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ON THE PREPARATIVE USE OF RECOMBINANT β(1-3)GALACTOSYL-TRANSFERASE

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ABSTRACT

A number of non-natural N-acyl derivatives of glucosamine is incubated with a recombinant β(1-3)galactosyl-transferase and UDP-galactose. Surprisingly, the enzyme recognizes the non-natural acceptors as substrates and transfers galactose onto the 3-OH group in a β-mode to give a series of Lewis^c-(type 1) disaccharides.

Mammalian cell-surface oligosaccharides are involved in a variety of adhesion phenomena[1,2]. A proper understanding of their biological roles especially in terms of their interactions with particular receptors necessitates a rapid access to a wide range of carbohydrate epitopes[3]. Thus natural and non-natural oligosaccharides can be investigated as potential drug candidates[4].

In nature oligosaccharides are assembled highly regio- and stereospecifically by glycosyl-transferases[5]. The enzymatic synthesis of complex carbohydrates[6], therefore, offers a versatile and simple alternative to the cumbersome, conventional chemical routes[7]. We could recently show that β(1-4)galactosyl-transferase[8], recombinant α(2-3)sialyl-transferase[9] and recombinant fucosyl-transferase III and VI[10,11] can be successfully employed to synthesize efficiently a large array of non-natural oligosaccharides. Here we wish to report our results on the preparative use of recombinant β(1-3)galactosyl-transferase[12]. *In vivo*, the enzyme transfers a galactose unit from uridine-diphosphate galactose (UDP-gal)[13] to the 3-OH group of a terminal N-acetyl glucosamine moiety in a β-mode resulting in the formation of a Lewis^c-disaccharide **3** (see figure). The parent compound (R_1 = Ac, R_2 = Lem) has been synthesized via a multistep protocol[14] and served in a protected form as a precursor for the preparation of human blood group antigens.

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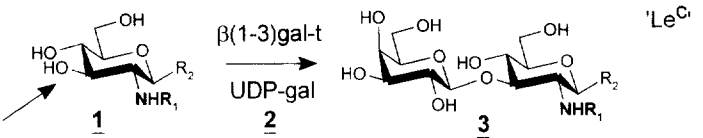
A more efficient approach towards the preparation of this disaccharide is the transferase-catalyzed pathway outlined in the scheme below.



Scheme: Enzymatic galactosylations with recombinant β(1-3)galactosyl-transferase.

We incubated[15] the parent compound **1** (R_1 = acetyl) with a recombinant β(1-3)galactosyl-transferase in the presence of UDP-gal **2** on a preparative scale. The usual work-up[8] gives a sugar whose MS-spectrum displayed the correct mass (553.6 [M]) of the expected disaccharide **3** (R_1 = acetyl). The stereochemical integrity has further been proven by extensive NMR measurements (see table for reporter group signals). In the ^{1}H NMR-spectrum a second doublet (~ 4.3 ppm) with a large coupling constant (~ 7.2 Hz) confirmed the β-attachment of a galactose unit to the N-acetylglucosamine moiety. The large low-field shift in the ^{13}C NMR-spectra of C-3 of the N-acetylglucosamine moiety (entry 1) indicated a (1→3)disaccharide linkage. This was unambiguously confirmed by irradiation experiments of H-3 of the glucosamine moiety and H-1 of the galactose unit (ROESY- and NOESY-spectra).

Consequently we probed the transferase on a preparative scale with a series of non-natural acceptors **1**[8]. All the compiled compounds (see table) are recognized by the enzyme as acceptor substrates. Surprisingly, even polar (entry 5) and charged (entry 6) replacements of the N-acetyl group are tolerated despite their close proximity to the reactive OH-functionality, and presumably the catalytic site of the enzyme. Omission of the aglycon part of the acceptors **1** (see entries 2,7,8) are also tolerated by this enzyme. In addition large lipophilic N-acyl residues (entry 8) are accepted.

