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Recognition of synthetic analogues of the acceptor, β-D-Gal*p*-OR, by the blood-group H gene-specified glycosyltransferase

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Abstract

The acceptor–substrate specificity of a cloned α-(1 → 2) fucosyltransferase has been explored using structural analogues of octyl β-D-galactopyranoside (**4**). This monosaccharide is the minimum acceptor–substrate for the H-transferase, one of two enzymes responsible for the biosynthesis of the O blood-group antigen, which terminates in the sequence α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*. Galactoside **4** has a K_m of 6 mM with this enzyme. Eighteen analogues of **4** have been prepared, including those where the hydroxyl groups at C-3, C-4, and C-6 have been replaced, independently, with deoxy, fluoro, *O*-methyl, amino, and acetamido functionalities. The C-3 and C-4 epimers have been prepared as has the C-5 de(hydroxymethyl)ated derivative. These compounds were screened as potential acceptors and inhibitors of the fucosyltransferase. The C-6 analogues that do not possess a charge show substrate activity with relative rates in the range of 27–316% that of **4**. The C-3 modified analogues are inhibitors with estimated K_i values of 0.9–43 mM. Those analogues with modifications at C-4 were both poor inhibitors and acceptors.

1. Introduction

The A, B, and O (H) blood-group antigens are ubiquitous biological oligosaccharide structures. Though originally described as constituents on the surfaces of red blood cells in 1900 by Landsteiner [1], these antigens have also been shown to be present in a wide range of other tissue cells and in soluble form in the

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cytoplasm [2]. Using crude human serum as a source of enzyme and a panel of synthetic disaccharides as potential substrates, we have recently reported our findings from probing the active sites of the enzymes which make the A and B blood-group antigens [3,4]. Using a similar panel of modified monosaccharide derivatives, we report here the results from probing the active site of a cloned H-transferase, one of the glycosyltransferases that synthesizes the O (or H) blood-group antigen.

The disaccharide, α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-OR (**3**), where R is a glycoconjugate, is the O blood-group antigen [2]. The biosynthesis of this antigenic determinant is shown in Fig. 1. Two distinct α-(1 → 2) fucosyltransferases, the H-enzyme and the Se-enzyme (EC 2.4.1.69) are known to synthesize this carbohydrate epitope in humans [5]. The former is believed to be responsible for the production of these antigens on red-blood cells, while the latter is thought to synthesize these structures in secretory tissues [6]. These enzymes act by transferring fucose from GDP-fucose (**2**) to the C-2 hydroxyl group of galactose residues located at the terminal ends of glycoconjugates (**1**) (ref. [6]). The transfer of the fucose residue proceeds with inversion of configuration at the anomeric center, i.e., the β-sugar nucleotide yields the α-glycosidic linkage. This disaccharide O antigen serves as a substrate for the glycosyltransferases that make the trisaccharide A and B blood-group antigens.

The mechanism of transfer is not known, but it has been hypothesized [7,8] that the observed inversion of configuration results from a direct S_N2-type displacement of the guanosine diphosphate (GDP) from the sugar nucleotide by the acceptor hydroxyl group. Possible assistance by a base on the enzyme to help deprotonate the incoming alcohol has also been proposed (Fig. 1).

Like a number of other carbohydrate antigens, it is well documented that these blood-group determinants serve as important recognition factors for a number of biological recognition processes, both normal and abnormal. In particular, it is now understood that they serve as important antigens in fetal development and organ differentiation [6], in addition to their well known critical importance in transplant operations and blood transfusions [9]. Recently, O antigens have been discovered on the von Willebrand factor, a glycoprotein involved in the blood clotting process, although their exact role on this protein is not known [10]. Furthermore, like other carbohydrate antigens, the expression of the O antigen on cell surfaces has been shown to be modulated during the onset of cancer [11–15]. The aberrant expression of these structures is thought to be the result of changes in the activities of the glycosyltransferases responsible for producing the structures. Therefore, the development of specific glycosyltransferase inhibitors for these enzymes may lead to new methods of cancer therapy. It is this area in which our research is directed [3,4,8,16–18].

Very little information is known about the substrate specificity of these α-(1 → 2) fucosyltransferases. No studies have been carried out concerning the donor specificity of either the H or Se-transferase. Although deoxygenated analogues of GDP-fucose have been tested as substrates for another fucosyltransferase [19], these derivatives have not been tested with either α-(1 → 2) fucosyltransferase.

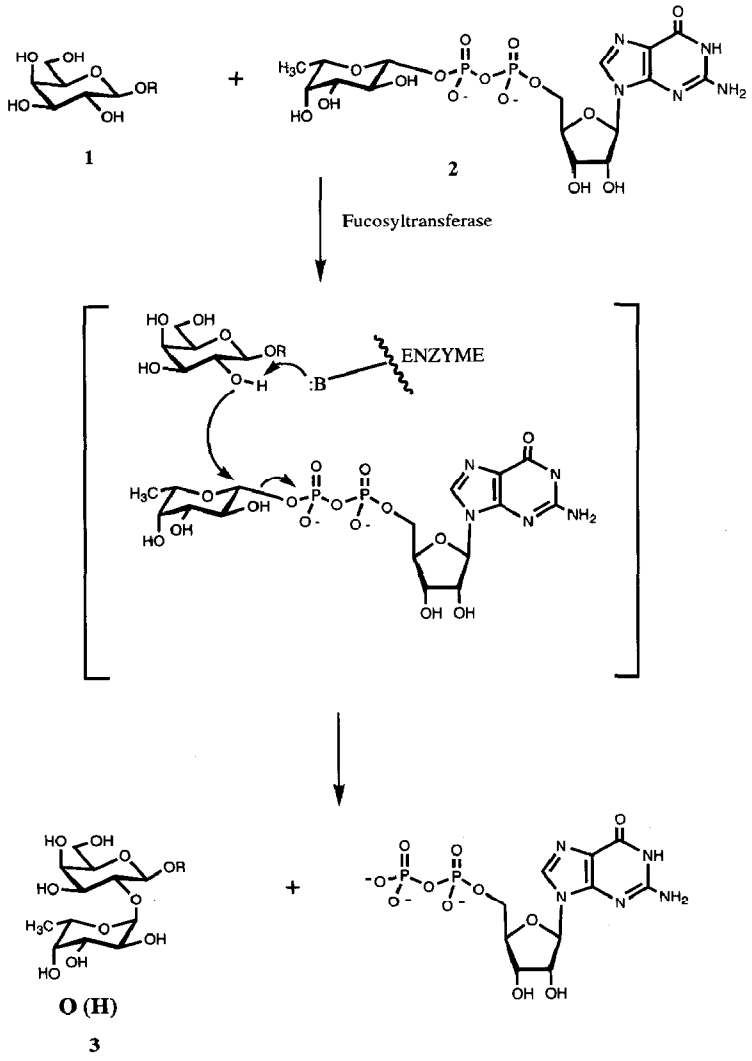


Fig. 1. Formation of the O (H) antigen from glycoconjugates with terminal β-linked galactose residues. The posulated mechanism for direct displacement reactions is shown in brackets.

