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Synergistic Inhibition of Human α-1,3-Fucosyltransferase V

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Abstract: Human α-1,3-fucosyltransferase V (FucT V), which catalyzes the transfer of L-fucose moiety from guanosine diphosphate β-L-fucose (GDP-Fuc) to an acceptor sugar to form sialyl Lewis x (sLe^x), was shown to proceed through an ordered, sequential mechanism by product inhibition studies. The designed azatrisaccharide propyl 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-3-*O*-(2-(*N*-(β-L-homofuconojirimycinyl))ethyl)-α-D-glucopyranoside (**2**), prepared by covalently linking the N-group of β-L-homofuconojirimycin (**1**) to the 3-OH of LacNAc through an ethylene unit, in the presence of GDP was found to be an effective inhibitor of FucT V. In the presence of 30 μM GDP, the concentration of **2** necessary to cause 50% inhibition was reduced 77-fold to 31 μM. Presumably, the azatrisaccharide and GDP form a complex which mimics the transition state of the enzymatic reaction. Given the low affinity of FucT V for its substrate LacNAc (K_m = 35 mM), the designed azatrisaccharide in the presence of GDP represents the most potent synergistic inhibitor complex reported so far.

Introduction

Many antigenic oligosaccharides on the cell surface are fucosylated. These fucose-containing structures are regarded as oncodevelopmental antigens since they accumulate in a large variety of human cancers.[1] The biosynthesis of these structures requires the action of several glycosyltransferases, of which fucosylation by a class of fucosyltransferases (FucT) is the last and critical step.[2] α-L-Fucosidase[3] is a degradative enzyme which is involved in the hydrolytic removal of fucose residue from these glycoconjugates. Studies have indicated that increased activities of fucosyltransferase and α-L-fucosidase are responsible for the abnormal expression of these fucose-containing antigens in endometrial carcinoma.[4] Therefore, inhibitors of Fuc-T and α-fucosidase are potentially useful as anti-inflammatory and anti-tumor agents.

Fucosyltransferases catalyze the transfer of the L-fucose moiety from guanosine diphosphate β-L-fucose (GDP-Fuc) to the corresponding glycoconjugate acceptors to form an α-1,2, α-1,3/4, α-1,3 or α-1,6 linkage.[5] Five human α-1,3-fucosyltransferases have been cloned and mapped on chromosomes;[6] among them, α-1,3-fucosyltransferase V (FucT V) has been shown to be responsible for the production of sialyl Lewis x (sLe^x, Figure 1), a ligand for *E*-selectin involved in inflammatory process and tumor development.[7] This enzyme has been studied for acceptor specificity and used in the chemoenzymatic synthesis of sLe^x.[8,9] This enzyme accepts both *N*-acetyllactosamine (LacNAc) and sialyl LacNAc as the substrates with K_m values of 35 and 100 mM, respectively. The related enzyme α-1,2-fucosyltransferase has been hypothesized to proceed through a simple ion-pair transition state which results in the inversion of anomeric configuration,[10] α-1,3-fucosyltransferases may operate *via* a similar mechanism involving a displacement of GDP by the acceptor hydroxyl group assisted by a base on the enzyme (Figure 2). To date there is no X-ray crystal structure of any glycosyltransferase reported and none of the glycosyltransferases have been studied in detail with respect to their mechanism, though the kinetic mechanisms of a limited

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(1) (a) Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257. (b) Hakomori, S.; Nudelman, E.; Levery, S. B.; Kannagi, R. *J. Biol. Chem.* **1984**, *259*, 4672. (c) Feizi, T. *Nature* **1985**, *314*, 53.

(2) (a) Natsuka, S.; Lowe, J. B. *Curr. Opin. Struct. Biol.* **1994**, *4*, 683. (b) Holme, E. H.; Ostrander, G. K.; Hakomori, S. *J. Biol. Chem.* **1986**, *261*, 3737. (c) Hanisch, F. G.; Mitsakos, A.; Schroten, H.; Uhlenbruck, G. *Carbohydr. Res.* **1988**, *178*, 23. (d) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631.

(3) Johnson, S. W.; Alhadeff, J. A. *Comp. Biochem. Physiol.* **1991**, *99B*, 479.

(4) (a) Wang, J.-W.; Ambros, R. A.; Weber, P. B.; Rosano, T. G. *Cancer Res.* **1995**, *55*, 3654. (b) Thompson, S.; Cantwell, B. M. J.; Matta, K. L.; Turner, G. A.; *Cancer Lett.* **1992**, *65*, 115. (c) Chandrasekaran, E. V.; Jain, R. K.; Matta, K. L. *J. Biol. Chem.* **1992**, *267*, 23806. (d) Hutchinson, W. L.; Johnson, P. J.; Du, M.-Q.; Williams, R. *Clin. Sci.* **1991**, *81*, 177.

(5) (a) Field, M. C.; Wainwright, L. J. *Glycobiology* **1995**, *5*, 463. (b) Palcic M. M. *Methods Enzymol.* **1994**, *230*, 301.

(6) (a) de Vries, T.; Srnka, C. A.; Palcic, M. M.; Swiedler, S. J.; van den Eijnden, D. H.; Macher, B. A. *J. Biol. Chem.* **1995**, *270*, 8712. (b) McCurley, R. S.; Recinos III, A.; Olsen, A. S.; Gingrich, J. C.; Szczepaniak, D.; Cameron, H. S.; Krauss, R.; Weston, B. W. *Genomics* **1995**, *26*, 142. (c) Kukowska-Latallo, J. F.; Larsen, R. D.; Nair, R. P.; Lowe, J. B. *Genes Dev.* **1990**, *4*, 1288. (d) Natsuka, S.; Gersten, K. M.; Zenita, K.; Kannagi, R.; Lowe, J. B. *J. Biol. Chem.* **1994**, *269*, 16789. (e) Sasaki, K. Kurata, K.; Funayama, K.; Nagata, M.; Watanabe, E.; Ohta, S.; Hanai, N.; Nishi, T. *J. Biol. Chem.* **269**, 14730. (f) Lowe, J. B.; Kukowska-Latallo, J. F.; Nair, R. P.; Larsen, R. D.; Marks, R. M.; Macher, B. A.; Kelly, R. J.; Ernst, L. K. *J. Biol. Chem.* **1991**, *266*, 17467. (g) Goelz, S. E.; Hession, C.; Goff, D.; Giffiths, B.; Tizard, R.; Newman, B.; Chi-Rosso, G.; Lobb, R. *Cell* **1990**, *63*, 1349. (h) Kumar, R.; Potvin, B.; Muller, W. A.; Stanley, P. *J. Biol. Chem.* **1991**, *266*, 21777. (i) Weston, B. W.; Nair, R. P.; Larsen, R. D.; Lowe, J. B. *J. Biol. Chem.* **1992**, *267*, 4152. (j) Weston, B. W.; Smith, P. L.; Kelly, R. J.; Lowe, J. B. *J. Biol. Chem.* **1992**, *267*, 24575. (k) Koszdin, K. L.; Bowen, B. R. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 152.

(7) (a) Phillips, M. L.; Nudelman, E.; Gaeta, F. C.; Perez, M.; Singhal, A. K.; Hakomori, S. Paulson, J. C. *Science* **1990**, *250*, 1132. (b) Walz, G.; Aruffo, A. Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1130. (c) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell*, **1990**, *63*, 475.

(8) Wong, C.-H.; Dumas, D. P.; Ichikawa, Y.; Koseki, K.; Danishefsky, D. J.; Weston, B. W.; Lown, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 7321.

(9) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

(10) Palcic, M. M.; Heerze, L. D.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174.

S0002-7863(96)00274-0 CCC: $12.00

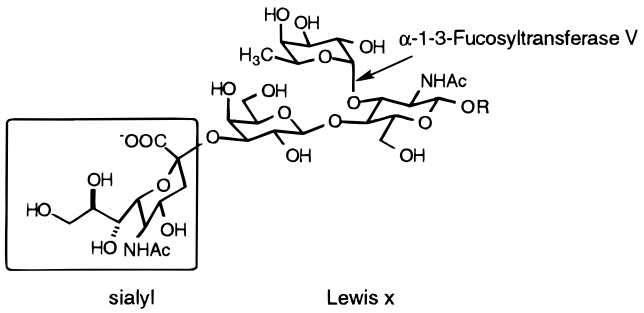


Figure 1. Structure of sialyl Lewis x. α-1,3-Fucosyltransferase V catalyzes the addition of fucose to LacNAc or sialyl LacNAc.

number of enzymes have been studied.[11−14] For example, β-1,4-galactosyltransferase,[12] β-D-xylosyltransferase,[13] and β-1,2-*N*-acetylglucosaminyl transferase II[14] have been reported to catalyze by an ordered, sequential manner while α-1,2-fucosyltransferase and UDP-glucuronosyltransferase are proposed to have a random binding mechanism.[10,11c] The kinetic mechanism of FucT V has not been reported to date.

The catalytic residues responsible for abstraction of the proton from the acceptor OH in most glycosyltransfer reactions remain elusive. Oligosaccharyl transferase has been shown to have a catalytic base with a $pK_a = 6.0$ which was suggested to be a histidine residue.[11c] The enzyme was found to be unstable at pH values greater than its optimal pH, 7.0. Most galactosyl transferases are also active in the pH range from 6 to 8.[11e] Glycosyltransferases require metal cofactors, typically manganese.[11a,c,d,13,14] The divalent manganese cofactor of β-1,4-galactosyltransferase has been shown to bind to the enzyme before the donor nucleotide sugar, UDP-galactose, and release from the galactosyltransferase in the form of a Mn^{2+}·UDP complex.[11a]

To date only limited success has been achieved in the development of inhibitors of these enzymes. Most efforts have focused on the production of unreactive structural analogs of GDP-Fuc.[15] A bisubstrate inhibitor of α-1,2-fucosyltransferase containing the acceptor structure and GDP portion of the donor substrate has also been reported.[10] However, these approaches only produced inhibitors with either comparable or slightly increased activity compared to that of the product inhibitor GDP. Very recently, trisubstrate analogs of α-1,3-fucosyltransferase containing D-glucose or *N*-acetyl-D-glucosamine, L-fucose analogs, and GDP have been synthesized as potential inhibitors, but no inhibition analysis was reported.[16]

A number of aza sugars have been found to be inhibitors of glycosidases and glycosyltransferases. Certain aza sugars are not only inhibitors of α-fucosidase but also moderate inhibitors of fucosyltransferases; in addition, preliminary studies indicate that these aza sugars synergistically inhibit fucosyltransferase in the presence of GDP.[8,9] Since both glycosidation and glycosyltransfer reactions are believed to involve transition states with substantial oxocarbenium ion character, it has been proposed that the protonated aza sugars may mimic the charge distribution of the glycosyl cation developed in the transition state.[17] The synergistic inhibition, on the other hand, indicates a possible interaction of aza sugars, GDP, and the acceptor sugar in the active site of the enzyme to mimic the transition state of the fucosyl transfer reaction (Figure 3). One advantage of using this type of synergistic inhibition is that a relatively high concentration of GDP is present in cells. The cellular concentration of GTP has been reported to be 0.3−0.5 mM in human cell lines[18] and that of GDP is 5−10% of the GTP concentration.[19] This translates into a 0.015−0.050 mM cellular concentration of GDP in these human cell lines, which argues for the *in vivo* relevance of the synergism between GDP and aza sugars in the inhibition of FucT V. A similar strategy has been used in the inhibition of UDP-GlcNAc enolpyruvoyl transferase of the antibiotic fosfomycin,[20] which requires the presence of UDP-GlcNAc *in vivo* for synergistic inhibition. Monosaccharide aza sugar inhibitors are, however, not specific because they only interfere with the binding of sugar nucleotide to the enzyme, not with the acceptor substrate which determines the specificity of the enzyme.

