Chemoenzymic synthesis of β-D-Gal(6-SO_4)-(1→4)-D-GlcNAc, β-D-Gal-(1→4)-D-GlcNAc(6-SO_4) and β-D-GlcNAc-(1→4)-D-GlcNAc(6-SO_4) and their roles as fucosyl acceptors in reactions catalysed by human α-3-fucosyltransferases

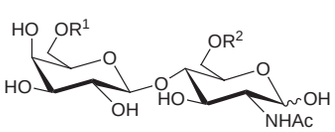
Chuong Hao Tran,[a] Peter Critchley,[a] David H. G. Crout,[a,*] Christopher J. Britten,[b] Sara J. Witham[b] and Michael I. Bird[b]

[a] *Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL*
[b] *Glycobiology Research Unit, Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, UK SG1 2NY*

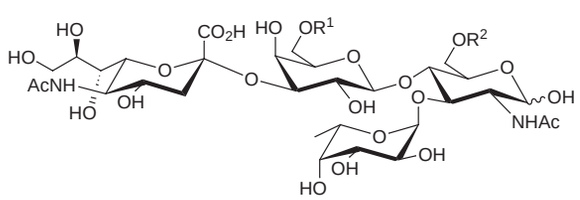
***N*-Acetyllactosamine 5 is obtained by transglycoslyation between *p*-nitrophenyl β-D-galactopyranoside 3 and *N*-acetyl-D-glucosamine 4 by using the β-galactosidase from *Bacillus circulans* as the biocatalyst. Sodium salts of β-Gal(6-SO_4)-(1→4)-GlcNAc 1 and β-Gal-(1→4)-GlcNAc(6-SO_4) 2 have been prepared from *N*-acetyllactosamine 5 in five and three steps respectively. β-D-GlcNAc-(1→4)-D-GlcNAc(6-SO_4) (di-*N*-acetylchitobiose 6-sulfate) 10 is synthesised from chitin in three steps. The products have been tested as fucosyl acceptors in reactions catalysed by recombinant human fucosyltransferases III to VII.**

Introduction

Selectins are carbohydrate-binding proteins located at cell membranes which mediate adhesion between leukocytes and endothelial cells during the recruitment of leukocyte to sites of inflammation.[1–3] A variety of oligosaccharides have been reported to be recognised by the selectins.[2] The common feature of most of those oligosaccharides is a backbone of either β-Gal(1→3)GlcNAc or β-Gal(1→4)GlcNAc.[2] Recent studies have underlined the importance of sulfate esters on the backbone of these oligosaccharides for their affinity for selectins.[3–10] Because of their significant biological interest, these complex carbohydrates have attracted a great deal of synthetic effort.[4,6,8,11–15]

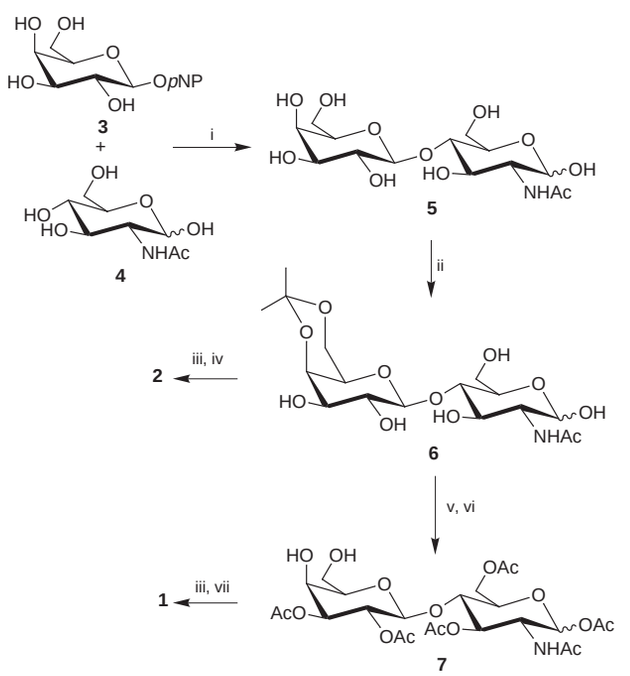


1 $R^1 = SO_3^-$, $R^2 = H$
2 $R^1 = H$, $R^2 = SO_3^-$



Sialyl Lewis^X 6-sulfate, $R^1 = H$, $R^2 = SO_3H$
Sialyl Lewis^X 6′-sulfate, $R^1 = SO_3H$, $R^2 = H$