Inhibition studies have been carried out with an α-(1 → 2) fucosyltransferase from rat intestine using a variety of GDP analogues [20]. It was determined that the enzyme is inhibited by GDP, GMP, GTP, and GDP-Man.

More is known about the acceptor specificity of the H-transferase. The fucose can be transferred to a terminal Gal residue that is linked to either GlcNAc, GalNAc, Gal or Glc through either the C-3 or C-4 hydroxyl group. The structures, known as Type I–VI structures [21], have been shown to be, at least to some

extent, tissue and glycoconjugate specific [15]. Moreover, the H and Se enzyme show differential recognition of these structures [22–24]. However, the required groups on the Gal residue itself were unknown except for the report that D-fucose (6-deoxy-D-galactose) is a substrate for the H-transferase [25].

The H-transferase has been cloned [26] and has been shown to be morphologically similar to other known glycosyltransferases. The enzyme possesses an amino-terminal cytoplasmic tail (8 amino acids), a transmembrane domain (16 amino acids), and a carboxy-terminal catalytic region (340 amino acids), located inside the Golgi apparatus [27]. The gene responsible for the expression of the enzyme is located on chromosome 19.

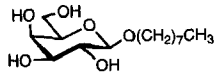
As a minimum acceptor structure, both the H and Se enzymes recognize the monosaccharide β-D-Gal*p*-OR (**1**) [6]. In the present work we chose R to be octyl (**4**) because the presence of this hydrophobic aglycon simplifies the enzymatic assays in that reverse-phase (C_{18}) cartridges can be used to separate and quantitate the product [28]. The monosaccharide analogues chosen were those where the hydroxyl groups at the C-3, C-4, and C-6 positions in the galactosyl residue were replaced, independently, with H (**5–7**), F (**8–10**), *O*–methyl (**11–13**), NH_2 (**16–18**), and NHAc (**19–21**). These modifications can be divided into two groups: (1) those that probe hydrogen bonding interactions, and (2) those that probe steric interactions.

Substitutions of hydroxyl groups by either H or F are sterically conservative modifications and can thus provide insights into whether the hydroxyl group removed was involved in critical hydrogen-bond interactions with the protein combining site. Deoxygenation provides information about whether that hydroxyl group is acting either as a hydrogen-bond acceptor or donor with the enzyme [8]. Substitution with fluorine, which can act as a hydrogen-bond acceptor but not a hydrogen-bond donor, would give insight into whether the hydroxyl group was acting as a hydrogen-bond acceptor [29,30].

The amino compounds were synthesized to probe for the existence of a negatively charged amino acid residue in the active site. At physiological pH, the amine would be protonated, and therefore it was expected that if there were a negatively charged group (base) near the positively charged amine, a strong ionic interaction might result. Such a compound could serve as a potent inhibitor of the enzyme via a tightly held enzyme–substrate complex [4].

Methylation of a hydroxyl group probes not only the hydrogen bonding requirements of the enzyme, but also provides insight into whether the enzyme can tolerate groups of larger steric bulk at that position [31]. Carbohydrates with an acetamido group at the C-2 position (GlcNAc and GalNAc) are widely occurring structures in mammalian systems. We synthesized these acetamido derivatives to investigate the effect of this group at other positions on the ring, as well as to further probe the steric constraints of the enzyme. Additionally, if the enzyme would tolerate the acetamido derivative, it would probably also tolerate a bromo- or iodo-acetamido derivative, which could act as an alkylating agent for a possible active-site nucleophile or base.

Another probe of steric constraints involved the synthesis of compounds where the configuration of the C-3,4 diol had been altered. Thus compounds containing



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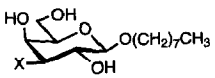
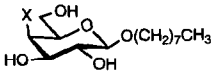
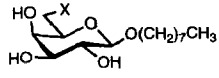


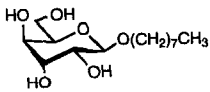
5 X = H
8 X = F
11 X = OCH_3
16 X = NH_2
19 X = NHAc



6 X = H
9 X = F
12 X = OCH_3
17 X = NH_2
20 X = NHAc



7 X = H
10 X = F
13 X = OCH_3
18 X = NH_2
21 X = NHAc



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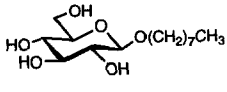

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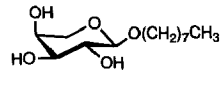

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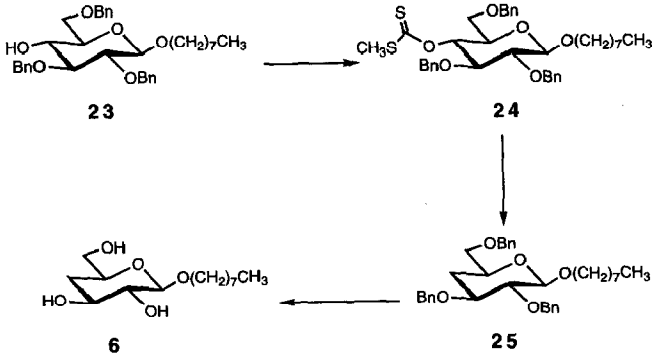
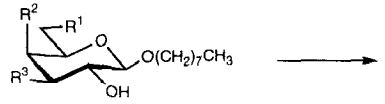


23 24 25 6

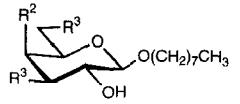
the C-3 and C-4 epimers of D-galactose, i.e. D-gulose (**14**) and D-glucose (**15**) were prepared. Finally, spurred by a report [32] that an L-arabinose-binding protein also recognizes D-galactose, the L-arabinose derivative **22** was synthesized.

