Enzymatic Synthesis of Oligosaccharide Analogues: Evaluation of UDP-Gal Analogues as Donors for Three Retaining α-Galactosyltransferases

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Abstract: A series of deoxygenated uridine 5′-diphosphogalactose (UDP-Gal) derivatives are evaluated as donors for three different retaining galactosyltransferases using capillary electrophoresis with laser-induced fluorescence detection. The enzymes investigated were calf thymus α(1→3) galactosyltransferase (E.C. 2.4.1.151), blood group B α(1→3) galactosyltransferase (E.C. 2.4.1.37) and *Neisseria meningitidis* α(1→4) galactosyltransferase. UDP-2-deoxy-Gal and UDP-6-deoxy-Gal were found to be active as donors for all three enzymes. Preparative syntheses utilizing these UDP-Gal derivatives were performed on mg scales, affording deoxygenated trisaccharide analogues in 5−100% yields.

Introduction

Oligosaccharide sequences, usually two to six residues in size, frequently act as recognition molecules in crucial cellular interactions. Such molecules are known to be involved in cell-signaling pathways and can act as ligands in cell−cell adhesion events as diverse as fertilization, cancer, and inflammation.[1] Oligosaccharides also frequently serve as receptors for the attachment of bacteria, toxins, and viruses to human cell surfaces.[1−4] Synthetic inhibitors of these events have important pharmaceutical potential.

The synthesis of complex oligosaccharide sequences containing two to six sugar units, both in solution and on the solid phase, represents a major challenge in organic chemistry.[5−8] Their synthesis remains inherently much more complex than that of oligonucleotides and peptides by virtue of the fact that new stereochemistry (either α or *β*) is introduced when coupling two sugar residues together through a glycosidic linkage. Mixtures are often obtained. In addition, most sugar units have multiple hydroxyl groups necessitating complex reaction sequences for their selective protection prior to glycosylation. New increasingly efficient glycosylation and protection strategies are actively being sought out to simplify the available chemical-synthetic protocols.[9−14]

There are two principle reasons why the use of enzymes in oligosaccharide synthesis has recently gained a high level of interest in the chemistry community: the enzymatic synthesis of a glycosidic linkage is always stereospecific, and no protecting groups are required. Both glycosidases (running in the "reverse" direction) and glycosyltransferases have been used.[15−17] Glycosidases are often readily available but frequently suffer from low yields of product formed in equilibrium reactions and the formation of multiple products due to the relaxed regiospecificity inherent to many of these enzymes. Glycosyltransferases, on the other hand, are enzymes of choice since they are highly regiospecific, yielding single products in quantitative yields.[18] These enzymes are, however, difficult to isolate and stabilize in a synthetic laboratory, but they are being cloned in large numbers and are increasingly becoming commercially available.[19]

The glycosyltransferases transfer sugars from the appropriate sugar nucleotides (many of the natural ones are commercially available) with either complete retention or complete inversion

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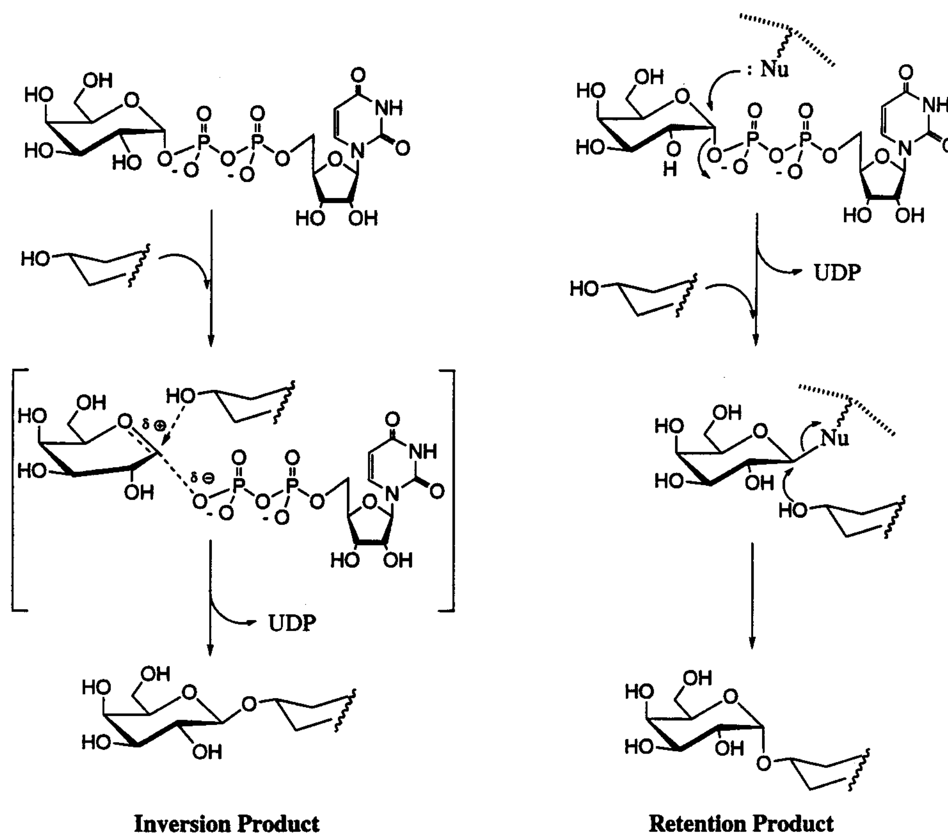

Figure 1. Hypothetical reaction pathways for enzymatic glycosyl-transfer proceeding with either inversion or retention at the anomeric center of the sugar being transferred.

at the anomeric center of the sugar being transferred (Figure 1). Any naturally occurring oligosaccharide sequence can in principle be prepared using the glycosyltransferase, the acceptor oligosaccharide, and the sugar nucleotide involved in its biosynthesis. As noted above, the cloned enzymes can be produced in large quantity and the sugar nucleotides can be purchased on a gram scale. For very large scale reactions, elegant enzyme recycling systems have been developed that produce the required sugar nucleotides in situ from the free monosaccharides, allowing the production of kilogram amounts of oligosaccharides in an economically viable manner.[15−17]

Glycosyltransferases are also being assessed as synthetic tools for the preparation of oligosaccharide *analogues*.[20] Both unnatural acceptors and unnatural donors have been reported to act as substrates for some glycosyltransferases. Many successful syntheses using modified acceptor structures and the natural sugar nucleotides have been reported.[20] Far fewer examples of the use of unnatural sugar nucleotides exist, and these reports consist solely of examples where the glycosyltransferases transfer the sugar with inversion of configuration at the anomeric center, presumably via a direct displacement mechanism proceeding by way of an oxocarbenium ion intermediate (Figure 1). The mechanism for the transfer of retaining glycosyltransferases is presumably more complex, proceeding via a double inversion mechanism involving a covalent glycosyl-enzyme intermediate (Figure 1).

In the present work, we extend previous studies on the glycosyltransferase-catalyzed synthesis of oligosaccharide analogues to include glycosyltransferases that transfer sugars with net *retention* of configuration. The enzymes selected were as follows: (1) α(1→3) galactosyltransferase (calf thymus α(1→3) GalT, E.C. 2.4.1.151),[21] which catalyzes the transfer of D-galactopyranose from UDP-Gal to the 3′-hydroxyl group of *β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*(NAc)-OR; (2) blood group B α(1→3) galactosyltransferase (blood group B α(1→3) GalT, E.C. 2.4.1.37),[22] which catalyzes the transfer of D-galactopyranose from UDP-Gal to the 3-hydroxyl group of α-L-Fuc*p*-(1→2)-*β*-D-Gal*p*-OR; and (3) *Neisseria meningitidis* α(1→4) galactosyltransferase (α(1→4) GalT),[23] which catalyzes the transfer of D-galactopyranose from UDP-Gal to the 4′-hydroxyl group of *β*-D-Gal*p*(1→4)-*β*-D-Glc*p*-OR.

