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The design and synthesis of a selective inhibitor of fucosyltransferase VI

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Inversion of configuration of the C-2′ hydroxyl of methyl *N*-acetyllactosamine was accomplished by a two-step procedure involving oxidation to a ketone followed by reduction with $NaBH_4$. After deprotection, the resulting derivative **2** was examined as a substrate for α-(2,6)- and α-(2,3)-sialyltransferase and fucosyltransferase III, IV, V and VI. It was found that none of these enzymes could glycosylate **2**. However, it showed exquisite selectivity for inhibition of fucosyltransferase VI. The kinetic data support an unusual mechanism in which the inhibitor can bind to the GDP-fucose complex as well as another enzyme form.

Introduction

Protein- and lipid-bound oligosaccharides play critical roles in a diverse range of biological processes such as embryogenesis, fertilization, neuronal development, hormonal activities, cell proliferation and cells' organization into specific tissues.[1,2] They are also important in health science and are involved in the invasion and attachment of pathogens, inflammation, metastasis, and xenotransplantation. Not surprisingly, considerable effort has been dedicated to the design and synthesis of inhibitors of carbohydrate processing enzymes.[3,4] Remarkable progress has been made in the development of glycosidase inhibitors and some of these compounds have entered advance clinical trials.[5] On the other hand, the search for glycosyltransferase inhibitors has been slow. This problem is mainly due to intrinsic features of these enzymes such as a complex four-partner transition state (sugar donor, acceptor, metal, nucleotide), weak binding of the enzyme with their natural substrates (usual K_m values are in the mM or high μM range) and limited structural data. Moreover, many aspects of the catalytic mechanisms of glycosyltransferases are still unknown, thus complicating the rational design of inhibitors. Nevertheless, several strategies for inhibitor design have been pursued with some success. The most important approaches involve acceptor and donor analogues, and transition state mimetics.[6–10]

N-Acetyllactosamine is a substrate for a wide range of glycosyltransferases. For example fucosyltransferase III, IV, V and VI catalyze the transfer of a fucosyl residue from GDP-fucose to the C-3 hydroxyl of *N*-acetyllactosamine,[11] whereas α-(2,6)-sialyltransferase and α-(2,3)-sialyltransferase transfer an *N*-acetyl neuraminic acid (Neu5Ac) moiety from CMP-Neu5Ac to the C-6′ and C-3′ hydroxyl of LacNAc, respectively.[12] The substrate specificity of these enzymes has been probed by using modified LacNAc derivatives. These studies have revealed that the C-6′ hydroxyl and acetamido group of LacNAc are essential for sialylation by rat liver α-(2,6)-sialyltransferase.[13] Furthermore, glycosylation of the C-3′, C-4′ and C-3 hydroxyl lead to inactivation of the substrate. Other transferases have different acceptor requirements. For example, α-(2,3)-sialyltransferase requires the 3-OH, 4-OH and 6-OH of Gal for recognition[13] whereas all three fucosyltransferases (FucTs III, IV and V) had an absolute requirement for a hydroxyl at C-6 of galactose in addition to the accepting hydroxyl at C-3 or C-4 of GlcNAc.[14,15] Furthermore, FucT VI accepts replacements and modification of the *N*-acetyl group of the GlcNAc unit.[16–18]

Recently, we found that rat liver α-(2,6)- and α-(2,3)-sialyltransferase and fucosyltransferase III, V and VI allow modifications at the C-2′ and C-6 hydroxyl of LacNAc, however, different enzymes responded differently.[19] For example, methylation of the C-6 and C-2′ hydroxyls of LacNAc had only a minimal effect on α-2,3-sialyltransferase, thus indicating that these hydroxyls make marginal interactions with the binding site. On the other hand, a significant loss of catalytic efficiency was observed when the same substrate was employed for rat liver α-2,6-sialyltransferase. Fucosyltransferase IV was not affected by methylation of the C-6 and C-2 hydroxyls, whereas the catalytic efficiency for α-1,3-fucosyltransferases VI was slightly increased. These findings indicate that in some cases, functionalities at C-6 and C-2′ hydroxyl can make interactions with the periphery of the binding site. It is to be expected that the improved selectivities may be exploited in the design of selective inhibitors for particular transferases.

