicity may not be the single determinant for acceptor – substrate binding affinity, as the results from Table 1 would suggest. Although the biphenyl group in **17** is slightly more hydrophobic than the naphthyl group in **18**, its K_m is about three times higher. If the naphthalene group is properly positioned with a three-atom linker (as in **19**) the K_m value becomes 0.064 mM, the lowest value of the LacNAc-aglycons in Table 1. A six-atom linker for the naphthalene group (as in **20**) increases the K_m value to 0.076 mM, indicating that the preferred linker length is around three atoms. Ideally, the experiment would make use of linkers that increase in length by single atoms. However, low solubilities of the LacNAc-aglycons became a problem if alkyl linkers were used, so ethylene glycol linkages were chosen to promote solubility.

One difference between the enzyme kinetics and the cell-based experiments is that U937 cells express FucT IV and VII enzymes,[13] while the enzyme kinetics experiment made use of FucT VI. We re-tested some of the deacetylated LacNAc-aglycons (**16**, **18**, and **19**) on the more relevant FucT IV (gift from the Consortium for Functional Glycomics and Neose Tech. Inc.) because it has been shown that LacNAc-aglycons are substrates for FucT IV and not for FucT VII.[13] The K_m values for **16**, **18**, and **19**, as measured for FucT IV, are 0.65 ± 0.08, 0.14 ± 0.04, and 0.09 ± 0.02 mM, respectively. In general, these values are somewhat higher than those measured for FucT VI. However, they displayed the same trend, showing greater enzyme-binding affinity with greater hydrophobicity of the aglycon moiety. These results are in agreement with the findings that FucT IV and VI share similar substrate specificity with the polymeric tri-LacNAc substrate.[27]

Although not definitive, the high-affinity LacNAc aglycons most probably inhibit cell-surface expression of sLe^x by usurping the cellular content of fucose substrates, since these acceptor decoys target FucT IV instead of FucT VII. FucT VII has a strong preference for sialyl LacNAc substrates and is believed to play the predominant role in synthesizing cell-surface sLe^x.[28] The enzyme kinetics experiments with FucT IV and VI show that LacNAc aglycons with naphthyl moieties (**18**, **19**, and **20**) are higher affinity substrates for the enzyme than substrates with less hydrophobic aglycon groups (**14**, **15**, and **16**). The experiment supports the observation made in the cell-based assay that peracetylated LacNAc aglycons with naphthyl moieties were better inhibitors of cell-surface expression of sLe^x.

Experimental Section

Cell culture: U937 (human histiocytic lymphoma, CRL 1593) cells were purchased from the American Type Culture Collection (Rockville, MD) and grown in suspension in RPMI 1640 medium, supplemented with fetal bovine calf serum (FBS, 10% (v/v); Hyclone

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Laboratories, Logan, UT), glutamine (0.3 g L^{-1}), streptomycin sulfate (100 μg mL^{-1}), and penicillin (100 units mL^{-1}). The cells were maintained at 37 °C in a humidified incubator containing 5% CO_2 and 95% air and passaged by dilution every 3–4 days.

Sialyl Lewis x expression: To measure the inhibition of sLe^x expression, the cells were treated with monoclonal antibody (mAb, CSLEX-1). U937 cells ($\approx 5 \times 10^4$) were grown in the presence and in the absence of peracetylated LacNAc analogues **1**–**7**, respectively, (0 to 50 μmol) for 4 days in 6-well plates. Cells were washed with PBS and fixed with 1% *p*-formaldehyde in phosphate buffer saline (PBS, 5 min, RT). The fixed cells were rinsed and resuspended (1×10^5) in 1% bovine serum albumine(BSA)/PBS (100 μL). CSLEX-1 (CD15 s, 0.8 μg mL^{-1}; Becton Dickinson) and anti-mouse IgM coupled to horseradish peroxidase (1:100) were added to the cells and incubated (1 h, RT). A control incubation (1 h, 37 °C) contained *Arthrobacter ureafaciens neuraminidase* (AUS, 20 mU/1×10^6 cells; Calbiochem) in 0.05 M Hepes (100 μL, pH 6.9). After incubation, the cells were washed with 1% BSA/PBS and developed with 150 μL developing buffer (19.5 g citric acid, 28.5 g Na_2HPO_4 pH 5.5 in 1 L H_2O) containing *o*-phenylenediamine (OPD) solution (20 mL citrate phosphate for 1 tablet OPD (10 mg) and 50 μL of 30% H_2O_2). The reaction was quenched by addition of 3 M H_2SO_4 (100 μL, 5–10 min), and the absorbance of solution was measured at 490 nm.

