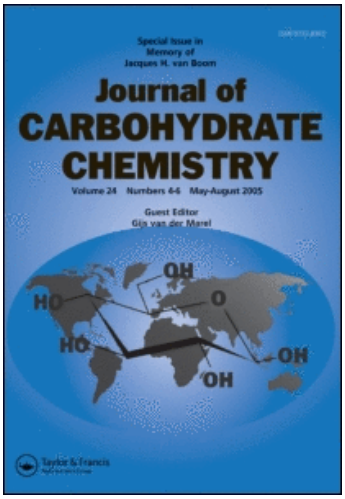

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Various fundamental biologic roles of milk oligosaccharides have been recognized; however, their structure-affinity relationship is still not fully revealed. Herein, we describe the synthesis of the fluorescent-labeled milk oligosaccharides 3-(5-dimethylaminonaphthalene-1-sulfonylamino)propyl β-D-galactopyranosyl-(1 → 3)-[α-L-fucopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) and 3-(5-dimethylaminonaphthalene-1-sulfonylamino)propyl β-D-galactopyranosyl-(1 → 3)-[α-L-fucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**2**) as useful tools for synthetic, analytic, and biologic applications. For the fucosylation of lactose and lacto-*N*-biose, the chemical and the enzymatic syntheses using fucosyltransferase III were compared.

Keywords Chemo-enzymatic synthesis, Fucosyltransferase III, Fluorescent-labeled milk oligosaccharides

INTRODUCTION

In human milk a great structural variety of oligosaccharides exists, which ranges from simple lactose to complex neutral or charged oligosaccharides containing lactose, lactosamine, and lacto-*N*-biose disaccharides, which may be connected in various ways, fucosylated, or sialylated. So far, more than a hundred different structures have been isolated and characterized.[1]

Although it has been traditionally observed that breastfeeding of infants is advantageous for their healthy development, only recently the biologic importance of milk oligosaccharides has been recognized.[1a] Among numerous other biologic roles, it has been demonstrated that they can inhibit bacterial adhesion to epithelial cells, and thus exert a protective action against infections.[1a,2] However, the oligosaccharidic structures responsible for these processes are yet to be identified and characterized.

Within a project devoted to the characterization of human milk oligosaccharides, different structures have been synthesized by chemical and chemo-enzymatic methods.[3] There, hydrolases have been exploited for the selective protection of specific hydroxyl groups in different building blocks, thus facilitating their manipulation for the subsequent chemical glycosylation reactions.

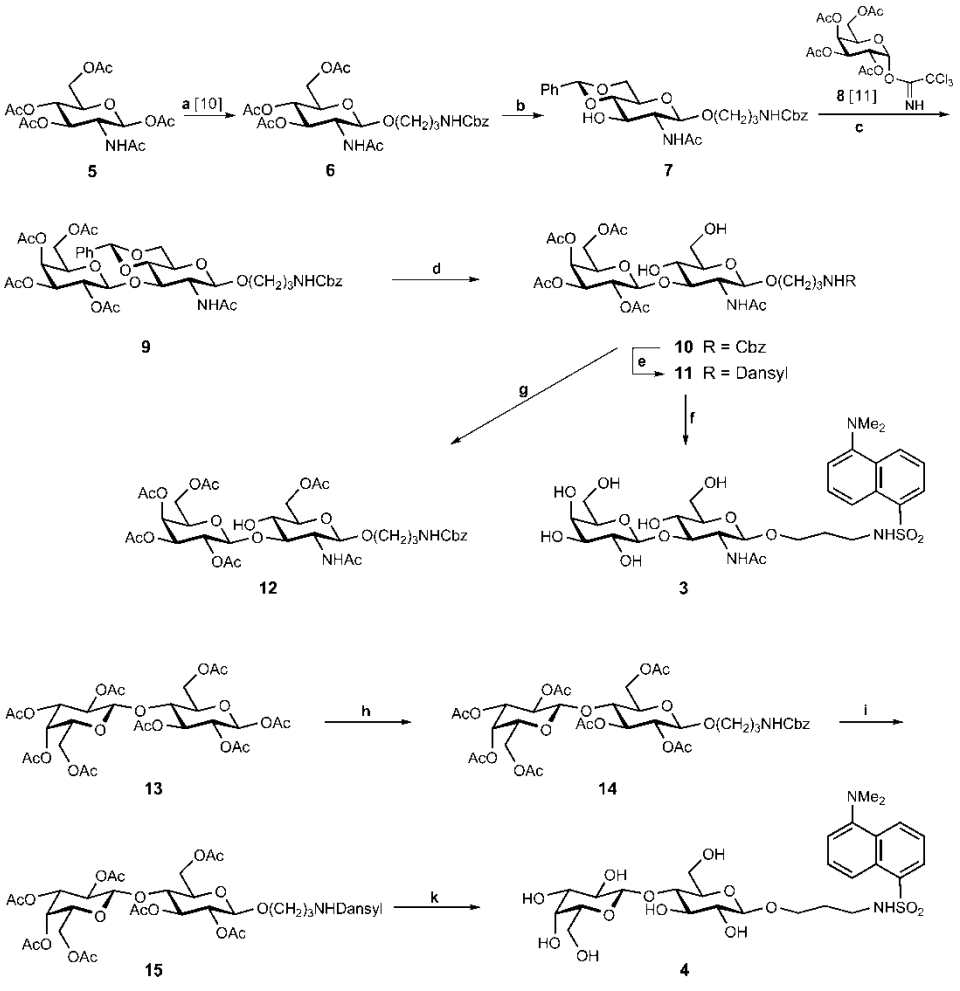
A second class of enzymes, which proved to be extremely useful for oligosaccharide preparation, are the glycosyltransferases devoted to the synthesis of glycosidic linkages *in vivo*. Scope and limitations of these enzymes have been extensively explored.[4] In particular, it has been demonstrated that some fucosyltransferases not only efficiently process natural substrates,[4a] but also are quite tolerant toward structural variations of the acceptor molecules. In addition, they also exhibit a broad tolerance toward modifications of the nucleotide sugar donor.[4b] This raised the question of whether fucosyltransferases could also be applied to the enzymatic fucosylation of fluorescent-labeled milk oligosaccharides.

Fluorescent labels are an extremely useful tool, because they allow the monitoring of low concentration of the chemical species of interest involved in biochemical processes. Fluorescence is commonly used for analytical purposes to visualize oligosaccharides after cleavage from glycoconjugates, to facilitate identification of particular oligosaccharides,[5] or to allow monitoring the progress of enzymatic reactions.[6] Fluorescent labeling has also been widely used for the determination of the cellular distribution of bioactive compounds, including carbohydrates,[7] and in the present case could be exploited to obtain information on adhesion phenomena between sugars and pathogenic microorganisms. In addition, fluorescent-labeled milk oligosaccharides would allow the elucidation of their role in cell recognition and to gain further information on their biodistribution. These findings could prove to be extremely useful for future applications in drug targeting.[8]

