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Carbohydrate Research 286 (1996) 87–105

CARBOHYDRATE RESEARCH

Recognition of β-D-Gal *p*-(1 → 3)-β-D-Glc *p*NAc-OR acceptor analogues by the Lewis α-(1 → 3/4)-fucosyltransferase from human milk

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Received 29 September 1995; accepted 25 January 1996

Abstract

The Lewis α-(1 → 3/4)-fucosyltransferase (E.C. 2.4.1.65) transfers L-fucose from GDP-fucose to OH-4 of the Glc *p*NAc residue in the disaccharide β-D-Gal *p*-(1 → 3)-β-D-Glc *p*NAc-OR [R = $(CH_2)_8COOMe$] (**1**) to give the Lewis-A blood group determinant β-D-Gal *p*-(1 → 3)-[α-L-Fuc *p*-(1 → 4)]-β-D-Glc *p*NAc-OR. Five deoxy analogues of **1**, as well as its *N*-propionyl derivative, were chemically synthesized and kinetically evaluated as both substrates and inhibitors for the enzyme from human milk. The unmodified acceptor **1** had $K_m = 640$ μM with V_{max} set arbitrarily to 100. The 6-deoxy ($K_m = 400$ μM, $V_{max} = 90$) and *N*-propionyl compounds ($K_m = 330$ μM, $V_{max} = 170$) remained excellent substrates while the 4-deoxy compound was a very weak competitive inhibitor with $K_i = 9$ mM. Deoxygenation of OH-2′ and OH-4′ (of the Gal residue) in **1** had little effect on the activity. The OH-6 group of the Gal residue proved to be critical for recognition by the enzyme since substitution of this group with hydrogen led to an inactive compound. © 1996 Elsevier Science Ltd.

Keywords: Fucosyltransferase; Oligosaccharide analogue; Deoxy; Substrate recognition

1. Introduction

Mammalian oligosaccharides usually occur attached to glycoproteins and glycolipids where they can act as recognition markers in biological processes [1]. The Lewis

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PII S0008-6215(96)00047-X

α-(1 → 3/4)-fucosyltransferase [2,3] is one of the enzymes involved in the terminal glycosylation of such oligosaccharides. This enzyme, most readily available from human milk [4], transfers fucose from guanosine 5′-diphosphofucose (GDP-Fuc) to both type-1 [β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc] (Fig. 1) and type-2 [β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc) disaccharides. In addition to their role in defining blood group antigens, the fucosylated structures synthesized by this (and related) enzymes are characteristic of the aberrant glycosylation that accompanies cancers [5]. This enzyme, and several related fucosyltransferases, have been cloned [3].

Efforts to develop specific glycosyltransferase inhibitors [6–13] as tools for studying the effects of aberrant glycosylation require a detailed understanding of substrate recognition. In this paper, we report the chemical synthesis of analogues of the type-1 acceptor **1** and their enzymatic evaluation as substrates or inhibitors for the α-(1 → 3/4)-fucosyltransferase. It was known [2,3] that OH-3′ of **1** could be sialylated without adversely affecting its activity as a substrate for this enzyme, so modification at this position was not investigated. The other five hydroxyl groups were replaced by hydrogen, one at a time, to produce the five analogues **17**, **18**, **30**, **31**, and **36**. The use of deoxy analogues for probing the recognition of carbohydrates by protein combining sites is widely accepted as a method for establishing which of the OH groups are critically involved in hydrogen-bonding networks [14–18]. In order to determine if a change to the acetamido region of the molecule affected the binding, the *N*-propionyl derivative **23** was also prepared. The disaccharide derivatives were prepared as 8-methoxycarbonyloctyl glycosides because this hydrophobic aglycon simplifies the enzymatic assays by allowing the use of reversed-phase (C_{18}) cartridges [19] to separate and quantitate the

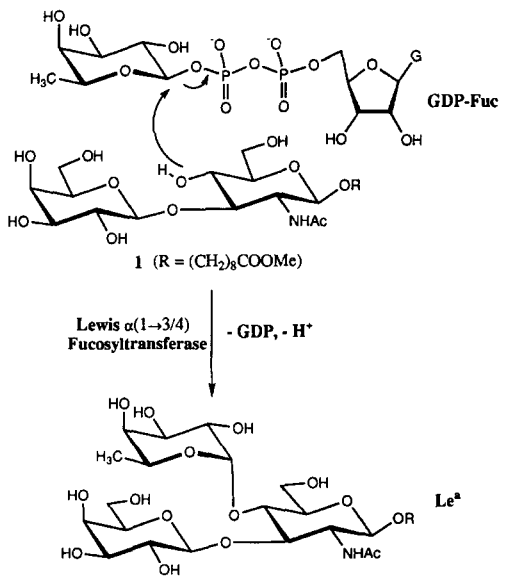
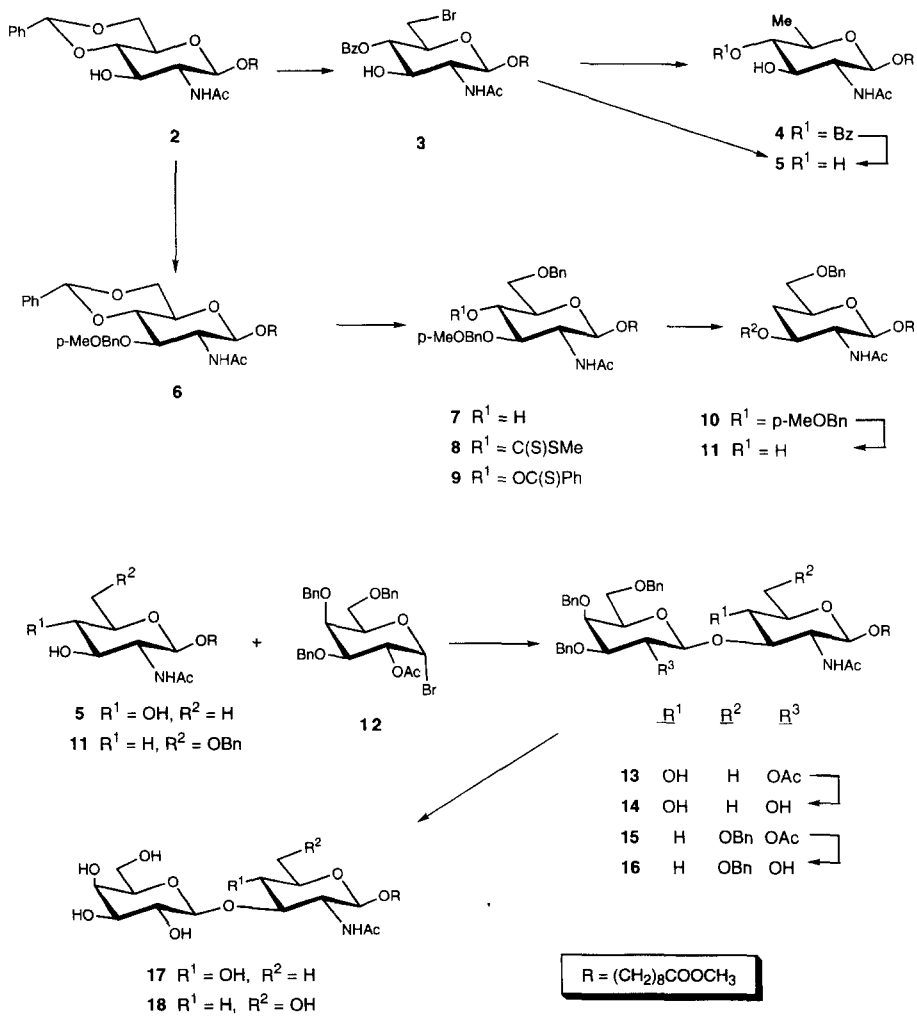


Fig. 1. Net inversion of configuration during transfer of L-fucose from GDP-fucose to the synthetic acceptor **1** catalyzed by the Lewis fucosyltransferase.

products. The long-term goal of this work is the design of inhibitors for this fucosyltransferase.