Recently, sulfated constituents of GlyCAM-1 (previously known as Sgp50), a mucin-like glycoprotein with sulfated, sialylated and fucosylated *O*-linked oligosaccharide chains, have been identified as Gal(6-SO_4), GlcNAc(6-SO_4), β-Gal-(6-SO_4)-(1→4)-GlcNAc and β-Gal-(1→4)-GlcNAc(6-SO_4).[5,6] Here we describe a simple method for the synthesis of β-Gal-(6-SO_4)-(1→4)-GlcNAc **1** and β-Gal-(1→4)-GlcNAc(6-SO_4) **2** which could be useful intermediates for the enzymic synthesis of sialyl Lewis^X 6- and 6′-sulfate[8,15] and of di-*N*-acetylchitobiose 6-sulfate **10** (see Scheme 2).



Scheme 1 *Reagents and conditions*: i, β-galactosidase from *Bacillus circulans*; ii, $MeC(OMe)_2MeH^+$; iii, SO_3–pyridine complex, 5 °C; iv, aq. H^+; v, Ac_2O pyridine; vi, MeOH, aq. H^+; vii, MeONa, MeOH. *p*NP = *p*-nitrophenyl.

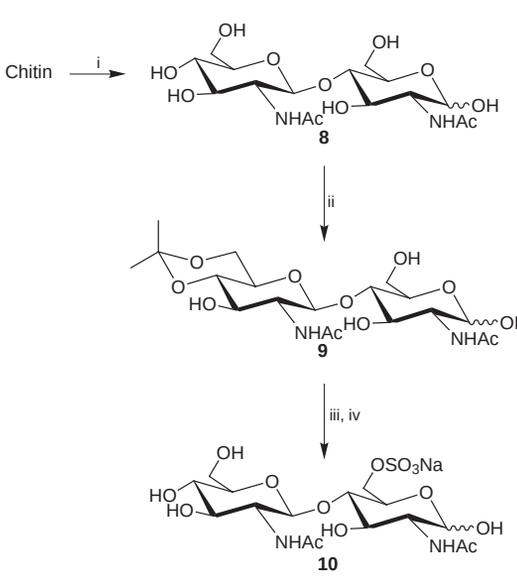
Results

A strategy for the synthesis of the sulfated disaccharides is outlined in Scheme 1. In order to minimise the number of synthetic steps, an enzymic technique was employed to prepare the *N*-acetyllactosamine **5**. The β-galactosidase from *Bacillus circulans* has been used successfully in the preparation of a variety of lactosamine derivatives using *p*-nitrophenyl β-D-galactopyranoside **3** as the glycoside donor,[16,17] based on the original procedure of Sakai *et al.*[18] in which the donor was lactose. An interesting result has been reported by Vetere and Paoletti who used lactose as the galactosyl donor and free *N*-acetylglucosamine **4** (GlcNAc) as the acceptor.[19] They showed

that low temperature favoured both the transglycosylation and the formation of *N*-acetyllactosamine **5**, and the competing glycohydrolase activity of the enzyme tended to grow significantly only after a few hours. *N*-Acetyllactosamine **5** was prepared in our laboratory in 67% yield by incubation of the donor **3** with 10 equivalents of acceptor GlcNAc **4** in the presence of the β-galactosidase from *B. circulans* at rt for 2 h. The unchanged acceptor **4** was separated from *N*-acetyllactosamine **5** by use of a charcoal–Celite column. *N*-Acetyllactosamine **5** was easily converted into its isopropylidene derivative **6** by treating it with 2,2-dimethoxypropane in the presence of PTSA.

Sulfation of compound **6** at low temperature followed by deprotection gave the sulfated disaccharide **2** in a reasonable yield (51%). This step showed the advantage of carrying out the sulfation at low temperature in that only minimum protection was required. For the synthesis of compound **1**, the disaccharide **6** was acetylated and then deprotected to give dihydroxy product **7** in 53% yield as a mixture of α and β anomers. Sulfation followed by deacetylation provided the disaccharide **1** in 76% yield. The number of sulfate groups in regioisomers **1** and **2** were confirmed by electrospray mass spectrometry (ES-MS), and their positions were confirmed by distortionless enhancement by polarisation transfer (DEPT) experiments. C-6′ in **1** and C-6 in **2** showed downfield shifts of 6.7 and 6.2 ppm, respectively. The results described here provide a simple method for the preparation of two sulfated *N*-acetyllactosamines (**1** and **2**) which are more efficient than previously published methods[20] owing to the introduction of the enzymic step.

