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Acceptor–substrate recognition by *N*-acetylglucosaminyltransferase-V: Critical role of the 4″-hydroxyl group in β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*(1 → 6)-β-D-Glc*p*-OR

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ABSTRACT

The enzyme *N*-acetylglucosaminyltransferase-V (GlcNAcT-V) transfers GlcNAc from UDP-GlcNAc to the OH-6′ group of oligosaccharides terminating in the sequence β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p* (or Man*p*)-OR (**5**, R = $(CH_2)_7CH_3$) to yield the sequence β-D-Glc*p*NAc-(1 → 2)-[β-D-Glc*p*NAc-(1 → 6)]-α-D-Man*p*-(1 → 6)-β-D-Glc*p* (or Man*p*)-OR. Biosynthetically, if β-(1 → 4)-galactosyltransferase acts first on **5**, the product β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p* (or Man*p*)-OR (**7**) is no longer a substrate for GlcNAcT-V even though it retains the active OH-6′ group. The reason for this loss in activity is examined in this paper.

Six analogues of the acceptor trisaccharide **5**, all with the reducing-end D-*gluco* configuration, were chemically synthesized. A key feature of the synthetic scheme is the use of 1,2-diaminoethane for the efficient removal of *N*-phthalimdo protecting groups. In these analogues OH-4 of the terminal sugar unit, the site of galactosylation by GalT in the normal GlcNAc-terminating trisaccharide **5**, was systematically replaced by OMe, F, NH_2, NHAc, and H, as well as inverted to the *galacto* configuration. The interactions of the resulting trisaccharide analogues with GlcNAcT-V from hamster kidney were then evaluated kinetically. All six compounds were found to be essentially inactive either as acceptors or as inhibitors of GlcNAcT-V. The conclusion is reached that galactosylation of natural acceptors for GlcNAcT-V destroys acceptor activity, not by introduction of the steric bulk of an added sugar residue, but by destroying an important hydrogen-bonding interaction of terminal OH-4 of the GlcNAc residues with the enzyme. This OH-4 group is therefore designated as a key polar group for GlcNAcT-V.

INTRODUCTION

The glycosyltransferase termed *N*-acetylglucosaminyltransferase-V (EC 2.4.1.155) transfers a GlcNAc residue from UDP-GlcNAc to complex biantennary asparagine-linked chains such as **1** to generate the additional β-(1 → 6) branch in the product **2**. A strong correlation between an increase in the activity of this enzyme and the increased metastatic potential of several cancer cell lines has been

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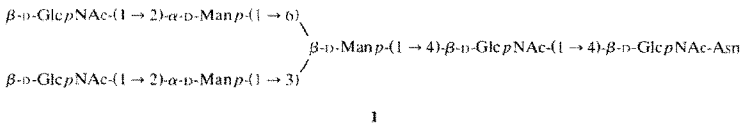
demonstrated[1–3]. This enzyme has, therefore, become a prime target for the development of a glycosyltransferase inhibitor.

Over 12 different GlcNAc-transferases use UDP-GlcNAc as the donor in mammalian cells[4,5]. We have, therefore, concentrated on a strategy of inhibition[6] which relies on the specific recognition of the acceptor rather than of the donor by the enzyme combining site. The minimum acceptor substrate acted on biosyntheti-



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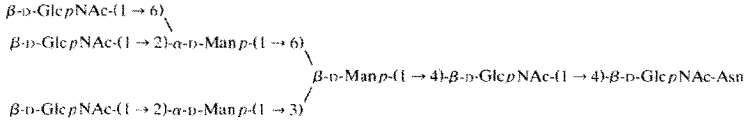

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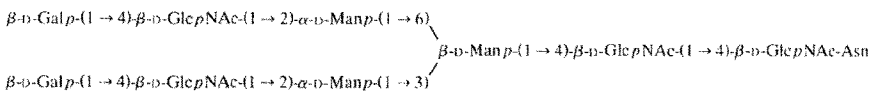

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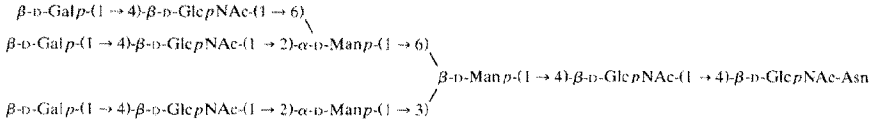

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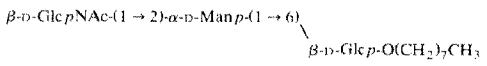

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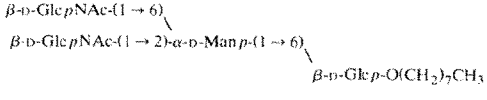

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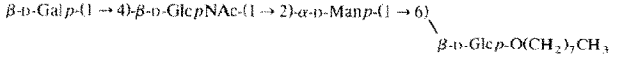

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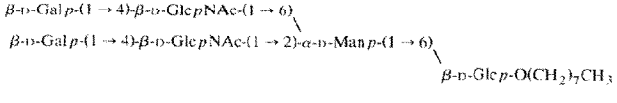

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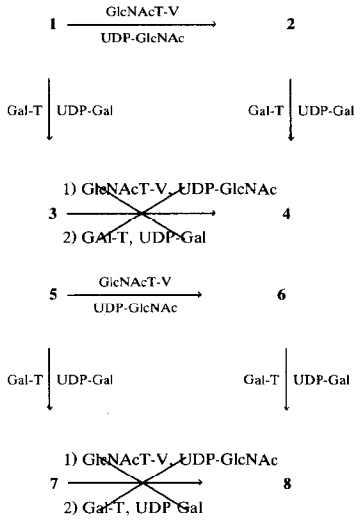
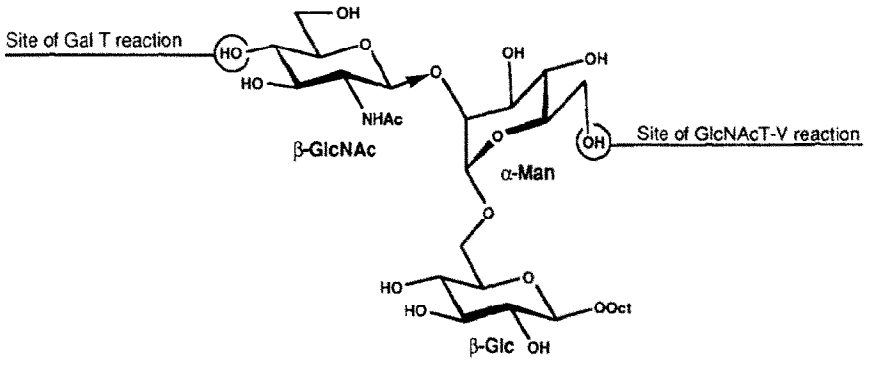


Fig. 1. Biosynthetic scheme for the synthesis of a triantennary complex carbohydrate chain compared with the corresponding enzymatic reactions on synthetic substrate analogues.

cally by GlcNAcT-V contains the heptasaccharide sequence shown in **1**. Of importance to the work reported here, galactosylation of **1** by a Golgi-localized β-(1 → 4)-galactosyltransferase (GalT) produces **3**, which is no longer an acceptor for GlcNAcT-V[7]. Triantennary oligosaccharides such as **4** must, therefore, be formed by the sequential ordered actions of GlcNAcT-V and GalT.

Several explanations may be advanced as to why galactosylation of **1** to produce **3** destroys its reactivity towards GlcNAcT-V. The simplest explanation is that the steric bulk of the added β-Gal residue interferes with substrate binding by the enzyme. Alternate explanations include a critical recognition of OH-4 of the GlcNAc residue in **1** by the enzyme active site through hydrogen-bonding interactions. Galactosylation of that OH-4 would, therefore, destroy this interaction, as might other substitutions. In this work, we examine these possibilities through the chemical synthesis and enzymatic evaluation of a series of potential oligosaccharide acceptors for GlcNAcT-V that have been systematically modified at that key OH-4 group of the terminal GlcNAc residue.

The synthetic trisaccharide derivative **5** (ref 8), a compound much more amenable to chemical modification than the complex natural sequence **1**, has previously been shown[9,10] to be an acceptor specific for GlcNAcT-V which converts it to **6** (Fig. 1). Similarly to **1**, galactosylation of **5** produces **7**, which is inactive as a GlcNAcT-V substrate (*vide infra*). Galactosylation of **6** produces **8** as expected. Acceptor **5** was, therefore, considered a valid model compound for evaluating the role of OH-4 of the GlcNAc residue in **1** in recognition by this enzyme. The role of OH-4 group was assessed by synthesizing six analogues of **5**, namely **9–14** (Fig. 2), where this group was sequentially replaced by either OMe, F,



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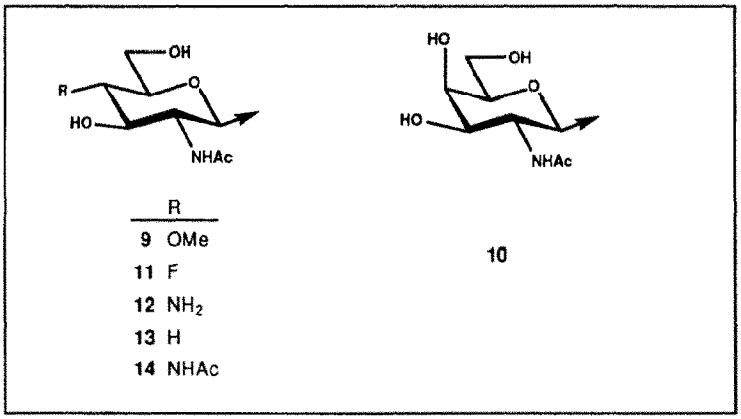


R	
9	OMe
11	F
12	NH_2
13	H
14	NHAc

10

Fig. 2. Structures of the target synthetic trisaccharide analogues. The sites of glycosylation of both GalT and GlcNAcT-V on the parent acceptor (**5**) are also shown.

NH_2, H, NHAc, or was configurationally inverted. The synthetic strategy was to prepare the disaccharide acceptor **15** which could then be glycosylated with the five monosaccharide donors **16**–**20**. Subsequent deprotection would then give the target trisaccharide derivatives. The 6′-*O*-allyl protecting group in **15** was included since this could then allow access to the reactive OH group glycosylated by GlcNAcT-V, with the possibility of providing subsequent inhibitors. In the present study, only acceptor analogues were prepared, and this group was therefore simply treated as a protecting group which was removed at the end of the synthesis.

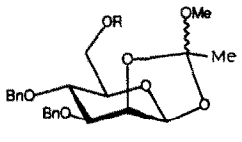
RESULTS AND DISCUSSION

Synthesis of disaccharide acceptor **15**.—*O*-Deacetylation of the 1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose derivative (**21**)[11] gave the alcohol **22** which was directly *O*-allylated to provide **23** (85%). Reaction of **23** with chlorotrimethylsi-

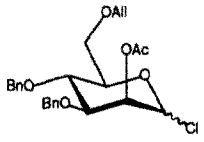
lane then gave the chloride **24**. Glycosylation of alcohol **25** (ref. 8) with donor **24** in the presence of the silver trifluoromethanesulfonate (triflate) and tetramethylurea gave the α-(1 → 6)-linked disaccharide **26** (95%). The $^1J_{C\text{-}1,H\text{-}1}$ value for the mannose residue was 171.1 Hz, which was in agreement with the expected α-mannosidic linkage[12]. *O*-Deacetylation of **26** then provided the disaccharide acceptor **15**.