The two α(1→3) GalTs have 30% sequence homology despite acting on different acceptors. This might reflect similarities in their sugar nucleotide combining sites. The bacterial α(1→4) GalT is unrelated. These enzymes thus represent a good study system for evaluating similarities and differences in glycosyltransferases for utility in analogue synthesis. We chose to examine whether these enzymes could transfer galactose residues where sterically conservative structural changes were made on each of the carbon atoms (either deoxygenation or hydroxymethyl group removal). Five analogues were evaluated. The required UDP-Gal analogues were prepared chemically as anomeric mixtures using a very convenient coupling between UDP and the per-trimethylsilylated glycosyl iodide of the commercially available unprotected galactose analogue.[29]

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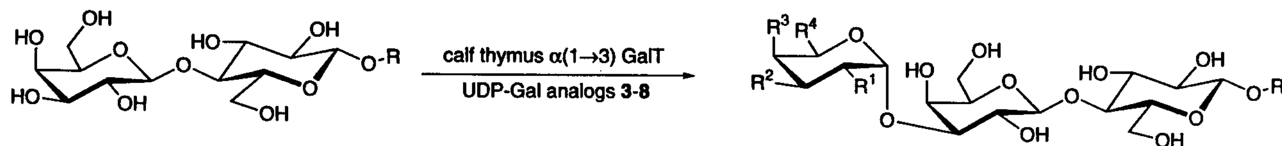
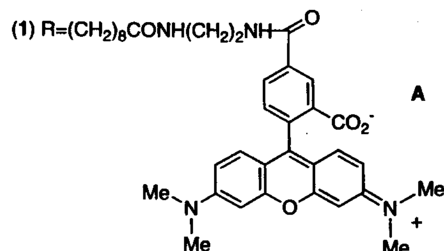
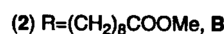
Scheme 1







	R^1	R^2	R^3	R^4	R
9	OH	OH	OH	CH_2OH	A
10	H	OH	OH	CH_2OH	A
11	OH	H	OH	CH_2OH	A
12	OH	OH	H	CH_2OH	A
13	OH	OH	OH	CH_3	A
14	OH	OH	OH	H	A
15	H	OH	OH	CH_2OH	B
16	OH	H	OH	CH_2OH	B
17	OH	OH	H	CH_2OH	B
18	OH	OH	OH	CH_3	B
19	OH	OH	OH	H	B

A rapid assay was required to determine whether the enzymes could transfer the modified galactosyl residues from their sugar nucleotides. Several methods to determine the activity of glycosyltransferases have been developed, including radiochemical assays,[24] ELISA assays,[25] and coupled enzyme assays.[26,27] For comparing the rates of transfer of modified sugars from sugar nucleotides, which are not commercially available in radiolabeled form, the use of coupled enzyme assays with spectroscopic analysis has been favored. This method, however, requires a relatively large amount of substrate and the addition of two or more coupling enzymes.[26] We therefore employed capillary electrophoresis (CE), which is capable of detecting 100 molecules of oligosaccharide product by laser-induced fluorescence,[28] to quantify the relative rates of transfer of the Gal-analogues. When a reasonable rate of transfer was observed, preparative syntheses were performed and the structures of the isolated products were confirmed by ^{1}H NMR spectroscopy and mass spectrometry.

Results and Discussion

Enzymatic assays utilizing capillary electrophoresis. UDP-Gal analogues (**3**−**8**) were synthesized as described[29] (Scheme 2), and the resulting mixture of α/β anomers was screened for activity as donors for the galactosyltransferases. To obtain the relative rate of transfer for each donor, tetramethylrhodamine (TMR)-labeled acceptors (**1**[30] for calf thymus α(1→3) GalT and α(1→4) GalT, **20**[31] for blood group B α(1→3) GalT) were used to enable laser-induced fluorescence detection. As shown in Schemes 1, 4, and 5, these acceptors were incubated with the synthetic donor analogues in the presence of each enzyme. Although all synthetic donor analogues were mixtures of the α and β anomers, the concentration of each α anomer (the configuration of the natural UDP-Gal) was adjusted to be identical. At the same time, commercially available UDP-α-Gal was also incubated as a standard with the three galactosyltransferases. After incubation, TLC was performed on the incubation mixture, permitting the visual observation of both starting materials and potential new fluorescent products. Only one product, which had a slightly lower R_f than that of the starting acceptor, was observed in each case. No degradation or side reaction of the starting acceptor was detected. Each reaction was stopped by 300-fold dilution, and the resulting mixture was quickly purified by adsorption onto a Sep-Pak Plus C18 cartridge, followed by washing with water and eluting with methanol. The sample thus obtained was analyzed by CE using

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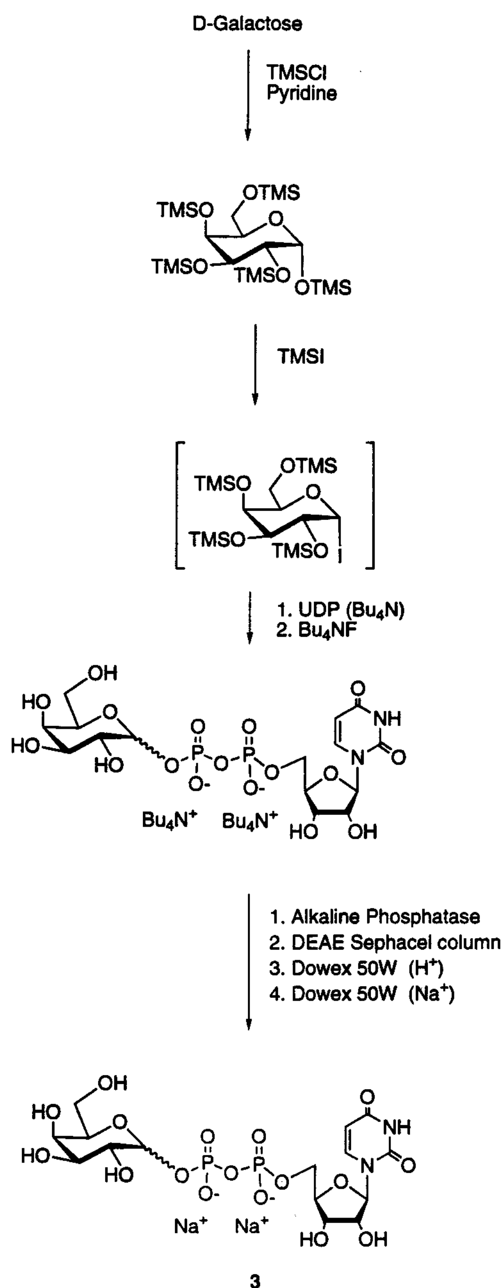
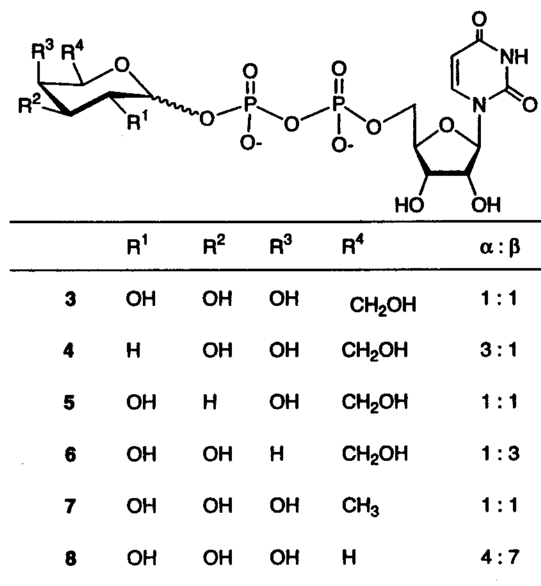
Scheme 2



Scheme 3



	R¹	R²	R³	R⁴	α : β
3	OH	OH	OH	CH₂OH	1 : 1
4	H	OH	OH	CH₂OH	3 : 1
5	OH	H	OH	CH₂OH	1 : 1
6	OH	OH	H	CH₂OH	1 : 3
7	OH	OH	OH	CH₃	1 : 1
8	OH	OH	OH	H	4 : 7

a miceller electrokinetic chromatography system.[32] Though this system is able to detect 100 TMR-labeled molecules,[28] 3.1 amol were injected to obtain a precise integration value for each peak. New peaks that had faster migration times than those of the starting disaccharides were assumed to be the enzymatically produced trisaccharides (Figure 2). The ratios of the resulting trisaccharides (**9**−**14**, **22**−**27**, **30**−**35**) to the sum of trisaccharide and starting disaccharide (**1**, **20**) were calculated on the basis of the integration of each peak and corresponded to the percent of transfer that had occurred. For the donors showing very slow transfer, a large amount of enzyme and long incubation times were employed, and the reaction rates were calculated assuming that initial velocity of the reaction was proportional to both the amount of enzyme and the incubation time. The relative rates obtained for the donor analogues as well as for the standard, which was set at 100, are summarized in Table 1.

