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Enzymatic Fucosylation of Non-Natural Trisaccharides with Cloned Fucosyltransferase VI

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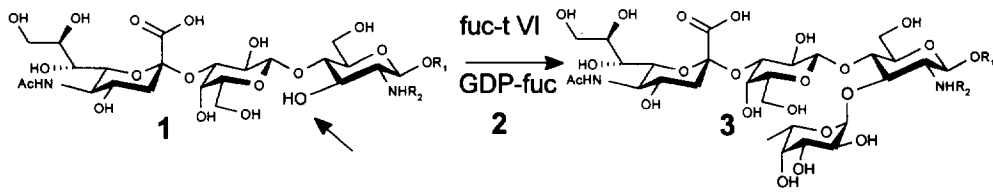
ABSTRACT

Non-natural sialyl-lactosamine derivatives are subjected to enzymatic fucosylation with cloned fucosyltransferase VI. The enzyme tolerates a wide range of acceptors, spanning from small and bulky aliphatic as well as charged and sulfonamide replacements of the natural N-acetyl residue.

The biological importance of oligosaccharides in adhesion phenomena and cell-cell recognition involved in the development of whole organisms is well documented[1]. For the treatment of disorders based on those interactions oligosaccharide-derived drugs offer a novel therapeutic approach[2)3)4)5]. The chemical synthesis of oligosaccharides by multistep procedures is possible. A more recent and less tedious approach relies on enzymatic oligosaccharide synthesis[6].

In nature highly regio- and stereospecific glycosylations are catalyzed by glycosyltransferases [7)8]. Cloning and overexpression has added galactosyltransferase[9)10)11], α(2-3)sialyltransferase[12)13], and fucosyltransferases[14)15)16] to the synthetic tools of the chemist. However, the synthetic potential of these biocatalysts has not been fully evaluated and could be greatly enhanced if non-natural acceptors were substrates[17]. We therefore explored the scope of cloned, human fucosyltransferase VI (fuc-t)[18] (EMBL accession no. LO1698) (see scheme) for the synthesis of sialyl-Lewis^x derivatives **3**, which differ in the acyl-substituent R_2.



Scheme : Enzymatic fucosylation; R_1=$(CH_2)_8COOMe$ for present study.

Fuc-t VI transfers a fucose-unit from the donor GDP-fucose **2** onto the 3-OH group of N-Ac-lactosamine or the sialylated compound **1** (R_2=Ac) in an α-mode[16]. Although there are a limited number of acceptor studies on fucosyltransferases III-V[19)20)21)22], only one reported that minor variations of the N-acyl-group in the N-acetylglucosamine moiety are tolerated[23]. For fuc-t VI, however, no acceptor studies were available[16].

A number of sialylated trisaccharides **1** (see scheme) had been synthesized unambiguously via transferases previously[17]. As shown in the table, both small (entries 1,2,3) and large (entries 17, 18, 20) lipophilic acyl-groups are tolerated by fuc-t VI. In addition, positively and negatively charged residues (entries 5, 14, 15, 21) are accepted in the proximity of the reaction site. Surprisingly the carbonyl-oxygen can be replaced by a sulfur (entries 7, 8). The amide-functionality can even be exchanged by sulfonamides (entries 19, 20) without any adverse effects. All compounds have been prepared on a preparative scale. Their structural integrity has been proven by MS- and ^{1}H and ^{13}C NMR data. The chemical shifts of some typical groups are compiled in the table. These data are consistent with those obtained for the parent tetrasaccharide **3** (R_2 = Ac)[24)25)]. Additionally some structures **3** have been verified by extensive NMR-measurements.

In conclusion our findings render fuc-t VI a versatile and stereochemically reliable tool for the preparation of non-natural sialyl-Lewis^{x}-derivatives and will contribute to a more wide-spread use of this class of stable biocatalysts in synthesis. The pronounced ease of the synthesis of a tetrasaccharide is described below. Further evaluations are in progress and will be reported in due course.