2. Results and discussion

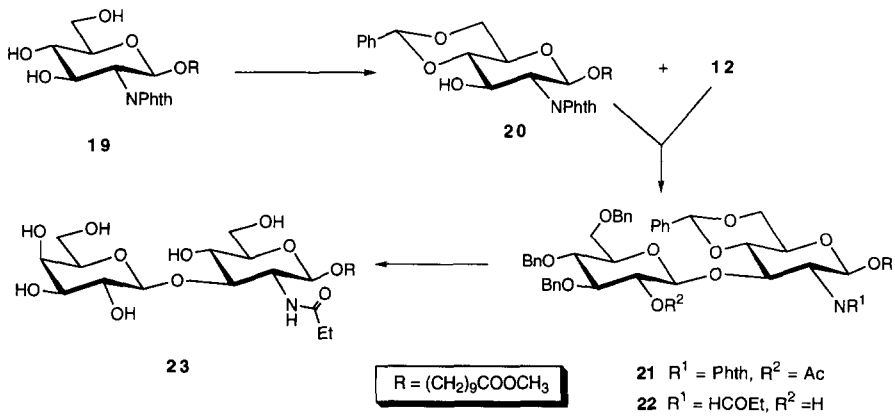
Chemical synthesis.—For the preparation of the 6-deoxy and 4-deoxy analogues **17** and **18**, 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**2**) [20,21] was treated with *N*-bromosuccinimide in the presence of barium carbonate [22] to give the 6-bromo derivative **3** (40%). Reduction of **3** with tributyltin hydride gave **4** (81%), which was debenzoylated to give diol **5** (95%).



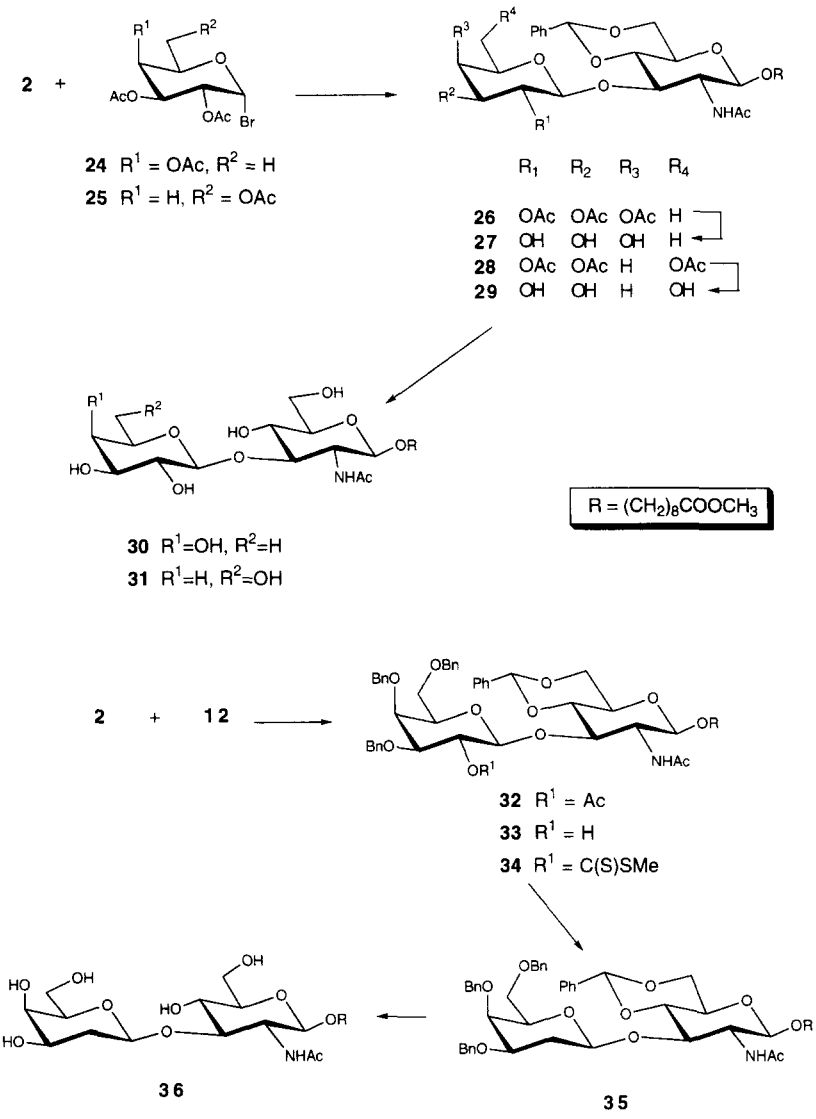
Compound **3** could also be converted directly to **5** (73%) by hydrogenolysis. For the preparation of the 4-deoxy monosaccharide **11**, *p*-methoxybenzylation of **2** gave **6** (68%). Regioselective benzylidene ring opening with sodium cyanoborohydride [23] provided the OH-4 derivative **7** (83%), which was then converted to either xanthate **8** (88%) or thionocarbonate **9** (73%). Reduction of either compound using tributyltin hydride then produced the 4-deoxy derivative **10** (90%). The *p*-methoxybenzyl group in **10** was then removed using ammonium cerium(IV) nitrate to afford **11** (95%) with OH-3 free for subsequent glycosylation.

The coupling reactions of alcohol **5** and **11** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl bromide **12** [24], promoted by mercuric cyanide, gave yields of 91% and 96%, respectively. The disaccharides **13** and **15** thus obtained were *O*-deacetylated with NaOMe, and finally the benzyl groups were removed by hydrogenolysis to provide the target compounds **17** and the previously described **18** [25].

The synthesis of the *N*-propionyl derivative of **1** began with 9-methoxycarbonylnonyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**), which was available from previous work (prepared as for the preparation of 8-methoxycarbonyloctyl 2-deoxy-2-phthalimido-β-D-glucopyranoside [20,21]). The OH-4 and OH-6 groups were selectively protected by benzylidenation (84%), and the product **20** was then coupled with bromide **12** to give the disaccharide **21** (69%). Treatment of **21** with ethylenediamine in butanol [26], followed by *N*-propionylation, gave **22**, which was isolated in 27% yield. Loss of the methyl ester during the phthalimido group removal was the major side reaction. Hydrogenolysis of **22** then provided **23** (93%).



To prepare the 6′- and 4′-deoxy derivatives **30** and **31**, the tri-*O*-acetyl-deoxyglycopyranosyl bromides **24** and **25** were first prepared by reported procedures [27,28]. The glycosylations of alcohol **2** were promoted by silver trifluoromethanesulfonate furnishing disaccharides **26** (65%) and **28** (63%). Zemplén deacetylation, followed by hydrogenolysis, afforded **30** and **31**.



For the synthesis of the 2′-deoxy analogue **36**, disaccharide **32** was prepared by condensation of alcohol **2** and bromide **12** (83%). *O*-Deacetylation, followed by reaction with carbon disulfide and methyl iodide in the presence of sodium hydride, gave **34** (33%). Reduction of **34** provided the 2′-deoxy derivative **35**, which was deprotected to afford the known compound **36** [25]. Key ^{1}H and ^{13}C NMR data for the deprotected compounds **17**, **18**, **23**, **30**, **31**, and **36** are presented in Tables 1 and 2.

