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Fucosyltransferase-catalyzed formation of L-galactosylated Lewis structures

Katja Stangier [a,b], Monica M. Palcic [a], David R. Bundle [a], Ole Hindsgaul [a], Joachim Thiem [b,*]

[a] *Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*
[b] *Institut für Organische Chemie, Universität Hamburg, Hamburg D-20146, Germany*

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Abstract

The Lewis (α1-3/4) fucosyltransferase isolated from human milk could be used for preparative fucosylations of the disaccharide acceptors Gal(β1-3)GlcNAc(β1-O)R (at position OH-4) and Gal(β1-4)GlcNAc(β1-O)R (at position OH-3) [R = $(CH_2)_8COOMe$]. As donors GDP-L-Gal and deoxygenated derivatives were used to lead to a series of novel modified trisaccharides of the Lewis[a] and the Lewis[x] type, respectively. © 1998 Elsevier Science Ltd.

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1. Introduction

L-Fuc represents an important sugar building unit in glycolipids and glycoproteins, and often terminally fucosylated derivatives function as decisive patterns in recognition processes [1]. They differentiate complex carbohydrate antigens such as the blood group antigens [2] and apparently must be discussed in connection with abnormal glycosylation patterns accompanying aberrant cell growth [3]. Thus, in mammalian cancer tissue considerably higher fucosylation rates and increased activities of fucosyltransferase (FucT) could be demonstrated [4]. There are a number of fucosyltransferases in the mammalian organism with quite different acceptor substrate specificity some of which have been cloned [5]. As donor substrate, all of them require guanosine 5′-diphosphofucose (GDP-Fuc) which biochemically is formed by phosphorylation of L-fucose with ATP and fucokinase to give the β-fucopyranosyl phosphate and its further transformation with GTP and GDP-fucose pyrophosphorylase [6–9]. Further, a de novo biochemical formation of GDP-Fuc from D-Glc or D-Man via guanosine 5′-diphosphomannose (GDP-Man) could be identified in various biological species including mammals as well as plants [10–14].

Fucosyltransferases characterized to date require disaccharide acceptor of the LacNAc, Gal(β1-4)GlcNAc, or the *iso*-LacNAc type, Gal(β1-3)GlcNAc, Lewis[c] [15,16]. For this study, a mixture of Lewis-(α1-3) and Lewis-(α1-3/4) fucosyltransferases [17] was isolated from human milk [5] following the purification approach reported [18]. The further separation of the two FucT with 51 and 53 KD could be performed previously [5], however, since it

* Corresponding author. Fax: +49-40-4123-4325; e-mail: thiem@chemie.uni.hamburg.de.

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is rather difficult and not necessary for the preparative use, this was omitted.

Previous work employing Lewis-(α1-3/4) FucT (EC 2.4.1.65) for preparative syntheses were reported by Palcic et al. [19] and Gokhale et al. [20] who were also the first to demonstrate the use of a complex modified GDP-Fuc derivative [21].

Both, interest in the preparation of modified Lewis structures in connection with the question of fucosyltransferase inhibition and also the width of acceptance of modified donor substrates for these FucT, led us to synthesize some modified GDP-Fuc derivatives and test their transfer to suitable acceptor substrates.

2. Results and discussion

The chemical syntheses of the modified GDP-Fuc components GDP-L-Gal (**1**), GDP-3-deoxy-L-Gal (**2**), and GDP-3,6-dideoxy-L-Gal (**6**) along classical routes was recently reported [22]. Acceptor disaccharide structures were the hydrophobic 8-methoxycarbonyloctyl disaccharides **3** Gal(β1-3)GlcNAc(β1-O)R and 7 Gal(β1-4)GlcNAc(β1-O)R [23].

Before preparative experiments, kinetic data for the transfer of the modified donor substrates to acceptor molecules were studied. By this assay [24] based on previous work [25], the formation of GDP during the transferase reaction is determined. With phosphoenol pyruvate in the presence of pyruvate kinase pyruvate is obtained, which in turn with NADH and lactate dehydrogenase gives NAD^+ the formation of which is easily followed at 340 nm.