The results obtained using this CE-assay are as follows: (1) For the three galactosyltransferases examined, the synthetic UDP-Gal (**3**) employed, which is a mixture of α and β anomers, showed almost the same rate of transfer as that of the commercial pure α anomer. This means inhibition by the β anomer of UDP-Gal was negligible. (2) UDP-2-deoxy-Gal showed very high relative rates of transfer compared to pure UDP-Gal in the case of the two α(1→ 3) GalTs. Since this donor does not possess an electron-withdrawing hydroxyl group at C-2, the formation of an oxocarbenium ion, which can be considered to be an intermediate of the reaction (Figure 1), is thought to be much easier than with the 2-hydroxy sugar. Preliminary kinetic evaluations employing capillary electrophoresis analysis of the calf thymus α(1→ 3) Gal reaction indicate that the V_{max} for UDP-2-deoxy-Gal (1:1 anomeric mixture) is much greater (10-fold) than for UDP-Gal while the K_M's are the same (65 μM) for both donors. This is in contrast to previous kinetic evaluations with UDP-2-deoxy-Gal and an inverting β(1→4) GalT from bovine milk that showed no effect on either V_{max} or donor K_M compared to UDP-Gal.[33a] (3) Hydroxyl groups at both the 3 and 4 positions of Gal were found to be very important for recognition by all three GalTs, and donors lacking these hydroxyl groups were very unreactive. The inverting β(1→4) GalT enzyme also exhibits poor reactivity with 3- and 4-deoxy-Gal donors.[33b,c] For UDP-4-deoxy-Gal, the rate reduction for β(1→4) GalT was due solely to a reduction in V_{max} to 6% of UDP-Gal, while the apparent K_M for modified donor (78 μM) was the same as that of UDP-Gal (95 μM).[33c] (4) UDP-6-deoxy-Gal showed reduced activity that was still high enough for practical preparative syntheses. For the blood group B α(1→3) GalT, preliminary kinetic evaluations suggest the rate reduction is due to a decrease in V_{max} to 5% of that of UDP-Gal, while the effect on K_M is not as great, 55 μM compared to 95 μM for UDP-Gal. UDP-L-Ara showed lower activity than UDP-6-deoxy-Gal for all three retaining galactosyltransferases. Similar rate reductions have been seen for β(1→4) GalT with UDP-L-Ara due to a reduction in V_{max} to 4% of UDP-Gal with only a minor effect on K_M.[33c] While relative rate data in two substrate systems at subsaturating substrate concentrations should be

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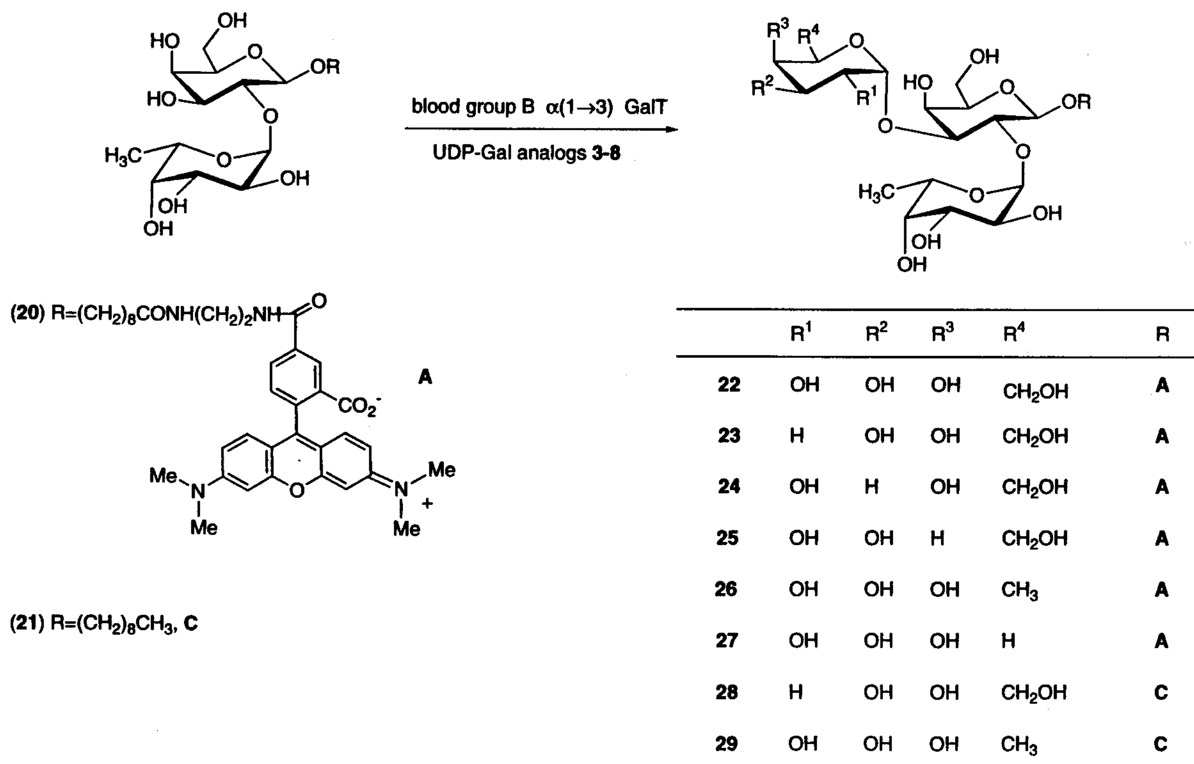
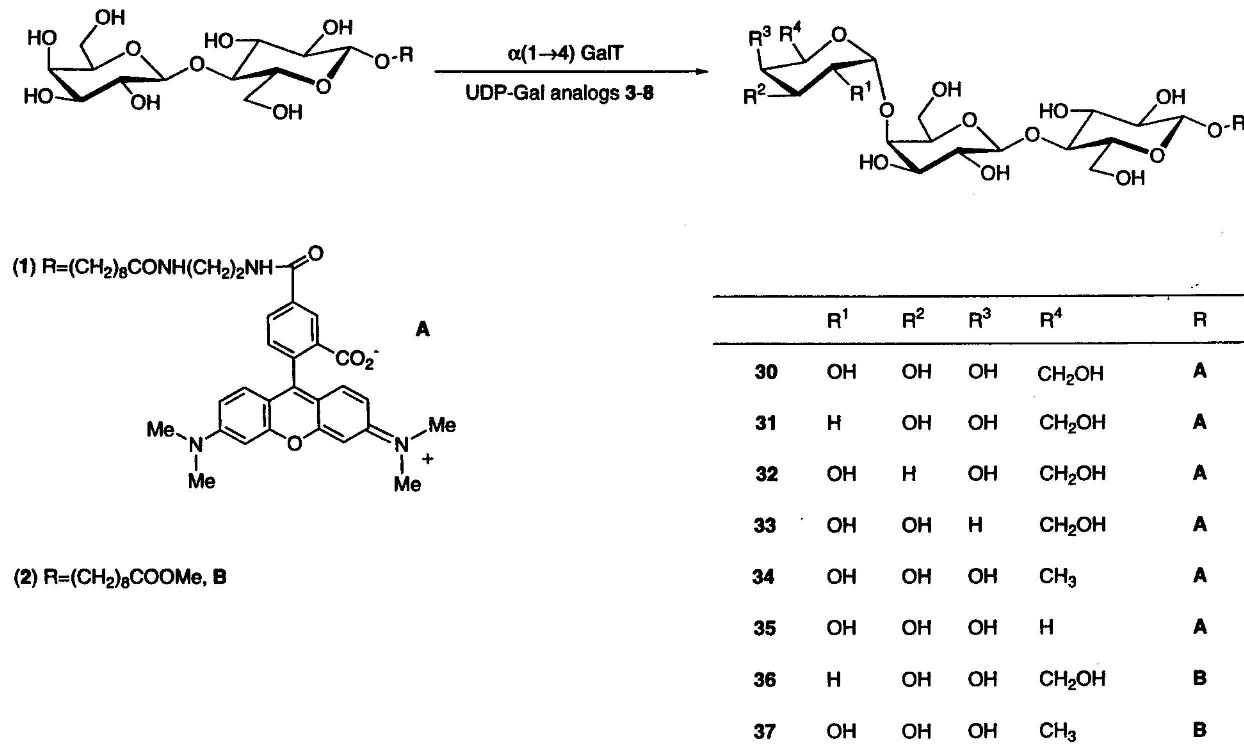
Scheme 4



