Chemoenzymatic Synthesis of α-(1→3)-Gal(NAc)-Terminating Glycosides of Complex Tertiary Sugar Alcohols

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Abstract: The scope of glycosyltransferases in the synthesis of oligosaccharide analogues has been expanded to include the use of "retaining" enzymes acting on complex tertiary *C*-branched disaccharide acceptors. The enzymes studied were α1,3-galactosyltransferase (α1,3-GalT, EC 2.4.1.151), which uses Galβ1→4Glcβ-OR as the acceptor, and recombinant blood group A [α1,3-GalNAc transferase (GTA), EC 2.4.1.40] and B [α1,3-Gal transferase (GTB), EC 2.4.1.37] glycosyltransferases, which use Fucα1→2Galβ-OR as the acceptor in their biosynthetic reactions. Chemical synthesis produced acceptor analogues in which the ring carbon bearing the OH groups undergoing enzymatic glycosylation in the natural reactions had their C−H bond replaced with a *C*-methyl or *C*-propyl bond. The tertiary *C*-methyl alcohols proved to be kinetically competent substrates which permitted the enzymatic preparation of the expected product trisaccharides in near quantitative yields.

Introduction

Oligosaccharides on cell surfaces play an important role in many biological recognition events including cell−cell adhesion, bacterial attachment, and viral infection.[1] The availability of oligosaccharides and their analogues as inhibitors can provide insight into their biological roles and might lead to the discovery of novel carbohydrate-based therapeutics.[2] Despite many advances in the chemical synthesis of oligosaccharides, it is still time-consuming and often difficult to synthesize these highly asymmetric and densely functionalized molecules.[3] As well, no replication system currently exists for amplifying minute quantities of carbohydrates, nor is instrumentation commercially available for the solid-phase synthesis of oligosaccharides.

Progress in recombinant DNA technology has made it possible to express large quantities of almost any enzyme, including glycosyltransferases that biosynthesize oligosaccharides. These enzymes catalyze the transfer of a single pyranosyl residue from a sugar nucleotide donor to a growing carbohydrate chain. Many glycosyltransferases, isolated and cloned, are now readily available in quantities sufficient for use in synthesis.[4] The use of glycosyltransferases to prepare oligosaccharides offers a number of advantages: high regio- and stereoselectivity, no requirement for chemical protection and deprotection, and very mild reaction conditions.[5] Numerous examples have been reported in which glycosyltransferases tolerate structural changes on both donor and acceptor substrates, thus making enzymatic synthesis a promising alternative for the preparation of both natural and *unnatural* oligosaccharides.[6]

Since structural information on protein (including enzyme) binding sites is not generally available, chemical approaches using engineered ligands are still necessary to study carbohydrate−protein interactions.[7] Most of the ligand analogues used have been acceptor analogues with hydroxyl groups selectively deoxygenated, derivatized, or substituted with other functional groups.[6,8] Chemical mapping studies using these analogues help elucidate hydrogen-bonding patterns and delineate the topology of the binding site. However, there are only a few examples known in which analogues having a C-bonded hydrogen atom modified (termed C-branched sugars) were used.[9] The scope of chemical mapping on glycosyltransferases and enzymatic synthesis has not yet included such sugar analogues. Probing protein binding domains with C-branched analogues can provide more information on the three-dimensional aspects of carbo-

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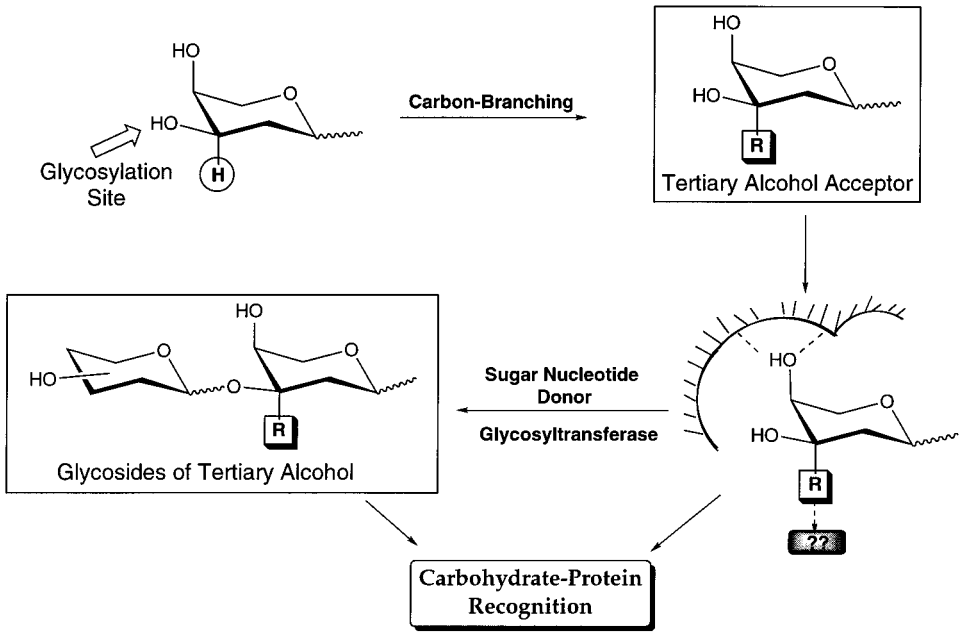
Scheme 1



hydrate−protein interactions (Scheme 1), which complements studies using analogues with modified hydroxyl groups. Functional groups replacing hydrogens could, for example, serve as molecular probes to explore which face of a given substrate is making contact with the binding site and how close the contact is (Scheme 1).

Tertiary alcohols of C-branched sugar residues, in particular those resulting from branching at the enzymatic reaction center, will also be useful for probing the active sites of glycosyltransferases and for studying how steric hindrance affects the binding. Since carbon-branched sugars are found only in plants and microorganisms,[10,11] it will be interesting to see whether mammalian glycosyltransferases are able to recognize them. Very few examples on chemical glycosylation of tertiary alcohols have been reported since chemical glycosylation of such hindered molecules is extremely difficult and frequently impossible.[12] Should glycosyltransferses be able to overcome the steric limitations inherent in the glycosylation of the tertiary alcohols of C-branched sugar residues, this enzymatic approach would constitute a novel method for the preparation of glycosides of complex tertiary alcohols. The resulting glycosides are expected to have less flexible glycosidic linkages and would potentially be useful in carbohydrate−protein recognition studies.[9a,13]

Our surprising finding of enzymatic fucosylation of a complex tertiary alcohol by human milk α1,3/4-fucosyltransferase (α1,3/4-FucT) demonstrated that it is possible for mammalian glycosyltransferases to catalyze the formation of glycosides of tertiary alcohols.[14] In light of this discovery, we decided to investigate the scope of using mammalian glycosyltransferases to form other types of glycosidic linkages with tertiary alcohol acceptors. α1,3-Galactosyltransferase (α1,3-GalT, EC 2.4.1.151)[15] and human blood group A and B glycosyltransferases (GTA and GTB, EC 2.4.1.40 and EC 2.4.1.37)[16] were chosen for this study because all three enzymes catalyze the formation of an α(axial)-glycosidic linkage as does the α1,3/4-FucT. However, these enzymes belong to a class of "retaining" enzymes which are different from α1,3/4-FucT and other "inverting" glycosyltransferases. They transfer the sugar residue from the nucleotide donor with overall retention, instead of inversion, of configuration at the anomeric center. Chemical mapping studies with the tertiary alcohol acceptor analogues will help us to understand the molecular specificity of these glycosyltransferases and increase the scope of enzymatic preparation of novel *unnatural* oligosaccharides. The enzyme kinetic properties of these analogues should also shed light on the molecular mechanisms of α-glycosidic linkage formation by "retaining" glycosyltransferases.

In this paper, a series of C-alkyl-branched substrate analogues were chemically synthesized and evaluated as acceptors for α1,3-GalT, GTA, and GTB. Preparative enzymatic synthesis of five α-(1→3)-Gal- or α-(1→3)-GalNAc-terminating glyco-

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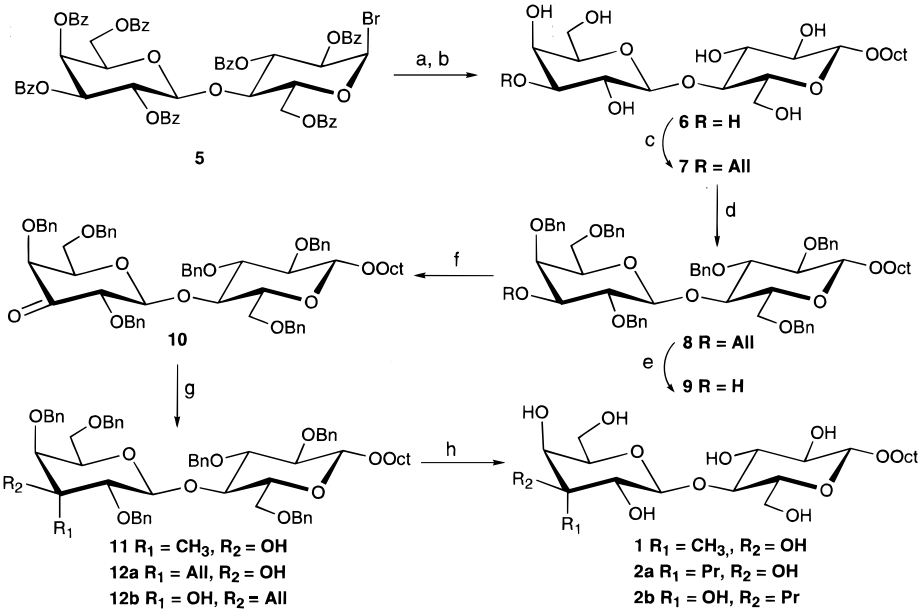
Scheme 2[a]



[a] Reagents and conditions: (a) octanol, AgOTf, 4 Å MS, CH_2Cl_2, −50 to 0 °C, 4 h; (b) NaOMe, MeOH, 20 h, 37% (two steps); (c) (i) Bu_2SnO, benzene, reflux, 24 h, (ii) AllBr, Bu_4NI, reflux, 14 h, 53%; (d) BnBr, NaH, DMF, 0 °C, 2 h, then room temperature, 14 h, 80%; (e) $PdCl_2$, MeOH, 4 h, 83%; (f) Dess−Martin periodinane, CH_2Cl_2, 1.5 h, 72%; (g) **11**, MeLi, THF, −78 to −40 °C, 1 h, 90%; **12**, AllMgBr, THF, 0 °C, 4 h, 39% **12a**, 51% **12b**; (h) H_2, 20% $Pd(OH)_2$/C, MeOH, quantitative.