The results are shown in Table 1 in comparison to literature values. The data for the genuine donor GDP-Fuc to the natural disaccharide Gal(β1-3)GlcNAc (K_m = 10.5 μM) is considerably lower [5] than to the hydrophobic β-8-methoxycarbonyloctyl glycoside **3** (K_m = 68 μM [19]). For the modified donor GDP-L-Gal (**1**) a value of K_m = 219 μM was obtained which indicates this to be a worse substrate by the factor three. With the 3-deoxygenated GDP-L-Gal (**2**), however, no reaction was observed, even at the edge of the solubility of **2** (4.8 mM).

For the transfer of fucose from GDP-Fuc to *N*-acetyllactosamine, Gal(β1-4)GlcNAc, a K_m = 5.0 μM was reported [5], and again the β-8-methoxycarbonyloctyl glycoside **4** showed to be an about ten times less effective substrate [19]. By going to the unnatural donor GDP-L-Gal (**1**) again, a decrease in substrate efficiency by about a factor 4 (K_m = 185 μM) was observed. In this case, the 3,6-dideoxy GDP-L-Gal (**6**) could be employed as donor, and the K_m = 556 μM revealed an about ten times worse donor substrate for the Lewis (α1-3/4) fucosyltransferase.

With these data at hand preparative fucosylations employing a concentrated solution of fucosyltransferase (~ 50 mU/mL) could be performed. The stability of the enzyme did not allow reactions exceeding seven days at 37 °C. Concentrations of the acceptor disaccharide glycosides **3** and **7** were 3 μmol each, and the donor was added in excess (9 μmol). In order to secure enough of the hydrolytically labil donor in the mixture and to overcome the expected product inhibition of the released GDP, the addition was done in 0.25 mg quantities over a period of 5 days.

Thus, the enzymatic preparation of the modified Lewis[a] trisaccharide structure **4** in 95% yield could be realized from GDP-L-Gal (**1**) and the Lewis[c] disaccharide glycoside **3**. As expected based on the kinetic studies, there was no transfer observed for 3-deoxy GDP-L-Gal (**2**) to **3** to give the trisaccharide **5**.

Table 1
Evaluation of modified glycosyl donors for human milk α1-3/4-fucosyltransferase

Donor	Acceptor	K_m (μM)	Reference
GDP-L-Fuc	Gal(β1-3)GlcNAc	10.5	[5]
GDP-L-Fuc	Gal(β1-3)GlcNAc(β1-O)R (**3**)	68	[19]
GDP-L-Gal (**1**)	Gal(β1-3)GlcNAc(β1-O)R (**3**)	219	
GDP-3*d*-L-Gal (**2**)	Gal(β1-3)GlcNAc(β1-O)R (**3**)	No reaction	
GDP-L-Fuc	Gal(β1-4)GlcNAc	5.0	[5]
GDP-L-Fuc	Gal(β1-4)GlcNAc(β1-O)R (**4**)	45	[19]
GDP-L-Gal (**1**)	Gal(β1-4)GlcNAc(β1-O)R (**4**)	185	
GDP-3,6-d_2-L-Gal (**6**)	Gal(β1-4)GlcNAc(β1-O)R (**4**)	556	

R = $(CH_2)_8COOCH_3$

With the *N*-acetyllactosamine glycoside **7**, each of the GDP donors GDP-L-Gal (**1**), GDP-3-deoxy-L-Gal (**2**), and GDP-3,6-dideoxy-L-Gal (**6**) showed transfer reactions. In convincing yields of 84–93%, enzymatic syntheses of the modified Lewis^x trisaccharides **8**, **9**, and **10** could be performed.

Work-up and separation for these preparative enzymatic syntheses followed the Sep-Pak procedure [26] and allowed a favorable and fast isolation of the pure trisaccharides. Their structural identity could be unequivocally assigned by NMR spectroscopy and matches with the data reported [27] for chemically synthesized Lewis^a derivatives [23]. In the 4-α-fucosylated Gal(β1-3)GlcNAc structure, the H-5 of the fucose unit shows an unusual shift of 4.87 ppm, and in fact, trisaccharide **4** shows H-5″ at δ 4.94 ppm. Furthermore, the anomeric proton of L-Gal was observed at $\delta = 5.18$ ppm with $J_{1'',2''} = 4.2$ Hz. The key NMR data of the modified Lewis^x trisaccharide structures (Table 2) are similarly in keeping with the structural requirements. (Schemes 1 and 2).