Our approach to the construction of fucosyltransferase-specific inhibitors is based on mimicking the proposed transition state of the fucosyl transfer reaction by covalently linking an aza sugar to the acceptor substrate. A designed inhibitor is shown in Figure 4 in which β-L-homofuconojirimycin (**1**),[21] a potent inhibitor of α-fucosidase ($K_i = 5.6$ nM), is linked to the 3-position of the acceptor substrate LacNAc *via* an ethylene spacer to form the azatrisaccharide **2**. This azatrisaccharide could then form a complex with GDP to mimic the transition state of the enzymatic reaction and thereby inhibit the enzyme in a sequence-specific, synergistic manner. The ethylene spacer is used to mimic the partially-forming glycosidic linkage; the flexibility of the linker may allow the aza analog of L-fucose and LacNAc residues to adopt the correct relative orientation for maximal enzymatic recognition. In this paper, we reported our kinetic investigation studies of FucT V, chemoenzymatic synthesis of inhibitors **1** and **2** for the inhibition studies of FucT V, and elucidation of the enzyme mechanism.

Results and Discussion

Kinetic Mechanism of FucT V. The product inhibition studies were conducted with purified FucT V. The inhibition mode was determined by inspection of the inhibition pattern of the double reciprocal analysis and statistical analysis of the nonlinear, least squares fit of the data to the equations for competitive, noncompetitive, and uncompetitive inhibition (see the Experimental Section). Double reciprocal analysis of FucT V as a function of GDP and GDP-Fuc exhibited a competitive pattern. Analysis of the data with the Compo FORTRAN

(11) (a) Tsopanakis, A. D.; Herries, D. G. *Eur. J. Biochem.* **1978**, *83*, 179−188. (b) Nakazawa, K.; Furukawa, K.; Narimatsu, K.; Kobata, A. *J. Biochem.* **1993**, *113*, 747−753. (c) Yin, H.; Bennett, G.; Jones, J. P. *Chem.-Biol. Interactions* **1994**, *90*, 47−58. (d) Breuer, W.; Bause, E. *Eur. J. Biochem.* **1995**, *228*, 689−696. (e) Ram, B. P.; Munjal, D. D. *CRC Crit. Rev. Biochem.* **1987**, *17*, 257−311.

(12) (a) Khatra, B. S.; Herries, D. G.; Brew, K. *Eur. J. Biochem.* **1974**, *44*, 537. (b) Kim, C. K.; Singh, A. N.; Raushel, F. M. *Arch. Biochem. Biophys.* **1988**, *267*, 54−59. (c) Ats, S.-C; Lehmann, J.; Petry, S. *Carbohydr. Res.* **1992**, *233*, 125−139.

(13) Kearns, A. E.; Campbell, S. C.; Westley, J.; Schwartz, N. B. *Biochemistry* **1991**, *30*, 7477.

(14) Bendiak, B.; Schachter, H. *J. Biol. Chem.* **1987**, *262*, 5784.

(15) (a) Cai, S.; Stroud, M. R.; Hakomori, S.; Toyokuni, T. *J. Org. Chem.* **1992**, *57*, 6693. (b) Luengo, J. T.; Gleason, J. G. *Tetrahedron Lett.* **1992**, *33*, 6911.

(16) (a) Heskamp, B. M.; Veeneman, G. H.; van der Marel, G. A.; van Boeckel, C. A. A.; van Boom, J. H. *Tetrahedron* **1995**, *51*, 8397. (b) Heskamp, B. M.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1995,** *14*, 1265.

(17) (a) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y; Kajimoto, T. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 521. (b) Jeong, J.-H.; Murray, B. W.; Takayama, S.; Wong, C.-H. *J. Am. Chem. Soc.*, in press. (c) Ganem, B. *Acc. Chem. Res.*, in press. (d) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182. (e) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199.

(18) Yabaluri, N.; Medaihradsky, F. *Mol. Pharmacol.* **1995**, *48*, 690.

(19) Gamberucci, A.; Innocenti, B.; Fulceri, R.; Banhegyi, G.; Giunti, R.; Poxxan, T.; Benedetii, A. *J. Biol. Chem.* **1994**, *269*, 23597.

(20) Marquardt, J. L.; Brown, E. D.; Lane, W. S.; Haley, T. M.; Ichikawa, Y.; Wong, C.-H.; Walsh, C. T. *Biochemistry* **1994**, *33*, 10646−10651.

(21) For chemical synthesis, see: Fleet, G. W. J.; Namgoong, S. K.; Barker, C.; Baines, S.; Jacob, G. S.; Winchester, B. *Tetrahedron Lett.* **1989**, *30*, 4439.

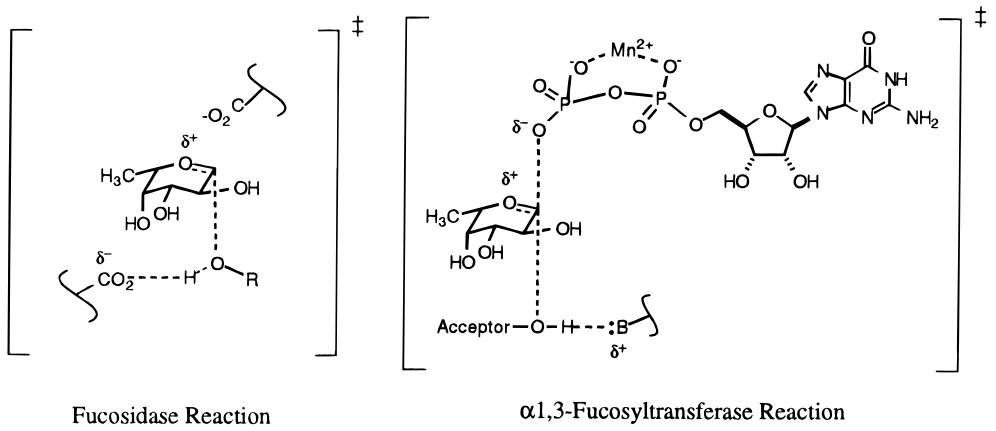


Figure 2. Proposed mechanisms of hydrolysis by fucosidase and fucosylation by α-1,3-fucosyltransferase V.

program[22] yielded a K_i of 29 μM for GDP. The fucosylation product Lewis x (Le^x) was found to be a moderate inhibitor with a K_i = 52.3 mM as determined by Dixon analysis. Le^x-β-*O*-$(CH_2)_5CO_2CH_3$[23] was found to be a noncompetitive inhibitor with respect to LacNAc and a noncompetitive inhibitor with respect to GDP-Fuc. FucT V was reported to be subject to potent substrate inhibition by GDP-fucose at concentrations greater than 0.2 mM in the presence of 10 mM LacNAc.[9] These results, coupled with the reported noncompetitive inhibition mode of GDP[9] with respect to LacNAc (K_{ii} = 0.12 mM, K_{is} = 0.16 mM), are consistent with an ordered, sequential mechanism[24] (Figure 5). The general rate equations for an ordered, sequential, BiBi mechanism including product inhibition terms have been described (eqs 1 and 2)[25] Equation 1 describes the

$$v = V_{max}A/\{K_{m,A}(1 + Q/K_{i,Q})(1 + K_{i,A}K_{m,B}/K_{m,A}B) + A(1 + K_{m,B}/B)\} \quad (1a)$$

$$v = V_{max}B/\{K_{m,B}[1 + (K_{i,A}/A)(1 + Q/K_{i,Q})] + B[1 + (K_{m,A}/A)(1 + Q/K_{i,Q})]\} \quad (1b)$$

velocity (v) in the presence of only GDP, eq 1a as a function of GDP-fucose concentration, and eq 1b as a function of LacNAc concentration. V_{max} is the maximal initial velocity, $K_{i,A}$ is an inhibition constant, and $K_{m,A}$ and $K_{m,B}$ are Michaelis constants. Equation 2 is the rate equation for the velocity in the presence of only Lewis x, eq 2a as a function of GDP-fucose concentration, and eq 2b as a function of LacNAc concentration.

$$v = V_{max}A/\{K_{m,A}[1 + (K_{i,A}K_{m,B}/K_{m,A}B)(1 + P/K_{i,Q})] + A[1 + (K_{m,B}/B)(1 + P/K_{i,Q}) + P/K_{i,P}]\} \quad (2a)$$

$$v = V_{max}B/\{K_{m,B}[(1 + K_{i,A}/A)(1 + P/K_{i,Q})] + B[1 + K_{m,B}/A + P/K_{i,P}]\} \quad (2b)$$

A = [GDP-fucose] B = [LacNAc]

P = [Lewis x] Q = [GDP]

Synthesis of 1 and 2. β-L-Homofuconojirimycin (**1**) was prepared conveniently by an aldolase-based strategy[26] as shown in Scheme 1. The acceptor substrate for aldolase was synthesized from commercially available 2-butyn-1-al diethyl acetal (**3**). Hydrogenation of **3** catalyzed by nickel (P2-Ni)[27] generated *in situ* gave the *cis*-olefin **4** (81%), which was then oxidized with *m*-CPBA to give epoxide **5** (71%). Stereoselective azide opening[28] of the epoxide followed by acidic hydrolysis provided the (±)-*threo*-azidoaldehyde **6**. FDP aldolase-catalyzed aldol condensation of **6** with dihydroxyacetone phosphate[29] (DHAP) followed by dephosphorylation with acid phosphatase afforded the desired enantiomerically pure azidoketose **7** (66%). Hydrogenation of **7** in the presence of Pd/C produced β-L-homofuconojirimycin (**1**) as the only product (100%) as shown by NMR spectroscopy. The high stereoselectivity of the hydrogenation is consistent with the early observations that hydrogen is delivered from the less hindered side of the possible imine intermediate during the reductive amination process.[17d]

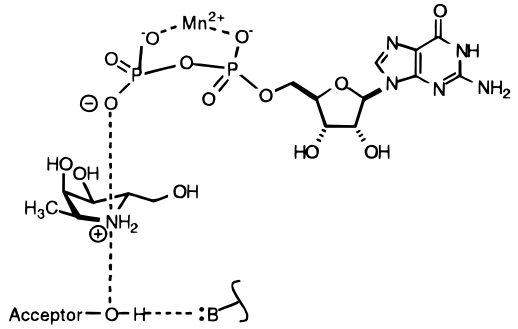


Figure 3. Proposed model for the synergistic inhibition of α-1,3-fucosyltransferase V by the combination of an aza sugar and GDP.