2. Results and discussion

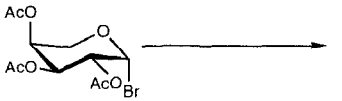
Chemical synthesis.—The chemical synthesis of monosaccharides **4–22** was achieved, for the most part, in the course of preparing the disaccharides required for the A- and B-transferases [3,4]. The preparation of the following derivatives have been previously published: parent monosaccharide **4**, 3-deoxy **5**, 3-fluoro **8**,



	R_1	R_2	R_3
16	OH	OH	NH_2
17	OH	NH_2	OH
18	NH_2	OH	OH



	R_1	R_2	R_3
19	OH	OH	NHAc
20	OH	NHAc	OH
21	NHAc	OH	OH



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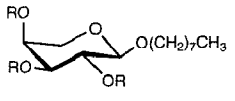


32 R = Ac
22 R = H

4-fluoro **9**, 6-fluoro **10**, 3-*O*-methyl **11**, 3-epimer **14**, and 4-amino **17**. The 4-epimer, octyl β-D-glucopyranoside (**15**), is commercially available and was purchased from Sigma Chemical Company, St. Louis, MO. The preparation of the remaining analogues is discussed in the following sections.

The 4-deoxy derivative was prepared from alcohol **23** (ref. [3]) in three steps. Conversion of **23** to the 4-xanthate (**24**, 61%) and then radical deoxygenation gave **25** in 82% yield. The final product **6** was obtained by hydrogenation (81%).

Treatment of dibenzoate **26** (ref. [3]), with sodium methoxide gave the 6-deoxy monosaccharide **7** in 96% yield.

The preparation of the 4-*O*-methyl and 6-*O*-methyl derivatives, respectively **12** and **13**, were obtained by hydrogenation of the corresponding dibenzyl ethers (ref. [4]). Thus **27** afforded **12** in 86% yield, and **13** was obtained from **28** by quantitative debenzylation.

The 3-amino derivative **16** was obtained in 73% yield from the fully deprotected azide **29** (ref. [4]) by hydrogenation.

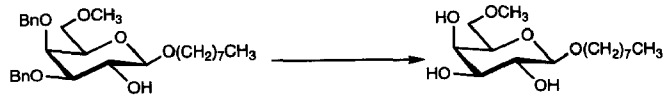
The preparation of the 6-amino derivative **18** began with alcohol **30** (ref. [4]). Treatment with hydrazine acetate to remove the phthalimido group, followed by hydrogenolysis of the benzyl ethers, gave the product in 70% overall yield.

The acetamido derivatives were easily prepared from the amino derivatives. Thus, acylation of **16**, **17** or **18** with acetic anhydride and sodium bicarbonate in methanol, provided the acetamido derivatives **19**, **20**, **21** in quantitative yield by TLC.

The known [33] bromide **31** was used as the starting material for the synthesis of the arabino analogue **22**. Treatment of **31** with octanol and silver triflate gave the protected octyl glycoside **32**, which was deprotected to **22** by Zemplén deacetylation.

26 7

27 12



28 13

29 16

30 18

Phth = phthalimido

Enzymatic testing.—Compared to the A- and B-transferases [3,4], this α-(1 → 2) fucosyltransferase (H-transferase) shows a less clear-cut acceptor specificity pattern. This could be due, in part, to the fact that although octyl β-D-galactopyranoside **4** is a substrate, it is a weak one with a K_m in the millimolar range. Much stronger recognition is achieved with the Type I–VI disaccharide structures discussed in the introduction. We anticipated that, as a result of the relatively weak substrate ability of **4**, the monosaccharide analogues synthesized would have K_i's too high to be useful inhibitors. Therefore we decided to determine only relative acceptor and inhibitor activities and not to carry out more detailed kinetic calculations. Nevertheless, we felt that this initial probing study would be best

Table 1
Relative acceptor activity of monosaccharides **4–22** towards the blood group H (Fuc)-transferase

Substrate	Activity [a] (%) H-transferase
Native (**4**)	100
3-Deoxy (**5**)	< 5
4-Deoxy (**6**)	< 5
6-Deoxy (**7**)	64
3-Fluoro (**8**)	< 5
4-Fluoro (**9**)	< 5
6-Fluoro (**10**)	93
3-*O*-Methyl (**11**)	< 5
4-*O*-Methyl (**12**)	123
6-*O*-Methyl (**13**)	316
3-Epimer (**14**)	< 5
4-Epimer (**15**)	< 5
3-Amino (**16**)	< 5
4-Amino (**17**)	9
6-Amino (**18**)	7
3-NHAc (**19**)	< 5
4-NHAc (**20**)	< 5
6-NHAc (**21**)	125
Ara (**22**)	27

[a] Compounds **4–22** were present at a concentration of 6.4 mM. Experiments were performed in duplicate with variation in replicates of 10% or less.

done at the monosaccharide level where the analogues could be easily prepared. Probing the H-transferase substrate specificity with disaccharide analogues would have been a much larger undertaking, The information presented here will be useful in that it can be used as a starting point to make analogues of the more complex Type I–VI structures. The K_m value for the Type I disaccharide with an α-(1 → 2) fucosyltransferase from porcine submaxillary gland, is 200 μM [8,34]. Therefore, inhibitors based on this structure should be more useful than the monosaccharide analogues.

The H-transferase used in these studies was a Protein A-chimera containing the putative Golgi lumenal portion of the cloned α-(1 → 2) fucosyltransferase. The experimental section contains details about the cloning procedure used. In order to assure that the enzyme was similar to the natural protein before screening these compounds with the enzyme, a K_m was determined for the parent substrate, octyl β-D-galactoside (**4**). The value obtained, 6.4 ± 1.2 mM, is roughly twice that of the K_m of phenyl β-D-galactoside with a purified H-transferase from human serum [23]. This close agreement in K_m suggests that the cloned enzyme is at least similar to the natural protein. The V_{max} for **4** was determined to be 3.03 ± 0.2 pmol/min under the standard conditions.