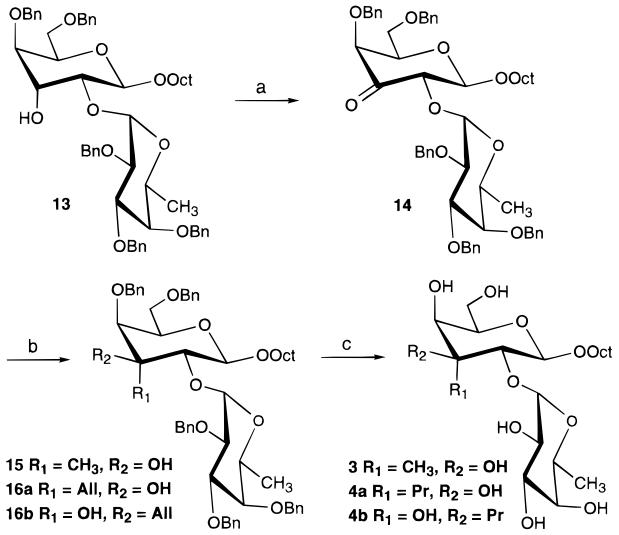
sides of complex tertiary sugar alcohols could thus be accomplished.

Results and Discussion

Chemical Synthesis. The general strategy for the preparation of the required tertiary alcohol substrates involved the selective protection of the parent disaccharide, permitting the oxidation of the required ring carbon to the ketone. Nucleophilic addition of the alkyl group from organometallic reagents followed by deprotection gave the C-branched compounds. The products were synthesized as their octyl glycosides, allowing the use of a well-established radioactive "Sep-Pak assay"[17] for determining kinetic parameters and simplifying the isolation of enzymatic products by absorption onto reverse-phase (C18) cartridges.

As described in Scheme 2, the key step in the synthesis of 3′-*C*-branched lactosides **1** and **2** was the regioselective stannylation of unprotected disaccharide **6**, resulting in selective 3′-*O*-alkylation. Thus, octyl lactoside **6** was prepared by glycosylation of bromide **5** with octanol followed by deacylation. Regioselective allylation at O-3′ with dibutyltin oxide and allyl bromide gave **7** in 53% yield.[18] Benzylation, followed by removal of the allyl group with $PdCl_2$,[19] provided **9** with OH-3′ free. Oxidation of **9** was achieved with Dess−Martin periodinane[20] to provide **10** in 72% yield. Nucleophilic addition of methyllithium to ketone **10** gave exclusively the product of axial attack (**11**) in excellent yield (95%), while the reaction of allylmagnesium bromide with **10** provided a mixture of both epimers (**12a** and **12b**) in almost a 1:1 ratio. Hydrogenolysis with $Pd(OH)_2$/C gave unprotected 3′-*C*-branched lactosides **1** and **2**. NOE studies employing one-dimensional TROESY experiments[21] showed that there are significant NOEs between the 3′-*C*-methyl protons in **1** (3′-*C*-CH_2CH_2CH_3 protons in **2a**) and H-1 of the Gal residue, while there are no NOEs between the 3′-*C*-propyl protons and H-1′ in **2b**.

Scheme 3[a]



[a] Reagents and conditions: (a) Dess−Martin periodinane, CH_2Cl_2, 2 h, 61%; (b) **15**, MeLi, THF, −78 to −40 °C, 1.5 h, 55%; **16**, AllMgBr, THF, 0 °C, 2 h, 33% **16a**, 29% **16b**; (c) H_2, 20% $Pd(OH)_2$/C, MeOH, 95% (for **3**), 94% (for **4a**), 92% (for **4b**).

Analogues **3** and **4** with carbon-branching at the 3-position of Fucα1→2Gal were prepared from the known precursor **13**,[22] following the same oxidation−nucleophilic addition sequence (Scheme 3). Disaccharide **13** was oxidized with Dess−Martin periodinane to provide **14** (61%). Nucleophilic attack of MeLi

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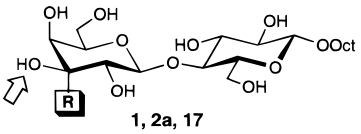
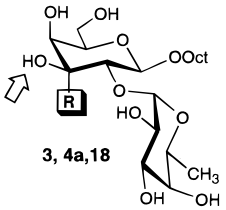
Table 1



1, 2a, 17



3, 4a,18

R	analogue	α1,3-GalT[a]	analogue	GTB[b]	GTA[c]
H	**17**	$K_m = 1.5 \pm 0.1$ mM $V_{max} = 100\%$	**18**	$K_m = 76 \pm 13$ μM $V_{max} = 100\%$	$K_m = 17 \pm 2$ μM $V_{max} = 100\%$
CH_3	**1**	$K_m = 23 \pm 5$ mM $V_{max} = 38\%$	**3**	$K_m = 209 \pm 15$ μM $V_{max} = 60\%$	$K_m = 39 \pm 3$ μM $V_{max} = 64\%$
Pr	**2a**	inactive	**4a**	$K_m = 2200 \pm 200$ μM $V_{max} = 8\%$	$K_m = 2800 \pm 300$ μM $V_{max} = 26\%$

[a] For assay conditions, see ref 15g. [b] Reactions were incubated at 37 °C for 30 min in 20 μL total volume with 35 mM sodium cacodylate buffer (pH 7.0), 20 mM $MnCl_2$, 1 mg/mL BSA, 200 μM UDP-Gal, UDP-[6-^{3}H]Gal (about 75 000 dpm), 20 μU GTB. [c] Reactions were incubated at 37 °C for 20 min in 20 μL total volume with 35 mM sodium cacodylate buffer (pH 7.0), 20 mM $MnCl_2$, 1 mg/mL BSA, 70 μM UDP-GalNAc, UDP-[6-^{3}H]GalNAc (about 75 000 dpm), 9 μU GTA.

on the carbonyl group of **14** provided the axial attack product *C*-methyl **15** in 61% yield, while reaction of allylmagnesium bromide with **15** again afforded epimers **16a** and **16b** in a 1:1 ratio. Hydrogenolysis of **15** and **16** gave the deprotected 3-*C*-branched disaccharides **3** and **4**. The configurations at the branching carbons were assigned on the basis of observed strong NOEs between H-1 of Gal and the 3-*C*-methyl protons in **3** (3-*C*-C*H*$_2$CH$_2$CH$_3$ protons in **4a**) and strong NOEs between H-2 of Gal and 3-*C*-C*H*$_2$CH$_2$CH$_3$ protons in **4b**.

Enzymatic Assays. α1,3-GalT, which is absent in humans, catalyzes the formation of Galα1→3Gal epitopes, the major xenoactive antigen which causes hyperacute rejection during xenotransplantation.[23] The enzyme can use both Gal*β*1→4GlcNAc*β*-OR and Gal*β*1→4Glc*β*-OR as acceptors.[15] Acceptor hydroxyl group mapping studies[15g] showed that Gal OH-4 is, according to the terminology of Lemieux,[24] a key polar group essential for substrate recognition. Substitution of Gal OH-3 with an amino group produces an inhibitor for this enzyme.[15d] These results suggested that OH-3′ is not essential for binding to the enzyme, although it is the site of glycosylation. Surprisingly, the carbon-branched analogue 3′-*C*-methyl lactoside **1** was found to be a substrate in the radioactive Sep-Pak assay. The K_m for **1** was 23 mM, about 15 times higher than that for the parent *n*-octyl lactoside **17** (K_m = 1.5 mM). The maximal rate of transfer (V_{max}) decreased to 38% of that of **17** (Table 1). However, replacement of the hydrogen with a bulkier propyl group produced an inactive compound **2a** when tested as an acceptor at a concentration of 2 mM in a 6-h incubation and as an inhibitor at a concentration of 5 mM.

As mentioned earlier, GTA and GTB are similar to α1,3-GalT and catalyze the formation of α1→3 galactosidic linkage with retention of configuration. GTA catalyzes the transfer of GalNAc from UDP−GalNAc to the blood group (O)H precursor structure Fucα1→2Gal*β*-OR to give the blood group A trisaccharide GalNAcα1→3[Fucα1→2]Gal*β*-OR. GTB uses the same acceptor substrate but catalyzes the transfer of Gal from UDP−Gal to produce the blood group B trisaccharide Galα1→3-[Fucα1→2]Gal*β*-OR. Systematic studies have revealed that Gal OH-3 is not essential for recognition by both enzymes, as observed in the case of α1,3-GalT.[22,25] Furthermore, GTA and GTB are highly homologous glycosyltransferases and differ by only four amino acid residues.[16a] Human GTB exhibits about 40% sequence identity with α1,3-GalT.[26] It would thus be reasonable to expect that both GTA and GTB could also tolerate carbon-branching at the 3-position of the Gal residue. In fact, both 3-*C*-methyl- and 3-*C*-propyl-branched analogues, **3** and **4a**, were found to be acceptors for these two enzymes. As shown in Table 1, replacement of the hydrogen atom at the site of the enzymatic reaction with a methyl group did not significantly influence the K_m and V_{max} values. However, substitution with a larger propyl group dramatically altered the kinetic properties. The K_m values were almost 30 and 165 times higher and V_{max} decreased to 8% and 26%, respectively.