The convergent strategy for the synthesis of compound **2** involved a coupling of protected **1** and a LacNAc derivative. Benzylation of **1** with benzyl bromide and sodium hydride generated the fully benzylated compound **8** (Scheme 2). The well-resolved spectrum of **8** combined with proton decoupling experiments allowed the unequivocal determination of the aza sugar configuration. The pertinent coupling constants, $J_{2,3}$ (8.0 Hz), $J_{3,4}$ (8.0 Hz), and $J_{4,5}$ (2.9 Hz), clearly indicate the *trans*-diaxial orientation between H 2−3, and H 3−4, as well as the

(22) Cleland, W. W. *Method Enzymol.* **1979**, *63*, 103.

(23) Lin, C.-C.; Shimazaki, M.; Heck, M.-P.; Aoki, S.; Wang, R.; Kimura, T.; Ritzen, H.; Takayama, S.; Wu, S.-H.; Weitz-Schmidt, G.; Wong, C.-H. *J. Am. Chem. Soc.*, submitted.

(24) (a) Cleland, W. W. *The Enzymes*, 3rd ed.; Academic Press: New York, 1970; Vol. 2, pp 1−65. (b) Goldsmith, J. O.; Kuo, K. C. *J. Biol. Chem.* **1993**, *268*, 18485.

(25) Segal, I. H. In *Enzyme Kinetics, Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; John Wiley and Sons, Inc.: New York, 1975; p 560−590.

(26) For a recent review for aldolase-catalyzed reactions see: Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 4442−473.

(27) Brown, C. A.; Ahuja, V. K. *J. Org. Chem.* **1973**, *38*, 2226.

(28) The position of the hydroxyl group of the product was determined by a proton decoupling experiment. Also see: Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696.

(29) Jung, S.-H.; Jeong, J.-H.; Miller, P.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 7182.

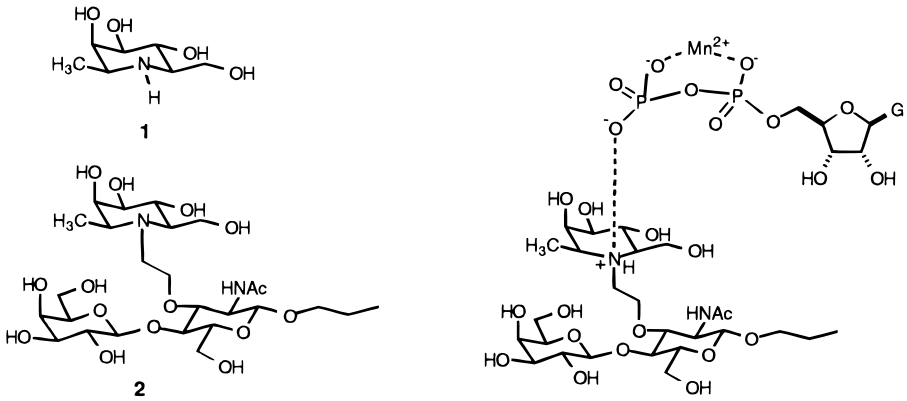


Figure 4. Structures of aza sugars **1** and **2** and possible mode of inhibition caused by **2** (right). In the presence of 0.03 mM GDP, the concentration of **2** necessary to achieve 50% inhibition of FucT V is 0.031 mM and that for **1** is 1.54 mM.

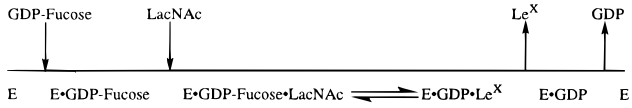
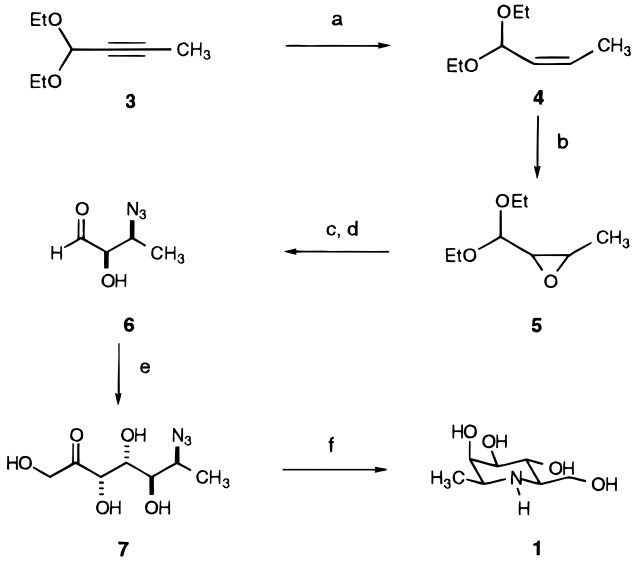


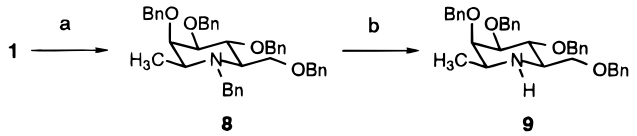
Figure 5. α-1,3-Fucosyltransferase V has an ordered, sequential mechanism as determined by product inhibition patterns. GDP-fucose is the donor sugar, *N*-acetyllactosamine (LacNAc) is the acceptor sugar, and Lewis x (Le^x) is the trisaccharide product.

Scheme 1[a]



[a] Reagents and conditions: (a) $Ni(OAc)_4 \cdot 4H_2O$, $NaBH_4$, $NH_2(CH_2)_2NH2$, then H_2, 1 atm, 81%; (b) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 71%; (c) NaN_3, NH_4Cl, EtOH:H_2O, 90 °C, 44%; (d) 0.1 N HCl, 50 °C, 5 h; (e) (i) DHAP, FDP aldolase, pH 6.7, 25 °C; (ii) acid phosphatase, pH 4.7, 37 °C, 66%; (f) Pd/C, H_2, 50 psi, 94%.

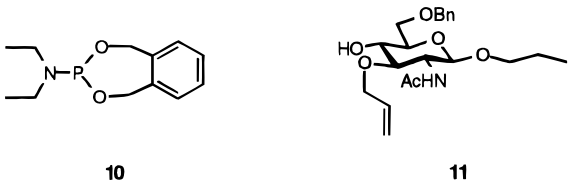
Scheme 2[a]



[a] Reagents and conditions: (a) NaH, BnBr, −40 to 25 °C, 53%; (b) $Pd(OH)_2$/C (Pearlman's catalyst), H_2, 55 psi, 96%.

cis-relationship between H 4−5. Selective *N*-debenzylation[30] of **8** by hydrogenolysis catalyzed by $Pd(OH)_2$ on carbon (Pearlman's catalyst) provided free amine **9** ready for coupling (51%, two steps).

Preparation of the protected LacNAc involved a glycosylation with a novel bicyclic glycosyl phosphite **12**. This convenient and improved phosphite methodology is different from the ones originally introduced by us[31] and Schmidt[32] with dibenzyl and diethyl glycosyl phosphite employed as the glycosyl donors, respectively. The bicyclic glycosyl phosphite **12** was obtained easily by condensation of commercially available *N,N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine (**10**) with



2,3,4,5-tetra-*O*-acetyl-D-galactopyranose[33] in the presence of 1*H*-tetrazole (92%) (Scheme 3).

Compound **11** was chosen as the glycosyl acceptor and was prepared by a standard reaction sequence.[34] The 3-allyl group not only activates the 4-OH for glycosylation but it also can be manipulated further to allow the attachment of the aza sugar moiety. Glycosylation of acceptor **11** with glycosyl phosphite **12** catalyzed by TMSOTf (0.3 equiv) at room temperature produced disaccharide **13** (38%); reactions with the corresponding trichloroacetimidate as donor substrate gave slightly lower yields (30%). Ozonolysis of the double bond followed by reduction of the intermediate aldehyde produced alcohol **14** (69%). One-pot triflate formation of alcohol **14** and *N*-alkylation[30a] of aza sugar **9** provided the target skeleton **15** (63%). Although it appeared that the direct reductive amination of the aldehyde intermediate obtained by ozonolysis with aza sugar **1** could give the precursor to **2**, this approach proved to be problematic under a variety of reaction conditions. Finally, saponification of the esters followed by hydrogenolysis of the benzyl groups provided compound **2** (91%).

(30) (a) van den Broek, L. A. G. M.; Vermaas, D. J.; Heskamp, B. M.; van Boeckel, C. A. A.; Tan, M. C. A. A.; Bolscher, J. G. M.; Ploegh, H. L.; van Kemenade, F. J.; de Goede, R. E. Y.; Miedema, F. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 82. (b) Bernotas, R. C.; Cube, R. V. *Synth. Commun.* **1990**, *20*, 1209.

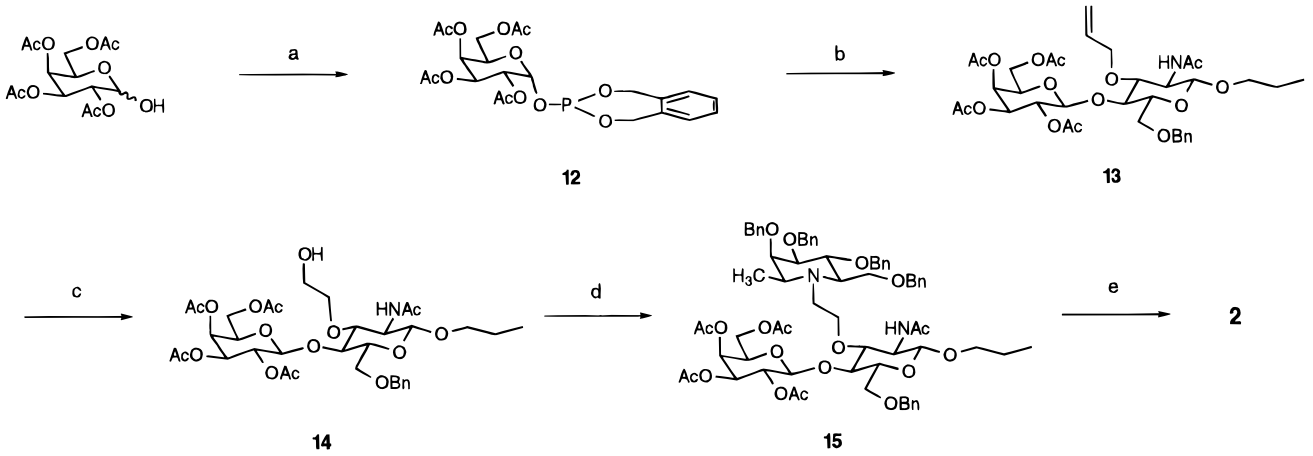
(31) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *57*, 2943.

(32) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123.

(33) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.

(34) Compound **11** was prepared from *N*-acetyl-D-glucosamine by glycosylation, benzylidene formation, allylation, and regioselective opening of the benzylidene ring. (a) For glycosylation see: Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076. (b) For benzylidene formation see: Evans, M. E. *Carbohydr. Res.* **1972**, *21*, 473. (c) For allylation see: Durette, P. L.; Meitzner, E. P. *Carbohydr. Res.* **1981**, *89*, 279. (d) For reductive benzylidene ring opening see: Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, C10.