Synthesis of N-*acetylglucosamine derivatives modified at OH-4.*—Compound **27** was selected as the starting material to produce the derivatives **29**, **31**, **34**, **36**, and **39**. The first monosaccharide synthesized was the *O*-methylated derivative **29**. Alcohol **27** was treated with diazomethane and borontrifluoride etherate in dichloromethane[13] to give **28** (71%). Other standard methods such as silver oxide–methyl iodide or sodium hydride–methyl iodide in *N*,*N*-dimethylformamide yielded less than 40% product. The allyl glycoside **28** was then converted to the reducing sugar **29** using Wilkinson's catalyst, followed by hydrolysis (84%)[14].

To obtain the galactosamine derivative **31**, as well as the fluoro- (**34**) and azido- (**36**) glucosamine derivatives, inversion at C-4 was required. Thus, the triflate of **27** was prepared (trifluoromethanesulfonic anhydride–pyridine) and was displaced using tetraethylammonium acetate. Deallylation then gave **31** (ref 15). Saponification of the acetate **30** yielded **32**, which was used for subsequent inversion reactions. Treatment of the alcohol **32** with diethylaminosulfur trifluoride[16] provided the 4-deoxy-4-fluoro-D-glucosamine derivative **33** (74%). The equatorial orientation of the fluorine atom in **33** was evident from in NMR spectra which showed $J_{3,4}$ 8.1, $J_{4,5}$ 9.3, and $^3J_{3,F}$ 15.1 Hz[17]. The allyl glycoside was then deblocked to give **34** (60%). Triflation of the alcohol **32**, followed by treatment with sodium azide, yielded the 4-azido glucosamine derivative **35** (87%). The characteristic infrared absorption at 2109 cm^{-1} corresponding to the azido group, and the ^1H NMR coupling constants ($J_{3,4}$ 9.0, $J_{4,5}$ 10.0 Hz) are consistent with the proposed structure. Glycoside **35** was converted to the corresponding hemiacetal



R	
21	Ac
22	H
23	All



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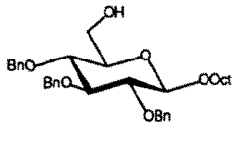

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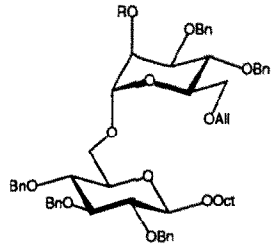
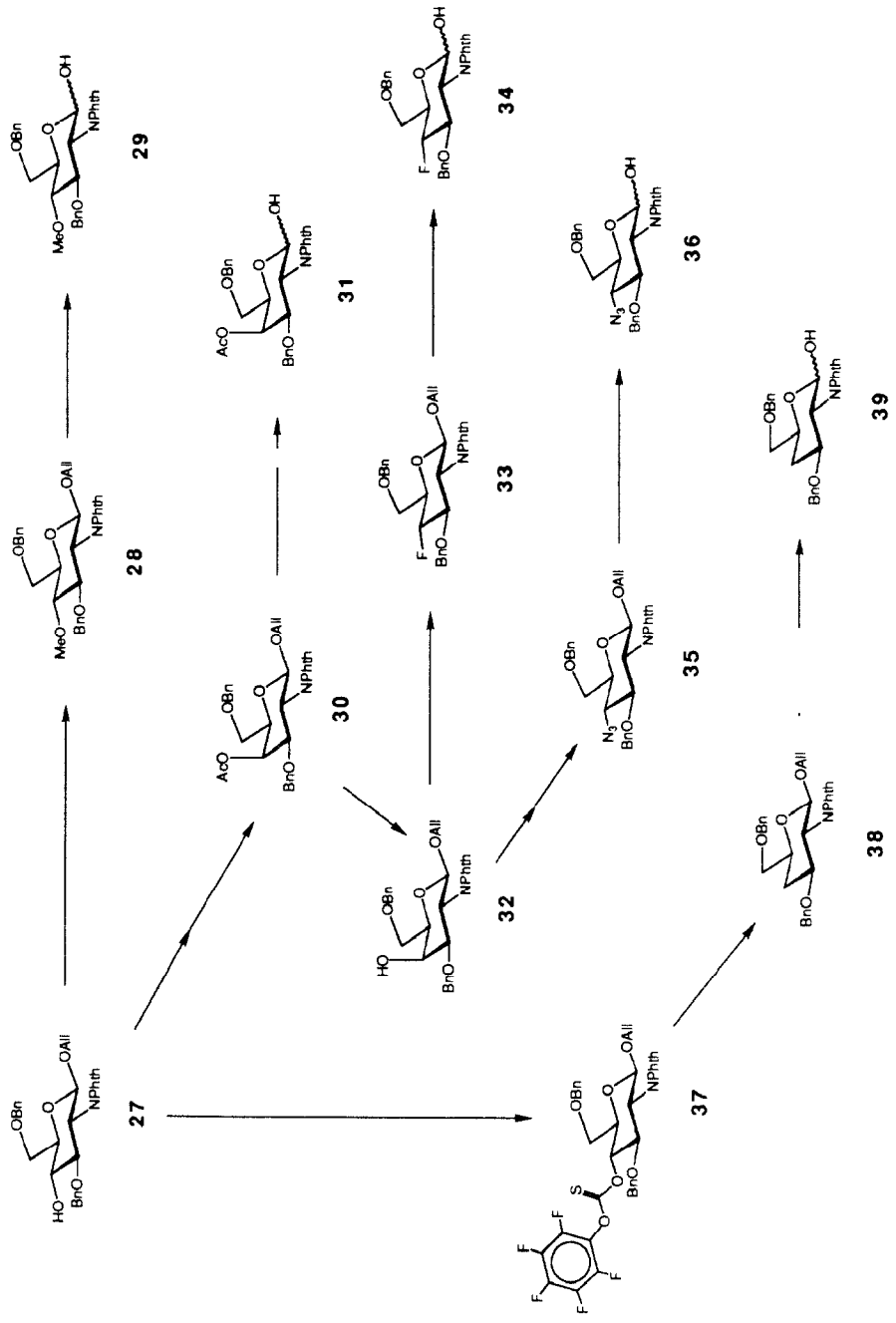


R	
26	Ac
15	H


27
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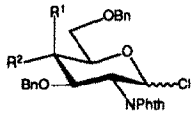
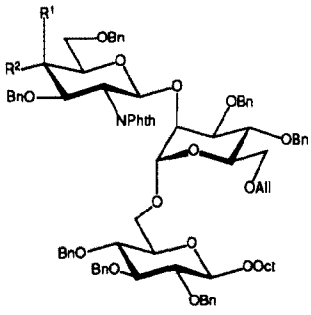
36 (77%) by treatment with palladium(II) chloride in acetic acid buffer[18], which was used because of the instability of the azido group to Wilkinson's catalyst.

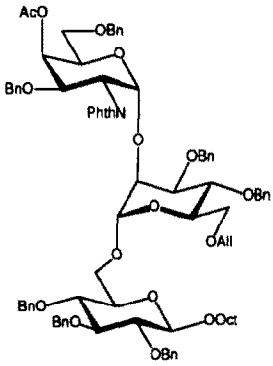
Finally, the pentafluorophenyloxythionocarbonyl ester[19] **37** was prepared from the OH-4 derivative **27** in 69% yield and was reduced by tributyltin hydride to give the 4-deoxy product **38** (92%). This excellent yield contrasts with that obtained for the attempted reduction of the pentafluorophenylthionocarbonyl ester of the



	R^1	R^2
16	H	OMe
17	OAc	H
18	H	F
19	H	N_3
20	H	H



	R^1	R^2
40	H	OMe
41	OAc	H
43	H	F
44	H	N_3
45	H	H



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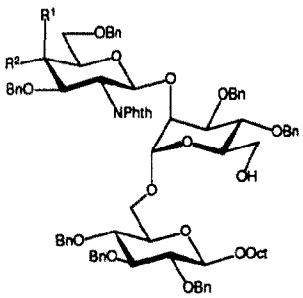
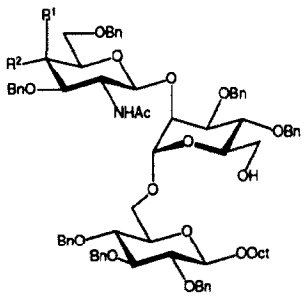


	R^1	R^2
46	H	OMe
47	OAc	H
48	H	F
49	H	N_3
50	H	H



	R^1	R^2
51	H	OMe
52	OH	H
53	H	F
54	H	N_3
55	H	H

D-*galacto* derivative **32**, which was only 42% (along with ~ 50% of **32** which was regenerated under the reaction conditions). Compound **38** was then converted to the reducing sugar **39** (68%).

Synthesis of protected trisaccharides.—Glycosyl chlorides (**16–20**) used to form trisaccharide derivatives were prepared from hemiacetals (**29**, **31**, **34**, **36**, and **39**) by reaction with *N*,*N*-dimethyl(chloromethylene)ammonium chloride[20] in almost quantitative yield. Glycosylation of disaccharide acceptor **15** with glycosyl donors **16–20** was performed in the presence of silver triflate and *s*-collidine to yield trisaccharides **40** (R^1 = H, R^2 = OMe; 76%), **41** (R^1 = OAc, R^2 = H; 41% along with 27% of α isomer **42**), **43** (R^1 = H, R^2 = F; 77%), **44** (R^1 = H, R^2 = N_3; 69%), and **45** (R^1 = R^2 = H; 81%). The anomeric configuration of these products were confirmed by their ^{1}H NMR data (see Experimental). In the case of the glycosidation of the GalNAc derivative, acetonitrile and nitromethane were examined as solvents (instead of dichloromethane) in an attempt to improve β-selectivity. These conditions, however, did not improve the $\alpha:\beta$-ratio. The 4-*O*-*p*-nitrobenzoyl-GalNAc derivative was also prepared from **27** by a procedure similar to that used for the synthesis of GalNAc donor **17**. Its use as a glycosyl donor, however, resulted in the preferential formation of the α-linked trisaccharide [$\alpha:\beta$ = 4:1, ^{1}H NMR data; α anomer: δ 6.03 (br s, 1 H, H-4″) and 5.30 (d, 1 H , $J_{1'',2''}$ 3.1 Hz, H-1″); β anomer: 5.96 (br d, 1 H, $J_{3'',4''}$ 3.2 Hz, H-4″) and 5.36 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1″).

Deprotection of trisaccharides.—Allyl, benzyl, and phthaloyl group removal were required to yield the target six trisaccharide derivatives **9–14**. Removal of allyl groups was achieved using palladium(II) chloride[18] yielding **46–50** in 58, 58, 62, 56, and 73% yields, respectively. The variation in yields was due to the formation of an oxidized byproduct[21,22] as a competing reaction.