It is quite remarkable that all three enzymes can tolerate the introduction of large substituents at the carbon bearing the hydroxyl group that undergoes glycosylation, although it is not required for the recognition. However, as revealed by kinetic data, the steric increase does make it more difficult for the enzymes to bind and transfer. The larger the alkyl group, the higher the K_m and the lower the V_{max} values. Among the three enzymes, the introduction of an alkyl substituent has more impact on K_m and V_{max} for α1,3-GalT than for GTA and GTB since the V_{max}/K_m value for **1** was only 2.5% of that for **17** with α1,3-GalT, while the V_{max}/K_m value for **3** was only decreased to 22−28% of that for **18**[25] with GTA and GTB. This is also in agreement with the observation that no detectable transfers to the 3′-*C*-propyl-branched analogue **2a** was observed with α1,3-GalT, while **4a** still displayed activity with GTA and GTB. The three enzymes seem to have similar molecular specificity with respect to the *β*-Gal residue: OH-4 is required for binding while OH-3 is not essential and *C*-methyl-branching is tolerated at C-3 of the *β*-Gal residue. The similar molecular specificity and the homology of these enzymes indicate that they may have similar three-dimensional structure at the active sites.

A two-step double-displacement mechanism involving the formation and breakdown of glycosyl−enzyme intermediate is most likely involved for the retaining glycosyltransferases[25] as well as for glycosidases.[27] Since a direct S_N2-type substitution is unlikely to occur in the second step for the very hindered tertiary alcohols investigated here, we propose that the break-

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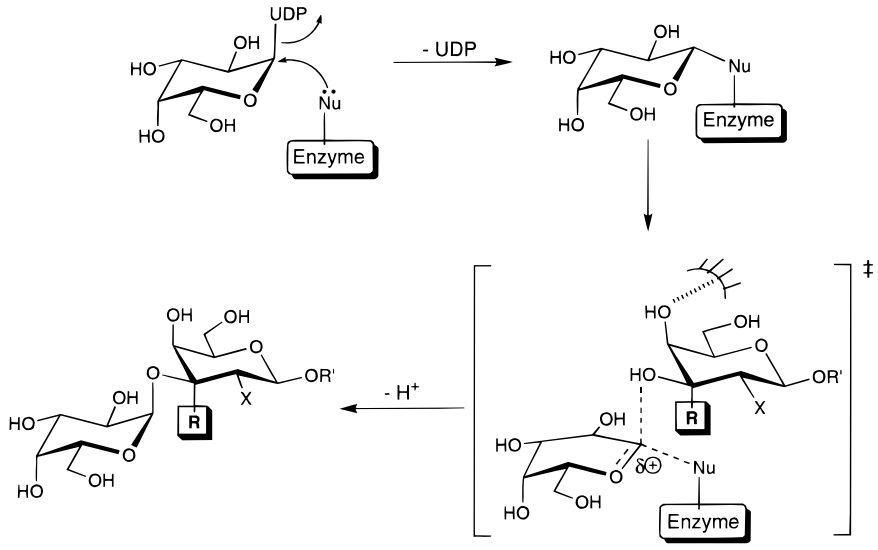
Scheme 4

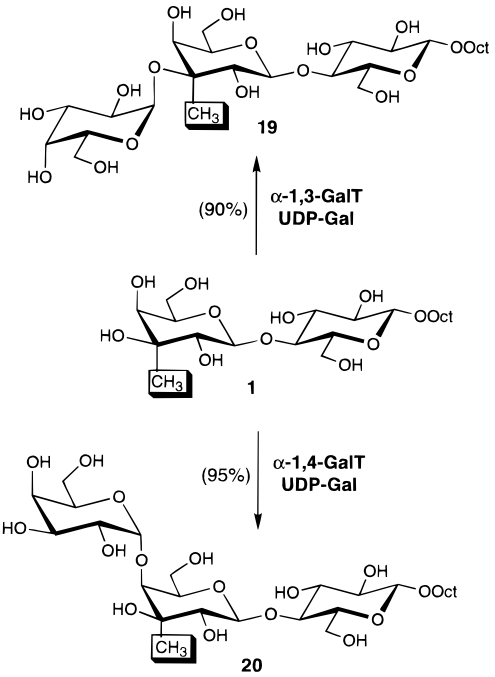


down of the glycosyl−enzyme intermediate begins prior to the nucleophilic attack by OH. The process would thus have significant S_N1 character (Scheme 4), as suggested for α1,3-FucT V[28] and α-glucosyltransferase.[29] It is interesting that the four enzymes, α1,3/4-FucT,[14] α1,3-GalT, GTA, and GTB, which all catalyze the formation of an α(axial)-glycosidic linkage with either retention or inversion of configuration, tolerate C-branching at the glycosylation site.

Preparative Synthesis and Characterization of Enzymatic Products. To be certain that the analogues found to be acceptors in radioactive enzymatic assays were indeed glycosylated in the expected manner, preparative reactions of **1**, **3**, and **4a** using the corresponding enzymes were performed. Mass spectrometry and extensive NMR studies were employed to confirm the structures of the enzymatic products.

Trisaccharide **19** was obtained in 90% yield from the reaction of **1** with UDP-Gal catalyzed by α1,3-GalT (Scheme 5). As expected, protons of the 3-*C*-methyl group were shifted downfield from 1.24 to 1.37 ppm.[30] The ^{13}C chemical shift of the quaternary branching carbon (C-3′) in **19** also shifted downfield from 74.7 to 80.9 ppm with no significant chemical shift change in other carbon resonances. Moreover, to exclude the possibility of incorrect linkage formation (1→4 instead of 1→3) in **19**, trisaccharide **20** was prepared in 95% yield with the use of α1,4-galactosyltransferase[31] (α1,4-GalT, Scheme 5). This precaution was necessary in view of the conformational peculiarities of Galα1→3Gal linkages, which result in much stronger nuclear Overhauser enhancements between αGal-H1 and *β*Gal-H4 compared to αGal-H1:*β*Gal-H3.[32,33] As can be seen in Figure 1,

Scheme 5



trisaccharide **19** shows the expected H1″/3′-*C*-methyl NOE as well as an even stronger H1″/H4′ interaction, which is consistent with earlier observations in Galα1→3Gal*β*1→4Glc[32] and the A and B trisaccharides,[33] whereas the 1→4-linked trisaccharide **20** shows the H1″/H4′ interaction only. These NOE results, together with the αGal-H1:*β*Gal-C3 correlation in HMBC[34] studies of **19**, prove unambiguously the 1→3 linkage of **19**.

As shown in Scheme 6, the A and B trisaccharide analogues **21**−**24** were obtained in excellent yields using GTA and GTB with UDP-GalNAc or UDP-Gal as donors. Complete conversion of the poor substrate **4a** was achieved by increasing the amounts of enzyme and donor and the reaction time. Detailed NMR studies revealed the NOE pattern characteristic for the A and B trisaccharides:[33] a stronger NOE from H-1 (α-Gal or α-GalNAc) to H-4 (*β*-Gal) than from H-1 (α-Gal or α-GalNAc) to protons of alkyl substituents at C-3. Significant downfield shifts for the

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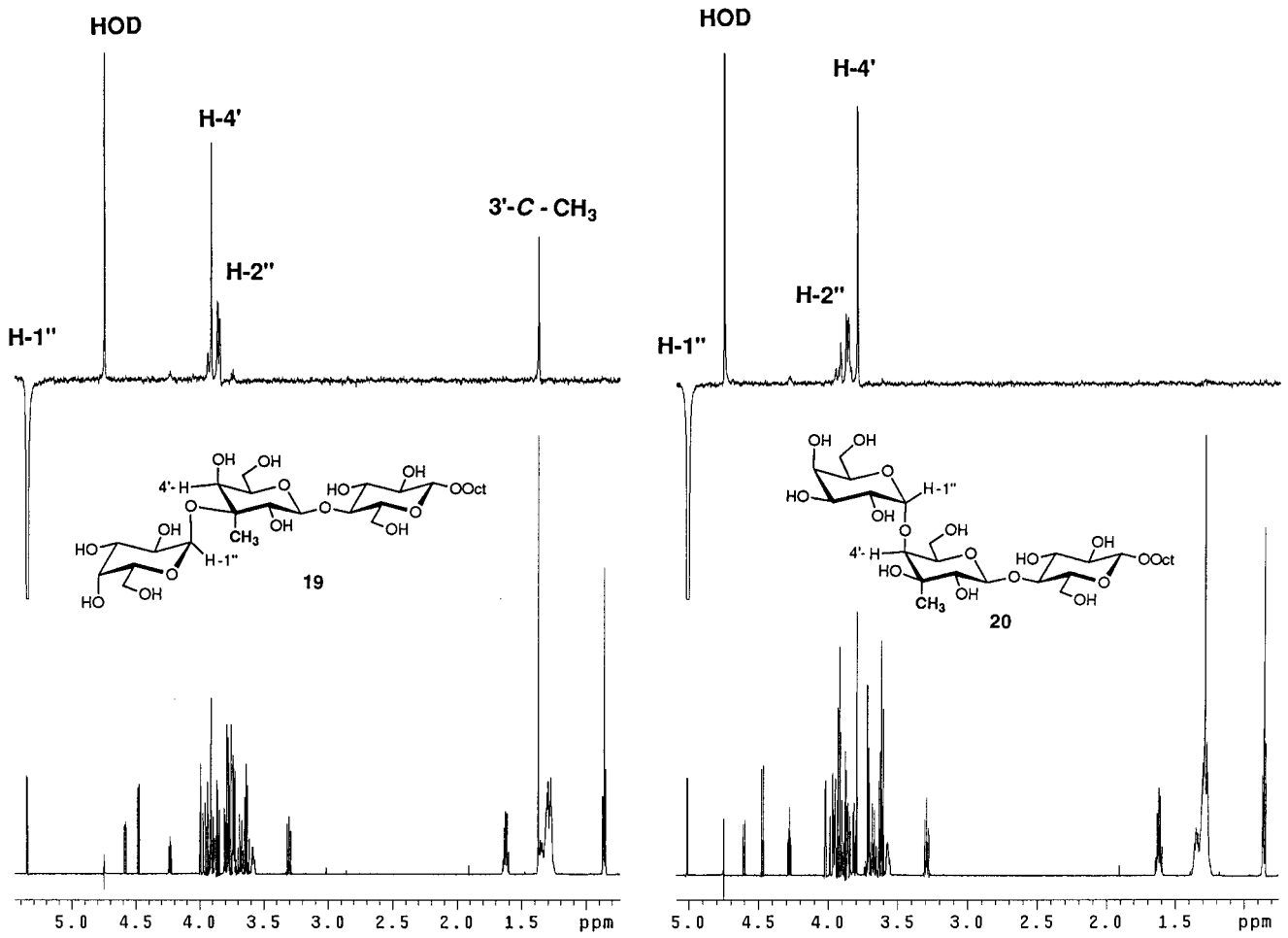
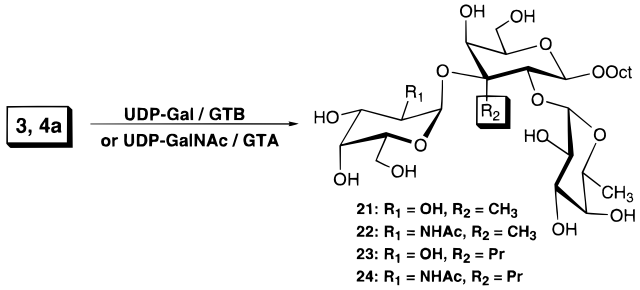