Lin *et al*.[21] have described an elegant enzymic synthesis of di-*N*-acetylchitobiose 6-sulfate **10**, a component of Nod factors, from di-*N*-diacetyl chitobiose **8**. In their synthesis seven enzymes were used because, in addition to the sulfotransferase, an enzyme system is required for the *in situ* regeneration of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) which served as the sulfate donor for the sulfotransferase. However, the key enzymes in their synthesis (sulfotransferase, ATP sulfurylase and APS kinase) are not commercially available and are rather difficult to extract and assay although they have been produced by genetically engineering the genes from rhizobia into *Escherichia coli*.[21] An alternative route was developed based on the high selectivity achievable using chemical sulfation with careful control of reaction temperature. Combined with the use of the isopropylidene group to block the 6′-OH group, this sulfation procedure made possible highly selective sulfation of the C-6 hydroxymethyl group. Di-*N*-acetylchitobiose **8** was obtained by hydrolysis of colloidal chitin by using the chitinase[22] from *Streptomyces griseus* (Scheme 2). The isopropylidene



Scheme 2 *Reagents and conditions*: i, chitinase from *Streptomyces griseus*; ii, $MeC(OMe)_2Me$, H^+; iii, SO_3-pyridine complex, 5 °C; iv, aq. H^+

derivative **9** was obtained by reaction of chitobiose **8** with 2,2-dimethoxypropane in the presence of PTSA. The identity of the compound was confirmed by NMR spectroscopy. Synthesis of di-*N*-acetylchitobiose 6-sulfate **10** was completed by sulfation followed by deprotection. The identity of the product was confirmed by NMR spectroscopy and electrospray 9.4 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry.

The method described here provides a simple alternative to the enzymic synthesis of *N*,*N*′-diacetylchitobiose 6-sulfate **10**. It has potential for the economical synthesis of a range of selectively sulfated carbohydrates.

The sulfated disaccharides were tested as acceptors of fucosyl transfer catalysed by recombinant human fucosyltransferases III, IV, V, VI and VII (FT-III to -VII, respectively) (Table 1). The results obtained with the sulfated acceptors provide an interesting insight into the acceptor specificity of the human α3 fucosyltransferases. None of the sulfated acceptors was fucosylated by FT-III. This is somewhat surprising given a previous report[23] which suggested that FT-III displayed more activity with the 3′-sulfated derivative of lacNAc [β-D-Gal-(1→4)-D-GlcNAc] than lacNAc (*N*-acetyllactosamine) alone. In our hands, lacNAc is generally a relatively weak, but still measurable, acceptor for FT-III, with β-D-Gal-(1→3)-D-GlcNAc being the preferred acceptor (data not shown). FT-IV displays limited activity with the sulfated acceptors, but this would be expected, given the clear preference of FT-IV for neutral acceptor sugars.[24] The results obtained with both FT-V and -VI are interesting in that both enzymes display similar activities with both (6′-SO_4)- and (6-SO_4)-lacNAc. Indeed, the activity of FT-V with (6-SO_4)lacNAc was previously found to be useful in the synthesis of a sulfated sialyl Lewis X saccharide.[8] The preference of FT-V, over FT-VI, for (3′-SO_4)-lacNAc is unexpected, as both enzymes will use 3′-sialyl-lacNAc, in addition to other acceptors, with similar efficiencies.[25] The activity of FT-VI with β-D-GlcNAc-(1→4)-D-(6-SO_4)GlcNAc is a novel observation and is the only known example of fucosylation of a chitobiose disaccharide by a human α3 fucosyltransferase. The reduced activity of FT-VII with the sulfated acceptors is most probably owing to the lack of sialic acid α-(2→3)-linked to the galactose. Our own studies[26] and several published reports[27,28] have clearly shown that FT-VII requires this sialic acid for acceptor recognition. It is interesting to note that when FT-VII is presented with the α-(2→3)-sialylated derivative of 6-(SO_4)lacNAc it readily forms the fucosylated 6-sulfosialyl Lewis X antigen, a potential L-selectin-binding saccharide.[29]