The results of the initial evaluation of monosaccharides **4–22** are shown in Table 1. The enzyme appears to recognize, to some degree, compounds with modifications at all three positions. Those analogues with modifications at C-3

show the least activity (0–2.5%), and in all cases for a series of analogues, the C-4 modified analogue is more active than the analogue modified at C-3. Additionally, with the exception of the amino series, the C-6 modified analogues are more active than the C-4 derivatives. Interestingly, three of the analogues, the 4-*O*-methyl **12**, 6-*O*-methyl **13**, and 6-acetamido **21** derivatives are actually better substrates than the parent substrate **4**. The 6-fluoro analogue **10** also retains most of the activity of **4**, and the 6-deoxy compound **7** is also a reasonable substrate. This latter observation confirms previously reported results [25]. The sterically less demanding arabino derivative **22** is a modest substrate. Only the 6-amino analogue **18** is a poor substrate.

These results suggest that in the active site the area occupied by OH-4 and OH-6 is relatively nonpolar, and that the replacement of the polar OH with a nonpolar methyl group enhances recognition by the enzyme. A hydrophobic region in this area would also explain the relatively low activity of **18** which shows an activity of only 6.7%. The positioning of a charge from the protonated amine in a hydrophobic area is likely to be disfavored resulting in the analogue being a poor substrate. Also, the observation that the 6-acetamido derivative is recognized suggests that the enzyme will tolerate substitutions of large steric bulk at C-6. It is important to note, however, that the presence of a much bulkier hydrophobic aglycon might have a significant effect on the acceptor specificity and/or the inhibition kinetics.

Those analogues not showing significant activity as substrates were tested for potential inhibitory activity. The results are presented in Table 2 where both percent inhibition and estimated K_i values are shown. The K_i values were estimated by assuming that the inhibition was competitive. However, the reader is

Table 2
Relative inhibitor activity and estimated K_i's of monosaccharides **4–22** towards the blood group H (Fuc)-transferase

Substrate	Inhibition [a] (%)	Estimated K_i [b] (mM)
3-Deoxy (**5**)	20	12.8
4-Deoxy (**6**)	8	36.8
3-Fluoro (**8**)	36	5.7
4-Fluoro (**9**)	0	NA
3-*O*-Methyl (**11**)	20	12.8
3-Epimer (**14**)	78	0.9
4-Epimer (**15**)	0	NA
3-Amino (**16**)	56	2.5
4-Amino (**17**)	0	NA
6-Amino (**18**)	0	NA
3-NHAc (**19**)	35	5.9
4-NHAc (**20**)	7	42.5

[a] Concentration of potential inhibitor was 6.4 mM with acceptor **4** at 6.4 mM. Experiments were performed in duplicate with variation in replicates of 10% or less.

[b] Estimated K_i assuming the inhibition is competitive and calculated from the equation $i = [I]/\{[I] + K_i(1 + [S]/K_m)\}$, where i is the fractional inhibition, [I] the inhibitor concentration, and [S] the substrate concentration [40]. NA = not applicable.

cautioned that we have no evidence that this is true for all analogues, and some (or all) may not follow this mode of inhibition. Only two of the Gal OH-4 modified derivatives (**6** and **20**) have any inhibitory activity. The inhibition for these compounds is very small and the estimated K_i's are an order of magnitude larger than the K_m of **4**. All of the Gal OH-3 modified derivatives possess inhibitory activity. The most potent inhibitor is the 3-epimeric analogue **14** with an estimated K_i of 900 μM. Another potent inhibitor is the 3-amino derivative **16** which has an estimated K_i of 2.5 mM.

In conclusion it appears that, in general, Gal OH-3 and OH-4 are required for the compound to have a reasonable activity as a substrate with this fucosyltransferase. However, the 4-*O*-methyl compound is an exception to this generalization, which could possibly be explained by the favorable interaction of this hydrophobic group with a lipophilic region in the active site. Additionally, the 6-OH group can be replaced with little adverse effect provided that the substitution does not introduce a charge into the C-6 position.

All analogues modified at Gal OH-3 are inhibitors of the enzyme. The ability of the enzyme to recognize a wide range of substrates at this position indicates that even this close to the site of reactivity the enzyme is tolerant of functional group modifications. Detailed kinetics carried out with similar analogues of the more potent Type I–VI disaccharides should establish the mechanism of inhibition.

3. Experimental

General Methods.—Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2°C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with sulfuric acid. All commercial reagents were used as supplied, and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (E. Merck, 40–60 μM, Darmstadt). Millex-GV (0.22 μM) filter units were from Millipore (Missisuaga, ON), C_{18} Sep-Pak sample preparation cartridges were from Waters Associates (Missisuaga, ON) and Ecolite scintillation cocktail was from ICN Radiochemicals (St. Laurent, PQ). GDP-[1-^{3}H]-Fuc (specific activity 6.7 Ci/mmol) was from New England Nuclear (Wilmington, DE). GDP-Fuc was prepared as previously described [34]. ^{1}H NMR spectra were recorded at 360 MHz (Bruker WM 360) or 300 MHz (Bruker AM 300) with either internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or DOH (δ 4.80, D_2O). ^{13}C NMR spectra were recorded either at 75.5 MHz (Bruker AM 300) or 125.7 MHz (Varian Unity 500) with internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or external 1,4-dioxane (δ 67.4, D_2O). ^{1}H data are reported as though they were first order. All ^{13}C shift assignments are tentative and were assigned based on comparison with published spectra [37,38]. Unless otherwise stated, all reactions were carried out at room temperature, and in the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were dried (sodium sulfate) prior to concentration under vacuum at < 40°C (bath

temperature). Microanalyses were carried out by the analytical services at this department, and all samples submitted for elemental analyses were dried overnight under vacuum with phosphorous pentoxide at 56°C (refluxing Me_2CO). Fast-atom-bombardment mass spectra were recorded on samples suspended in Cleland's matrix using a Kratos AEIMS9 instrument with Xe as the bombarding gas.