In summary, modified GDP-Fuc derivatives could be used in preparative enzymatic glycosylation employing Lewis (α1-3/4) fucosyltransferase from human milk. Thus, an objective of this study was to show that modification in donor substrates are acceptable even for complex enzymes such as these transferases. With these enzyme-assisted syntheses, access to novel oligosaccharide analogs could be demonstrated.

Table 2
Selected ^{1}H chemical shifts δ (ppm) and coupling constants J (Hz) for modified Lewis^a and Lewis^x trisaccharides[1]

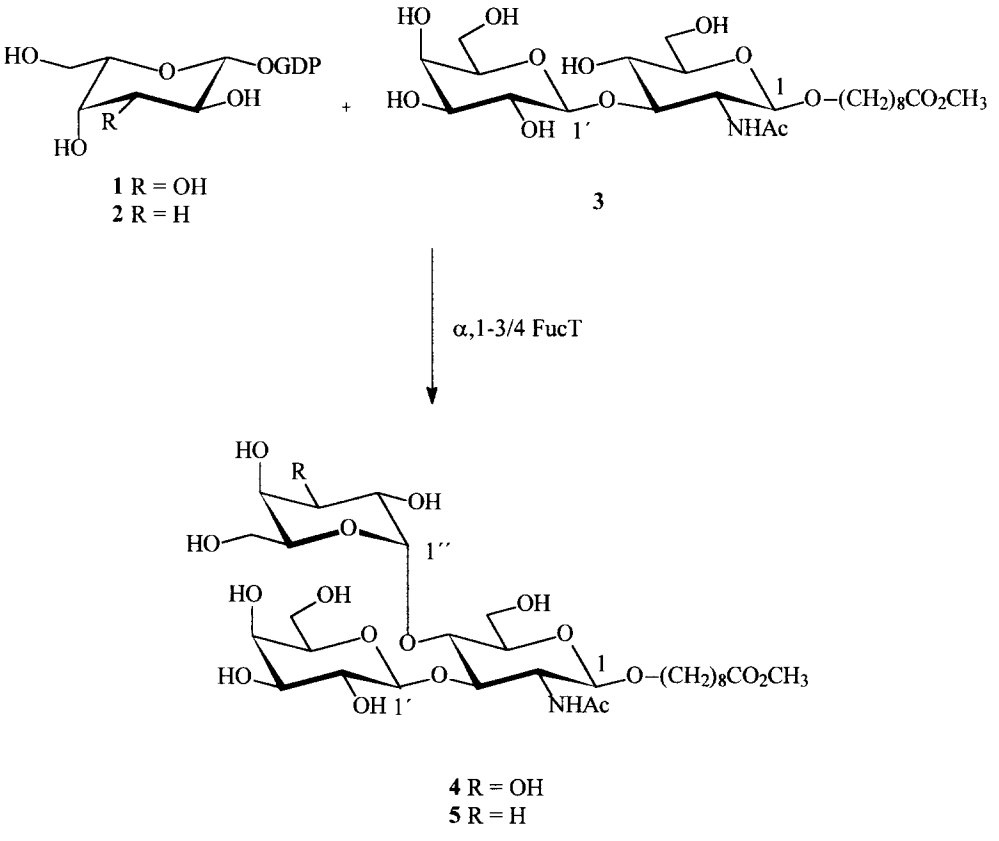
Compound	**4**	**8**	**9**	**10**
H-1 ($J_{1,2}$)	4.56 (8.6)	4.53 (8.4)	4.53 (8.1)	4.52 (8.0)
H-1′ ($J_{1',2'}$)	4.53 (8.0)	4.46 (7.6)	4.49 (7.7)	4.48 (7.4)
H-1″ ($J_{1'',2''}$)	5.18 (4.2)	5.20 (4.0)	5.12 (4.1)	5.09 (3.9)
H-5″ ([2])	4.94 (7.0)	4.79 (7.0)	4.70 (6.9)	4.74 (6.4)
H-6″	–	–	–	1.20
$COOCH_3$	3.64	3.69	3.67	3.68
$NHCOCH_3$	2.05	2.03	2.07	2.04

[1]500 MHz in deuterium oxide; [2]$J_{5'',6a''} \approx J_{5'',6b''}$.

3. Experimental

For general methods cf. Ref. [22].

The purification of human milk followed the reported procedure [25] with minor advantageous modifications [19]. The enzyme material obtained showed



Scheme 1.

