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Enzymatic α(2-3)-Sialylation of Non-Natural Disaccharides with Cloned Sialyl-transferase

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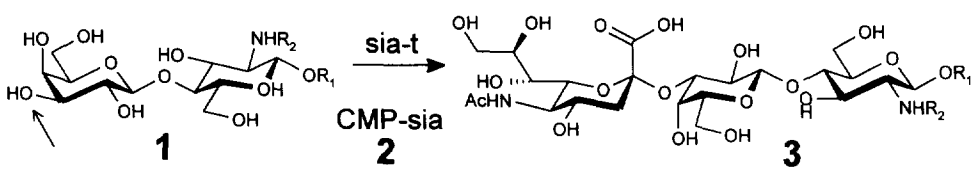
ABSTRACT

Cloned α(2-3)sialyltransferase from rat liver is used to sialylate lactosamine derivatives. The enzyme accepts a broad range of N-acetyl substituants. The residues encompass small and bulky aliphatic replacements as well as charged and sulfonamide groups. Copyright © 1996 Elsevier Science Ltd

Carbohydrates play a key role in cell-adhesion and developmental processes[1)2)3)] and therefore aroused a recent interest by the pharmaceutical industry. However, the methods for the chemical synthesis of such compounds are still insufficiently developed[4)5)]. A chemo-enzymatic approach may be useful in this aspect. *In vivo* the highly regio- and stereospecific assemblage of oligosaccharides is catalyzed by glycosyltransferases. Due to recent progress in cloning and overexpression, more glycosyltransferases will be available as efficient tools in synthetic carbohydrate chemistry[6)]. The synthetic value of those catalysts would be greatly enhanced if their specific action *in vivo* could additionally be applied to non-natural substrates *in vitro*[7)].

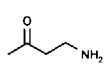
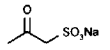
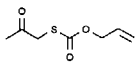
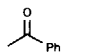
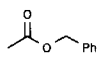
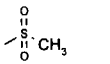
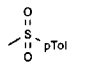
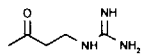
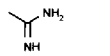
α(2-3)-(N)Sialyltransferase, originally isolated from rat liver[8)], has been cloned[9)] (EMBL accession no. M97754). We have produced a soluble version of this enzyme in insect cells and have purified it to homogeneity. The enzyme transfers a sialic acid unit from CMP-activated sialic acid **2** (the donor) to the 3-OH group of the galactose residue **1** (the acceptor) - in an α-mode[10)] (see scheme). Some kinetic studies were reported probing dif-ferent deoxy-galactose moieties of type I (galβ(1-3)glcNAc) and type II (galβ(1-4)glcNAc) as acceptors[11)]. A small number of variations involving the aglycon part R_1 have also been tolerated by the enzyme [12)]. The acyl-substituent R_2 on the glucosamine unit, the natural one being acetyl, has not been investigated systematically; it has, however, been found that only the amine (R_2=H) and the propylamide (R_2=C(O)CH_2CH_3) were moderate acceptors[11)].



1 **2** **3**

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

entry	R_2 (**1** or **3**)	% (mg)	glcN: H-1**	gal: H-1**	sia: H-2eq; Ac;**	glcN: C-1, 2, 4	gal: 1, 3, 4	sia: C-2, 3	C-others (aglycon, NHR)
1		71 (80.6)	4.48	4.52	2.73; 1.99	102.55; 56.56; 79.95	104.01; 77.00; 68.99	100.82; 41.00	71.90; 23.61
2		77 (30.7)	4.60	4.57	2.75; 2.02	102.94; 56.07; 80.37	104.73; 77.33; 69.62	101.96; 41.79	72.70; 166.69
3		77 (15.0)	4.29	4.34	2.70; 1.89	102.21; 55.69; 80.46	104.17; 76.37; 68.35	100.62; 41.32	70.24; 19.30
4		89 (35.9)	4.66	4.61	2.71; 2.11	102.97; 56.92; 80.49	104.64; 77.25; 69.57	101.91; 41.73	72.69; 63.13
5		49 (16.3)	4.50	4.56	2.76; 2.03	103.34; 57.94; 81.07	105.35; 78.24; 70.34	102.59; 42.70	73.32; 43.43
6		69 (23.8)	4.62	4.62	2.72; 2.00	101.73; 56.76; 80.78	104.68; 78.71; 68.77	100.68; 41.62	70.37; 119.85
7		77 24.2	4.36	4.40	2.77; 1.94	102.33; 61.44; 80.39	104.54; 77.28; 68.78	100.76; 41.61	70.29; 33.47
8***		65 (14.8)	4.28	4.37	2.71; 1.95	102.93; 59.64; 81.07	104.98; 77.65; 69.04	101.08; 42.05	70.71; 14.60
9		68 (14.9)	4.56	4.56	2.74; 2.15	103.10; 61.38; 79.66	103.60; 76.00; 68.81	101.13; 40.95	70.71; 14.95
10		72 (186)	4.38	4.45	2.71; 2.02	102.30; 57.56; 80.42	104.11; 76.14; 68.83	100.25; 41.07	69.90; 116.39
11		68 (20.4)	4.47	4.54	2.72; 2.02	104.81; 55.38; 81.60	105.86; 78.50; 70.80	103.12; 42.95	72.72; 17.20
12		95 (26.6)	4.48	4.54	2.73; 2.03	104.71; 60.10; 81.87	106.13; 78.77; 71.07	103.38; 43.21	74.33; 18.53
13		80 (10.7)	4.54	4.47	2.73; 2.00	105.34; 59.00; 81.58	105.63; 78.38; 70.06	102.87; 42.70	73.66; 17.50
14		69 (10.1)	4.31	4.38	2.76; 1.95	103.26; 56.98; 81.45	105.26; 77.98; 69.69	101.43; 42.76	71.16; 33.59