Octyl 2,3,6-tri-O-benzyl-4-O-[(methylthio)thiocarbonyl]-β-D-glucopyranoside (**24**). —To a solution of **23** (ref. [3], 305 mg, 0.54 mmol) in dry THF (5 mL) was added NaH (50 mg, 80% in oil, 1.66 mmol) and imidazole (10 mg). After stirring for one h CS_2 (330 μL, 5.40 mmol) was added and stirring continued for another h. At this point CH_3I (101 μL, 1.62 mmol) was added, and stirring was continued overnight. Evaporation of the solvent gave a yellow liquid that was chromatographed (9:1 hexane–EtOAc) to give **24** (216 mg, 61%) as an oil: $[\alpha]_D$ +16.5° (*c* 0.5 $CHCl_3$), R_f 0.65 (4:1 hexane–EtOAc). ^{1}H NMR ($CDCl_3$): δ 7.20–7.40 (m, 15 H, Ph), 5.97 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.92, 4.74, 4.70, 4.65, 4.57, 4.50 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2), 4.46 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.96 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH$_2$), 3.75 (t, 1 H, $J_{2,3} = J_{4,5}$ 9.5 Hz, H-3), 3.52–3.74 (m, 4 H, H-6a, H-6b, H-5, OCH_2CH$_2$), 3.51 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.51 (s, 3 H, SCH_3), 1.57–1.72 (m, 2 H, OCH$_2$CH_2), 1.20–1.45 (10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR ($CDCl_3$): δ 215.60 (C = S), 138.42, 138.11 (Ph quat.), 128.38, 128.34, 128.29, 128.15, 128.13, 127.74, 127.66, 127.61 (Ph methine), 103.52 (C-1), 82.02 (C-3), 81.93 (C-2), 79.37 (C-5), 75.33, 75.07 (PhCH_2), 73.77 (C-4), 73.74 (PhCH_2), 70.37 (OCH_2CH$_2$), 69.80 (C-6), 31.87, 29.81, 29.45, 29.30, 26.22, 22.70 (octyl CH_2), 19.37 (SCH_3), and 14.13 (octyl CH_3). Anal. Calcd for $C_{37}H_{48}O_6S_2$ (652.91): C, 68.07; H, 7.41; S, 9.82. Found: C 68.19; H, 7.59; S, 9.94.

Octyl 2,3,6-tri-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside (**25**).—Compound **24** (144 mg, 0.22 mmol) was dissolved in dry toluene (5 mL), and then tributylstannane (300 μL, 1.1 mmol) and AIBN (30 mg, 0.18 mmol) were added. The solution was heated under reflux for 2 h. Evaporation of the solvent, followed by chromatography (6:1 hexane–EtOAc) gave **25** (98 mg, 82%) as a colorless oil, $[\alpha]_D$ −3.7° (*c* 0.6, $CHCl_3$), R_f 0.60 (4:1 hexane–EtOAc). ^{1}H NMR ($CDCl_3$): δ 7.30–7.50 (m, 15 H, Ph), 4.93, 4.75 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2), 4.67 (s, 2 H, PhCH_2), 4.59, 4.54 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2), 4.33 (d, 1 H, J_{12} 7.5 Hz, H-1), 3.93 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH$_2$), 3.45–3.64 (m, 5 H, H-3, H-5, H-6a, H-6b, OCH_2CH$_2$), 3.31 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 2.11 (ddd, 1 H, $J_{3,4e}$ 5.5, $J_{4e,5}$ 1.5, $J_{4e,4a}$ 13 Hz, H-4e), 1.59–1.70 (m, 2 H, OCH$_2$CH_2), 1.20–1.50 (11 H, octyl CH_2, H-4a), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR ($CDCl_3$): δ 138.88, 138.72, 138.21 (Ph quat.), 128.43, 128.36, 128.29, 128.12, 127.72, 127.67, 127.55 (Ph methine), 103.90 (C-1), 82.96 (C-3), 78.31 (C-2), 74.98, 73.59, 72.62 (PhCH_2), 72.32 (C-6), 71.01 (C-5), 70.16 (OCH_2CH$_2$), 34.06 (C-4), 31.87, 29.88, 29.48, 29.29, 26.23, 22.69 (octyl CH_2), and 14.13 (octyl CH_3). Anal. Calcd for $C_{35}H_{46}O_5$ (546.75): C, 76.89; H, 8.48. Found: C, 76.92; H, 8.61.

Octyl 4-deoxy-β-D-xylo-hexopyranoside (**6**).—Compound **25** (201 mg, 0.37 mmol) was stirred in CH_3OH (10 mL) with 5% Pd–C (100 mg) under H_2 overnight. The reaction was filtered, the solvent was evaporated, and the residue was chromatographed (19:1 CH_2Cl_2–CH_3OH) to give the product **6** (83 mg, 81%) as a

white solid, $[\alpha]_D$ −36.9° (c 1.1, CH_3OH), R_f 0.10 (19:1 CH_2Cl_2–CH_3OH). ^1H NMR (CD_3OD): δ 4.17 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.87 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.45–3.62 (m, 5 H, H-3, H-5, H-6a, H-6b, OCH_2CH_2), 3.06 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.5 Hz, H-2), 1.91 (ddd, 1 H, $J_{3,4e}$ 5, $J_{4e,5}$ 1.5, $J_{4e,4a}$ 12–5 Hz, H-4e), 1.54–1.67 (m, 2 H, OCH_2CH_2), 1.20–1.45 (m, 11 H, octyl CH_2, H-4a), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.68 (C-1), 76.93 (C-5), 73.83 (C-3), 72.24 (C-2), 70.84 (OCH_2CH_2), 65.57 (C-6), 36.48 (C-4), 32.99, 30.82, 30.56, 30.40, 27.10, 23.69 (octyl CH_2), and 14.42 (octyl CH_3). Anal. Calcd for $C_{14}H_{28}O_5$ (276.38): C, 60.84; H, 10.21. Found: C, 60.91; H, 10.08.