Scheme 3[a]



[a] Reagents and conditions: (a) **10**, 1*H*-tetrazole, THF, 25 °C, 92%; (b) **11**, TMSOTf (0.3 equiv) CH_2Cl_2, 25 °C, 38%; (c) O_3, CH_2Cl_2:MeOH, then Me_2S, −78 to 25 °C, then $NaBH_4$, EtOH, 25 °C, 69%; (d) diisopropylethylamine, Tf_2O, CH_2Cl_2, −20 °C, then **9**, −20 to 25 °C, 63%; (e) (i) NaOMe, MeOH; (ii) Pd(OH)2/C, (Degussa type), MeOH/AcOH, H_2, 1 atm, 91%.

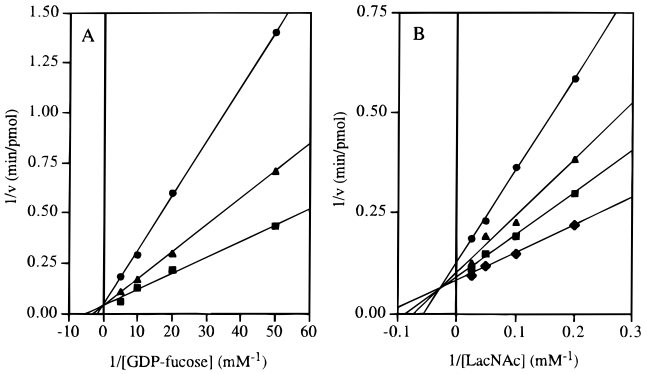
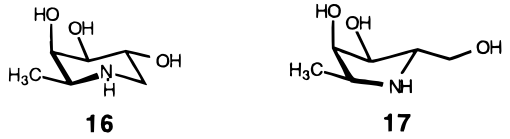


Figure 6. Inhibition of α-1,3-fucosyltransferase by **2**. (A) Inhibition with respect to the donor sugar, GDP-fucose (at 24 mM LacNAc). A competitive pattern is observed. Nonlinear regression analysis of the data with the Compo program reveals a $K_i = 2.7$ mM. Concentrations of inhibitor **2** were 0 (■), 4 (▲), and 8 mM (●). (B) Inhibition with respect to the acceptor sugar, LacNAc (at 0.05 mM GDP-fucose). Mixed inhibition is observed resulting in a $K_i = 2.4$ mM. Inhibitor concentrations were 0 (◆), 2 (■), 4 (▲), and 8 mM (●).

Inhibition Analysis. Inhibition studies with FucT V were conducted with purified enzyme. Double reciprocal analysis demonstrated that compound **2** had a competitive inhibition pattern with respect to GDP-fucose (Figure 6a), and nonlinear regression analysis[22] of the data yielded a $K_i = 2.7$ mM. The mode of inhibition of **2** with respect to LacNAc was linear mixed (Figure 6b) and the K_i was determined to be 2.4 mM. β-L-Homofuconojirimycin (**1**) was determined to have a K_i of 32.9 mM by Dixon analysis.[35] The K_m of LacNAc has been reported to be 35 mM.[9] Therefore, inhibitor **2** is an order of magnitude more potent than either of its components **1** and LacNAc.

Synergy of aza sugars in combination with GDP was evaluated for the inhibition of FucT V. Synergy is defined as an interaction between the two inhibitors on the enzyme such that the presence of one inhibitor decreases the dissociation constant of the other (and vice versa). It would seem reasonable to believe that both the fucose-type aza sugar **1** and GDP bind to FucT V at the GDP-fucose site in the enzyme active site. At their K_i levels, individually **1** and GDP caused 17% and 25% inhibition in the presence of 24 mM LacNAc, respectively; combination of **1** and GDP resulted in 79% inhibition of the enzyme. Evaluation of the synergistic effect of **2** and GDP demonstrated that combination of **2** and GDP at their K_i levels resulted in 80% inhibition in the presence of 24 mM LacNAc, while individually only 15% and 29% inhibitions were observed, respectively.

Table 1.



16 **17**

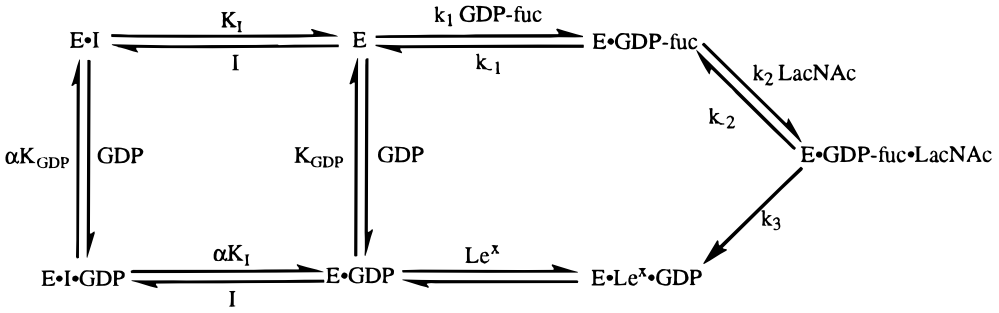
compound	K_m, mM	K_i, mM (IC_{50})
LacNAc	35	
GDP-fucose	0.009	
Lewis x		52.3 (84.0)
GDP		0.0290 (0.067)
azatrisaccharide **2**		2.40 (5.7)
azatrisaccharide **2**[a]		− (0.031)
homofuconojirimycin **1**		32.9 (71.5)
homofuconojirimycin **1**[a]		− (1.54)
deoxyfuconojirimycin **16**		45.3 (73.1)
deoxyfuconojirmycin[a] **16**		− (3.55)
aza sugar **17**		− (80)

[a] IC_{50} with all assays containing 0.030 mM GDP.

Apparent IC_{50} values were determined for **1**, **2**, and deoxyfuconojirimycin (**16**) in the presence of GDP. The percent of inhibition of FucT V by the azasaccharides in the presence of 0.030 mM GDP, 5 mM LacNAc, and 0.025 mM GDP-fucose was determined *relative to a solution that contained* 0.030 mM GDP, 5 mM LacNAc, and 0.025 mM GDP-fucose. The absolute amount of inhibition of FucT V at the apparent IC_{50} was greater than 50% because GDP is an inhibitor, but was factored out by the experimental design. The IC_{50} values of **1**, **2**, **16** were determined to be 71.5, 5.7, and 73.1 mM, respectively. In the presence of 0.030 mM GDP, the apparent IC_{50} values of **1**, **2**, and **16** were 1.54, 0.031, and 3.55 mM, respectively. The synergism of GDP, aza sugar and acceptor sugar was responsible for a 22−77-fold decrease in the concentration of aza sugar necessary to cause 50% inhibition (IC_{50}) (Table 1).

The kinetic proof of synergistic inhibition is illustrated in Scheme 4. Since GDP and fucose-type aza sugars are competitive inhibitors with respect to GDP-fucose, they both bind only free enzyme; this is analogous to the single substrate system described by Yonetani and Theorell.[36] GDP is a product inhibitor of FucT V and the kinetics of such a system has been

Scheme 4. Kinetic Mechanism for FucT V Including Synergistic Inhibition by the Combination of GDP and an Aza Sugar (I)[a]



[a] FucT V has an ordered, sequential BiBi mechanism.

described (eq 1).[25] Fucose-type aza sugars should interact with FucT V in a mode similar to that of GDP. Therefore, we can extend the rate equation for GDP inhibition (eq 1) to an equation for synergistic inhibition (eq 3) by including terms for aza sugar inhibition ([aza sugar]/$K_{i,aza\ sugar}$) and for the formation of the GDP−aza sugar−enzyme complex ([aza sugar][GDP]/$\alpha K_{i,aza\text{-}sugar}K_{i,GDP}$). The data obtained can be fitted to the following equation:

$$v = V_{max}[\text{GDP-fucose}]/[K_m,\text{GDP}_{fuc}I^*\{1 + K_{i,GDP-fuc}K_{m,LacNAc}/[K_{m,GDP-fuc}[\text{LacNAc}])\} + [\text{GDP-fuc}](1 + K_{m,LacNAc}/[\text{LacNAc}])]$$

$$I^* = 1 + \frac{[\text{GDP}]}{K_{i,GDP}} + \frac{[\text{aza sugar}]}{K_{i,aza\ sugar}} + \frac{[\text{GDP}][\text{aza sugar}]}{\alpha K_{i,GDP}K_{i,aza\ sugar}} \quad (3)$$

The variable α is the interaction constant of the two inhibitors, aza sugar and GDP. If $\alpha > 1$, the binding of one inhibitor hinders the binding of the other (antisynergy); if $\alpha = 1$, the binding of one inhibitor has no effect on the binding of the other; and if $\alpha < 1$, the inhibitors bind synergistically. Equation 3 can be rearranged into a linear expression in the form of Dixon plots. At constant substrate concentration, plots of velocity versus the inhibitor concentration, [I], at different fixed concentrations of the other inhibitor, X, give lines that intersect at a *y*-value less than $1/V_{max}$ for $\alpha > 1$ (antisynergy), lines that intersect at a *y*-value equal to $1/V_{max}$ for $\alpha = 1$, or lines that intersect at a *y*-value greater than $1/V_{max}$ for $\alpha < 1$ (synergy). Yonetani-Theorell plots for the combination of GDP and **2** showed a pattern of nonexclusive, synergistic inhibition; a family of lines intersecting at a *y*-value greater than $1/V_{max}$ and $[I] = -\alpha K_i$ (Figure 7A). The interaction constant α was determined to be 0.18, showing that the presence of one inhibitor increases the affinity of the other by a factor of 6-fold (1/α); and the $-\alpha K_i$ was determined to be 0.45 mM.

The role of the acceptor sugar in the synergistic inhibition of FucT V was investigated by monitoring FucT V activity as a function of LacNAc concentration at fixed GDP-fucose, GDP, and aza sugar concentrations. The concentrations of LacNAc used in the study were from 5 to 40 mM. The effect of the individual inhibitors on FucT V as a function of LacNAc was modest, which is in agreement with the inhibition studies at 24 mM LacNAc. The combination of the aza sugars with GDP was subject to a substantial acceptor sugar effect. The combination of 2.8 mM **2** and 0.030 mM GDP produced a linear effect in the double reciprocal plot despite the fact that **2** contains a LacNAc moiety (Figure 7B). The apparent K_m of

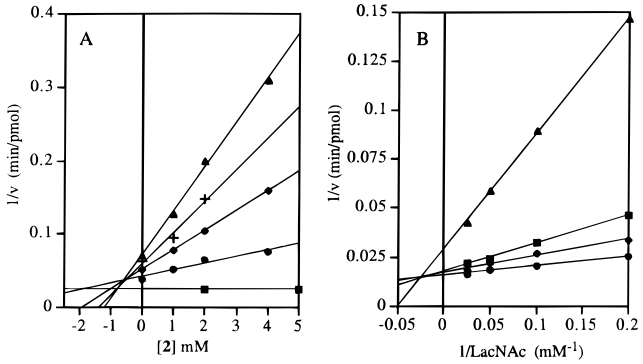


Figure 7. Multiple inhibition studies of FucT V. (A) the Yonetani−Theorell plot reveals nonexclusive binding by the pattern of intersecting lines. The intersection point (αK_i) reveals that $\alpha < 1$, indicative of synergistic inhibition. LacNAc (24 mM) and GDP-fucose (0.05 mM) were held at fixed concentrations. GDP concentrations were as follows: 0.0 mM (●), 0.05 mM (◆), 0.10 mM (+), 0.025 mM (▲), and $1/V_{max}$ (■). (B) Role of the acceptor sugar, LacNAc, in the synergistic inhibition of Fuc-T V by the combination of the **2** and GDP. The acceptor sugar had a modest effect on the inhibition of single inhibitors but had a substantial effect on the inhibition of a combination of **2** and GDP. GDP-fucose was held constant at 0.05 mM. No inhibitors (●), 0.03 mM GDP (◆), 2.8 mM **2** (■) and 0.03 mM GDP + 2.8 mM **2** (▲).