RESULTS AND DISCUSSION

As a consequence of its reported high substrate tolerance,[4] fucosyltransferase III (FucT III, EC2.4.1.65)[For the cDNA of FucT III see Ref.[9]] was selected for the enzymatic synthesis of fluorescent-labeled milk oligosaccharides, that is, the Lewis^a and Lewis^x derivatives **1** and **2** (Sch. 2). For this approach, the fluorescent-labeled disaccharides **3** and **4** (Sch. 1) were chemically synthesized and subsequently enzymatically or chemically fucosylated.[4] The *N*-acetylglucosamine derivative **6**, which can easily be obtained from peracetylated glucosamine **5**,[10] was used as starting material for the synthesis of lacto-*N*-biose derivative **3**. Deacetylation and benzylidene formation[11] afforded compound **7** in a state ready for glycosylation with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trichloroacetimidate (**8**) [12] in the presence of catalytic amounts of $BF_3 \cdot OEt_2$ ($\rightarrow$**9**). Acidic hydrolysis of the benzylidene protecting group ($\rightarrow$**10**) and removal of the carbobenzoxy protecting group by hydrogenolysis yielded the free primary amine, which was directly dansylated to afford the fluorescent-labeled compound **11**. Finally, the target molecule **3** was obtained by Zémplen deacetylation. Since attempts to perform a selective enzymatic acetylation on the 6-OH of the Glc*N*Ac moiety using a lipase failed,[3] the starting material **12** for the chemical fucosylation was obtained by regioselective acetylation of the 6-position at low temperature. The lactose derivative **4** (Sch. 1) was synthesized starting from the commercially available lactose octaacetate **13** by direct glycosylation of 3-benzyloxycarbonylamino-1-propanol, promoted by $BF3 \cdot OEt_2$ as Lewis acid. **14** was obtained stereoselectively, although in moderate yield. Deprotection of the primary amine followed by dansylation ($\rightarrow$**15**) and Zemplén deacetylation provided the lactose derivative **4**.

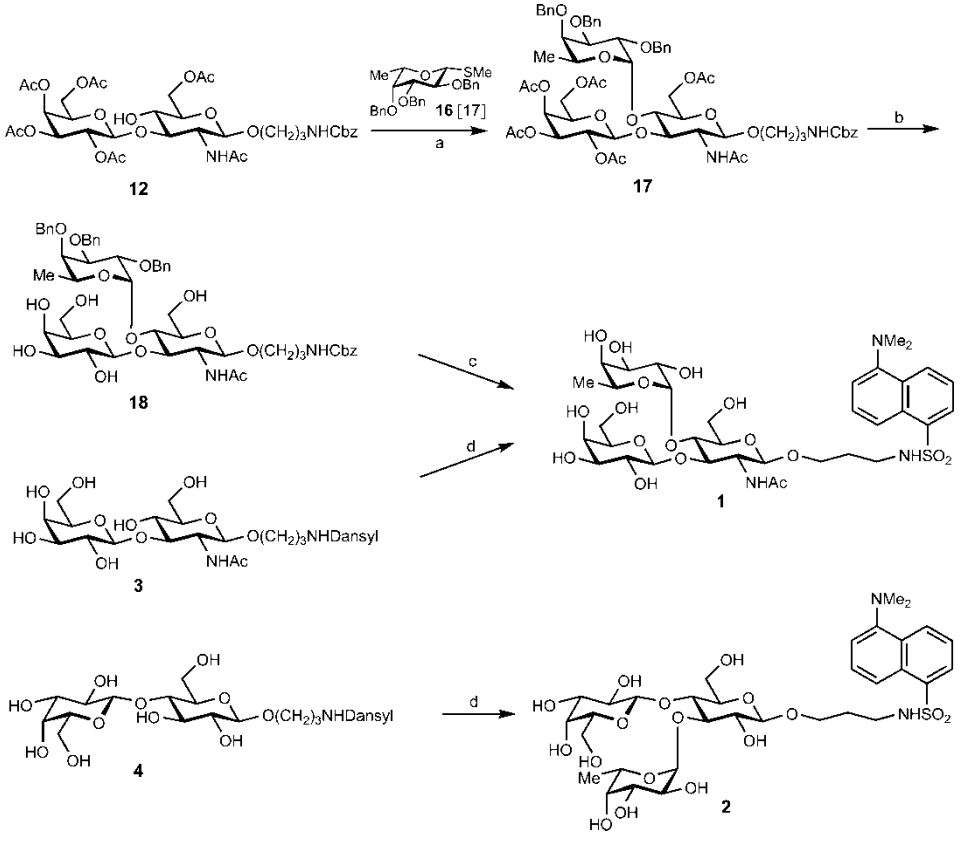
In the next step, type 1 and type 2 disaccharides **3** and **4** were analyzed for their ability to act as acceptors for FucT III using ^{14}C-labeled GDP-fucose.[13] For this purpose, we expressed a new construct of FucT III [Other constructs



Scheme 1: Synthesis of fluorescent-labeled lacto-*N*-biose **3** and lactose **4**: a) $HO(CH_2)_3NHCbz$, TMSOTf, $Cl(CH_2)_2Cl$, reflux, 88% (10); b) MeONa, MeOH, rt, then benzaldehyde-dimethylacetal, cat. pTsOH, DMF, 81%; c) 8 (11), $BF3{\bullet}OEt_2$, CH_2Cl_2, 0°C to rt, 78%; d) TFA/H_2O 7 : 3, CH_2Cl_2, 0°C, 83%; e) Pd/C, H_2, MeOH, rt, then dansyl chloride, TEA, CH_2Cl_2, rt, 72%; f) MeONa, MeOH, 57%; g) AcCl, pyridine, CH_2Cl_2, -78°C, 74%; h) $HO(CH_2)_3NHCbz$, $BF_3{\bullet}OEt_2$, CH_2Cl_2, rt, 41%; i) Pd/C, H_2, MeOH, rt, then dansyl chloride, TEA, CH_2Cl_2, rt, 58%; k) MeONa, MeOH, rt, 77%.

of FucT III have already been expressed in various expression systems][14] in a secretable form. FucT III cDNA[9] lacking the first 35 amino acids of the coding region was fused to a secretory sequence and a protein A binding domain. This construct was used for the transfection of *Sf*9 cells leading to the secreted enzyme, which could easily be purified by affinity chromatography. The enzyme shows predominantly $\alpha(1-4)$-activity (see lacto-*N*-biose, Table 1). With the nonnatural substrate **3**, an improved synthetic productivity

compared to the natural substrate lacto-*N*-biose was observed. The productivity with Lac*N*Ac and the type 2 derivative **4** is comparable. The maximum rate possible, when both donor and acceptor are saturated (k_{cat}), is often affected by rate-limiting substrate binding or product release steps. The specificity constant (k_{cat}/K_m) combines the effects of both rate and binding and therefore gives a better indication of the synthetic productivity of the enzyme. Since the major factor governing specificity is the stability of the enzyme-bound transition state, the specificity constant reflects how well the transition states, which are formed during the conversion of the substrates, are stabilized by the protein.[15] As expected for substrate **3**, the specificity constant is in the same order as the one for the natural acceptor substrate lacto-*N*-biose. The specificity constant for substrate **4**, however, is only 5% compared to the natural substrate (see Table 1), but is expected to still be



Scheme 2: Chemical and enzymatic fucosylation of fluorescent-labeled lacto-*N*-biose **3** and lactose **4**: a) $CuBr_2$, Bu_4NBr, MS 3 Å, DMF/CH_2Cl_2 1:1, rt, 96%; b) MeONa, MeOH, rt, quant.; c) $Pd(OH)_2$, H_2, MeOH, then dansyl chloride, TEA, MeOH, 52%; d) FucT III, GDPfucose, MnCl2, Na-cacodylate buffer, BSA, CIAP, 37°C, overnight (**3** → 1 (88%); **4** → **2** (54%)).

sufficient for a preparative application.[16] These data therefore prompted us to apply the enzymatic fucosylation reaction for the preparative synthesis of the fluorescent-labeled target molecules **1** and **2** (Sch. 2).