Octyl 6-deoxy-β-D-galactopyranoside (**7**).—Octyl 3,4-di-*O*-benzoyl-6-deoxy-β-D-galactopyranoside **26** (ref. [3], 103 mg, 0.21 mmol) was dissolved in CH_3OH (10 mL), and $NaOCH_3$ (10 mg) was added. After stirring overnight, the solution was neutralized by the addition of CH_3CO_2H. The solvent was evaporated, and the residue was chromatographed (19:1 CH_2Cl_2–CH_3OH) to give the product **7** (55 mg, 95%) as a white solid: $[\alpha]_D$ −20.2° (*c* 0.4 $CHCl_3$), R_f 0.15 (19:1 CH_2Cl_2–CH_3OH). ^1H NMR (CD_3OD): δ 4.18 (m, 1 H, H-1), 3.85 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.58–3.70 (m, 2 H, H-4, H-5), 3.52 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.40–3.50 (m, 2 H, H-2, H-3), 1.54–1.70 (m, 2 H, OCH_2CH_2), 1.28–1.45 (m, 10 H, octyl CH_2), 1.26 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (CD_3OD): δ 104.77 (C-1), 75.17 (C-5), 73.02 (C-3), 72.29 (C-2), 71.80 (C-4), 70.75 (OCH_2CH_2), 32.97, 30.82, 30.52, 30.37, 27.08, 23.67 (octyl CH_2), 16.74 (C-6), and 14.40 (octyl CH_3). Anal. Calcd for $C_{14}H_{28}O_5$ (276.38): C, 60.84; H, 10.21. Found: C, 60.95; H, 10.44.

Octyl 4-O-methyl-β-D-galactopyranoside (**12**).—Octyl 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-galactopyranoside **27** (ref. [4], 48 mg, 0.098 mmol) was dissolved in CH_3OH (5 mL), 5% Pd–C (20 mg) added, and the reaction was allowed to stir overnight under a flow of H_2. The catalyst was filtered away, the solvent was evaporated, and the residue was redissolved in water and then passed through a C_{18} Sep-Pak, washing first with water and then with CH_3OH. The CH_3OH eluant was evaporated, redissolved in water, filtered and lyophilized to give **12** (26 mg, 86%) as a white solid. ^1H NMR (CD_3OD): δ 4.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.85 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.68 (d, 2 H, H-6a, H-6b), 3.55 (s, 3 H, OCH_3), 3.41–3.54 (m, 5 H, H-2, H-3, H-4, H-5, OCH_2CH_2), 1.55–1.65 (m, 2 H, OCH_2CH_2), 1.25–1.42 (10 H, octyl CH_2), 0.90 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR (CD_3OD): δ 104.97 (C-1), 80.20 (C-4), 76.51 (C-5), 75.60 (C-2), 72.85 (C-3), 70.90 (OCH_2CH_2), 62.01 (OCH_3), 61.70 (C-6), 32.98, 30.80, 30.54, 30.38, 27.07, 23.69 (octyl CH_2), and 14.40 (octyl CH_3). FABMS: *m/z* 345 $[M+K]^+$, 329 $[M+Na]^+$, and 307 $[M+H]^+$ ($C_{15}H_{30}O_6$ requires *m/z* 306).

Octyl 6-O-methyl-β-D-galactopyranoside (**13**).—Octyl 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-galactopyranoside [4] (**28**, 37 mg, 0.77 mmol) was dissolved in CH_3OH (10 mL) and 5% Pd–C (20 mg) added. The reaction was allowed to stir overnight under a flow of H_2. After filtration of the catalyst, the product was purified as described for **12** to give **13** (23 mg, quantitative) as a white solid: R_f 0.22 (19:1 CH_2Cl_2–CH_3OH). ^1H NMR (CD_3OD): δ 4.12 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.78 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 4.71 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1 Hz, H-4),

3.49–3.58 (m, 3 H, H-5, H-6a, H-6b), 3.35–3.48 (m, 3 H, H-2, H-3, OCH_2CH_2), 3.31 (s, 3 H, OCH_3), 1.50–1.59 (m, 2 H, OCH_2CH_2), 1.16–1.35 (10 H, octyl CH_2), and 0.83 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR (CD_3OD): δ 104.96 (C-1), 74.94 (C-5), 74.85 (C-3), 73.02 (C-6), 72.52 (C-2), 70.91 (OCH_2CH_2), 70.52 (C-4), 59.44 (OCH_3), 33.00, 30.84, 30.54, 30.40, 27.10, 23.70 (octyl CH_2), and 14.40 (octyl CH_3). FABMS: m/z 345 $[M + K]^+$, 329 $[M + Na]^+$, and 307 $[M + H]^+$ ($C_{15}H_{30}O_6$ requires m/z 306).

Octyl 3-amino-3-deoxy-β-D-galactopyranoside (**16**).—Azido-galactoside **29** (ref. [4], 60 mg, 0.15 mmol) was dissolved in EtOH (10 mL) and stirred overnight under a flow of H_2 in the presence of 5% Pd–C (35 mg) and HCl (0.30 mmol). Filtration of the catalyst, followed by chromatography (10:4:1 CH_2Cl_2–CH_3OH–NH_4OH), gave the product **16** (31 mg, 72%) as a white solid: R_f 0.55 (10:4:1 CH_2Cl_2–CH_3OH–NH_4OH). ^1H NMR (D_2O): δ 4.47 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.12 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 3.95 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.74–3.84 (m, 3 H, H-5, H-6a, H-6b), 3.71 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.66 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10.5 Hz, H-2), 3.47 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3 Hz, H-4), 1.55–1.70 (m 2 H, OCH_2CH_2), 1.20–1.40 (10 H, octyl CH_2), and 0.88 (t, 3H, J_{vic} 7 Hz, octyl CH_3). ^{13}C NMR (D_2O): δ 104.06 (C-1), 77.38 (C-5), 72.36 (C-4), 71.39 (OCH_2CH_2), 69.17 (C-4), 61.82 (C-6), 56.20 (C-3), 31.92, 29.58, 29.28, 29.20, 25.87, 22.84 (octyl CH_2), and 14.23 (octyl CH_3). FABMS: m/z 314 $[M + Na]^+$ and 292 $[M + H]^+$ ($C_{14}H_{29}NO_5$ requires m/z 291).