Figure 1. One-dimensional 600-MHz spectra of trisaccharides **19** and **20** (bottom) together with 1D-TROESY spectra obtained from selective excitation of the H-1″ resonance (top).

Scheme 6



Acceptor	Enzyme (mU)	Donor	Product	Time	Yield
3	GTB (750)	UDP-Gal (1.6 eq)	**21**	2 days	quant
3	GTA (1.5)	UDP-GalNAc (3 eq)	**22**	4 days	92%
4a	GTB (2000)	UDP-Gal (6 eq)	**23**	22 days	quant
4a	GTA (6000)	UDP-GalNAc (8 eq)	**24**	26 days	94%

protons (~0.15 ppm) of 3-*C*-methyl or 3-*C*-C*H*$_2$CH$_2$CH$_3$ and for the carbon resonance C-3 (~7 ppm) were also observed, compared to the starting disaccharides. In addition, conformation-independent proof for correct linkages in trisaccharides **21**−**24** was obtained by HMBC experiments[34] through ^{13}C/^{1}H correlations across the glycosidic linkage.

Conclusions

In summary, efficient chemical routes were developed leading to axially tertiary C-branched alcohols of disaccharide acceptors for three related "retaining" α1,3-glycosyltransferases. Despite the potential complexity of the mechanisms of these enzymes, they all tolerated the replacement of a C−H bond by a C−CH$_3$ bond directly at the site of glycosylations, permitting the preparative synthesis and characterization of the expected product trisaccharides. Larger *C*-propyl derivatives were not preparatively useful. The trisaccharides produced by this chemoenzymatic approach are analogues of naturally occurring carbohydrate antigens and should find application in the study of carbohydrate−protein recognition. In particular, such *C*-methyl compounds should reveal the steric requirements of the binding sites of antibodies, lectins (selectins), glycosyltransferases, and glycosidases near the OH group where the substitution has occurred. Of potentially greater interest is that the C-branched oligosaccharides should be conformationally more restricted due to the increased steric interaction between sugar rings and may thus be useful for evaluating entropic contributions to protein−oligosaccharide binding.

Experimental Section

Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40−63 μm, Darmstadt). Iatrobeads (beaded silica gel 6RS-8060) were from Iatron Laboratories (Tokyo). Millex-GV (0.22 μm) filter units were from Millipore (Missisauga, ON, Canada), and C18 Sep-Pak sample preparation cartridges were from

Waters (Missisauga, ON, Canada). ^{1}H NMR spectra were recorded at 300 MHz (Varian Inova 300), 360 MHz (Bruker AM 360), 500 MHz (Varian Unity 500), or 600 MHz (Varian Inova 600). ^{13}C NMR spectra were recorded at 75 MHz (Bruker AM 300) or 125 MHz (Varian Unity 500). Chemical shifts were referenced to solvent peaks for solutions in $CDCl_3$, CD_2Cl_2, and CD_3OD, or external 1% acetone (^{1}H, δ 2.225; ^{13}C, δ 31.07) for solutions in D_2O. High-resolution electrospray mass spectra were recorded on a Micro-mass ZabSpec Hydroid Sector-TOF. α1,3-GalT was isolated from calf thymus.[15e] GTA and GTB were expressed and cloned in *E. coli*.[16c,d] α1,4-GalT was a generous gift from Dr. Warren W. Wakarchuk.[31] Alkaline phosphatase (calf intestine) was from Boehringer Mannheim. Enzymatic assays were performed according to a well-established radioactive Sep-Pak assay method.[17,22,25] Kinetic parameters, K_m and V_{max}, were derived from the best fit of the Michaelis−Menten equation using nonlinear regression analysis with the SigmaPlot 4.1 program. The maximal velocity (V_{max}) was arbitrarily set to 100% for the parent acceptors **17** and **18**.

Octyl β-D-Galactopyranosyl-(1→4)-β-D-glucopyranoside (6). Bromide **5** (2 g, 1.76 mmol) and ground 4Å molecular sieves in CH_2Cl_2 (20 mL) were stirred under argon at room temperature for 20 min and cooled to −50 °C. Octanol (0.45 mL, 2.82 mmol) and silver triflate (725 mg, 2.82 mmol) were added. The reaction mixture was stirred for 4 h while it was allowed to warm to 0 °C. After neutralization with Et_3N, the mixture was filtered through Celite, washed with CH_2Cl_2, and concentrated. The partially purified disaccharide (obtained by column chromatography using 3:1 hexanes/EtOAc) was dissolved in MeOH, and a freshly prepared methanolic solution of NaOMe was added. The mixture was stirred at room temperature for 27 h, neutralized with Amberlite IR-120 (H^+), filtered, concentrated, and recrystallized from MeOH to yield **6** (295 mg, 37% for two steps) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 4.48 (d, 1H, J = 8.0 Hz), 4.45 (d, 1H, J = 8.0 Hz), 3.98 (dd, 1H, J = 12.1, 2.1 Hz), 3.94−3.88 (m, 2H), 3.82−3.75 (m, 3H), 3.74−3.62 (m, 5H), 3.60−3.56 (m, 1H), 3.54 (dd, 1H, J = 10.0, 7.8 Hz), 3.30 (m, 1H), 1.62 (m, 2H), 1.39−1.23 (m, 10H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 103.8, 102.8, 79.3, 76.2, 75.6, 75.3, 73.7, 73.4, 71.8, 71.6, 69.4, 61.8, 61.0, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 14.2; HR-ESMS calcd for $C_{20}H_{38}O_{11}Na$ (M + Na^+) 477.2311, found 477.2315.

Octyl 3-*O*-Allyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (7). A suspension of **6** (454 mg, 1 mmol) in benzene (25 mL) was refluxed for 24 h in the presence of Bu_2SnO (300 mg, 1.2 mmol) in a flask equipped with a Dean−Stark separator. Allyl bromide (1.6 mL, 18.5 mmol) and tetrabutylammonium iodide (185 mg, 0.5 mmol) were added. The resulting solution was refluxed for 14 h. After evaporation of solvent, the residue was dissolved in hot MeOH. On cooling, a crystalline solid was obtained on filtration, and the filtrate was concentrated. Purification on a column of Iatrobeads (10:1 CH_2Cl_2/MeOH) yielded **7** (260 mg, 53%) as a white solid: ^{1}H NMR (360 MHz, CD_3OD) δ 5.98 (dddd, 1H, J = 17.0, 10.5, 5.5, 5.5 Hz), 5.32 (dddd, 1H, J = 17.0, 1.5, 1.5, 1.5 Hz), 5.15 (dddd, 1H, J = 10.5, 1.5, 1.5, 1.5 Hz), 4.37 (d, 1H, J = 7.8 Hz), 4.27 (d, 1H, J = 7.8 Hz), 4.22 (dddd, 1H, J = 13.0, 5.5, 1.5, 1.5 Hz), 4.12 (dddd, 1H, J = 13.0, 5.5, 1.5, 1.5 Hz), 3.99 (dd, 1H, J = 3.0, 0.5 Hz), 3.92−3.81 (m, 3H), 3.77 (dd, 1H, J = 11.4, 7.4 Hz), 3.69 (dd, 1H, J = 11.4, 4.7 Hz), 3.62 (dd, 1H, J = 9.7, 7.8 Hz), 3.59−3.47 (m, 4H), 3.38 (m, 1H), 3.32 (m, 1H), 3.23 (dd, 1H, J = 8.8, 7.8 Hz), 1.61 (m, 2H), 1.43−1.23 (m, 10H), 0.89 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 136.5, 117.4, 105.1, 104.3, 82.2, 80.9, 76.9, 76.5, 75.4, 74.8, 71.8, 71.7, 71.0, 67.1, 62.5, 62.1, 33.0, 30.8, 30.6, 30.4, 27.1, 23.7, 14.4; HR-ESMS calcd for $C_{23}H_{42}O_{11}Na$ (M + Na^+) 517.2625, found 517.2625.