In addition, the Lewis^a derivative **1** was also synthesized by a chemical glycosydation (Sch. 2) using thioglycoside **16**[17] as donor. In the first attempt, the electrophile was generated by the conditions described by Ogawa et al.[18] using cupric bromide as thiophilic promoter. Although the presence of an acylamino function is known to be potentially problematic, the fucosylation worked successfully, giving the Lewis^a derivative **17** in almost quantitative yield. Unfortunately, the product retained traces of the copper salt, which could not be removed by washing with EDTA or concentrated ammonia solutions or by repeated filtrations through silica gel. Because of the paramagnetic properties of copper, the ^{1}H NMR spectrum contained broad lines and was impossible to interpret, whereas the ^{13}C NMR spectrum was in good agreement with the expected structure. For the final confirmation of the structure of the trisaccharide **17**, the glycosylation was repeated using NIS-TfOH as promoter. This change of the conditions resulted in a much lower yield (26%), but allowed the full characterization of **17**. After deacetylation leading to **18**, the removal of the copper contamination was readily achieved. Therefore, the cupric bromide-promoted glycosylation turned out to be the method of choice, despite the inconvenience of the contamination with copper salts. Final deprotection (i.e., removal of the benzyl and the carbobenzoxy protecting groups by hydrogenolysis) followed by dansylation afforded fluorescent-labeled Lewis^a trisaccharide **1**.

CONCLUSION

With FucT III, an excellent specificity constant for type 1 substrate **3** (137% with respect to the natural substrate lacto-*N*-biose) was obtained, leading to **1** in a high yield (see Sch. 1 Table 1). The enzyme, however, exhibits only a weak affinity for the lactose derivative **4** (5% compared to the natural substrate lacto-*N*-biose). However, the kinetic parameters proved to be sufficient for the preparative synthesis of the Lewis^x derivative **2**. The specificity constant for **4**

Table 1: Kinetic parameters of FucT III for the natural substrates lacto-*N*-biose and *NAc*-lactosamine and the non-natural derivatives **3** and **4**

Substrate	k_{cat} (min^{-1})	Km (μM)	k_{cat}/K_m ($mM^{-1} \cdot min^{-1}$)
Lacto-*N*-biose (type 1)	6.09	182	33.46
Lac*NAc* (type 2)	0.82	691	1.19
3	8.92	194	45.97
4	7.06	4470	1.58

is comparable to the one for Lac*N*Ac, indicating that the *N*-acetyl group is not contributing to the stabilization of the transition state formed during the conversion of the enzyme-bound substrate to the product.

In conclusion, a comparison of the two approaches applied to the synthesis of trisaccharide **1** indicates that overall the purely chemical and the chemo-enzymatic approaches are equally efficient. However, the main differences are as follows: in the chemical approach the fluorescent label is introduced in the last step, whereas in the chemo-enzymatic approach the fluorescently labeled acceptor is enzymatically fucosylated. The latter furnishes us with the possibility of using a variety of GDP-fucose analogs[19] to form a library of trisaccharides in one final step. Moreover, FucT III shows a remarkable substrate tolerance for structurally modified type 1 disaccharides. Although the reaction rates with type 2 or lactose derivatives are much lower, they are still sufficient for preparative applications.

EXPERIMENTAL

Dry solvents and liquid reagents were distilled prior to use: dichloromethane and pyridine were distilled from calcium hydride; 1,2-dichloroethane, DMF, and methanol were dried on 4Å molecular sieves; all reaction vessels, after being dried, were kept under argon. Organic solutions were dried over anhydrous sodium sulfate, and the solvent was evaporated at reduced pressure below 40°C. All reactions were monitored by TLC on silica gel 60 F−254 plates (Merck), spots being developed with 5% sulfuric acid in methanol/water (1 : 1) or with phosphomolybdate-based reagent. Silica gel Merck 60 (230−400 mesh) was used for flash chromatography. Optical rotations were measured at rt with a Perkin-Elmer 241 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 300 and Varian Gemini 200 spectrometers in solution. Chemical shifts are given in ppm (δ), relative to $SiMe_4$ as internal standard; J values are given in Hz. IR spectra were measured by a Perkin-Elmer 1420 spectrophotometer (NaCl crystal windows). Mass spectrometry was performed on a Thermo Quest Finningan LCQ^{TM} DECA spectrometer. Mass spectra were recorded using negative and positive electrospray (ES) as indicated. Elemental analyses were performed using the Carlo Erba elemental analyzer 1108.

3-(Benzyloxycarbonylamino)propyl 2-acetylamino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (7)

Compound **6** (2.42 g, 4.50 mmol) was dissolved in anhydrous methanol (10 mL) and 0.5M sodium methoxide (2.7 mL) was added. After 2 h the reaction mixture was neutralized with IR 120 resin, H^+ form filtered, and concentrated. The crude material was dissolved in DMF (20 mL), benzaldehyde

dimethylacetal (0.65 g, 4.28 mmol) and *p*-toluenesulfonic acid (0.01 g) were added, the solution was distilled under vacuum at 60°C until TLC (ethyl acetate) revealed the disappearence of the starting material, and it was concentrated. The crude material was diluted with dichloromethane and then washed with 5% $NaHCO_3$ solution. The organic layer was dried and concentrated to afford the title compound **7** (1.74 g, 81%) as a foam. $[\alpha]_D$ −38.1 (*C* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.40–1.95 (m, 2H, CH_2), 2.10 (s, 3H, CH_3CONH), 2.92–3.12 (m, 1H, CH_aH$_b$NH), 3.16–3.40 (m, 2H, H-2, CH$_a$$H_b$NH), 3.55–3.86 (m, 5H, H-4, H-5, H-6a, CH_2O), 3.90–4.04 (dt, 1H, $J_{2,3} = J_{3,4}$ 9.2 Hz, $J_{3,OH}$ 3.4 Hz, H-3), 4.16 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.30 (dd, 1H, $J_{6a,6b}$ 10.3 Hz, $J_{6b,5}$ 4.9 Hz, H-6b), 4.90–5.20 (m, 3H, CH_2Ph, NH), 5.57 (s, 1H, benzylidene), 7.25–7.60 (m, 10H, Ph), 7.70 (d, 1H, J 7.7 Hz, N*H*COCH$_3$); ^{13}C NMR ($CDCl_3$) δ 23.3 (s, CH_3CONH), 30.9 (t, CH_2), 37.0 (t, CH_2NH), 59.6 (d, C-2), 66.6, 66.8, 67.8 (3t, C-6, CH_2O, CH_2Ph), 69.2, 74.8, 82.5 (3d, C-3, C-4, C-5,), 102.1, 102.63 (C-1, CHPh), 127.1–129.8 (10d, CHAr), 137.2, 137.9 (2s, CqAr), 158.0 (s, NHCO), 174.9 (s, CH_3CO); IR (nujol): 3300, 1690 cm^{-1}; ESI/MS (positive ion mode), *m/e* 501 $[M + 1]^+$; Anal. Calcd for $C_{26}H_{32}N_2O_8$: C, 62.39; H, 6.44; N, 5.60. Found: C, 62.61; H, 6.12; N, 5.33.

