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ENZYMATIC SYNTHESIS OF SIALYL-LEWIS^a-LIBRARIES WITH TWO NON-NATURAL MONOSACCHARIDE UNITS

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ABSTRACT

A series of sialylated type-I sugars, which have the natural N-acetyl group of the glucosamine moiety replaced by a wide range of amides, is incubated with recombinant fucosyl-transferase III and non-natural guanosine-diphosphate activated donor-sugars. Surprisingly, the enzyme tolerates the simultaneous alterations on the donor and acceptor to form a wide array of sialyl-Lewis^a-analogues.

It is now generally accepted that carbohydrates are an integrative part of various glycoconjugates and are relevant in a number of biologically important recognition and adhesion events[1,2]. Recent interest has been focused mainly on selectin carbohydrate interactions[3] and on the search for biologically more effective carbohydrate analogues than the natural sialyl-Lewis^x or sialyl-Lewis^a-tetrasaccharides[4]. Both tetrasaccharides contain an α-linked fucose unit. Fucosylated structures have also been found to participate in a number of other physiological and pathological processes in mammalian and human tissues[5]. This is substantiated by the large number of fucosyl-transferases encoded by the human genome[6]. *In vivo* they transfer a fucose unit from guanosine-diphosphate activated fucose onto a growing oligosaccharide chain.

In an ongoing program to investigate the application of carbohydrate-based drugs[7] we explore the synthetic value of recombinant fucosyl-transferases as preparative tools[8,9]. So we could recently show that fuc-t III and fuc-t VI unexpectedly recognize a wide range of GDP-fuc analogues as substrates, in which either the guanosine moiety[10] or the fucose part[11] have been replaced by non-natural structures. Both transferases also tolerate non-natural acceptors, in which the natural N-acetyl group of the glcNAc-moiety in type-I and type-II sugars can be replaced by various N-acyl groups[12,13]. Furthermore, we could show that even a combination of non-natural donors and non-natural acceptors is recognized by recombinant fuc-t VI to produce sLe^x-libraries[14]. Here we wish to report our findings concerning the preparative use of recombinant fuc-t III[15] (EMBL accession

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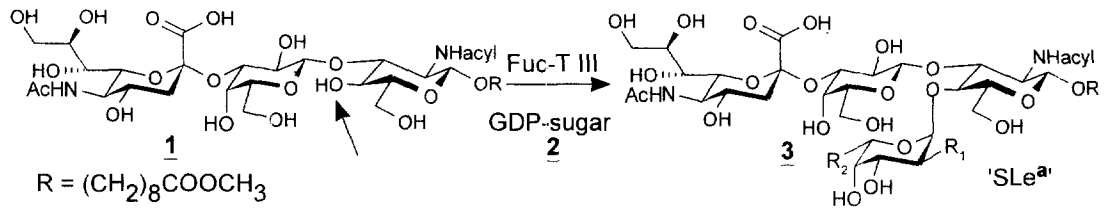
no. X53578). *In vivo* this enzyme transfers a fucose unit from GDP-fuc onto the 4-OH group of the glcNAc-moiety of a sialylated or non-sialylated type-I sugar in an α-mode (see scheme) to form Le^a- or sLe^a-oligosaccharides, respectively.

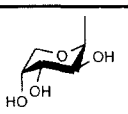
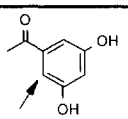
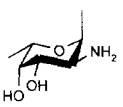
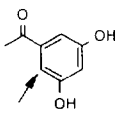
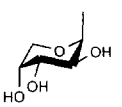
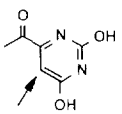
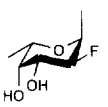
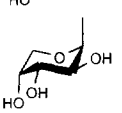
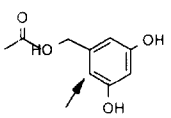
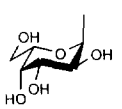
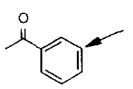
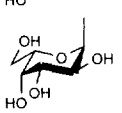
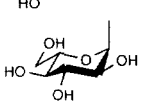
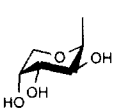
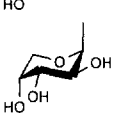
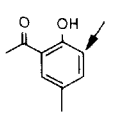
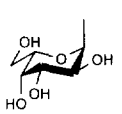
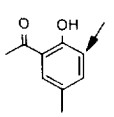
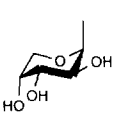


Scheme: Enzymatic transfer of fucose analogues.