To further explore the effect of modifying the C-2′ hydroxyl of LacNAc, we synthesized derivative **2**, in which the C-2′ hydroxyl of LacNAc was epimerized (Fig. 1). It was found that this modification abolished catalytic activity for α-(2,6)- and α-(2,3)-sialyltransferase and fucosyltransferase III and V. Interestingly, compound **2** is a remarkably selective inhibitor for the α-1,3-fucosyltransferase VI. Detailed analysis of inhibition data revealed that **2** can bind to the GDP-fucose complex as well as to another enzyme form.

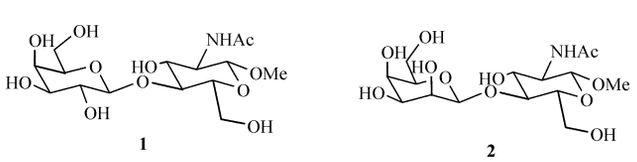

Fig. 1 Compounds **1** and **2**.

Results and discussion

Epimerization of the C-2′ hydroxyl of **3**[20] was achieved through a two-step procedure involving oxidation to a ketone followed by a stereoselective reduction. Thus, the acetyl group of **3** was removed by treatment with sodium methoxide and methanol to give compound **4** in an almost quantitative yield (Scheme 1).

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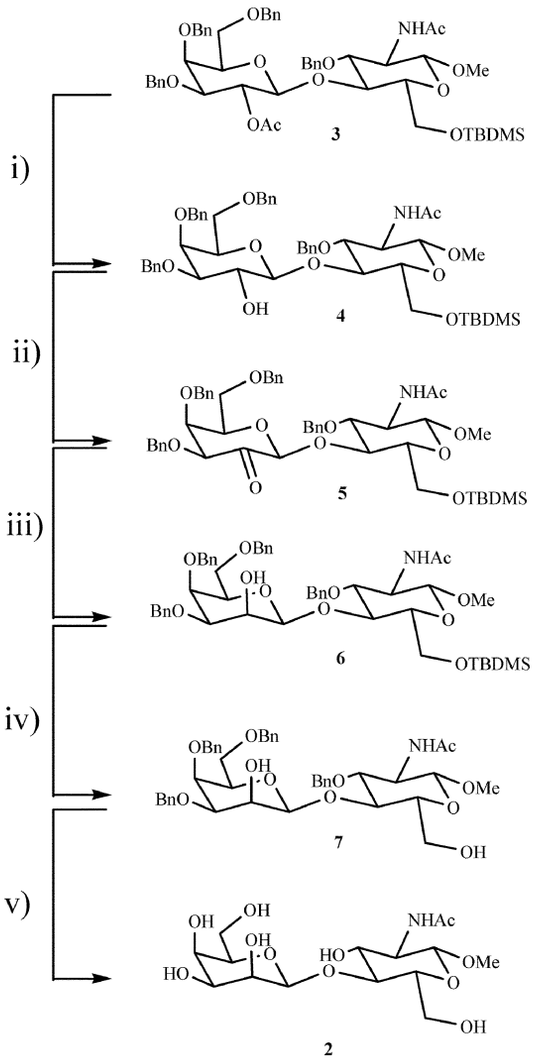

Scheme 1 *Reagents and conditions*: i) NaOMe, MeOH; ii) Ac_2O, DMSO; iii) $NaHB_4$, DCM/MeOH; iv) HBF_4, MeCN; v) $Pd(OH)_2$, H_2, EtOH.