3-(Benzyloxycarbonylamino)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (9)

Compound **7** (1.00 g, 2.0 mmol) and donor **8** (1.48 g, 3.0 mmol) were dissolved in dichloromethane (50 mL) under inert atmosphere, then at 0°C BF3 · OEt_2 (1 mL, 0.1M solution in dichloromethane) was added. After stirring for 3 h at rt, the solution was neutralized by addition of Et_3N and concentrated. Flash chromatography of the residue (ethyl acetate) gave **9** (1.30 g, 78%) as a foam. $[\alpha]_D$ − 16.2 (*C* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.65–1.90 (m, 2H, CH_2), 1.95–2.10 (5s, 15H, $COCH_3$), 3.15–3.40 (m, 3H, CH_2NH, H-2), 3.45–3.80 (m, 5H, H-4, H-5, H-6a, H-5′, CH_aH$_b$O), 3.85–4.00 (m, 2H, H-6′a, CH$_a$$H_b$O), 4.10 (m, 1H, H-6′b), 4.30 (dd, 1H, $J_{6a,6b}$ 10.3 Hz, $J_{6b,5}$ 4.6 Hz, H-6b), 4.45 (t, 1H, J 9.0 Hz, H-3), 4.75 (d, 1H, J 8.0 Hz, H- 1′), 4.90 (dd, 1H, $J_{3',4'}$ 10.3 Hz, $J_{3',2'}$ 3.3 Hz, H-3′), 5.05–5.25 (m, 5H, CH_2Ph, NH, H-1′, H-2′), 5.30 (d, 1H, H-4′), 5.50 (s, 1H, benzylidene), 6.10 (d, 1H, J 7.1 Hz, N*H*COCH$_3$), 7.25–7.60 (m, 10H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.5, 20.5, 20.5, 23.4 (5q, CH_3CO), 29.5 (t, CH_2), 37.9 (t, CH_2NH), 57.6 (d, C-2), 60.9 (t), 66.0 (d), 66.6 (t), 66.8 (d), 67.4 (t), 68.6 (t), 69.4 (d), 70.5 (d), 71.0 (d), 77.2 (d), 80.5 (d), 100.0, 100.4, 101.4 (3d, C-1, C-1′, CHPh), 126.0–129.1 (10d, CHAr), 136.6, 137.1 (2s, CqAr), 156.5 (s, NHCO), 169.4, 170.0, 170.0, 170.0, 170.8 (5s, CH_3CO); IR (nujol): 3400, 1740, 1700, 1650; ESI/MS (positive ion mode), *m/e* 831 $[M + 1]^+$; Anal. Calcd for $C_{40}H_{50}N_2O_{17}$: C, 57.83; H, 6.07; N, 3.37. Found C, 57.59; H, 5.80; N, 3.58.

3-(Benzyloxycarbonylamino)propyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside (10)

To a stirred solution of compound 9 (0.090 g, 0.11 mmol) in dichloromethane (1 mL) at 0°C trifluoroacetic acid (1 mL, 70% aqueous solution) was added. After stirring for 10 min at 0°C, the solution was neutralized by addition of satd. aqueous Na_2CO_3, extracted with dichloromethane (3 × 10 mL), and concentrated. Benzaldehyde was coevaporated with toluene. Flash chromatography (ethyl acetate) of the crude material afforded **10** (0.070 g, 83%) as a foam. $[\alpha]_D$ −0.2 (*C* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.68–1.75 (m, 2H, CH_2), 1.97, 1.97, 2.05, 2.07, 2.14 (5s, 15H, $COCH_3$), 3.15–3.60 (m, 6H, C*H*$_2$NH, H-3, H-3′, H-5, C*H*$_a$H$_b$O), 3.65–3.75 (m, 2H, CH$_a$*H*$_b$O, OH), 3.80–3.95 (m, 3H, 2H-6, OH), 3.95–4.05 (t, 1H, H-5′), 4.10–4.20 (m, 3H, 2H-6′, H-2), 4.55 (d, 1H, J 7.9 Hz, H-1′), 4.72 (d, 1H, J 8.3 Hz, H-1), 5.00 (dd, 1H, $J_{2',3'}$ 10.4 Hz, $J_{4',3'}$ 3.3 Hz, H-3′), 5.05–5.20 (m, 3H, CH_2Ph, NH), 5.23 (dd, 1H, H-2′), 5.35 (d, 1H, H-4′), 6.25 (d, 1H, $J_{2,NH}$7.7 Hz, N*H*COCH$_3$), 7.30–7.45 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.5, 20.5, 20.7, 23.4 (5q, CH_3CO), 29.7 (t, CH_2), 37.6 (t, CH_2NH), 56.5 (d, C-2), 61.5, 62.7 (2t, C-6, C-6′), 66.8 (2t, CH_2O, CH_2Ph), 66.9 (d), 68.9 (d), 70.1 (d), 70.7 (d), 71.1 (d), 75.3 (d), 83.8 (d), 99.7, 101.4 (2d, C-1, C-1′), 128.0, 128.2, 128.2, 128.6, 128.6 (5d, CH_{Ar}), 137.5 (s, CqAr), 156.5 (s, NHCO), 169.4, 170.0, 170.1, 170.5, 170.8 (5s, CH_3CO); IR (nujol): 3300, 1740, 1700 cm^{-1}; ESI/MS (positive ion mode), *m*/*e* 743 $[M + 1]^+$; Anal. Calcd for $C_{33}H_{46}N_2O_{17}$: C, 53.37; H, 6.24; N, 3.77. Found: C, 53.09; H, 6.51; N, 3.51.

3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (11)

Compound **10** (0.106 g, 0.14 mmol) was dissolved in methanol (4 mL), and 10% Pd-C (0.100 g) and glacial acetic acid (five drops) were added. The mixture was kept in a hydrogen atmosphere with vigorously stirring for 1 h. The mixture was filtered over a Celite pad and concentrated. The crude material was dissolved in 5 mL of dichloromethane/methanol (7 : 3), and dansyl chloride (0.039 g, 0.14 mmol) and Et_3N (0.024 mL, 0.172 mmol) were added. After stirring for 1 h at rt, the reaction was diluted with dichloromethane (20 mL) and washed with water (15 mL). The aqueous layer was separated and extracted with dichloromethane (3 × 15 mL), the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (ethyl acetate) affording the title compound **11** (0.087 g, 72%) as a yellow, amorphous solid. $[\alpha]_D$ −1.75 (*C* 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.60–1.70 (m, 2H, CH_2), 1.95, 1.98, 2.03, 2.06, 2.13 (5s, 15H, CH_3CO), 2.87 (s, 6H,

$N(CH_3)_2$), 2.98–3.05 (m, 2H, CH_2NH), 3.30 (m, 1H, H-2), 3.40–3.46 (m, 2H, 2H-6), 3.56–3.64 (m, 2H, CH_aH$_b$O), 3.71–3.77 (m, 1H, H-5′), 3.83–3.95 (m, 4H, H-4, CH$_a$$H_b$O, 2 H-6′), 3.98–4.03 (m, 1H, H-5′), 4.10–4.12 (m, 2H, 2H-6′), 4.18 (m, 1H, H-3), 4.57 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.78 (d, 1H, $J_{1',2'}$ 8.1 Hz H-1′), 5.00 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{4',3'}$ 3.5 Hz, H-3′), 5.20 (dd, 1H, H-2′), 5.35 (d, 1H, H-4′), 5.86 (t, 1H, J 5.9 Hz, $NHSO_2$), 6.46 (d, 1H, J 7.0 Hz, NH), 7.17 (d, 1H, J 7.6 Hz, H_{Ar}), 7.48–7.57 (m, 2H, H_{Ar}), 8.17–8.29 (m, 2H, 2H_{Ar}), 8.53 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.5, 20.5, 20.7, 23.6 (5q, CH_3CO), 29.0 (t, CH_2), 40.5 (t, CH_2NH), 45.4 (2q, $(CH_3)_2N$), 56.6 (d, C-2), 61.4 (t), 62.7 (t), 67.0 (t), 67.2 (t), 68.8 (t), 70.0 (d), 70.8 (d), 71.0 (d), 75.6 (d), 83.4 (d), 99.8, 101.4 (2d, C-1, C-1′), 115.3 (d, CH Dans), 118.7 (d, CH Dans), 123.2 (d, CH Dans), 128.4 (d, CH Dans), 129.2 (d, CH Dans), 129.6 (s, Dans), 129.9 (s, Dans), 130.5 (d, CH Dans), 134.8 (s, CSO_2), 152.0 (s, $CN(CH_3)_2$), 169.5, 170.0, 170.1, 170.5, 171.2 (5s, CH_3CO); IR (nujol): 3250, 1750 cm^{-1}; ESI/MS (positive ion mode), *m/e* 842 $[M+1]^+$; Anal. Calcd for $C_{37}H_{51}N_3O_{17}S$: C, 52.79; H, 6.11; N, 4.99. Found: C, 52.53; H, 6.35; N, 4.72.