Octyl 3-*O*-Allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). A solution of **7** (240 mg, 0.49 mmol) and benzyl bromide (0.5 mL, 3.92 mmol) in DMF (5 mL) was cooled to 0 °C. Sodium hydride (196 mg, 60% dispension in mineral oil, 4.9 mmol) was added, and the mixture was stirred at 0 °C for 2 h. The reaction was then slowly warmed to room temperature and stirred overnight. After quenching with MeOH, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water and brine and then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography (6:1 hexanes/EtOAc) to yield **8** (400 mg, 80%): ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50−7.10 (m, 30H), 5.93 (dddd, 1H, J = 17.0, 10.5, 5.5, 5.5 Hz), 5.32 (dddd, 1H, J = 17.0, 1.5, 1.5, 1.5 Hz), 5.18 (dddd, 1H, J = 10.5, 1.5, 1.5, 1.5 Hz), 5.02 (d, 1H, J = 10.8 Hz), 4.97 (d, 1H, J = 11.5 Hz), 4.92 (d, 1H, J = 10.8 Hz), 4.84−4.71 (m, 4H), 4.56 (d, 1H, J = 11.5 Hz), 4.54 (d, 1H, J = 12.0 Hz), 4.45 (d, 1H, J = 7.7 Hz), 4.44 (d, 1H, J = 10.8 Hz), 4.38 (d, 1H, J = 8.3 Hz), 4.34 (d, 1H, J = 11.8 Hz), 4.24 (d, 1H, J = 11.8 Hz), 4.17 (m, 2H), 3.99−3.86 (m, 3H), 3.83−3.67 (m, 3H), 3.61−3.48 (m, 3H), 3.45−3.28 (m, 5H), 1.64 (m, 2H), 1.46−1.22 (m, 10H), 0.89 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.3, 139.2, 139.0, 138.8, 138.6, 138.2, 135.1, 128.4, 128.3, 128.2, 128.1, 128.0 (2 × C), 127.9 (2 × C), 127.8, 127.7, 127.6, 127.5, 127.4 (2 × C), 127.1, 116.4, 103.7, 102.8, 83.1, 82.5, 81.9, 79.6, 77.0, 75.4, 75.0, 74.7, 73.6, 73.5, 73.1, 73.0, 71.5, 70.1, 68.5, 68.2, 31.9, 29.8, 29.5, 29.3, 26.2, 22.7, 14.1; HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ (M + Na^+) 1057.5442, found 1057.5435.

Octyl 2,4,6-Tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). To a solution of **8** (400 mg, 0.39 mmol) in MeOH (15 mL) was added $PdCl_2$ (34 mg, 0.19 mmol). The mixture was stirred at room temperature for 4 h. After evaporation of MeOH, the residue was purified by column chromatography (4:1 hexanes/EtOAc) to yield **9** (320 mg, 83%) as a syrup: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50−7.10 (m, 30H), 5.06 (d, 1H, J = 10.8 Hz), 4.96 (d, 1H, J = 10.8 Hz), 4.86 (d, 1H, J = 11.5 Hz), 4.85−4.76 (m, 3H), 4.75 (d, 1H, J = 11.5 Hz), 4.69 (d, 1H, J = 11.2 Hz), 4.63 (d, 1H, J = 11.5 Hz), 4.52−4.39 (m, 4H), 4.32 (d, 1H, J = 11.8 Hz), 4.05−3.94 (m, 2H), 3.91−3.76 (m, 3H), 3.66−3.51 (m, 5H), 3.51−3.39 (m, 4H), 1.69 (m, 2H), 1.50−1.22 (m, 10H), 0.92 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.2, 138.8 (2 × C), 138.5, 138.4, 138.1, 128.5, 128.4, 128.3, 128.1 (2 × C), 128.0, 127.9, 127.8, 127.7, 127.6 (2 × C), 127.5, 127.1, 103.7, 102.7, 82.9, 81.8, 80.7, 76.8, 76.0, 75.3, 75.2, 75.1, 75.0, 74.2, 73.4, 73.3, 73.2, 70.1, 68.4, 68.0, 31.9, 29.8, 29.5, 29.3, 26.2, 22.7, 14.1; HR-ESMS calcd for $C_{62}H_{74}O_{11}Na$ (M + Na^+) 1017.5129, found 1017.5137.

Octyl 2,4,6-Tri-*O*-benzyl-β-D-*xylo*-hex-3-ulopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10). A solution of Dess−Martin periodinane (190 mg, 0.45 mmol) in CH_2Cl_2 (6 mL) was added to a stirred solution of **9** (300 mg, 0.30 mmol) in CH_2Cl_2 (7 mL), and stirring was continued for 1.5 h. The mixture was poured into a saturated aqueous $NaHCO_3$ solution containing $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated, and submitted to column chromatography (5:1 hexanes/EtOAc) to yield **10** (215 mg, 72%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.10 (m, 30H), 4.93 (d, 1H, J = 10.4 Hz), 4.89 (d, 1H, J = 10.4 Hz), 4.79 (d, 1H, J = 11.0 Hz), 4.69 (d, 1H, J = 11.0 Hz), 4.64 (d, 1H, J = 7.6 Hz), 4.63 (d, 1H, J = 11.5 Hz), 4.48 (d, 1H, J = 12.0 Hz), 4.46 (d, 1H, J = 12.0 Hz), 4.44, (d, 1H, J = 11.0 Hz), 4.40 (d, 1H, J = 12.0 Hz), 4.37 (d, 1H, J = 8.3 Hz), 4.34 (d, 1H, J = 12.0 Hz), 4.31 (d, 1H, J = 11.0 Hz), 4.30 (d, 1H, J = 7.6 Hz), 4.20 (d, 1H, J = 12.0 Hz), 3.96−3.82 (m, 3H), 3.80−3.70 (m, 2H), 3.68−3.46 (m, 3H), 3.44−3.32 (m, 4H), 1.62 (m, 2H), 1.44−1.20 (m, 10H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.3, 139.1, 138.7, 138.4, 138.1, 137.4, 137.0, 128.5, 128.4 (2 × C), 128.3, 128.2, 128.1 (3 × C), 128.0, 127.8, 127.6 (2 × C), 127.5, 127.2, 103.7, 103.6, 83.3, 83.1, 82.0, 81.0, 77.7, 75.3, 75.0, 74.9, 73.5, 73.3, 73.2, 73.1, 72.5, 70.1, 68.6, 66.8, 31.9, 29.8, 29.5, 29.3, 26.2, 22.7, 14.1; HR-ESMS calcd for $C_{62}H_{72}O_{11}Na$ (M + Na^+) 1015.4972, found 1015.4972.

Octyl 2,4,6-Tri-*O*-benzyl-3-*C*-methyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11). Compound **10** (85 mg, 0.09 mmol) was dissolved in THF (1.5 mL) and cooled under argon to −78 °C. Methyllithium (1.4 M, 0.1 mL) in diethyl ether was added with stirring. The reaction mixture was stirred for 1 h while it was allowed to warm to −40 °C. The reaction was poured into saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated, and submitted to column chromatography (5:1 hexanes/EtOAc) to yield **11** (78 mg, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.10 (m, 30H), 5.01 (d, 1H, J = 11.0 Hz), 4.89 (d, 1H, J = 11.0 Hz), 4.77 (d, 1H, J = 11.6 Hz), 4.76 (d, 1H, J = 11.0 Hz), 4.71 (d, 1H, J = 11.0 Hz), 4.67 (d, 1H, J = 11.6 Hz), 4.64 (d, 1H, J = 11.7 Hz), 4.60 (d, 1H, J = 11.7 Hz), 4.53 (d, 1H, J = 8.0 Hz), 4.46 (d, 1H, J = 12.3 Hz), 4.42 (d, 1H,

J = 12.3 Hz), 4.37 (d, 1H, J = 11.8 Hz), 4.34 (d, 1H, J = 7.7 Hz), 4.28 (d, 1H, J = 11.8 Hz), 3.97−3.86 (m, 2H), 3.74 (dd, 1H, J = 11.0, 4.2 Hz), 3.67 (dd, 1H, J = 11.0, 1.5 Hz), 3.64−3.46 (m, 4H), 3.44−3.28 (m, 5H), 1.62 (m, 2H), 1.44−1.18 (m, 10H), 1.14 (s, 3H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.4, 139.0, 138.8, 138.5, 138.3, 137.9, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9 (2 × C), 127.8, 127.7, 127.6, 127.4 (2 × C), 127.2, 127.1, 103.7, 101.9, 83.1 (2 × C), 82.1, 81.9, 77.3, 75.7, 75.2 (2 × C), 75.1, 75.0, 74.9, 73.5, 72.9, 71.9, 70.1, 68.5, 68.3, 31.9, 29.8, 29.5, 29.3, 26.2, 22.7, 19.2, 14.1; HR-ESMS calcd for $C_{63}H_{76}O_{11}Na$ ($M + Na^+$) 1031.5285, found 1031.5297.

Octyl 2,4,6-Tri-*O*-benzyl-3-*C*-allyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12a) and Octyl 2,4,6-Tri-*O*-benzyl-3-*C*-allyl-β-D-gulopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12b). To a solution of **10** (40 mg, 0.04 mmol) in THF (3 mL) at 0 °C was added allylmagnesium bromide (1.0 M, 0.1 mL) in diethyl ether with stirring under argon. The reaction mixture was stirred at 0 °C for 4 h, quenched with saturated NH_4Cl, and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated, and submitted to column chromatography (20:1 toluene/EtOAc) to yield **12a** (16 mg, 39%) and **12b** (21 mg, 51%).