The C-2′ hydroxyl of **4** was oxidized using the procedure of Albright and Goldman using DMSO and Ac_2O.[21] The resulting ketone **5** was immediately reduced with sodium borohydride in a mixture of dichloromethane and methanol to give, after purification by silica gel column chromatography, compound **6** in an overall yield of 60%. Finally, removal of the TBDMS protecting group, using standard conditions followed by catalytic hydrogenation over $Pd(OH)_2$, gave the target compound **2**. The appearance of H-1′ (4.58 ppm) and H-3′ (3.74 ppm) as singlets in the ^1H spectra of compound **2** confirmed the axial orientation of the C-2′ hydroxyl.

Next, the apparent kinetic parameters for the α-2,3- and α-2,6-sialyltransferase and fucosyltransferases III, IV, V and VI catalyzed transfer of CMP-[^{14}C]-Neu5Ac or GDP-[^{14}C]-fucose to acceptors **1** and **2** were determined using reported assays.[13,15,22,23] In each case, the K_m for **1** was in close agreement with previously reported data. Surprisingly, none of the enzymes showed any activity when the modified compound **2** was used.

It may be possible that **2** can be recognized by the glycosyltransferases, but is unable to accept the monosaccharide moiety from the sugar nucleotide. Thus, to investigate whether **2** exhibits inhibitory activity, relative rates of transfer were measured at different concentrations of **2** (0 to 1.5 mM) while a saturating concentration of GDP-fucose was used in combination with a fixed concentration of methyl LacNAc (**1**) that corresponded to the K_m value for each of the six enzymes. Surprisingly, only FucT VI showed a decrease in the relative rate of transfer indicating that only this enzyme can recognize compound **2**.

To explore in detail the mode of inhibition, kinetic parameters were determined at different concentrations of **2** with respect to both methyl LacNAc (**1**) and GDP-fucose. Concentrations of **2** were chosen in such a manner that the inhibition was between 15–75% for accurate data collection. With respect to methyl LacNAc (**1**), data were collected at 0, 0.2, and 0.4 mM, and fitted to the equations for competitive and mixed inhibition with COMPO and NCOMP.[24] The data were fitted all at once without inhibitor present and with all inhibitor concentrations. As can be seen in Fig. 2, a mixed inhibition model gave the best fit for the data of methyl LacNAc. From the data shown in Fig. 2A, the K_{is} value was determined to be 0.193 ± 0.056 mM and the K_{ii} value was 0.382 ± 0.116 mM. Compound **2** showed a weaker inhibitory effect with respect to GDP-fucose (Fig. 3). The data do not easily distinguish between competitive (dashed line) and mixed inhibition (solid line). However, the best fit was for a mixed inhibition model (Fig. 3A). The double reciprocal plot also showed better statistics for this mode of inhibition (Fig. 3B). Fitting the data to COMPO[24] gave a K_i value of 0.475 ± 0.096 mM.

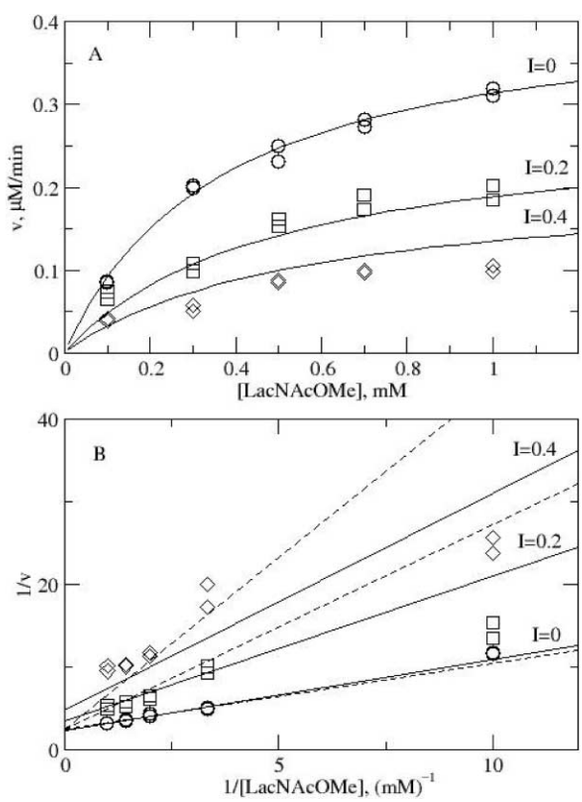

Fig. 2 Michaelis–Menten plot (A) and Lineweaver–Burk plot (B) for LacNAc-OMe at different inhibitor concentrations. The lines are the result of fitting the data to NCOMP. Competitive inhibition (dashed line) and mixed inhibition (solid line) fits.