Phthalimido groups are normally removed using hydrazine[23], and this reaction could also likely have been accomplished in the present deprotection sequence where the *O*-allyl groups were removed first. To retain flexibility, however, we decided not to use this reagent, which also causes reduction of *O*-allyl to *O*-propyl groups as a side reaction. Had modification to the OH-6 group of the α-D-Man*p* residue late in the reaction scheme been desired, a method for phthalimido group

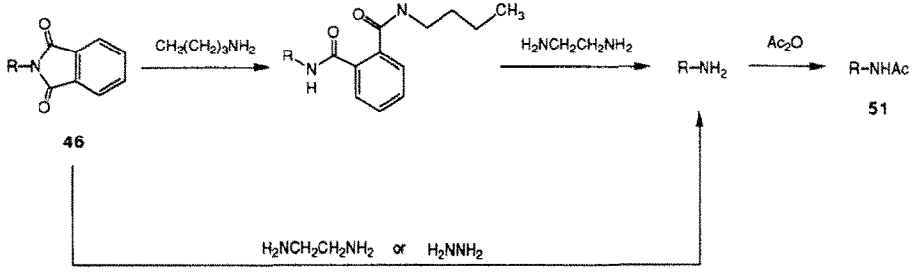


Fig. 3. Formation of a mixed amide during attempted removal of the phthalimido group from **46**.

removal compatible with alkenes would have to be used. Therefore, we selected the more generally applicable procedure[24] of butylamine in butanol. When these conditions were applied to **46**, the sole product obtained was the opened butyl–phthaloyl derivative (Fig. 3), the product of attack of butylamine at one of the phthalimide carbonyls (data not shown). Further prolonged heating could not complete the deprotection, and only the mixed diamide (Fig. 3) was observed. Reaction with 1,2-diaminoethane (ethylenediamine) proved very efficient and reproducible, however, and we propose the use of this reagent as a new and general method for phthalimido-group removal. When compounds **46–50** were treated with an excess ethylenediamine in butanol at 90°C followed by *N*-acetylation, **51–55** were isolated in 96, 67, 78, 96, and 75% yields, respectively. The stability of *O*-allyl ethers to these conditions was demonstrated in separate experiments not reported here.

Hydrogenation of **51–53** and **55** over 5% palladium-on-charcoal in ethanol smoothly removed the *O*-benzyl groups to give the four target compounds **9–11** and **13**. When the azide **54** was treated under the same conditions, however, both *N*-ethylated and *N,N*-diethylated products were observed. Carefully purified ethanol, which was redistilled from zinc–potassium hydroxide after refluxing (1 day)[25] was required as solvent in the presence of hydrochloric acid to protonate the amine. Purification of the crude product by high-performance liquid chromatography (HPLC) gave the amine **12** (52%). A sample of **12** was also *N*-acetylated to give **14**. Key ^{1}H and ^{13}C NMR data for **9–14** are presented in Table I.

Kinetic evaluation of acceptor analogues.—The octyl trisaccharide **5** is a known acceptor for GlcNAcT-V[8,9]. The hydrophobic octyl group in **5** furthermore permits the use of a very rapid enzyme assay procedure for GlcNAcT-V termed a "SepPak Assay"[26]. With the partially purified enzyme from hamster kidney used in the present study, **5** had a K_m of 0.034 mM (similar to previously observed values[6,10]) and a V_{max} of 796 pmol/h under standard assay conditions (see Table II and Experimental section). Only with high concentrations of **9–14**, and UDP-[^{3}H]GlcNAc of high specific activity could low, but significant, transfer of radioactivity to these compounds be detected. Because compounds **9–14** were so weakly active, it was difficult to distinguish whether indeed the transfer reaction was occurring in the expected manner. Either very small amounts of contaminating structures in **9–14** could have been responsible for the observed residual activities, or the modified acceptors might have been substrates for additional EDTA-insensitive enzymes present in our partially purified preparation of GlcNAcT-V. In fact, with these modified acceptors it was difficult to achieve either saturation of the enzyme or more than 1% turnover. The rate of transfer to these compounds was also decreased after 2 h. These observations suggested the presence of small amounts of contaminating acceptors in **9–14**. The possibility that this contaminant was **5** (potentially present since all of **9–14** ultimately came from the same GlcNAc-precursor **27** where the "normal" OH-4 group was present) was therefore assessed.

TABLE I

Selected chemical shifts δ and coupling constants (Hz) for compounds **9–14**

Compound	**9**	**10**	**11**	**12**	**13**	**14**
4″-substituent	OMe	epi OH	F	NH_2	H	NHAc
H-1 ($J_{1,2}$)	4.45(7.9)	4.46(7.8)	4.46(7.8)	4.46(7.8)	4.46(7.8)	4.46(7.8)
H-1′ ($J_{1',2'}$)	4.88(br s)	4.91(br s)	4.90(br s)	4.91(br s)	4.90(1.3)	4.89(br s)
H-1″ ($J_{1'',2''}$)	4.53(8.1)	4.52(8.6)	4.64(8.6)	4.55(8.2)	4.48(8.0)	4.58(8.2)
$OCOCH_3$	2.05	2.06	2.06	2.07	2.06	2.06, 203
CH_2CH_3	0.86	0.86	0.86	0.86	0.86	0.86
Other H	3.57(OCH_3)		4.41(ddd, 8.5, 9.2, 50.8 [a], H-4″)	2.75(t, 9.5, 9.5, H-4″)	2.03(br ddd, 5.0, 12.5, 1.1, H-4″*eq*), 1.47 (br q, H-4″*ax*)	
C-1	102.26	103.16	102.28	102.26	103.18	103.24
C-1′	99.33	100.86	99.24	99.62	100.86	100.32
C-1″	96.72	97.74	96.71	96.78	97.76	97.72
C-2″	55.33	52.20	54.88(5.5 [a])	55.74	57.75	56.81
$COCH_3$	174.76	174.40	174.79	174.76	175.75	174.75, 175.61
$COCH_3$	22.24	23.22	22.23	22.25	23.21	23.24
CH_2CH_3	13.35	14.23	13.34	13.35	14.23	14.32
Other C	60.23(OCH_3)		89.18(143.2 [a], C-4″)	52.81(C-4″)	35.21(C-4″)	52.98(C-4″)

[a] Coupling constant between fluorine atom and corresponding nucleus.

Acceptor **5** was first reacted with GalT and UDP-Gal to produce **7**, which was confirmed to be inactive as an acceptor for GlcNAcT-V in the present assays. Each of compounds **9–14** was treated similarly with GalT, and the resulting products were evaluated as acceptors for GlcNAcT-V. The rationale for these experiments was that if **5** was the sole contaminant responsible for the residual activity in **9–14**, then this "pretreatment" should render solutions of the analogues completely inactive. Decreases in activity of between 55 and 95% were indeed observed after the galactosylation reactions, but the activities were not completely abolished. If these decreases in activity were in fact due to the presence of contaminating **5**, then the level of contamination could be estimated from the kinetic parameters for

TABLE II

Kinetic parameters obtained for GlcNAcT-V acceptors, derived from rate data as described in the Experimental section

Acceptor	K_m (mM) [a]	V_{max} (pmol/h) [a]	V/K
5	0.043 ± 0.008	796 ± 30	18500
9	3.5 ± 1	15 ± 2	4.3
10	3.9 ± 0.5	90 ± 7	23
11	4.4 ± 0.4	86 ± 4	20
12	6 ± 2	100 ± 20	17
13	13.09 ± 0.04	109.8 ± 0.02	8.4
14	1.4 ± 0.2	7.6 ± 0.4	5.4

[a] $\pm$ Standard errors.

5 and those for analogues **9–14** (Table II), to be under 1%. Had contaminating **5** been the sole acceptor substrate in **9–14**, the V_{max} values for all the compounds should also have been the same, assuming no inhibition by any of the acceptors or products formed. Since the V_{max} values differ over 100-fold for GalT-treated acceptors (Table II), the presence of **5** cannot be solely responsible for the observed very weak activities. The Michaelis–Menten parameters derived for **5** and **9–14** are shown in Table II. These parameters show all six analogues of **5**, modified at OH-4", to be extremely poor acceptors with K_m values between 30 and 300-fold higher than **5** and V_{max} values between 7 and 105-fold slower. V/K values for these analogues, indicators of enzyme efficiency, were between 0.12 and 0.02% of the value obtained for **5**. The OH-4 group of the GlcNAc residue in **5** (and, by inference, also in the natural glycopeptide acceptor **1**) is therefore a critical recognition element for this enzyme active site.

The residual activities of **9–14** were so low that we consider the distinction between "extremely poor acceptors" and "non-acceptors" to border on semantics for the purpose of the present study. This study therefore concludes that the combining site of GlcNAcT-V extends at least from the reactive OH-6 of the α-D-Man residue to OH-4 of the non-reducing GlcNAc residue. Galactosylation of **1** (and **5**) destroys the acceptor activity of these compounds, not by introducing the steric bulk of an additional sugar residue, but by destroying a critical H-bonding interaction (or network) between the OH-4 group and the enzyme combining site. This OH-4 group of the terminal GlcNAc residue, according to the classification of Lemieux[28], can therefore be designated as a key polar group in the recognition of substrates by GlcNAcT-V.

EXPERIMENTAL

General methods.—TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by quenching of fluorescence, by charring with H_2SO_4, and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40–63 μm). HPLC was performed by using a Waters model 590 pump, model U6K injector, and model 481 UV detector. C_{18} Sep-Pak sample-preparation cartridges were from Waters Associates. Millex-GV (0.22 mm) filter units were from Millipore. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at $22 \pm 2°$. Melting points were measured with Fisher–Johns melting point apparatus. IR spectra were recorded with a Nicolet SX-20 FTIR by the spectral services laboratory of the Chemistry Department. ^1H NMR spectra were recorded at 300 MHz (Bruker AM 300), at 360 MHz (Bruker WM 360), or at 500 MHz (Varian UNITY 500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0), C_6D_6 (internal Me_4Si, δ 0) or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75 MHz, at 90 MHz, or at 125 MHz respectively, on the same instruments 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. The assignments of ^{13}C resonances are

tentative. FAB-mass spectra (FABMS) were obtained on a Kratos AEIMS9 instrument by the departmental microanalytical laboratory. Elemental analyses were carried out on a Carlo Erba EA1108.

Materials and methods.—6-*O*-Acetyl-3,4-di-*O*-benzyl-1,2-methoxyethylidene-β-D-mannopyranose (**21**)[11], octyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**25**)[8], allyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**27**)[8], allyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**32**)[15], and 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl chloride (**17**)[15] were prepared by literature methods. Ethylenediaminetetraacetic acid (EDTA), 2-[*N*-morpholino] ethanesulfonic acid (MES), uridine 5′-diphosphate (UDP), UDP-GlcNAc, bovine serum albumin, and bovine galactosyltransferase (EC 2.4.1.22) were obtained from Sigma Chemical Co. Triton X-100 was from Calbiochem or Sigma. Liquid scintillation cocktail was from ICN Biomedicals, Inc. [Ecolite(+)]. UDP-[4,5-^{3}H(N)]galactose was from NEN Dupont. UDP-[6-^{3}H(N)]GlcNAc was obtained from American Radiolabelled Chemicals, Inc. In order to reduce background values obtained in radioassays, this material was lyophilized, passed through a C_{18} SepPak cartridge pre-equilibrated with MeOH and water, and then relyophilized and dissolved in 70:30 EtOH–water for use. GlcNAcT-V was detergent-extracted from hamster kidneys and refined by affinity chromatography as previously described[6].

Synthesis of the disaccharide acceptor.