Chemical synthesis: All chemicals were purchased and used without further purification. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. Ether (Et_2O) was treated with sodium metal/benzophenone ketyl and distilled prior to use. Anhydrous dimethylformamide (DMF) was bought from Aldrich. Molecular sieves (MS) were AW 300 and flame-activated prior to use. Reactions were monitored by analytical thin layer chromatography (TLC) on EM silica gel 60 F254 plates and visualized under UV light (254 nm) and/or by staining with acidic ceric ammonium molybdate. Flash column chromatography was performed on Geduran silica gel 60 (3575 μm, EM Science). ^1H NMR spectra were recorded on Bruker DRX 500 (500 MHz) or 600 (600 MHz) spectrometers at 20 °C. Chemical shifts (δ, ppm) were assigned according to the internal standard signal of tetramethylsilane in $CDCl_3$ ($\delta = 0$ ppm) or spiked acetone in D_2O ($\delta = 2.04$ ppm). ^{13}C Attached Proton Test NMR (^{13}C-Apt) spectra were obtained on Bruker DRX 500 or 600 (125 MHz) spectrometers and are reported in the δ ppm scale with the signal of $CDCl_3$ ($\delta = 77.00$ ppm) or spiked acetone in D_2O ($\delta = 29.8$ ppm) as reference. Peracetylated LacNAc **4**[14] and LacNAc **14**[16] were prepared as previously described. ^1H and ^{13}C-Apt NMR spectra for compounds **1**–**3**, **5**–**7**, and **17**–**20** can be found in the supporting information.

General procedure for the synthesis of peracetyl LacNAc analogues 1–7: Glucosaminyl donor **8** (1 equiv.), aglycon alcohol **9** (1.5 equiv.), and MS were suspended in dry CH_2Cl_2 and stirred under argon at RT for 1 h. The reaction solution was cooled to −23 °C, and TMSOTf (0.1 equiv.) was added. The mixture was stirred at −23 °C for 4 h and quenched by the addition of triethylamine (TEA). After removal of the molecular sieves by filtration, the reaction solution was washed with sat. aq. $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, concentrated, and purified by column chromatography to yield **10** (60 to 80%).

The compound **10** (1 equiv.) was dissolved in 80% aq. acetic acid and the solution was stirred at 80 °C for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2, which was washed with H_2O, dried over $MgSO_4$, and concentrated for subsequent silylation.

The crude residue was dissolved in dry THF, and this was followed by the addition of *tert*-butyldimethylsilyl chloride (TBDMSCl, 1 equiv.) and imidazole (Im, 1.2 equiv.). The mixture was stirred at RT for 3 h and was then quenched with MeOH, followed by removal of solvent under reduced pressure. The concentrated residue was dissolved in CH_2Cl_2 and was washed with 1 N aq. HCl, H_2O, and brine, dried over $MgSO_4$, concentrated, and purified by column chromatography to give **11** in 60–80% yield.

The compound **11** (2 equiv.), 2,3,4,6-tetra-*O*-acetyl-galactopyranosyl trichloroacetimidate (**12**, 1 equiv.), and MS were suspended in dry CH_2Cl_2 and stirred at RT under Ar for 1 h. The reaction mixture was cooled to −23 °C, and TMSOTf (0.1 equiv.) was added. The mixture was stirred for 5 h at −23 °C and was quenched by the addition of triethylamine (TEA). The solution was filtered and washed with sat. aq. $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, concentrated, and purified by flash column chromatography to give **13** (40 to 60%).