Fucosyltransferases have been shown to obey an ordered, sequential BiBi mechanism,[25] with GDP-fucose being the first substrate to bind to the free enzyme and GDP the last product released. Normally, the acceptor sugar binds as the second substrate exclusively to the GDP-fucose–enzyme complex. However, the mixed inhibition observed for **2** when the concentration of methyl LacNAc was varied indicates that this compound can bind to the GDP-fucose complex as well as to another enzyme form. There is reasonable agreement in the values of K_i (0.475 mM) and K_{is} (0.193 mM), which are the apparent dissociation constants of **2** for binding to the free enzyme. With respect to GDP-fucose, the best fit was for mixed inhibition, which would indicate that **2** can also bind to the E-GDP-Fuc complex substrate as well as the E-GDP product

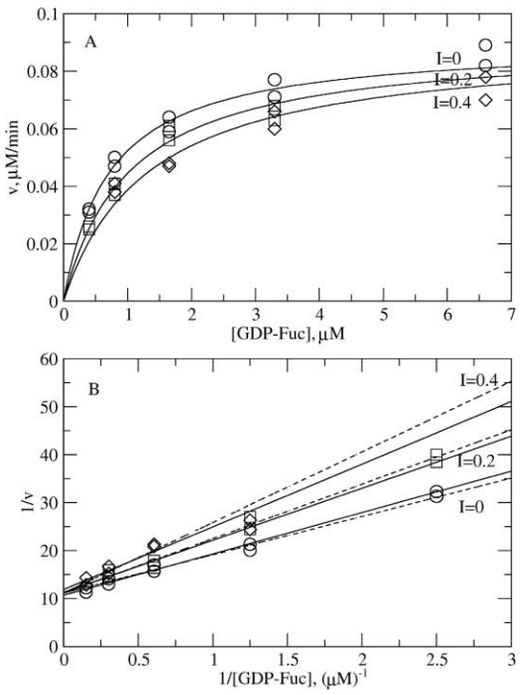

Fig. 3 Michaelis–Menten plot (A) and Lineweaver–Burk plot (B) for GDP-fucose at different inhibitor concentrations. The lines are the result of fitting the data to COMPO. Competitive inhibition (dashed line) and mixed inhibition (solid line) fits.

complex. However, a competitive inhibition model could not be excluded and in this case the inhibitor can also bind to the free enzyme.

Observations of structural studies provide a possible rationale for the sequential BiBi mechanism. A disordered loop in the vicinity of the donor/acceptor-binding site is a common feature of glycosyltransferases. The binding of a nucleotide sugar structures the loop by making a direct hydrogen bond with the phosphate groups. It has been suggested that the ordered loop provides a monosaccharide-sized pocket centered directly over the catalytic base and the C-1 of the nucleotide. Furthermore, ordering of the loop may also be a means of protecting the bound nucleotide sugar from hydrolysis and, thus, be important for product release. The fact that compound **2** can be bound to FucT VI in the absence of bound GDP-Fuc shows that ordering of the loop is not important for the binding of this compound. The analysis of the inhibitory data suggests that compound **2** can be bound to FucT VI without prior binding to GDP-Fuc. Furthermore, the compound is probably complexed in a different fashion than the natural substrate LacNAc since it is unable to accept the fucosyl moiety.