Octyl 6-amino-6-deoxy-β-D-galactopyranoside (**18**).—Monosaccharide **30** (ref. [4], 58 mg, 0.096 mmol) was dissolved in CH_3OH (15 mL), and hydrazine acetate (258 mg, 2.88 mmol) was added. The solution was refluxed for 3 h, then another portion of hydrazine acetate was added (258 mg, 2.88 mmol), and refluxing was continued for a total of 24 h. The solution was cooled, diluted with CH_2Cl_2 and washed with water and brine. The product was not further purified but was immediately redissolved in CH_3OH, and 5% Pd–C (50 mg) and HCl (0.192 mmol) were added. The solution was stirred under a flow of H_2 overnight. After filtration of the catalyst, the product was purified as described for **12** to give **18** (19 mg, 70%) as a white solid: ^1H NMR (D_2O): δ 4.33 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.83–3.94 (m, 2 H, H-4, OCH_2CH_2), 3.41–3.67 (m, 4 H, H-2, H-3, H-5, OCH_2CH_2), 2.92 (dd, 1 H $J_{5,6a}$ 7.5, $J_{6a,6b}$ 13 Hz, H-6a), 2.84 (dd, 1 H, $J_{5,6b}$ 5, $J_{6a,6b}$ 13 Hz, H-4), 1.57–1.71 (m, 2 H, OCH_2CH_2) 1.21–1.41 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).^{13}C NMR (D_2O): δ 103.46 (C-1), 73.30 (C-5), 71.41 (C-3), 71.17 (C-2), 71.38 (OCH_2CH_2), 69.99 (C-4), 40.85 (C-6), 31.94, 29.55, 29.37, 29.25, 25.93, 22.84 (octyl CH_2), and 14.22 (octyl CH_3). FABMS: m/z 314 $[M + Na]^+$ and 292 $[M + H]^+$ ($C_{14}H_{29}NO_5$ requires m/z 291).

Octyl 3-acetamido-3-deoxy-β-D-galactopyranoside (**19**).—Octyl 3-amino-3-deoxy-β-D-galactopyranoside (**16**, 1 mg, 2.28 μmol) was dissolved in CH_3OH (500 μL). Acetic anhydride (1 μL) and $NaHCO_3$ (2 mg) were added, and the reaction mixture was stirred for 2 h, at which point TLC indicated quantitative conversion of the amino derivative to the acetamido derivative. The solvent was then evaporated, and the product was purified by redissolution in water and then passing the solution through a Waters C_{18} Sep-Pak cartridge. The cartridge was washed with

water, and then the product was eluted with CH_3OH. The CH_3OH eluant was evaporated, the residue redissolved in water, filtered through a 0.22 μM filter and lyophilized to give 19, as a white solid. ^{1}H NMR (CD_3OD): δ 4.19 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.82 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.71–3.80 (m, 2 H H-3, H-4), 3.64 (dd, 1 H, $J_{5,6a}$ 7, $J_{6a,6b}$ 12 Hz, H-6a), 3.60 (dd, 1 H, $J_{5,6b}$ 5, $J_{6a,6b}$ 12 Hz, H-6b), 3.39–3.51 (m, 3 H, H-2, H-5, OCH_2CH_2), 2.03 (s, 3 H, acetate CH_3), 1.58–1.70 (m, 2 H, OCH_2CH_2), 1.20–1.46 (m, 10 H, octyl CH_2), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3);

Octyl 4-acetamido-4-deoxy-β-D-galactopyranoside (**20**).—Octyl 4-amino-4-deoxy-β-D-galactopyranoside [4] (**17**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **19** to provide the product **20** as a white solid. ^{1}H NMR (D_2O): δ 4.20 (m, 2 H, H-1, H-4), 4.05 (dd, 1 H $J_{2,3}$ 10 Hz, $J_{3,4}$ 4.5 Hz, H-3) 3.63–3.99 (m, 3 H, OCH_2CH_2, H-3, H-5), 3.45 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.0 Hz, H-2), 3.46 (dd, 1 H, $J_{5,6a}$ 7, $J_{6a,6b}$ 12 Hz, H-6a), 3.34 (dd, 1 H, $J_{5,6b}$ 5, $J_{6a,6b}$ 12 Hz, H-6b), 3.20 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 2.07 (s, 3 H, acetate CH_3), 1.55–1.70 (m, 2 H, OCH_2CH_2), 1.20–1.40 (m, 10 H, octyl CH_2), 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

Octyl 6-acetamido-6-deoxy-β-D-galactopyranoside (**21**).—Octyl 6-amino-6-deoxy-β-D-galactopyranoside (**18**, 1 mg, 2.28 μmol) was acetylated in quantitative yield (by TLC) as described above for the preparation of compound **19** to provide the product **21** as a white solid. ^{1}H NMR (D_2O): δ 4.17 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.99 (d, 1 H, $J_{3,4}$ 5 Hz, H-4), 3.86–3.92 (m, 2 H, OCH_2CH_2, H-3), 3.58–3.78 (m, 4 H, H-5, H-6a, H-6b, OCH_2CH_2), 3.21 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10 Hz, H-2), 1.99 (s, 3 H, acetate CH_3), 1.56–1.70 (m, 2 H, OCH_2CH_2), 1.20–1.40 (m, 10 H, octyl CH_2), and 0.86 (t, 3 H, J_{vic} 7 Hz, octyl CH_3).