6-O-Allyl-3,4-di-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranose (**23**). —6-*O*-Acetyl-3,4-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (**21**; 3.39 g, 7.39 mmol) was treated with NaOMe (100 mg) in dry methanol (100 mL) for 1 h. TLC [R_f 0.24 (2:1 hexane–EtOAc)] indicated complete conversion into the corresponding alcohol (**22**) which was not further characterized. The mixture was taken to dryness and the residue was dissolved in DMF (20 mL) and NaH (50% oil suspension, 532 mg, 11.1 mmol) was added. After stirring for 30 min, allyl bromide (0.83 mL, 9.6 mmol) was added to the mixture, which was then stirred at room temperature for 14 h. Methanol was next added to destroy the excess NaH, then the mixture was concentrated to dryness. The resulting residue was dissolved in CH_2Cl_2 and washed with water, and the organic solution was then dried (Na_2SO_4) and concentrated to give a syrup. Compound **23** was crystallized from hexane–ether (1.82 g, 53.9%). The mother liquor was purified by column chromatography using 2:1 hexane–EtOAc yielding more **23** (1.04 g, total 84.7%); R_f 0.45 (2:1 hexane–EtOAc); $[\alpha]_D$ + 7.0° (*c* 1.6 $CHCl_3$); mp 71–72°C. NMR data ($CDCl_3$): ^{1}H (300 MHz), δ 7.43–7.26 (m, 10 H, Ar), 5.88 (m, 1 H, CH_2=C*H*CH_2O), 5.33 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 5.27 and 5.15 (2 m, each 1 H, C*H*$_2$=CHCH_2O), 4.94 and 4.67 (2 d, each 1 H, J_{gem} 10.9 Hz, PhC*H*), 4.78 (s, 2 H, PhC*H*), 4.39 (dd, 1 H, $J_{2,3}$ 3.9 Hz, H-2), 4.06 and 3.99 (2 m, each 1 H, CH_2=CHC*H*$_2$O), 3.90 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.72 (dd, 1 H, H-3), 3.70 (dd, 1 H, $J_{5,6a}$ 4.3, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.66 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 3.38 (ddd, 1 H, H-5), 3.29 (s, 3 H, C*H*$_3$O), 1.75 [s, 3 H, C*H*$_3$C(OR)$_3$]; ^{13}C (75 MHz), δ 123.98 [CH_3C(OR)$_3$], 116.65

(CH_2=CH-CH_2O), 97.57 (C-1), 49.73 (CH_3O), and 24.41 [$CH_3C(OR)_3$]. Anal. Calcd for $C_{26}H_{32}O_7$: C, 68.40; H, 7.06. Found: C, 68.19; H, 7.18.

Octyl 6-O-(2-O-acetyl-6-O-allyl-3,4-di-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**26**).—To a solution of **23** (1.23 g, 2.7 mmol) in dry CH_2Cl_2 (30 mL), chlorotrimethylsilane (3.4 mL, 27 mmol) was added at 5°C, and the solution was stirred for 10 min. Concentration, followed by co-evaporation with toluene, left a syrup that consisted of one major product (**24**) [R_f 4.9 (8:1 toluene–EtOAc)], which was not further purified nor characterized. To a solution of octyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**25**; 750 mg, 1.33 mmol) in dry CH_2Cl_2 (10 mL) were added silver trifluoromethanesulfonate (907.4 mg, 3.5 mmol), *N*,*N*,*N*′,*N*′-tetramethylurea (0.43 mL, 3.5 mmol), and 4A molecular sieves (1 g). To the resulting mixture was added dropwise a solution of the glycosyl chloride **24** (~2.7 mmol) in dry CH_2Cl_2 (10 mL) at −5°C. The mixture was allowed to warm to room temperature. After 14 h of stirring, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$, and water, and then dried over Na_2SO_4, filtered, and concentrated. The residual oil was purified by column chromatography using 4:1 hexane–EtOAc as eluent to provide **26** (1.25 g, 95%); R_f 4.9 (8:1 toluene–EtOAc); $[\alpha]_D$ + 15° (*c* 0.3, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (300 MHz), δ 7.40–7.18 (m, 15 H, Ar), 5.88 (m, 1 H, CH_2=C*H*CH_2O), 5.43 (dd, 1 H, $J_{1',2'}$ 1.7, $J_{2',3'}$ 2.9 Hz, H-2′), 5.25 and 5.14 (2 m, each 1 H, CH_2=$CHCH_2O$), 4.96, 4.95, 4.90, 4.86, 4.77, 4.72 × 2, 4.57, 4.52, and 4.50 (9 d, 10 H, J_{gem} 10.9 Hz, PhCH_2), 4.89 (d, 1 H, H-1′), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.06 (m, 1 H, one of CH_2=CHCH_2O), 2.15 (s, 3 H, CH_3CO), 0.86 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75 MHz), δ 170.36 (CO), 116.92 (CH_2=$CHCH_2O$), 103.52 ($^1J_{C,H}$ 160.8 Hz, C-1′), 97.90 ($^1J_{C,H}$ 171.1 Hz, C-1′), 63.24 (C-2′), 21.13 (CH_3CO), and 14.11 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{60}H_{74}O_{12}$: C, 73.00; H, 7.56. Found: C, 73.12; H, 7.59.

Octyl 6-O-(6-O-allyl-3,4-di-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**15**).—Compound **26** (1.2 g, 1.22 mmol) was treated with methanolic NaOMe (10%, 200 mL) at room temperature for 6 h. Neutralization with Amberlite IR-120 (H^+) resin, filtration of the resin, and evaporation left a syrup which was purified by column chromatography using 3:1 hexane–EtOAc as eluent to provide **15** (1.1 g, 96%); R_f 0.33 (4:1 toluene–EtOAc); $[\alpha]_D$ + 25° (*c* 1, $CHCl_3$); mp 67–68°C NMR data ($CDCl_3$): ^{1}H (300 MHz), δ 7.42–7.18 (m, 25 H, Ar), 5.87 (m, 1 H, CH_2=C*H*CH_2O), 5.24 and 5.13 (2 m, each 1 H, CH_2=$CHCH_2O$), 4.97 (br s, 1 H, H-1′), 4.35 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.08 (br s, partially overlapped with one of CH_2=CHCH_2O, H-2′), 4.04 and 3.92 (2 m, each 1 H, CH_2=CHCH_2O), 2.39 (br, 1 H, OH), and 0.87 [t, 1 H, $CH_3(CH_2)_7O$]; ^{13}C (75 MHz), δ 116.98 (CH_2=CH-CH_2O), 103.58 (C-1′), 99.56 (C-1), and 14.10 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{58}H_{72}O_{11}$: C, 73.70; H, 7.68. Found: C, 73.88; H, 7.59.

Syntheses of the monosaccharide derivatives.

Allyl 3,6-di-O-benzyl-2-deoxy-4-O-methyl-2-phthalimido-β-D-glucopyranoside (**28**).—Borontrifluoride etherate in dry CH_2Cl_2 (1/100 v/v, 1 mL) was added dropwise

with stirring to a solution of **27** (960 mg, 1.81 mmol) in dry CH_2Cl_2 (12 mL) at 0–5°C, followed by the addition of ~1% diazomethane in dry CH_2Cl_2 until a yellow color persisted. The periodic addition of the borontrifluoride solution and diazomethane solution were continued until TLC showed the conversion of the starting material into a single product. The reaction mixture was filtered to remove a white solid that was washed with CH_2Cl_2. The combined filtrate was washed successively with satd $NaHCO_3$ and water, and dried (Na_2SO_4). Evaporation of the solvent left a syrup which was purified by column chromatography using 2:1 hexane–EtOAc, to give **28** (700 mg, 71.0%); R_f 0.50 (2:1 hexane–EtOAc), $[\alpha]_D$ + 6.8° (*c* 0.9, $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.85–6.82 (m, 14 H, Ar), 5.67 (m, 1 H, CH_2=C*H*-CH_2O), 5.15 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 5.08 and 4.99 (2 m, each 1 H, CH_2=CH-CH_2O), 4.80 and 4.45 (2 d, each 1 H, PhC*H*), 4.70 and 4.61 (2 d, each 1 H, PhC*H*), 4.24 and 3.99 (2 m, each 1 H, CH_2=CH-CH_2O), 4.24 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 7.8 Hz, H-3), 4.16 (dd, 1 H, H-2), 3.81 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.77 (dd, 1 H, $J_{5,6b}$ 3.7 Hz, H-6b), 3.55 (ddd, 1 H, $J_{4,5}$ 10.0 Hz, H-5), 3.54 (s, 3 H, CH_3O), and 3.46 (dd, 1 H, H-4); ^{13}C (75.5 MHz), δ 168.10 and 167.80 (CO), 117.22 (*C*H_2=CH-CH_2O), 97.34 (C-1), 60.63 (*C*H_3O), and 55.79 (C-2). Anal. Calcd for $C_{31}H_{33}NO_7$: C, 70.70; H, 6.12; N, 2.58. Found: C, 70.89; H, 5.72; N, 2.50.

3,6-Di-O-benzyl-2-deoxy-4-O-methyl-2-phthalimido-α,β-D-glucopyranose (**29**).—A solution of **28** (100 mg, 0.18 mmol), tris(triphenylphosphine)rhodium(I) chloride (25 mg, 0.03 mmol) and 1,8-diazabicyclo[2.2.2]-octane (9 mg, 0.08 mmol) in 7:3:1 EtOH–benzene–water (7 mL) was heated at reflux for 30 h. The solvent was removed, and the residue was dissolved in acetone (3 mL) containing mercuric oxide (1 mg, 0.005 mmol). To this solution was added a solution of mercuric chloride (300 mg, 6.01 mmol) in 9:1 acetone–water (3 mL), and the mixture was stirred for 2 h. Following solvent evaporation the residue was dissolved in CH_2Cl_2 and washed with 30% KI, followed by water. Evaporation left a residue which was purified by column chromatography using 3:2 hexane–EtOAc as eluent, to give **29** (77.8 mg, 84.0%); R_f 0.23 (2:1 hexane–EtOAc). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.78–6.82 (m, 14 H, Ar), 5.34 (dd, 1 H, $J_{1,2}$ 8.7, $J_{1,OH}$ 7.7 Hz, H-1), 4.79 and 4.44 (2 d, each 1 H, PhC*H*), 4.65 and 4.55 (2 d, each 1 H, PhC*H*), 4.28 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 8.6 Hz, H-3), 4.06 (dd, 1 H, H-2), 3.74–3.70 (m, 2 H, H-6s), 3.68 (d, 1 H, OH), 3.60 (dt, 1 H, $J_{4,5}$ 10.0, $J_{5,6a} = J_{5,6b}$ = ~3.5 Hz, H-5), 3.51 (s, 3 H, CH_3O), and 3.45 (dd, 1 H, H-4); ^{13}C (75.5 MHz), δ 92.68 (C-1), 60.46 (*C*H_3O), and 57.28 (C-2).

