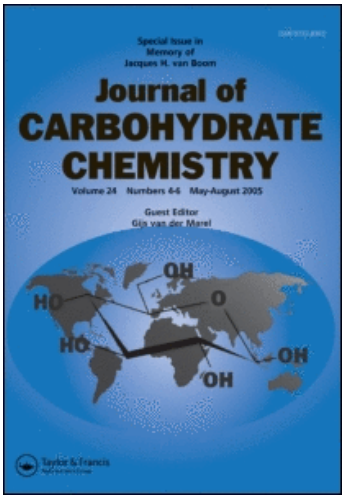

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Substrate Specificity and Preparative Use of Recombinant Rat ST3Gal III

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ABSTRACT

Recombinant rat $\alpha(2\rightarrow3)$-sialyltransferase (ST3Gal III, EC 2.4.99.6) was expressed from baculovirus infected *Sf 9* insect cells in preparative amounts. In order to elucidate substrate tolerances of ST3Gal III, the natural type I and type II substrates **21** and **22** as well as several non-natural sugars were investigated. The non-natural Gal-$\beta(1\rightarrow3)$-Gal(*N*) (type III) disaccharides **11**, **13**, and **16** turned out to be surprisingly good substrates for ST3Gal III. All sialylations were run in preparative scale and kinetic parameters (V_{max} and K_m) were determined.

Key Words: ST3Gal III; Gangliosides; CMP-NeuAc; Myelin-associated glycoprotein; Enzymatic sialylation.

INTRODUCTION

Carbohydrates from glycolipids and glycoproteins with terminal sialic acids are involved in a broad variety of biological recognition and adhesion phenomena.[1,2]

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Gangliosides, a group of sialylated glycosphingolipids widely present in mammalian tissues, have been recognized to play an important role in many biological processes such as cell–cell recognition, cell growth, differentiation, transformation, and neural functions.[3–6] Thus, the gangliosides GM1b, GD1a, and GT1b (Fig. 1) have been shown to bind with high affinity to the myelin-associated glycoprotein (MAG), a member of the siglec family, which is a potent inhibitor of neurite outgrowth of most neurons.[7–9]

The common element of gangliosides binding to MAG is the terminal NeuAc-$\alpha(2\rightarrow3)$-Gal-$\beta(1\rightarrow3)$-Gal*N*Ac partial structure, suggesting a special importance of this trisaccharide subunit.

In order to investigate their binding affinity to MAG and other siglecs, large numbers of natural and modified oligosaccharides have to be made available for extensive biological screening. Although numerous glycosylation methods are known, the chemical synthesis of complex oligosaccharides is still not a routine procedure.[10]

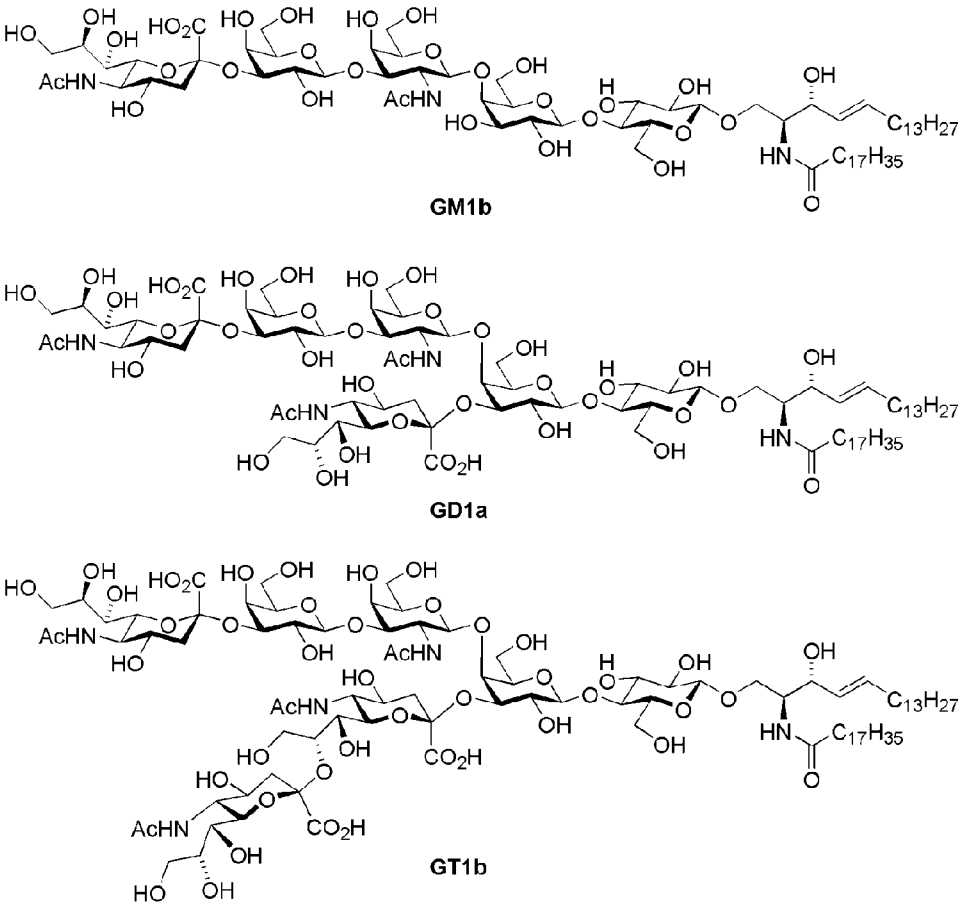

Figure 1. The gangliosides GM1b, GD1a, and GT1b.

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Difficulties often occur from time-consuming protecting group manipulations. Cumbersome separation of the desired product from the anomeric mixtures is often necessary. Finally, chemical sialylations suffer from poor stereoselectivity due to the lack of neighboring group assistance of the sialic acid.[10] A convenient alternative is the application of sialyltransferases (STs).[11,12] Depending on their specificity, these enzymes catalyze the transfer of a sialic acid moiety from cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) α-selectively to the 3-OH or 6-OH of a terminal galactose residue, or to the 8-OH or 9-OH of a sialic acid unit, depending on the specificity of the particular enzyme.

The mammalian ST family consists of more than 20 STs, which can be divided into three sub-families. The members of each sub-family differ in their substrate specificity. *ST8Sia I-V* and *ST9Sia* mediate the transfer of a sialic acid moiety to the 8-OH and 9-OH, respectively. *ST6Gal I* and *ST6GalNAc I–VI* catalyze the α-sialylation of the 6-OH of a terminal galactose (Gal) residue or a terminal or internal *N*-acetylgalactosamine group, respectively.[13,14]

To date, six different $\alpha(2\rightarrow3)$-STs have been characterized. *ST3Gal I*[15] and *ST3Gal II*[16] mediate the transfer of sialic acid residues to the galactose unit of terminal Gal-$\beta(1\rightarrow3)$-Gal*N*Ac oligosaccharides found on glycolipids or glycoproteins. Several groups have reported the successful syntheses of $\alpha(2\rightarrow3)$-sialylated *O*-linked oligosaccharides employing ST3Gal I.[17] *ST3Gal III*[18] acts on type I and type II chains [Gal-$\beta(1\rightarrow3/4)$-Glc*N*Ac] and was, therefore, used for the synthesis of sialyl Lewis^a and sialyl Lewis^x epitopes[12,19,20] as well as for the preparation of ganglioside GM3.[21] Recombinant soluble *ST3Gal IV*[22] accepts as substrates glycolipids or glycoproteins containing either terminal Gal-$\beta(1\rightarrow4)$-Glc*N*Ac or Gal-$\beta(1\rightarrow3)$-Gal*N*Ac motifs. Recombinant human *ST3Gal V* was shown to sialidate only lactosylceramide [Gal-$\beta(1\rightarrow4)$-Glc-β-Cer], whereas the purified rat liver enzyme exhibited a broader substrate tolerance utilizing also galactosylceramide (Gal-β-Cer) and asialoganglioside GA2 [Gal*N*Ac-$\beta(1\rightarrow4)$-Gal-$\beta(1\rightarrow4)$-Glc-β-Cer] as substrates.[23,24] Finally, human *ST3Gal VI*[25] accepts almost exclusively the Gal-$\beta(1\rightarrow4)$-Glc*N*Ac motif on glycoproteins and glycolipids.

Our investigations are aiming at the substrate specificity of STs in general and their use in preparative scale. In previous studies on the substrate specificity of recombinant rat ST3Gal III (EC 2.4.99.6), it was found that all of the hydroxyl groups of the galactose unit are essential for substrate recognition by the enzyme.[26] The enzyme does, however, accept a wide variety of acyl groups on the glucosamine nitrogen of type I and type II acceptors.[12,27–29] The replacement of the *N*-acetyl group by azide, aromatic or heteroaromatic moieties, charged residues, bulky polar uronic acids or sulfonamides is well tolerated. Thus, libraries of sialylated type I and type II sugars, the immediate precursors of the sialyl Lewis^a and sialyl Lewis^x tetrasaccharides are produced rapidly with good overall yields. In order to further elucidate the substrate specificity of recombinant rat ST3Gal III, we investigated in this study its tolerance towards several type III [Gal-$\beta(1\rightarrow3)$-Gal(*N*)] disaccharides. The physiological enzymes for the $\alpha(2\rightarrow3)$-sialylation of these sugars are ST3Gal I, II, and IV (EC 2.4.99.4).

Recombinant rat ST3Gal III[30] (The ST3Gal III construct was obtained from M. Streiff, Novartis Pharma Inc., Basel) was expressed in preparative amounts (9 U/L) from baculovirus infected *Sf 9* insect cells[31] and applied to enzymatic sialylation for natural and non-natural disaccharides.