LacNAc under the experimental conditions is 35 mM and the maximum LacNAc concentration (40 mM) was not saturating. The mono aza sugars were subject to an acceptor sugar effect. Both β-L-homofuconojirimycin (**1**) and the 5-membered fucose-type aza sugar **17**[8] in combination with 0.05 mM GDP produced a substantial, linear acceptor sugar effect in the double reciprocal plot (Figure 8). These studies indicate a role for the acceptor sugar in the synergistic inhibition of FucT V through a quaternary complex of FucT V, acceptor sugar, GDP, and aza sugar, and suggest that certain aza sugars which inhibit glycosidases may also inhibit glycosyltransferases *in vivo*.

Mechanism. The synergistic inhibition of fucosyltransferase displayed by aza sugars and GDP indicates the interaction of the positively charged aza sugars with both the negatively charged GDP and the bound acceptor sugar in the active site, which may mimic the transition state of the fucosyltransfer reaction as shown in Figure 2. The increased inhibitory potency of **2** over simple aza sugars such as **1** provides further indication. The synergism of GDP and **2** was responsible for an additional 77-fold enhancement in inhibitory potency. Since transition state analogs are expected to bind tightly to the enzymes,[37] the moderate inhibition of **2** may reflect the nonideal length of the linker moiety, which resulting in the less than perfect orientation of the LacNAc or **1** in their respective binding pockets. Initial

(35) Dixon, M.; Webb, E. C. In *Enzymes*, 3rd ed.; Academic Press: New York, 1979; p 350−351

(36) Yonetani, T.; Theorell, H. *Arch. Biochem. Biophys.* **1964**, *106*, 243.

(37) (a) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375. (b) Pauling, L. *Am. Scientist* **1948**, *36*, 51.

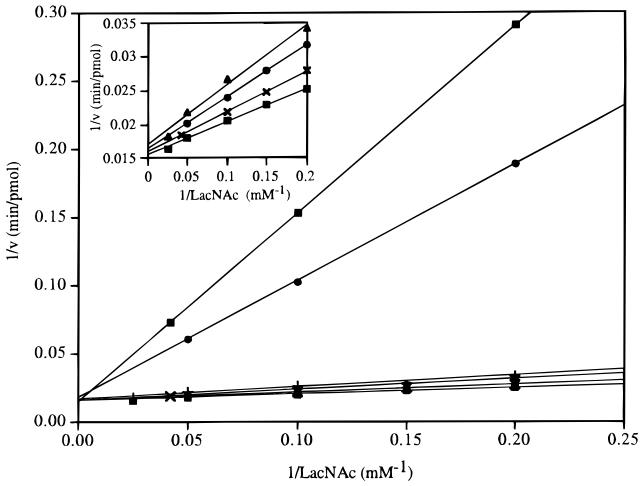


Figure 8. Role of the acceptor sugar (LacNAc) in the synergistic inhibition of FucT V with the combination of aza monosaccharides and GDP. The effect of the individual inhibitors on FucT V was modest. The combination of the aza sugars with GDP was subject to a substantial acceptor sugar effect. GDP-fucose and GDP were both held constant at 0.05 mM. The concentrations are as follows: 33 mM **1** + 0.050 mM GDP (■), **17** + 0.030 mM GDP (●). (Inset) The concentrations of the inhibitors are as follows: no inhibitors (■), 33 mM **1** (×), 80 mM **17** (●), 0.050 mM GDP (▲).

computer modeling studies reveal that **2** is not an ideal Le^x mimetic due to the length of the linker; therefore, variation of the linker could lead to better inhibitors. Acceptor specificity studies have demonstrated that FucT V has unusually high K_m values for all substrates studied (e.g. LacNAc, $K_m = 35$ mM);[7a,8,9] therefore, it is likely that more potent inhibition and profound synergistic inhibition may occur with an inhibitor comprised of a more specific substrate than LacNAc, as illustrated in other studies.[38−42]

In our recent study on the specificity and mechanism of FucT V,[43] a pH−rate profile revealed one catalytically relevant residue with a p$K_a = 4.1$. This result is consistent with a carboxylic, general-base-catalyzed mechanism. FucT V was shown to be subject to a solvent isotope effect ($D_{V/K} = 2.1$, $D_V = 2.9$) and a proton inventory study that revealed that one exchangeable proton was involved in the catalytic mechanism.[43] Detailed kinetic studies on FucT V yielded the following parameters:[43] $k_{cat} = 40$ min^{-1}, $K_{i,GDP-fuc} = 0.0062$ mM, $K_{m,GDP-fuc} = 0.060$ mM, $K_{m,LacNAc} = 8.8$ mM. The enzyme proficiency of FucT V, $(k_{cat}/K_{i,GDP-fuc}K_{m,LacNAc})/k_{non}$, is estimated to be 1.2×10^{10} M^{-1} and the transition-state affinity is therefore 8.6×10^{-11} M.[43] This places FucT V at the lower end of the range of transition state affinities (5.3×10^8 to 2.0×10^{23} M^{-1}),[44] which may contribute to the difficulty in the development of potent fucosyltransferase inhibitors. However, given the low affinity of FucT V for its substrate ($K_m = 35$ mM for LacNAc), the designed azatrisaccharide in the presence of GDP represents the most potent transition-state analog inhibitor ($IC_{50} = 31$ μM) reported so far.

(38) Gruys, K. J.; Walker, M. C.; Sikorski, J. A. *Biochemistry* **1992**, *31*, 5535.
(39) Seidel, H. M.; Knowles, J. R. *Biochemistry*, **1994**, *33*, 5641.
(40) Fletcher, R. S.; Arion, D.; Borkow, G.; Wainberg, M. A.; Dmitrienko, G. I.; Parniak, M. A. *Biochemistry* **1995**, *34*, 10106.
(41) England, P.; Herve, G. *Biochemistry* **1994**, *33*, 3913.
(42) (a) Pfuetzner, R. A.; Chan, W. W.-C. *J. Biol. Chem.* **1988**, *263*, 4056. (b) Xue, Y.; Huang, S.; Liang, J.-Y.; Zhao, Y.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12482. (c) Janc, J. W.; O'leary, M. H.; Cleland, W. W. *Biochemistry*, **1992**, *31*, 6421.
(43) Murray, B. W.; Takayama, S.; Wong, C.-H. *Biochemistry*, submitted.
(44) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93.

Experimental Section

General Methods. All processes involving air or moisture sensitive reactants were done under an atmosphere of dry argon using oven-dried glassware. Reagents and solvents were reagent grade and used as supplied unless otherwise mentioned. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (<0.1 Torr) to constant sample weight. High resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE instrument under fast atom bombardment (FAB) conditions. ^{1}H NMR spectra were obtained at 400 MHz and ^{13}C NMR at 100 MHz on a Bruker AM-400 instrument. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the solvent resonance as the reference: $CDCl_3$ δ 7.26, HDO δ 4.60; ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.0. Purified soluble α-1,3-Fuc-T V was a generous gift from the Cytel Corporation (La Jolla, CA). *N*-Acetyllactosamine, guanosine 5′-diphosphate, guanosine-5′-diphospho-*β*-fucose, adenosine 5′-triphosphate, and FDP aldolase were purchased from Sigma. Guanosine-5′-diphospho-L-[U-^{14}C]fucose was purchased from Amersham Life Science. ScintiVerseI scintillation cocktail was purchased from Fisher. Scintillation counting was performed on a Beckman LS 3801 instrument.

***cis*-2-Buten-l-al Diethyl Acetal (4).** Sodium borohydride (147 mg, 3.9 mmol) in EtOH (4 mL) was added dropwise to a solution of nickel acetate tetrahydrate (0.98 g, 3.9 mmol) in EtOH (40 mL). The resulting mixture was stirred for a an additional 10 min and then ethylene diamine (0.52 mL, 7.8 mmol) and butyn-1-al diethyl acetal (5.0 g, 35.1 mmol) were added. The mixture was stirred under a H_2 atmosphere for 6 h, and then filtered though a pad of Celite. To the filtrate was added H_2O and it was extracted with ether (60 mL × 3). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated below 20 °C to give olefin **4** (4.1 g, 81%) as a colorless and volatile oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.75−5.65 (m, 1 H, H-2), 5.50−5.42 (m, 1 H, H-3), 5.20 (d, $J = 7.0$ Hz, 1 H, H-1), 3.68−3.58 (m, 2 H, OC*H*$_2$CH$_3$), 3.50−3.42 (m, 2 H, OC*H*$_2$CH$_3$), 1.70 (dd, $J = 7.0$, 1.5 Hz, 3 H, C*H*$_3$), 1.22 (t, $J = 7.0$ Hz, 6 H, 2 × OCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 128.99, 128.16, 97.47, 60.49, 15.29, 13.61.

***cis*-2,3-Epoxybutyraldehyde Diethyl Acetal (5).** A mixture of olefin **4** (211 mg, 1.46 mmol), *m*-CPBA (503 mg of a solid of 57−86% purity, 2 mmol), and $NaHCO_3$ (132 mg, 1.57 mmol) in CH_2Cl_2 (25 mL) was stirred at room temperature for 12 h. Saturated Na_2SO_3 solution (10 mL) was then added slowly and the resulting mixture was stirred for 20 min. The organic layer was separated, washed with saturated $NaHCO_3$, H_2O, and brine, and dried ($MgSO_4$). The solvent was removed *in vacuo* below 20 °C to give epoxide **5** (165 mg, 71%) as a volatile oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.30 (d, $J = 6.5$ Hz, 1 H, H-1), 3.80−3.52 (m, 4 H, 2 × OC*H*$_2$CH$_3$), 3.13−3.02 (m, 2 H, H-2, H-3), 1.25 (t, $J = 7.0$ Hz, 3 H, OCH$_2$C*H*$_3$), 1.22 (t, $J = 7.0$ Hz, 3 H, OCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 100.33, 62.08, 56.37, 51.87, 15.33, 15.25.

