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ENZYMATIC SYNTHESIS OF SIALYL Le^x AND DERIVATIVES BASED ON A RECOMBINANT FUCOSYLTRANSFERASE

David P. Dumas, Yoshitaka Ichikawa and Chi-Huey Wong*
Department of Chemistry, The Scripps Research Institute
La Jolla, CA 92037

John B. Lowe*†§ and Rajan P. Nair†
†Howard Hughes Medical Institute and §Department of Pathology,
University of Michigan Medical Center
Ann Arbor, MI 48109-0650

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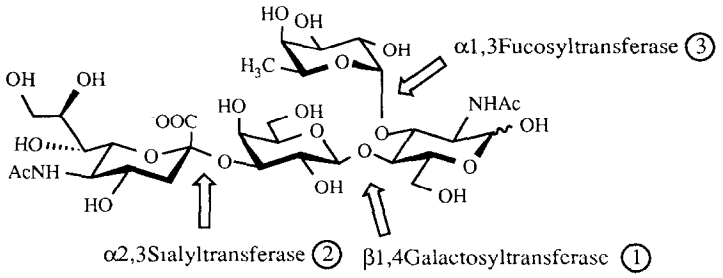
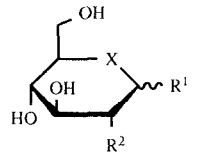
Abstract: A recombinant human Lewis α(1,3/1,4)fucosyltransferase has been studied for its acceptor substrate specificity and used in the synthesis of sialyl Le^x and derivatives.

The tetrasaccharide sialyl Lewis x (Le^x) (Neuα2,3Galβ1,4(Fucα1,3)GlcNAc) has been identified as a ligand for endothelial leukocyte adhesion molecule-1 (ELAM-1),[1] a glycoprotein synthesized in response to inflammatory agents during tissue injury to promote adhesion of neutrophils, monocytes and a subpopulation of lymphocytes.[2] Sialyl Le^x has also been found on the surface of some tumor and cancer cells. Sialyl Le^x and analogs or mimetics may therefore offer new opportunities for tumor diagnosis and for the treatment of inflammation. The biosynthetic glycosidic bond formations of sialyl Le^x have been determined, with three glycosyltransferases acting in sequence to form the molecule (see the indicated sequence).[1a]

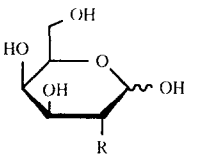
Two groups[3,4] have recently reported the total chemical synthesis of sialyl Le^x. We describe in this letter our preliminary studies on the acceptor specificity of a recombinant Lewis α(1,3/1,4)fucosyltransferase (FucT)[5] and its application to the synthesis of sialyl Le^x and derivatives. The enzyme FucT catalyzes the transfer of fucose (Fuc) from GDP-Fuc to position 3 or 4 of the GlcNAc moiety of an acceptor oligosaccharide such as β-galactosyl-N-acetylglucosamine.[6] The substrate specificity of this enzyme, however, is not well understood.



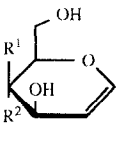
Sialyl Le^x



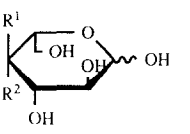
	X	R^1	R^2
11	O	OH	OH
12	O	OH	NHAc
13	O	β-OAllyl	OH
14	S	OH	OH
15	NH	H	OH



	R
16	OH
17	NHAc



	R^1	R^2
18	H	OH
19	OH	H



	R^1	R^2
20	OH	H
21	H	OH

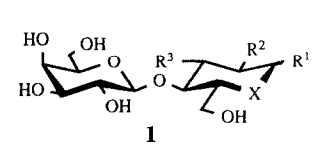
Table 1: Relative Activity of Acceptors and Inhibitors for FucT[a]

Compound	Relative Activity	Compound	Relative Activity
1a	100[b]	**11**	5
1b	IC_{50}>125 mM	**12**	3
1c	310	**13**	<1
1d	IC_{50}=40 mM	**14**	7
1e	120	**15**	5
3	IC_{50}>125 mM	**16**	6
4	580	**17**	4
5	23	**18**	7
6	27	**19**	2
7	60	**20**	3
9	250	**21**	7
10	13		