Kinetic evaluation of acceptor analogues.—The "native" disaccharide **1** and its analogues **17**, **18**, **23**, **30**, **31**, and **36** were kinetically evaluated as both substrates and inhibitors of α-(1 → 3/4)-fucosyltransferase, partially purified from human milk, using

Table 1
Selected ^{1}H chemical shifts δ (ppm) and coupling constants J (Hz) for the deprotected compounds

Compound	**17**	**18**	**23**	**30**	**31**	**36**
H-1 ($J_{1,2}$)	4.51(8.5)	4.47(8.5)	4.55(8.0)	4.54(8.0)	4.55(8.0)	4.57(8.0)
H-1′ ($J_{1',2'}$)	4.40(7.8)	4.46(7.8)	4.41(7.8)	4.35(8.0)	4.40(7.8)	4.70(8.0)
OCH_3	3.67	3.70	3.69	3.73	3.69	3.71
$COCH_2$	2.37	2.40	2.38	2.35	2.40	2.40
$COCH_3$	2.00	2.04	2.03	2.03	2.07	
Other H	1.31(d, 6.0 H-6′)	2.24(dd, 12.5, 4.0, H-4eq) 1.53(q, 12.5, H-4ax)	2.29(q, 7.5 $COCH_2$) 1.15(t, 7.5 $COCH_2CH_3$)	1.24(d, 6.5, H-6′)	1.97(dd, 12.5, 5.0, H-4′eq) 1.46(q, 12.5, H-4′ax)	1.93(ddd, 12.5, 5.0, H-2′eq) 1.72(dd, 8.0 12.5, H-2′ax)

Table 2
Selected ^{13}C chemical shifts δ (ppm) for the deprotected compounds

Compound	**17**	**18**	**23**	**30**	**31**	**36**
C-1	104.31	105.19	104.30	104.32	104.34	101.71
C-1′	101.56	102.17	101.54	101.73	101.72	101.43
CO	178.58	178.70	178.88	178.39	178.52	
CON	175.14	175.35	175.06	175.13	174.97	
C-6	17.70	64.48	61.86	61.61	64.27	62.28
C-6′	61.81	61.78	61.46	16.17	61.62	61.60
C-2	55.67	56.58	55.45	55.47	55.42	55.61
OCH_2	71.29	71.20	71.21	71.26	71.28	71.36
OCH_3	52.80	52.90	52.79	52.77	52.79	52.89
$COCH_2$	34.49	34.54	34.53	34.49	34.50	34.53
$COCH_3$	23.05	23.13	23.05	23.06	22.93	
Other C		35.37 (C-4)	30.22 ($COCH_2$) 10.30 (CH_3)		34.79 (C-4′)	34.59 (C-2′)

Table 3
Evaluation of disaccharide analogues as acceptors and inhibitors of human milk fucosyltransferase [a]

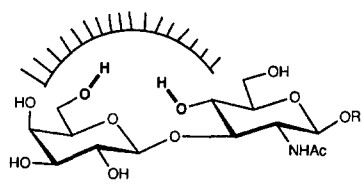
Compound	K_m (μM)	V_{max} (pmol/min μL)
1	640	1.8
17 (6-deoxy)	400	1.6
18 (4-deoxy)	**9.0** (K_i) [b]	
23 (*N*-propionyl)	330	3.0
30 (6′-deoxy)	inactive	inactive
31 (4′-deoxy)	850	1.6
36 (2′-deoxy)	730	3.2

[a] Errors were within 15% in K_m and V_{max}.
[b] Inhibition was competitive with **1**.

a previously described radioactive assay [4,8,19]. The results are shown in Table 3. The K_m value determined for **1** was 640 μM. Deoxygenation at C-6 or substitution of the *N*-acetyl group by an *N*-propionyl group decreased the K_m values to 400 and 330 μM, respectively, i.e., the compounds were better ligands for the enzyme. As expected [8], removal of the OH-4 in **18** yielded a compound that was not fucosylated but nevertheless remained a very weak competitive inhibitor of the enzyme with a K_i value of 9.0 mM. Deoxygenation at either C-2′ or C-4′ had little effect on acceptor activity since the K_m values of **31** (850 μM) and **36** (730 μM) are both similar to that of **1**. The OH-6′ group of the Gal residue is the only hydroxyl group essential for substrate binding since the 6′-deoxy analogue **30** showed no detectable activity.

3. Conclusions

In summary, the human milk Lewis α-(1 → 3/4)-fucosyltransferase appears to recognize type-1 acceptors exclusively from the "back side" as summarized in Fig. 2. Only the reactive OH group (OH-4 of the GlcNAc residue) and OH-6′ of the Gal residue, which are proximal in the minimum energy conformation [29] of disaccharide **1** illustrated in Fig. 2, are required for efficient recognition by the enzyme. An identical recognition pattern was recently also reported in a study of recombinant fucosyltransferases FucT-III and FucT-V where a panel of type-1 disaccharides, present as both the



1 (R = $(CH_2)_8COOMe$)

Fig. 2. Schematic representation of the critical recognition of HO-4 and HO-6′ of acceptor **1** by the α-(1 → 3/4)-fucosyltransferase.

8-methoxycarbonyloctyl and methyl glycosides, were used to map out the active site requirements, though the kinetic parameters were not determined in that study [30]. These two key recognition elements, OH-4 and OH-6″, should therefore be present in any acceptor based inhibitor designed for this enzyme.

4. Experimental

General methods.—Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2 °C. Melting points were measured with a Fisher–Johns melting point apparatus. TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by charring with H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–63 μm, E. Merck, Darmstadt). Hydrogenation reactions were preformed under an atmospheric pressure of hydrogen. C_{18} Sep-Pak sample-preparation cartridges were from Waters Associates (Missisauga, ON). Millex-GV (0.22 mm) filter units were from Millipore (Missisauga, ON). ^{3}H-GDP-fucose was from Dupont (Boston, MA). GDP-fucose [31] was available from previous work. Ecolite scintillation cocktail was from ICN Radiochemicals (Costa Mesa, CA). ^{1}H NMR spectra were recorded at 360 MHz (Bruker AMR 360) or at 300 MHz (Bruker AM 300) on solutions in $CDCl_3$ (internal Me_4Si, δ 0), or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$ (internal Me_4Si, δ 0), or D_2O (internal 1,4-dioxane, δ 67.4). Only partial NMR data are reported as the other data were in accord with the proposed structures. FAB mass spectra were obtained on a Kratos AEIMS9 instrument using glycerol as the matrix. Elemental analyses were carried on a Carlo Erba EA 1108 instrument by the departmental microanalytical laboratory.

*8-Methoxycarbonyloctyl 2-acetamido-4-O-benzoyl-6-bromo-2,6-dideoxy-β-*D*-gluco-pyranoside* (**3**).—To a suspension of 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**2**) [24] (300 mg, 0.62 mmol) in carbon tetrachloride (9 mL) and 1,1,2,2-tetrachloroethane (0.5 mL) was added *N*-bromosuccinimide (128 mg, 0.70 mmol), followed by barium carbonate (100 mg). The mixture was refluxed for 5 h and filtered through a pad of Celite. After evaporation of the solvent, the residue was applied to a column of silica gel (2:1 hexane–acetone) to provide **3** (140 mg, 40%) as a white solid; R_f 0.21 (19:1 CH_2Cl_2–MeOH); mp 144–145 °C (EtOAc–hexane); $[\alpha]_D$ ± 14.6° (*c* 0.8, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 8.00, 7.60–7.40 (m, 5 H, Ph), 6.11 (d, 1 H, $J_{NH,2}$ 6.5 Hz, NH), 5.05 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.76 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 3.69 (d, 1 H, $J_{3,OH}$ 3.5 Hz, OH), 4.21 (dt, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 3.68 (s, 3 H, OCH_3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.01 (s, 3 H, $COCH_3$), Anal. Calcd for $C_{25}H_{36}BrNO_8$: C, 53.77; H, 6.50; N, 2.51. Found: C, 53.96; H, 6.57; N, 2.51.

*8-Methoxycarbonyloctyl 2-acetamido-4-O-benzoyl-2,6-dideoxy-β-*D*-glucopyranoside* (**4**).—A solution of tributyltin hydride (43 μL) in dry toluene (0.95 mL) was added dropwise to a gently refluxing solution of **3** (30 mg, 53.7 μmol) in toluene (0.95 mL). The reaction mixture was then refluxed for 22 h. The solvent was evaporated, and the residue was applied to a column of silica gel (3:1 → 2:1 hexane–acetone) to provide **4** as a white solid (21 mg, 81%); R_f 0.42 (19:1 CH_2Cl_2–MeOH); mp 117–118 °C (flakes

from EtOAc–hexane); $[\alpha]_D$ −3.2° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 8.00, 7.60–7.35 (m, 5 H, Ph), 5.98 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 5.05 (t, 1 H, $J_{3,4} \sim J_{4,5}$ 9.5 Hz, 10.0 Hz, H-4), 4.60 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 3.64 (s, 3 H, OCH_3), 2.28 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.84 (s, 3 H, $COCH_3$), 1.39 (d, 3 H, $J_{5,6}$ 6.5 Hz, H_3-6). Anal. Calcd for $C_{25}H_{37}NO_8$: C, 62.61; H, 7.77; N, 2.92. Found: C, 62.15; H, 8.00; N, 2.92.