***threo*-3-Azido-2-hydroxybutyraldehyde Diethyl Acetal (6).** A mixture of epoxide **5** (2.4 g, 15 mmol), sodium azide (4.9 g, 75 mmol), and ammonium chloride (4.0 g, 75 mmol) in EtOH (135 mL) and H_2O (15 mL) was heated at 90 °C for 20 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was concentrated *in vacuo* to about 50 mL and then extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Filtration through a short silica gel columm (EtOAc:hexane 1:10 to 1:5) and concentration gave the title compound (1.35 g, 44%) as an yellowish volatile oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.48 (d, $J = 6.5$ Hz, 1 H, H-1), 3.82−3.69 (m, 2 H, OC*H*$_2$CH$_3$), 3.60−3.48 (m, 3 H, H-3, OC*H*$_2$CH$_3$), 3.41 (dd, $J = 6.5$, 2.0 Hz, H-2), 1.41 (d, $J = 7.0$ Hz, 3 H, CH$_3$), 1.20 (t, $J = 7.0$ Hz, 3 H, OCH$_2$C*H*$_3$), 1.16 (t, $J = 7.0$ Hz, 3 H, OCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 102.90, 74.75, 64.46, 63.48, 56.43, 15.78, 15.41, 15.34.

A small amount of the title compound was acetylated with acetic anhydride and pyridine to give the corresponding acetate: ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 4.92 (dd, $J = 7.0$, 2.0 Hz, 1 H, H-2), 4.59 (d, $J = 7.0$ Hz, 1 H, H-1), 3.80−3.50 (m, 5 H, 2 × OC*H*$_2$CH$_3$, H-3), 2.10 (s, 3 H, COC*H*$_3$), 1.26−1.10 (m, 9 H, 3 × C*H*$_3$).

6-Azido-6,7-dideoxy-L-*galacto*-heptulose (7). A mixture of *threo*-3-azido-2-hydroxybutyraldehyde diethyl acetal (1.14 g, 5.63 mmol) in

HCl (0.1 N, 30 mL) was heated at 50 °C for 5 h. The reaction mixture was cooled to room temperature and the pH was adjusted to 7.0 with 5.0 N NaOH. Dihydroxyacetone phosphate[29] (5.62 mL of a 0.50 M solution, 2.81 mmol) was added and the pH was adjusted to 6.7 with 1 N NaOH. FDP aldolase (1134 units) was added, and the reaction mixture was stirred at room temperature. After 12 h, the pH of the reaction mixture (6.2) was adjusted to 6.7 with 1 N NaOH and then the reaction mixture was stirred for an additional 28 h. The pH of the reaction mixture was adjusted to 4.7 with 1.0 N HCl, and acid phosphatase (750 units) was added. The mixture was incubated at 37 °C for 22 h. The reaction mixture was neutralized to pH = 7.0 with 1.0 N NaOH and concentrated *in vacuo* below 30 °C. The residue obtained was extracted with MeOH (15 mL × 4), and the combined extracts were concentrated. Flash chromatography (silica, CH_2Cl_2:CH_3-OH, 15:1) of the residue gave **7** (407 mg, 66%), as a yellowish oil, and the other diastereomer (130 mg, 21%).

For **7**: ^{1}H NMR (D_2O, 400 MHz) δ 4.20−4.10 (m, 2 H), 3.70−3.50 (m, 4 H), 1.30 (m, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 83.38, 78.92, 77.91, 66.18, 58.88, 17.42; HRMS (LSIMS$^+$) *m/z* calcd for $C_7H_{13}N_3O_5 + Na^+$ 242.0753, found 242.0748.

2,6,7-Trideoxy-2,6-imino-L-*glycero*-D-*manno*-heptitol (*β*-L-Homofuconojirimycin) (1). A solution of azidoketose **7** (410 mg, 1.87 mmol) in methanol (30 mL) was hydrogenated at 50 psi in the presence of 10% Pd/C (40 mg) for 24 h. The catalyst was removed by filtration through a pad of Celite. Concentration of the filtrate *in vacuo* gave aza sugar **1** (310 mg, 94%) as a foam: ^{1}H NMR (D_2O, 400 MHz) δ 3.80−3.70 (m, 3 H, H-5, H-1a, H-1b), 3.56−3.54 (m, 2 H, H-3, H-4), 2.85 (dd, J = 7.0, 7.0 Hz, 1 H, H-6), 2.52−2.48 (m, 1 H, H-2), 1.10 (d, J = 7.0 Hz, 3 H, C*H*$_3$); ^{13}C NMR (D_2O, 100 MHz) δ 77.42, 74.81, 70.25, 62.95, 62.27, 54.63, 18.61; HRMS (LSIMS$^+$) *m/z* calcd for $C_7H_{15}NO_4 + H^+$ 178.1079, found 178.1083.

1,3,4,5-Tetra-*O*-benzyl-2,6-(*N*-benzylimino)-2,6,7-trideoxy-L-*glycero*-D-*manno*-heptitol (8). At −50 °C, NaH (164 mg of a 60% dispersion in oil, 4.11 mmol, washed with dry hexane before use) was added to a solution of aza sugar **1** (112 mg, 0.633 mmol) in dry DMF (2.5 mL). After being stirred for 5 min, the reaction mixture was allowed to warm to 0 °C, and benzyl bromide (0.70 mL, 5.88 mmol) was added. After being stirred at 0 °C for 5 h and room temperature for 4 h, the reaction mixture was diluted with EtOAc (20 mL) and washed with H_2O and brine, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography (silica, hexane:EtOAc, gradient, 30:1 to 10:1) gave compound **8** (209 mg, 53%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.15 (m, 25 H, ArH), 4.92 (d, J = 11.8 Hz, 1 H, OC*H*HPh), 4.80 (d, J = 11.0 Hz, 1 H, OCH*H*Ph), 4.75 (ABq, J = 11.8 Hz, 2 H, OC*H*$_2$Ph), 4.65 (d, J = 11.8 Hz, 1 H, OC*H*HPh), 4.55 (d, J = 11.0 Hz, 1 H, OCH*H*Ph), 4.20 (ABq, J = 12.0 Hz, 2 H, OC*H*$_2$Ph), 4.15 (d, J = 16.6 Hz, 1 H, NC*H*HPh), 4.05 (dd, J = 8.0, 8.0 Hz, 1 H, H-3), 3.98 (d, J = 16.5 Hz, 1 H, NCH*H*Ph), 3.80 (t, J = 2.8 Hz, 1 H. H-1a), 3.70 (m, 2 H, H-5, H-1b), 3.61 (dd, J = 8.0, 2.9 Hz, 1 H, H-4), 3.00−2.90 (m, 2 H, H-2, H-6), 1.08 (d, 3 H, J = 7.0 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.97, 139.23, 138.84, 138.77, 138.40, 128.34, 128.28, 128.24, 128.21, 128.15, 127.92, 127.86, 127.76, 127.73, 127.44, 127.35, 127.29, 126.15, 84.29, 78.44, 77.35, 77.03, 76.71, 75.40, 74.13, 73.84, 72.87, 72.55, 68.75, 64.04, 56.98, 52.55, 16.65; HRMS (LSIMS$^+$) *m/z* calcd for $C_{42}H_{45}NO_4 + Cs^+$ 760.2403, found 760.2425.

1,3,4,5-Tetra-*O*-benzyl-2,6-imino-2,6,7-trideoxy-L-*glycero*-D-*manno*-heptitol (9). A mixture of compound **8** (209 mg, 0.333 mmol) and 20% Pd(OH)$_2$/C (Pearlman's catalyst, 108 mg) in EtOAc:EtOH (18:3 mL) was hydrogenated at 55 Psi for 16 h. Filtration and concentration gave amine **9** (172 mg, 96%) as an oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.15 (m , 20 H, ArH), 5.05 (d, J = 11.5 Hz, 1 H, OC*H*HPh), 4.89 (d, J = 11.0 Hz, 1 H, OCH*H*Ph), 4.73 (ABq, J = 11.5 Hz, 2 H, OC*HH*Ph), 4.67 (d, J = 11.5 Hz, 1 H, OC*H*HPh), 4.51 (d, J = 11.5 Hz, 1 H, OCH*H*Ph), 4.48 (d, J = 11.0 Hz, 1 H, OCH*H*Ph), 4.42 (d, J = 11.5 Hz, 1 H, OCH*H*Ph), 3.91 (dd, J = 9.5, 9.5 Hz, 1 H, H-3), 3.71−3.68 (m, 2 H, H-5, H-1a), 3.61 (dd, J = 9.0, 2.5 Hz, 1 H, H-1b), 3.54 (dd, J = 9.5, 2.0 Hz, 1 H, H-4), 2.75−2.65 (m, 2 H, H-2, H-6), 1.09 (d, J = 6.7 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.65, 128.40, 128.32, 128.16, 128.13, 127.82, 127.64, 127.55, 127.50, 127.35, 86.36, 77.74, 76.62, 75.27, 74.27, 73.16, 72.29, 69.40, 59.58, 53.29, 18.10; HRMS (LSIMS$^+$) *m/z* calcd for $C_{35}H_{39}NO_4 + H^+$ 538.2957, found 538.2945.

Propyl 2-Acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (11). The title compound was prepared from *N*-acetyl-D-glucosamine by a reaction sequence involving glycosylation, benzylidenation, allylation, and reductive ring opening of the benzylidene ring.[34] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.20 (m, 5 H, ArH), 5.95−5.85 (dddd, J = 17.2, 10.3, 5.8, 5.6 Hz, 1 H, OCH$_2$C*H*=CH$_2$), 5.84−5.82 (br s, 1 H, NH), 5.26 (d, J = 17.2 Hz, 1 H, OCH$_2$CH=C*H*H), 5.17 (d, J = 10.3 Hz, 1 H, OCH$_2$CH=CH*H*), 4.90 (d, J = 8.2 Hz, 1 H, H-1), 4.58 (ABq, J = 12.0 Hz, 2 H, OC*H*$_2$Ph), 4.25 (dd, J = 12.6, 5.6 Hz, 1 H, OC*H*HCH=CH$_2$), 4.18 (dd, J = 12.6, 5.8 Hz, 1 H, OCH*H*CH=CH$_2$), 3.95 (dd, J = 10.2, 8.3 Hz, 1 H, H-3), 3.82−3.72 (m, 3 H, H-6a, OC*H*$_2$CH$_2$CH$_3$), 3.58 (br m, 2 H, H-4, H-6b), 3.41 (ddd, J = 9.6, 6.9, 6.9 Hz, 1 H, H-5), 3.15 (ddd, J = 10.1, 8.04, 8.04 Hz, 1 H, H-2), 3.10 (br s, 1 H, OH), 1.98 (s, 3 H, C*H*$_3$CO), 1.58 (dq, J = 7.4, 7.2 Hz, 2 H, OCH$_2$C*H*$_2$CH$_3$), 0.88 (t, J = 7.4 Hz, 3 H, OCH$_2$-CH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.51, 137.72, 134.99, 128.44, 127.80, 127.74, 117.21, 99.56, 80.13, 73.66, 73.56, 73.35, 73.26, 71.35, 70.65, 57.58, 23.62, 22.74, 10.34; HRMS (LSIMS$^+$) *m/z* calcd for $C_{21}H_{31}O_6N + Na^+$ 416.2049, found 416.2032.