The compound **13** was dissolved in dry THF, and HF·py (10 equiv.) was added. The reaction mixture was stirred at RT under Ar for 1 h and quenched by the addition of sat. aq. $NaHCO_3$. The desilylated product was extracted with CH_2Cl_2 (×3) and the organic phase was washed with H_2O, dried over $MgSO_4$, and concentrated. The concentrated residue, together with a catalytic amount of DMAP, was dissolved in CH_2Cl_2/Ac_2O (1:1), and acid-washed zinc dust was added. The mixture was stirred at RT for 4 h and filtered. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was washed with 1 N aq. HCl and H_2O, dried over $MgSO_4$, and concentrated for flash column chromatography to give the target peracetylated LacNAc analogues **1**–**7**, respectively. Spectra and spectral assignments of these compounds can be found in the Supporting Information.

General procedure for the deprotection of peracetyl LacNAc analogues: Peracetylated LacNAc analogues **2**–**4**, **6**, and **7** were deprotected by standard transesterification with NaOMe in MeOH/CH_2Cl_2 (1:1) to give deprotected the LacNAc analogues **16**–**20**, respectively. Spectra and spectral assignments of these compounds can be found in the Supporting Information.

Enzyme assay: The fucosyltransferase activity was assayed by use of the coupling enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH). The assay conditions are as follows: 50 mM KHepes pH 7.4, 150 mM KCl, 10 mM $MgCl_2$, 0.5 mM PEP, 20 units mL^{-1} PK, 20 units mL^{-1} LDH, and 0.033 mM NADH. The concentrations of LacNAc and LacNAc aglycons were varied from approximately 0.1 to 10 times the K_m value, while the GDP-fucose concentration was held constant at 100 μM. GDP-fucose was varied between 0.5 to 10 times the K_m value, while the LacNAc aglycon was held constant at twice its K_m value. FucT was added last to start the reaction and the reaction was monitored fluorometrically at 460 nm emission (340 nm excitation) on a Hitachi F-2000 fluorescence spectrophotometer. One unit of activity is equal to the conversion of 1 μmol of substrate to product per minute at 37 °C. The assay mixture was held constant at 37 °C during the reaction, since fluorescence can be highly dependent on changes in temperature. This assay was adapted from Murray et al.[29]. The molecular weight of 42 000 g mol^{-1} for FucT VI was used for rate calculations.[30]

Data processing: All data fitting was performed by use either of pro Fit (Quantum Soft) or of GraFit (Erithacus Software) software packages. Initial velocity patterns were fitted to Eq. (1) or Eq. (2), where ν is the measured velocity, V is the maximum velocity, A (GDP-Fucose) and B (LacNAc) are the substrate concentrations, K_a and K_b are the respective Michaelis constants, and K_{ia} is the dissociation constant of A.

$$\nu = \frac{VA}{K_a + A} \quad (1)$$

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$$\nu = \frac{VAB}{K_{ia}K_b + K_a B + K_b A + AB} \quad (2)$$