*8-Methoxycarbonyloctyl 2-acetamido-2,6-dideoxy-β-*D*-glucopyranoside* (**5**).—(*a*) From **4**: Compound **4** (20 mg, 41.8 μmol) was treated with 0.07 N methanolic NaOMe (2 mL) for 0.5 h. Deionization with Amberlite IRC-50 (H^+) and evaporation gave **5** (15 mg, 96%). An analytical sample was obtained by column chromatography (6:1 EtOAc–MeOH); R_f 0.22 (6:1 EtOAc–MeOH); $[\alpha]_D$ −63.9° (*c* 0.3, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 6.45 (bd, 1 H, NH), 4.38 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.65 (s, 3 H, OCH_3), 3.21 (t, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 2.29 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.04 (s, 3 H, $COCH_3$), 1.33 (d, 3 H, $J_{5,6}$ 6.0 Hz, H_3-6). Anal. Calcd for $C_{18}H_{33}NO_7$: C, 57.58; H, 8.86; N, 3.69. Found: C, 57.61; H, 8.84; N, 3.69.

(*b*) From **3**: Compound **3** (90 mg, 0.16 mmol) in MeOH (6 mL) and Et_3N (0.6 mL) was hydrogenated in the presence of 5% Pd/C (100 mg) for 2 days. Filtration of the catalyst, followed by evaporation of the solvents, left a residue that was purified by chromatography on silica gel (6:1 EtOAc–MeOH) to yield **5** (44 mg, 73%), which was identical with **5** prepared by method *a*.

*8-Methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-*p*-methoxybenzyl)-β-*D*-glucopyranoside* (**6**).—Sodium hydride (56 mg, 1.4 mmol, 60% in mineral oil) was added in small portions to a solution of **2** (500 mg, 1.06 mmol) in DMF (4.5 mL) at −40 to −50 °C under nitrogen. After stirring for 0.5 h, *p*-methoxybenzyl chloride (0.14 mL, 1.10 mmol) was added, and the reaction mixture was allowed to reach room temperature. Stirring was continued for 1 h during which time one more addition of *p*-methoxybenzyl chloride (30 μL, 0.24 mmol) was made. MeOH was added to decompose the remaining sodium hydride, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and filtered. After evaporation of the solvent, the crude product was applied to a column of silica gel (9:4 hexane–acetone) to provide **6** (430 mg, 68%) as a white solid; R_f 0.35 (2:1 hexane–acetone); mp 190–192 °C (EtOAc–hexane); $[\alpha]_D$ −11.9° (*c* 0.3, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.60–7.35 (m, 5 H, Ph), 7.24 and 6.85 (2 d, 4 H, *p*-MeOPh), 5.60 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 5.58 (s, 1 H, CHPh), 5.00 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.81 and 4.59 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 3.80 and 3.66 (2 s, each 3 H, $2CH_3O$), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.90 (s, 3 H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 174.30 (CO), 101.23, 100.41 (C-1, PhCH), 74.17 ($PhCH_2$), 70.16 (OCH_2), 69.90 (C-6), 58.21(OCH_3), 55.30 (C-2), 51.48 (OCH_3), 34.08 ($COCH_2$), 23.61 ($COCH_3$). Anal. Calcd for $C_{33}H_{45}NO_9$: C, 66.09; H, 7.56; N, 2.34. Found: C, 65.91; H, 7.62; N, 2.33.

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-*p*-methoxybenzyl)-β-*D*-glucopyranoside* (**7**).—Diethyl ether saturated with HCl was added to a mixture of **6** (380 mg, 0.63 mmol), sodium cyanoborohydride (820 mg, 13.17 mmol), a few crystals of methyl orange, and 3 Å molecular sieves (550 mg) in dry THF (17 mL) at 0 °C under nitrogen until the color of the indicator turned red. Then stirring was continued for 40 min, and the reaction mixture was poured into aq $NaHCO_3$. The organic layer was washed with water, dried, and evaporated. The crude product was purified by column

chromatography (2:1 hexane–acetone) to provide **7** (321 mg, 83%) as a white solid. An analytical sample was recrystallized from EtOAc–hexane to give flakes of **7**; mp 93–95 °C; R_f 0.21 (19:1 CH_2Cl_2–MeOH); $[\alpha]_D$ −18.8° (*c* 0.6, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.60–7.35 (m, 5 H, Ph), 7.24 and 6.85 (2 d, 4 H, *p*-MeOPh), 5.71 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 4.82 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.71 and 4.65 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 3.97 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 10.0 Hz, H-3), 3.80 and 3.66 (2 s, each 3 H, $2CH_3O$), 2.81 (d, 1 H, $J_{OH,4}$ 1.5 Hz, OH), 2.24 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.92 (s, 3 H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 174.17 (CO), 100.56 (C-1), 73.62, 73.43 ($2PhCH_2$), 70.50 (OCH_2), 69.48 (C-6), 55.82, 55.16 (C-2 and OCH_3), 51.29 (OCH_3), 33.92 ($COCH_2$), 23.34 ($COCH_3$). Anal. Calcd for $C_{33}H_{47}NO_9$: C, 65.87; H, 7.87; N, 2.33. Found: C, 65.69; H, 7.83; N, 2.38.

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-*p*-methoxybenzyl)-4-*O*-(methylthio)thiocarbonyl-β-*D*-glucopyranoside* (**8**).—A solution of **7** (100 mg, 0.16 mmol) and imidazole (2 mg) in THF (1 mL) was refluxed under nitrogen. Sodium hydride (10 mg, 0.26 mmol, 50% in mineral oil) was added in small portions. After refluxing for 0.5 h, carbon disulfide (93 μL) was added, followed by methyl iodide (93 μL) after 0.5 h. Refluxing was continued for 0.5 h, the reaction mixture was cooled, and MeOH was added. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and filtered. The filtrate was evaporated, and the residue was chromatographed on a column of silica gel (2:1 hexane–acetone) to yield **8** (98 mg, 88%) as a white solid. An analytical sample was recrystallized from EtOAc–hexane; mp 83–84 °C; R_f 0.35 (19:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +31.7° (*c* 0.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.35 (m, 5 H, Ph), 7.20 and 6.86 (2 d, 4 H, *p*-MeOPh), 5.95 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 5.74 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.06 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.59 and 4.42 (ABq, 2 H, J_{AB} 11.0 Hz, $PhCH_2$), 4.56 and 4.51 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 3.79 and 3.66 (2 s, each 3 H, $2CH_3O$), 2.54 (s, 3 H, SCH_3), 2.30 (t, 2 H, *J* 7.0 Hz, CH_2CO), 1.91 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{35}H_{49}NO_9S_2$: C, 60.71; H, 7.13; N, 2.02. Found: C, 60.62; H, 7.21; N, 2.06.

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-*p*-methoxybenzyl)-4-*O*-phenoxythiocarbonyl-β-*D*-glucopyranoside* (**9**).—A mixture of **7** (110 mg, 0.18 mmol), phenyl chlorothionocarbonate (0.53 mL, 3.79 mmol), and 4-dimethylaminopyridine (770 mg, 6.31 mmol) in acetonitrile (9 mL) was refluxed under nitrogen for 9 h and left at room temperature for 13 h. After evaporation, the residue was dissolved in CH_2Cl_2 and washed with water, 1% aq HCl, and aq $NaHCO_3$. Following solvent evaporation, the residue was purified by chromatography on silica gel (5:2 hexane–acetone) to provide **9** (99 mg, 73%) as a white solid; R_f 0.36 (5% MeOH in CH_2Cl_2); mp 135–137 °C (EtOAc–hexane); $[\alpha]_D$ +24.2° (*c* 0.6, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.70–7.40 and 6.88 (m, 14 H, 2 Ph and *p*-MeOPh), 5.77 (bd, 1 H, NH), 5.58 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 5.05 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.71 and 4.56 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 4.62 and 4.56 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 4.49 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.79 and 3.67 (2 s, each 3H, $2CH_3O$), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.93 (s, 3 H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 194.42 (CS), 174.37 (CO), 99.41 (C-1), 81.92 (C-4), 74.03, 73.72 ($2PhCH_2$), 70.02 (OCH_2), 69.80 (C-6), 57.82 (OCH_3), 55.33 (C-2), 51.49 (OCH_3), 34.11 ($COCH_2$), 23.61 ($COCH_3$). Anal. Calcd for $C_{40}H_{51}NO_{10}S$: C, 65.03; H, 6.96; N, 1.90. Found: C, 65.25; H, 7.52; N, 1.94.