Allyl 3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-2-phthalimido-β-D-glucopyranoside (**33**).—Diethylaminosulfur trifluoride (0.61 mL, 4.53 mmol) was added to a solution of **32** (485 mg, 0.92 mmol) at −10°C, and the reaction mixture was allowed to warm to room temperature. After stirring for 14 h, the solution was cooled to 0°C, and MeOH (1 mL) was added, then evaporated. The residue was then purified by column chromatography using 3:1 hexane–EtOAc as eluent to give **33** (360 mg, 73.9%); R_f 0.50 (2:1 hexane–EtOAc); $[\alpha]_D$ + 61.5° (*c* 0.2, $CHCl_3$). NMR data

($CDCl_3$): ^1H (300 MHz), δ 7.82–6.89 (m, 14 H, Ar), 5.68 (m, 1 H, CH_2=C*H*-CH_2O), 5.19 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.10 and 5.02 (2 m, each 1 H, CH_2=CH-CH_2O), 4.80 and 4.45 (2 d, each 1 H, PhC*H*), 4.68 and 4.64 (2 d, each 1 H, PhC*H*), 4.64 (ddd, 1 H, $J_{3,4}$ 8.1, $J_{4,5}$ 9.3, $^2J_{4,F}$ 50.1 Hz, H-4), 4.40 (ddd, 1 H, $J_{2,3}$ 10.2, $^3J_{3,F}$ 15.1 Hz, H-3), 4.26 and 4.01 (2 m, each 1 H, CH_2=CH-C*H*$_2O$), 4.21 (ddd, 1 H, $^4J_{2,F}$ 1.1 Hz, H-2), and 3.88–3.71 (m, 3 H, H-5 and H-6s); ^{13}C (90 MHz), δ 117.49 (CH_2=CH-CH_2O), 97.25 (C-1), 91.63 ($^1J_{C,F}$ 183.1 Hz, C-4), 75.99 ($^2J_{C,F}$ 17.1 Hz, C-3) 73.23 ($^2J_{C,F}$ 24.4 Hz, C-5), and 55.03 ($^3J_{C,F}$ 9.8 Hz, C-2).

3,6-Di-O-benzyl-2,4-dideoxy-4-fluoro-2-phthalimido-α,β-D-glucopyranose (**34**).—Compound **33** (342 mg, 0.643 mmol) was treated with tris(triphenylphosphine)rhodium(I) chloride (75 mg, 0.08 mmol) and 1,8-diazabicyclo[2.2.2]octane (27 mg, 0.12 mmol) in 7:3:1 EtOH–benzene–water (20 mL), followed by mercuric oxide (3 mg, 0.015 mmol) and mercuric chloride (800 mg, 17.23 mmol) in 9:1 acetone–water (20 mL) as described for the preparation of **29**. The resulting residue was purified by column chromatography using 3:2 hexane–EtOAc as eluent, to give **34** (191 mg, 60.4%); R_f 0.11 (8:1 toluene–EtOAc). ^1H NMR data ($CDCl_3$, 300 MHz), δ 7.85–6.89 (m, 14 H, Ar), 5.37 (dd, 1 H, $J_{1,2}$ 8.6, $J_{1,OH}$ 7.5 Hz, H-1), 4.80 and 4.44 (2 d, each 1 H, PhC*H*), 4.64 and 4.59 (2 d, each 1 H, PhC*H*), 4.63 (ddd, 1 H, $J_{3,4}$ 8.3, $J_{4,5}$ 9.7, $^2J_{4,F}$ 50.6 Hz, H-4), 4.46 (ddd, 1 H, $J_{2,3}$ 10.6, $^3J_{3,F}$ 15.5 Hz, H-3), 4.12 (ddd, 1 H, $^4J_{2,F}$ 1.1 Hz, H-2), 3.87–3.68 (m, 3 H, H-5 and H-6s), and 3.58 (d, 1 H, OH). Anal. Calcd for $C_{28}H_{26}FNO_6$: C, 68.42; H, 5.33; N, 2.85. Found: C, 68.64; H, 5.33; N, 2.85.

Allyl 4-azido-3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-glucopyranoside (**35**).—To a solution of **32** (691 mg, 1.31 mmol) in dry CH_2Cl_2 (10 mL) and dry pyridine (0.55 mL) was added dropwise a solution of trifluoromethanesulfonic anhydride (0.53 mL, 3.2 mmol) in dry CH_2Cl_2 (1 mL) at −5°C. The reaction mixture allowed to come to room temperature and was then stirred for 30 min, diluted with CH_2Cl_2 (10 mL) and washed with 0.5% HCl and water. Evaporation left a foam [R_f 0.51 (2:1 hexane–EtOAc)], presumably the triflate of **32**. The foam was dissolved in dry DMF (2 mL), and then sodium azide (424 mg, 6.52 mmol) was added. The resulting mixture was stirred at room temperature for 6 h. Solvent was evaporated, and the residue was suspended in CH_2Cl_2 and washed with 0.5% HCl, satd $NaHCO_3$, then water. Solvent removal left a syrup which was purified by column chromatography using 3:1 hexane–EtOAc as eluent, to give **35** (630 mg, 87%) as a syrup; R_f 0.59 (2:1 hexane–EtOAc); $[\alpha]_D$ + 8.8° (*c* 2.2, $CHCl_3$). FTIR ($CDCl_3$): 2109 (N_3) and 1713 cm^{-1} (CO); NMR data: ^1H (C_6D_6, 300 MHz), δ 7.43–6.65 (m, 14 H, Ar), 5.58 (m, 1 H, CH_2=C*H*-CH_2O), 5.29 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.00 and 4.81 (2 m, each 1 H, CH_2=CH-CH_2O), 4.75 and 4.40 (2 d, each 1 H, PhC*H*), 4.65 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 4.45 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 4.43 and 4.38 (2 d, each 1 H, PhC*H*), 4.14 and 3.86 (2 m, each 1 H, CH_2=CH-C*H*$_2O$), 3.63 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 3.56 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.53 (dd, 1 H, $J_{5,6b}$ 3.9 Hz H-6b), and 3.21 (ddd, 1 H, H-5); ^{13}C ($CDCl_3$, 90 MHz), δ 117.41 (CH_2=CH-CH_2O), 97.27 (C-1), 63.55 (C-4), and 55.65 (C-2).

4-Azido-3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-α,β-D-glucopyranose (**36**).—To a solution of compound **35** (223.7 mg, 0.40 mmol), a mixture of palladium(II) chloride (213 mg, 1.20 mmol) and NaOAc (197 mg, 2.4 mmol) in 20:1 acetic acid–water (10 mL), which was previously degassed and flushed with Ar, was added under Ar at room temperature. The reaction mixture was stirred at room temperature for 48 h, then toluene (50 mL) and water (50 mL) were added, and the resulting mixture was vigorously stirred for 30 min. After filtration of insoluble materials, the organic phase was separated, and the aqueous solution was extracted with toluene four times. The organic extracts were combined and concentrated to give a syrup which was purified by column chromatography using 5:1 toluene–EtOAc, to yield **36** (159 mg, 76.6%; R_f 0.3 (2:1 hexane–EtOAc); mp 126–128°C. ^{1}H NMR data ($CDCl_3$, 360 MHz), δ 7.82–6.85 (m, 14 H, Ar), 5.30 (dd, 1 H, $J_{1,2} = J_{1,OH} = 8.3$ Hz, H-1), 4.86 and 4.44 (2 d, each 1 H, PhC*H*), 4.68 and 4.59 (2 d, each 1 H, PhC*H*), 4.33 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 4.12 (dd, 1 H, H-2), 3.85–3.72 (m, 3 H, H-4 and H-6s), 3.55 (ddd, 1 H, $J_{4,5}$ 10.2, $J_{5,6a}$ 2.3, $J_{5,6b}$ 3.8 Hz, H-5), and 3.26 (d, 1 H, OH). Anal. Calcd for $C_{28}H_{26}N_4O_6$: C, 65.36; H, 5.09; N, 10.89. Found: C, 65.10; H, 5.11; N, 10.82.

Allyl 3,6-di-O-benzyl-2-deoxy-4-O-pentafluorophenoxythionocarbonyl-2-phthalimido-β-D-glucopyranoside (**37**).—Pentafluorophenylchlorothionoformate (192.5 μL, 1.20 mmol) was added with stirring to a solution of **27** (210.9 mg, 0.40 mmol) in dry CH_2Cl_2 (15 mL) containing 4-(dimethylamino)pyridine (243 mg, 1.99 mmol) at 0°C. The reaction temperature was allowed to increase to room temperature, and after 14 h the reaction mixture was extracted with CH_2Cl_2. The extract was washed with 0.5% HCl, satd $NaHCO_3$ and water, then dried (Na_2SO_4), and concentrated to a syrup, which was purified by column chromatography using 3:1 hexane–EtOAc to yield **37** (262.2 mg, 68.8%); R_f 0.55 (8:1 toluene–EtOAc); $[\alpha]_D$ + 8.6° (*c* 2.5 $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (360 MHz), δ 7.82–6.80 (m, 14 H, Ar), 5.65 (dd, 1 H, $J_{3,4}$ 7.6, $J_{4,5}$ 9.7 Hz, H-4), 5.62 (m, 1 H, CH_2=C*H*-CH_2O), 5.18 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 5.03 and 4.96 (2 m, each 1 H, CH_2=CH-CH_2O), 4.69 and 4.26 (2 d, each 1 H, PhC*H*), 4.55 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 4.53 (s, 2 H, PhC*H*), 4.25 (dd 1 H, H-3), 4.19 and 3.95 (2 m, each 1 H, CH_2=CH-CH_2O), 3.80 (ddd, 1 H, $J_{5,6a}$ 3.1, $J_{5,6b}$ 5.6 Hz, H-5), 3.70 (dd, 1 H, $J_{6a,6b}$ 11.4 Hz, H-6a), and 3.60 (dd, 1 H, H-6b); ^{13}C (125 MHz), δ 191.10 (C=S), 117.62 (CH_2=CH-CH_2O), 97.23 (C-1), 55.35 (C-2). Anal. Calcd for $C_{38}H_{30}F_5NO_8S$: C, 60.40; H, 4.00; N, 1.85; S, 4.24. Found: C, 60.41; H, 3.88; N, 1.91; S, 4.39.

Allyl 3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-xylo-hexopyranoside (**38**).—To a solution of **37** (218.7 mg, 0.23 mmol) in toluene (15 mL), tributyltin hydride (307.7 μL, 1.14 mmol) and azobis(isobutyronitrile) (AIBN) (18.8 mg, 0.11 mmol) were added under Ar at room temperature. The mixture was refluxed for 4 h at 130°C (bath temp.). Evaporation left a syrup, which was dissolved in MeOH, treated with Amberlite IR-410 (OH^-) resin to remove the pentafluorophenol, washed with MeOH, filtered, and concentrated. The resulting syrup was then purified by column chromatography using 3:1 hexane–EtOAc as eluent, to give **38**

(108.6 mg, 92.4%); R_f 0.48 (2:1 hexane–EtOAc); $[\alpha]_D$ +3.9° (*c* 1.4, $CHCl_3$). NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.85–7.00 (m, 14 H, Ar), 5.70 (m, 1 H, CH_2=C*H*-CH_2O), 5.15 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 5.11 and 5.01 (2 m, each 1 H, CH_2=CH-CH_2O), 4.61 (s, 2 H, PhCH_2), 4.59 and 4.29 (2 d, each 1 H, PhC*H*), 4.30 (dt, 1 H, $J_{2,3} = J_{3,4ax} = 10.2$, $J_{3,4eq}$ 5.5 Hz, H-3), 4.26 and 4.02 (2 m, each 1 H, CH_2=CH-CH_2O), 4.14 (dd, 1 H, H-2), 3.80 (dddd, 1 H, $J_{4ax,5}$ 10.5, $J_{4eq,5} \sim 1$, $J_{5,6a}$ 5.7, $J_{5,6b}$ 5.1 Hz, H-5), 3.68 (dd, 1 H, $J_{6a,6b}$ 10.1 Hz, H-6a), 3.58 (dd, 1 H, H-6b), 2.30 (ddd, 1 H, H-4*eq*), and 1.56 (q, 1 H, H-4*ax*). Anal. Calcd for $C_{31}H_{31}NO_6$: C, 72.50; H, 6.08; N, 2.73. Found: C, 72.57; H, 5.99 N, 2.70.