Recombinant Fuc-Ts share significant amino acid sequence homology,[26] despite differences in acceptor specificity. DNA sequence analysis of α-1,3-fucosyltransferase III, V and VI revealed greater than 85% homology, suggesting that these enzymes evolved from common ancestor genes.[27] The hypervariable region containing 85–90% of the amino acid differences between FucT III, V and VI[28] and approximately 60% of the amino acid differences for FucT-IV[14] is confined to the N-terminal part of the proteins.[26] It has been suggested that this domain controls the acceptor specificity and the differences in this region may be important for selective inhibition of FucT VI by compound **2**.

Conclusion

Although the C-2′ hydroxyl of LacNAc is not essential for sialyl- and fucosyltransferase mediated glycosylations, inversion of the configuration led to a compound that was not accepted as a substrate by α-2,3- and α-2,6-sialyltransferase and α-1,3-fucosyltransferase III, IV, V and VI. Surprisingly, fucosyltransferase VI was the only enzyme that was inhibited by **2**. Detailed kinetic analysis revealed that the inhibition was of a mixed type with respect to LacNAc. The data fit a model in which compound **2** can bind the GDP-fucose complex as well as another enzyme form. Compound **2** is the first inhibitor that displays exquisite selectivity for a particular fucosyltransferase. Furthermore, the fact that FucT VI was inhibited is highly relevant given that this enzyme is involved in the biosynthesis of sialyl Lewis^x. The over-expression of this oligosaccharide has been implicated in numerous diseases, such as cancer and inflammation.[29]

Experimental

General methodology for synthesis

Chemicals were purchased from Aldrich and Fluka and used without further purification. Molecular sieves were activated at 350 °C for 3 h *in vacuo*. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. All reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Flash chromatography was performed on silica gel (Merck, mesh 70–230). Extracts were concentrated under reduced pressure at <40 °C (bath). ^{1}H NMR (1D, 2D) and ^{13}C NMR spectra were recorded on a Varian Merc300 spectrometer and Varian 500, 600 and 800 MHz spectrometers equipped with Sun workstations. For ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$, chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H, $\delta = 7.26$; ^{13}C, $\delta = 77.3$) as an internal standard for the protected compounds and using NH proton (1.91 ppm) and OMe carbon (56.83 ppm) as internal standard for deprotected molecules. Negative ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid matrix. High-resolution mass spectra were obtained using a Voyager delayed extraction STR with 2,5-dihydroxybenzoic acid as an internal calibration matrix. Optical rotations were measured on a Jasco P-1020 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^3 g^{-1} at 26 °C, 50 mm cell. Human recombinant α-1,3-fucosyltransferases V and VI, rat liver α-2,6-sialyltransferase, CTP, CMP-Neu5Ac and calf alkaline phosphatase were purchased from Calbiochem. α-2,3-Sialyltransferase was obtained from Sigma. Human recombinant α-1,3-fucosyltransferases III and IV were a generous gift from Dr. Theodora de Vries. ACS liquid scintillation cocktail was obtained from Fisher Scientific.

Methyl 2-acetamido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside 4. Sodium methoxide (0.5 mg, 0.01 mmol) was added to a stirred solution of **3** (50 mg, 0.055 mmol) in methanol (7 mL). The mixture was left stirring at room temperature for 48 hours. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The mixture was neutralized with Dowex 50H$^+$ resin, and then filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (flash silica gel, gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **4** (42.5 mg, 86%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.40–7.10 (m, 20H, arom), 5.58 (d, 1H, $J_{NH,2}$ 8.0), 4.83, 4.80 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.68, 4.60 (AB q, 2H, J_{AB} 12.1, OCH_2Ph), 4.63, 4.46 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.54 (d, 1H, $J_{1,2}$ 7.4, H-1), 4.41 (d, 1H, $J_{1,2}$ 7.4, H-1′), 4.15, 4.10 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 3.95 (d, 1H, $J_{3'-4'}$ 2.75, H-4′), 3.98 (dd, 1H, $J_{2'3'}$ 11.8 H-3′), 3.90–3.80(m, 4H, H-2′, H-5′, H-6b′, H6b), 3.50 (t, 1-H, $J_{3,4}$ 8.0, H-3), 3.49–3.40 (m, 4H, H-6a, H-6a′, H-5, H-2), 3.43 (s, 3H, OCH_3), 1.97 (s, 3H, AcNH), 0.81 (s, 9H, Si(CH_3)$_3$), −0.03, −0.05 (2s, 6H,