	R^1	R^2	R^3	R^4	R
22	OH	OH	OH	CH_2OH	A
23	H	OH	OH	CH_2OH	A
24	OH	H	OH	CH_2OH	A
25	OH	OH	H	CH_2OH	A
26	OH	OH	OH	CH_3	A
27	OH	OH	OH	H	A
28	H	OH	OH	CH_2OH	C
29	OH	OH	OH	CH_3	C

Scheme 5



	R^1	R^2	R^3	R^4	R
30	OH	OH	OH	CH_2OH	A
31	H	OH	OH	CH_2OH	A
32	OH	H	OH	CH_2OH	A
33	OH	OH	H	CH_2OH	A
34	OH	OH	OH	CH_3	A
35	OH	OH	OH	H	A
36	H	OH	OH	CH_2OH	B
37	OH	OH	OH	CH_3	B

cautiously interpreted in the absence of detailed kinetic evaluations, the preliminary data on our retaining and on an inverting β(1→4) GalT suggest that deoxygenation effects are more pronounced on V_{max}. Further support for this comes from the observations that UDP is a competitive inhibitor of all galactosyltransferases with K_i values generally in the range of K_M's for UDP-Gal donors, indicating strong recognition of the nucleotide moiety. This is routinely exploited in enzyme purification employing affinity chromatography on UDP supports.

Correlation of Donor Specificity with Sequence. Recently, Breton et al.[34] reviewed sequence-function relationships of prokaryotic and eukaryotic galactosyltransferases. Galactosyltransferases were grouped into five families based on a computer-assisted systematic search for local homology in amino acid sequence. As a result of the study, calf thymus α(1→3)

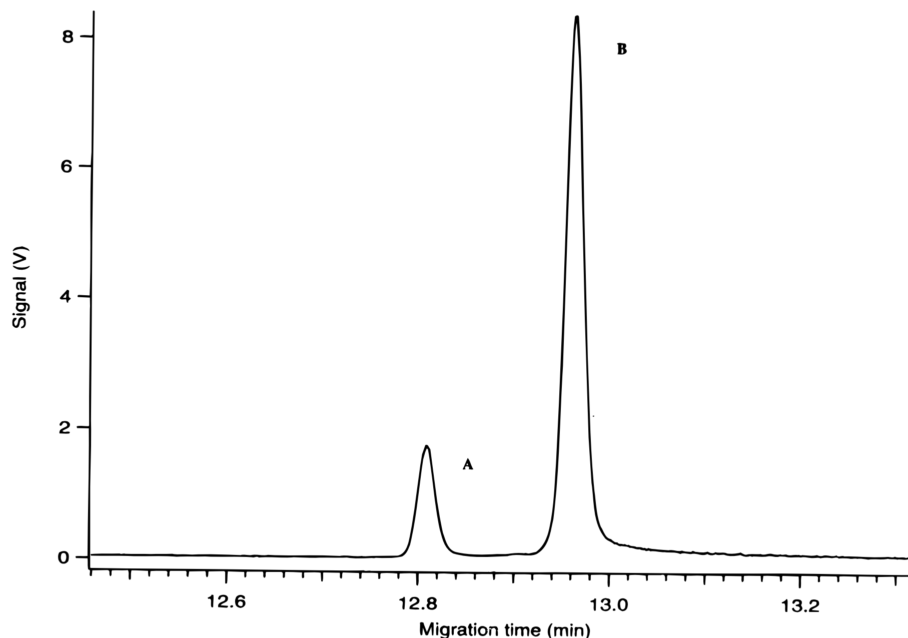


Figure 2. Electropherogram obtained from capillary electrophoresis with laser induced fluorescence detection analysis of a blood group B α(1→3) galactosyltransferase reaction. The incubation mixture containing enzyme and 390 μM each of UDP-Gal donor and Fuc-Gal-TMR acceptor **22** was partially purified after reaction on a reversed-phase cartridge and diluted with CE running buffer to 204 nM TMR as described in the text. For analysis, sample was injected in a volume of 15 pL (1 kV for 5 s) containing 3.1 amol total TMR-labeled molecules. The peaks in the electropherogram are the trisaccharide product **22** (**A**) and disaccharide acceptor **20** (**B**).

Table 1. Relative Rates of Transfer of UDP-deoxy-Gal Analogues

		calf thymus α(1→3) GalT	blood group B α(1→3) GalT	*Neisseria meningitides* α(1→4) GalT
	(α:β)	acceptor: lactose-TMR (**1**)	acceptor: Fuc-Gal-TMR (**20**)	acceptor: lactose-TMR (**1**)
UDP-Gal (Sigma)	(α)	100	100	100
UDP-Gal (**3**)	(1:1)	105	65	78
UDP-2-deoxy-Gal (**4**)	(3:1)	342	173	28
UDP-3-deoxy-Gal (**5**)	(1:1)	0.20	0.12	0.0
UDP-4-deoxy-Gal (**6**)	(1:3)	0.61	0.21	1.8
UDP-6-deoxy-Gal (**7**)	(1:1)	1.53	18	42
UDP-L-Ara (**8**)	(4:7)	0.77	0.11	6.9

GalT and blood group B α(1→3) GalT, together with β(1→4) GalT, were in the same family, which comprises the eukaryotic α(1→3) and β(1→4) galactosyltransferases. All of these enzymes have three conserved motifs that are called regions I, II, and III. We have already suggested that segments around amino acid residues 266 and 268 in blood group B α(1→3) GalT could be critical for binding UDP-Gal.[22b,c] These residues correspond to two of the four amino acids that are different in the blood group B and A enzymes. Indeed, these two residues were in region II as defined by Breton et al. This suggests that not only blood group B α(1→3) GalT but also calf thymus α(1→3) GalT and β(1→4) GalT utilize region II for binding UDP-Gal. On the other hand, α(1→4) GalT is in another family along with other bacterial and plant galactoslytransferases, and these enzymes likely possess different amino acid residues that interact with UDP-Gal. The data in Table 1 shows some interesting differences between the two α(1→3) GalTs and α(1→4) GalT. For example, both α(1→3) GalTs show higher transfer rates with UDP-2-deoxy-Gal compared to UDP-Gal. In contrast, α-(1→4) GalT has a relative transfer rate of 28% with UDP-2-deoxy-Gal compared to UDP-Gal. For UDP-3-deoxy-Gal, the two α(1→3) GalTs show weak activity with this donor while α(1→4) GalT has no detectable activity. In addition, the activity with UDP-6-deoxy-Gal and UDP-L-Ara are considerably higher for α(1→4) GalT than the two α-(1→3) GalTs. These differences are attributed to differences in the three-dimensional structures of the two α(1→3) GalTs and α(1→4) GalT.