8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-2,4-dideoxy-3-O-(4-methoxybenzyl)-β-D-xylopyranoside (**10**).—(*a*) From **8**: Compound **8** (74 mg, 0.11 mmol) in toluene (1.6 mL) was stirred at 80 °C under nitrogen. α,α′-Azobisisobutyronitrile (19.8 mg, 0.12 mmol) was added, followed by tributyltin hydride (0.43 mL, 1.62 mmol). After 2 h at 80 °C, the reaction mixture was concentrated, and the residue was applied to a column of silica gel (2:1 hexane–acetone) to yield **10** (60 mg, 94%); R_f 0.20 (2:1 hexane–acetone); $[\alpha]_D$ +2.4° (*c* 0.3, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.35 (m, 5 H, Ph), 7.20 and 6.85 (2 d, 4 H, *p*-MeOPh), 5.54 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 4.84 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.61 and 4.56 (ABq, 2 H, J_{AB} 11.0 Hz, $PhCH_2$), 4.57 and 4.40 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 4.10 (dt, 1 H, $J_{2,3} = J_{3,4ax} = 10.0$, $J_{3,4eq}$ 5.0 Hz, H-3), 3.80 and 3.66 (2 s, each 3 H, 2 CH_3O), 2.29 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.19 (dd, 1 H, $J_{4ax,4eq}$ 12.5, $J_{3,4eq}$ 5.0 Hz, H-4eq), 1.94 (s, 3 H, $COCH_3$), 1.40 (q, 1 H, $J_{4ax,5} = J_{3,4ax} = 11.0$ Hz, H-4ax). ^{13}C NMR ($CDCl_3$): δ 174.36 (CO), 100.10 (C-1), 73.54, 72.63 (2 $PhCH_2$), 70.87 (OCH_2), 69.62 (C-6), 58.94, 51.48 (2 CH_3O), 55.33 (C-2), 34.29 (C-4), 34.13 ($COCH_2$), 23.77 ($COCH_3$). Anal. Calcd for $C_{35}H_{47}NO_8$: C, 67.67; H, 8.09; N, 2.39. Found: C, 67.63; H, 8.25; N, 2.41.

(*b*) From **9**: Compound **9** (20 mg, 28.4 μmol) in toluene (1.8 mL) was heated at 80 °C. α,α′-Azobisisobutyronitrile (4.75 mg, 29 μmol) was added, followed by tributyltin hydride (0.1 mL, 39.8 μmol). After 2 h at 80 °C, the reaction mixture was concentrated, and the residue was purified on silica gel (2:1 hexane–acetone) to provide **10** (15 mg, 90%).

2-acetamido-6-O-benzyl-2,4-dideoxy-β-D-xylopyranoside (**11**).—A solution of **10** (52 mg, 88.8 μmol) and ammonium cerium(IV) nitrate (95 mg, 176 μmol) in 9:1 acetonitrile–water (0.8 mL) was stirred for 0.5 h. CH_2Cl_2 was added, and the organic layer was washed with aq $NaHCO_3$, water, and concentrated. The crude material was purified on silica gel (20:1 EtOAc–MeOH) to provide **11** (39 mg, 95%) as a white solid; R_f 0.28 (93:7 CH_2Cl_2–MeOH); mp 85–86 °C (EtOAc–hexane); $[\alpha]_D$ −58.6° (*c* 0.4, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.35–7.25 (m, 5 H, Ph), 5.79 (d, 1 H, NH), 4.60 and 4.56 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 4.28 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.67 (s, 3 H, OCH_3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.09 (ddd, 1 H, $J_{4ax,4eq}$ 12.0, $J_{3,4eq}$ 4.0, $J_{4eq,5}$ 1.5 Hz, H-4eq), 2.07 (s, 3 H, $COCH_3$), 1.49 (q, 1 H, $J_{4ax,5} = J_{3,4ax} = 11.5$ Hz, H-4ax). ^{13}C NMR ($CDCl_3$): δ 100.72 (C-1), 73.48 ($PhCH_2$), 72.37 (OCH_2), 69.45 (C-6), 51.46 (OCH_3), 35.76 (C-4), 34.03 ($COCH_2$). Anal. Calcd for $C_{25}H_{39}NO_7$: C, 64.49; H, 8.44; N, 3.01. Found: C, 64.30; H, 8.65; N, 3.03.

8-Methoxycarbonyloctyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2,6-dideoxy-β-D-glucopyranoside (**13**).—A solution of 2-*O*-acetyl 3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl bromide (**12**) [24] (95 mg, 0.17 mmol) in nitromethane–toluene (1:1, 0.5 mL) was added to a stirred mixture of **5** (40 mg, 0.11 mmol), mercuric cyanide (36.7 mg, 0.24 mmol), calcium sulfate (40 mg), and 4 Å molecular sieves (83 mg) in the same solvent (1.6 mL) under nitrogen. Stirring was continued for 2 h, and the reaction mixture was filtered. After concentration of the filtrate, the residue was purified on silica gel (5:2 hexane–acetone) to provide **13** (83 mg, 91%) as a white solid; R_f 0.32 (2:1 hexane–acetone); $[\alpha]_D$ +7.1° (*c* 0.2, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.35–7.20 (m, 15 H, 3 Ph), 5.72 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.34 (t, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 4.93 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.89

and 4.54 (ABq, 2 H, J_{AB} 12.0 Hz, CH_2Ph), 4.66 and 4.50 (ABq, 2 H, J_{AB} 12.0 Hz, CH_2Ph), 4.46 and 4.42 (ABq, 2 H, J_{AB} 12.0 Hz, CH_2Ph), 4.40 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.31 (t, 1 H, $J_{2,3} = J_{3,4} = 10.5$, 9.5 Hz, H-3), 4.26 (d, 1 H, $J_{OH,4}$ 1.5 Hz, OH), 3.87 (d, 1 H, $J_{3',4'}$ 2.5 Hz, H-4'), 3.65 (s, 3 H, OCH_3), 3.50 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 2.5 Hz, H-3'), 3.16 (dt, 1 H, H-4), 2.90 (m, 1 H, H-2), 2.29 (t, 2 H, J 7.5 Hz, CH_2CO), 2.03 and 1.95 (2s, each 3 H, $2COCH_3$), 1.33 (d, 3 H, $J_{5,6}$ 6.0 Hz, H_3-6). Anal. Calcd for $C_{47}H_{63}NO_{13}$: C, 66.41; H, 7.47; N, 1.65. Found: C, 66.70; H, 7.47; N, 1.73.

*8-Methoxycarbonyloctyl 3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-2,6-dideoxy-β-*D*-glucopyranoside* (**14**).—Compound **13** (80 mg, 0.094 mmol) was treated with 0.045 N methanolic NaOMe (2.2 mL) for 6 h. After neutralization with Amberlite IRC-50 (H^+) and evaporation of the solvent, the crude product was purified on a short column of silica gel (1:2 EtOAc–hexane) to yield **14** (61 mg, 80%); R_f 0.22 (2:1 hexane–acetone); $[\alpha]_D$ −13.1° (*c* 0.7, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.35–7.20 (m, 15 H, 3Ph), 6.13 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 4.83 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.84 and 4.53 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.70 and 4.66 (ABq, 2 H, J_{AB} 12.0 Hz, CH_2Ph), 4.45 and 4.37 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.27 (bd, 1 H, OH), 4.22 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 3.67 (s, 3 H, OCH_3), 3.04 (bd, 1 H, OH), 2.30 (t, 2 H, J 7.5 Hz, CH_2CO), 1.96 (s, 3 H, $COCH_3$), 1.34 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-3, -6). Anal. Calcd for $C_{45}H_{61}NO_{12}$: C, 66.89; H, 7.51; N, 1.73. Found: C, 66.90; H, 7.63; N, 1.77.