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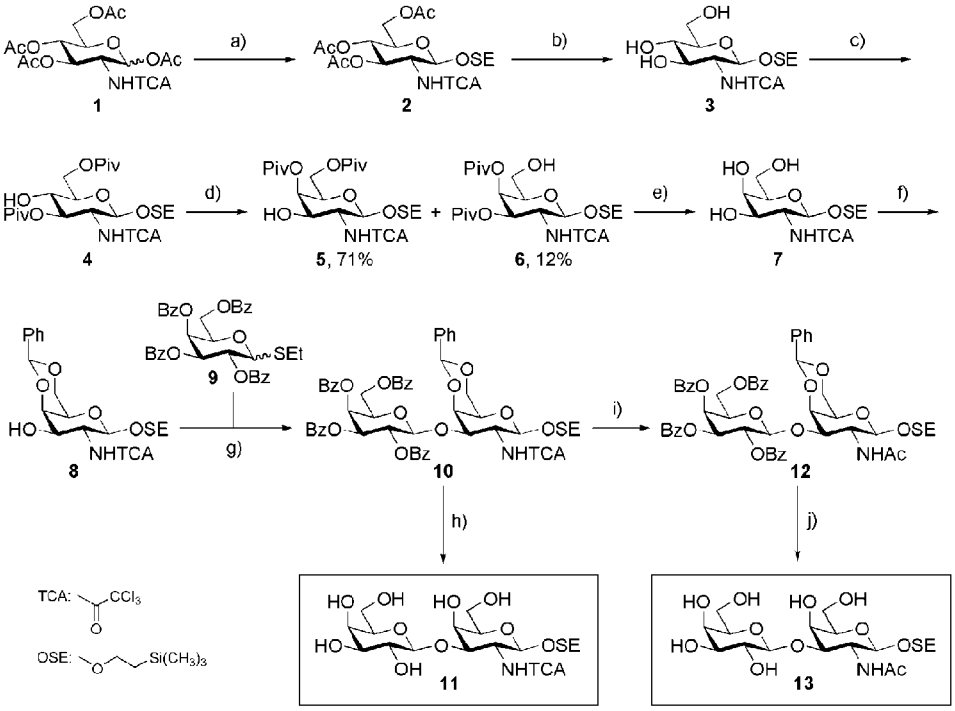
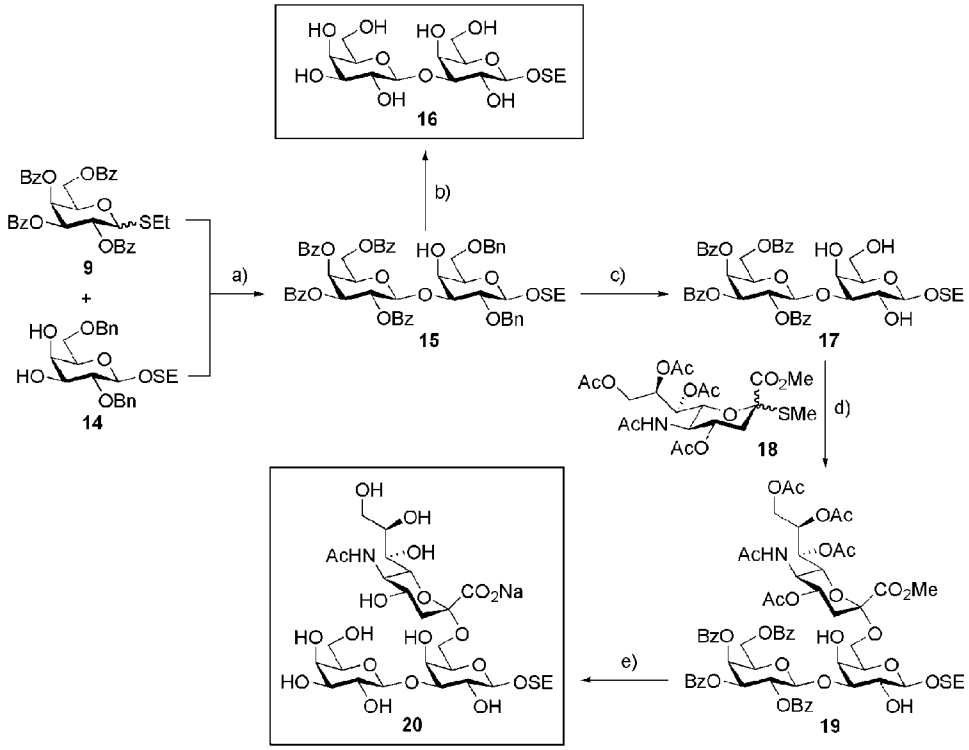
RESULTS AND DISCUSSION

The syntheses of the Gal-Gal*N* and Gal-Gal substrates (type III) are shown in Schs. 1 and 2. Gal-$\beta(1\rightarrow3)$-Gal*N*TCA-β-OSE **11** and Gal-$\beta(1\rightarrow3)$-Gal*N*Ac-β-OSE **13** were easily available from the galactosamide derived building block **8** (Sch. 1).

Compound **8** was synthesized in seven steps from per-*O*-acetylated *N*-trichloroacetyl glucosamide **1**[32a] following a procedure by Jacquinet et al.[32b] After the quantitative transformation of **1** into the corresponding bromide, glycosylation with 2-(trimethylsilyl)-ethanol (HOSE) was reached in the presence of $Hg(CN)_2$ as promoter to give β-glucoside **2** in 83% yield. Subsequent *O*-deacetylation afforded **3**. Treatment with pivaloyl chloride (2.5 eq. $-30^{\circ}C$, pyridine) gave the 3,6-bispivaloate **4** in 89% yield. The 4-hydroxy group was then transformed into the corresponding triflate, which in the presence of water at $80^{\circ}C$ furnished galactosamines **5** and **6** in 83% overall yield.[32b] Transesterification with sodium methoxide gave **7** (96%), which was transformed into the corresponding benzylidene derivative **8** with 2,2-dimethoxytoluene in the presence of cat. *p*-TsOH.



Scheme 1. (a) i. HBr/AcOH, rt, 17 hr, ii. HOSE, $Hg(CN)_2$, MS 3 Å, toluene, rt, 3 d (89%); (b) cat. NaOMe/MeOH, rt, 16 hr (quant); (c) 2.5 eq PivCl, cat. DMAP, pyridine, $-30^{\circ}C$, 20 hr (89%); (d) Tf_2O, DCE/pyridine 2 : 1, $0^{\circ}C$, 4 hr, then H_2O, $80^{\circ}C$, 2 hr (83%); (e) 0.1 M NaOMe/MeOH, rt, 7 hr, (96%); (f) $PhCH(OMe)_2$, cat. *p*-TsOH, MeCN, rt, 4 hr (86%); (g) NIS, TfOH, CH_2Cl_2, $-25^{\circ}C$, 1.5 d (64% β); (h) i. cat. NaOMe/MeOH, rt, 18 hr, ii. 80% aq. AcOH, $80^{\circ}C$, 2 hr (46%); (i) Bu_3SnH, AIBN, benzene, $80^{\circ}C$, 3 hr (93%); (j) i. 80% aq. AcOH, $50^{\circ}C$, 4.5 hr, ii. cat. NaOMe/MeOH, rt, 21 hr (64%).



Scheme 2. (a) DMTST, CH_2Cl_2, 7°C, 16 hr (87%); (b) i. NaOMe, MeOH, rt, 2 hr, ii. 10% Pd/C, H_2, MeOH, rt, 3 hr (75%); (c) 10% Pd/C, H_2 (4 bar), MeOH/dioxane, rt, 9 d (67%); (d) **18**, NIS, TfOH, CH_2Cl_2, −30°C, 16 hr (45% α); (e) NaOMe, MeOH, rt, 7 hr, then aq. NaOH, rt, 16 hr (90%).

Galactosylation of **8** using the donor **9**[33] promoted by *N*-iodosuccinimide (NIS)/TfOH at −25°C gave predominantly disaccharide **10** as 4 : 1-mixture. The separation of the anomers was achieved by crystallization from *i*-Pr_2O yielding pure β-**10** in 64%. Removal of the benzoyl groups using NaOMe and subsequent cleavage of the benzylidene group with 80% aqueous acetic acid gave Gal-β(1→3)-Gal*N*TCA-β-OSE (**11**) in 46% yield.

For the synthesis of Gal-β(1→3)-Gal*N*Ac-OSE (**13**), the *N*-trichloroacetyl group in **10** was reduced to the *N*-acetyl group with Bu_3SnH/AIBN in refluxing benzene[32] to give **12** (93%), followed by *O*-debenzoylation yielding **13** (64%).

Gal-β(1→3)-Gal-β-OSE (**16**) and Gal-β(1→3)-[NeuAc-α(2→6)]-Gal-β-OSE (**20**) were synthesized starting from the known galactose derivative **14**[34,35] (Sch. 2).

The coupling of **14**[34,35] with donor **9** was promoted by dimethyl(methylthio) sulfonium triflate (DMTST),[36,37] giving the β-galactoside **15** in excellent yield. After removal of the benzoate and benzyl protection by transesterification and hydrogenation, respectively, Gal-β(1→3)-Gal-β-OSE (**16**) was isolated in 75% yield.

Catalytic hydrogenation of compound **15** turned out to be slow and incomplete, presumably because of steric hindrance by the benzoyl groups. Therefore, disaccharide **17**

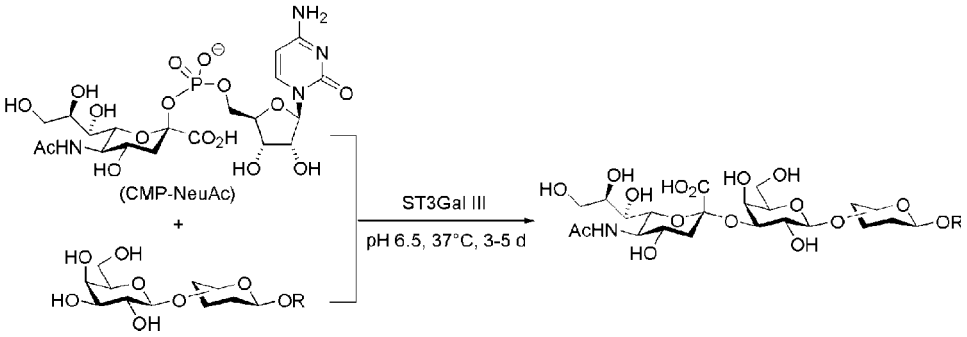
was obtained only in 67%. Sialylation of **17** with the sialic acid donor **18**[37] in the presence of NIS/TfOH afforded the corresponding α-glycoside **19** in 45% yield. Subsequent saponification of **19** using first sodium methoxide and then sodium hydroxide gave trisaccharide **20** in 90%.

Following standard sialylation protocols,[12,29,38,39] the saccharides **11**, **13**, **16**, and **20**, and, as control experiments, the natural substrates **21**[40] and **22**[41] were then incubated in preparative scale with CMP-NeuAc and recombinant rat ST3Gal III (EC 2.4.99.6, 9 U/L) (Sch. 3). This enzyme transferred a sialic acid moiety from CMP-NeuAc regio- and α-stereoselectively onto the 3-OH group of a terminal galactose.

In order to determine the optimal reaction conditions, the activity of the ST3Gal III over time at 37°C was investigated (Fig. 2). Aliquots of the enzyme in sodium cacodylate buffer were incubated at 37°C for 0, 6, 9, 12, 24, 60, and 84 hr, then acceptor **22** and donor CMP-NeuAc were added and the activity of the ST was determined. As shown in Fig. 2, the activity of the enzyme remained practically unchanged for the initial 9 hr. Incubation for 12 and 24 hr, however, led to a significant decrease of activity. It is noteworthy, that even after 84 hr of incubation at 37°C about 20% of the original enzyme activity was still preserved.