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Enzymatic Synthesis of Trisaccharide Analogues. Trisaccharides **15**−**19** were synthesized using α/β mixtures of the UDP-Gal analogues.[29] Since only limited quantities of these donors and enzymes were available, the reactions could not be driven to completion except for the synthesis of **15**, which utilizes the very reactive UDP-2-deoxy-Gal. Though the materials obtained were mixtures of starting acceptor (**2**) and trisaccharides, we could confirm by ^{1}H NMR spectroscopy and mass spectrometry that the new linkages formed were α(1→3) linkages and that the products were trisaccharide analogues.

Next, the syntheses of **15** and **18** were repeated utilizing partially purified donors **4** and **7**. The synthesis of sugar nucleotides is known to be time consuming, involving several chromatographic steps to purify the final products. For this reason, we attempted to utilize crude or partially purified donors for the trisaccharide syntheses. For the synthesis of **15**, the donor was prepared by the coupling of 2-deoxy-3,4,6-tris(trimethylsilyl)-α-D-galactopyranosyl iodide and UDP n-Bu_4N salt followed by the deprotection with n-Bu_4NF as shown in Scheme 2.[29] The crude mixture, containing the desired UDP-2-deoxy-α-Gal together with UDP-2-deoxy-β-Gal, remaining unreacted UDP, and Bu_4NI was first passed through an ion-exchange column in order to exchange all n-Bu_4N^+ ions to Na^+ ions. The eluate was lyophilized, and the resulting powder was then treated with alkaline phosphatase to degrade the remaining UDP, which is an inhibitor of calf thymus α(1→3) GalT. The resulting solution was used directly in enzymatic synthesis where the desired trisaccharide was obtained in quantitative yield. For the synthesis

of **18**, UDP-6-deoxy-Gal was also prepared by the coupling of 6-deoxy-2,3,4-tris(trimethylsilyl)-α-D-galactopyranosyl iodide and UDP *n*-Bu_4N salt followed by removal of the silyl groups with *n*-Bu_4NF. The resulting crude mixture was lyophilized and mixed with acceptor (**2**) in the presence of calf thymus α(1→3) GalT and alkaline phosphatase. After incubation for 20 days at room temperature, trisaccharide **18** was obtained in 86% yield. Compounds **15**−**19** are analogues of α-D-Gal*p*-(1→3)-*β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc-OR, which are potential inhibitors of both the anti-α-Gal antibody[35] and toxin A of *Clostridium difficile*.[36]

The chemically prepared UDP-deoxy-Gal analogues were also used to synthesize deoxygenated blood group B trisaccharide analogues **28** and **29**.[37] For the synthesis of **28**, UDP-2-deoxy-Gal was made by coupling of the glycosyl iodide and UDP as shown in Scheme 2. The resulting donor was then directly used in the enzymatic reaction without any purification. After 12 days of incubation at room temperature, the desired product was obtained in 65% yield. In this case, there was irreversible loss of enzyme activity after 8 days by some component in the reaction that could not be restored by the addition of more enzyme. Since UDP-2-deoxy-Gal is less stable than UDP-Gal, and enzymes are generally more stable in the presence of their substrates, we speculate that the blood group B α(1→3) GalT had lost its activity in the absence of intact donor. It was also found that this enzymatic reaction was complete in 2 days when the *n*-Bu_4N^+ ion in the synthesized donor was exchanged with Na^+ ion prior to mixing with enzyme and acceptor **21**. In a similar manner, utilization of UDP-6-deoxy-Gal after ion exchange gave a better result for the synthesis of **29** compared to the reaction with the donor directly from the organic synthesis without purification.

The next targets of our chemi-enzymatic oligosaccharide synthesis were analogues of globotriose α-D-Gal*p*(1→4)-*β*-D-Gal*p*(1→4)-*β*-D-Glc*p*-OR,[38] the known receptor for Shiga and Shiga-like toxins.[39] In the case of the synthesis of the 2”-deoxy analogue **36**, reaction with purified UDP-2-deoxy-Gal gave a low (11%) yield of the desired product. TLC showed that a large amount of the UDP-2-deoxy-Gal had decomposed after 1 day of reaction verifying that the donor is not stable under these enzymatic incubation conditions. However, crude UDP-6-deoxy-Gal could be used in an enzymatic reaction to give the 6”-deoxy analogue **37** in 95% yield after 13 days of incubation.

In summary, we have developed a sensitive method for the determination of glycosyltransferase activity with donor substrate analogues. Utilizing CE with laser induced fluorescence detection, the rates of transfer from modified donors was obtained with an injection of amol of TMR-labeled oligosaccharide reaction product. The rates of transfer of deoxy sugar from UDP-deoxy-Gal analogues were variable depending on the position of deoxygenation. Among all of the UDP-deoxy-Gals studied, UDP-2- and 6-deoxy-Gal were effectively utilized by all three retaining α-galactosyltransferases. On the other hand, UDP-3-deoxy- and 4-deoxy-Gals showed relatively low rates of transfer, indicating that both the 3- and 4-hydroxyl groups of galactose in UDP-Gal are important for recognition by the enzymes. Differences in the protein sequences between the two α(1→3) GalTs and α(1→4) GalT could account for the differences in the rates of transfer of the deoxy-Gal analogues. Chemically synthesized UDP-deoxy-Gal analogues are very useful for enzymatic synthesis of oligosaccharides. In addition, deoxygenated analogues of the α-Gal antigen, blood group B antigens, and globotriose were easily synthesized. We have also demonstrated that complete purification of chemically synthesized donors is not always necessary for the enzymatic reaction to proceed, and even crude donors were useful enough for the preparation of the desired oligosaccharides.

Experimental Section

Materials. Calf thymus α(1→3) GalT was isolated according to literature procedures.[21] Recombinant blood group B α(1→3) GalT[22] and recombinant *Neisseria meningitidis* α(1→4) GalT[23] were prepared as previously described. Lactose-TMR (**1**) and Fuc-Gal-TMR (**20**) were synthesized from **2** and 8-methoxycarbonyloctyl α-L-fucopyranosyl-(1→2)-*β*-D-galactopyranoside, respectively, as described.[28] UDP-Gal (Na^+ salt) from Sigma was used as a standard in assays. TLC was conducted on glass plates precoated with 250 μm layers of Silica Gel 60-F254 (Merck, Darmstadt). Sep-Pak Plus C18 cartridges were from Waters, and each cartridge was preequilibrated with MeOH (10 mL) and then water (10 mL) before use. Millex-GV filters (0.22 μm) were from MILLIPORE. ^{1}H NMR spectroscopy was performed on a Varian UNITY 500 (500 MHz) or an INOVA 600 (600 MHz) instrument. Only partial NMR data are reported; the remaining data were in accordance with the proposed structures. Mass spectra were recorded on a Micromass ZabSpec Hybrid Sector-TOF by positive mode electrospray ionization using a 1% solution of acetic acid in methanol/water 1:1 as the liquid carrier.

The activity of the enzymes was estimated by radiochemical assay with product isolation on Sep-Pak reversed-phase cartridges.[24b] One mU of enzyme activity is defined as the amount producing 1 nmol of product/min. For calf thymus α(1→3) GalT, 8-methoxycarbonyloctyl *β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranoside was employed as the standard acceptor substrate; the activity of lactose derivative **2** is 30% of this standard.[40] For assaying α(1→4) GalT and blood group B α(1→3) GalT, lactose derivative **2** and Fuc-Gal derivative **20** were used as acceptors, respectively. Buffer A (30 mM sodium cacodylate, 20 mM $MnCl_2$, 0.1% Triton X-100, 0.8 mg/mL BSA, pH 6.5), B (50 mM sodium cacodylate, 20 mM $MnCl_2$, 1 mg/mL BSA, pH 6.8), and C (50 mM Hepes, 0.1 mg/mL BSA, pH 7.5) were used for calf thymus α(1→3) GalT, blood group B α(1→3) GalT, and α(1→4) Gal T, respectively.