*8-Methoxycarbonyloctyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-6-*O*-benzyl-2,4-dideoxy-β-*D*-xylo-hexopyranoside* (**15**).—This compound was prepared by reaction of **11** (23 mg, 0.049 mmol) and **12** (50 mg, 0.091 mmol) in the presence of mercuric cyanide (15 mg, 0.059 mmol) as described for the preparation of **13**. Chromatography on silica gel (2:1 hexane–acetone) yielded **15** (44 mg, 96%); R_f 0.29 (2:1 hexane–acetone); $[\alpha]_D$ −2.0° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.35–7.20 (m, 20 H, 4Ph), 5.71 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.29 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2'), 4.91 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.90 (d, 1 H, from ABq, J_{AB} 11.5 Hz, CH_2Ph), 4.66–4.40 (m, 8 H, 4 CH_2Ph and H-1'), 3.91 (d, 1 H, $J_{3',4'}$ 2.6 Hz, H-4'), 3.66 (s, 3 H, OCH_3), 2.29 (t, 2 H, J 7.5 Hz, CH_2CO), 2.15 (dd, 1 H, $J_{4eq,4ax}$ 12.5, $J_{3,4eq}$ 5.0 Hz, H-4eq), 2.01 and 1.93 (2 s, each 3 H, $2COCH_3$), 1.50 (q, 1 H, J 12.5 Hz, H-4ax). Anal. Calcd for $C_{53}H_{67}NO_{13}$: C, 68.73; H, 7.29; N, 1.51. Found: C, 68.96; H, 7.71; N, 1.55.

*8-Methoxycarbonyloctyl 3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-6-*O*-benzyl-2,4-dideoxy-β-*D*-xylo-hexopyranoside* (**16**).—Compound **15** (42 mg, 0.045 mmol) was treated with 0.045 N methanolic NaOMe (1 mL) for 24 h. Neutralization with Amberlite IRC-50 (H^+) and evaporation of the solvent left crude **16**, which was purified by column of silica gel (2:1 hexane–acetone) to provide a white solid (37 mg, 93%); R_f 0.24 (2:1 hexane–acetone); $[\alpha]_D$ −10.0° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.35–7.20 (m, 20 H, 4 Ph), 6.13 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 4.85 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.84 and 4.58 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.70 and 4.65 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.56 and 4.51 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.45 and 4.39 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.29 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 3.67 (s, 3 H, OCH_3), 2.99 (br, 1 H, OH), 2.30 (t, 2 H, J 7.2 Hz, CH_2CO), 2.20 (dd, 1 H, $J_{4eq,4ax}$ 12.5, $J_{3,4eq}$ 4.0 Hz, H-4eq), 1.94 (s, 3 H, $COCH_3$), 1.51 (q, 1 H, J 12.5 Hz, H-4ax). FABMS, *m/z*: 920 (8.2%, $[M + Na]^+$), 898 (3.8%, $[M + H]^+$).

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 3)-2-acetamido-2, 6-dideoxy-β-D-glucopyranoside (**17**).—Compound **14** (50 mg, 0.061 mmol) in MeOH (3 mL) was hydrogenated in the presence of 5% Pd/C (50 mg) for 4 h. The catalyst was removed by filtration, and the solvent was evaporated. The solid that remained was adsorbed onto a Sep-Pak C_{18} cartridge in water, the cartridge was washed with water, and eluted with MeOH. Evaporation of the eluant, filtration through a Millex filter, and lyophilization from water gave **17** (27 mg, 82%) as a light white powder; R_f 0.77 (6:3:1 EtOAc–MeOH–water); $[\alpha]_D$ −36.9° (*c* 0.2, MeOH). NMR data are reported in Tables 1 and 2. FABMS, *m/z*: 560 (4.2%, $[M + Na]^+$), 538 (20.3%, $[M + H]^+$).

9-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 3)-2-acetamido-2,4-dideoxy-β-D-xylo-hexopyranoside (**18**).—Compound **16** (30 mg, 0.034 mmol) in MeOH (4 mL) was hydrogenated in the presence of 5% Pd/C (30 mg) for 3 h. Filtration of the catalyst, followed by evaporation, left a white solid that was purified, as described for the preparation of **17**, to give **18** (17 mg, 93%) as a powder; R_f 0.47 (60:30:3 EtOAc–MeOH–water); $[\alpha]_D$ −22° (*c* 0.2, MeOH). NMR data are reported in Tables 1 and 2. FABMS, *m/z*: 560 (9.5%, $[M + Na]^+$), 538 (25%, $[M + H]^+$).

9-Methoxycarbonylnonyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**20**).—A mixture of 9-methoxycarbonylnonyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**) (200 mg, 0.41 mmol), α,α-dimethoxytoluene (0.13 mL, 0.88 mmol), and *p*-toluenesulfonic acid monohydrate (15 mg) in acetonitrile (4 mL) was kept at room temperature for 1 h. Et_3N was added, and the reaction mixture was evaporated. The residue was purified on silica gel (5:2 hexane–acetone) to yield **20** (198 mg, 84%) as a syrup; R_f 0.36 (1:1 EtOAc–hexane); $[\alpha]_D$ −39.4° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.85 and 7.65 (2 m, 4 H, phthalimido), 7.45 and 7.35 (2 m, 5 H, Ph), 5.55 (s, 1 H, CHPh), 5.26 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.24 (dd, 1 H, $J_{1,2}$ 8.5 Hz, H-2), 3.66 (s, 3 H, CH_3O), 2.54 (d, 1 H, $J_{OH,3}$ 2.5 Hz, OH), 2.25 (t, 2 H, *J* 7.5 Hz, CH_2CO). ^{13}C NMR ($CDCl_3$): δ 174.29 (CO), 168.13 (CO), 101.93 (PhCH), 98.94 (C-1), 70.06 (OCH_2), 68.74 (C-6), 56.71 (C-2), 51.44 (OCH_3), 34.06 ($COCH_2$). Anal. Calcd for $C_{32}H_{39}NO_9$: C, 66.34; H, 7.10; N, 2.42. Found: C, 66.08; H, 6.76; N, 2.41.

9-Methoxycarbonylnonyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**21**).—This compound was prepared from **20** (65 mg, 0.11 mmol) and **12** (82 mg, 0.15 mmol) in the presence of mercuric cyanide (59 mg, 0.24 mmol) as described for the preparation of **13**. Chromatography on silica gel (1:2 EtOAc–hexane) yielded **21** (82 mg, 69%); R_f 0.44 (1:2 EtOAc–hexane); $[\alpha]_D$ −4.6° (*c* 0.4, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.75 and 7.45 (2 m, each 2 H, phthalimido), 7.40–7.10 (m, 20 H, 4 Ph), 5.50 (s, 1 H, PhCH), 5.30 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.0 Hz, H-2′), 5.13 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.67 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 3.92 (d, 1 H, $J_{3',4'}$ 2.5 Hz, H-4′), 3.68 (s, 3 H, OCH_3), 2.28 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.98 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{61}H_{69}NO_{15}$: C, 69.36; H, 6.58; N, 1.33. Found: C, 69.50; H, 6.66; N, 1.09.