3-(2,3,4,6-Tetra-*O*-acetyl-D-galactopyranosyl)-1,5-dihydro-2,4,3-benzodioxaphosphepin (12). At room temperature, *N*,*N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine (**10**) (370 μL, 1.72 mmol) was added to a solution of D-galactose tetraacetate (300 mg, 0.862 mmol) and 1*H*-tetrazole (241 mg, 3.45 mmol) in dry THF (3.0 mL) under an argon atmosphere. After the reaction mixture was stirred for 2.5 h, CH_2Cl_2 (20 mL) and saturated $NaHCO_3$ (20 mL) were added. The organic layer was separated, washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography (silica, CH_2Cl_2:MeOH:Et_3N, 125:1:0.25) gave phosphite **12** (397 mg, 92%) as a mixture of α,β-anomers as well as fractions containing pure α-anomer.

For α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25−7.18 (m, 4 H, Ar*H*), 5.81−5.76 (m, 2 H, H-1, OC*H*HAr), 5.70 (dd, J = 13.4, 10.8 Hz, 1 H, OC*H*HAr), 5.49 (br d, J = 2.5 Hz, 1 H, H-4), 5.40 (dd, J = 10.8, 3.3 Hz, 1 H, H-3), 5.22 (dd, J = 10.8, 3.5 Hz, 1 H, H-2), 4.65 (dd, J = 13.4, 9.7 Hz, 1 H, OC*H*HAr), 4.57 (dd, J = 13.4, 9.9 Hz, 1 H, OC*H*HAr), 4.48 (br t, J = 6.4 Hz, 1 H, H-5), 4.14 (dd, J = 11.3, 6.4 Hz, 1 H, H-6a), 4.08 (dd, J = 11.3, 6.9 Hz, 1 H, H-6b), 2.13 (s, 3 H, C*H*$_3$CO), 2.06 (s, 3 H, C*H*$_3$CO), 1.99 (s, 3 H, C*H*$_3$CO), 1.98 (s, 3 H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.38, 170.17, 170.14, 170.06, 137.74 (d, J = 4.0 Hz), 128.40 (d, J = 2.0 Hz), 128.20, 91.48 (d, J = 21.6 Hz), 67.94 (d, J = 4.0 Hz), 67.74, 67.50, 67.41, 64.89 (d, J = 150 Hz), 61.52, 20.67, 20.62; ^{31}P NMR ($CDCl_3$, 162 MHz) δ 131.98; HRMS (LSIMS$^+$) *m/z* calcd for $C_{22}H_{27}O_{12}P + Cs^+$ 647.0294, found 647.0271.

Propyl 2-*N*-Acetamido-3-*O*-allyl-6-*O*-benzyl-2-*O*-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranoside (13). At room temperature, TMSOTf (22.0 μL, 0.112 mmol) was added dropwise to a solution of alcohol **11** (147 mg, 0.375 mmol) and phosphite **12** (224 mg, 0.45 mmol) in dry CH_2Cl_2 (1.2 mL) over a period of 1.5 h. The reaction mixture was stirred at room temperature for 18 h and CH_2Cl_2 (20 mL) was then added. The resulting solution was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (silica, CH_2Cl_2:MeOH, gradient, 100:1 to 80:1) gave disaccharide **13** (103 mg, 38%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.25 (m, 5 H, ArH), 6.11 (d, J = 8.4 Hz, 1 H, NH), 5.85 (dddd, J = 17.2, 10.3, 5.8, 5.6 Hz, 1 H, OCH$_2$C*H*=CH$_2$), 5.32 (d, J = 2.8 Hz, 1 H, H-4′), 5.22 (dd, J = 17.2 , 1.6 Hz, 1 H, OCH$_2$CH=C*H*H), 5.15−5.05 (M, 2 H, H-2′, OCH$_2$CH=CH*H*), 4.90 (dd, J = 10.5, 3.4 Hz, 1 H, H-3′), 4.72 (d, J = 5.7 Hz, 1 H, H-1′), 4.65 (d, J = 12.0 Hz, 1 H, OC*H*HPh), 4.50−4.46 (m, 2 H, H-1, OCH*H*Ph), 4.20−4.05 (m, 4 H), 3.90−3.55 (m, 8 H), 3.35 (dt, J = 9.4, 6.8 Hz, 1 H), 2.12 (s, 3 H, CH_3CO), 2.02−1.96 (4 × s, 4 × COC*H*$_3$), 1.55 (sextet, J = 7.0 Hz, OCH$_2$C*H*$_2$CH$_3$), 0.87 (t, J = 7.0 Hz, 3 H, OCH$_2$CH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.29, 170.18, 170.01, 169.89, 137.98, 134.87, 128.48, 127.91, 116.58, 99.76, 99.73, 76.69, 75.28, 74.10, 73.50, 72.03, 70.99, 70.60, 70.56, 69.17, 68.85, 66.82, 60.93, 53.15, 23.38, 22.72, 20.83, 20.65, 20.55, 10.43; HRMS (LSIMS$^+$) *m/z* calcd for $C_{35}H_{49}NO_{15} + Cs^+$ 856.2157, found 856.2183.

Propyl 2-Acetamido-3-*O*-(2-hydroxyethyl)-6-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranoside (14). At −78 °C, ozone was bubbled through a solution of olefin **13** (325 mg, 0.449 mmol) in CH_2Cl_2 (20 mL) until a blue color persisted. This solution was kept at −78 °C for an additional 5 min and then purged with O_2 to remove the excess O_3. Methyl sulfide (0.42 mL) was added, and the resulting mixture was kept at −78 °C for 1 h and room temperature for 1 h. The mixture was then concentrated *in vacuo* and the residue was dissolved in EtOH (3 mL). $NaBH_4$ (8.2 mg, 0.216 mmol) in H_2O (0.3 mL) was added to the solution and the mixture was stirred at room temperature. After 20 min, 1 N HCl (0.3 mL) was added and the resulting mixture was stirred for an additional 5 min. Concentration *in vacuo* and purification by flash chromatography (silica, CH_2Cl_2:MeOH, 35:1) gave alcohol **14** (225 mg, 69%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.25 (m, 5 H, ArH), 6.05 (d, J = 8.1 Hz, 1 H, NH), 5.20 (d, J = 2.4 Hz, 1 H, H-4′), 5.10 (dd, J = 10.4, 8.1 Hz, 1 H, H-2′), 4.84 (dd, J = 10.4, 3.4 Hz, 1 H, H-3′), 4.79 (d, J = 6.4 Hz, 1 H, H-1), 4.70 (d, J = 12 Hz, 1 H, OC*H*HPh), 4.48 (d, J = 8.0 Hz, 1 H, H-1′), 4.46 (d, J = 12.0 Hz, 1 H, OC*H*HPh), 4.16 (dd, J = 11.3, 6.4 Hz, 1 H, H-6′a), 4.08 (dd, J = 11.3, 7.0 Hz, 1 H, H-6′b), 3.90−3.34 (m, 13 H), 3.18 (br s, 1 H, OH), 2.13 (s, 3 H, C*H*$_3$CO), 2.03 (s, 3 H, C*H*$_3$CO), 1.99 (s, 6 H, 2 × C*H*$_3$CO), 1.96 (s, 3 H, C*H*$_3$CO), 1.56 (sextet, J = 7.4 Hz, 2 H, $OCH_2CH_2CH_3$), 0.87 (t, J = 7.4 Hz, 3 H, C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.60, 170.30, 170.00, 169.44, 137.75, 128.56, 128.05, 128.00, 127.81, 99.81, 99.53, 77.66, 75.12, 74.11, 73.84, 73.55, 71.28, 70.80, 70.64, 69.10, 68.23, 66.72, 61.66, 60.79, 23.37, 22.64, 20.74, 20.64, 20.54, 10.36; HRMS (LSIMS$^+$) *m/z* calcd for $C_{34}H_{49}NO_{16}$ + Cs^+ 860.2106 found 860.2126.

Propyl 2-Acetamido-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-6-*O*-benzyl-3-*O*-(2-(*N*-(1,3,4,5-tetra-*O*-benzyl-*β*-L-homofuconojirimycinyl))ethyl)-α-D-glucopyranoside (15). A solution of alcohol **14** (115 mg, 0.158 mmol) and diisopropylethylamine (0.2 mL, 1.14 mmol) in dry CH_2Cl_2 (2.0 mL) was cooled to −20 °C. Tf_2O (35 μL, 0.205 mmol) was added slowly, and the resulting mixture was kept at −20 °C for 5 h. An additional portion of Tf_2O (10 μL, 0.06 mml) was then added, and the reaction mixture was stirred at −20 °C for an additional 20 min. Aza sugar **9** (56 mg, 0.104 mmol) in CH_2Cl_2 (0.25 mL) was added dropwise, and the resulting mixture was allowed to warm to room temperature. After the reaction mixture was stirred for 20 h, CH_2Cl_2 (10 mL), 0.5 N NaOH (2 mL), and saturated $NaHCO_3$ (10 mL) were added. The organic layer was separated and washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography gave **15** (81 mg, 63%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.08 (m, 25 H, Ar*H*), 6.25 (d, J = 3.1 Hz, 1 H), 5.05 (dd, J = 10.2, 8.2 Hz, 1 H), 4.94 (d, J = 8.2 Hz, 1 H), 4.81−4.40 (m, 12 H), 4.30−3.24 (m, 23 H), 2.30 (s, 3 H), 2.12 (s, 3 H), 2.02 (s, 3 H), 1.96 (s, 3 H), 1.95 (s, 3 H), 1.58−1.50 (m, 2 H), 1.27 (d, J = 6.2 Hz, 3 H), 0.82 (t, J = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.32, 170.12, 168.66, 165.96, 137.83, 137.64, 137.27, 137.01, 128.62, 128.58, 128.47, 128.42, 128.37, 128.16, 128.12, 128.01, 127.95, 127.84, 127.67, 99.69, 99.51, 80.79, 77.48, 77.20, 76.12, 75.33, 74.86, 73.78, 73.56, 73.33, 73.04, 71.96, 71.69, 71.01, 70.45, 69.47, 69.05, 67.62, 67.33, 66.85, 63.78, 62.15, 61.59, 60.81, 29.68, 22.81, 20.67, 20.62, 20.57, 16.87, 14.00, 10.40; HRMS (LSIMS$^+$) *m/z* calcd for $C_{69}H_{86}N_2O_{19}$ + H^+ 1347.5903, found 1347.5950.

Propyl 2-Acetamido-2-deoxy-4-*O*-(*β*-D-galactopyranosyl)-3-*O*-(2-(*N*-(*β*-L-homofuconojirimycinyl))ethyl)-α-D-glucopyranoside (2). To a solution of compound **15** (17 mg, 13.6 μmol) in dry MeOH (2.0 mL) was added NaOMe (30 μL of a 0.5 M solution in MeOH, 15 μmol). The resulting mixture was stirred at room temperature for 2.5 h and then concentrated to give a glassy residue: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.20 (m, 25 H, Ar*H*), 4.65−4.35 (m, 12 H), 4.15−3.16 (m, 32 H), 1.89 (s, 3 H), 1.43−1.38 (m, 2 H), 1.18 (d, 3 H, J = 6.8 Hz), 0.75 (t, 3 H, J = 7.2 Hz); HRMS (LSIMS$^+$) *m/z* calcd for $C_{61}H_{78}N_2O_{15}$ + H^+ 1079.5480, found 1079.5526.