Calf Thymus α(1→3) GalT CE Assay. UDP-Gal analogues (**3**−**8**, 20 nmol of α-anomer, UDP-Gal from Sigma was used as a standard) and lactose-TMR (**1**, 10 nmol) were incubated with calf thymus α(1→3) GalT (54−280 μU) in buffer A (20 μL) at 37 °C. The progress of the reactions was monitored by TLC (65:35:7 $CHCl_3/MeOH/H_2O$). Incubations were stopped after 6−215 min by 300-fold dilution of a 10 μL aliquot of the assay mixtures. The resulting solutions were loaded onto Sep-Pak Plus C18 cartridges. The cartridges were washed with water (20 mL), and then TMR-labeled sugars were eluted with MeOH (5 mL). These MeOH eluates were concentrated to dryness, and the resulting residues were dissolved in water (3 mL). These solutions were passed through a Millex-GV filter (0.22 μm), and the filtrate was lyophilized. The residues were dissolved in water to a concentration of 1 μM TMR. Sample (5 μL) was mixed with CE running buffer (10 mM phosphate, 2.5 mM sodium borate, 10 mM sodium dodecyl sulfate, and 10 mM phenyl boronic acid, pH 9, 45 μL) for injection in CE.

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Blood Group B α(1→3) GalT CE Assays. UDP-Gal analogues (**3**−**8**, 13 nmol of α-anomer, UDP-Gal from Sigma was used as a standard) and Fuc-Gal-TMR (**20**, 13 nmol) were incubated with blood group B α(1→3) GalT (177−1446 μU) in buffer B (33 μL) at 37 °C. The progress of the reactions was monitored by TLC (65:35:5 $CHCl_3$/MeOH/H_2O). Incubations were stopped after 10−215 min by 300-fold dilution of 10 μL aliquots of the assay mixtures. Samples for CE analysis were prepared as described for the calf thymus α(1→3) GalT CE assay.

α(1→4) GalT CE Assays. UDP-Gal analogues (**3**−**8**, 20 nmol of α-anomer, UDP-Gal from Sigma was used as a standard) and lactose-TMR (**1**, 20 nmol) were incubated with α(1→4) GalT (184−1470 μU) in buffer C (20 μL) at 37 °C. The progress of the reactions was monitored by TLC (65:35:7 $CHCl_3$/MeOH/H_2O). Incubations were stopped after 200−320 min by 300-fold dilution of 10 μL aliquots of the assay mixtures. Samples for CE analysis were prepared as described for the calf thymus α(1→3) GalT CE assay.

Analysis of Relative Rate by Capillary Electrophoresis with Laser-Induced Fluorescence Detection (CE-LIF). A locally constructed capillary electrophoresis system was used for the analysis.[28] A 5 mW helium−neon laser beam, $\lambda = 543.5$ nm, was focused into a postcolumn sheath flow cuvette. Separation was carried out in a 57 cm long, 10 μm i.d. fused silica capillary with an electric field of 400 V/cm. The aqueous electrophoresis buffer contained 10 mM phosphate, 2.5 mM borate, 10 mM sodium dodecyl sulfate, and 10 mM phenyl boronic acid (pH 9). Electrokinetic injection was performed by applying a 1 kV potential for 5 s to the sample. After injection, the sample was replaced with fresh running buffer. Separation was performed at room temperature. Data were processed with IGOR PRO. The ratios of product trisaccharide to starting disaccharide were determined by integration of each peak.

Preparative Synthesis, 8-Methoxycarbonyloctyl 2-Deoxy-α-D-galactopyranosyl-(1→3)-*β*-D-galactopyronosyl-(1→4)-*β*-D-glucopyranoside (15). Procedure A. A mixture of lactose derivative **2** (0.2 mg, 0.40 μmol), UDP-2-deoxy-Gal **4** (0.66 μmol of the active α-anomer in 4 μL H_2O), calf thymus α(1→3) GalT (7.1 mU/mL in buffer A, 100 μL, 0.71 mU), and alkaline phosphatase (1 U/μL, 1 μL) was incubated at room temperature, rotating gently. After 7 h, additional calf thymus α(1→3) GalT (100 μL, 0.71 mU) and UDP-2-deoxy-Gal **4** (0.66 μmol of the active α-anomer in 4 μL of H_2O) were added to the reaction mixture, and it was incubated for an additional 22 h until TLC showed complete conversion to product (40:10:1 EtOAc/MeOH/H_2O). The reaction mixture was loaded onto a Sep-Pak Plus C18 cartridge. The cartridge was washed with 100 mL water, and the product was eluted with 30% MeOH (100 mL). This eluate was concentrated, and the product was dissolved in water (5 mL). This solution was passed through a Millex-GV filter, and the filtrate was lyophilized to yield a fluffy white powder. NMR showed conversion was complete. **Procedure B.** UDP-2-deoxy-Gal (**4**) was synthesized by coupling 2-deoxy-3,4,6-tris(trimethylsilyl)-α-D-galactopyranosyl iodide (300 μmol) and UDP (300 μmol).[29] After deprotection with tetrabutylammonium fluoride, the reaction mixture was passed through an ion-exchange column (BIO-RAD AG50W-X8, 100-200 mesh, Na^+ form, 85 mL) and lyophilized to yield 337 mg of white powder. This resulting crude material (75 mg, 20 μmol of the active α-anomer) was treated with alkaline phosphatase (1 U/μL, 300 μL) in buffer (1.1 mL, 136 mM sodium cacodylate, 68 mM $MnCl_2$, 1.1% Triton X-100, 1.4 mg/mL BSA, pH 6.1) for 24 h until all remaining UDP was converted to uridine. To this solution were added lactose derivative (**2**, 2.9 mg, 6.0 μmol) and calf thymus α(1→3) GalT (3 mL, 9.3 mU), and the mixture was incubated at room temperature, rotating gently for 12 days. The reaction mixture was loaded onto a Sep-Pak Plus C18 cartridge. The cartridge was washed with 100 mL of water, and the product was eluted with MeOH (20 mL). This eluate was concentrated and again mixed with additional UDP-2-deoxy-Gal (20 μmol of the active α-anomer, prepared as described above) and calf thymus α(1→3) GalT (7.9 mU) in buffer (5 mL, 100 mM sodium cacodylate, 50 mM $MnCl_2$, 0.8% Triton X-100, 1 mg/mL BSA, pH 6.1) and incubated at room temperature for 19 h. The reaction mixture was loaded onto two Sep-Pak Plus C18 cartridges. The cartridges were washed with 100 mL water then 10% MeOH (20 mL). The product was eluted with 40% MeOH (100 mL). The eluate was concentrated and dissolved in water (5 mL). This solution was passed through a Millex-GV filter (0.22 μm), and the filtrate was lyophilized to yield a fluffy white powder (4.0 mg, 6.0 μmol, 100%): R_f 0.19 (75:25:2 CH_2Cl_2/MeOH/H_2O); NMR (D_2O) δ 5.25 (d, 1H, $J_{1,2a}$ = 3.2 Hz, H-1 (α-2-deoxy-Gal)), 4.51 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 3.68 (s, 3H, CO_2Me), 3.32−3.28 (m, 1H), 2.39 (t, 2H, J = 7.5 Hz), 1.98 (ddd, 1H, J_{2a2b} = 12.5 Hz, $J_{2a,3}$ = 12.5 Hz, H-2a (α-2-deoxy-Gal)), 1.91 (dd, 1H, $J_{2b,3}$ = 5.7 Hz, H-2b (α-2-deoxy-Gal)), 1.65−1.57 (m, 4H), 1.37−1.28 (m, 8H); HRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.2946, found 681.2940.