[a]**Conditions:** The assay procedure was essentially the same as described previously[5] with some minor modifications. A stock mixture containing 0.25 mM ^{14}C-GDP-fucose (5000 cpm/μL), 6.25 mM ATP, 25 mM $MnCl_2$, and 62.5 mM sodium cacodylate buffer, pH 6.2 was mixed fresh the day of use and stored on ice. To this solution, FucT was added immediately before use, and the reaction was initiated by combination of 16 μL of this mixture with 4 μL of 100 mM acceptor substrate and incubated at 37°C for 30 to 240 min depending upon the acceptor under study. Separate assays in the absence of acceptor were used to correct for background hydrolysis of GDP-fucose. Upon completion of incubation, 400 μL of a 25% (v/v) suspension of QAE-Sephadex was added. These suspensions were gently mixed at room temperature for 10 min before centrifugation at 13,000 rpm for 1 min. From the supernatant fluid, 200 μL was extracted and mixed with 10 mL scintillation cocktail. The radioactive content was measured on a scintillation counter. Care was taken to be sure that less than 10% of the enzymatic reaction had taken place over the incubation period. Compounds **1b-1d** and **3** were prepared as previously described (Wong, C.-H.; Ichikawa, Y.; Krach, T.; Gautheron, C.; Dumas, D.; Look, G. *J. Am Chem Soc.*, in press). Compounds **1a, 1e, 4-7, 9** and **10** are commercially available. Inhibition was measured in an analogous fashion in the presence of 2 mM **1a**, 0.2 mM GDP-fucose, and varying concentrations of inhibitor.

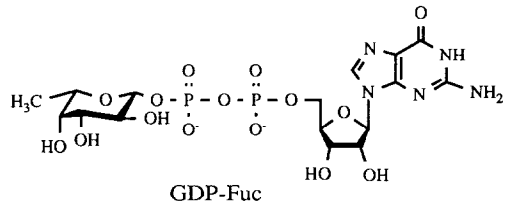
[b] 2 U/mg (1U = 1 μmol of GDP-Fuc consumed per hr).

Scheme 1. Disaccharides and derivatives as acceptor substrates or inhibitors for FucT.



1

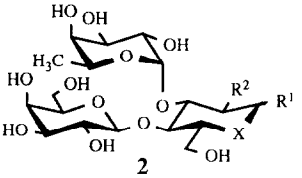
	X	R^1	R^2	R^3
a	O	OH	NHAc	OH
b	O	β-OAllyl	NHAc	H
c	S	OH	OH	OH
d	NH	H	OH	OH
e	O	OH	OH	OH



GDP-Fuc

1a, 1c, 1e — Fuc T → GDP

2a, 2c, 2e



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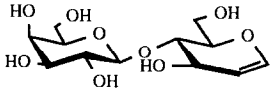

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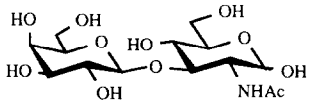

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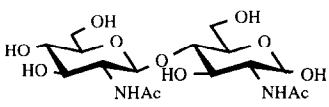

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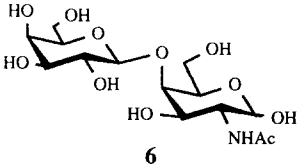
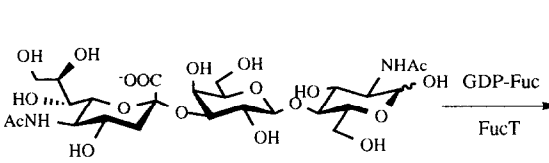


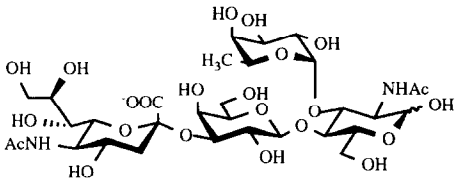
6

Scheme 2. Trisaccharides as acceptor substrates for FucT.