9-Methoxycarbonylnonyl 3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-propionamido-β-D-glucopyranoside (**22**).—A mixture of **21** (46 mg, 0.044 mmol) and ethylenediamine (1.8 mL) in butanol (9.6 mL) was kept at 90 °C under nitrogen for 20 h. The solvent was evaporated to give a syrup that was dissolved in MeOH (6 mL) and reacted with propionic anhydride (1.6 mL) and Et_3N (0.16 mL)

for 16 h. After evaporation, the residual syrup was chromatographed on silica gel (1:1 EtOAc–hexane) to yield **22** (11 mg, 27%); R_f 0.43 (19:1 CH_2Cl_2–MeOH); $[\alpha]_D$ −31.4° (*c* 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.50–7.20 (m, 20 H, 4Ph), 6.02 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.50 (s, 1 H, PhCH), 4.95 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.87 and 4.59 (ABq, 2 H, J_{AB} 11.5 Hz, $PhCH_2$), 4.65 and 4.59 (ABq, 2 H, J_{AB} 12.0 Hz, $PhCH_2$), 4.39 and 4.33 (ABq, 2 H, overlapped, $PhCH_2$), 4.36 (d, 1 H, overlapped, H-1′), 3.66 (s, 3 H, OCH_3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.13 (q, 2 H, *J* 7.5 Hz, $COCH_2CH_3$), 1.07 (t, 3 H, *J* 7.5 Hz, $COCH_2CH_3$). ^{13}C NMR ($CDCl_3$): δ 174.81, 174.41 (2 CO), 103.29, 101.40, 101.08 (PhCH, C-1 and C-1′), 74.65, 73.48, 72.39 (3 $PhCH_2$), 70.23 (OCH_2), 68.83, 68.30 (C-6 and C-6′), 57.46 (C-2), 51.51 (OCH_3), 34.14, 29.86 (2 $COCH_2$), 9.70 (CH_3). Anal. Calcd for $C_{54}H_{69}NO_{13}$: C, 68.99; H, 7.40; N, 1.50. Found: C, 68.28; H, 7.60; N, 1.50.

*9-Methoxycarbonylnonyl β-*D*-galactopyranosyl-(1 → 3)-2-deoxy-2-propionamido-β-*D*- glucopyranoside* (**23**).—Compound **22** (9 mg, 9.72 μmol) and 5% Pd/C (10 mg) in MeOH (0.6 mL) were hydrogenated for 5 h. Filtration of the catalyst, followed by evaporation of the solvent, left a white solid that was purified as described for the preparation of **17** to give **23** (5.1 mg, 93%) as a white powder; R_f 0.52 (60:30:3 EtOAc–MeOH–water); $[\alpha]_D$ −22° (*c* 0.1, MeOH). FABMS, *m/z*: 604 (22.7%, $[M + Na]^+$), 582 (6.0%, $[M + H]^+$). NMR data are reported in Tables 1 and 2.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-acetyl-6-deoxy-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**26**).—Compound **2** (50 mg, 0.10 mmol), silver trifluoromethanesulfonate (29.5 mg, 0.11 mmol), *sym*-collidine (10.99 μL, 0.083 mmol), and 4 Å molecular sieves (130 mg, powdered) were stirred under nitrogen at −30 to −40 °C in a mixture of toluene and nitromethane (1:1, 0.4 mL). A solution of 2,3,4-tri-*O*-acetyl-6-*O*-deoxy-α-D-galactopyranosyl bromide (**24**) [27] (59 mg, 0.167 mmol) in the same solvent (0.2 mL) was added. After 1.5 h at 0 °C to −30 °C, more *sym*-collidine (13 μL) was added and stirring was continued for 45 min. The mixture was then diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was washed sequentially with water, 1 N HCl, water, and concentrated. The residue was purified on silica gel (2:1 hexane–acetone, containing 0.3% Et_3N) to provide **26** (51 mg, 65%); R_f 0.26 (19:1 CH_2Cl_2–MeOH); mp 145–146.5 °C (needles from EtOAc–hexane); $[\alpha]_D$ −19.4° (*c* 0.3, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.50–7.30 (m, 5 H, Ph), 5.84 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.50 (s, 1 H, CHPh), 5.18 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.12 (dd, 1 H, $J_{1',2'}$, 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 5.09 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 4.87 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.5 Hz, H-3′), 4.70 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 3.66 (s, 3 H, OCH_3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.13–1.95 (4 s, 12 H, 4 $COCH_3$), 1.01 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-3, -6′). Anal. Calcd for $C_{37}H_{53}NO_{15}$: C, 59.11; H, 7.11; N, 1.86. Found: C, 59.13; H, 7.14; N, 1.73.

*8-Methoxycarbonyloctyl 6-deoxy-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**27**).—Compound **26** (34 mg, 0.045 mmol) was treated with 0.08 N methanolic NaOMe (0.7 mL) for 4 h. Neutralization with Amberlite IRC-50 (H^+) resin and evaporation of the solvent left a white residue that was purified by chromatography on silica gel (6:1 EtOAc–MeOH, containing 0.3% Et_3N). Compound **27** (26 mg, 93%) was obtained as a white solid; R_f 0.20 (6:1 EtOAc–MeOH); mp 262–263 °C (MeOH); $[\alpha]_D$ −43.4° (*c* 0.3, CH_2Cl_2–MeOH). ^{1}H

NMR ($CDCl_3$ + CD_3OD): δ 7.50–7.30 (m, 5 H, Ph), 5.55 (s, 1 H, CHPh), 4.67 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.32 (dd, 1 H, $J_{6,6}$ 10.5, $J_{5,6}$ 4.9 Hz, H-6), 4.28 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.20 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.66 (s, 3 H, OCH_3), 3.12 (q, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 2.32 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.97 (s, 3 H, $COCH_3$), 1.24 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-3, H-6'). Anal. Calcd for $C_{31}H_{47}NO_{12}$: C, 59.51; H, 7.57; N, 2.24. Found: C, 59.51; H, 7.70; N, 2.13.

*8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-deoxy-β-*D*-xylo-hexopyranosyl-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**28**).—A solution of 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranosyl bromide **25** [28] (30 mg, 0.085 mmol) in dry CH_2Cl_2 (0.3 mL) was added to a mixture of **2** (27 mg, 0.057 mmol), silver trifluoromethanesulfonate (24 mg, 0.093 mmol), 4 Å molecular sieves (60 mg), and tetramethylurea (11.9 μL, 0.10 mmol) in CH_2Cl_2 (0.3 mL) at −30 °C under nitrogen. After 1 h at −30 to −10 °C, diisopropylethylamine (50 μL) was added, and the reaction mixture was filtered and evaporated. Column chromatography of the residue (1:1 EtOAc–hexane, then 2:5 EtOAc–CH_2Cl_2) yielded, **28** (27 mg, 63%); R_f 0.48 (19:1 CH_2Cl_2–MeOH); $[\alpha]_D$ −23.8° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.45–7.35 (m, 5 H, Ph), 5.90 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.52 (s, 1 H, CHPh), 5.22 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.90 (dt, 1 H, $J_{2',3'}$ 9.5, $J_{3',4'}$ 5.5 Hz, H-3'), 4.84 (dd, 1 H, $J_{1',2'}$, 8.0, $J_{2',3'}$ 9.5 Hz, H-2'), 4.69 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 3.66 (s, 3 H, OCH_3), 3.02 (m, 1 H, H-2), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.05 (m, 1 H, overlapped, H-4'eq), 2.02, 2.01, 1.95, and 1.90 (4 s, 12 H, 4 $COCH_3$), 1.65 (q, 1 H, overlapped, H-4'ax). ^{13}C NMR ($CDCl_3$): δ 174.30, 170.64, 170.32, and 169.78 (4 CO), 100.59, 99.49, and 99.37 (C-1, C-1' and PhCH), 70.28 (OCH_2), 68.89 and 65.27 (C-6 and C-6'), 58.67 (C-2), 51.47 (OCH_3), 34.06 ($COCH_2$), 32.36 (C-4'), 20.90 and 20.75 (2 $COCH_3$). Anal. Calcd for $C_{37}H_{53}NO_{15}$: C, 59.11; H, 7.11; N, 1.86. Found: C, 58.65; H, 7.00; N, 1.90.

*8-Methoxycarbonyloctyl 4-deoxy-β-*D*-xylo-hexopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**29**).—Compound **28** (20 mg, 0.027 mmol) was treated with 0.08 N methanolic NaOMe (0.5 mL) for 14 h. Neutralization with Amberlite IR 50 (H^+) and solvent evaporation left a solid that was purified on silica gel (6:1 EtOAc–MeOH) to provide **29** (13 mg, 78%); R_f 0.31 (19:1 CH_2Cl_2–MeOH); $[\alpha]_D$ −52.6° (*c* 0.2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 7.50, 7.40 (2 m, 5 H, Ph), 6.10 (d, 1 H, $J_{NH,2}$ 8.0 Hz, NH), 5.55 (s, 1 H, CHPh), 5.90 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.35 (d, 1 H, overlapped, H-1'), 3.66 (s, 3 H, OCH_3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.05 (s, 3 H, $COCH_3$), 1.85 (ddd, 1 H, $J_{4'ax,4'eq}$ 12.0, $J_{3',4'eq}$ 5.0, $J_{4'eq,5'}$ 1.0 Hz, H-4'eq), ~ 1.50 (q, 1 H, overlapped, H-4'ax). FABMS, *m/z*: 648 (6.4%, $[M + Na]^+$), 626 (8.8%, $[M + 1]^+$).