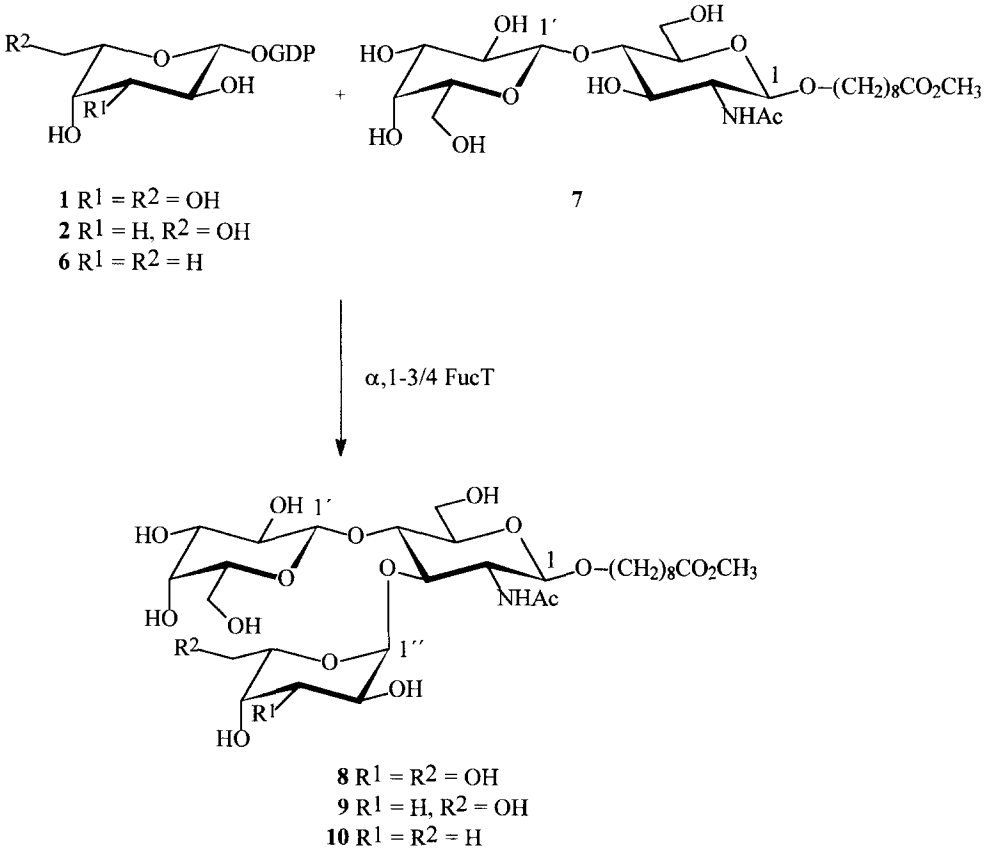
an activity of 1–2 U/mg protein. It is stored in 25 mM sodium cacodylate buffer (pH 6.5) containing 5 mM manganese(II) chloride and 25% (v/v) glycerol (50 U/mL) at 4 °C and stable for at least 12 months.

Continuous spectroscopic assay for FucT.—The assay was done on microtiter plates with 96 wells. Each well contained pyruvate kinase (7.5 U), lactate dehydrogenase (15 U), manganese(II) chloride (0.7 mM), BSA (0.13%), phosphoenol pyruvate (16.8 μg), NADH (58.2 μg), potassium chloride (0.56 mg), 200 mM Hepes buffer, pH 7.0 (10 μL); GDP-L-Gal (**1**) or GDP-3*d*-L-Gal (**2**) or GDP-3,6-d_2-L-Gal (**6**) (9.4–600 μM); acceptor disaccharide glycosides **3** or **7** (1.08 mM); (α1-3/4) FucT (0.6 mU). The final volume to 150 μL was made up with bidest. water. Incubation for 10–15 min at 37 °C was in the Elisa reader and the decrease of absorbance at 340 nm continuously followed.