According to these results, the conditions for the reactions in preparative scale were optimized for the type III disaccharide **13**, and were then applied for all the other substrates (Sch. 3): Substrates and CMP-NeuAc were incubated with ST3Gal III for 3–5 days at 37°C in a mixture of 50 mM sodium cacodylate buffer (pH 6.5), 60 mM $MnCl_2$-solution and water containing BSA and CIAP[21] (EC 3.1.3.1). After 17–24 hr of incubation, an additional aliquot of transferase was added (except for the natural substrates **21** and **22**). Further addition of ST3Gal III and CMP-NeuAc did not increase the yield of products. The reactions were monitored by TLC (silica gel, dichloromethane (DCM)/MeOH/H_2O 10 : 4 : 0.8). Isolated yields, recovered starting material, and kinetic data (V_{max} and K_m) are summarized in Table 1.

As shown before,[29] the natural substrates **21** (type II disaccharide) and **22** (type I) were converted quantitatively into the corresponding trisaccharides **23** and **24** (entries 1 and 2 in Table 1). Although the physiological enzymes for the sialylation of terminal Gal-β(1→3)-Gal*N*Ac subunits are ST3Gal I, II, and IV (EC 2.4.99.4), compounds **11**, **13**, and **16** also were sialylated by ST3Gal III giving the trisaccharides **25**–**27** in



Scheme 3. Enzymatic sialidation using ST3Gal III and CMP-NeuAc.

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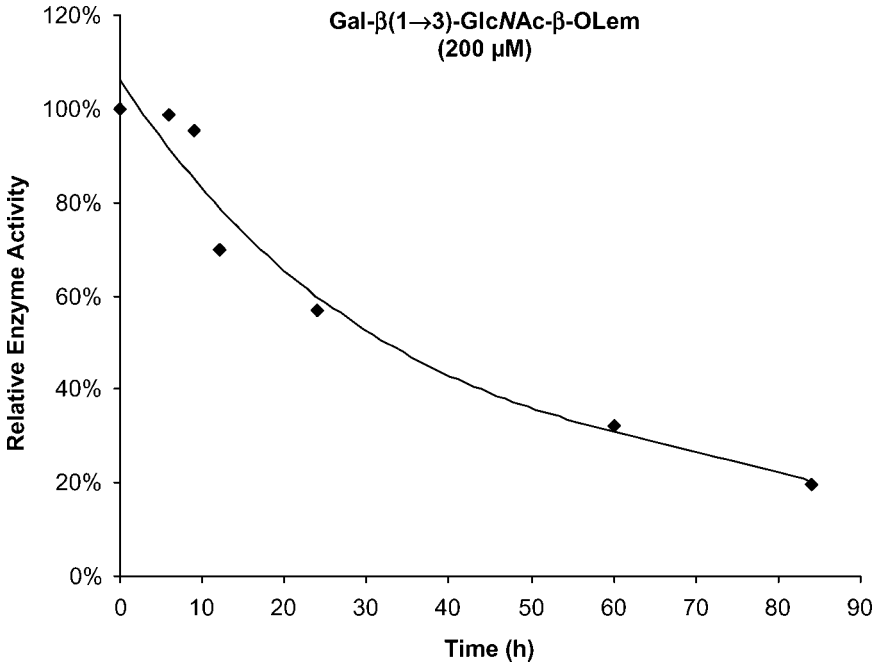

Figure 2. Activity over time of ST3 Gal III in the sialidation of Gal-β(1→3)-Glc*N*Ac-β-OLem **22** at 37°C.

acceptable yields. In all cases, the unreacted starting materials could be recovered almost completely. The kinetic data for the sialylation reactions indicate that the activity of ST3Gal III towards the non-natural substrates **11**, **13**, and **16** is reduced only about 10-fold compared to its natural substrates **21** and **22**. As expected, the transfer efficiency V_{max}/K_m is much lower for the unnatural acceptors as for the natural substrates **21** and **22**. This explains the incomplete, but still preparatively useful, conversion of the type III disaccharides. 6-*O*-Sialylated compound **20**, however, was not tolerated as substrate by ST3Gal III, presumably due to the bulky substituent in the 6-position of galactose. Sialylation of a derivative of trisaccharide **20** was recently reported using the physiological enzyme ST3Gal I.[17e]

Comparative NMR investigations (COSY, HSQC, and TOCSY) of the starting sugars and the isolated products confirm the attachment of a sialic acid unit. Additional signals in the ^{13}C NMR spectra at approximately 100 and 40 ppm are characteristic for the C-2 and C-3 atoms of an α-linked sialic acid.[20a,29,42] Simultaneous down-field shifts (~4 ppm) of the galactose C-3 atom in the ^{13}C NMR spectra and about 0.6 ppm of the galactose H-3 in the ^{1}H NMR spectra confirm the introduction of sialic acid onto the 3-OH group of the galactose moiety.[29]

In conclusion, our investigations show, in addition to previous reports,[12,29] an unexpected high substrate tolerance of recombinant ST3Gal III. The enzyme accepts the replacement of the natural Glc*N*Ac moiety by Gal, Gal*N*Ac and Gal*N*TCA. Thus, the preparative applicability of the transferase has been substantially extended.

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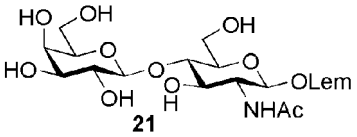
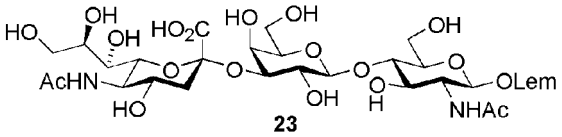
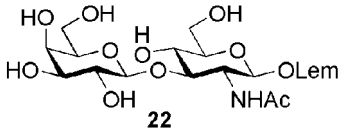
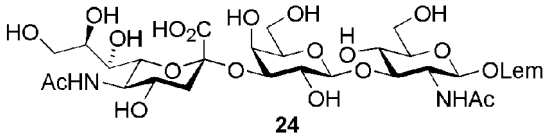
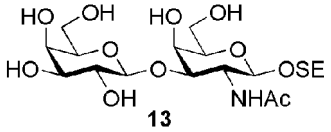
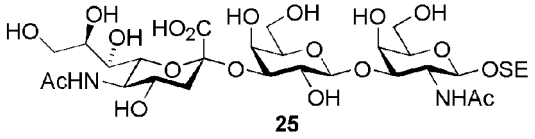
Table 1. Isolated yields and kinetic data of the enzymatic sialidations.

Entry	Acceptor	Product	Isolated product (%)	Recovered acceptor (%)	V_{max} (nmol/ mL min)	K_m (μm)
1	 **21**	 **23**	76	—	3.19	87.61
2	 **22**	 **24**	90	—	3.42	66.08
3	 **13**	 **25**	56	44	1.11	995.55

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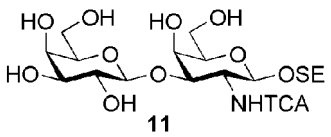
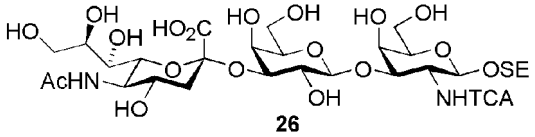
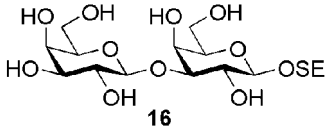
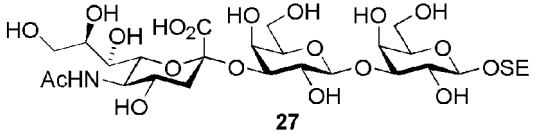
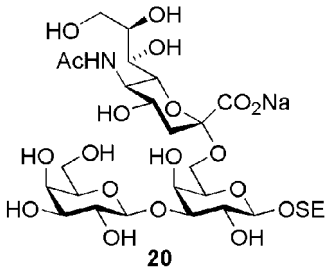
4	 **11**	 **26**	36	62	0.89	811.64
5	 **16**	 **27**	59	40	0.43	990.51
6	 **20**	No reaction	—	95	—	—

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EXPERIMENTAL

General Methods

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, TOCSY). Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD, and HDO as references. Optical rotations were measured using a Perkin–Elmer Polarimeter 241. Low resolution LC/MS analyses were carried out using a Waters Alliance 2690 LC system equipped with a photodiode array detector and a Micromass Quattro II mass spectrometer. The spectra were recorded in positive EI mode. The LC method consisted of a separation column (YMC ODS AQ 12.5 cm length, 2 mm i.d.) held at 40°C. The mobile phase was MeCN/H_2O with the addition of 0.05% trifluoroacetic acid using a flow rate of 0.5 mL/min. The linear gradient ran from 0% to 100% MeCN in 15 min followed by 5 min at 100% MeCN before returning to initial conditions. The LC/HRMS analysis were carried out using an Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. All spectra were recorded in positive EI mode. The LC method consisted of a separation column (YMC ODS AQ 12.5 cm length, 2 mm i.d.) held at rt. The mobile phase was MeCN/H_2O with the addition of 0.5% formic acid using a flow rate of 0.2 mL/min. The linear gradient ran from 5% to 95% MeCN in 10 min followed by 8 min at 95% MeCN before returning to initial conditions. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck, CH-8953 Dietikon, Switzerland) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Uetikon, 40–60 mesh). Methanol (MeOH) was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine was freshly distilled under argon over KOH. Dichloromethane, dichloroethane (DCE), acetonitrile (MeCN), toluene, and benzene were dried by filtration over Al_2O_3 (Fluka, CH-9471 Buchs, Switzerland, type 5016 A basic). Molecular sieves (3 Å) were activated in vacuo at 500°C for 2 hr immediately before use.

Expression of Sialyltransferase ST3Gal III (EC 2.4.99.6)[30,31]

ST3Gal III (EC 2.4.99.6) was expressed using a recombinant baculovirus expression system. A DNA fragment coding for the chimeric protein containing the untranslated γ-interferon secreting sequence and the sequence for the catalytic domain was cloned into the commercially available vector pVL1392, a baculovirus expression system transfer vector (PharMingen). After transfection of Sf-9 cells in monolayer culture with the recombinant virus (MOI of 20 pfu/cell) soluble $\alpha(2\rightarrow3)$-STs were produced. Complete medium was removed from confluent cells, and fresh medium containing 20 plaque forming units per cell was added. After 1 hr incubation at rt, inoculum was removed, fresh medium was added, and the culture was returned to the incubator (27°C). Supernatant was collected 72 hr postinfection and spun and filtrated through a membrane

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(0.2 μm). The enzyme activity was measured by the ST activity assay using CMP-[^{14}C]-NeuAc.

