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ENZYMATIC FUCOSYLATIONS OF NON-NATURAL ACCEPTORS WITH NON-NATURAL DONOR-SUGARS

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ABSTRACT

Non-natural sialyl-lactosamine derivatives are treated with recombinant fucosyl-transferase VI in the presence of non-natural guanosine diphosphate-activated donor sugars. The biocatalyst transfers the non-natural donor sugars with the same regio- and stereospecificity as the parent fucose to the non-natural acceptors yielding sialyl-Le^x-oligosaccharide libraries.

Oligosaccharides play a key role in adhesion and cell-cell recognition phenomena[1]. Therefore, there is a need for an efficient and predictive production of a wide variety of oligosaccharides[2] to investigate disorders based on those carbohydrate-protein interactions[3,4,5].

Although the chemical synthesis of carbohydrates is well developed, the preparation of a particular oligosacharide still remains a cumbersome challenge[6]. In nature highly regio- and stereospecific glycosylations are catalyzed by glycosyl-transferases, which sequentially transfer a monosaccharide unit from a nucleotide-activated donor sugar to a growing oligosaccharide chain[7]. A combined use of chemical and enzymatic methods *in vitro*[8], therefore, offers the opportunity of a rapid and unambiguous assemblage of carbohydrate libraries.

We could recently show that galactosyl-transferase[9], recombinant α(2-3)sialyl-transferase[10] and fucosyl-transferase VI[11] can be successfully used to glycosylate an array of non-natural acceptors very efficiently. It has also been shown that Fuc-T's (virtually a mixture of two enzymes) isolated from human milk transfer modified fucose donors to their natural N-acetyl lactosamine acceptor[12,13]. A convenient procedure for the preparation of a large number of purine-diphosphate activated donors[14]

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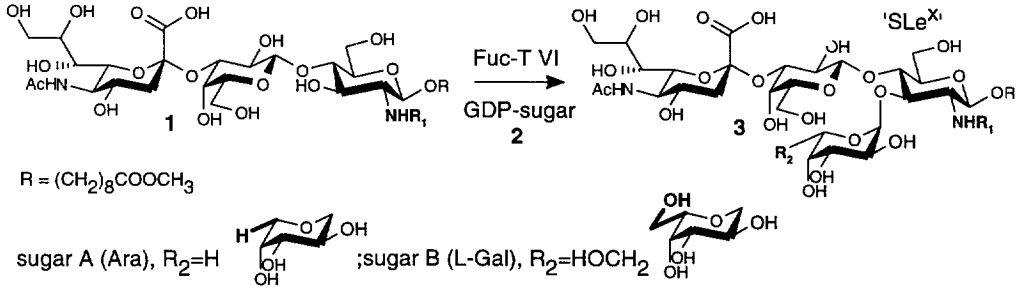
allowed us to investigate the preparative usefulness of recombinant, well-characterized Fuc-T VI (EMBL accession no. LO 1698)[15]. This enzyme transfers a fucose or a fucose-analog to the 3-OH-group of N-acetyl lactosamine or its sialylated congener **1** (see scheme) in an α-mode[16].



Scheme: Enzymatic fucosylations with recombinant fucosyl-transferase VI.

We incubated[17] Fuc-T VI with a series of non-natural sialylated N-acyl lactosamine derivatives **1**[10,19] and non-natural donors **2** (see scheme and table) in order to obtain sialyl-Lewis^x compounds. All the isolated saccharides **3** contain an additional α-linked sugar. Extensive ^{1}H NMR and ^{13}C NMR measurements proved further that all these sugars are attached to the 3-OH group of the N-acylglucosamine moiety in the same way as fucose (R_1 = Ac, R_2 = CH_3) in the parent sialyl-Lewis^x tetrasaccharide despite the high steric demand of R1 next to the 'fucosylation'-site in some of the examples. All compounds have been prepared on a preparative scale and gave correct MS-spectra. The chemical shifts of some reporter signals (all measurements in CD_3OD-D_2O with D_2O as internal standard) are compiled in the table confirming the structural integrity of the novel tetrasaccharides.

In conclusion our studies render Fuc-T VI a valuable biocatalyst. The enzyme can be used to rapidly assemble sialyl-Lewis^x libraries, giving sialyl-Lewis^x congeners altered at two positions simultaneously. The sugars are probed in high through-put screens in the selectin area[18,19]

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17) **Representative incubation procedure**: 10.5 mg (11.0 µmol) of sialylated trisaccharide **1** (entry 7), 11.3 mg (18.0 µmol) guanosine-diphosphate arabinose and 1.8 mg bovine serum albumine (Boehringer) are added to a mixture of 830 µl bidistilled water, 100 µl of a 250 mM $MnCl_2$-solution and 470 µl (250 mM) sodium cacodylate-buffer (pH = 6.52). The slightly turbid mixture is then incubated with 30 U (2 µl) calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/498 µl) and 0.8 U (40 µl) of a Fuc-T VI solution at 37° C for 48 h. The mixture is then passed over a short C-18 reversed phase column, washed with water and eluted with methanol and then lyophilized from water. The residue is finally purified on silica gel (eluent: dichloromethane - methanol - water mixtures). The product-containing fractions are combined, evaporated to dryness and lyophilized from dioxane - water to give 10.0 mg (81%) of the tetrasaccharide **3** (entry 7) as a white, fluffy powder.

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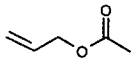
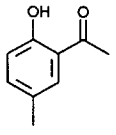
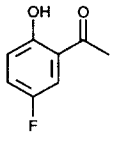
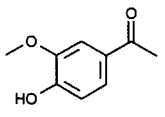
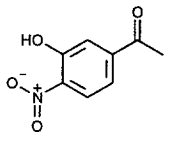
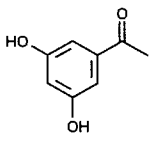
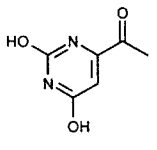
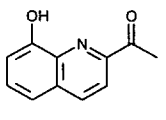
entry	R1	sugar	%(mg) of 3	sugar H-1*	GlcNac C-1	Gal C-1	Sia C-2	sugar C-1	other C
1		L-Gal	66(18.0)	5.11	102.8	104.0	100.9	100.4	117.4
2		Ara	66(8.0)	5.10	103.6	103.9	101.9	100.8	119.9
3		L-Gal	29(13.0)	5.08	102.1	104.0	100.9	100.1	164.5
4		Ara	54(6.0)	5.06	102.6	103.8	100.9	99.9	20.6
5		L-Gal	82(14.0)	5.08	102.5	104.1	100.9	100.0	20.6
6		Ara	46(8.0)	5.08	102.6	103.8	100.8	100.0	114.5
7		Ara	81(10.0)	5.10	102.6	103.8	100.9	99.9	151.7
8		L-Gal	68(8.0)	5.04	102.6	104.1	100.9	99.9	151.6
9		L-Gal	77(6.0)	5.04	102.4	104.1	100.9	100.2	142.3
10		Ara	87(11.0)	5.08	102.5	103.8	100.9	99.9	106.9
11		L-Gal	70(9.0)	5.04	102.4	104.1	100.9	99.9	107.0
12		L-Gal	53(7.0)	5.03	101.1	104.1	101.0	100.5	112.2
13		Ara	62(8.0)	5.02	101.8	103.3	100.4	99.7	112.3
14		L-Gal	24(5.0)	5.13	102.9	104.1	100.9	100.0	113.8

Table: Yields and NMR-data[20] of tetrasaccharides **3**; * doublet (J ≈ 4.3 Hz).

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