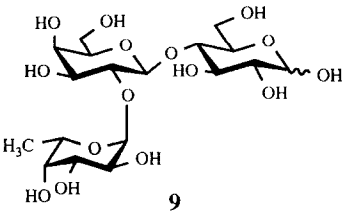


7

GDP-Fuc, FucT



8 Sialyl Le^x



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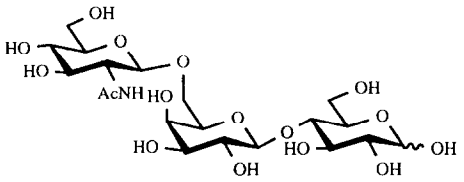

10

We first examined several disaccharides (β-galactosides) as acceptor substrates for FucT (Scheme 1). As indicated in Table 1, the enzyme accepts the galactosides of GlcNAc with β1,3- or β1,4-linkages as good substrates. The β1,3-linked disaccharide **4** and 5-thio-N-acetyllactosamine **1c** are better substrates than N-acetyllactosamine **1a** (rel rates, 580% and 310% compared to **1a**). Lactal **3** and the β-O-allylglycoside **1b**, however, are not acceptable. Compound **1a** is an inhibitor of FucT with an IC_{50} ~40 mM. For trisaccharides (Scheme 2), 3'-sialyl N-acetyllactosamine **7** and 2'-fucosyllactose **9** are good substrates (rel rates, 60% and 250% compared to compound **1a,** respectively). Compound **10** is a weak substrate. All the monosaccharides and derivatives examined, however, are poor substrates for the enzyme.

With this specificity information in hand, we have prepared three fucosyl oligosaccharides including Le^x (**2a**), sialyl Le^x and 3-fucosyl-5-thiolactose (**2c**) on 10-50 mg scales.[6] Work is in progress to develop the practical synthesis of fucosyl oligosaccharides with in situ regeneration of GDP-Fuc.

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1. (a) Lowe, J.B.; Stoolman, L M.; Nair, R.P.; Larsen, R.D.; Berhend, T.L.; Marks, R.M. *Cell* **1990**, *63*, 475 (b) Phillips, M.L.; Nudelman, E.; Gaeta, F.C.A.; Perez, M.; Singhal, A.K.; Hakomori, S.I.; Paulson, J.C. *Science* **1990**, *250*, 1130. (c) Tiemeyer, M.; Swiedler, S. J.; Ishihara, M.; Moreland, M.; Schweingruber, H.; Hirtzer, P.; Brandley, B. K. *Proc Natl Acad Sci USA* **1991**, *88*, 1138. (d) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
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6. For a representative synthesis of **2b**, a solution of **1c** (3 mg, 8.4 μmol), GDP-Fuc (6 mg, 8.4 μmol) and FucT (0.05 U) in Na cacodylate buffer (540 μL; 50 mM, pH 6.2) containing 5 mM ATP and 20 mM $MnCl_2$ was stirred for 2 days at room temperature. The R_f values of **1c** and **2c** were 0.39 and 0.31, respectively, in EtOAc/AcOH/H_2O 3:2:1 on silica TLC. The reaction mixture was applied directly to a column of Sephadex G-25 Superfine (1.5 x 30 cm), and eluted with water. The fractions containing the product were pooled and successively passed through columns of QAE-Sephadex and Dowex 50-X8 [H^+] with water. The effluent was pooled and lyophilized. ^1H NMR (D_2O, 20°C)δ 1.13 (3H, d, J=6.7 Hz, 6-CH_3 of Fuc), 3.40 (1H, dd, J=6.4 and 11.7 Hz), 3.60 (1H, dd, J=3.6 and 11.7 Hz), 4.52 (1H, d, J=7.9 Hz), 4.95 (1H, J=2.6Hz), 5.34 (1H, d, J=3.8 Hz). Le^x and sialyl Le^x were prepared similarly. The physical data (^1H- and ^{13}C-NMR) are the same as reported.[4]