For **12a**: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.40−7.20 (m, 30H), 5.86 (dddd, 1H, J = 17.0, 10.0, 9.6, 4.8 Hz), 5.06 (ddd, 1H, J = 10.0, 1.5, 1.5 Hz), 5.02 (d, 1H, J = 11.0 Hz), 4.88 (d, 1H, J = 11.0 Hz), 4.84 (ddd, 1H, J = 17.0, 1.5, 1.5 Hz), 4.78−4.62 (m, 7H), 4.53 (d, 1H, J = 12.3 Hz), 4.45 (d, 1H, J = 12.0 Hz), 4.43 (d, 1H, J = 12.0 Hz), 4.36 (d, 1H, J = 8.0 Hz, H-1), 4.33 (d, 1H, J = 12.3 Hz), 3.94 (t, 1H, J = 9.4 Hz), 3.90 (dt, 1H, J = 9.6, 6.5 Hz), 3.82 (dd, 1H, J = 10.8, 3.8 Hz), 3.68 (dd, 1H, J = 10.8, 1.4 Hz), 3.64−3.39 (m, 7H), 3.38−3.28 (m, 2H), 2.76 (dddd, 1H, J = 15.0, 4.8, 1.5, 1.5 Hz), 2.44 (d, 1H, J = 1.5 Hz), 1.97 (bdd, 1H, J = 15.0, 9.6 Hz), 1.64 (m, 2H), 1.44−1.22 (m, 10H), 0.88 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.9, 139.4, 139.0, 138.8, 138.7, 133.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3 (2 × C), 128.2, 128.1 (2 × C), 128.0, 127.9, 127.8 (2 × C), 127.7, 127.4, 127.3, 118.3, 104.1, 101.8, 83.7, 83.2, 82.1, 78.7, 77.3, 76.4, 75.9, 75.8, 75.3, 75.2, 75.0, 73.6, 73.1, 71.7, 70.3, 68.8, 68.7, 36.3, 32.2, 30.2, 29.8, 29.7, 26.6, 23.1, 14.3; HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ ($M + Na^+$) 1057.5442, found 1057.5440.

For **12b**: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.40−7.10 (m, 30H), 5.97 (dddd, 1H, J = 17.0, 10.0, 6.4, 3.7 Hz), 5.18−5.08 (m, 2H), 4.88 (d, 1H, J = 11.0 Hz), 4.87 (d, 1H, J = 7.9 Hz), 4.86 (d, 1H, J = 11.0 Hz), 4.71 (d, 1H, J = 11.0 Hz), 4.69 (d, 1H, J = 11.0 Hz), 4.60 (d, 1H, J = 11.0 Hz), 4.59 (d, 1H, J = 11.0 Hz), 4.50−4.45 (m, 3H), 4.37 (d, 1H, J = 12.0 Hz), 4.36 (d, 1H, J = 7.8 Hz), 4.13 (dd, 1H, J = 8.3, 6.2 Hz), 3.96 (t, 1H, J = 8.5 Hz), 3.90 (dt, 1H, J = 9.5, 6.5 Hz), 3.77 (dd, 1H, J = 10.8, 4.0 Hz), 3.72 (dd, 1H, J = 10.8, 2.0 Hz), 3.62−3.43 (m, 5H), 3.40−3.33 (m, 2H), 3.30 (dd, 1H, J = 9.2, 7.8 Hz), 2.62 (dddd, 1H, J = 14.5, 6.4, 1.5, 1.5 Hz), 2.54 (bdd, 1H, J = 14.5, 7.7 Hz), 2.18 (s, 1H), 1.64 (m, 2H), 1.44−1.20 (m, 10H), 0.89 (t, 3H, J = 7.0 Hz); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.9, 139.5, 139.1, 138.8 (2 × C), 138.6, 134.2, 128.7 (2 × C), 128.6 (2 × C), 128.5 (2 × C), 128.4, 128.2 (3 × C), 128.0, 127.9, 127.8 (2 × C), 127.7 (2 × C), 127.4, 118.8, 104.0, 101.5, 83.1, 81.9, 80.6, 77.6, 76.7, 75.8, 75.4 (2 × C), 75.1, 75.0, 73.7, 73.5, 72.3, 70.3, 68.8, 68.6, 39.8, 32.3, 30.2, 29.8, 29.7, 26.6, 23.1, 14.3; HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ ($M + Na^+$) 1057.5442, found 1057.5450.

Octyl 3-*C*-Methyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1). The benzyl-protected **11** (21 mg, 21 μmol) was dissolved in MeOH (10 mL), and the solution was stirred under a stream of H_2 in the presence of 20% $Pd(OH)_2$/C (25 mg) for 10 h. The catalyst was removed by filtration through a Millex-GV 0.22-μm filter and the solvent evaporated. The product was purified by redissolution in water and then passing the solution through a Waters C18 Sep-Pak cartridge. The cartridge was washed with water and eluted with gradient MeOH−H_2O 1:4 to 2:1. The elulant containing the compound was concentrated, redissolved in water, filtered through a Millex-GV 0.22-μm filter, and lyophilized to yield **1** (9.5 mg, quant) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 4.52 (d, 1H, J = 8.1 Hz), 4.47 (d, 1H, J = 8.0 Hz), 3.96 (dd, 1H, J = 12.2, 2.2 Hz), 3.94−3.87 (m, 2H), 3.81−3.72 (m, 3H), 3.67 (dt, 1H, J = 10.0, 7.0 Hz), 3.65−3.55 (m, 5H), 3.30 (m, 1H), 1.62 (m, 2H), 1.38−1.24 (m, 10H), 1.24 (s, 3H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 102.8, 102.7, 79.4, 75.5, 75.4, 75.3, 74.7, 74.1, 74.0, 73.6, 71.6, 62.2, 61.0, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 18.6, 14.2; HR-ESMS calcd for $C_{21}H_{41}O_{11}$ ($M + H^+$) 469.2649, found 469.2648.

Octyl 3-*C*-Propyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2a) and Octyl 3-*C*-Propyl-β-D-gulopyranosyl-(1→4)-β-D-glucopyranoside (2b). Compounds **12a** (7 mg, 6.8 μmol) and **12b** (9 mg, 8.7 μmol) were treated in the same fashion as for **1** to give, respectively, **2a** (3.3 mg) and **2b** (4.1 mg) in quantitative yields.

For **2a**: ^{1}H NMR (600 MHz, D_2O) δ 4.58 (d, 1H, J = 8.2 Hz), 4.47 (d, 1H, J = 7.9 Hz), 3.96 (dd, 1H, J = 12.3, 2.2 Hz), 3.91 (dt, 1H, J = 10.0, 7.0 Hz), 3.85 (dd, 1H, J = 7.4, 4.8 Hz), 3.82 (bs, 1H), 3.78 (dd, 1H, J = 12.3, 5.0 Hz), 3.75 (dd, 1H, J = 11.8, 7.4 Hz), 3.73 (dd, 1H, J = 11.8, 4.8 Hz), 3.67 (dt, 1H, J = 10.0, 7.0 Hz), 3.65−3.60 (m, 3H), 3.57 (m, 1H), 3.30 (m, 1H), 1.73 (m, 1H), 1.62 (m, 2H), 1.56 (m, 1H), 1.41 (m, 2H), 1.38−1.22 (m, 10 H), 0.92 (t, 3H, J = 7.0 Hz), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 102.8, 102.5, 79.4, 76.3, 75.5, 75.4, 74.9, 74.6, 73.6, 71.6, 70.0, 62.2, 61.0, 33.0, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 15.9, 14.8, 14.2; HR-ESMS calcd for $C_{23}H_{44}O_{11}Na$ ($M + Na^+$) 519.2781, found 519.2786.

For **2b**: ^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, 1H, J = 8.0 Hz), 4.47 (d, 1H, J = 8.0 Hz), 4.06 (ddd, 1H, J = 8.0, 4.1, 0.8 Hz), 3.97 (dd, 1H, J = 12.2, 4.2 Hz), 3.91 (dt, 1H, J = 10.0, 7.0 Hz), 3.81 (dd, 1H, J = 12.2, 5.1 Hz), 3.75 (dd, 1H, J = 12.0, 8.0 Hz), 3.73 (dd, 1H, J = 12.0, 4.1 Hz), 3.67 (dt, 1H, J = 10.0, 7.0 Hz), 3.65−3.60 (m, 3H), 3,57 (m, 1H), 3.38 (d, 1H, J = 8.0 Hz), 3.30 (m, 1H), 1.76 (m, 1H), 1.66−1.60 (m, 3H), 1.40−1.22 (m, 12H), 0.92 (t, 3H, J = 7.0 Hz), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 102.9, 102.6, 79.9, 76.6, 75.5, 75.4 (2 × C), 73.7, 72.8, 71.6, 69.6, 62.2, 61.1, 36.4, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 15.4, 14.6, 14.2; HR-ESMS calcd for $C_{23}H_{44}O_{11}Na$ ($M + Na^+$) 519.2781, found 519.2786.

Octyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-4,6-di-*O*-benzyl-β-D-*xylo*-hexopyranosid-3-ulose (14). Compound **13** (135 mg, 0.15 mmol) was oxidized in the same fashion as for **10**. The crude product was purified by column chromatography (24:1 toluene/EtOAc) to yield **14** (81 mg, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50−7.20 (m, 25H), 5.05 (d, 1H, J = 3.7 Hz), 4.99 (d, 1H, J = 11.5 Hz), 4.98 (d, 1H, J = 11.5 Hz), 4.92 (d, 1H, J = 11.6 Hz), 4.73 (d, 1H, J = 11.6 Hz), 4.70 (d, 1H, J = 7.9 Hz), 4.69 (d, 1H, J = 11.5 Hz), 4.64 (d, 1H, J = 11.6 Hz), 4.60 (d, 1H, 7.9 Hz), 4.54 (d, 1H, J = 11.8 Hz), 4.52 (d, 1H, J = 11.8 Hz), 4.46 (d, 1H, J = 11.8 Hz), 4.41 (d, 1H, J = 11.8 Hz), 4.21 (bq, 1H, J = 6.6 Hz), 4.10 (dd, 1H, J = 10.2, 3.5 Hz), 3.96 (dd, 1H, J = 10.2, 2.9 Hz), 3.90 (dt, 1H, J = 9.4, 6.4 Hz), 3.87 (d, 1H, J = 1.4 Hz), 3.78−3.72 (m, 2H), 3.70 (m, 1H), 3.61 (bd, 1H, J = 2.9 Hz), 3.42 (dt, 1H, J = 9.4, 6.4 Hz), 1.52 (m, 2H), 1.30−1.18 (m, 10H), 1.08 (d, 3H, J = 6.6 Hz), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.4, 139.2, 138.8, 138.5, 137.9, 136.6, 128.7, 128.6, 128.5 (2 × C), 128.4, 128.3 (3 × C), 128.2, 127.8, 127.7, 127.6, 127.5, 127.4 (2 × C), 102.6, 96.0, 80.5, 79.1, 78.3, 77.6, 76.1, 74.9, 73.8, 73.7, 73.5, 72.6 (2 × C), 70.1, 67.8, 66.8, 31.9, 29.7, 29.5, 29.4, 26.2, 22.7, 16.5, 14.1; HR-ESMS calcd for $C_{55}H_{66}O_{10}Na$ ($M + Na^+$) 909.4554, found 909.4547.

Octyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-4,6-di-*O*-benzyl-3-*C*-methyl-β-D-galactopyranoside (15). Compound **14** (50 mg, 56 μmol) was treated with MeLi in the same fashion as for **11**. The crude product was purified by column chromatography (25:1 toluene/EtOAc) to yield **15** (28 mg, 55%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.20 (m, 25H), 5.33 (d, 1H, J = 3.5 Hz), 4.94 (d, 1H, J = 11.6 Hz), 4.82 (d, 1H, J = 11.6 Hz), 4.77 (d, 1H, J = 11.6 Hz), 4.74 (d, 1H, J = 11.6 Hz), 4.72 (d, 1H, J = 11.6 Hz), 4.70 (d, 1H, J = 11.6 Hz), 4.62 (d, 1H, J = 11.6 Hz), 4.61 (d, 1H, J = 11.6 Hz), 4.48 (d, 1H, J = 11.6 Hz), 4.44 (d, 1H, J = 11.6 Hz), 4.26 (d, 1H, J = 7.9 Hz), 4.16 (bq, 1H, J = 6.5 Hz), 4.02 (dd, 1H, J = 10.1, 3.5 Hz), 3.96 (dd, 1H, J = 10.1, 2.7 Hz), 3.82−3.76 (m, 2H), 3.74 (d, 1H, J = 7.9 Hz), 3.62 (dd, 1H, J = 2.7, 1.0 Hz), 3.60−3.57 (m, 2H), 3.41 (d, 1H, J = 0.9 Hz), 3.35 (dt, 1H, J = 9.3, 7.0 Hz), 1.53 (m, 2H), 1.28−1.19 (m, 13H), 1.08 (d, 3H, J = 6.5 Hz), 0.85 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.9, 138.4, 138.2, 138.0, 129.0, 128.7, 128.5, 128.4 (2 × C), 128.3 (2 × C), 128.2 (3 × C), 128.1, 127.9 (2 × C), 127.8 (2 × C), 127.7, 127.5 (2 × C), 101.5, 98.7, 81.8,

79.8, 79.4, 78.0, 77.2, 75.8, 75.7, 74.8, 73.7, 73.6, 72.9, 72.7, 70.1, 69.5, 66.8, 31.9, 29.8, 29.5, 29.3, 26.1, 22.7, 20.3, 16.7, 14.1; HR-ESMS calcd for $C_{56}H_{70}O_{10}Na$ ($M + Na^+$) 925.4867, found 925.4872.

Octyl α-L-Fucopyranosyl-(1→2)-3-*C*-methyl-β-D-galactopyranoside (3). The benzyl-protected **15** (13 mg, 14 μmol) was treated in the same fashion as for **1** to give **3** (6 mg, 95%): ^{1}H NMR (600 MHz, D_2O) δ 5.35 (d, 1H, $J = 4.0$ Hz), 4.53 (d, 1H, $J = 8.1$ Hz), 4.37 (bq, 1H, $J = 6.6$ Hz), 3.91 (dt, 1H, $J = 9.6, 6.6$ Hz), 3.89 (dd, 1H, $J = 10.5, 3.4$ Hz), 3.84−3.77 (m, 3H), 3.76 (dd, 1H, $J = 11.5, 7.2$ Hz), 3.72 (dd, 1H, $J = 11.5, 3.8$ Hz), 3.66 (d, 1H, $J = 8.1$ Hz), 3.64 (dt, 1H, $J = 9.6, 6.6$ Hz), 3.55 (bs, 1H), 1.60 (m, 2H), 1.36−1.24 (m, 13H), 1.21 (d, 1H, $J = 6.6$ Hz), 0.86 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 102.6, 99.5, 78.3, 76.3, 74.8, 74.5, 72.8, 71.5, 70.3, 69.4, 67.5, 62.1, 31.9, 29.8, 29.3, 29.2, 26.2, 22.8, 19.3, 16.2, 14.2; HR-ESMS calcd for $C_{21}H_{40}O_{10}Na$ ($M + Na^+$) 475.2519, found 475.2526.

Octyl α-L-Fucopyranosyl-(1→2)-3-*C*-propyl-β-D-galactopyranoside (4a) and Octyl α-L-Fucopyranosyl-(1→2)-3-*C*-propyl-β-D-gulopyranoside (4b). Compound **14** (20 mg, 22 μmol) was treated with allylmagnesium bromide as described for the preparation of **12**. The crude product was purified by column chromatography (8:1 hexanes/EtOAc) to yield **16a** (7 mg, 33%) and **16b** (6 mg, 29%). Hydrogenolysis of **16a** (7 mg, 7.5 μmol) and **16b** (4 mg, 4.3 μmol) as described for **1** gave **4a** (3.4 mg, 94%) and **4b** (1.9 mg, 92%).

For **4a**: ^{1}H NMR (600 MHz, D_2O) δ 5.32 (d, 1H, $J = 4.0$ Hz, H-1′), 4.60 (d, 1H, $J = 8.2$ Hz, H-1), 4.36 (bq, 1H, $J = 6.6$ Hz), 3.91 (dt, $J = 9.9, 6.6$ Hz), 3.89 (dd, 1H, $J = 10.4, 3.3$ Hz), 3.81−3.73 (m, 5H), 3.72 (dd, 1H, $J = 11.3, 5.0$ Hz), 3.69 (d, 1H, $J = 8.2$ Hz), 3.65 (dd, 1H, $J = 9.9, 6.6$ Hz), 1.92 (m, 1H), 1.67−1.58 (m, 3H), 1.42 (m, 1H), 1.37−1.24 (m, 11H), 1.22 (d, 3H, $J = 6.6$ Hz), 0.93 (t, 3H, $J = 7.0$ Hz), 0.87 (t, 3H, $J = 7.0$ Hz); HR-ESMS calcd for $C_{23}H_{44}O_{10}Na$ ($M + Na^+$) 503.2832, found 503.2838.

For **4b**: ^{1}H NMR (600 MHz, D_2O) δ 5.01 (d, 1H, $J = 3.8$ Hz), 4.77 (d, 1H, $J = 8.0$ Hz), 4.38 (bq, 1H, $J = 6.6$ Hz), 4.00 (dd, $J = 7.2, 5.1$ Hz), 3.89 (dd, 1H, $J = 10.5, 3.3$ Hz), 3.86 (m, 1H), 3.83−3.79 (m, 2H), 3.76 (dd, 1H, $J = 11.7, 7.5$ Hz), 3.73−3.68 (m, 2H), 3.65 (bs, 1H), 3.42 (d, 1H, $J = 8.0$ Hz), 1.92 (m, 1H), 1.72−1.60 (m, 3H), 1.42−1.26 (m, 12H), 1.20 (d, 3H, $J = 6.6$ Hz), 0.93 (t, 3H, $J = 7.0$ Hz), 0.86 (t, 3H, $J = 7.0$ Hz); HR-ESMS calcd for $C_{23}H_{44}O_{10}Na$ ($M + Na^+$) 503.2832, found 503.2838.

Octyl α-D-Galactopyranosyl-(1→3)-β-D-3-*C*-methylgalactopyranosyl-(1→4)-β-D-glucopyranoside (19). Disaccharide **1** (3.4 mg, 7.3 μmol), UDP-Gal (8.9 mg, 14.6 μmol), and 10 μL of alkaline phosphatase (1 U/μL) were added to a 0.5-mL solution of calf thymus α1,3-GalT (120 mU in 30 mM sodium cacodylate, 20 mM $MnCl_2$, 0.1% Triton X-100, pH 6.5). The mixture was incubated with rotation at room temperature for 5 days. The reaction mixture was filtered through glass wool and loaded onto two sequential C18 Sep-Pak cartridges. The cartridges were washed with water (100 mL) and 10% aqueous methanol (40 mL) and then eluted with 50% aqueous MeOH (40 mL). The 50% eluant was concentrated, redissolved in water, filtered through a Millex-GV 0.22-μm filter, and lyophilized to yield **19** (4.1 mg, 90%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.35 (d, 1H, $J = 4.0$ Hz), 4.58 (d, 1H, $J = 8.2$ Hz), 4.48 (d, 1H, $J = 8.0$ Hz), 4.23 (m, 1H), 4.00−3.96 (m, 2H), 3.94 (dd, 1H, $J = 10.2, 3.3$ Hz), 3.92−3.87 (m, 3H), 3.86 (dd, 1H, $J = 10.2, 4.0$ Hz), 3.81−3.70 (m, 6H), 3.68 (dt, 1H, $J = 10.0, 6.8$ Hz), 3.64−3.59 (m, 2H), 3.58 (m, 1H), 3.30 (m, 1H), 1.63 (m, 2H), 1.38−1.24 (m, 13H), 0.86 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 102.8, 102.4, 93.1, 80.9, 79.7, 75.5, 75.4, 74.9, 73.6, 73.3, 71.9, 71.6, 71.4, 70.2, 70.0, 69.0, 62.2, 61.9, 61.1, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 16.0, 14.2; HR-ESMS calcd for $C_{27}H_{50}O_{16}Na$ ($M + Na^+$) 653.2997, found 653.2998.