*8-Methoxycarbonyloctyl 6-deoxy-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**30**).—A mixture of **27** (24 mg, 0.038 mmol) and 5% Pd/C (25 mg) in 95% EtOH (3 mL) was hydrogenated for 5 h. Filtration of the catalyst, followed by concentration left a white solid that was purified as described for the preparation of **17** to give **30** (20 mg, 96%) as a white solid; R_f 0.64 (6:3:1 EtOAc–MeOH–water); $[\alpha]_D$ −26.8° (*c* 0.2, MeOH). NMR data are reported in Tables 1 and 2. FABMS, *m/z*: 560 (56%, $[M + Na]^+$), 538 (6.5%, $[M + H]^+$).

*8-Methoxycarbonyloctyl 4-deoxy-β-*D*-xylo-hexopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**31**).—A mixture of **29** (10 mg, 0.016 mmol) and 5% Pd/C

(10 mg) in MeOH (0.6 mL) was hydrogenated for 7 h. Filtration of the catalyst, followed by evaporation of the solvent left a white solid that was purified as described for the preparation of **17** to give **31** (6.6 mg, 77%) as a white powder; R_f 0.48 (60:30:3 EtOAc–MeOH–water); $[\alpha]_D$ −33° (*c* 0.2, MeOH). NMR data are reported in Tables 1 and 2. FABMS, *m/z*: 560 (3.3%, $[M + Na]^+$), 538 (8.1%, $[M + H]^+$).

8-Methoxycarbonyloctyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**32**).—Compound **2** (100 mg, 0.21 mmol) was reacted with **12** (222 mg, 0.40 mmol) in the presence of mercuric cyanide (109 mg, 0.43 mmol) as described for the preparation of **13**. Chromatography (2:1 hexane–acetone), yielded **32** (166 mg, 83%) as a foam; R_f 0.55 (19:1 CH_2Cl_2–MeOH); mp 75–77 °C (acetone–hexane); $[\alpha]_D$ −7.9° (*c* 0.2, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.45–7.15 (m, 20 H, 4Ph), 5.81 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.44 (s, 1 H, PhCH), 5.25 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 5.22 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.87 and 4.55 (ABq, 2 H, J_{AB} 11.5 Hz, CH_2Ph), 4.62 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.61 and 4.43 (ABq, 2 H, J_{AB} 12.0 Hz, CH_2Ph), 4.24 (s, 2 H, $PhCH_2$), 3.67 (s, 3 H, OCH_3), 2.95 (m, 1 H, H-2), 2.30 (t, 2 H, *J* 7.5 Hz, $COCH_2$), 1.97 and 1.96 (2 s, each 3 H, 2 $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 174.33, 170.66, and 169.65 (3 CO), 101.33, 100.42, and 99.31 (C-1, C-1′ and PhCH), 74.47, 73.50, and 71.90 (3 $PhCH_2$), 70.29 (OCH_2), 68.91 and 68.44 (C-6 and C-6′), 58.85 (C-2), 51.50 (OCH_3), 34.09 ($COCH_2$), 32.36 (C-4′), 23.72 and 21.09 (2 $COCH_3$). Anal. Calcd for $C_{54}H_{67}NO_{14}$: C, 67.98; H, 7.08; N, 1.47. Found: C, 67.74; H, 7.16; N, 1.37.

Preparation of 8-methoxycarbonyloctyl 3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**33**).—Compound **32** (140 mg, 0.15 mmol) was treated with 0.045 N methanolic NaOMe (3 mL) and CH_2Cl_2 (1 mL) for 20 h. Neutralization with Amberlite 50 (H^+) and evaporation of the solvent left a solid that was purified by column chromatography (2:1 hexane–acetone) to provide **33** (125 mg, 93%) that was identical to material previously prepared [25].

8-Methoxycarbonyloctyl 3,4,6-tri-O-benzyl-2-O-(methylthio)thiocarbonyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**34**).—Compound **33** (64 mg, 0.07 mmol) was reacted with sodium hydride (4 mg, 60% suspension in mineral oil, 0.1 mmol), imidazole (1 mg), and carbon disulfide (50 μL), followed by methyl iodide (50 μL) in THF, following the same procedure as for the preparation of **8**. Compound **34** (23 mg, 33%) was obtained after column chromatography (2:1 hexane–acetone); R_f 0.46 (2:1 hexane–acetone); $[\alpha]_D$ −18.7° (*c* 0.5, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): δ 7.45–7.15 (m, 20 H, 4 Ph), 6.32 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 5.94 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.48 (s, 1 H, PhCH), 5.25 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.56 (d, 1 H, overlapped, H-1′), 4.89, 4.62-4.47 (3 ABq, 6 H, 3 $PhCH_2$), 4.27 (s, 2 H, $PhCH_2$), 3.67 (s, 3 H, OCH_3), 2.59 (s, 3 H, SCH_3), 2.31 (t, 2 H, *J* 7.5 Hz, CH_2CO), 1.99 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{54}H_{67}NO_{13}S_2$: C, 64.71; H, 6.74; N, 1.39. Found: C, 64.33; H, 6.13; N, 1.44.

Preparation of 8-methoxycarbonyloctyl 3,4,6-tri-O-benzyl-2-deoxy-β-D-lyxo-hexopyranosyl-(1 → 3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**35**).—Compound **34** (18 mg, 0.018 mmol) in toluene (0.4 mL) was heated at 80 °C under nitrogen. α,α′-Azobisisobutyronitrile (6 mg, 0.037 mmol) was added, followed by tributyltin hydride (132 μL, 0.49 mmol). After 2 h, the solvent was evaporated, and

the residue was purified on silica gel (2:1 hexane–acetone) to provide **35** (12 mg, 75%); $[\alpha]_D$ −33.3° (*c* 0.5, CH_2Cl_2) identical to material previously prepared [24].

*Preparation of 8-Methoxycarbonyloctyl 2-deoxy-β-*D*-lyxo-hexopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**36**).—Compound **35** (12 mg, 0.013 mmol) in MeOH (1 mL) was hydrogenated in the presence of Pd/C (5%, 15 mg) for 32 h. Filtration of the catalyst, followed by evaporation of the solvents, left a white solid that was purified as described for the preparation of **17** to yield previously reported **36** [25] (5 mg, 71%) as a white powder. NMR data are reported in Tables 1 and 2.

Measurement of enzyme kinetics.—Radiochemical assays were performed as previously described [4,8,19]. The fucosyltransferase was isolated from human milk as reported [4]. All the assays were carried out in a total volume of 31.2 μL with 20 mM Hepes buffer, pH 7.0, containing 20 mM $MnCl_2$, 64 μM GDP-fucose, 0.02 μCi ^{3}H-GDP-fucose, and 0.5 μL fucosyltransferase from human milk (35 mU/mL). The incubation time was 30 min at 37 °C, after which time the reaction was quenched by the addition of EDTA (100 μL of a 32 mM solution). The mixture was then applied to a pre-equilibrated C_{18} Sep-Pak cartridge [19] that was washed with water (50 mL). The radiolabelled product was eluted with MeOH (3 mL) and quantitated by liquid scintillation counting. For the determination of K_m values, the following concentrations of acceptors were used: 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 2.0, and 4.0 mM. Determination of the K_i for compound **18** was done using inhibitor concentrations of 4 and 8 mM, and **18** was determined to be a competitive inhibitor by kinetic analysis as previously described [7,8].

Acknowledgements

We thank Professor Monica M. Palcic and Dr. Chris Scaman for a generous gift of the fucosyltransferase and for assistance with the enzyme assays, and Professor R.U. Lemieux for a gift of compound **19**. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

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