8-Methoxycarbonyloctyl 3-Deoxy-α-D-galactopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside (16). A mixture of lactose derivative **2** (0.2 mg, 0.40 μmol), UDP-3-deoxy-gal (**5**, 1.7 μmol of the active α-anomer), calf thymus α(1→3) GalT (3.3 mU) in buffer A (containing 25% glycerol), and alkaline phosphatase (1 U/μL, 2 μL) was incubated at room temperature for 17 days, rotating gently. NMR showed 5% of disaccharide (**2**) was converted to trisaccharide (**16**) after purification as described for the synthesis of **15**: R_f 0.31 (75:25:2 CH_2Cl_2/MeOH/H_2O); NMR (D_2O) δ 5.08−5.06 (br, 1H, H-1(α-3-deoxy-Gal)); LRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.3, found 681.3.

8-Methoxycarbonyloctyl 4-Deoxy-α-D-galactopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside (17). A mixture of lactose derivative **2** (0.2 mg, 0.40 μmol), UDP-4-deoxy-gal (**6**, 0.72 μmol of the active α-anomer), calf thymus α(1→3) GalT (4.3 mU) in buffer A (containing 25% glycerol), and alkaline phosphatase (1 U/μL, 2 μL) was incubated at room temperature for 17 days, rotating gently. NMR showed 50% of disaccharide (**2**) was converted to trisaccharide (**17**) after purification as described for the synthesis of **15**: R_f 0.27 (77:25:2 CH_2Cl_2/MeOH/H_2O); NMR (D_2O) δ 5.14 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1 (α-4-deoxy-Gal)), 4.51 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1); LRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.3, found 681.3.

8-Methoxycarbonyloctyl 6-Deoxy-α-D-galactopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside (18). Procedure A. A mixture of lactose derivative (**2**, 0.2 mg, 0.40 μmol), UDP-6-deoxy-gal (**7**, 1.2 μmol of the active α-anomer), α(1→3) GalT (9.3 mU) in buffer A, and alkaline phosphatase (1 U/μL, 2 μL) was incubated at room temperature, rotating gently at 37 °C for 10 days. By NMR analysis 34% of disaccharide (**2**) was converted to trisaccharide (**18**) after purification as described for the synthesis of **15**. **Procedure B.** UDP-6-deoxy-Gal (**7**) was synthesized by coupling 6-deoxy-2,3,4-tris-(trimethylsilyl)-α-D-galactopyranosyl iodide (100 μmol) and UDP (100 μmol) followed by deprotection with tetrabutylammonium fluoride.[29] The resulting crude material was lyophilized to yield a white powder (15 μmol of the active α-anomer). This powder was mixed with lactose derivative (**2**, 2.0 mg, 3.9 μmol), alkaline phosphatase (1 U/μL, 50 μL), and calf thymus α(1→3) GalT (41 mU) in buffer A and incubated at room temperature, rotating gently. After 19 h, the reaction mixture was filtered through a Millex-GV filter (0.22 μm), and an additional 18 mU of calf thymus α(1→3) GalT was added. This reaction mixture was incubated at room temperature, rotating gently for 20 days, with the addition of 8.1 mU aliquots of α(1→3) GalT after 1 and 3 days. The reaction mixture was passed through an ion-exchange column (BIO-RAD AG50W-X8, 100-200 mesh, Na^+ form, 20 mL) and then loaded onto two Sep-Pak Plus C18 cartridges. The cartridges were washed with 100 mL of water and then 10% MeOH (20 mL). The product was eluted with MeOH (50 mL). This eluate was concentrated and loaded on a column of Iatrobeads (5 g) prepared with CH_2Cl_2/MeOH 9:1. Product was eluted with CH_2Cl_2/MeOH 4:1. Fractions containing product were collected and concentrated. The residue was dissolved in water (10 mL) and loaded onto a Sep-Pak Plus C18 cartridge. The cartridge was washed with 50 mL of water, and then product was eluted with MeOH (50 mL). This eluate was concentrated, and the resulting residue was dissolved in water (5 mL). This solution was passed through a Millex-GV filter (0.22 μm), and the filtrate was lyophilized to yield a fluffy white powder (2.2 mg, 3.4 μmol, 86%): R_f 0.25 (75:25:2 CH_2Cl_2/MeOH/H_2O); NMR (D_2O) δ 5.05 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1 (α-6-deoxy-Gal)), 4.51 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.48 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.31 (dd, 1H, $J_{4,5}$ = 13.4 Hz, $J_{5,6}$ = 6.7 Hz, H-5 (α-6-deoxy-Gal)), 4.14 (d, 1H, J = 3.1 Hz), 3.69 (s, 3H, CO_2Me), 3.33−3.27 (m, 1H), 2.38 (t, 2H, J = 7.0 Hz), 1.64−1.56 (m, 4H), 1.37−1.28 (m, 8H),

1.20 (d, 3H, H-6 (α-6-deoxy-Gal)); HRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.2946, found 681.2940.

8-Methoxycarbonyloctyl *β*-L-Arabinopyranosyl-(1→3)-*β*-D-galactopyranosyl(1→4)-*β*-D-glucopyranoside (19). A mixture of lactose derivative **2** (0.2 mg, 0.40 *μ*mol), UDP-Ara **8** (2.3 *μ*mol of the active α-anomer), calf thymus α(1→3) GalT (9.3 mU) in buffer A, and alkaline phosphatase (1 U/*μ*L, 2 *μ*L) was incubated at 37 °C, rotating gently for 10 days. NMR analysis showed that 8% of the disaccharide (**2**) was converted to trisaccharide (**19**) after purification as described in the synthesis of **15** (procedure A): R_f 0.13 (75:25:2 CH_2Cl_2/MeOH/H_2O); NMR (D_2O) δ 5.11 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1 (*β*-L-Ara)); LRMS calcd for $[M + Na]^+$ $C_{27}H_{49}O_{17}$ 645.3, found 645.3.

Octyl α-L-Fucopyranosyl-(1→2)-[2-deoxy-α-D-galactopyranosyl-(1→3)]-*β*-D-galactopyranoside (28). Procedure A. UDP-2-deoxy-Gal **4** was synthesized by coupling 2-deoxy-3,4,6-tris(trimethylsilyl)-α-D-galactopyranosyl iodide (100 *μ*mol) and UDP (100 *μ*mol). After deprotection with tetrabutylammonium fluoride, the reaction mixture was filtered and lyophilized (30 *μ*mol of the active α-anomer). This crude material was mixed with Fuc-Gal acceptor (**21**, 3.1 mg, 7.1 *μ*mol), blood group B α(1→3) GalT (195 mU), and alkaline phosphatase (1 U/*μ*L, 50 *μ*L) in buffer B (1.6 mL). This reaction was incubated at room temperature for 12 days. During incubation, additional blood group B α(1→3) GalT (117 mU) was added to the reaction mixture after 8 days. Pure trisaccharide **36** (2.7 mg, 4.6 *μ*mol, 65%) was obtained by the procedure as described for the synthesis of **18** (procedure B). **Procedure B.** UDP-2-deoxy-Gal (**4**) was prepared as described for the synthesis of **15** (procedure B), and 75 mg of crude material (20 *μ*mol of the active α-anomer) was treated with alkaline phosphatase (1 U/*μ*L, 200 *μ*L) in buffer B (2 mL) at room temperature for 5 h, turning gently. To this mixture were added Fuc-Gal acceptor (**21**, 3.2 mg, 7.3 *μ*mol) and blood group B α(1→3) GalT (320 mU) in buffer B (2.5 mL), and the reaction mixture was incubated at room temperature for 46 h. Pure trisaccharide **28** (4.1 mg, 7.0 *μ*mol, 96%) was obtained as described for the synthesis of **18** (procedure B): R_f 0.29 (40:10:2 EtOAc/MeOH/H_2O); NMR (D_2O) δ 5.32 (d, 1H, $J_{1,2a}$ = 3.8 Hz, H-1 (α-2-deoxy-Gal)), 5.30 (d, $J_{1,2}$ = 2.8 Hz, H-1(α-Fuc)), 4.52 (d, $J_{1,2}$ = 7.8 Hz, H-1(*β*-Gal)), 4.46 (dd, 1H, $J_{5,6}$ = 6.6 Hz, $J_{4,5}$ = 13.1 Hz, H-5(α-Fuc)), 4.24 (d, 1H, *J* = 3.1 Hz), 4.14 (dd, 1H, *J* = 4.9, 7.6 Hz), 4.08 (ddd, 1H, $J_{3,4}$ = 3.1 Hz, $J_{2b,3}$ = 6.1 Hz, $J_{2a,3}$ = 11.1 Hz, H-3 (α-2-deoxy-Gal)), 1.95 (dt 1H, H-2a (α-2-deoxy-Gal)), 1.91 (dd, H-2b(α-2-deoxy-Gal)) 1.64−1.55 (m, 2H), 1.36−1.24 (m, 10H), 1.21 (d, 3H, H-6(α-Fuc)), 0.86 (t, 3H, *J* = 6.7 Hz, Me); HRMS calcd for $[M + Na]^+$ $C_{26}H_{48}O_{14}Na$ 607.2942, found 607.2944.