Octyl α-D-Galactopyranosyl-(1→4)-β-D-3-*C*-methylgalactopyranosyl-(1→4)-β-D-glucopyranoside (20). The reaction mixture contained disaccharide **1** (1.8 mg, 3.8 μmol), UDP-Gal (5.0 mg, 8.2 μmol), 20 mU of α1,4-GalT (in 200 μL containing 52 mM Hepes, 10 mM ammonium acetate, pH 7.5), and 10 μL of alkaline phosphatase (1 U/μL). The reaction was incubated with rotation at room temperature for 4 days. The mixture was purified as described for **19** to yield **20** (2.3 mg, 95%) as a while solid: ^{1}H NMR (600 MHz, D_2O) δ 5.01 (d, 1H, $J = 4.0$ Hz), 4.60 (d, 1H, $J = 8.0$ Hz), 4.47 (d, 1H, $J = 8.0$ Hz), 4.28 (bt, 1H, $J = 6.5$ Hz), 4.02 (bd, 1H, $J = 3.3$ Hz), 3.99−3.79 (m, 9H), 3.73−3.60 (m, 6H), 3.57 (m, 1H), 3.29 (m, 1H), 1.62 (m, 2H), 1.39−1.24 (m, 13H), 0.86 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 103.1, 102.8, 101.8, 84.0, 80.0, 75.7, 75.5, 75.4, 74.7, 74.3, 73.7, 72.4, 71.6, 69.8 (2 × C), 69.3, 61.6, 61.5, 61.0, 31.9, 29.5, 29.2, 29.1, 25.8, 22.8, 19.3, 14.2; HR-ESMS calcd for $C_{27}H_{50}O_{16}Na$ ($M + Na^+$) 653.2997, found 653.3002.

Octyl α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-*C*-methyl-β-D-galactopyranoside (21). The reaction mixture contained disaccharide **3** (2.4 mg, 5.3 μmol), UDP-Gal (5.0 mg, 8.2 μmol), 750 mU of blood group B transferase (in 150 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 40 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), and 4 μL of alkaline phosphatase (1 U/μL). The reaction was incubated with rotation at room temperature for 2 days. The mixture was purified as described for **19** to yield **21** (3.2 mg, quantitative) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.38 (d, 1H, $J = 4.0$ Hz), 5.32 (d, 1H, $J = 4.0$ Hz), 4.60 (d, 1H, $J = 8.0$ Hz), 4.46 (bq, 1H, $J = 6.6$ Hz), 4.14 (m, 1H), 4.03 (d, 1H, $J = 3.1$ Hz), 3.98 (s, 1H), 3.93 (dt, 1H, $J = 9.8, 6.6$ Hz), 3.90−3.69 (m, 11H), 3.65 (dt, 1H, $J = 9.8, 6.6$ Hz), 1.60 (m, 2H), 1.48 (s, 3H), 1.37−1.24 (m, 10H), 1.22 (d, 3H, $J = 6.6$ Hz), 0.87 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 101.1, 99.3, 93.3, 83.8, 76.4, 74.3, 72.8, 72.6, 71.5, 70.6, 70.3, 70.2, 69.9, 69.1, 68.8, 67.5, 62.2, 61.9, 31.9, 29.8, 29.4, 29.2, 26.3, 22.8, 17.2, 16.2, 14.2; HR-ESMS calcd for $C_{27}H_{50}O_{15}Na$ ($M + Na^+$) 637.3047, found 637.3057.

Octyl α-D-2-Acetamido-2-deoxygalactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-*C*-methyl-β-D-galactopyranoside (22). The reaction mixture contained disaccharide **3** (1.2 mg, 2.7 μmol), UDP-GalNAc (5.4 mg, 8.3 μmol), 1.45 mU of blood group A transferase (in 490 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 80 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), and 10 μL of alkaline phosphatase (1 U/μL). The reaction was incubated with rotation at 37 °C for 4 days, and the reaction mixture was purified as described for **19** to yield **22** (1.6 mg, 92%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.36 (d, 1H, $J = 3.8$ Hz), 5.32 (d, 1H, $J = 3.7$ Hz), 4.60 (d, 1H, $J = 8.2$ Hz), 4.50 (bq, 1H, $J = 6.6$ Hz), 4.22−4.17 (m, 2H), 4.06 (d, 1H, $J = 2.4$ Hz), 4.00 (s, 1H), 3.94 (dt, 1H, $J = 9.8, 6.6$ Hz), 3.90−3.84 (m, 2H), 3.84−3.70 (m, 8H), 3.66 (dt, 1H, $J = 9.8, 6.6$ Hz), 2.02 (s, 3H), 1.49 (s, 3H), 1.61 (m, 2H), 1.38−1.26 (m, 10H), 1.24 (d, 3H, $J = 6.6$ Hz), 0.87 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.5, 101.1, 99.1, 92.7, 83.9, 76.2, 75.1, 72.8, 72.0, 71.6, 70.3, 69.8, 69.2, 69.0 (2 × C), 67.6, 62.2, 61.7, 50.7, 31.9, 29.8, 29.4, 29.2, 26.3, 22.9, 22.8, 18.9, 16.2, 14.2; HR-ESMS calcd for $C_{29}H_{53}NO_{15}Na$ ($M + Na^+$) 678.3313, found 678.3310.

Octyl α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-*C*-propyl-β-D-galactopyranoside (23). The reaction mixture contained disaccharide **4a** (1.2 mg, 2.5 μmol), UDP-Gal (3.1 mg, 5.1 μmol), 0.5 U of blood group B transferase (in 100 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 35 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), 5 μL of alkaline phosphatase (1 U/μL), and 200 μL of water. The reaction was incubated with rotation at 37 °C for 22 days. Additional UDP-Gal (6.2 mg), B transferase (1.5 U), and alkaline phosphatase (10 μL) were added during the incubation period. The reaction mixture was purified as described for **19** to yield **23** (1.6 mg, quantitative) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.43 (d, 1H, $J = 4.2$ Hz), 5.29 (d, 1H, $J = 4.0$ Hz), 4.68 (d, 1H, $J = 7.9$ Hz), 4.44 (bq, 1H, $J = 6.6$ Hz), 4.19−4.16 (m, 2H), 4.06 (d, 1H, $J = 3.3$ Hz), 3.92 (dt, 1H, $J = 9.9, 6.6$ Hz), 3.91−3.86 (m, 3H), 3.84−3.69 (m, 8H), 3.66 (dt, 1H, $J = 9.9, 6.6$ Hz), 2.02 (m, 1H), 1.77 (m, 1H), 1.66−1.56 (m, 3H), 1.40−1.24 (m, 11H), 1.22 (d, 3H, $J = 6.6$ Hz), 0.90 (t, 3H, $J = 7.0$ Hz), 0.87 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 101.3, 99.5, 93.5, 84.9, 77.5, 74.1, 72.8, 72.4, 71.5, 70.4, 70.3, 69.8, 69.2, 69.1, 67.6 (2 × C), 62.1, 61.4, 34.9, 31.9, 29.8, 29.4, 29.2, 26.3, 22.8, 16.7, 16.2, 15.2, 14.2; HR-ESMS calcd for $C_{29}H_{54}O_{15}Na$ ($M + Na^+$) 665.3360, found 665.3355.

Octyl α-D-2-Acetamido-2-deoxygalactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-*C*-propyl-β-D-galactopyranoside (24). The reaction mixture contained disaccharide **4a** (1.2 mg, 2.5 μmol), UDP-

GalNAc (3.3 mg, 5.1 μmol), 1.4 U of blood group A transferase (in 200 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 10 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), 10 μL of alkaline phosphatase (1 U/μL), and 220 μL of water. The reaction was incubated with rotation at 37 °C for 26 days. Additional UDP-GalNAc (9.3 mg), A transferase (4.5 U), and alkaline phosphatase (10 μL) were added during the incubation period. The reaction mixture was purified as described for **19** to yield **24** (1.6 mg, 94%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.36 (d, 1H, J = 4.0 Hz), 5.32 (d, 1H, J = 4.0 Hz), 4.67 (d, 1H, J = 7.9 Hz), 4.43 (bq, 1H, J = 6.6 Hz), 4.22−4.17 (m, 2H), 4.10 (bs, 1H), 4.08 (d, 1H, J = 3.1 Hz), 3.93 (dt, 1H, J = 9.7, 6.8 Hz), 3.91 (d, 1H, J = 7.9 Hz), 3.88 (dd, 1H, J = 10.4, 3.3 Hz), 3.85−3.81 (m, 2H), 3.79−3.70 (m, 6H), 3.66 (dt, 1H, J = 9.7, 6.8 Hz), 2.03 (s, 3H), 1.89 (m, 1H), 1.79 (m, 1H), 1.65−1.54 (m, 3H), 1.38−1.16 (m, 14 H), 0.91−0.85 (m, 6H); ^{13}C NMR (125 MHz, D_2O) δ 175.4, 101.7, 99.0, 92.5, 84.8, 77.9, 74.3, 72.8, 72.0, 71.7, 70.5, 69.3, 69.0 (2 × C), 67.8, 66.8, 62.0, 61.3, 51.1, 34.7, 31.9, 29.8, 29.4, 29.2, 26.3, 23.0, 22.8, 16.2 (2 × C), 14.9, 14.2; HR-ESMS calcd for $C_{31}H_{57}NO_{15}Na$ ($M + Na^+$) 706.3626, found 706.3621.

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Supporting Information Available: ^{1}H NMR spectra for compounds **1**−**4**, **6**−**12**, **14**, **15**, **19**−**24** and HMBC spectra for compounds **19**−**24** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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