3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (3)

To a stirred solution of compound **11** (0.087 g, 0.10 mmol) in methanol (1 mL), 1M sodium methoxide in anhydrous methanol (0.155 mL) was added. After stirring for 1 h at rt, the solution was neutralized by addition of IRC-86 resin, H^+ form; the resin was filtered off and washed with methanol and the filtrate was concentrated. Flash chromatography of the residue (ethyl acetate/methanol/water, 7 : 2 : 1) afforded **3** (0.040 g, 57 %) as a yellow solid. $[\alpha]_D$ −2.4 (*C* 0.25, CD_3OD); ^{1}H NMR (CD_3OD) δ 1.59–1.66 (m, 2H, CH_2), 1.89 (s, 3H, $NHCOCH_3$), 2.90 (s, 6H, $N(CH_3)_2$), 2.92–2.96 (m, 2H, *CH*$_2$NH), 3.18–3.90 (m, 14H, CH_2O, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2H-6′), 4.27 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.39 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 7.29 (d, 1H, J 7.0 Hz, H_{Ar}), 7.58–7.63 (m, 2H, H_{Ar}), 8.20 (dd, 1H, J 7.0 Hz, J 1.0 Hz, H_{Ar}), 8.37 (d, 1H, J 8.7 Hz, H_{Ar}), 8.58 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR (CD_3OD) δ 23.5 (q, CH_3CONH), 31.2 (t, CH_2), 41.4 (t, CH_2NH), 46.1 (2q, $(CH_3)_2N$)), 56.6 (d, C-2), 62.8, 63.1, 68.0 (3t, C-6, C-6′, CH_2O), 70.6 (d), 71.0 (d), 72.7 (d), 75.0 (d), 77.4 (d), 77.9 (d), 85.5 (d), 102.6, 105.9 (2d, C-1, C-1′), 116.8 (d, CH Dans), 120.9 (d, CH Dans), 124.6 (d, CH Dans), 129.4 (d, CH Dans), 130.3 (d, CH Dans), 131.3 (s, Dans), 131.4 (d, CH Dans), 131.5 (s, Dans), 131.4 (s, CSO_2), 153.6 (s, $CN(CH_3)_2$), 174.5 (s, CH_3CONH); IR (nujol): 3300, 1630, 1570, 1320 cm^{-1}; ESI/MS (negative ion mode), *m/e* 672 $[M-1]^-$; Anal. Calcd for $C_{29}H_{43}N_3O_{13}S$: C, 51.70; H, 6.43; N, 6.24. Found: C, 51.48; H, 6.62; N, 6.09.

3-(Benzyloxycarbonylamino)propyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside (12)

To a stirred solution of compound **10** (0.460 g, 0.62 mmol) in dichloromethane (7.5 mL) at −78°C pyridine (0.250 mL, 3.10 mmol) and acetyl chloride (0.140 mL, 1.86 mmol) were added. The solution was stirred at −78°C for 10 min, then quenched by addition of methanol and concentrated. The residue was purified by flash chromatography (ethyl acetate/methanol, 9:1), affording the title compound **12** (0.360 g, 74%) as a white, amorphous solid. $[\alpha]_D$ −1.3 (*C* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.62–1.82 (m, 2H, CH_2), 1.95–2.14 (6s, 18H, CH_3CO), 3.14–3.24 (m, 2H, CH_2NH), 3.34–3.52 (m, 4H, H-3, H-4, H-5, CH_aH_bO), 3.82–3.92 (m, 2H, CH_aH_bO, OH), 4.00 (m, 1H, H-5′), 4.09–4.18 (m, 3H, H-2, 2 H-6′), 4.23 (d, 1H, $J_{6a,6b}$ 12.0 Hz, $J_{6a,5}$ 5.0 Hz, H-6a), 4.40 (d, 1H, H-6b), 4.54 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.69 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.99 (dd, 1H, $J_{3',2'}$ 11.0 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.02–5.12 (m, 3H, CH_2Ph, NH), 5.21 (dd, 1H, H-2′), 5.36 (d, 1H, H-4′), 6.10 (d, 1H, $NHCOCH_3$), 7.30–7.45 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.5–23.4 (6q, CH_3CO), 30.4 (t, CH_2), 38.4 (t, CH_2NH), 57.6 (d, C-2), 62.2, 63.9 (2t, C-6, C-6′), 67.4, 67.6 (2t, CH_2O, CH_2Ph), 69.5 (2d), 69.9 (d), 71.5 (d), 71.9 (d), 74.2 (d), 84.4 (d), 100.0, 102.4 (2d, C-1, C-1′), 128.7–129.3 (5d, CHAr), 137.6 (s, CqAr), 157.6 (s, NHCO), 169.4–170.8 (6s, CH_3CO); IR (nujol): 3300, 1750, 1660 cm^{-1}; ESI/MS (positive ion mode), *m/e* 785 $[M+1]^+$; Anal. Calcd for $C_{35}H_{48}N_2O_{18}$: C, 53.57; H, 6.17; N, 3.57. Found: C, 53.78; H, 6.29; N, 3.34.

3-(Benzyloxycarbonylamino)propyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (14)

To a stirred solution of octa-*O*-acetyl-β-lactose **13** (0.548 g, 0.81 mmol) and 3-benzyloxycarbonylamino-1-propanol (0.203 g, 0.97 mmol) in dichloromethane (11.5 mL) at 0°C $BF_3 \cdot OEt_2$ (0.200 mL, 1.61 mmol) was added. The solution was stirred at rt for 17 h, then Et_3N was added until neutralization. The solvent was removed under reduced pressure. Flash chromatography (hexane/ethyl acetate, 4:6) afforded **14** (0.274 g, 41%) as a foam. $[\alpha]_D$ −6.6 (C 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.75–1.77 (m, 2H, CH_2), 1.95, 2.00, 2.03, 2.03, 2.04, 2.07, 2.13 (7s, 21H, CH_3CO), 3.15–3.18 (m, 2H, CH_2NH), 3.52–3.60 (m, 2H, CH_aH_bO, H-5), 3.76 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 3.80–3.90 (m, 2H, H-5′, CH_aH_bO, 4.03–4.20 (m, 3H, H-6a, 2H-6′), 4.40–4.55 (m, 3H, H-1, H-1′, H-6b), 4.87 (dd, 1H, $J_{1,2}$ 9.5 Hz, $J_{2,3}$ 8.0 Hz, H-2), 4.95 (dd, 1H, $J_{2',3'}$ 10. 5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.00–5.21 (m, 4H, CH_2Ph,