This report describes a synthesis of a series of 6-sulfated disaccharides that can be used as acceptors for the human α3 fucosyltransferases. It should be noted that these results simply give an indication of whether the acceptors can be used as substrates by the α3 fucosyltransferases. No account is taken of the likely differences in K_m/V_{max} for each acceptor which could have significant implications for the efficiency of fucose transfer. Nevertheless, further analysis of the activities of the enzymes with these sugars could be useful in the determination of the likely order of fucosylation and sulfation during the biosynthesis of complex carbohydrates *in vivo*.[23,30]

Experimental

β-Galactosidase from *B. circulans* 'Biolacta' (E.C. 3.2.1.23) was a gift from Daiwa Kasei Co. Ltd., Osaka, Japan. Acceptor saccharides lacNAc and lacNac 3-sulfate were obtained from Dextra Laboratories, Reading, UK. GDP-[^{3}H]fucose (2.22 Tbq $mmol^{-1}$) was obtained from Amersham International (Amersham, UK). NMR spectra were recorded on a Bruker AC-250 or 400 spectrometer. *J*-values are quoted in Hz. Optical rotations were determined using an AA-1000 polarimeter (Optical Activity Ltd) with a 2 dm cell. Specific optical rotations $[\alpha]_D$ are given in units of 10^{-1} deg cm^2 g^{-1}. Low-resolution

Table 1 Relative activities of sulfated acceptors as α3 fucosyltransferase substrates.

Acceptor	FT-III	FT-IV	FT-V	FT-VI	FT-VII
β-D-Gal-(1→4)-D-GlcNAc	100	100	100	100	
α-NeuAc-(2→3)-β-D-Gal-(1→4)-D-GlcNAc					100
β-D-Gal(3-SO_4)-(1→4)-D-GlcNAc	0	11	68	22	21
β-D-Gal(6-SO_4)-(1→4)-D-GlcNAc	0	7	22	22	7
β-D-Gal-(1→4)-D-GlcNAc(6-SO_4)	0	2	27	23	10
β-D-GlcNAc-(1→4)-D-GlcNAc(6-SO_4)	0	2	0	40	0

Saccharides were tested as fucose acceptors by incubation with the five different fucosyltransferases plus GDP-fucose. Results are expressed as % control where 100% enzyme activity is that seen with β-D-Gal-(1→4)-D-GlcNAc as the acceptor for FT-III through -VI, and α-NeuAc-(2→3)-β-D-Gal-(1→4)-D-GlcNAc as the acceptor for FT-VII.

mass spectra were determined using a VG Analytical Quattra 2 spectrometer, and high-resolution mass spectra on a VG Analytical ZAB-E mass spectrometer or a Bruker Electrospray BioApex 9.4 T FTICR mass spectrometer. Carbon–Celite columns were prepared by mixing equal parts by weight of activated carbon (Darco G-60, 100 mesh, Aldrich Chemical Company) and Celite (Celite 535 [Fluka]) in water. The mixture was packed into a glass column under pressure. TLC was performed in 25 mm E. Merck silica gel plates (60F-254) and compounds were detected by spraying the plates with 12% aq. H_2SO_4 and heating. For column chromatography, Silica Gel 60 (Merck), particle size 0.040–0.063 mm, was used.

2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose 5

N-Acetylglucosamine **2** (7.4 g, 33.48 mmol) was dissolved in acetate buffer (50 mM, pH 5.0; 25 cm^3). Gal*p*NP **3** (1.0 g, 3.32 mmol) and β-galactosidase from *B. circulans* (50 mg) were added and the resulting mixture was stirred at rt for 1.5 h. The reaction was quenched by heating the mixture at 100 °C for 3 min. The mixture was then applied to a carbon–Celite column. The column was eluted successively with water (1 dm^3) and 5% aq. ethanol (5 dm^3) to remove the unchanged GlcNAc. It was then eluted with 15% aq. ethanol (5 dm^3). This fraction was concentrated by evaporation under reduced pressure and the residue was freeze dried to give *N*-acetyllactosamine **5** as a crystalline solid (0.86 g, 67%). NMR data corresponded to those reported previously;[31,32] δ_H(250 MHz; D_2O) 1.97 (3 H, s, $COCH_3$), 3.44–3.92 (12 H, m), 4.40 (1 H, d, *J* 7.6, H-1′), 4.65 (0.4 H, d, *J* 7, H-1β) and 5.13 (0.6 H, d, *J* 2, H-1α); δ_C(63 MHz; D_2O) 22.73, 23.01, 54.55, 57.03, 60.78, 60.90, 61.86, 69.39, 70.11, 71.09, 71.80, 73.33, 75.68, 76.18, 79.15, 79.59, 91.36, 95.70, 103.70, 103.76, 175.27 and 175.57.