Octyl 2,3,4-tri-O-acetyl-α-L-arabinopyranoside (**32**).—Silver triflate (1.140 g, 4.43 mmol, dried in vacuo over P_2O_5 for 1 h), was stirred with collidine (400 μL, 2.96 mmol) and octanol (1.4 mL, 8.87 mmol) in CH_2Cl_2 (10 mL) containing crushed 3Å molecular sieves (1.0 g) under N_2 at −30°C for 20 min. To this solution was added dropwise 2,3,4-tri-*O*-acetyl β-L-arabinopyranosyl bromide [33] (**31**, 1.0 g, 2.96 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred under N_2 and warmed to room temperature. After stirring for 90 min, the reaction was quenched with collidine (400 μL), and the mixture was filtered and evaporated. The residue was then taken up in CH_2Cl_2 and washed with water, then 5% HCl, $NaHCO_3$ and finally brine. After solvent evaporation, the resulting clear oil was chromatographed (4:1 hexane–EtOAc) to give **32** (791 mg, 69%) as an oil: $[\alpha]_D$ +4.2° (*c* 0.9, $CHCl_3$), R_f 0.42 (4:1 hexane–EtOAc). ^{1}H NMR ($CDCl_3$): δ 5.26 (br. s, 1 H, H-4), 5.16 (dd, 1 H, $J_{2,3}$ 9.0, $J_{1,2}$ 7.0 Hz, H-2), 5.04 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 3.5 Hz, H-3), 4.41 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.02 (dd, 1 H, $J_{5a,5b}$ 12.5, $J_{4,5a}$ 3.5 Hz, H-5a), 3.85 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 3.63 (dd, 1 H, $J_{5a,5b}$ 12.5, $J_{4,5a}$ 1.5 Hz, H-5a), 3.45 (dt, 1 H, J_{gem} 10, J_{vic} 7 Hz, OCH_2CH_2), 2.13, 2.06, 2.03 (s, 3 H, acetate CH_3), 1.50–1.64 (m, 2 H, OCH_2CH_2), 1.19–1.39 (m, 10 H, octyl CH_2), and 0.88 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR ($CDCl_3$): δ 170.31, 170.14, 169.36 (C = O), 100.95 (C-1), 70.22 (C-4), 69.77 (OCH_2CH_2), 69.28 (C-3), 67.73 (C-2), 63.05 (C-5), 31.79, 29.44, 29.28, 29.23, 25.88, 22.62 (octyl CH_2), 20.90, 20.74, 20.66

(acetate CH_3), and 14.06 (octyl CH_3). Anal. Calcd for $C_{19}H_{32}O_8$ (388.46): C, 58.75; H, 8.30. Found: C, 58.90; H, 8.50.

Octyl α-L-arabinopyranoside (**22**).—Arabinoside **32** (791 mg, 2.04 mmol) was dissolved in CH_3OH (50 mL), and $NaOCH_3$ (20 mg) was added. After stirring for 1 h, the solution was neutralized by the addition of acetic acid. The solvent was evaporated, and the residue was chromatographed (19:1 CH_2Cl_2–CH_3OH) to give **22** (480 mg, 90%) as a white solid: $[\alpha]_D$ −6.0° (*c* 1.0, CH_3OH). ^1H NMR (CD_3OD): δ 4.09 (m, 1 H, H-1), 3.65–3.80 (m, 3 H, H-5a, H-4, OCH_2CH_2), 3.37–3.48 (m, 4 H, H-5b, H-3, H-2, OCH_2CH_2), 1.45–1.58 (m, 2 H, OCH_2CH_2), 1.12–1.34 (m, 10 H, octyl CH_2), and 0.80 (t, 3 H, J_{vic} 7 Hz, octyl CH_3); ^{13}C NMR (CD_3OD): δ 104.81 (C-1), 74.30 (C-4), 72.42 (C-3), 70.61 (OCH_2CH_2), 69.61 (C-2), 66.75 (C-5), 33.00, 30.80, 30.54, 30.40, 27.15, 23.71 (octyl CH_2), and 14.43 (octyl CH_3). Anal. Calcd for $C_{13}H_{26}O_5$ (262.37): C, 59.52; H, 9.99. Found: C, 59.66; H, 10.01.

Cloning of the H-transferase.—The H-fucosyltransferase used in this study was cloned in a manner similar to that of Larsen et al. [26] with the plasmid used by Wei et al. [39] The enzyme used here differed from the one cloned by Larsen et al. in that this enzyme was a fusion protein with Protein A attached through the second histidine following the transmembrane domain [26]. The beads containing the cloned protein immobilized to IgG were stored at 4°C in a buffer of 10 mM Tris containing 150 mM sodium chloride and 25% glycerol, pH 7.4.

Elution of enzyme from beads.—The immobilized enzyme beads (20 μL) were initially washed by mixing them in a 600 μL centrifuge tube with 100 μL of a mM Tris buffer containing 150 mM sodium chloride, pH 8.0. This suspension was spun down in a microcentrifuge, and the supernatant was aspirated from the tube. The cleavage was carried out by adding, to the resin beads, 50 μL of a buffer containing 0.1 M sodium citrate and 0.1 M citric acid, pH 4.4. The beads were quickly mixed in the buffer three times with a micropipette, the solution spun down and the supernatant was directly added to 20 μL of a quench buffer containing M Tris, pH 8.2. This cleavage–quenching procedure was repeated five times. Finally, to each of the quenched cleavage solutions was added 70 μL of a storage buffer containing 5 mM $MnCl_2$, 25 mM sodium cacodylate, and 25% glycerol, pH 6.5. The individual fractions were assayed for activity, using the assay described below. Typically between three and five of the cleavage fractions contained active enzyme.

Assay conditions.—All assays were carried out in a total volume of 40 μL with 20 mM HEPES buffer, pH 7.0, containing 20 mM $MnCl_2$, 0.2% BSA, 50 μM GDP-Fuc, 0.05 μCi GDP-[1-^{3}H]-Fuc and 3 μL of the enzyme in the aforementioned storage buffer (1 mU/mL solution). Under these conditions, the rate of product formation with the native monosaccharide **4** was shown to be linear up to a time of 90 min. Incubations were carried out at 37°C for 60 min and then quenched by the addition of water. The reaction mixtures were transferred to pre-equilibrated C_{18} Sep-Pak cartridges, and the unreacted radiolabelled donor was removed by washing with water until background counts were obtained [28]. The radiolabelled product was eluted with methanol (1 × 3 mL) and quantitated by

liquid scintillation. The K_m of **4** was determined to be 6.4 mM under these conditions. Assays to test activity as an acceptor were carried out at concentrations of 6.4 mM. In a typical experiment, 6219 DPM were obtained for **4** with a background of 55 DPM. The results are presented in Table 1. To test for inhibitory activity, the potential inhibitor (6.4 mM) was added to 4 at 6.4 mM. The results are recorded in Table 2. The K_m determination for compound **4**, was carried out at the following concentrations: 17.5, 15.0, 10.0, 7.5, 5.0, 2.5, 1.25 mM. Rate data were fit to the Michaelis–Menten equation using unweighted nonlinear regression with the SigmaPlot 4.0 program to estimate the kinetic parameters.

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