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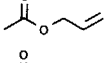
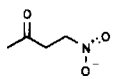
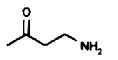
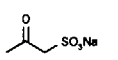
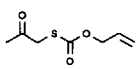
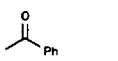
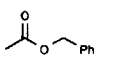
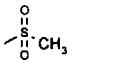
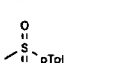
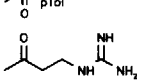
entry	R_2 (in **3**)	% (mg)	glcN: H-1*	gal: H-1*	sia:H-2eq**	fuc: H-1*, H-6*	glcN: C-1, 2, 4***	gal: 1, 3***	sia: C-2, 3***	fuc: C-1,6***	C-others*** (aglycon, NHR)
1		74 (8.2)	4.30	4.41	2.78	1.08 4.96	102.39; 56.50; 75.00	103.70; 76.17	100.61; 42.64	99.50; 16.59	70.76; 23.17
2		77 (18.4)	4.49	4.49	2.70	1.13 5.10	101.99; 55.81; 74.56	102.88; 76.20	100.92; 41.05	99.44; 16.55	71.90; 165.83
3		78 (15.2)	4.57	4.52	2.65	1.12 5.10	102.41; 53.97; 75.35	103.88; 76.27	100.88; 42.27	99.91; 16.55	70.61; 19.61
4		64 (14.8)	4.63	4.57	2.75	1.20 5.11	102.21; 56.89; 75.48	103.91; 77.27	100.88; 42.28	100.15; 16.57	70.66; 62.96
5		61 (8.5)	4.55	4.51	2.81	1.18 5.05	102.77; 57.49; 75.15	103.48; 77.23	100.42; 42.05	100.42; 16.56	70.94; 442.05
6		96 (26.1)	4.57	4.56	2.75	1.16 5.01	102.02; 57.88; 75.10	103.82; 77.29	100.89; 42.22	100.09; 16.55	70.76; 119.84
7		82 (21.2)	4.60	4.52	2.74	1.18 4.95	102.09; 62.30; 75.77	103.90; 76.50	100.86; 42.24	99.67; 16.56	70.64; 33.84
8		50 (7.3)	4.29	4.40	2.75	1.04 5.01	103.34; 61.72; 75.00	103.81; 76.75	100.88; 42.32	99.37; 16.58	70.98; 14.79
9		88 (59.9)	4.48	4.52	2.72	1.14 5.22	102.79; 53.95; 75.50	103.83; 76.71	100.87; 42.22	100.01; 16.55	70.74; 117.37
10		70 (16.2)	4.29	4.42	2.77	1.08 5.02	102.83; 59.20; 75.26	103.83; 77.17	100.87; 42.23	100.03; 16.55	70.72; 15.08
11		50 (12.6)	4.54	4.54	2.77	1.18 5.12	102.25; 58.58; 74.66	102.91; 76.26	101.00; 41.12	99.62; 16.55	70.63; 16.24
12		83 (8.1)	4.32	4.41	2.78	1.06 5.06	103.72; 60.05; 75.00	103.84; 76.71	100.88; 42.33	99.74; 16.55	70.73; 15.86

13		87 (11.1)	4.58	4.53	2.77	1.18 5.13	102.39; 57.45; 75.01	103.89; 77.32	100.90; 42.31	100.13; 16.57	70.77; 33.88
14		60 (13.6)	4.48	4.48	2.76	1.09 5.02	102.28; 56.96; 75.24	103.93; 77.35	100.88; 42.33	100.60; 16.65	70.59; 37.34
15		45 (11.3)	4.50	4.47	2.72	1.24 5.23	101.77; 57.01; 74.14	102.48; 76.06	100.51; 40.64	99.18; 16.12	69.55; 58.28
16		78 (14.3)	4.67	4.62	2.78	1.18 5.12	102.23; 57.74; 75.04	103.09; 77.23	100.81; 42.12	100.04; 16.55	71.06; 119.67
17		81 (16.5)	4.51	4.45	2.79	1.08 4.90	102.47; 58.03; 75.44	103.90; 77.30	100.89; 42.29	99.86; 16.54	70.68; 132.73
18		89 (15.4)	4.30	4.41	2.78	1.08 5.06	102.78; 59.36; 75.29	103.86; 76.75	100.86; 42.30	99.97; 16.54	70.77; 67.59
19		91 (23.0)	4.42	4.26	2.79	1.08 5.48	102.67; 61.60; 75.29	103.92; 77.18	100.86; 42.23	99.71; 16.55	71.07; 42.71
20		97 (12.0)	4.41	4.10	2.79	1.09 5.52	102.45; 61.61; 75.29	103.89; 77.12	100.89; 42.67	99.52; 16.57	70.67; 22.58
21		69 (15.5)	4.50	4.50	2.73	1.14 5.04	101.62; 56.69; 74.22	102.84; 77.00	100.53;40.64	99.46; 16.60	70.12; 37.99

* relative to D_2O (4.80 ppm), dublett each, **relative to D_2O, J ~ 13.4 and 6.5 Hz (dublett of dublett), ***relative to CD_3OD (77.00 ppm);

Table : Yields and confirmative NMR-data of fucosylated tetrasaccharides.

*Representative experimental procedure: 15 mg (16 μmol) of trisaccharide **1** (entry 18), 25 mg of GDP-fucose (~ 60%)[26] and 4 mg of bovine serum albumine (Boehringer) were added to a mixture of 160 μl of a 250 mM $MnCl_2$-solution, 550 μl of a 250 mM Na-cacodylate-buffer (pH = 6.48) and 800 μl deionized water in a plastic tube. After addition of 2 μl (30 U) of calf intestine alkaline phosphatase[27] (Boehringer No. 108146, 7500U/498μl) and 200 μl (400 mU) of a fuc-t VI-solution[28], the mixture was briefly vortexed and incubated at 37°C with stirring[29]. After a TLC ($CHCl_2$-MeOH-H_2O-mixtures) showed the consumption of the starting sugar, the turbid mixture was centrifuged and the resulting supernatant passed over a short C-18 column[17)25]. Lyophilization gave a white powder which was dissolved in water and passed over a Na^+-column, lyophilized once more and chromatographed on silica gel (eluent v.s.). A final lyophilization from dioxane yielded the desired tetrasaccharide as a white powder (15.4 mg, 89%), which was pure according to MS, ^1H and ^{13}C NMR analysis.*

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