2-Acetamido-2-deoxy-4-*O*-(4,6-*O*-isopropylidene-β-D-galactopyranosyl)-D-glucopyranose 6

N-Acetyllactosamine **5** (0.4 g, 1.04 mmol) was dissolved in dry DMF (13 cm^3). To the solution were added 2,2-dimethoxypropane (1.3 g, 12.5 mmol) and then PTSA (30 mg). The mixture was stored at rt and the reaction was followed by TLC (*n*-PrOH–$MeNO_2$–water, 10:9:2, v/v). After 2.5 h, saturated aq. $NaHCO_3$ (5 cm^3) and water (10 cm^3) were added. The solution was concentrated by evaporation under reduced pressure. The residue was dissolved in water and applied to a carbon–Celite column (45 cm × 3 cm). The column was eluted successively with 15% aq. ethanol (1 dm^3), 20% aq. ethanol (500 cm^3) and 25% aq. ethanol (7 dm^3). The 25% ethanol fraction was concentrated and freeze dried to give the isopropylidene derivative **6** as a powder (33 mg, 76%) (Found: $[M + H]^+$, 424.1826. $C_{17}H_{30}NO_{11}$ requires *m/z*, 424.1919); $[\alpha]_D^{23}$ +6.0 (*c* 0.5, water); δ_H(400 MHz; D_2O) 1.39 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 2.00 (3 H, s, $COCH_3$), 3.55–3.94 (10 H, m), 4.21 (1 H, d, *J* 12.9, 4.29 (1 H, d, *J* 3.3), 4.45 (1 H, d, *J* 7.9, H-1′), 4.68 (0.4 H, d, *J* 7.9, H-1β) and 5.17 (0.6 H, d, *J* 2.9, H-1α); δ_C(100 MHz; D_2O) 18.46, 22.57, 22.87, 28.82, 54.35, 56.79, 60.48, 60.62, 63.10, 66.95, 69.01, 69.77, 70.94, 71.75, 73.04, 75.50, 79.64, 79.91, 91.13, 95.55, 100.50, 103.52, 175.09 and 175.36; *m/z* (relative abundance) [FAB, polyethylene glycol/*m*-nitrobenzyl alcohol (NOBA)] 424 [$(M + H)^+$, 5%], 415 (100) and 412 (5).

Alternatively, *N*-acetyllactosamine **5** (857 mg, 2.24 mmol) was dissolved in dry DMF (20 cm^3). 2,2-Dimethoxypropane (2.5 g, 24 mmol) and PTSA (100 mg) were added. After 2 h, saturated aq. $NaHCO_3$ (10 cm^3) and water (30 cm^3) were added. The mixture was evaporated and the residue was applied to a Sephadex G25 column (2.5 cm × 90 cm). The column was eluted with water. Fractions containing the desired product were combined, concentrated and freeze dried to give title compound **6** as a powder (836 mg, 88%).

Sodium 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose 6-sulfate 2

Protected disaccharide **6** (74 mg, 0.17 mmol) was dissolved in anhydrous pyridine (8 cm^3) at 0 °C. SO_3–pyridine complex (110 mg, 0.69 mmol) was added and the resulting mixture was stirred at 5 °C for 18 h. Water (10 cm^3) was added to quench the reaction. The excess of pyridine was removed by co-evaporation with water (<35 °C). The residue was diluted with water (10 cm^3). Conc. HCl (40 mm^3) was added and the mixture was kept at rt. The reaction was followed by TLC (*n*-PrOH–$MeNO_2$–water, 10:9:2, v/v). After 2 h, the mixture was applied to an anion-exchange column (Dowex 1X2-400, Cl^--form). The column was washed with water (50 cm^3) and eluted with aq. NaCl (0.2 M). The NaCl eluate was concentrated under reduced pressure (<35 °C). The residue was applied to a Sephadex G25 column (2.5 cm × 90 cm) which was then eluted with water. Fractions containing the desired product were combined, concentrated and freeze dried to provide the sulfated *disaccharide* **2** as a powder (43 mg, 51%) {Found: (ES-MS) $[M + Na]^+$, 508.0684. $C_{14}H_{24}NNa_2O_{14}S$ requires *m/z*, 508.0707}; $[\alpha]_D^{22}$ +16.6 (*c* 1, (water); δ_H(400 MHz; D_2O) 2.03 (3 H, s, $COCH_3$), 3.49–3.54 (1 H, m), 3.64–3.94 (8.8 H, m), 4.17 (0.6 H, m, H-5), 4.27–4.39 (2 H, m, H-6), 4.517 [0.4 H, d, *J* 7.6, H-1′ (β-anomer)], 4.524 [0.6 H, d, *J* 7.9, H-1′ (α-anomer)] and 5.19 (0.6 H, d, *J* 2.9, H-1α); δ_C(63 MHz; water) 22.81, 23.09, 54.4, 56.92, 61.92, 67.25, 67.33, 69.15, 69.46, 70.08, 71.82, 73.17, 73.31, 73.45, 76.22, 78.19, 78.57, 91.51, 95.78, 103.39, 175.32 and 175.63; *m/z* (relative abundance) (ES-MS) 462 ($[M - Na]^-$, 30%) and 97 (100).