Sialyltransferase Enzyme Assay

The assay mixture (20 μL) containing the acceptor substrate (free disaccharide), ST3Gal III (0.015 mU), 0.1 mM CMP-NeuAc (2 nmol) and 60,000 cpm of CMP-[^{14}C]-NeuAc in 100 mM sodium cacodylate buffer at pH 6.0 with 0.3% Triton X-100 and 10 mM $MgCl_2$ was incubated at 37°C for 1 hr. The reaction mixture was diluted with water to 5 mL and applied to SepPac (Waters, CH-5102 Rupperswil, Switzerland) C_{18}-cartridges. The column was washed twice with water (5 mL) and eluted with methanol (6 mL). Incorporation of [^{14}C]-NeuAc was determined by liquid scintillation counting of the eluate. One unit of activity throughout this report refers to the amount of enzyme catalyzing the transfer of 1 μmol NeuAc to the acceptor per min using the standard incubation conditions (37°C and pH 6.0).

Determination of the Relative Enzyme Activity at 37°C

Aliquots (20 μL) of ST3Gal III (0.015 mU) in 100 mM sodium cacodylate buffer at pH 6.0 with 0.3% Triton X-100 and 10 mM $MgCl_2$ were incubated at 37°C for 0 (control experiment), 6, 9, 12, 24, 60, and 84 hr, respectively. Then the acceptor substrate Gal-β-(1→3)-Glc*N*Ac-β-OLem (**22**, 200 μM), CMP-NeuAc (0.1 mmol) and 60,000 cpm of CMP-[^{14}C]-NeuAc were added and the enzyme activity was determined as described above. The enzyme activity for each sample was calculated as a percentage relative to the control experiment ($t = 0$ hr).

Determination of Kinetic Parameters

Enzyme kinetics of ST3Gal III were determined in eluates as described above. Relative rates for each substrate were calculated as a percentage of the incorporation of NeuAc into the D-Gal-containing disaccharide. Under the above conditions, the formation of products was shown to be linear in time. For the K_m value determination 8–9 different acceptor concentrations were used. Kinetic constants were obtained from double-reciprocal plots by linear regression analysis of Lineweaver–Burk plots.

2-(Trimethylsilyl)ethyl 3,4,6-tri-*O*-Acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (2)

To an ice-cooled solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-glucopyranose **1**[32] (2.32 g, 4.71 mmol) in DCM (30 mL) was added dropwise HBr in acetic acid (4.1 M, 10 mL). After stirring for 17 hr at rt, the mixture was carefully added to DCM (100 mL) and saturated aqueous $KHCO_3$ (200 mL) under ice-cooling and stirring, followed by extraction with DCM (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. To the residue (2.39 g)

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and 2-(trimethylsilyl)ethanol (1.32 mL, 9.27 mmol) dissolved in toluene, molecular sieves (3 Å, 4 g) and mercuric cyanide (2.34 g, 9.27 mmol) were added. The mixture was stirred at rt for 3 d under light exclusion. The suspension was filtered through a pad of Celite, which was washed with toluene (3 × 20 mL). The combined filtrates were evaporated to dryness yielding a residue, which was purified by silica gel chromatography (petroleum ether/EtOAc 2 : 1) to afford **2** (2.28 g, 89%) as a colorless foam.

$[\alpha]_D$ −10.5 ($c = 0.45$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.93 (m, 2H, H-2′), 2.03, 2.04, 2.10 (3s, 9H, $3CH_3$), 3.58 (m, 1H, H-1′a), 3.74 (ddd, $J = 2.5$, 4.8, 9.9 Hz, 1H, H-5), 3.90 (dt, $J = 8.5$, 10.7 Hz, 1H, H-2), 3.97 (m, 1H, H-1′b), 4.16 (dd, $J = 2.5$, 12.3 Hz, 1H, H-6a), 4.29 (dd, $J = 4.8$, 12.3 Hz, 1H, H-6b), 4.72 (d, $J = 8.3$ Hz, 1H, H-1), 5.11 (dd, $J = 9.3$, 9.9 Hz, 1H, H-4), 5.38 (dd, $J = 9.3$, 10.7 Hz, 1H, H-3), 6.81 (d, $J = 8.7$ Hz, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.0 (C-2′), 20.6, 20.6, 20.7 ($3CH_3$), 56.2 (C-2), 62.1 (C-6), 67.8 (C-1′), 68.5 (C-4), 71.5 (C-3), 71.9 (C-5), 92.3 (CCl_3), 99.8 (C-1), 161.8, 169.3, 170.7, 170.8 (4CO); HRMS (FAB) Calcd for $C_{19}H_{30}Cl_3NO_9Si$ $[M - NH_4]^+$: 567.1099; Found: 567.1087.

2-(Trimethylsilyl)ethyl 2-Deoxy-2-trichloroacetamido-β-D-glucopyranoside (3)

A solution of **2** (2.27 g, 4.12 mmol) in methanol (15 mL) was treated with 1 M methanolic NaOMe (1 mL) at rt for 16 hr. The reaction mixture was neutralized with Dowex 50X8 (H^+) ion-exchange resin and filtered through a pad of Celite. The Celite was washed with methanol (3 × 5 mL), and the combined filtrates were evaporated to dryness. The crude residue (1.79 g, quant) was used without further purification.

^{1}H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.93 (m, 2H, H-2′), 3.27–3.34 (m, 2H, H-4, H-5), 3.58 (ddd, $J = 6.1$, 9.5, 10.7 Hz, 1H, H-1′a), 3.61 (dd, $J = 10.4$, 10.5 Hz, 1H, H-3), 3.63 (dd, $J = 8.0$, 10.4 Hz, 1H, H-2), 3.69 (dd, $J = 5.5$, 12.0 Hz, 1H, H-6a), 3.89 (dd, $J = 2.0$, 12.0 Hz, 1H, H-6b), 3.99 (ddd, $J = 5.9$, 9.6, 10.7 Hz, 1H, H-1′b), 4.57 (d, $J = 8.0$ Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ −1.5 ($SiMe_3$), 19.2 (C-2′), 59.3 (C-2), 62.8 (C-6), 68.3 (C-1′), 72.4 (C-4), 75.3 (C-3), 78.0 (C-5), 101.7 (C-1), 164.4 (CO); HRMS (FAB) Calcd for $C_{13}H_{24}Cl_3NO_6Si$ $[M - NH_4]^+$: 441.0782; Found: 441.0783.

2-(Trimethylsilyl)ethyl 2-Deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-glucopyranoside (4)

To a solution of **3** (2.89 g, 6.80 mmol) and DMAP (20 mg) in pyridine (30 mL) was added dropwise pivaloyl chloride (2.08 mL, 17.0 mmol) at −30°C. After stirring for 20 hr at −30°C, the reaction was quenched with methanol (2 mL). After removal of the solvents under reduced pressure, the residue was redissolved in DCM (100 mL) and the solution was washed with 5% aqueous HCl (20 mL) and saturated aqueous $KHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 2 : 1) to give **4** (3.58 g, 89%) as a colorless foam.

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$[\alpha]_D$ −32.6 ($c = 0.50$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.94 (m, 2H, H-2′), 1.21, 1.24 (2s, 18H, $2CMe_3$), 3.25 (d, $J = 5.8$ Hz, 1H, OH), 3.50–3.60 (m, 2H, H-4, H-1′a), 3.61 (ddd, $J = 2.5$, 5.7, 9.8 Hz, 1H, H-5), 3.90 (ddd, $J = 8.3$, 9.0, 10.7 Hz, 1H, H-2), 3.94 (m, 1H, H-1′b), 4.37 (dd, $J = 5.7$, 12.1 Hz, 1H, H-6a), 4.44 (dd, $J = 2.4$, 12.1 Hz, 1H, H-6b), 4.65 (d, $J = 8.3$ Hz, 1H, H-1), 5.41 (dd, $J = 9.0$, 10.7 Hz, 1H, H-3), 7.05 (d, $J = 9.0$ Hz, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.1 (C-2′), 27.0, 27.1 ($2CMe_3$), 38.9, 39.0 ($2CMe_3$), 56.0 (C-2), 63.3 (C-6), 67.5 (C-1′), 69.9 (C-4), 74.0, 74.1 (C-3, C-5), 92.5 (CCl_3), 100.0 (C-1), 161.9, 179.1, 179.9 (3CO); HRMS (FAB) Calcd for $C_{23}H_{40}Cl_3NO_8Si$ $[M - NH_4]^+$: 609.1933; Found: 609.1909.

2-(Trimethylsilyl)ethyl 2-Deoxy-4,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside (5) and 2-(Trimethylsilyl)ethyl 2-Deoxy-3,4-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside (6)

Trifluoromethane sulfonic anhydride (1.48 mL, 8.99 mmol) was added at 0°C to a solution of **4** (3.55 g, 5.99 mmol) and DMAP (20 mg) in pyridine (7 mL) and DCE (25 mL). After stirring for 4 hr at this temperature, water (3 mL) was added and the mixture was stirred for additional 2 hr at 80°C. After cooling to rt, DCM (25 mL) was added, followed by washing with 5% aqueous HCl (20 mL) and saturated aqueous $KHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc, gradient 3 : 1 to 1 : 1) to give 4,6-dipivaloate **5** (2.52 g, 71%) and 3,4-dipivaloate **6** (422 mg, 12%) as colorless foams.

5: $[\alpha]_D$ −13.8 ($c = 0.94$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.96 (m, 2H, H-2′), 1.19, 1.26 (2s, 18H, $2CMe_3$), 2.78 (d, $J = 4.3$ Hz, 1H, OH), 3.55 (ddd, $J = 6.4$, 8.3, 10.7 Hz, 1H, H-2), 3.57 (m, 1H, H-1′a), 3.94 (m, 1H, H-5), 3.97 (m, 1H, H-1′b), 4.11 (dd, $J = 6.3$, 11.2 Hz, 1H, H-6a), 4.18 (dd, $J = 7.3$, 11.2 Hz, 1H, H-6b), 4.40 (ddd, $J = 3.7$, 4.2, 10.7 Hz, 1H, H-3), 4.82 (d, $J = 8.3$ Hz, 1H, H-1), 5.31 (dd, $J = 1.2$, 3.7 Hz, 1H, H-4), 6.91 (d, $J = 6.3$ Hz, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.2 (C-2′), 27.1, 27.2 ($2CMe_3$), 38.7, 39.3 ($2CMe_3$), 57.2 (C-2), 61.7 (C-6), 67.7 (C-1′), 68.8 (C-3), 69.2 (C-4), 71.2 (C-5), 92.3 (CCl_3), 98.9 (C-1), 162.7, 177.9, 178.5 (3CO); HRMS (FAB) Calcd for $C_{23}H_{40}Cl_3NO_8Si$ $[M - NH_4]^+$: 609.1933; Found: 609.1872.