15		61 (19.0)	4.52	4.52	2.76; 2.04	101.89; 55.92; 79.59	103.71; 76.55; 68.50	100.83; 40.74	71.38; 36.67
16		92 (23.4)	4.49	4.56	2.75; 2.03	103.93; 58.54; 81.15	105.51; 78.10; 70.42	102.18; 42.55	73.80; 63.01
17		56 (18.3)	4.49	4.49	2.70; 1.96	102.72; 57.24; 80.70	104.68; 76.98; 69.10	102.32; 41.91	71.52; 120.07
18		86* (19.9)	4.39	4.45	2.76; 1.93	102.35; 56.51; 80.71	104.42; 76.54; 68.41	100.47; 41.51	70.15; 132.21
19		73* (16.5)	4.55	4.63	2.94; 2.18	103.10; 58.72; 80.62	104.51; 76.78; 68.88	101.05; 41.65	71.11; 67.56
20		92 (34.0)	4.44	4.20	2.74; 1.92	102.33; 60.17; 79.80	103.85; 76.24; 68.36	100.51; 40.99	70.83; 42.47
21		66* (15.2)	4.36	4.22	2.78; 1.95	102.99; 60.93; 80.90	104.88; 77.23; 69.10	100.02; 42.09	70.73; 21.54
22		86 (20.3)	4.50	4.54	2.73; 2.02	102.89; 56.81; 81.71	105.29; 77.30; 70.88	100.99;41.96	70.88; 39.08
23		41 (8.8)	4.54	4.54	2.75; 2.03	103.13; 59.87; 80.22	104.59; 77.13; 69.33	101.72; 41.55	72.76; 159.77

* addition of DMSO; ** relative to water, J ~ 7.2 Hz (dublett each), J ~ 13.4 and 6.5 Hz (dublett of dublett); *** data of main isomer.

*Representative experimental procedure: 15.0 mg (25.2 μmol) of disaccharide **1** (entry 18) was added to a mixture of 2 ml of a $MnCl_2$-solution (0.06 M), 2 ml of Na-cacodylate-buffer (0.05 M, pH = 6.45) and 1.3 ml water (in this case, further 0.5 ml DMSO was added). The mixture is briefly sonicated to give a slightly turbid solution. Subsequently 28.2 mg (42.8 μmol) of CMP-sia **2** (~90%)[17], 2.6 mg of bovine serum albumine, 200 μl (1.4 U) of sialyltransferase and 2 μl of calf intestine alkaline phosphatase[18] (Boehringer No. 108146, 7500U/498μl) were added and the mixture incubated at 37°C with stirring. After a TLC (CH_2Cl_2-MeOH-H_2O-mixtures) showed the consumption of all the starting acceptor, the turbid solution was centrifuged and the clear supernatant passed over a C-18 column[10] followed by silica-gel chromatography. 19.9 mg (86%, entry 18) of a white powder was obtained after lyophilization from dioxane.*

In connection with an ongoing investigation[13] we were interested in both the glycosyltransferases as synthetic tools and in the scope of their applicability. Although the sialylation takes place at the terminal galactose (s. scheme) we could not expect the sialyltransferase (E.C. 2.4.99.6) to tolerate broad variations in R_2 in advance[10]. Most transferases often recognize the overall structures of the acceptors[14].

The substrates **1** which have been successfully sialylated on a preparative scale are compiled in the table. (For their synthesis see the preceeding paper.) We observed that large aromatic residues (entries 18,19, 21) were as easily accepted as small aliphatic ones (entries 1-14). Variations in the acylgroup, e.g. a urea (entry 13), a thiourea (entry 9) or urethanes (entries 10, 11, 19) are well tolerated by the enzyme; even sulfonamides (entries 20, 21) have been sialylated in high yields. More surprisingly, both positive and negative charges on R_2 were tolerated to give the trisaccharides **3** (entries 5, 15, 16, 22, 23). All structures have been proven by ^1H-, ^{13}C- and MS-spectra respectively. The NMR-data are in agreement with those of the 'parent'-compound (entry 1); selected data of significant reporter groups[10] are included in the table.

In some cases (entries 18, 19, 21) DMSO had to be added to obtain a sufficient solubility. α (2-3)-sialyltransferase is able to act with additional organic solvents without any adverse effects.

Together with the studies concerning galactosyl-transferase[13], our findings should contribute to a more wide-spread and stereochemically reliable use of α(2-3)sialyltransferase in the synthesis of non-natural carbohydrates. Especially the low yields of chemical sialylations may thus be circumvented efficiently[15][16].

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