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose 7

Disaccharide **6** (83 mg, 1.96 mmol) was dissolved in ahydrous pyridine (10 cm^3). Acetic anhydride (5 cm^3) and DMAP (50 mg) were added. The resulting mixture was stored at rt for 5 h and concentrated by evaporation under reduced pressure. The residue was diluted with ethyl acetate (150 cm^3) and the solution was washed successively with water (20 cm^3), saturated aq. $NaHCO_3$ (5 × 15 cm^3), water (15 cm^3) and brine (15 cm^3). It was then evaporated under reduced pressure to give a brown syrup, which was dissolved in methanol (30 cm^3). Water (20 cm^3) and conc. HCl (400 mm^3) were added. The mixture was kept at rt and the reaction was followed by TLC (CH_2Cl_2–MeOH, 50:4, v/v). After 5 h, saturated aq. $NaHCO_3$ (6 cm^3) was added to quench the reaction. The mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2–

MeOH, 50:4, v/v) to give a syrup, which was dissolved in water (15 cm^3) and freeze dried to give the *disaccharide* **7** as a powder (61 mg, 53%) (Found: $[M + Na]^+$ 616.1849. $C_{24}H_{35}NNaO_{16}$ requires *m/z*, 616.1853); $[\alpha]_D^{23}$ +50.1 (*c* 1, water); δ_H(400 MHz; D_2O) 1.95 (3 H, s, $COCH_3$), 2.08–2.20 [15 H, m, 5 × $COCH_3$ (α- and β-anomer)], 3.75 (2 H, br s, H-6′), 4.07–4.22 (3 H, m), 4.37–4.48 (1.75 H, m), 4.68 (1 H, d, *J* 7, H-1′), 4.98–5.07 (2 H, m), 5.18–5.30 (1 H, m), 5.76 (0.25 H, d, *J* 8.9, H-1β) and 6.03 (0.75 H, d, *J* 3.7, H-1α); δ_C(100 MHz; D_2O) 20.89, 21.11, 21.18, 21.32, 22.33, 22.55, 50.93, 53.20, 60.98, 63.02, 66.98, 71.02, 71.10, 71.64, 73.60, 73.76, 74.19, 75.55, 76.45, 91.18, 92.58, 101.41, 172.47, 172.77, 173.39, 173.54, 173.94, 174.06, 174.30, 175.02 and 175.18; *m/z* (relative abundance) (FAB-NOBA) 616 ($[M + Na]^+$, 90%), 594 ($[M + H]^+$, 10) 556 (35) and 534 (100).

Sodium 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranoyl)-D-glucopyranose 6′sulfate 1