6: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (m, 2H, H-2′), 1.15, 1.29 (2s, 18H, $2CMe_3$), 2.57 (m, 1H, OH), 3.47 (m, 1H, H-6a), 3.72 (m, 1H, H-6b), 3.59 (m, 1H, H-1′a), 3.78 (m, 1H, H-5), 3.97 (m, 1H, H-1′b), 4.22 (m, 1H, H-2), 4.69 (d, $J = 8.3$ Hz, 1H, H-1), 5.29–5.38 (m, 2H, H-3, H-4), 6.75 (m, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.3 (C-2′), 27.0, 27.5 ($2CMe_3$), 38.9, 39.2 ($2CMe_3$), 53.2 (C-2), 60.4 (C-6), 67.1 (C-1′), 67.8 (C-4), 69.9 (C-3), 73.8 (C-5), 92.4 (CCl_3), 100.6 (C-1), 161.8, 178.2, 178.5 (3CO).

2-(Trimethylsilyl)ethyl 2-Deoxy-2-trichloroacetamido-β-D-galactopyranoside (7)

Compound **5** (2.49 g, 4.19 mmol) was treated with 1 M NaOMe/MeOH (2 mL) in methanol (20 mL) at rt. After 7 hr, the mixture was neutralized with Dowex 50 X 8 (H^+) ion-exchange resin and filtered through a pad of Celite. The Celite was washed with

methanol (3 × 5 mL) and the combined filtrates were concentrated in vacuo to give galactoside **7** (1.71 g, 96%) as a colorless solid.

$[\alpha]_D$ −3.3 ($c = 0.59$, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.92 (m, 2H, H-2′), 3.51 (ddd, $J = 1.2$, 5.4, 6.8 Hz, 1H, H-5), 3.58 (ddd, $J = 6.2$, 9.6, 10.7 Hz, 1H, H-1′a), 3.74 (dd, $J = 5.4$, 11.3 Hz, 1H, H-6a), 3.77 (dd, $J = 3.2$, 10.9 Hz, 1H, H-3), 3.78 (dd, $J = 6.8$, 11.3 Hz, 1H, H-6b), 3.85 (dd, $J = 1.3$, 3.5 Hz, 1H, H-4), 3.96 (ddd, $J = 8.5$, 9.0, 11.0 Hz, 1H, H-2), 3.99 (ddd, $J = 6.0$, 9.5, 10.7 Hz, 1H, H-1′b), 4.54 (d, $J = 8.4$ Hz, 1H, H-1), 8.62 (d, $J = 9.0$ Hz, 1H, NH); ^{13}C NMR (125 MHz, CD_3OD): δ −1.5 ($SiMe_3$), 19.0 (C-2′), 56.2 (C-2), 62.5 (C-6), 67.8 (C-1′), 69.6 (C-4), 72.1 (C-3), 76.5 (C-5), 94.1 (CCl_3), 101.9 (C-1), 164.3 (CO); HRMS (FAB) Calcd for $C_{13}H_{24}Cl_3NO_6Si$ $[M - NH_4]^+$: 441.0782; Found: 441.0780.

2-(Trimethylsilyl)ethyl 4,6-*O*-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (8)

To a solution of **7** (1.69 g, 3.98 mmol) and benzaldehyde dimethyl acetal (895 μL, 5.97 mmol) in acetonitrile (30 mL) was added *p*-toluene sulfonic acid monohydrate (75.7 mg, 0.398 mmol) at rt. The mixture was stirred for 4 hr before NEt_3 (2 mL) was added. The solvent was removed, and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc, gradient 1 : 1 to 1 : 2) to yield product **8** (1.75 g, 86%) as a colorless foam.

$[\alpha]_D$ −7.2 ($c = 1.00$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.96 (m, 2H, H-2′), 2.76 (m, 1H, OH), 3.54 (m, 1H, H-5), 3.56 (m, 1H, H-1′a), 3.74 (ddd, $J = 7.1$, 8.3, 10.1 Hz, 1H, H-2), 4.03 (m, 1H, H-1′b), 4.10 (dd, $J = 1.9$, 12.5 Hz, 1H, H-6a), 4.25 (m, 2H, H-3, H-4), 4.36 (dd, $J = 1.6$, 12.5 Hz, 1H, H-6b), 4.84 (d, $J = 8.3$ Hz, 1H, H-1), 5.59 (s, 1H, PhC*H*), 6.87 (d, $J = 7.1$ Hz, 1H, NH), 7.36–7.38, 7.50–7.52 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.4 ($SiMe_3$), 18.1 (C-2′), 57.0 (C-2), 66.6 (C-5), 67.3 (C-1′), 69.1 (C-3), 69.1 (C-6), 75.0 (C-4), 92.5 (CCl_3), 99.0 (C-1), 101.3 (Ph*C*H), 126.4, 128.2, 129.3, 137.3 (C_6H_5), 162.4 (CO); HRMS (FAB) Calcd for $C_{20}H_{28}Cl_3NO_6Si$ $[M - NH_4]^+$: 529.1095; Found: 529.1078.

2-(Trimethylsilyl)ethyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-Benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (10)

A solution of acceptor **8** (500 mg, 0.975 mmol) and ethyl 1-thio-D-galactopyranoside **9**[33] (920 mg, 1.44 mmol) in DCM (10 mL) containing 3 Å molecular sieves (2 g) was stirred at −25°C under argon. After 1.5 hr, *N*-iodosuccinimide (NIS; 660 mg, 2.93 mmol) was added, and stirring was continued for 30 min. Trifluoromethane sulfonic acid (TfOH; 25.5 μL, 0.293 mmol) in DCM (200 μL) was then added dropwise at −25°C. The reaction mixture was stirred for additional 1.5 hr at −25°C, diluted with DCM (20 mL), and filtered through a pad of Celite. The Celite was washed with DCM, and the combined filtrates were extracted with 10% aqueous $Na_2S_2O_3$ (10 mL) and saturated aqueous $KHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/

EtOAc, gradient 5 : 1 to 2 : 1) to yield the β-disaccharide **10** (998 mg, 94%) as a 4 : 1 mixture with its α-anomer. After a final crystallization from $(i\text{-Pr})_2O$, the pure β-anomer **10** was isolated as a colorless solid (655 mg, 64%).

$[\alpha]_D$ +77.6 ($c = 0.50$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ −0.03 (s, 9H, $SiMe_3$), 0.89 (m, 2H, H-2″), 3.25 (m, 1H, H-5), 3.51 (m, 1H, H-1″a), 3.67 (ddd, $J = 6.5$, 8.2, 11.2 Hz, 1H, H-2), 3.82 (dd, $J = 1.8$, 12.3 Hz, 1H, H-6a), 3.95 (m, 1H, H-1″b), 4.23 (dd, $J = 1.6$, 12.3 Hz, 1H, H-6b), 4.35 (ddd, $J = 1.1$, 4.8, 7.6 Hz, 1H, H-5′), 4.41 (m, 1H, H-4), 4.43 (dd, $J = 4.8$, 11.6 Hz, 1H, H-6′a), 4.73 (dd, $J = 7.6$, 11.6 Hz, 1H, H-6′b), 4.78 (dd, $J = 3.6$, 11.2 Hz, 1H, H-3), 5.09 (d, $J = 8.2$ Hz, 1H, H-1), 5.28 (d, $J = 7.9$ Hz, 1H, H-1′), 5.51 (s, 1H, PhC*H*), 5.57 (dd, $J = 3.4$, 10.4 Hz, 1H, H-3′), 5.87 (dd, $J = 7.9$, 10.4 Hz, 1H, H-2′), 5.96 (dd, $J = 1.1$, 3.4 Hz, 1H, H-4′), 7.02 (d, $J = 6.4$ Hz, 1H, NH), 7.30–7.34, 7.37–7.49, 7.56–7.61, 7.73–7.76, 7.89, 8.04–8.05 (m, 25H, $5C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.1 (C-2″), 55.6 (C-2), 62.7 (C-6′), 66.5 (C-5), 67.4 (C-1″), 68.2 (C-4′), 68.9 (C-6), 69.8 (C-2′), 71.6 (C-5′), 71.8 (C-3′), 74.1 (C-3), 76.2 (C-4), 92.1 (CCl_3), 97.6 (C-1), 100.5 (C-1′), 101.2 (Ph*C*H), 126.1, 128.1, 128.2, 128.4, 128.5, 128.6, 128.6, 128.8, 128.8, 129.1, 129.3, 129.7, 129.7, 129.8, 129.9, 133.3, 133.4, 133.6, 137.8 ($5C_6H_5$), 162.1, 165.1, 165.2, 165.9 (5CO); HRMS (FAB) Calcd for $C_{54}H_{54}Cl_3NO_{15}Si$ $[M\text{-}NH_4]^+$: 1107.2672; Found: 1107.2655.

2-(Trimethylsilyl)ethyl β-D-Galactopyranosyl-(1→3)-2-Deoxy-2-trichloroacetamido-β-D-galactopyranoside (11)

Compound **10** (48.5 mg, 44.5 μmol) was treated with 1 M NaOMe/MeOH (0.5 mL) in methanol (5 mL) at rt. After 18 hr, the mixture was neutralized with Dowex 50 X 8 (H^+) ion-exchange resin and filtered through a pad of Celite. The Celite was washed with methanol (3 × 3 mL), and the combined filtrates were concentrated at reduced pressure. The residue was dissolved in 80% aqueous acetic acid (3 mL) and stirred for 2 hr at 80°C. The solvent was evaporated in vacuo, and the remaining solid was purified by column chromatography on silica gel (DCM/MeOH 10 : 1) to give disaccharide **11** (12.1 mg, 46%) as a colorless solid.

$[\alpha]_D$ −3.4 ($c = 0.25$, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.92 (m, 2H, H-2″), 3.42 (dd, $J = 2.8$, 9.7 Hz, 1H, H-3′), 3.49 (m, 1H, H-5′), 3.53–3.56 (m, 2H, H-5, H-2′), 3.59 (m, 1H, H-1″a), 3.69 (dd, $J = 4.8$, 11.4 Hz, 1H, H-6′a), 3.73–3.78 (m, 3H, H-6, H-6′b), 3.80 (m, 1H, H-4′), 3.99 (m, 1H, H-1″b), 4.00–4.01 (m, 2H, H-2, H-3), 4.13 (m, 1H, H-4), 4.38 (d, $J = 7.6$ Hz, 1H, H-1′), 4.65 (d, $J = 8.0$ Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ −1.3 ($SiMe_3$), 17.9 (C-2″), 55.0 (C-2), 61.3 (C-6), 61.4 (C-6′), 66.9 (C-1″), 68.3 (C-4), 69.2 (C-4′), 71.7 (C-3′), 71.8 (C-2′), 74.9 (C-5), 75.2 (C-5′), 78.7 (C-3), 101.4 (C-1), 105.1 (C-1′); HRMS (FAB) Calcd for $C_{19}H_{34}Cl_3NO_{11}Si$ $[M - NH_4]^+$: 603.1310; Found: 603.1280.