The residue obtained above was dissolved in a solution of MeOH: AcOH (2:0.75 mL), and then $Pd(OH)_2$/C (Degussa type, 16 mg) was added under an argon atmosphere. The mixture was hydrogenated at 1 atm for 18 h. Filtration through a pad of Celite and concentration *in vacuo* gave a residue. Filtration through a Bio-gel P-2 column gave **2** (12 mg, 91%) as a glassy solid: ^{1}H NMR (D_2O, 400 MHz) δ 4.50−4.20 (m, 4 H), 4.02−3.45 (m, 21 H), 3.20−3.05 (m, 2 H), 2.15 (s, 3 H), 1.65 (sextet, J = 7.1 Hz, 2 H), 1.39 (d, J = 6.7 Hz, 3 H), 0.90 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (D_2O, 100 MHz) δ 175.91, 105.30, 101.25, 78.35, 77.85, 76.28, 75.47, 74.93, 73.62, 72.26, 71.09, 67.88, 63.68, 63.60, 63.29, 62.50, 61.86, 60.58, 59.86, 57.60, 57.32, 57.01, 24.56, 22.56, 16.45, 12.01.

Inhibition Studies: A. α-1,3-fucosyltransferase V. Soluble FucT V was expressed in the filamentous fungus, *Aspergillus niger* var. *awamori*. The strain was chromosomally integrated with a gene construct containing the FucT V catalytic domain[7i] fused to the glucoamylase promoter and coding region.[45] FucT V was purified from fungal supernatants by 20−60% ammonium sulfate precipitation and phenyl sepharose column chromatography. Approximately 300 units of FucT V can be prepared per liter of fermentation solution (1 unit of enzyme will consume 1 μmol of GDP-Fuc per minute). The enzyme was stored at −20 °C in 150 mM NaCl, 50 mM MOPS, pH 7.0, 10 mM $MnCl_2$, and 50% glycerol.

The activity of FucT V was detected by the assay described previously.[46] The assay pH was varied from 6 to 8. The pH optimum was found to be 6.2. All assays contained 10 mM $MnCl_2$, 10 mM ATP, 2.1 munit of purified FucT V (except where noted), and 25 mM cacodylate buffer (pH 6.2) in a total assay volume of 0.05 mL. Assays were performed at 25 °C. Reactions were halted with the addition of 0.5 mL of distilled, deionized water. GDP-fucose was separated from the product, Lewis x, with a 1.0 mL Dowex1-X8 pipet column. The reaction mixtures were applied to the column and washed with 0.3 mL distilled, deionized water three times. The flow through and the column washes were collected in 10 mL of ScintiVerse I scintillation cocktail. Control reactions, without enzyme, were used to establish the background, nonenzymatic cleavage rate. A typical control reaction of 32 672 cpm guanosine-5′-diphospho-L-[U-^{14}C]fucose would result in 173 cpm of nonenzymatic column flow through.

An initial experiment with 24 mM LacNAc and 0.05 mM GDP-Fuc (0.67 Ci/mol) was used to define the time course of the reaction. Aliquots (0.05 mL) of the 0.25 mL enzymatic reaction were taken at 60, 90, 155, and 330 min. Time points at 1 h or less were judged to be in the linear portion of the velocity−time profile. All subsequent data was obtained with 1 h of reaction time. Kinetic parameters for α-1,3-fucosyltransferase V were in agreement with the literature values. Data was subjected to nonlinear least squares fit of the Michaelis−Menton equation with the Hypero FORTRAN program of Cleland.[22] Concentrations of LacNAc were 5−80 mM at 0.05 mM GDP-Fuc. The K_m of LacNAc was determined to be 31.1 ± 6.2 mM while the literature value is 35 mM.[8,9] The $1/V_{max}$ was determined to be 0.0241. The apparent K_m of GDP-Fuc was determined at 24 mM LacNAc and 0.025−0.4 mM GDP-Fuc to be 0.078 ± 0.032 mM. Initial K_i values were obtained with Dixon analysis at 24 mM LacNAc and 0.05 mM GDP-Fucose. GDP was evaluated at 0, 0.05, 0.25 mM with 24 mM LacNAc and 0.025, 0.050, 0.10, 0.20, and 0.40 mM GDP-fucose. The K_i of Le^x was determined by Dixon analysis at 0.05 mM GDP-fucose, 24 mM LacNAc, and variable Lewis x (0, 10, 20, 30, and 40 mM). Le^x with a β-*O*-$(CH_2)_5CO_2CH_3$[23] at the reducing end (K_i = 0.125 ± 0.0142 mM) was used to determine the mode of inhibition of Le^x at 0, 0.4, and 1.0 mM with 0.05 mM GDP-Fuc. This Le^x derivative (0, 0.2, 0.4 mmol) was analyzed at 20 mM LacNAc and at variable GDP-Fuc concentrations (0.025, 0.050, 0.10, and 0.20 mmol) and it did not inhibit bovine β-1,4-galactosyltransferase. Compound **1** was evaluated at 0, 5, 20, 48, and 90 mM and **2** was evaluated at 0, 1, 2, 3, 6, and 15 mM by Dixon analysis at 0.05 mM GDP-fucose and 24 mM LacNAc. The mode of inhibition was established with double reciprocal plots at inhibitor levels of K_i and greater. Statistical analysis of the fit of the product inhibition data to the equations for competitive, noncompetitive, and uncompetitive inhibition was used to confirm the mode of inhibition. This was accomplished with the Compo (eq 4), Ncomp

(45) Dunn-Coleman, N. S.; Bloebaum, P.; Berka, R. M.; Bodie, E.; Robinson, N.; Armstrong, G.; Ward, M.; Przetak, M.; Carter, G. L.; LaCost, R.; Wilson, L. J.; Kodama, K. H.; Baliu, E. F.; Bower, B.; Lamsa, M.; Heinsohn, H. *Bio/Technology*, **1991**, *9*, 976.

(46) Sarnesto, A.; Köhlin, T.; Hindsgaul, O.; Vogele, K.; Blaszcyk-Thurin, M.; Thurin, J. *J. Biol. Chem.* **1992**, *267*, 2745.

(eq 5), and Uncomp (eq 6) FORTRAN nonlinear least square programs adapted for the Apple Macintosh. Compound **2** was evaluated at 0.05 mM GDP-Fuc, variable LacNAc (5, 10, 20, and 40 mM), and fixed **2** (0, 2, 4, 6, 8 mM) and evaluated with the Ncomp (eq 5) FORTRAN program. Compound **2** was also evaluated at 24 mM LacNAc, variable GDP-Fuc (0.02, 0.05, 0.1, and 0.2 mM) at fixed **2** (0, 4, 8 mM). The K_i value was derived from nonlinear, least squares fit to the kinetic equation for competitive inhibition by the Compo FORTRAN program (eq 4).

$$\text{Compo: } \upsilon = V_{max}*[S]/([S] + K_m*(1 + [I]/K_i)) \quad (4)$$

$$\text{Ncomp: } \upsilon = V_{max}*[S]/([S](1 + [I]/K_i) + K_m*(1 + [I]/K_i)) \quad (5)$$

$$\text{Uncomp: } \upsilon = V_{max}*[S]/([S](1 + [I]/K_i) + K_m*) \quad (6)$$

*apparent kinetic constants

Synergistic Inhibition. Synergistic inhibition of was initially probed by monitoring the percent inhibition of FucT V (0.05 mM GDP-Fuc, 24 mM LacNAc) with the individual inhibitors and combination of GDP and the aza sugars: **1** (33 mM), **2** (2.5 mM), GDP (0.05 mM), the combination of **1** and GDP, and the combination of **2** and GDP. Yonetani−Theorell analysis[36] was used next to address synergism of multiple inhibitors. LacNAc and GDP-Fuc were held constant at 24 and 0.05 mM, respectively. **2** was varied (0, 1, 2, 4 mM) at fixed concentrations of GDP (0, 0.05, 0.1, 0.25 mM). A steady-state derivation for the synergistic inhibition of FucT V (Scheme 4) in the absence of Lewis x was undertaken. The kinetic constants are as follows: $K_{i,GDP-fuc} = k_{-1}/k_1$, $k_{m,GDP-fuc} = k_3/k_1$, $k_{m,LacNAc} = (k_{-2} + k_3)/k_2$ and $V_{max} = k_3E_t$. The resulting equation (eq 3) is in a form similar to the equation found in the literature for inhibition by the second product released (GDP) in an ordered, sequential BiBi mechanism (eq 1). The data obtained was fitted to eq 3.

The role of the acceptor sugar in the synergistic inhibition was evaluated by monitoring FucT V activity as a function of LacNAc concentration at fixed GDP-Fuc (0.05 mM), GDP (0.03 mM), **2** (2.8, 14 mM), and the combination of GDP (0.03 mM) and **2** (2.8, 14 mM). Reactions contained 0.3 munits FucT V and were allowed to react at 25 °C for 30 min. Mono aza sugars were evaluated in a similar manner. The inhibition of the following inhibitor(s) was evaluated: no inhibitors, GDP only, aza sugar only, and the combination of GDP and an aza sugar. Compound **1**, 5-membered fucose-type aza sugar, trans 5-membered aza sugar, GDP, and GDP-fucose were held constant at 33, 80, 34, 0.05, 0.05 mM respectively. Reactions contained 0.3 munits with the LacNAc concentration varied from 5 to 40 mM.

B. α-L-Fucosidase. Inhibition studies of α-L-fucosidase were carried out on bovine epididymus α-L-fucosidase monitoring the hydrolysis of *p*-nitrophenyl α-L-fucoside in a 50 mM sodium acetate buffer (pH 6.5) at 37 °C. Liberation of *p*-nitrophenolate anion was monitored in a 1.0 mL assay over the course of 2 min at 400 nm on a Beckman DU-6 spectrophotometer. Determinations were the average of at least three measures. Enzyme kinetic parameters were calculated by nonlinear regression analysis with the Hypero program. Inhibition constants were first estimated by the method of Dixon at a K_m level of substrate.[35] The mode of inhibition of **1** was evaluated by double reciprocal analysis of data derived from monitoring the velocity as a function of *p*-nitrophenyl α-L-fucoside (0.075, 0.15, 0.30, 0.60, 1.28 mM) at fixed **1** concentrations (0, 4, 8, 16 nM). A precise inhibition constant was derived for **1** from a best fit of the initial velocity data to the kinetic equation for competitive inhibition by a nonlinear, least squares method, the Compo FORTRAN program (eq 4).

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **1**, **2**, **7**, **8**, **9**, **12**, **13**, **14**, **15**, and inhibition study of α-fucosidase (17 pages). See any current masthead page for ordering and internet access instructions.

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