Octyl α-L-Fucopyranosyl-(1→2)-[6-deoxy-α-D-galactopyranosyl-(1→3)]-*β*-D-galactopyranoside (29). Procedure A. UDP-6-deoxy-Gal **7** was synthesized by coupling 6-deoxy-2,3,4-tris(trimethylsilyl)-α-D-galactopyranosyl iodide (100 *μ*mol) and UDP (100 *μ*mol) followed by deprotection with tetrabutylammonium fluoride.[29] The reaction mixture was lyophilized to give a white powder, which was mixed with Fuc-Gal acceptor (**20**, 2.6 mg, 6.0 *μ*mol), alkaline phosphatase (1 U/*μ*L, 50 *μ*L), and blood group B α(1→3) GalT (390 mU) in buffer B (1.8 mL). After incubation for 1 day, the reaction mixture was filtered, and additional blood group B α(1→3) GalT (180 mU) in buffer B (1.5 mL) was added. The reaction mixture was incubated at room temperature for 15 days. Pure trisaccharide **29** (2.0 mg, 3.4 *μ*mol, 56%) was obtained by the procedure described for the synthesis of **18** (procedure B). **Procedure B.** UDP-6-deoxy-Gal **7** was synthesized by coupling of 6-deoxy-2,3,4-tris(trimethylsilyl)-α-D-galactopyranosyl iodide (100 *μ*mol) and UDP (100 *μ*mol) followed by deprotection with tetrabutylammonium fluoride.[29] This reaction mixture was passed through an ion-exchange column (BIO-RAD AG50W-X8, 100−200 mesh, Na^+ form, 26 mL) and lyophilized to yield 325 mg of white powder. This resulting crude material (86 mg, 8 *μ*mol of the active α-anomer) was mixed with Fuc-Gal acceptor (**20**, 2.6 mg, 6.0 *μ*mol), blood group B α(1→3) GalT (400 mU), and alkaline phosphatase (1 U/*μ*L, 50 *μ*L) in buffer B (1.0 mL). The reaction mixture was incubated at room temperature for 18 days. Pure trisaccharide **29** (3.4 mg, 5.9 *μ*mol, 98%) was obtained by the procedure as described for the synthesis of **15** (procedure A): R_f 0.28 (40:10:2 EtOAc/MeOH/H_2O); NMR (D_2O) δ 5.29 (d, 1H, $_{1,2}$ = 3.8 Hz, H-1), 5.17 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.53 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1(*β*-Gal)), 4.48 (dd, 1H, $J_{4,5}$ = 13.3 Hz, $J_{5,6}$ = 6.6 Hz, H-5), 4.33 (dd, 1H, $J_{4,5}$ = 13.1 Hz, $J_{5,6}$= 6.4 Hz, H-5), 4.22 (d, 1H, *J* = 3.1 Hz), 1.64−1.56 (m, 2H), 1.24−1.36 (m, 10H), 1.21 (d, 3H, H-6), 1.19 (d, 3H, H-6), 0.86 (t, 3H, *J* = 7.0 Hz, Me); HRMS calcd for $[M + Na]^+$ $C_{26}H_{48}O_{14}Na$ 607.2942, found 607.2948.

8-Methoxycarbonyloctyl 2-Deoxy-α-D-galactopyranosyl-(1→4)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside (36). α(1→4) GalT solution (100 *μ*L, 50 mM ammonium acetate, pH 7.5), Hepes buffer (125 *μ*L, 400 mM, pH 7.5), 100 mM DTT solution (25 *μ*L), BSA solution (100 mg/mL, 5 *μ*L), and water (245 *μ*L) were combined and left to stand at room temperature for 1 h to activate the enzyme (569 mU/mL, 500 *μ*L, 285 mU). To this enzyme solution were added lactose derivative (**2**, 2.2 mg, 4.3 *μ*mol), alkaline phosphatase (1U/*μ*L, 5 *μ*L), and UDP-2-deoxy-Gal **4** (12 *μ*mol of the active α-anomer). This reaction mixture was incubated at room temperature for 90 h. During this incubation, additional α(1→4) GalT (140 mU) and UDP-2-deoxy-Gal **4** (12 *μ*mol of the active α-anomer) were added to the reaction mixture after 46 h. Pure trisaccharide **36** (0.3 mg, 0.46 *μ*mol, 11%) was obtained as described for the synthesis of **18** (procedure B): R_f 0.18 (40:10:2 EtOAc/MeOH/H_2O); NMR (D_2O) δ 5.04 (t, 1H, $J_{1,2a,}$ $J_{1,2b}$ = 2.5 Hz, H-1 (α-2-deoxy-Gal)), 4.49 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1), 4.48 (d, 1H, $J_{1,2}$ =3.1 Hz, H-1), 3.69 (s, 3H, CO_2Me), 2.39 (t, *J* = 7.7 Hz), 1.97−1.94 (m, 2H, H-2a, 2b), 1.66−1.58 (m, 4H), 1.30−1.38 (m, 8H); HRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.2946, found 681.2953.

8-Methoxycarbonyloctyl 6-Deoxy-α-D-galactopyranosyl-(1→4)-*β*-galactopyranosyl-(1→4)-*β*-D-glucopyranoside (37). α(1→4) GalT (800 *μ*L, 50 mM ammonium acetate, pH 7.5), Hepes (500 *μ*L, 400 mM, pH 7.5), 100 mM DTT solution (200 *μ*L), and water (2.5 mL) were combined and left to stand at room temperature for 1 h to activate the enzyme (18 mU/mL, 4 mL, 72 mU). To this enzyme solution were added BSA solution (40 *μ*L, 100 mg/mL), lactose derivative (**2**, 2.1 mg, 4.0 *μ*mol), alkaline phosphatase (50 *μ*L, 1 U/*μ*L), and the crude UDP-6-deoxy-Gal **8** (15 *μ*mol of the active α-anomer), which was prepared as described for the synthesis of **19** (procedure B). This reaction mixture was gently turned at room temperature for 13 days. During this incubation, additional α(1→4) GalT (36 mU after 5 h, 18 mU after 4 days, and 18 mU after 9 days) was added to the reaction mixture. Pure trisaccharide **37** (2.5 mg, 3.8 *μ*mol, 95%) was obtained as described for the synthesis of **18** (procedure A): R_f 0.22 (40:10:2 EtOAc/MeOH/H_2O); NMR (D_2O) δ 4.86 (d, 1H, $J_{1,2}$ = 4.1 Hz, H-1 (α-6-deoxy-Gal)), 4.51 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.48 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 4.46 (dd, 1H, $J_{4,5}$ = 12.2, $J_{5,6}$ = 7.5 Hz, H-5 (α-6-deoxy-Gal)), 3.69 (s, 3H, CO_2Me), 3.32−3.26 (m, 1H), 2.39 (t, *J* = 6.7 Hz), 1.56−1.66 (m, 4H), 1.38−1.28 (m, 8 H), 1.18 (d, 3H, H-6 (α-6-deoxy-Gal)); HRMS calcd for $[M + Na]^+$ $C_{28}H_{50}O_{17}Na$ 681.2946, found 681.2961.

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