H-2′, NH), 5.17 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.34 (d, 1H, H-4′), 7.15–7.40 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.3–20.6 (7q, CH_3CO), 29.5 (t, CH_2), 38.1 (t, CH_2NH), 60.8, 61.9 (2t, C-6, C-6′), 66.4 (t), 66.7 (d), 67.3 (t), 69.1 (d), 69.7 (d), 70.6 (d), 70.9 (d), 71.6 (d), 72.7 (d), 76.1 (d), 100.3, 100.9 (2d, C-1, C-1′), 127.9, 127.9, 127.9 (3d, CHAr), 128.3, 128.3 (2d, CHAr), 136.7 (s, CqAr), 156.4 (s, NHCO), 168.9, 169.4, 169.6, 169.8, 169.9, 170.1, 170.2 (7s, CH_3CO); IR (nujol): 3391, 1751, 1725 cm^{-1}; ESI/MS (positive ion mode), *m/e* 845 $[M + 18]^+$; Anal. Calcd for $C_{37}H_{49}NO_{20}$: C, 53.69; H, 5.97; N, 1.69. Found: C, 53.42; H, 5.81; N, 1.57.

3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (15)

Compound **14** (0.340 g, 0.41 mmol) was dissolved in methanol (4 mL), and 10% Pd-C (0.400 g) and glacial acetic acid (two drops) were added. The mixture was kept in a hydrogen atmosphere with vigorous stirring for 1 h. The mixture was filtered over a Celite pad and concentrated. The crude material was dissolved in dichloromethane (5 mL), and dansyl chloride (0.110 g, 0.41 mmol) and Et_3N (0.07 mL, 0.49 mmol) were added. After stirring for 2 h at rt the reaction was quenched by addition of methanol, then diluted with dichloromethane (20 mL) and washed with water (15 mL). After phase separation the aqueous layer was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (hexane/ethyl acetate, 1:1) affording **15** (0.220 g, 58%) as an amorphous solid. $[\alpha]_D$ −15.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.60–1.70 (m, 2H, CH_2), 1.90–2.20 (7s, 21H, CH_3CO), 2.90 (s, 6H, 2 $N(CH_3)_2$), 2.92–3.05 (m, 2H, CH_2NH), 3.45–3.60 (m, 2H, CH_aH_bO, H-5), 3.72–3.91 (m, 3H, CH_aH_bO, H-4, H-5′), 4.04–4.15 (m, 3H, H-6a, 2H-6′), 4.37 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.50–4.57 (m, 2H, H-6b, H-1′), 4.80 (t, 1H, $J_{1,2} = J_{2,3}$ 8.8 Hz, H-2), 4.97 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$ 3.7 Hz, H-3′), 5.07–5.22 (m, 3H, H-2′, H-3, NH), 5.35 (d, 1H, H-4′), 7.19 (d, 1H, J 7.5 Hz, H_{Ar}), 7.48–7.62 (m, 2H, H_{Ar}), 8.23 (d, 1H, J 7.1 Hz, H_{Ar}), 8.31 (d, 1H, J 8.6 Hz, H_{Ar}), 8.54 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR ($CDCl_3$) δ 20.4–20.7 (7_2, CH_3CO), 29.4 (t, CH_2), 40.4 (t, CH_2NH), 45.4, 45.4 (2q, $(CH_3)_2N$), 60.8, 61.8 (2t, C-6, C-6′), 66.6 (d), 67.1 (t, CH_2O), 69.1 (d), 70.7 (d), 71.0 (d), 71.6 (d), 72.8 (d), 76.1 (d), 77.0 (d), 100.3, 101.0 (2d, C-1, C-1′), 115.1 (d, CH Dans), 118.9 (d, CH Dans), 123.2 (d, CH Dans), 128.2 (d, CH Dans), 129.4 (d, CH Dans), 129.9 (2s, Dans), 130.3 (d, CH Dans), 135.0 (s, CSO2), 152.0 (s, $CN(CH_3)_2$), 169.0–170.5 (7s, CH_3CO); IR (nujol): 3370, 1750, 1650, 1375 cm^{-1}; ESI/MS (positive ion mode), *m/e* 927 $[M + 1]^+$; Anal. Calcd for $C_{41}H_{54}N_2O_{20}S$: C, 53.13; H, 5.87; N, 3.02. Found: C, 52.88; H, 6.13; N, 3.21.

3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl β-D-galactopyranosyl-(1→4)- β-D-glucopyranoside (4)

Compound **15** (0.086 g, 0.09 mmol) was dissolved in methanol (2 mL), and 1M sodium methoxide in anhydrous methanol (0.220 mL) was added. After stirring for 2 h at rt, the solution was neutralized by addition of IRC-86 resin, H^+ form; the resin was filtered off and the filtrate was concentrated. The residue was purified by flash chromatography (ethyl acetate/methanol/water, 7 : 2 : 1), affording 4 (0.045 g, 77%) as a yellow foam. $[\alpha]_D$ −2.0 (C 1.4, CH_3OH); ^1H NMR (CD_3OD) δ 1.65 (m, 2H, CH_2), 2.87 (s, 6H, $N(CH_3)_2$), 2.99 (t, 2H, J 6.6 Hz, CH_2NH), 3.14–3.83 (m, 14H, CH_2O, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2H-6′), 4.14 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.35 (d, 1H, $J_{1',2'}$ 7.0 Hz, H-1′), 7.25 (d, 1H, J 7.5 Hz, H_{Ar}), 7.51–7.62 (m, 2H, H_{Ar}), 8.18 (d, 1H, J 6.8 Hz, H_{Ar}), 8.35 (d, 1H, J 8.6 Hz, H_{Ar}), 8.54 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR (CD_3OD) δ 30.9 (t, CH_2), 41.1 (t, CH_2NH), 45.9, 45.9 (2q, $(CH_3)_2N$), 62.0, 62.5 (2t, C-6, C-6′), 68.0 (t, CH_2O), 70.3 (d), 72.6 (d), 74.7 (d), 74.9 (d), 76.4 (d), 76.4 (d), 77.1 (d), 80.8 (d), 104.1, 105.2 (2d, C-1, C-1′), 116.5 (d, CH Dans), 120.6 (d, CH Dans), 124.3 (d, CH Dans), 129.1 (d, CH Dans), 130.2 (d, CH Dans), 131.2 (d, CH Dans), 131.3 (2s, Dans), 137.1 (s, CSO_2), 153.3 (s, $CN(CH_3)_2$); IR (nujol): 3320, 1310; ESI/MS (positive ion mode), m/e 633 $[M + 1]^+$; Anal. Calcd for $C_{27}H_{40}N_2O_{13}S$: C, 51.26; H, 6.37; N, 4.43. Found: C, 51.49; H, 6.51; N, 4.18.