*3,6-Di-*O*-benzyl-2,4-dideoxy-2-phthalimido-*D*-xylo-hexopyranose* (**39**).—Compound **38** (95.8 mg, 0.19 mmol) was treated with a mixture of palladium(II) chloride (101 mg, 0.57 mmol) and NaOAc (92 mg, 1.12 mmol) in 20:1 acetic acid–water (5 mL) as described for the preparation of **36**. Then the resulting residue was purified by column chromatography using 5:1 toluene–EtOAc to yield **39** (61.3 mg, 67.9%); R_f 0.05 (2:1 hexane–EtOAc). ^1H NMR data ($CDCl_3$, 360 MHz), δ 7.85–6.96 (m, 14 H, Ar), 5.34 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.57 and 4.53 (2 d, each 1 H, PhC*H*), 4.56 and 4.28 (2 d, each 1 H, PhC*H*), 4.37 (dt, 1 H, $J_{2,3} = J_{3,4ax} = 10.3$, $J_{3,4eq}$ 4.8 Hz, H-3), 4.03 (dd, 1 H, H-2), 3.83 (m, 1 H, H-5), 3.62 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.51 (dd, 1 H, $J_{5,6b}$ 4.3 Hz, H-6b), 2.23 (ddd, 1 H, $J_{4ax,4eq}$ 12.3, $J_{4eq,5}$ 1.5 Hz, H-4*eq*), and 1.55 (q, 1 H, $J_{4ax,5}$ 12.2 Hz, H-4*ax*).

Preparation of donors.

*3,6-Di-*O*-benzyl-2-deoxy-4-*O*-methyl-2-phthalimido-α,β-*D*-glucopyranosyl chloride* (**16**).—A solution of 0.1 M *N,N*-dimethyl(chloromethylene)ammonium chloride in $CHCl_3$ (6.3 mL, 0.63 mmol) was added to a chloroform solution of **29** (107 mg, 0.21 mmol) at room temperature. After stirring for 1 h, the mixture was diluted with CH_2Cl_2, and washed with 0.5% HCl, satd $NaHCO_3$ and water, then dried and concentrated to a syrup [R_f 0.57 and 0.51 (8:1 toluene–EtOAc)], which was not further purified and was used for the next glycosylation reaction. ^1H NMR data ($CDCl_3$, 360 MHz), δ 6.21 (d, 0.46 H, $J_{1,2}$ 3.9 Hz, H-1 α), and 5.95 (d, 0.54 H, $J_{1,2}$ 9.2 Hz, H-1 β).

*3,6-Di-*O*-benzyl-2,4-dideoxy-4-fluoro-2-phthalimido-α,β-*D*-glucopyranosyl chloride* (**18**).—Compound **34** (106.4 mg, 0.22 mmol) was treated with 0.1 M *N,N*-dimethyl(chloromethylene)ammonium chloride solution in $CHCl_3$ (6.5 mL, 0.65 mmol) in the same manner as described for the preparation of **16**; R_f 0.55 and 0.52 (2:1 hexane–EtOAc). ^1H NMR data ($CDCl_3$, 360 MHz), δ 6.21 [m (second order), 0.45 H, $J_{1,2}$ 3.4 Hz, H-1 α] and 5.96 (m, 0.55 H, $J_{1,2}$ 8.9 Hz, H-1 β).

*4-Azido-3,6-di-*O*-benzyl-2,4-dideoxy-2-phthalimido-α,β-*D*-glucopyranosyl chloride* (**19**).—Compound **36** (131.8 mg, 0.26 mmol) was treated with 0.1 M *N,N*-dimethyl(chloromethylene)ammonium chloride in $CHCl_3$ (7.8 mL, 0.78 mmol) in a same manner as described for the preparation of **16**; R_f 0.77 and 0.61 (7:1 toluene–acetone). ^1H NMR data ($CDCl_3$, 360 MHz), δ 6.20 (d, 0.46 H, $J_{1,2}$ 3.8 Hz, H-1α), 5.92 (d, 0.54 H, $J_{1,2}$ 9.0 Hz, H-1β).

*3,6-Di-O-benzyl-2,4-dideoxy-2-phthalimido-α,β-*D-xylo*-hexopyranosyl chloride* (**20**).—Compound **39** (50 mg, 0.10 mmol) was treated with 0.1 M *N,N*-dimethyl(chloromethylene)ammonium chloride in $CHCl_3$ (3.8 mL, 0.38 mmol) in a same manner as described for the preparation of **16**: R_f 0.67 and 0.58 (2:1 hexane–EtOAc). ^{1}H NMR data ($CDCl_3$, 360 MHz), δ 6.33 (d, 0.3 H, $J_{1,2}$ 3.6 Hz, H-1 α), 5.98 (d, 0.7 H, $J_{1,2}$ 9.0 Hz, H-1 β).

Synthesis of protected trisaccharides.

*Octyl 6-O-[6-O-allyl-3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2-deoxy-4-O-methyl-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-2,3,4-tri-O-benzyl-β-*D*-glucopyranoside* (**40**).—To a solution of **15** (160 mg, 0.17 mmol) in dry CH_2Cl_2 (1 mL) were added silver trifluoromethanesulfonate (71.3 mg, 0.275 mmol), collidine (54.5 μL, 0.41 mmol), and 4A molecular sieves (300 mg). To the resulting mixture was added dropwise a solution of the glycosyl chloride **16** (~0.21 mmol) in dry CH_2Cl_2 (1 mL) at −40°C. The mixture was allowed to warm to room temperature. After 14 h of stirring excess tetraethylammonium chloride was added, and the mixture stirred for another 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$ and water, and then dried over Na_2SO_4, filtered, and concentrated. The residual oil was purified by column chromatography using a toluene–EtOAc gradient (20:1 → 4:1) as eluent to provide **40** (184 mg, 76%); R_f 0.58 (4:1 toluene–EtOAc); $[\alpha]_D$ + 9.6° (*c* 0.5, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (360 MHz), δ 7.80–6.86 (m, 39 H, Ar), 5.52 (m, 1 H, CH_2=C*H*-CH_2O), 5.26 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.20 (br t, $J_{1',2'} \approx J_{2',3'}$ = 2.1 Hz, H-2′), 3.56 (s, 3 H, CH_3O), and 0.88 [t, 3 H, CH_3(CH_2)$_7$O]; ^{13}C (90 MHz), δ 116.07 (*C*H_2=CH-CH_2O), 103.74 (C-1), 97.57 (C-1′), 96.44 (C-1″), 60.61 (*C*H_3O), 55.57 (C-2″), and 14.10 [*C*H_3(CH_2)$_7$O]. Anal. Calcd for $C_{87}H_{99}NO_{17}$: C, 73.04; H, 6.97; N, 0.98. Found: C, 72.72; H, 7.22; N, 1.04.

*Octyl 6-O-[2-O-(4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-6-O-allyl-3,4-di-O-benzyl-α-*D*-mannopyranosyl]-2,3,4-tri-O-benzyl-β-*D*-glucopyranoside* (**41**).—Glycosylation of **15** (141.3 mg, 0.15 mmol) using the glycosyl chloride **17** (~0.184 mmol) was carried out in the presence of silver trifluoromethane sulfonate (65.8 mg, 0.254 mmol), collidine (50.2 μL, 0.38 mmol), and 4A molecular sieves (500 mg) in dry CH_2Cl_2 (3 mL) as described for the preparation of **40**. After workup, the residual oil was purified by column chromatography using a toluene–EtOAc gradient (20:1 → 4:1) as eluent to provide **41** (89.2 mg, 41%); R_f 0.41 (8:1 toluene–EtOAc); $[\alpha]_D$ + 5.7° (*c* 1.1, $CHCl_3$). Then eluted the α isomer **42** (59.7 mg, 27.4%); R_f 0.48 (8:1 toluene–EtOAc); $[\alpha]_D$ + 78.6° (*c* 1.2, $CHCl_3$).

Compound **41**: NMR data ($CDCl_3$): ^{1}H (360 MHz), δ 7.84–6.89 (m, 39 H, Ar), 5.60 (dd, 1 H, $J_{3'',4''}$ 2.8, $J_{4'',5''}$ < 1 Hz, H-4″), 5.53 (m, 1 H, CH_2=C*H*-CH_2O), 5.28 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1″), 4.20 (br t, $J_{1',2'} \approx J_{2',3'}$ 2.1 Hz, H-2′), 2.14 (s, 3 H, CH_3CO), and 0.85 [t, 3 H, CH_3(CH_2)$_7$O]; ^{13}C (75.5 MHz), δ 170.56, 168.51, and 167.58 (CO), 116.17 (*C*H_2=CH-CH_2O), 103.76 (C-1), 97.78 (C-1′), 96.84 (C-1″),

52.87 (C-2″), 21.05 (CH_3CO), and 14.13 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{88}H_{99}NO_{18}$: C, 72.46; H, 6.84; N, 0.96. Found: C, 72.28; H, 6.96; N, 1.01.

α Isomer **42**: ^{1}H NMR data ($CDCl_3$, 360 MHz), δ 7.78–6.88 (m, 39 H, Ar), 5.98 (m, 1 H, CH_2=C*H*-CH_2O), 5.77 (dd, 1 H, $J_{3'',4''}$ 2.5, $J_{4'',5''}$ < 1 Hz, H-4″), 5.42 (dd, 1 H, $J_{2'',3''}$ 11.5, $J_{3'',4''}$ 2.7 Hz, H-3″), 5.21 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1″), 4.38 (dd, 1 H, H-2″), 2.06 (s, 3 H, CH_3CO), and 0.84 [t, 3 H, $CH_3(CH_2)_7O$]. Anal. Found for **42**: C, 72.22; H, 7.04; N, 1.01.

Octyl 6-O-[6-O-allyl-3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**43**).—Glycosylation of **15** (170 mg, 0.18 mmol) using the chloride **18** (~ 0.22 mmol) was carried out in the presence of silver trifluoromethanesulfonate (71.3 mg, 0.275 mmol), collidine (54.4 μL, 0.41 mmol), and 4A molecular sieves (500 mg) in dry CH_2Cl_2 (3 mL) according to the procedure described for the preparation of **40**. After workup, the residual oil was purified by column chromatography using a toluene–EtOAc gradient (20:1 → 4:1) as eluent, to provide compound **43** (196.3 mg, 77%); R_f 0.40 (8:1 toluene–EtOAc); $[\alpha]_D$ + 11° (*c* 0.5, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (300 MHz), δ 7.79–6.86 (m, 39 H, Ar), 5.52 (m, 1 H, CH_2=C*H*-CH_2O), 5.29 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.19 (dd, 1 H $J_{1',2'}$ 1.5, $J_{2',3'}$ 2.7 Hz, H-2′), and 0.85 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (90 MHz), δ 116.25 (CH_2=CH-CH_2O), 103.80 (C-1), 97.73 (C-1′), 96.38 (C-1″), 54.81 ($^3J_{C,F}$ 9.8 Hz, C-2″), and 14.08 [$CH_3(CH_2)_7O$].