$Si(CH_3)_2$). MALDI-TOF: *m/z* 896.4 $[M + Na]^+$. Anal. calcd for $C_{49}H_{65}NO_{11}Si$: C, 67.48; H, 7.51; N, 1.61; O, 20.18; Si, 3.22; found: C, 67.56; H, 7.49; N, 1.69%. $[\alpha]_D = -8.5$ (*c* 0.32, DCM).

Methyl 2-acetamido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-talopyranosyl)-β-D-glucopyranoside 6. A solution of **4** (8.72 mg, 0.01 mmol) in acetic anhydride/dimethyl sulfoxide (1/2, v/v, 12 ml) was left stirring at room temperature overnight. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The mixture was concentrated *in vacuo* to give compound **5** as a white solid. Without further purification, sodium borohydride (3.8 mg, 0.1 mmol) was added to a stirred solution of **5** in DCM/MeOH (6 ml, 1/1, v/v). The mixture was left stirring at room temperature for 4 h. After completion of the reaction, the mixture was diluted with dichloromethane (20 mL), washed successively with water, citric acid (5% in water), a saturated solution of $NaHCO_3$ (5 mL), water (2 × 5 mL) and brine (5 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography (flash silica gel, gradient hexane/ethyl acetate, 3/1 to 1/1, v/v) to afford compound **6** (5.2 mg, 60% over 2 steps) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.40–7.10 (m, 20H, arom), 6.10 (d, 1H, $J_{NH,2}$ 7.8), 4.98, 4.58 (AB q, 2H, J_{AB} 10.7, OCH_2Ph), 4.81, 4.57 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.78, 4.50 (AB q, 2H, J_{AB} 11.7, OCH_2Ph), 4.52 (s, 1H, H-1′), 4.51 (d, 1H, $J_{1,2}$ 7.2, H-1), 4.44, 4.38 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.08 (m, 1H, H-2′), 3.96 (d, 1H, $J_{3'-4'} < 2$, H-4′), 3.87 (m, 1H, H-4), 4.00–3.80 (m, 3H, H-6b, H-6b′–H-5′), 3.79 (m, 1H, H-2), 3.60–3.50 (m, 2H, H-3, H-5), 3.51 (m, 1H, H-6a′), 3.46 (m, 1H, H-6a), 3.45–3.34 (m, 2H, H-3, H-3′), 3.39 (s, 3H, OCH_3), 1.96 (s, 3H, AcNH), 0.82 (s, 9H, Si(CH_3)$_3$), −0.02, −0.04 (2s, 6H, Si(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz); δ 170.26, 139.03, 138.04, 137.91, 128.76, 128.68, 128.62, 128.45, 128.37, 128.20, 128.10, 128.05, 127.85, 127.56, 101.63, 101.32. 78.25, 76.79, 75.88, 75.66, 74.98, 74.09, 73.75, 73.32, 69.94, 69.55, 68.06, 62.55, 56.34, 53.34, 29.91, 26.10, 26.06, 23.57, 18.45, 0.20, −4.90. HRMS: calculated *m/z* 894.4225 $[M + Na]^+$; observed 894.4247. $[\alpha]_D = -31$ (*c* 0.09, DCM).