2-(Trimethylsilyl)ethyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (12)

A solution of **10** (60.0 mg, 55.0 μmol), tributyltin hydride (58.0 μL, 220 μmol), and AIBN (2 mg) in dry benzene (2 mL) was stirred for 45 min at rt under argon, then heated under reflux for 2.5 hr, cooled, and concentrated under reduced pressure. The remains were

dissolved in acetonitrile (10 mL) and washed with hexane (5 mL). The acetonitrile was evaporated, and the residue was precipitated from EtOAc/hexane to give **12** (50.4 mg, 93%) as a colorless solid.

$[\alpha]_D$ +80.1 ($c = 0.50$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ −0.04 (s, 9H, $SiMe_3$), 0.80–0.91 (m, 2H, H-2″), 1.34 (s, 3H, CH_3), 3.26–3.31 (m, 2H, H-2, H-5), 3.45 (m, 1H, H-1″a), 3.67 (A of AB, $J = 12.1$ Hz, 1H, H-6A), 3.94 (m, 1H, H-1″b), 4.17 (B of AB, $J = 12.1$ Hz, 1H, H-6B), 4.35–4.41 (m, 3H, H-4, H-5′, H-6′a), 4.74 (dd, $J = 6.6$, 10.6 Hz, 1H, H-6′b), 4.82 (dd, $J = 3.5$, 11.0 Hz, 1H, H-3), 5.07 (m, 2H, H-1, H-1′), 5.41 (d, $J = 6.6$ Hz, 1H, NH), 5.46 (s, 1H, PhC*H*), 5.61 (dd, $J = 3.3$, 10.3 Hz, 1H, H-3′), 5.84 (dd, $J = 8.0$, 10.2 Hz, 1H, H-2′), 5.96 (m, 1H, H-4′), 7.21–7.24, 7.34–7.62, 7.76–7.77, 7.94–7.95, 8.02–8.07 (m, 25H, $5C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.4 ($SiMe_3$), 17.9 (C-2″), 23.1 (CH_3), 54.9 (C-2), 62.7 (C-6′), 66.4 (C-5), 66.8 (C-1″), 68.2 (C-4′), 69.1 (C-6), 69.9 (C-2′), 71.4 (C-5′), 71.7 (C-3′), 76.0 (C-4), 76.2 (C-3), 98.0 (C-1), 100.7 (C-1′), 102.5 (Ph*C*H), 126.3, 128.1, 128.3, 128.5, 128.6, 128.7, 128.9, 129.2, 129.7, 129.7, 130.0, 133.3, 133.4, 133.5, 133.6, 138.0 ($5C_6H_5$), 164.9, 165.9, 166.0, 171.0 (5CO); HRMS (FAB) Calcd for $C_{54}H_{57}NO_{15}Si$ $[M - NH_4]^+$: 1005.3841; Found: 1005.3825.

2-(Trimethylsilyl)ethyl β-D-Galactopyranosyl-(1→3)-2-Acetamido-2-deoxy-β-D-galactopyranoside (13)

A solution of **12** (50.0 mg, 50.6 μmol) in 80% aqueous acetic acid (3 mL) was stirred for 4.5 hr at 50°C. The solvent was evaporated in vacuo, and the remaining solid was treated with 1 M NaOMe/MeOH (0.4 mL) in methanol (4 mL) at rt. After 21 hr, the mixture was neutralized with Dowex 50 X 8 (H^+) ion-exchange resin and filtered through a pad of Celite. The Celite was washed with methanol (3 × 3 mL), and the combined filtrates were concentrated at reduced pressure. The residue was purified by column chromatography on RP-18 (H_2O/MeOH, gradient 1 : 0 to 1 : 1) to give disaccharide **13** (15.7 mg, 64%) as a colorless solid.

$[\alpha]_D$ −7.8 ($c = 0.25$, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.02 (s, 9H, $SiMe_3$), 0.86 (ddd, $J = 5.4$, 9.7, 14.0 Hz, 1H, H-2″a), 0.96 (ddd, $J = 7.0$, 10.2, 14.0 Hz, 1H, H-2″b), 1.95 (s, 3H, CH_3), 3.45 (dd, $J = 3.3$, 9.7 Hz, 1H, H-3′), 3.48–3.58 (m, 4H, H-5, H-2′, H-5′, H-1″a), 3.71 (dd, $J = 5.1$, 11.4 Hz, 1H, H-6′a), 3.72–3.78 (m, 4H, H-3, H-6, H-6′b), 3.82 (m, 1H, H-4′), 3.99 (dd, $J = 8.5$, 10.5 Hz, 1H, H-2), 4.03 (m, 1H, H-1″b), 4.10 (m, 1H, H-4), 4.31 (d, $J = 7.6$ Hz, 1H, H-1′), 4.49 (d, $J = 8.5$ Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ −1.3 ($SiMe_3$), 18.8 (C-2″), 23.3 (CH_3), 53.1 (C-2), 62.5, 62.6 (C-6, C-6′), 67.7 (C-1″), 69.7 (C-4), 70.3 (C-4′), 72.5 (C-3′), 74.6 (C-2′), 76.3, 76.8 (C-5, C-5′), 81.9 (C-3), 102.2 (C-1), 106.7 (C-1′), 174.2 (CO); HRMS (FAB) Calcd for $C_{19}H_{37}NO_{11}Si$ $[M - H]^-$: 484.2214; Found: 484.2219.

2-(Trimethylsilyl)ethyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-di-*O*-Benzyl-β-D-galactopyranoside (15)

A mixture of 2-(trimethylsilyl)ethyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (**14**,[17,18] 300 mg, 0.651 mmol), ethyl 1-thio-D-galactopyranoside **9**[33] (500 mg,

0.780 mmol) and activated powdered molecular sieves 3 Å (2.0 g) in DCM was stirred for 6 hr at rt under argon. At 0°C DMTST (504 mg, 1.95 mmol) was then added in one portion. The reaction mixture was stirred at 7°C for additional 16 hr, diluted with DCM (10 mL), and filtered through a pad of Celite. The Celite was washed with DCM (3 × 10 mL), and the combined filtrates were washed with saturated aqueous $KHCO_3$ (2 × 20 mL) and H_2O (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether/EtOAc 5 : 1) to afford disaccharide **15** (588 mg, 87%) as a colorless foam.

$[\alpha]_D$ +56.4 ($c = 1.00$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (m, 2H, H-2″), 3.54 (ddd, $J = 5.8, 9.6, 11.5$ Hz, 1H, H-1″a), 3.58–3.62 (m, 2H, H-2, H-5), 3.71 (dd, $J = 5.1, 10.1$ Hz, 1H, H-6a), 3.75 (dd, $J = 6.6, 10.1$ Hz, 1H, H-6b), 3.84 (dd, $J = 3.3, 9.4$ Hz, 1H, H-3), 4.00 (ddd, $J = 6.1, 9.6, 11.4$ Hz, 1H, H-1″b), 4.13 (d, $J = 2.8$ Hz, 1H, H-4), 4.28 (m, 1H, H-5′), 4.34 (d, $J = 7.8$ Hz, 1H, H-1), 4.36 (A of AB, $J = 11.1$ Hz, 1H, $PhCH_2$), 4.45 (dd, $J = 6.1, 11.4$ Hz, 1H, H-6′a), 4.55 and 4.59 (A, B of AB, $J = 11.9$ Hz, 2H, $PhCH_2$), 4.65 (dd, $J = 7.0, 11.4$ Hz, 1H, H-6′b), 4.72 (B of AB, $J = 11.0$ Hz, 1H, $PhCH_2$), 5.27 (d, $J = 8.0$ Hz, 1H, H-1′), 5.62 (dd, $J = 3.5, 10.5$ Hz, 1H, H-3′), 5.89 (dd, $J = 8.0, 10.5$ Hz, 1H, H-2′), 6.00 (dd, $J = 0.8, 3.5$ Hz, 1H, H-4′), 7.16–7.18, 7.25–7.32, 7.35–7.37, 7.43–7.47, 7.53–7.68, 7.78–7.80, 7.87–7.89, 8.03–8.05, 8.12–8.14 (m, 30H, $6C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.5 ($SiMe_3$), 18.4 (C-2″), 62.0 (C-6′), 67.3 (C-1″), 68.1 (C-4′), 68.9 (C-4), 69.5 (C-6), 69.9 (C-2′), 71.4, 71.5 (C-3′, C-5′), 73.2 (C-5), 73.6 (Ph*C*H_2), 74.7 (Ph*C*H_2), 78.9 (C-2), 80.8 (C-3), 101.6 (C-1′), 102.9 (C-1), 127.5–138.5 (36C, $6C_6H_5$), 165.5, 165.5, 165.6, 165.9 (4CO); HRMS (FAB) Calcd for $C_{59}H_{62}O_{15}Si$ $[M - NH_4]^+$: 1056.4202; Found: 1056.4198.

2-(Trimethylsilyl)ethyl β-D-Galactopyranosyl-(1→3)-β-D-Galactopyranoside (16)

To a solution of **15** (83.0 mg, 79.9 μmol) in methanol (5 mL) was added 1 M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred at rt under argon for 2 hr, then neutralized with Amberlite IRC 50 ion-exchange resin, filtered and concentrated under reduced pressure. The residue was dissolved in MeOH (2.5 mL) containing a catalytic amount of acetic acid and hydrogenolyzed (1 bar H_2) in the presence of 10% Pd–C (15 mg) for 3 hr at rt. The reaction mixture was filtered through a pad of Celite, which was washed with MeOH (5 mL). The combined filtrates were concentrated, and the residue was purified by silica gel chromatography (DCM/MeOH 3 : 1) to afford disaccharide **16** (26.6 mg, 75%) as a colorless foam.

$[\alpha]_D$ −2.3 ($c = 1.00$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.94 (m, 1H, H-2″a), 1.02 (m, 1H, H-2″b), 3.46 (dd, $J = 3.2, 9.7$ Hz, 1H, H-3′), 3.49–3.52 (m, 2H, H-5, H-5′), 3.55–3.74 (m, 8H, H-2, H-3, H-6, H-2′, H-6′, H-1″a), 3.75 (d, $J = 3.2$ Hz, 1H, H-4′), 3.98 (ddd, $J = 5.7, 9.6, 11.5$ Hz, 1H, H-1″b), 4.08 (d, $J = 3.0$ Hz, 1H, H-4), 4.26 (d, $J = 7.7$ Hz, 1H, H-1), 4.45 (d, $J = 7.6$ Hz, 1H, H-1′); ^{13}C NMR (125 MHz, CD_3OD): δ −1.5 ($SiMe_3$), 19.0 (C-2″), 62.4 (2C, C-6, C-6′), 67.9 (H-1″), 69.7, 70.1 (C-4, C-4′), 71.4, 72.9 (C-2, C-2′), 74.5, 76.1 (C-5, C-5′), 76.7 (C-3′), 85.0 (C-3), 103.8 (C-1), 106.3 (C-1′); HRMS (FAB) Calcd for $C_{17}H_{34}O_{11}Si$ [M-Na]$^+$: 465.1768; Found: 465.1762.