Octyl 6-O-[6-O-allyl-2-O-(4-azido-3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**44**).—Glycosylation of **15** (200 mg, 0.21 mmol) using the chloride **19** (~ 0.26 mmol) was carried out in the presence of silver trifluoromethane sulfonate (86.9 mg, 0.34 mmol), collidine (66.3 μL, 0.50 mmol), and 4A molecular sieves (500 mg) in dry CH_2Cl_2 (3 mL) as described for the preparation of **40**. After workup the residual oil was purified by column chromatography using a toluene–EtOAc gradient (20:1 → 4:1) as eluent to provide **44** (211 mg, 69.2%); R_f 0.46 (8:1 toluene–EtOAc); $[\alpha]_D$ + 30.6° (*c* 0.9, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (360 MHz), δ 7.80–6.83 (m, 39 H, Ar), 5.55 (m, 1 H, CH_2=C*H*-CH_2O), 5.24 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), and 0.86 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz): δ 116.22 (CH_2=CH-CH_2O), 103.87 (C-1), 97.77 (C-1′), 96.41 (C-1″), 63.76 (C-4″), 55.61 (C-2″), and 14.16 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{86}H_{96}N_4O_{16}$: C, 71.64; H, 6.71; N, 3.89. Found; C, 71.44; H, 6.61; N, 3.66.

*Octyl 6-O-[6-O-allyl-3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-*xylo*-hexopyranosyl)-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside* (**45**).—Glycosylation of **15** (600 mg, 0.64 mmol) using the chloride **20** (~ 0.1 mmol) was carried out in the presence of silver trifluoromethane sulfonate (55.5 mg, 0.22 mmol), collidine (28.5 μL, 0.22 mmol), and 4A molecular sieves (500 mg) in dry CH_2Cl_2 (3 mL) as described for the preparation of **40**. After workup the residual oil was purified by column chromatography using a toluene–EtOAc gradient (20:1 → 4:1) as eluent to provide **45** (117 mg, 81%; based on the donor

20); R_f 0.42 (8:1 toluene–EtOAc); $[\alpha]_D$ + 30° (*c* 1.2, $CHCl_3$). NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.85 (m, 39 H, Ar), 5.55 (m, 1 H, CH_2=C*H*-CH_2O), 5.25 [m (second order), 1 H, $J_{1'',2''}$ 8.3 Hz, H-1''], 4.20 (br t, H-2'), 2.34 (br d, $J_{4''ax,4''eq}$ 12.1 Hz, H-4''*ax*), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 116.08 (CH_2=CH-CH_2O), 103.75 (C-1), 97.71 (C-1'), 96.89 (C-1''), 58.86 (C-2''), 34.40 (C-4''), and 14.10 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{86}H_{97}O_{16}$: C, 73.74; H, 6.98; N, 1.00. Found: C, 73.44; H, 6.78; N, 1.09.

Deprotection of the allyl group.

Octyl 6-O-[3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2-deoxy-4-O-methyl-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**46**).—Compound **40** (89 mg, 0.062 mmol) was treated with a mixture of palladium(II) chloride (33.7 mg, 0.19 mmol) and NaOAc (31.2 mg, 0.38 mmol) in 20:1 acetic acid–water (2 mL) as described for the preparation of **36**. The resulting residue was purified by column chromatography using 4:1 toluene–EtOAc as eluant, to yield **46** (50 mg, 57.8%); R_f 0.37 (4:1 toluene–EtOAc); $[\alpha]_D$ + 5.9° (*c* 0.5, $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.83–6.86 (m, 39 H, Ar), 5.15 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1''), 4.14 (br t, 1 H, $J_{1',2'} \approx J_{2',3'} = 2.3$ Hz, H-2'), 3.50 (s, 3 H, CH_3O), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 103.80 (C-1), 97.57 (C-1'), 96.54 (C-1''), 60.79 (CH_3O), 55.74 (C-2''), and 14.17 [$CH_3(CH_2)_7O$].

Octyl 6-O-[2-O-(4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**47**).—Compound **41** (101.7 mg, 0.072 mmol) was treated with a mixture of palladium(II) chloride (38.3 mg, 0.22 mmol) and NaOAc (34.5 mg, 0.42 mmol) in 20:1 acetic acid–water (2 mL) as described for the preparation of **36**. The resulting residue was purified by column chromatography using 5:1 toluene–EtOAc, to yield **47** (54 mg, 54.5%); R_f 0.46 (4:1 toluene–EtOAc); $[\alpha]_D$ + 0.4° (*c* 0.8, $CHCl_3$). NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.87–6.93 (m, 39 H, Ar), 5.61 (br d, 1 H, $J_{3'',4''}$ 3.1, $J_{4'',5''} \sim 1$ Hz, H-4), 5.17 (d, 1 H, $J_{1'',2''}$ 8.6 Hz, H-1''), 4.12 (br t, 1 H, $J_{1',2'} \approx J_{2',3'} = 2.3$ Hz, H-2'), 2.16 (s, 3 H, CH_3CO), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (125 MHz), δ 170.56, 168.51, and 167.72 (CO), 103.72 (C-1), 97.67 (C-1'), 97.12 (C-1''), 52.94 (C-2''), 21.04 (CH_3CO), and 14.10 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{85}H_{95}NO_{18}$: C, 71.96; H, 6.75; N, 0.99. Found: C, 71.90; H, 6.88; N, 1.04.

Octyl 6-O-[3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**48**).—Compound **43** (80 mg, 0.056 mmol) was treated with a mixture of palladium(II) chloride (30 mg, 0.17 mmol) and NaOAc (27.9 mg, 0.34 mmol) in 20:1 acetic acid–water (2 mL) as described for the preparation of **36**. The resulting residue was purified by column chromatography using 4:1 toluene–EtOAc, to yield compound **48** (48 mg, 61.7%); R_f 0.49 (7:1 toluene–EtOH); $[\alpha]_D$ + 15° (*c* 0.18, $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.88–6.82 (m, 39

H, Ar), 5.20 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1''), 4.15 (br t, 1 H, $J_{1',2'} \approx J_{2',3'} = 2.3$ Hz, H-2'), and 0.88 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 103.87 (C-1), 98.27 (C-1'), 97.19 (C-1''), 55.00 (C-2''), and 14.20 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{83}H_{92}FNO_{16}$: C, 72.31; H, 6.73; N, 1.02. Found: C, 72.27; H, 6.87; N, 1.08.

Octyl 6-O-[2-O-(4-azido-3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**49**).—Compound **44** (92.5 mg, 0.064 mmol) was treated with a mixture of palladium(II) chloride (34 mg, 0.19 mmol) and NaOAc (31.2 mg, 0.38 mmol) in 20:1 acetic acid–water (2 mL) as described for the preparation of **36**. The resulting residue was purified by column chromatography using 2:1 hexane–EtOAc to yield **49** (50.7 mg, 56.4%); R_f 0.48 (7:1 toluene–EtOH); $[\alpha]_D + 2.8°$ (*c* 0.1, $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.82–6.83 (m, 39 H, Ar), 5.13 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1''), 4.12 (dd, 1 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 2.3 Hz, H-2'), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (90 MHz), δ 103.80 (C-1), 97.62 (C-1'), 96.47 (C-1''), 63.65 (C-4''), 55.65 (C-2''), and 14.07 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{83}H_{92}N_4O_{16}$: C, 71.12; H, 6.62; N, 4.00. Found: C, 70.76; H, 6.25; N, 4.00.

Octyl 6-O-[3,4-di-O-benzyl-2-O-(3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-xylo-hexopyranosyl)-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**50**).—To a solution of **45** (110 mg, 0.076 mmol) was added a mixture of palladium(II) chloride (40.4 mg, 0.23 mmol) and NaOAc (38.6 mg, 0.47 mmol) in 20:1 acetic acid–water (2 mL) as described for the preparation of **36**. The product was purified by column chromatography using 5:1 toluene–EtOAc to yield **50** (75 mg, 72.5%); R_f 0.51 (4:1 toluene–EtOAc); $[\alpha]_D + 5.7°$ (*c* 0.3, $CHCl_3$). NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.88–7.02 (m, 39 H, Ar), 5.11 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.12 (br t, 1 H, H-2''), 2.33 (br ddd, 1 H, $J_{3'',4'',eq}$ 4.5, $J_{4''ax,4''eq}$ 12.4, $J_{4''eq,5''} \sim 1.3$ Hz, H-4''*eq*), and 0.86 [t, 3 H, $CH_3(CH_2)O_7$]; ^{13}C (90 MHz), δ 103.71 (C-1), 97.70 (C-1'), 97.13 (C-1''), 57.01 (C-2''), 34.41 (C-4''), and 14.08 [$CH_3(CH_2)_7O$].

Conversion of the phthalimido group into the acetamido group

Octyl 6-O-[2-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-methyl-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**51**).—Compound **46** (50 mg, 0.036 mmol) in butanol (8 mL) was added to ethylenediamine (1.5 mL) under Ar. The solution was stirred for 20 h at 90°C. Evaporation to dryness was followed by addition and evaporation twice of toluene, followed by EtOH, to give a yellow syrup [R_f 0.36 (7:1 toluene–EtOH); ninhydrin positive]. This was dissolved in MeOH (5 mL) to which Ac_2O (1 mL) and triethylamine (0.1 mL) were added. After stirring for 14 h at room temperature, EtOH (20 mL) and water (1 mL) were added, and the solution was concentrated to dryness. The residue was purified by column chromatography using 10:1 toluene–EtOH as eluent, to yield **51** (45 mg, 96%); $[\alpha]_D + 9.1°$ (*c* 0.5, $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.40–7.08 (m, 35 H, Ar), 5.45 (d, 1 H, $J_{2'',NH}$ 7.5 Hz, NH), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.18 (br t, 1 H, $J_{1',2'} \approx J_{2',3'} = 2.3$ Hz, H-2'), 3.51 (s, 3 H, CH_3O), 1.80 (s, 3 H, CH_3CO), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C

(75.5 MHz), δ 170.91 (CO), 103.81 (C-1), 98.92 (C-1′), 98.20 (C-1″), 60.49 (CH_3O), 56.90 (C-2″), 23.54 (CH_3CO), and 14.12 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{78}H_{95}NO_{16}$: C, 71.92; H, 7.35; N, 1.08. Found: C, 71.69; H, 7.36; N, 1.16.

*Octyl 6-O-[2-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-galactopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-*glucopyranoside (**52**).—Compound **47** (48 mg, 0.028 mmol) was treated with ethylenediamine (1 mL) in butanol (5 mL) as described for the preparation of **51** giving a syrup [R_f 0.04 (1:1 hexane–EtOAc); ninhydrin positive]. This was dissolved in pyridine (3 mL) and Ac_2O (0.5 mL) was added. After stirring for 16 h at 30°C, MeOH was added to the mixture which was then concentrated to dryness. The residue [R_f 0.56 (1:1 hexane–EtOAc)] was then treated with NaOMe (5 mg) in dry MeOH (3 mL) for 4 h at 40°C. Treatment with Amberlite IR-120 (H^+) resin, filtration of the resin, and concentration of solvent left a syrup that was purified by column chromatography using 25:1 CH_2Cl_2–MeOH as eluent to yield **52** (28 mg, 66.6%); R_f 0.21 (1:1 hexane–EtOAc); $[\alpha]_D$ + 9.9° (*c* 1.4, $CHCl_3$). NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.40-7.12 (m, 35 H, Ar), 5.76 (br d, 1 H, NH), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 1.84 (s, 3 H, CH_3CO), and 0.86 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 171.19 (CO), 103.78 (C-1), 98.63 (C-1′), 98.34 (C-1″), 53.89 (C-2″), 23.62 (CH_3CO), and 14.13 [$CH_3(CH_2)_7O$].