3-(Benzyloxycarbonylamino)propyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)- (1→3)-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→4))-6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside (17)

Compound **12** (0.176 g, 0.22 mmol) and fucosyl donor **16** (0.312 g, 0.67 mmol) were dissolved in dichloromethane (2 mL) and DMF (2 mL) under inert atmosphere; this solution was transferred into a flask containing powdered molecular sieves 3 Å (2.00 g), Bu_4NBr (0.361 mg, 1.12 mmol), and $CuBr_2$ (0.223 mg, 1.00 mmol). The mixture was stirred at rt for 2 h, then filtered over a Celite pad and washed with $NaHCO_3$ saturated aqueous solution and brine; after phase separation the aqueous layer was extracted with dichloromethane and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (dichloromethane/methanol, 20 : 1) affording the title compound **17** in almost quantitative yield (96%) as a foam. $[\alpha]_D$ −10.0 (C 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.23 (d, 3H, $J_{6'',5''}$ 6.8 Hz, H-6″), 1.60–1.80 (m, 2H, CH_2), 1.91–2.05 (6s, 18H, CH_3CO), 3.10–3.45 (m, 3H, CH_2NH, CH_aH_bO), 3.60–4.20 (m, 12H, H-2, H-3, H-4, H-5, CH_aH_bO, 2 H-6′, H-5′, H-2″, H-3″, H-4″, H-5″), 4.35–4.52 (m, 3H, 2 H-6, H-1″), 4.65–5.20 (m, 13H, H-1, H-1′, 4 CH_2Ph, H-3′, H-2′, NH), 5.33 (d, 1H,

J 4′,3′ 3.5 Hz, H-4′), 6.20 (d, 1H, N*H*COCH$_3$), 7.20–7.50 (m, 20H, Ph); ^{13}C NMR ($CDCl_3$) δ 14.0 (d, C-6″), 20.5–24.6 (6q, CH_3CO), 29.7 (t, CH_2), 37.7 (t, CH_2NH), 53.5 (d, C-2), 59.7, 60.5, 62.7 (3t, C-6, C-6′, CH_2O), 66.1 (t, CH_2Ph), 66.5 (t, CH_2Ph), 66.8 (d), 67.0 (d), 68.4 (d), 70.6 (d), 71.2 (2d), 71.9 (d), 72.6 (t, CH_2Ph), 74.4 (t, CH_2Ph), 75.5 (d), 76.8 (d), 77.0 (d), 80.3 (d), 96.1 (d, C-1″), 100.3 (2d, C-1, C-1′), 127.1–128.7 (20d, CHAr), 136.7, 137.9, 138.5, 138.7 (4s, CqAr), 156.8 (s, NHCO), 169.9–170.6 (6s, CH_3CO); IR (nujol): 3450, 1650 cm^{-1}. ESI/MS (positive ion mode), m/e 1218 $[M + 18]^+$; Anal. Calcd for $C_{62}H_{76}N_2O_{22}$: C, 61.99; H, 6.38; N, 2.33. Found: C, 62.21; H, 6.11; N, 2.12.

3-(Benzyloxycarbonylamino)propyl β-D-galactopyranosyl-(1→3)-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4))-2-acetamido-2-deoxy-β-D-glucopyranoside (18)

Compound **17** (0.096 g, 0.08 mmol) was dissolved in methanol (1 mL), and 1M sodium methoxide in anhydrous methanol (0.01 mL) was added. After stirring for 1 h at rt, the solution was neutralized by addition of IRC-86 resin, H^+ form; the resin was filtered off and the filtrate was concentrated. The residue was purified by flash chromatography (ethyl acetate/methanol, 8:2) affording **18** in quantitative yield as a foam. $[\alpha]_D$ −94.0 (c 1, CH_3OH); ^{1}H NMR (CD_3OD) δ 1.20 (d, 3H, $J_{6'',5''}$ 6.8 Hz, C-6″), 1.50–1.66 (m, 2H, CH_2), 1.83 (s, 3H, CH_3CONH), 2.90–3.05 (m, 2H, *C*H_2NH), 3.10–3.90 (m, 18H, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2 H-6′, H-2″, H-3″, H-4″, H-5″, CH_2O), 4.12 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.17 (d, 1H, $J_{1',2'}$ 7.0 Hz, H-1′), 4.34–4.67 (m, 6H, 3 CH_2Ph), 4.76–4.82 (m, 2H, CH_2Ph), 4.88 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 6.80–7.15 (m, 20H, Ph). ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-6″), 24.8 (q, CH_3CONH), 30.9 (t, CH_2), 38.7 (t, CH_2NH), 57.6 (d, C-2), 59.6, 61.3, 63.3, 67.4, 68.0, 68.2, 68.5 (7t, 4 CH_2Ph, C-6, C-6′, CH_2O), 72.4 (d), 73.4 (d), 73.9 (d), 74.8 (d), 75.8 (d), 76.4 (d), 77.0 (d), 77.4 (d), 78.3 (d), 79.8 (d), 80.9 (d), 98.6 (d, C-1″), 102.4, 104.9 (2d, C-1, C-1'), 128.5–130.0 (20d, CHAr), 139.5–140.4 (4s, CqAr), 158.9 (s, NHCO), 174.8 (s, CH_3CONH); IR (nujol): 3300, 1750, 1660 cm^{-1}; ESI/MS (positive ion mode), m/e 991 $[M + 1]^+$; Anal. Calcd for $C_{52}H_{66}N_2O_{18}$: C, 63.02; H, 6.71; N, 2.83. Found: C, 63.28; H, 6.49; N, 3.04.

3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl β-D-galactopyranosyl-(1→3)- (α-L-fucopyranosyl-(1 → 4))-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

Compound **18** (0.080 g, 0.08 mmol) was dissolved in methanol (0.8 mL), and 10% Pd(OH)2-C (0.080 g) and glacial acetic acid (two drops) were added. The mixture was kept in a hydrogen atmosphere with vigorous stirring for 96 h. The mixture was filtered over a Celite pad and concentrated. The residue was dissolved in methanol (2 mL), and dansyl chloride (0.024 g, 0.09 mmol)

and Et_3N (0.013 mL, 0.097 mmol) were added. After stirring for 56 h at rt, the solvent was removed. The crude product was purified by flash chromatography (ethyl acetate/methanol/water, 8 : 2.5 : 1) affording the title compound **1** (0.036 g, 52%) as an amorphous yellow solid. $[\alpha]_D$ −57.7 (*c* 0.5, CH_3OH); ^1H NMR (CD_3OD) δ 1.19 (d, 3H, $J_{6'',5''}$ 6.5 Hz, C-6″), 1.58–1.65 (m, 2H, CH_2), 1.85 (s, 3H, CH_3CONH), 2.89 (s, 6H, $N(CH_3)_2$), 2.92 (t, 2H, J 7.0 Hz, CH_2NH), 3.32–3.97 (m, 18H, CH_2O, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2H-6′, H-2″, H-3″, H-4″, H-5″), 4.36–4.37 (2d, 2H, J 7.8 Hz and J 8.0 Hz, H-1 and H-1′), 5.02 (d, 1H, J 4.0 Hz, H-1″), 7.28 (d, 1H, J 7.5 Hz, H_{Ar}), 7.56–7.63 (m, 2H, H_{Ar}), 8.19 (d, 1H, J 7.5 Hz, H_{Ar}), 8.35 (d, 1H, J 8.5 Hz, H_{Ar}); 8.55 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR (CD_3OD) δ 16.6 (q, C-6″), 23.2 (q, CH_3CONH), 30.9 (t, CH_2), 41.1 (t, CH_2NH), 45.8 (q, 2C, $(CH_3)_2N$), 57.2 (d, C-2), 61.3, 62.8, 67.6 (3t, C-6, C-6′, CH_2O), 69.8 (d), 70.0 (d), 71.1 (d), 72.3 (d), 73.6 (d),73.7 (d), 74.8 (d), 76.7 (d), 76.7 (d), 77.4 (d), 78.5 (d), 99.6 (d, C-1″), 102.3 and 104.9 (2d, C-1, C-1′), 116.4 (d, CH Dans), 120.8 (d, CH Dans), 124.4 (d, CH Dans), 129.2 (d, CH Dans), 130.1 (d, CH Dans), 130.9 (d, CH Dans), 131.1 (s, Dans), 131.2 (s, Dans), 137.0 (s, CSO_2), 153.2 (s, $CN(CH_3)_2$), 174.1 (s, CH_3CONH); IR (nujol): 3400, 1690 cm^{-1}; ESI/MS (positive ion mode), *m/e* 820 $[M + 1]^+$; Anal. Calcd for $C_{35}H_{53}N_3O_{17}S$: C, 51.27; H, 6.52; N, 5.13. Found: C, 51.02; H, 6.79; N, 4. 79.