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2-(Trimethylsilyl)ethyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-β-D-Galactopyranoside (17)

Compound **15** (100 mg, 96.2 μmol) was dissolved in methanol (2.0 mL) and dioxane (1.0 mL) containing a trace of acetic acid and hydrogenolyzed (4 bar H_2) in the presence of 10% Pd–C (150 mg) for 9 d. The reaction mixture was filtered through a pad of Celite, which was washed with DCM (5 mL). The combined filtrates were concentrated, and the residue was purified by silica gel chromatography (petroleum ether/EtOAc 2 : 1) to afford **17** (55.4 mg, 67%) as colorless oil, which was immediately used in the next step.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.97 (m, 2H, H-2″), 3.47 (t, J = 5.6 Hz, 1H, H-5), 3.54 (m, 1H, H-1″a), 3.65–3.68 (m, 2H, H-3, H-6a), 3.73 (m, 1H, H-2), 3.89 (dd, J = 6.6, 11.7 Hz, 1H, H-6b), 3.99 (m, 1H, H-1″b), 4.08 (d, J = 2.9 Hz, 1H, H-4), 4.22 (d, J = 7.6 Hz, 1H, H-1), 4.39 (m, 1H, H-5′), 4.48 (dd, J = 5.6, 11.5 Hz, 1H, H-6′a), 4.68 (dd, J = 7.2, 11.5 Hz, 1H, H-6′b), 5.16 (d, J = 7.9 Hz, 1H, H-1′), 5.68 (dd, J = 3.5, 10.5 Hz, 1H, H-3′), 5.81 (dd, J = 7.9, 10.4 Hz, 1H, H-2′), 6.01 (d, J = 3.2 Hz, 1H, H-4′), 7.26–7.29, 7.37–7.40, 7.45–7.48, 7.51–7.55, 7.59–7.62, 7.65–7.68, 7.80–7.81, 7.97–7.98, 8.03–8.05, 8.12–8.13 (m, 20H, $4C_6H_5$); MS (ESI) Calcd for $C_{45}H_{50}O_{15}Si$ $[M + Na]^+$: 881.6; Found: 881.6.

2-(Trimethylsilyl)ethyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosynate)]-(2→6)-β-D-Galactopyranoside (19)

To a solution of **17** (50.0 mg, 58.2 μmol) and methyl(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-2-nonulopyranosid)onate (**18**,[37] 60.7 mg, 0.116 mmol) in MeCN/DCM (3 : 1, 4 mL) was added activated powdered molecular sieves 3 Å (1.0 g). The reaction mixture was stirred at rt under argon for 6 hr and then cooled to −25°C. After the addition of NIS (52.4 mg, 0.23 mmol) and TfOH (2.0 μL, 0.02 mmol), stirring was continued for 16 hr at −25°C. Then the mixture was diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3 × 10 mL), and the combined filtrates were extensively washed with 20% aqueous $Na_2S_2O_3$ (30 mL), saturated aqueous $KHCO_3$ (2 × 20mL) and H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 60 : 1) to afford the trisaccharide **19** (35.0 mg, 45%) as a colorless foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.98 (m, 2H, H-2‴), 1.89, 2.02, 2.03, 2.12, 2.15 (5s, 15H, $5CH_3$), 1.94 (m, 1H, H-3″a), 2.59 (dd, J = 4.6, 12.8 Hz, 1H, H-3″b), 3.51–3.58 (m, 2H, H-5, H-1‴a), 3.65–3.68 (m, 2H, H-2, H-6a), 3.74 (dd, J = 3.2, 9.4 Hz, 1H, H-3), 3.81 (s, 3H, OCH_3), 3.88 (dd, J = 5.5, 9.6 Hz, 1H, H-6b), 3.98 (m, 1H, H-1‴), 4.06–4.13 (m, 4H, H-4, H-5″, H-6″, H-9″a), 4.19 (d, J = 7.7 Hz, 1H, H-1), 4.30–4.38 (m, 2H, H-9″b, H-5′), 4.42 (dd, J = 7.0, 11.1 Hz, 1H, H-6′a), 4.70 (dd, J = 6.2, 11.1 Hz, 1H, H-6′b), 4.86 (ddd, J = 4.6, 9.8, 12.2 Hz, 1H, H-4″), 5.24 (d, J = 9.6 Hz, 1H, NH), 5.30–5.34 (m, 2H, H-1′, H-7″), 5.36–5.40 (m, 1H, H-8″), 5.66 (dd, J = 3.5, 10.4 Hz, 1H, H-3′), 5.80 (dd, J = 8.0, 10.4 Hz, 1H, H-2′), 6.02 (d, J = 3.4 Hz, 1H, H-4′), 7.24–7.27,

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7.36–7.39, 7.43–7.47, 7.50–7.55, 7.57–7.60, 7.63–7.66, 7.78–7.80, 7.95–7.97, 8.01–8.03, 8.10–8.12 (m, 20H, $4C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ −1.4 ($SiMe_3$), 18.1 (C-2‴), 20.8, 20.8, 21.0, 23.2 ($5CH_3$), 37.6 (C-3″), 49.4 (C-5″), 52.9 (OCH_3), 61.7 (C-6′), 62.4 (C-9″), 62.6 (C-6), 67.1 (C-1‴), 67.5 (2C, C-4, C-7″), 67.6 (C-4′), 68.0 (2C, C-4″, C-8″), 68.9 (C-2′), 70.0 (C-2), 71.1, 71.4 (C-3′, C-5′), 72.3 (C-6″), 72.8 (C-5), 81.2 (C-3), 99.0 (C-2″), 101.9, 102.1 (C-1, C-1′), 128.3, 128.4, 128.5, 128.6, 128.7, 129.0, 129.2, 129.3, 129.7, 129.8, 130.0, 131.0, 133.3, 133.4, 133.6 (24C, $4C_6H_5$), 165.5, 165.6, 165.6, 169.0, 169.9, 170.1, 170.3, 170.7, 170.9 (10C, 10CO); HRMS (FAB) Calcd for $C_{65}H_{77}NO_{27}Si$ $[M - NH_4]^+$: 1349.4796; Found: 1349.4778.

2-(Trimethylsilyl)ethyl β-D-Galactopyranosyl-(1→3)-[Sodium (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosynate)]-(2→6)-β-D-Galactopyranoside (20)

To a solution of **19** (32.5 mg, 24.4 μmol) in methanol (1 mL) was added freshly prepared 1 M NaOMe/MeOH (1.0 mL). The mixture was stirred at rt under argon for 7 hr, then water (1 mL) was added and the mixture was stirred for another 16 hr at rt. The solution was concentrated, and the residue was purified by reverse phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **20** (17.8 mg, 90%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D$ −3.5 ($c = 1.00$, H_2O); ^1H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (ddd, $J = 5.2$, 12.9 Hz, 1H, H-2‴a), 1.04 (ddd, $J = 5.5$, 12.9 Hz, 1H, H-2‴b), 1.63 (t, $J = 12.2$ Hz, 1H, H-3″a), 2.00 (s, 3H, CH_3), 2.68 (dd, $J = 4.7$, 12.4 Hz, 1H, H-3″b), 3.55 (m, 1H, H-6″), 3.56–3.60 (m, 3H, H-2, H-2′, H-9″a), 3.61–3.64 (m, 2H, H-6a, H-3′), 3.66–3.68 (m, 3H, H-5′, H-4″, H-7″), 3.71 (m, 1H, H-6′a), 3.72–3.79 (m, 4H, H-3, H-6′b, H-8″, H-1‴a), 3.81–3.85 (m, 3H, H-5, H-6b, H-5″), 3.87–3.91 (m, 2H, H-4′, H-9″b), 4.00 (ddd, $J = 5.1$, 10.1, 12.7 Hz, H-1‴b), 4.20 (d, $J = 3.3$ Hz, 1H, H-4), 4.42 (d, $J = 8.0$ Hz, 1H, H-1), 4.55 (d, $J = 7.6$ Hz, 1H, H-1′); ^{13}C NMR (125 MHz, D_2O): δ −2.2 ($SiMe_3$), 17.1 (C-2‴), 39.9 (C-3″), 52.2 (C-5″), 61.4 (C-6′), 63.0 (C-6), 63.4 (C-9″), 68.5, 68.5 (C-5′, C-6″), 68.5 (C-1‴), 68.9 (2C, C-4, C-4′), 70.0 (C-2), 71.3 (2C, C-2′, C-7″), 72.0 (C-5), 72.9 (C-4″), 73.3 (C-8″), 75.4 (C-3′), 83.0 (C-3), 100.2 (C-2″), 102.1 (C-1), 105.0 (C-1′), 173.2, 175.4 (CO); MS (ESI) Calcd for $C_{28}H_{50}NNaO_{19}Si$ $[M + Na]^+$: 778.3; Found: 778.5 (100%).