Preparative fucosylation.—The incubation mixture contained one of the acceptor disaccharides glycoside **3** or **7** (1.5 mg, 0.27 mmol), one of the GDP donors **1**, **2**, or **6** (4.5 mg, 0.73, 0.75, or 0.77 mmol, respectively), and the buffer-stabilized fucosyltransferase mixture (60 mU). Incubation was for four to seven days at 37 °C. The labile donors were added continuously in 0.25 mg portions during the reaction time. Work-up followed the Sep-Pak method. First, the cartridges were eluted with methanol (10 mL) and water (20 mL). Following dilution with water (10 mL), the mixture was applied to the cartridge and eluted with water (20 mL). The trisaccharides were eluted with methanol (5 mL) and dried.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 3)[α-L-galactopyranosyl-(1 → 4)]-β-D-N-acetylglucosamine (**4**).—The preparative fucosylation of **3** with GDP-L-Gal (**1**) was done for seven days and monitored by TLC (dichloromethane–methanol–water 7:3:0.3). Purification was as described to give **4** (2 mg, 93%) as a colorless foam. ^{1}H NMR (D_2O): δ 5.18 (d, 1 H, $J_{1'',2''}$ = 4.2 Hz, H-1″), 4.94 (ddd ~ dt, 1 H, $J_{5'',6a''} \sim {}_{5'',6b''}$ = 7.0 Hz, H-5″), 4.56 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 4.53 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 3.64 (s, 3 H, $COOCH_3$), 2.34 (m, 2 H, $H_{8\text{-}CH2}$), 2.05 (s, 3 H, NAc), 1.62 (m, 2 H, $H_{7\text{-}CH2}$).

8-Methoxycarbonyloctyl β-D-galactopyrano-syl-(1 → 4)[α-L-galactopyranosyl-(1 → 3)]-β-D-N-acetylglucosamine (**8**).—The preparative fucosyla-



Scheme 2.

tion was as above for five days. By purification **8** (1.9 mg, 88%) was obtained as colorless foam. ^{1}H NMR (D_2O): δ 5.20 (d, 1 H, $J_{1'',2''} = 4.0$ Hz, H-1''), 4.79 (ddd ~ dt, 1 H, $J_{5'',6a'' \sim 5'',6b''} = 7.0$ Hz, H-5''), 4.53 (d, 1 H, $J_{1,2} = 8.4$ Hz, H-1), 4.46 (d, H-1, $J_{1',2'} = 7.6$ Hz, H-1'), 3.69 (s, 3 H, $COOCH_3$), 2.39 (m, 2 H, $H_{8\text{-}CH2}$), 2.03 (s, 3 H, NAc), 1.62 (m, 2 H, $H_{7\text{-}CH2}$).

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 4)[α-3-deoxy-L-xylohexopyranosyl-(1 → 3)]-β-D-N-acetylglucosamine (**9**).—Preparative fucosylation for seven days gave after work-up **9** (2 mg, 93%) as a colorless foam. ^{1}H NMR (D_2O): δ 5.12 (d, 1 H, $J_{1'',2''} = 4.1$ Hz, H-1''), 4.70 (ddd ~ dt, 1 H, $J_{5'',6a'' \sim 5'',6b''} = 6.9$ Hz, H-5''), 4.53 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1), 4.49 (d, 1 H, $J_{1',2'} = 7.7$ Hz, H-1'), 3.67 (s, 3 H, $COOCH_3$), 2.38 (m, 2 H, $H_{8\text{-}CH2}$), 2.17 (m, 1 H, H-3a''), 2.07 (s, 3 H, NAc), 1.91 (m, 1 H, H-3e''), 1.59 (m, 2 H, $H_{7\text{-}CH2}$).

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 4)[α-3,6-dideoxy-L-xylohexopyranosyl-(1 → 3)]-β-D-N-acetylglucosamine (**10**).—The preparative fucosylation was performed for seven days to give after work-up **10** as colorless foam (1.8 mg, 84%). ^{1}H NMR (D_2O): δ 5.09 (d, 1 H, $J_{1'',2''} = 3.9$ Hz, H-1''), 4.74 (ddd ~ dt, 1 H, $J_{5'',6a'' \sim 5'',6b''} = 6.4$ Hz, H-5''), 4.52 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.48 (d, 1 H, $J_{1',2'} = 7.4$ Hz, H-1'), 3.68 (s, 3 H, $COOCH_3$), 2.36 (m, 2 H, $H_{8\text{-}CH2}$), 2.14 (m, 1 H, H-3a''), 2.04 (s, 3 H, NAc), 1.81 (m, 1 H, H-3e''), 1.63 (m, 2 H, $H_{7\text{-}CH2}$), 1.20 (d, 3 H, H-6'').

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