[1] D. Vestweber, J. E. Blanks, *Physiol. Rev.* **1999**, *79*, 181 – 213.
[2] A. Etzioni, J. M. Harlan in *The Selectins*, *Vol. 3* (Ed.: D. Vestweber), Harwood, Amsterdam, **1997**, pp. 117 – 124.
[3] P. Maly, A. D. Thall, B. Petryniak, C. E. Rogers, P. L. Smith, R. M. Marks, R. J. Kelly, K. M. Gersten, G. Cheng, T. L. Saunders, S. A. Camper, R. T. Camphausen, F. X. Sullivan, Y. Isogai, O. Hindsgaul, U. H. von Andran, J. B. Lowe, *Cell* **1996**, *86*, 643 – 653.
[4] T. Krause, G. A. Turner, *Clin. Exp. Metastasis* **1999**, *79*, 183 – 192.
[5] W. Spevak, C. Foxall, D. H. Charych, F. Dasgupta, J. O. Nagy, *J. Med. Chem.* **1996**, *39*, 1018 – 1020.
[6] M. W. Cappi, W. J. Moree, L. Qiao, T. G. Marron, G. Weitz-Schmidt, C.-H. Wong, *Angew. Chem.* **1996**, *108*, 2501 – 2503; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2346—2348.
[7] S. A. DeFrees, L. Phillips, L. Guo, S. Zalipsky, *J. Am. Chem. Soc.* **1996**, *118*, 6101 – 6104.
[8] P. Compain, O. R. Martin, *Bioorg. Med. Chem.* **2001**, *9*, 3077 – 3092.
[9] M. L. Mitchell, T. Feng, V. L. Lee, C.-H. Wong, *Angew. Chem.* **2002**, *114*, 3167 – 3170; *Angew. Chem. Int. Ed.* **2002**, *41*, 3041 – 3044..
[10] M. Okayama, K. Kimata, K. Suzuki, *Biochem. J.* **1973**, *74*, 1069 – 1073.
[11] S.-F. Kuan, C. J. Byrd, C. Babaum, Y. S. Kim, *J. Biol. Chem.* **1989**, *264*, 19 271 – 19 277.
[12] A. K. Sarkar, T. A. Fritz, W. H. Taylor, J. D. Esko, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3323 – 3327.
[13] A. K. Sarkar, K. S. Rostand, R. K. Jain, K. L. Matta, J. D. Esko, *J. Biol. Chem.* **1997**, *272*, 25 608 – 25 616.
[14] A. K. Sarkar, J. R. Brown, J. D. Esko, *Carbohydr. Res.* **2000**, *329*, 287 – 300.
[15] Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734.
[16] T. K.-K. Mong, C.-Y. Huang, C.-H. Wong, *J. Org. Chem.* in press.
[17] P. J Garegg, *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179.
[18] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
[19] K. M. Koeller, C.-H. Wong, *Chem. Eur. J.* **2000**, *6*, 1243.
[20] B. S. Furniss, A. J. Hannaford, P. W. G. Smith, A. R. Tatchen in *Vogel's Textbook of Practical Organic Chemistry, 5th Ed.* **1989**, Longman Scientific & Technical, UK, p. 650.
[21] T. A. Fritz, F. N. Lugemwa, A. K. Sarkar, J. D. Esko, *J. Biol. Chem.* **1994**, *269*, 300 – 307.
[22] T. A. Fritz, P. K. Agrawal, J. D. Esko, N. R. Krishna, *Glycobiology* **1997**, *7*, 587 – 596.
[23] S. Takayama, M. Shimazaki, L. Qiao, C.-H. Wong, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1123 – 1126.
[24] B. W. Murray, S. Takayama, J. Schultz, C. H. Wong, *Biochemistry* **1996**, *35*, 11 183 – 11 195.
[25] T. de Vries, M. P. Palcic, P. S. Schoemakers, D. H. van den Eijnden, D. H. Joziasse, *Glycobiology* **1997**, *7*, 921 – 927.
[26] Y. Miura, S. Kim, J. R. Etchison, Y. Ding, O. Hindsgaul, H. Freeze, *Glycoconj. J.* **1999**, *16*, 725 – 730.
[27] S. Nishihara, H. Iwasaki, M. Kaneko, A. Tawada, M. Ito, H. Narimatsu, *FEBS Lett.* **1999**, *462*, 289 – 294.
[28] R. Niemela, J. Natunen, M. L. Majuri, H. Maaheimo, J. Helin, J. B. Lowe, O. Renkonen, R. Renkonen, *J. Biol. Chem.* **1998**, *273*, 4021 – 4026.
[29] B. W. Murray, V. Wittmann, M. D. Burkart, S. H. Hung, C. H. Wong, *Biochemistry* **1997**, *36*, 823 – 831.
[30] B. W. Weston, P. L. Smith, R. J. Kelly, J. B. Lowe, *J. Biol. Chem.* **1992**, *267*, 24 575 – 24 584.

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