Cloning, Expression, and Purification of Fucosyltransferase III (EC 2.4.1.65)

FucT III (EMBL accession no. X53578[14]) was expressed in S*f*9 insect cells using the baculovirus expression system. FucT III cDNA lacking in the first 35 amino acids of the coding region was fused to the untranslated γ-interferon secreting sequence and to the IgG binding domain of protein A. The construct was then cloned into baculovirus transfer vector pVL1392 (PharMingen). After transfection of S*f*9 cells in monolayer, recombinant virus was produced, and the titer was amplified to 1.10^9 pfu/mL. A confluent monolayer culture of S*f*9 insect cells (1×10^6 cells/mL) in SF900II medium supplemented with 5% fetal calf serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin was infected with recombinant baculovirus at the m.o.i of 5 and incubated at 27°C. The supernatant containing the secreted enzyme was collected 48 h post-infection, centrifuged, and filtered through a Millipore membrane (0.2 μm). The filtrate was adjusted to pH 7.6 with 2.5 M Tris-HCl solution and loaded onto an IgG-Sepharose-6 column (Amersham Biosciences) attached to an FPLC system (BioRad). Two washing steps with 10 bed volumes of buffer containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.05% Tween 20 followed by two bed volumes of 5 mM NH_4OAc (pH 5.0) were performed. The enzyme was eluted with 10 mL of 500 mM NH_4OAc (pH 3.4). The collected fractions were immediately neutralized with 2.5 M Tris-HCl solution and concentrated

by ultrafiltration (Amicon, YM 30) to a 50 μL volume. The protein concentration (325.5 μm/mL) was determined by Bradford assay using bovin serum albumin as standard. The enzyme activity (10.75 U/mg) was measured by the FucT III assay.

FucT III Assay

The assay format published by Palcic et al.[20] was slightly modified. The assay, run in duplicates, was conducted in a total volume of 30 μL containing 50 mM cacodylate (pH 6.5), 20 mM $MnCl_2$, 0.150 mM GDP-fucose, 55 000 cpm GDP-[^{14}C]fucose (287 mCi/mmol, Amersham Pharmacia), 3 μg BSA (Sigma), 0.01 to 4 mM acceptor substrate, and 3 mU of FucT III. After 30 min of incubation at 37°C, the reaction was stopped by addition of 1 mL water and loaded onto Sep-Pak-C18 cartridges (Waters). The cartridges were washed twice with 5 mL bi distilled water and eluted with 5 mL methanol. The transfer rate of [^{14}C]fucose onto the acceptor substrate was determined by liquid scintillation counting.

Determination of Kinetic Parameters for the Substrates Lacto-*N*-biose, Lac*N*Ac, 3 and 4

The kinetic parameters were determined using 0.150 mM of GDP-fucose and concentrations of the acceptor substrates ranging from 0.01 to 4 mM. The reaction mixtures were prepared as described above. Under the above conditions the formation of products was shown to be linear in time. Kinetic constants were obtained from double-reciprocal plots by linear regression analysis of Lineweaver-Burk plots.

Enzymatic Synthesis of 3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl β-D-Galactopyranosyl-(1→3)-(α-L-fucopyranosyl-(1→4))-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

The reaction was conducted in 500 μL total volume of bidistilled water containing Na-cacodylate buffer (150 μL, 250 mM, pH 6.5), $MnCl_2$-solution (50 μL, 250 mM), disaccharide **3** (2.3 mg, 3.4 μmol), GDP-fucose (4.1 mg, 6.9 μmol), and bovine serum albumin (0.7 mg, Fluka). The mixture was vortexed and incubated overnight at 37°C with fucosyltransferase III (150 μL, 500 mU) and calf intestine alkaline phosphatase (2 ml, 2U, Roche). After a TLC (CH_2Cl_2/methanol/water, 10 : 4 : 0.8) control indicated the total consumption of **3**, the turbid solution was centrifuged and the supernatant passed over RP-18 column (Sep-Pack-C_{18} cartridge, Waters). The column was washed with water and eluted with methanol. After evaporation of the solvent, the residue was chromatographed on RP-18 (H_2O/MeOH gradient 1 : 0 to 1 : 1) to

yield, after a final lyophilization from water, trisaccharide **1** (1.5 mg, 88%) as colorless powder with identical spectroscopic properties as **1** obtained by chemical means.

Enzymatic Synthesis of 3-(5-Dimethylamino-naphthalene-1-sulfonylamino)propyl β-D-Galactopyranosyl-(1→3)-(α-L-fucopyranosyl-(1→4))-β-D-glucopyranoside (2)

Compound **2** was synthesized according to the procedure described for **1**, except that the reaction time was increased to 48 h. Starting from disaccharide **4** (2.3 mg, 3.6 μmol) trisaccharide **2** (2.5 mg, 54%) was obtained. $[\alpha]_D$ −46.61 (*c* 0.14, CH_3OH); ^1H NMR (CD_3OD) δ 1.18 (d, 3H, J $_{6'',5''}$ 6.5 Hz, C-6″), 1.63–1.66 (m, 2H, CH_2), 2.89 (s, 6H, $N(CH_3)_2$), 2.94–3.00 (m, 2H, CH_2NH), 3.38–3.92 (m, 18H, CH_2O, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2H-6′;, H-2″, H-3″, H-4″, H-5″), 4.12 (d, 1H, J 7.8 Hz, H-1′), 4.39 (d, 1H, J 7.2 Hz, H-1), 5.43 (d, 1H, J 3.9 Hz, H-1″), 7.28 (d, 1H, J 7.5 Hz, H_{Ar}), 7.56–7.61 (m, 2H, H_{Ar}), 8.19 (d, 1H, J 7.3 Hz, H_{Ar}), 8.35 (d, 1H, J 8.7 Hz, H_{Ar}), 8.56 (d, 1H, J 8.5 Hz, H_{Ar}); ^{13}C NMR (CD_3OD) δ 17.0 (q, C-6″), 31.2 (t, CH_2), 41.4 (t, CH_2NH), 46.2 (2q, $(CH_3)_2N$), 61.3, 63.2, 67.8 (3t, C-6, C-6′, CH_2O), 68.3 (d), 70.4 (d), 70.8 (d), 71.6 (d), 73.3 (d), 74.2 (d), 74.9 (d), 75.3 (d), 76.8 (d), 77.1 (d), 77.6 (d), 79.5 (d), 100.9, 104.2, 104.6 (3C, C-1, C-1′, C-1″), 116.8 (d, CH Dans), 121.0 (d, CH Dans), 124.8 (d, CH Dans), 129.5 (d, CH Dans), 130.0 (d, CH Dans), 130.6 (d, CH Dans), 131.4 (s, Dans), 131.5 (s, Dans), 137.6 (s, CSO_2), 153.6 (s, $CN(CH_3)_2$); ESI/MS (positive ion mode), *m*/*e* 779 $[M + 1]^+$.

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