Sulfur trioxide–pyridine complex (110 mg, 0.69 mmol) was added to a solution of the disaccharide **7** (100 mg, 0.17 mmol) in anhydrous pyridine (5 cm^3) at 0 °C. The mixture was stirred at 5 °C for 20 h. Water (10 cm^3) was added and excess of pyridine was removed by co-evaporation with water under reduced pressure. The residue was diluted with water (10 cm^3) and the mixture was applied to an anion-exchange column (Dowex 1X2-400, Cl^--form). The column was washed with water (50 cm^3) and eluted with 0.2 M aq. NaCl. The NaCl eluate was concentrated and applied to a Sephadex G25 column (2.5 cm × 90 cm). Fractions containing the sugar were combined, concentrated and freeze dried to give a powder (93 mg), which was dissolved in anhydrous MeOH (10 cm^3) and NaOMe (31 mg) was added. The mixture was stirred at rt and the reaction was followed by TLC (*n*-PrOH–$MeNO_2$–water, 10:9:2, v/v). After 2 h the mixture was neutralised by passage through a cation-exchange column (Dowex 50W-X8, H^+-form). The neutralised eluate was applied to an anion-exchange column (Dowex 1X2-400, Cl^--form) which was eluted with water. The column was washed with water (70 cm^3) and eluted with aq. NaCl (0.15 M) (alternatively, the column could be eluted by gradient elution, 0→0.15 M). The eluate was concentrated, then applied to a Sephadex G25 column (2.5 cm × 90 cm). Fractions containing the desired product were combined, concentrated and freeze dried to give the *sulfated lactosamine* **1** as a powder (62 mg. 76%) {Found: (ES-MS) $[M + Na]^+$, 508.0706. $C_{14}H_{24}NNa_2O_{14}S$ requires *m/z*, 508.0707}; $[\alpha]_D^{22}$ +13.3 (*c* 1.25, water); δ_H(400 MHz; D_2O) 2.00 (3 H, s, $COCH_3$), 3.49–3.94 (10 H, m), 4.16–4.17 (2 H, m, H-6′), 4.47 (1 H, d, *J* 7.6, H-1′), 4.69 (0.4 H, d, *J* 7, H-1β) and 5.18 (0.6 H, d, *J* 2.3, H-1α); δ_C(100 MHz; D_2O) 22.61, 22.93, 54.40, 56.87, 60.72, 60.86, 67.94, 69.85, 68.96, 69.85, 70.83, 71.46, 72.97, 73.49, 75.42, 79.72, 80.26, 91.13, 95.55, 103.52, 103.72, 175.17 and 175.47; *m/z* (relative abundance) (ES-MS) 508 ($[M + Na]^+$, 100%) and 490 (30).

Di-*N*-acetylchitobiose 8

Colloidal chitin[33] (100 g containing more than 95% water) was suspended in acetic acid–phosphate buffer (0.1 M, pH, 6.3; 600 cm^3). Chitinase (26 mg) (Sigma) was added and the mixture was shaken at 37 °C. After 3 days, chitinase (7 mg) was added and the mixture was shaken for another 2 days. The mixture was filtered, the filtrate was applied to a carbon–Celite column, and the column was eluted with water (2.5 dm^3), 5% aq. ethanol (2 dm^3) and 10% aq. ethanol (6 dm^3). The 10% ethanol eluate was concentrated and freeze dried to give di-*N*-acetylchitobiose **8** (1.3 g).

2-Acetamido-4-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose 9

Di-*N*-acetylchitobiose **8** (0.3 g, 0.71 mmol) was dissolved in DMF (10 cm^3). 2,2-Dimethoxypropane (1.5 g, 14.42 mmol) and PTSA (30 mg) were added and the resulting mixture was stored at rt for 1 day. Saturated aq. $NaHCO_3$ (1 cm^3) was added. The mixture was diluted with water (30 cm^3) and applied to a carbon–Celite column, which was then eluted successively with water (400 cm^3), 10% ethanol in water (400 cm^3) and 20% ethanol in water (400 cm^3). The 20% aq. ethanol eluate was concentrated and freeze dried to give title compound **9** as a powder (0.2 g, 61%); $[\alpha]_D^{22}$ −11.4 (*c* 0.25, water) [Found: (FAB) $(M + Na)^+$, 487.1922. $C_{19}H_{32}N_2NaO_{11}$ requires *m/z*, 478.1904]; δ_H(400 MHz; D_2O) 1.40 (3 H, s, CH_3), 1.54 (3 H, s, CH_3), 1.99 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$), 3.40–3.98 (12 H, m, HC O), 4.59 [0.33 H, d, *J* 8.30, H-1′ (β anomer)], 4.60 [0.67 H, d, *J* 8.62, H-1′b (α anomer)], 4.64 [0.33 H, d, *J* 7.96, H-1b (β anomer)] and 5.13 [0.67 H, d, *J* 2.98, H-1 (α anomer)]; δ_C(62.9 MHz; D_2O) 19.24, 22.71, 22.96, 28.66, 54.64, 57.19, 57.29, 60.78, 61.92, 62.11, 67.42, 69.95, 70.91, 71.69, 73.19, 73.75, 75.43, 79.73, 80.11, 91.32, 95.59, 98.17, 101.79, 102.59, 175.27 and 175.44; *m/z* (relative abundance) (FAB-NOBA) 487 ($[M + Na]^+$, 100%), 465 ($[M + H]^+$, 25) and 244 (40).