Methyl 2-acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-talopyranosyl)-β-D-glucopyranoside 7. Tetrafluoroboric acid (48% in water, 2 μL, 0.015 mmol) was added to the stirred solution of **6** (4.1 mg, 0.005 mmol). The mixture was left stirring at room temperature for 5 minutes. TLC analysis (toluene/ethyl acetate, 1/1, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (4 μL, 0.03 mmol) and concentrated *in vacuo*. The residue was then diluted in dichloromethane (5 mL), washed successively with a saturated solution of $NaHCO_3$ (2 mL), water (2 × 2 mL) and brine (2 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography (flash silica gel, gradient hexane/ethyl acetate, 3/1 to 1/1, v/v) to afford compound **7** (3.7 mg, 94%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.40–7.10 (m, 20H, arom), 5.85 (d, 1H, $J_{NH,2}$ 7.9), 4.98, 4.58 (AB q, 2H, J_{AB} 10.7, OCH_2Ph), 4.81, 4.55 (AB q, 2H, J_{AB} 11.7, OCH_2Ph), 4.80, 4.51 (AB q, 2H, J_{AB} 11.7, OCH_2Ph), 4.63 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.51 (s, 1H, H-1′), 4.44, 4.35 (AB q, 2H, J_{AB} 11.9, OCH_2Ph), 4.14 (m, 1H, H-2′), 3.98 (t, 1-H, J_{3-4} 7.3, H-3), 3.94 (d, 1H, $J_{3'-4'} < 2$, H-4′), 3.88 (m, 1H, H-4), 3.88–3.80 (m, 3H, H-6b, H-6b′- H-5′), 3.70 (dd, 1H, H-2), 3.62 (m, 1H, H-5), 3.57 (m, 1H, H-6a′), 3.47 (s, 3H, OCH_3), 3.43 (ddd, 1H, J_{6a-5} 3.41, H-6a), 3.39 (s, 1-H, H-3′), 1.96 (s, 3H, AcNH). ^{13}C NMR ($CDCl_3$, 125 MHz); δ 170.42, 138.74, 137.99, 137.85, 137.80, 128.78, 128.69, 128.65, 128.52, 128.50, 128.24, 128.20, 128.13, 128.06, 127.85, 127.87, 101.99, 101.37, 78.91, 76.00, 79.00, 75.93, 75.02, 74.21, 73.77, 69.99, 68.91, 68.17, 62.54, 56.98, 54.74, 29.92, 16.44, 6.01. HRMS: calculated *m/z* 780.3360 $[M + Na]^+$; observed 780.3387. $[\alpha]_D = +4.3$ (*c* 0.12, DCM).

Methyl 2-acetamido-2-deoxy-4-*O*-(β-D-talopyranosyl)-β-D-glucopyranoside 2. Palladium hydroxide (4 mg) was added to a solution of **7** (3.5 mg, 0.0046 mmol) in ethanol (2 mL). The mixture was vigorously stirred under an atmosphere of hydrogen for 3 hours. TLC (chloroform/methanol, 9/1, v/v) indicated the completion of the reaction. After filtration on Celite and concentration, the crude material was purified by chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to afford compound **2** (1.8 mg, 98%) as an amorphous white solid. ^{1}H NMR (D_2O, 300 MHz); δ 4.58 (s, 1H, H-1′), 4.35 (d, 1H, $J_{1,2}$ 5.9, H-1), 3.91 (d, 1H, $J_{3',4'} < 0.2$, H-4′), 3.80 (dd, 1H, J_{6a-6b} 12.2, H-6a), 3.74 (s, 1H, H-3′), 3.64 (m, 1H, H-6b), 3.70–3.58(m, 3-H, H-4, H-3, H-2′), 3.54 (m, 1-H, H-5), 3.46 (m, 1-H, H-5′), 3.38 (s, 3H, OCH_3), 1.91 (s, 3H, AcNH). ^{13}C NMR (D_2O, 125 MHz); δ 101.70 (C-1), 100.65 (C-1′), 79.01 (C-4), 76.00 (C-5), 74.49 (C-5′), 72.25 (C-3), 70.55 (C-4′), 68.43 (C-3′), 68.02 (C-2′), 60.86 (C-6), 60.12 (C-6′), 56.83 (OCH_3), 54.64 (C-2), 21.92 ($NHCOCH_3$). HRMS: calculated *m/z* 397.1584 $[M + Na]^+$; observed 397.1569.