The structurally relevant NMR-shifts are observed in all the listed examples (see table) and all compounds **3** give correct MS-data. In some cases prolonged incubation caused a partial loss of the methyl ester group (entry 6), which may be attributed to minor catalytic impurities in the enzyme preparation.

The disaccharides **3** are valuable themselves as probes for assaying α(1-2)fucosyl-transferases[17] but can also serve as starting materials for the enzymatic synthesis of Lewis^a, sialyl-Lewis^a and several other blood group antigen mimetics[3].

In conclusion our findings show for the first time the usefulness of a recombinant β(1-3)galactosyl-construct for the preparative synthesis of the parent Lewis^c-disaccharide and several congeners, which bear non-natural replacements of the natural N-acetyl group on the acceptor substrate. Thus an additional, versatile transferase can be added to the synthetic arsenal of the glycobiologist. Further studies are in progress and will be reported in due course.

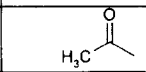
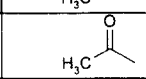
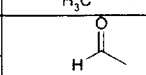
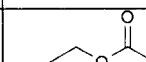
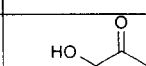
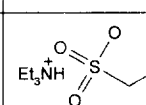
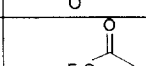
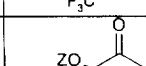
entry	R1	R2	% (mg)	Gal H-1[a] (J)	GlcNR H-1	Gal C-1	GlcNR C-1	GlcNR C-3
1		Lem	74 (35.8)	4.26 (7.2)	4.45 (8.7)	105.6	102.4	85.0
2		H	68 (15.8)	4.33 (7.3)	--	105.2	82.3	85.6
3[b]		Lem	29 (10.9)	4.22 (7.6)	4.31 (8.3)	105.0	101.8	84.0
4		Lem	34 (9.4)	4.24 (7.6)	4.32 (8.3)	105.3	102.8	84.7
5		Lem	61 (12.8)	4.20 (7.3)	4.41 (8.8)	104.9	101.9	83.5
6[c]		Lem	51 (23.4)	4.51[e] (7.6)	4.65[e] (7.6)	107.5	105.8	82.1
7		H	59 (13.0)	4.28 (7.6)	--	105.0	82.2	84.4
8[d]		H	85 (25.7)	4.25 (7.3)	--	103.9	81.5	83.9

Table: Yields and NMR-data (solvent: CD_3OD = int. ref.) of disaccharides **3**; a) all coupling constants in (Hz); b) major isomer; c) NMR in D_2O (= int. ref.); d) NMR in D_6-DMSO (= int. ref.); e) interconvertible.

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15) **Representative incubation procedure**: 34.5 mg (88.1 μmol) of monosaccharide **1** (entry 1), 70.0 mg (112.5 μmol) uridine-diphosphate galactose and 2.7 mg bovine serum albumine (Boehringer) are dissolved in 140 μl of a 0.5 M $MnCl_2$-solution and 3000 μl (50 mM) sodium cacodylate-buffer (pH = 6.44) containing 175 μl DMSO. The mixture is then incubated with 45 U (3 μl) calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/498 μl) and 40 mU (50 μl) of a β(1-3)Gal-T solution at 37° C for 48 h. The mixture is then passed over a short C-18 reversed phase column and lyophilized[8]. 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 35.8 mg (74%) of the disaccharide **3** (entry 1) as a white, fluffy powder. Nearly quantitative conversion is observed on a smaller scale. Depending on the substrate, a prolonged incubation is sometimes advisable.

16) We also used a homologous enzyme of the originally described transferase[12] found in human brain for galactosyl-transfer; for cloning and overexpression of this enzyme (termed 'β(1-3)Gal-T 2' by us) see, F. Kolbinger, M. Streiff, A. Katopodis* manuscript submitted to J. Biol. Chem. **1997**.

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