Sodium 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose 6-sulfate 10

A mixture of **9** (0.1 g, 0.22 mmol), SO_3–pyridine complex (0.1 g, 0.63 mmol) and anhydrous pyridine (8 cm^3) was stirred at 5 °C for 19 h. Water (10 cm^3) was added and the pyridine was removed by co-evaporation with water (<35 °C). The residue was re-dissolved in water (15 cm^3), and conc. HCl (40 cm^3) was added. The mixture was kept at rt and the reaction was followed by TLC (*n*-PrOH–$MeNO_2$–water, 10:9:2, v/v). After 2 h, the reaction mixture was applied to an anion-exchange column (Dowex 1X2-400, Cl^--form). The column was washed with water, then was eluted with 0.2 M aq. NaCl. The NaCl eluate was concentrated and applied to a Sephadex G25 column (2.5 cm × 90 cm) which was eluted with water to give the *product* **10** (80 mg, 69%); $[\alpha]_D^{30}$ +13.6 (*c* 1, water) [Found: (Bruker Electrospray BioApex 9.4 T FTICR mass spectrometer) $(M + Na)^+$, 549.0973. $C_{16}H_{27}N_2Na_2O_{14}S$ requires *m/z*, 549.0971]; δ_H(400 MHz; D_2O) 2.02 (3 H, s, $COCH_3$), 2.08 (3 H, s, $COCH_3$), 3.44–3.92 (10 H, m, HCO), 4.06–4.26 (2 H, m, H-6), 4.62 (1 H, d, *J* 8.42, H-1′), 4.71 (0.33 H, d, *J* 7.76, H-1b) and 5.18 (0.67 H, d, *J* 2.90, H-1a); δ_C(100 MHz; D_2O) 22.70, 23.03, 54.36, 56.29, 56.85, 61.26, 67.00, 67.09, 68.73, 70.03, 70.47, 73.12, 74.41, 76.67, 79.49, 79.98, 91.37, 95.80, 102.09, 175.23 and 175.60; *m/z* (relative abundance) (Electrospray) 503 ($[M - Na]^-$, 100%). The DEPT experiment showed a downfield shift of the signal attributable to C-6.

Expression of α3 fucosyltransferases[34]

All five human α3 fucosyltransferases were expressed by stable transfection of CHO DG44 cells. Briefly, the full-length coding sequences of FT-III to -VII were cloned into pCDNA3, and CHO cells were transfected using lipofectin. Stable transfectants were selected by growth in the presence of 0.8 mg cm^{-3} G418 and cloned by limiting dilution.

Enzyme extraction and assay

Cells expressing recombinant α3 fucosyltransferases were harvested and washed twice with phosphate-buffered saline (NaCl, 150 mM; KH_2PO_4, 1.5 mM; NaH_2PO_4, 8 mM; pH 7.2). Pelleted cells were re-suspended in solubilisation medium (Triton X-100) (2% [v/v]), glycerol (20% [v/v]), NaCl (150 mM), $MgCl_2$ (10 mM), DTT (1 mM), buffered to pH 6.5 with sodium cacodylate [50 mM]), at 10^7 cells cm^{-3}, and incubated for 15 min at 40 °C prior to probe sonication (4 × 15 s; MSE Soniprep 150).

α3 FT activity was assayed as previously described.[35] The assay medium contained GDP-β-fucose (10 μM), GDP-[^{3}H]-fucose (100 000 cpm), ATP (1 mM), $MnCl_2$ (4 mM), buffered to pH 7.0 with Hepes†-NaOH (50 mM). Acceptor saccharides,

† 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid.

dissolved in distilled water, were added to a final concentration of 5 mM. The reaction was started by the addition of enzyme (typically 10–30 μg protein) and transfer to a water-bath at 37 °C. After 30–60 min at 37 °C, the reaction was halted by the addition of a slurry of ion-exchange resin (Dowex 1-X8, Cl^--form) (1 part, w/v) in distilled water (4 parts), mixed on a vortex mixer and centrifuged. The radioactivity in the supernatant (0.600 cm^3) was measured by liquid scintillation counting. Non-specific breakdown of GDP-fucose was assessed by duplicating the enzyme assay in the absence of acceptor saccharide. No loss of acceptor saccharide was noted after Dowex treatment.

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