α-2,6- and α-2,3-Sialyltransferase assays

Reported methods[13,22,23,30] were employed for assaying sialyltransferase activity at different inhibitor concentrations. For studies of the relative rates of transfer, incubation mixtures contained saturating concentration of CMP[^{14}C]Neu5Ac (200 μM, 1655 cpm nmol^{-1}), substrate (1.6 mM for α-2,6-ST and 4 mM for α-2,3-ST) and different inhibitor concentration (0–1.5 mM), bovine serum albumin (1 mg ml^{-1}), 57 μU of α-2,6-Sialyltransferase and 370 μU of α-2,3-sialyltransferase in sodium cacodylate (50 mM, pH 6.5) containing 0.1% Triton X100 in a total volume of 60 μL were incubated at 37 °C for a period of 30 min. The radiolabeled product was isolated using a procedure modified by Horenstein *et al.*[30] based on Paulson's ion-exchange chromatography on a Dowex 1X8-200 (PO_4^{2-}, 100–200 mesh) Pasteur pipette column.[22] Columns (5 cm high) were eluted twice with 1 mM PO_4^{2-} (4 mL) buffer to ensure that no radiolabeled product was left on the column.

Fucosyltransferase assays

Reported methods[15,23]were employed for assaying fucosyltransferase activity. For studies of the relative rates, incubation mixtures contained a saturating concentration of GDP[^{14}C]-fucose (45 μM, 6532 cpm nmol^{-1}), substrate (100 nmol), inhibitor (0–1.5 mM) and an amount of enzyme corresponding to initial velocity for each fucosyltransferase (56 μU of FucT VI, 4 μL of FucT IV (6 μg protein μL^{-1}), 100 μU of FucT V and 10 μU of FucT III) assayed in sodium cacodylate (25 mM, pH 6.5) containing $MnCl_2$ (8 mM), ATP (1.6 mM) and NaN_3 (1.6 mM) in a total volume of 50 μL were incubated at 37 °C for a period of 60 min. The radiolabeled product was isolated using ion-exchange chromatography on a Dowex 1X8-200 (Cl^-, 100–200 mesh) Pasteur pipette column.[15] Columns (2.5 cm high) were eluted twice with ice cold water (1.5 mL) to ensure that no radiolabeled product was left on the column.

Inhibition studies

With respect to methyl LacNAc. Apparent kinetic parameters of the human recombinant FucT VI for synthetic acceptors were determined under the above standard conditions using a saturating concentration of GDP-fucose and different inhibitor concentrations (0, 0.2, 0.3 and 0.4 mM). Assays were performed in duplicate using the appropriate amount of enzyme. The concentration of oligosaccharide acceptor was varied around the K_m value (0.1–1.0 mM), whereas the concentration of GDP-fucose was kept constant at 45 μM. The time of incubation at 37 °C was varied to 15 min. to limit the GDP-[^{14}C]fucose consumption to 10–15 % to ensure initial rate conditions. The

kinetic parameters were determined by fitting the data to the Fortran programs of Cleland, COMPO and NCOMP.[24]

With respect to GDP-Fuc. Apparent kinetic parameters of the human recombinant FucT VI for GDP-Fuc were determined under the above standard conditions using a saturating concentration of LacNAc-OMe. Assays were performed in duplicate using the appropriate amount of enzyme (56 μU). The concentration of GDP-[^{14}C]fucose was varied around the K_m value (0.4–6.6 μM), whereas the concentration of LacNAc-OMe was kept constant at 1.5 mM and different inhibitor concentrations (0, 0.2 and 0.4 mM) were used. The time of incubation at 37 °C was varied to 15 min. The kinetic parameters were determined by fitting the data to the Fortran programs of Cleland, COMPO and NCOMP.[24]

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