Fuc-t III is quite flexible with respect to the GDP-sugar[11]. Consequently, we incubated the enzyme with non-natural donors **2**[16] together with non-natural acceptor-trisaccharides **1**[13]. As observed with recombinant fuc-t VI[14], also fuc-t III tolerates this combination and transfers fucose-analogues from the GDP-activated donors **2** onto the non-natural acceptors **1** in a preparative useful way[17] (compare table). Thus a large number of sLe^a-analogues **3** are obtained. They have e.g. the parent N-acetyl group of the glcNAc-unit replaced by a formamide (entry 4), carbamates (5, 7, 8, 15) or a thiocarbamate (13). The N-acetyl group may even be exchanged by bulky aromatics (1, 2, 5, 6, 10, 11) or heteroaromatics (3). Even polar or charged residues are tolerated at this position (12, 14) or the total replacement by a sulfonamide residue (9). In all cases non-natural sugar units, like D-arabinose (1, 3, 5, 9, 10, 12 - 14) - missing the 6-methyl group of the natural fucose -, L-galactose (6, 7, 11, 15) - having an additional OH-group at the C-6-atom - or its 4-epimer, L-glucose (8), or 2-amino- or 2-fluoro-fucose (2, 4) have been transferred from their respective GDP-activated donors. 'Pseudo-fucosylation' takes place exclusively at the 4-OH-group in an α-mode (see scheme). This is confirmed by ^{1}H NMR- and ^{13}C NMR-data of the isolated tetrasaccharides **3** (confer table for selected signals of reporter groups). The shifts correlate well with literature data for the parent tetrasaccharide[18]. The H-1 shifts of all fucose-substitutes appear at about 5 ppm (doublet, J ~ 4 - 4.5 Hz) which is consistent with an α-linked fucose. This linkage is corroborated by the respective shifts of the C-1atoms at about 100 ppm in the ^{13}NMR-spectra[18]. In addition, the transfer to the 4-OH-group of the glcNacyl-moiety is substantiated by a significant up-field shift of the C-3 atom from about 84 ppm to about 77 ppm when glycosylated at the 4-OH-group (compare also data in lit.[12, 19]).

In conclusion our investigations demonstrate the high promiscuity of recombinant fuc-t III with respect to a simultaneous conversion of donor- and acceptor-substrates. Despite these 'double-sided' alterations the enzyme reliably transfers the donor-sugar exclusively onto the desired OH-group of the acceptor with the expected α-selectivity. So a series of non-natural sLe^a-congeners is rapidly and unambiguously produced for biological

screenings. This renders fuc-t III a valuable biocatalyst and extends the synthetic arsenal of the carbohydrate chemist significantly[20]. Further applications are in progress and will be reported in due course.

entry	sugar	acyl	% (mg)	GlcNacyl: C-1	Gal: C-1	Sia: C-3	'Fuc': H-1, C-1	others: →
1			72 (10)	101.9	103.3	41.9	5.01, 100.0	106.9
2			32 (8)	101.8	103.2	41.9	5.26, 95.5	106.8
3			84 (11)	102.1	103.9	41.6	5.06, 100.2	104.1
4			73 (11)	103.1	105.1	42.6	5.15, 97.8	211.1*
5			64 (9)	102.2	103.3	42.1	4.98, 100.2	107.7
6			87 (18)	101.4	104.0	41.1	5.05, 99.6	128.4
7			94 (10)	102.3	104.4	41.9	4.99, 99.9	158.9
8			48 (5)	104.4	105.1	41.6	5.09, 101.0	15.5
9			67 (12)	102.9	103.9	42.5	5.02, 100.4	43.1
10			84 (14)	102.4	103.2	41.9	5.08, 100.4	117.1
11			84 (14)	102.2	103.7	41.1	5.09, 99.5	117.3
12			100 (14)	102.2	104.3	42.6	4.96, 100.3	62.8

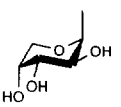
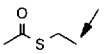
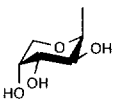
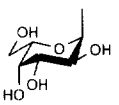
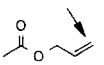
13			56 (14)	102.0	103.6	42.1	5.01, 100.3	16.7
14			96 (13)	102.2	104.9	42.5	4.98, 100.2	45.4
15			77 (27)	101.4	104.3	42.0	4.99, 99.8	117.7

Table: All measurements in D_2O-CD_3OD (400.1 MHz resp. 62.9 MHz, ref. D_2O: 4.80 ppm and CD_3OD: 49.00 ppm); *) ^{19}F NMR (376.5 MHz, ext. ref. $CFCl_3$), dd J = 22.6 Hz and 52.7 Hz.

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17) **Representative incubation procedure**: To a mixture of 600 µl of bidistilled water, 450 µl of Na-cacodylate buffer (250 mM, pH = 6.5) and 150 µl of a 250 mM $MnCl_2$-solution are added 11.9 mg (12.7 µmol) of trisaccharide **1**[13] (entry 1), 13.8 mg (27.3 µmol) of GDP-ara[16] and 1.6 mg of bovine serum albumine (Boehringer). The clear mixture is incubated at 37°C in a plastic tube with 120 µl (~ 6 U/ml) of a fuc-t III solution and 2 µl (34 U) of calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/ 498 µl). When a TLC (CH_2Cl_2 - Methanol - water mixtures ~ 10 - 4 - 0.8) shows the consumption of the starting acceptor **1**, the turbid solution is centrifuged and the clear supernatant passed over a C-18 reversed-phase column. The column is washed with water and then eluted with methanol. The methanol solution is evaporated and the residue purified over silica-gel (CH_2Cl_2 - Methanol - water mixtures as above). The product-containing fractions are combined, evaporated and lyophilized from water to give 9.7 mg (72%) of compound **3** (entry 1) as a white powder which is pure according MS-, ^1H- and ^{13}C NMR-analysis.
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