8-(Methoxycarbonyl)octyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-Galactopyranosyl-(1→4)-2-Acetamido-2-deoxy-β-D-glucopyranoside (23)

Disaccharide **21**[40] (3.0 mg, 5.4 μmol) and CMP-NeuAc (5.3 mg, 8.1 μmol) were dissolved in a mixture of 50 mM sodium cacodylate buffer pH 6.5 (0.6 mL), 60 mM aqueous $MnCl_2$ (0.6 mL) and deionized water (0.4 mL) containing BSA (0.4 mg, Fluka). The mixture was incubated at 37°C with CIAP (2 μL) and recombinant ST3Gal III (200 μL, 9 U/L). After 3 d TLC (silica gel, DCM/MeOH/H_2O 10 : 4 : 0.8) indicated complete consumption of **21**. The turbid solution was centrifuged, and the supernatant was passed over a RP-18 column, which was washed with water before the product was eluted

with MeOH. After evaporation of methanol, the residue was chromatographed on silica gel (DCM/MeOH/H_2O 10 : 4 : 0.4, then 6 : 4 : 1) to yield trisaccharide **23** (3.5 mg, 76%) as colorless powder after a final lyophilization from water. The spectroscopic data of **23** were in accordance to those reported.[20a,29a]

8-(Methoxycarbonyl)octyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-Galactopyranosyl-(1→3)-2-Acetamido-2-deoxy-β-D-glucopyranoside (24)

According to the procedure described for the synthesis of **23**, compound **22**[41] (3.0 mg, 5.4 μmol) was incubated with CMP-NeuAc (5.3 mg, 8.1 μmol) and ST3Gal III (150 μL, 9 U/L). Reaction control by TLC (silica gel, DCM/MeOH/H_2O 10 : 4 : 0.8) indicated complete consumption of **22** after 3 d. After work-up, the crude product was purified on RP-18 (H_2O/MeOH gradient 1 : 0 to 1 : 1) to yield trisaccharide **24** (4.1 mg, 90%) as colorless powder after a final lyophilization from water. The spectroscopic data of **24** were in accordance to those reported.[29b,42]

2-(Trimethylsilyl)ethyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-Galactopyranosyl-(1→3)-2-Acetamido-2-deoxy-β-D-galactopyranoside (25)

Disaccharide **13** (10.0 mg, 20.6 μmol) and CMP-NeuAc (20.4 mg, 31.1 μmol) were dissolved in a mixture of 50 mM sodium cacodylate-buffer pH 6.5 (2 mL), 60 mM aqueous $MnCl_2$ (2 mL) and deionized water (1.3 mL) containing BSA (2.0 mg). The mixture was incubated at 37°C with CIAP (2 μL) and recombinant ST3Gal III (500 μL, 9 U/L). After 17 hr, additional ST3Gal III (250 μL) was added, and the incubation was continued for 4 d. The turbid solution was centrifuged, and the supernatant was passed over a RP-18 column, which was washed with water before the crude product was eluted with MeOH. After evaporation of the solvent, the residue was chromatographed on RP-18 (H_2O/MeOH gradient 1 : 0 to 1 : 1) to yield trisaccharide **25** (9.0 mg, 56%) and starting material **13** (4.4 mg, 44%) as colorless powders after a final lyophilization from water.

$[\alpha]_D$ −13.1 ($c = 0.25$, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.86 (m, 1H, H-2‴a), 0.97 (m, 1H, H-2‴b), 1.78 (t, $J = 11.6$ Hz, 1H, H-3″a), 2.00, 2.02 (s, 6H, $2CH_3$), 2.74 (m, 1H, H-3″b), 3.54 (m, 1H, H-2′), 3.57–3.62 (m, 3H, H-5, H-6″, H-7″), 3.63–3.74 (m, 6H, H-6, H-6′a, H-4″, H-9″a, H-1‴a), 3.75–3.81 (m, 2H, H-5′, H-6′b), 3.82–3.87 (m, 4H, H-3, H-5″, H-8″, H-9″b), 3.92 (m, 1H, H-4′), 3.97–4.06 (m, 3H, H-2, H-3′, H-1‴b), 4.16 (m, 1H, H-4), 4.49 (d, $J = 7.6$ Hz, 1H, H-1′), 4.53 (d, $J = 8.3$ Hz, 1H, H-1); ^{13}C NMR (125 MHz, D_2O): δ −1.8 ($SiMe_3$), 17.6 (C-2‴), 22.4, 22.6 ($2CH_3$), 40.1 (C-3″), 51.6 (C-2), 52.2 (C-5″), 61.3, 61.4 (C-6, C-6′), 62.7 (C-9″), 67.7 (C-4′), 68.2 (C-4), 68.3 (C-7″), 68.5 (C-4″), 68.6 (C-1‴), 69.2 (C-2′), 72.2 (C-8″), 72.7 (C-6″), 75.0, 75.1 (C-5, C-5′), 76.1 (C-3′), 80.8 (C-3), 100.2 (C-1″), 100.8 (C-1), 104.9 (C-1′); HRMS (FAB) Calcd for $C_{30}H_{54}N_2O_{19}Si$ $[M - H]^-$: 775.3168; Found: 775.3110.

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2-(Trimethylsilyl)ethyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-Galactopyranosyl-(1→3)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (26)

According to the procedure described for the synthesis of **25**, compound **11** (12.0 mg, 20.4 μmol) was incubated with CMP-NeuAc (20.2 mg, 30.1 μmol), BSA (2.1 mg), CIAP (2 μL) and ST3Gal III (500 μL, 9 U/L). After 21 hr additional ST3Gal III (250 μL) was added, and the incubation was continued for 3 d. After work-up, the residue was chromatographed on silica gel (DCM/MeOH/H_2O 10 : 4 : 0.4, then 6 : 4 : 1) to yield starting material **11** (7.5 mg, 62%) and trisaccharide **26** (6.4 mg, 36%) as colorless powders after a final lyophilization from water.

$[\alpha]_D$ −8.5 ($c = 0.25$, H_2O); ^{1}H NMR (500 MHz, D_2O): δ −0.03 (s, 9H, $SiMe_3$), 0.89 (m, 1H, H-2‴a), 0.98 (m, 1H, H-2‴b), 1.75 (t, $J = 12.2$ Hz, 1H, H-3″a), 2.00 (s, 3H, CH_3), 2.72 (dd, $J = 4.6$, 12.4 Hz, 1H, H-3″b), 3.52 (dd, $J = 7.8$, 9.7 Hz, 1H, H-2′), 3.56 (dd, $J = 1.8$, 10.5 Hz, 1H, H-6″), 3.59–3.61 (m, 3H, H-5, H-6a, H-7″), 3.62–3.74 (m, 6H, H-6b, H-5′, H-6′a, H-4″, H-9″a, H-1‴a), 3.75–3.83 (m, 3H, H-6′b, H-5″, H-8″), 3.86 (dd, $J = 2.4$, 11.8 Hz, 1H, H-9″b), 3.90 (m, 1H, H-4′), 3.96–4.04 (m, 3H, H-2, H-3′, H-1‴b), 4.09 (dd, $J = 3.1$, 10.9 Hz, 1H, H-3), 4.18 (m, 1H, H-4), 4.53 (d, $J = 7.8$ Hz, 1H, H-1′), 4.68 (d, $J = 8.4$ Hz, 1H, H-1); ^{13}C NMR (125 MHz, D_2O): δ − 1.8 ($SiMe_3$), 17.8 (C-2‴), 22.2 (CH_3), 40.3 (C-3″), 52.4 (C-5″), 53.9 (C-2), 61.2, 61.4 (C-6, C-6′), 62.6 (C-9″), 67.5 (C-4′), 68.1 (C-4), 68.2 (C-7″), 68.7 (C-4″), 69.0 (C-1‴), 69.4 (C-2′), 69.5 (C-6″), 72.0 (C-8″), 75.2, 75.3 (C-5, C-5′), 76.1 (C-3′), 78.5 (C-3), 100.5 (2C, C-1, C-1″), 104.3 (C-1′); HRMS (FAB) Calcd for $C_{30}H_{51}Cl_3N_2O_{19}Si$ $[M - NH_4]^+$: 894.2264; Found: 894.2261.

2-(Trimethylsilyl)ethyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-Galactopyranosyl-(1→3)-β-D-galactopyranoside (27)

According to the procedure described for the synthesis of **25**, compound **16** (10.0 mg, 22.6 μmol) was incubated with CMP-NeuAc (22.3 mg, 33.9 μmol), BSA (2.3 mg), CIAP (2 μL) and ST3Gal III (500 μL, 9 U/L). After 17 hr additional ST3Gal III (250 μL) was added, and the incubation was continued for 3 d. After work-up, the residue was chromatographed on silica gel (DCM/MeOH/H_2O 10 : 4 : 0.4, then 6 : 4 : 1) to yield starting material **16** (4.0 mg, 40%) and trisaccharide **27** (9.7 mg, 59%) as colorless powders after a final lyophilization from water.

$[\alpha]_D$ +4.1 ($c = 0.63$, H_2O); ^{1}H NMR (500 MHz, D_2O): δ −0.02 (s, 9H, $SiMe_3$), 0.97 (m, 1H, H-2‴a), 1.07 (m, 1H, H-2‴b), 1.79 (t, $J = 12.1$ Hz, 1H, H-3″a), 2.02 (s, 3H, CH_3), 2.75 (dd, $J = 4.6$, 12.4 Hz, 1H, H-3″b), 3.58 (dd, $J = 1.5$, 9.2 Hz, 1H, H-7″), 3.59–3.66 (m, 4H, H-2, H-2′, H-6″, H-9″a), 3.66–3.77 (m, 8H, H-5, H-6, H-5′, H-6′, H-4″, H-1‴a), 3.80 (dd, $J = 3,4$, 9.9 Hz, 1H, H-3), 3.84–3.88 (m, 3H, H-5″, H-8″, H-9″b), 3.94 (d, $J = 3.0$ Hz, 1H, H-4′), 4.04 (ddd, $J = 5.2$, 10.0, 12.7 Hz, 1H, H-1‴b), 4.10 (dd, $J = 3.1$, 9.8 Hz, 1H, H-3′), 4.18 (d, $J = 3.3$ Hz, 1H, H-4), 4.45 (d, $J = 8.0$ Hz, 1H, H-1), 4.68 (d, $J = 7.8$ Hz, 1H, H-1′); ^{13}C NMR (125 MHz, D_2O): δ −2.0 ($SiMe_3$), 18.1 (C-2‴), 22.6 (CH_3), 40.2 (C-3″), 52.2 (C-5″), 61.4, 61.5 (C-6, C-6′), 63.1 (C-9″),

67.9 (C-4′), 68.6 (C-7″), 68.8 (C-4″), 68.9 (C-1‴), 69.0 (C-4), 70.1, 70.3 (C-2, C-2′), 72.4 (C-8″), 73.4 (C-6″), 75.2, 75.4 (C-5, C-5′), 76.1 (C-3′), 83.1 (C-3), 100.4 (C-1″), 102.3 (C-1), 104.6 (C-1′); HRMS (FAB) Calcd for $C_{28}H_{51}NO_{19}Si$ $[M - NH_4]^+$: 751.3186; Found: 751.3172.

Attempted Enzymatic Sialylation of 2-(Trimethylsilyl)ethyl β-D-Galactopyranosyl-(1→3)-[sodium (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosynate)]-(2→6)-β-D-Galactopyranoside (20)

According to the procedure described for the synthesis of **25**, trisaccharide **20** (10.0 mg, 13.2 μmol) was incubated with CMP-NeuAc (13.0 mg, 19.8 μmol), CIAP (2 μL) and ST3Gal III (500 μL, 9 U/L) in a mixture of 50 mM sodium cacodylate-buffer pH 6.5 (2 mL), 60 mM aqueous $MnCl_2$ (2 mL) and deionized water (1.3 mL) containing BSA (2.0 mg). After 24 hr, additional ST3Gal III (250 μL) was added, and the incubation was continued for 3 d. After work-up, the crude product was chromatographed on silica gel (DCM/MeOH/H_2O 10 : 4 : 0.8) to yield exclusively starting material **20** (9.5 mg, 95%) as a colorless powder after a final lyophilization from water.

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