Octyl 6-O-[2-O-(2-acetamido-3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**53**).—Compound **48** (48 mg, 0.035 mmol) was treated with ethylenediamine (1 mL) in butanol (5 mL) as described for the preparation of **51**. The intermediate amine had R_f 0.4 [(7:1 toluene–EtOH), ninhydrin positive] and was acetylated [Ac_2O (1 mL)–triethylamine (0.1 mL) in MeOH (5 mL)] as for **51**. Column chromatography using 1:1 toluene–EtOAc as eluent yielded compound **53** (35 mg, 77.7%); R_f 0.31 (1:1 toluene–EtOAc); $[\alpha]_D$ + 9.7° (*c* 0.4 $CHCl_3$). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.40–7.08 (m, 35 H, Ar), 5.58 (d, 1 H, $J_{2'',NH}$ 7.4 Hz, NH), 5.03 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1″), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.20 (br t, 1 H, $J_{1',2'} \approx J_{2',3'} = 1.9$ Hz, H-2′), 1.83 (s, 3 H, CH_3CO), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 170.98 (CO), 103.89 (C-1), 98.93 and 98.69 (C-1′ and C-1″), 56.82 (C-2″), 23.49 (CH_3CO), and 14.11 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{77}H_{95}FNO_{15}$: C, 71.49; H, 7.40; N, 1.08. Found: C, 71.78; H, 7.37; N, 1.17.

Octyl 6-O-[2-O-(2-acetamido-4-azido-3,6-di-O-benzyl-2,4-dideoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**54**).—Compound **49** (50.7 mg, 0.036 mmol) was treated with ethylenediamine (1 mL) in butanol (5 mL) and acetylated as described for **51**. The intermediate amine had R_f 0.44 (7:1 toluene–EtOH); ninhydrin positive. Column chromatography using 20:1 toluene–EtOH as eluent yielded **54** (45.6 mg, 96%); $[\alpha]_D$ + 35.7° (*c* 0.6, $CHCl_3$); R_f 0.45 (7:1 toluene–EtOH). NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.40–7.12 (m, 35 H, Ar), 5.64 (d, 1 H, $J_{2'',NH}$ 7.3 Hz, NH), 5.03 (d, 1 H, $J_{1'',2''}$ 8.3 Hz, H-1″), 4.35 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 1.80 (s, 3 H, CH_3CO), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (75.5 MHz), δ 171.25 (CO), 103.88 (C-1), 98.23 (C-1′), 98.02

(C-1″), 63.07 (C-4″), 57.86 (C-2″), 23.49 (CH_3CO), and 14.13 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{77}H_{92}N_4O_{15}$: C, 70.41; H, 7.06; N, 4.27. Found: C, 70.19; H, 7.08; N, 4.18.

*Octyl 6-O-[2-O-(2-acetamido-3,6-di-O-benzyl-2,4-dideoxy-β-*D*-xylo-hexopyranosyl)-3,4-di-O-benzyl-α-*D*-mannopyranosyl]-2,3,4-tri-O-benzyl-β-*D*-glucopyranoside* (**55**).—Compound **50** (63.3 mg, 0.047 mmol) in butanol (10 mL) was treated with ethylenediamine (2 mL). The intermediate amine had R_f 0.33 [(7:1 toluene–EtOH), ninhydrin positive]. This was acetylated [Ac_2O (1 mL)–triethylamine (0.1 mL) in MeOH (5 mL)] as described for the preparation of **51**. Column chromatography using 1:1 toluene–EtOAc as eluent yielded compound **55** (44.1 mg, 74.6%); R_f 0.15 (1:1 hexane–EtOAc); $[\alpha]_D$ + 4.8° (*c* 0.65, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (360 MHz), δ 7.37–7.12 (m, 35 H, Ar), 5.50 (d, 1 H, $J_{2'',NH}$ 7.6 Hz, NH), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.15 (t, 1 H, H-2′), 2.22 (ddd, 1 H, $J_{3'',4'',eq}$ 4.0, $J_{4''ax,4''eq}$ 11.6, $J_{4''eq,5''}$ ~1 Hz, H-4″*eq*), 1.89 (s, 3 H, CH_3CO), and 0.87 [t, 3 H, $CH_3(CH_2)_7O$]; ^{13}C (90 MHz), δ 103.77 (C-1), 99.80 (C-1′), 98.29 (C-1″), 57.68 (C-2″), 33.95 (C-4″), 23.62 (CH_3CO), and 14.08 [$CH_3(CH_2)_7O$]. Anal. Calcd for $C_{77}H_{93}NO_{15}$: C, 72.67; H, 7.37; N, 1.10. Found: C, 72.57; H, 7.35; N, 1.17.

Hydrogenolysis.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-4-O-methyl-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-glucopyranoside* (**9**).—Compound **51** (10 mg, 7.6 μmol) was dissolved in 95% EtOH (2 mL) containing 5% palladium-on-charcoal (20 mg) and stirred under H_2 for 16 h. Filtration of the catalyst, followed by evaporation, left a glass which was homogeneous by TLC. This material was adsorbed on to a SepPak C_{18} cartridge in water, the cartridge was washed with water (25 mL) and eluted with MeOH (10 mL). Evaporation of the eluant, filtration through a Millex filter, and lyophilization from water gave **9** (4.8 mg, 93%); R_f 0.73 (60:35:6 CH_2Cl_2–MeOH–water). NMR data are presented in Table I. FABMS: *m/z* 694 $(M + Na)^+$.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-glucopyranoside* (**10**).—Compound **52** (4.1 mg, 3.1 μmol) was dissolved in 95% EtOH (2 mL) containing 5% palladium-on-charcoal (10 mg) and stirred under H_2 for 40 h. Filtration of the catalyst, followed by evaporation, left a glass. This material was purified as described for the preparation of **9** to give **10** (2 mg, 95%); R_f 0.36 (60:35:6 CH_2Cl_2–MeOH–water). NMR data are presented in Table I. FAB MS: *m/z* 680 $(M + Na)^+$ and 658 $(M + H)^+$.

*Octyl 6-O-[2-O-(2-acetamido-2,4-dideoxy-4-fluoro-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-glucopyranoside* (**11**).—Compound **53** (10 mg, 7.7 μmol) was dissolved in 95% EtOH (2 mL) containing 5% palladium-on-charcoal (20 mg) and stirred under hydrogen for 16 h. Filtration of the catalyst, followed by evaporation, left a glass which showed a single spot on TLC. This material was purified as described for the preparation of **9**, to give **11** (5.0 mg, 98%); R_f 0.73 (60:35:6 CH_2Cl_2–MeOH–water). NMR data are presented in Table I. FAB MS: *m/z* 682 $(M + Na)^+$.

Octyl 6-O-[2-O-(2-acetamido-4-amino-2,4-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (**12**).—Compound **54** (15 mg, 0.0114 mmol) was dissolved in aldehyde free EtOH (95% EtOH was distilled from KOH and Zn) (8 mL) and 0.01 N HCl (2.28 mL, 0.0228 mmol) containing 5% palladium-on-charcoal (60 mg) and stirred under H_2 for 14 h. The catalyst was removed by filtration, and the solution was neutralized with 0.01 N NaOH for 30 min. Then CO_2 was bubbled through the solution. Evaporation left a glass, which showed a major spot on TLC [R_f 0.24 (60:35:6 CH_2Cl_2–MeOH–water)]. This material was passed through a SepPak C_{18} cartridge washing first with water then eluting with MeOH. Evaporation of the MeOH eluant, filtration through a Millex filter and lyophilization gave the crude product which was purified by HPLC. Purification was carried out on column of PARTISIL 5 PAC (4.7 mm i.d. × 110 mm; Whatman), eluted with 88:12 acetonitrile–water at a flow rate of 1 mL/min and a temperature of 25°C with detection at 202 nm. The retention time of **12** was 26.8 min. Evaporation, followed by filtration of the residue through a Millex filter, provided **12** (2.8 mg, 51.5%): NMR data are presented in Table I. FAB MS: m/z 679 $(M + Na)^+$ and 657 $(M + H)^+$.

Octyl 6-O-[2-O-(2-acetamido-2,4-dideoxy-β-D-xylo-hexopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (**13**).—Compound **55** (17 mg, 0.013 mmol) was dissolved in 95% EtOH (5 mL) containing 5% palladium-on-charcoal (20 mg) and stirred under H_2 for 16 h. Filtration of the catalyst, followed by evaporation, left a glass which was homogeneous by TLC. This material was purified as described for the preparation of **9** to give **13** (8.48 mg, 98.9%); R_f 0.73 (60:35:6 CH_2Cl_2–MeOH–water). NMR data are presented in Table I. FAB MS: m/z 664 $(M + Na)^+$, and 642 $(M + H)^+$.

Octyl 6-O-[2-O-(2,4-diacetamido-2,4-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (**14**).—To a solution of **12** (1 mg, 1.5 μmol) in dry MeOH (1 mL), Ac_2O (70 μL, 7.5 μmol) and triethylamine (15 μL, 0.11 μmol) were added at room temperature. The reaction mixture was stirred for 14 h, solvents were removed, and water, then EtOH, were added and evaporated. The resulting residue was purified as described for the preparation of **9** to give **14** (1.0 mg, 94%); R_f 0.41 (60:35:6 CH_2Cl_2–MeOH–water). NMR data are presented in Table I. FAB MS: m/z 721 $(M + Na)^+$ and 699 $(M + H)^+$.

Testing of 4-substituted GlcNAcT-V acceptor analogues.—4″-Substituted acceptor analogues **9–14** (3.6 nmol) were incubated for 26 h with GlcNAcT-V (9 μU) and UDP-GlcNAc (35.2 nmol, 38 300 dpm/nmol) in 50 mM sodium cacodylate, pH 6.5, with 20% glycerol, 10 mM EDTA, 1 mg/mL BSA and 0.1% Triton X-100. Reactions were quenched with water, and mixtures were transferred onto C_{18} SepPak cartridges. Unreacted radiolabelled donor was removed by washing with water until background counts were obtained. Radiolabelled product was eluted with MeOH (2 × 4 mL) and quantitated by liquid scintillation counting[26]. Apparent conversions of substrate to product ranged between 0.3 and 5.7% (0.5%, **9**; 5.7%, **10**; 5.3%, **11**; 5.3%, **12**; 2.5%, **13** and 0.3%, **14**), suggesting that the compounds were acceptor substrates for GlcNAcT-V.

The ability of β-(1 → 4)-galactosyltransferase to transfer galactose from UDP-galactose to the OH-4 position of the terminal GlcNAc residue of acceptor **5** was exploited to determine if there were small amounts of **5** contaminating the trisaccharides **9–14**. 4″-Substituted acceptors (80 nmol) were lyophilized with 7.2 nmol UDP-Gal (22000 dpm/nmol) in microfuge tubes. Trace amounts of normal acceptor **5** with a free OH-4″ group were then galactosylated by preincubation (for 10.6 h at 37°C) with 18 mU bovine milk β-(1 → 4)-galactosyltransferase (from Sigma) in 50 mM sodium cacodylate buffer, pH 7.2, with 1 mg/mL BSA, 20% glycerol and 2 mM $MnCl_2$ in a final volume of 10 μL. Preliminary experiments had shown that these conditions were sufficient to convert at least 1.6 nmol of trisaccharide **5** to galactosylated product **7**, which was no longer an acceptor for GlcNAcT-V. Fractions of turnover of the potential substrates varied over the range 0.2 to 2.6% (**9**, **10**, **11**, **12**, **13**, and **14** were 2.3, 2.6, 2.0, 0.2, 1.0, and 0.4% radiolabeled with galactosyltransferase, respectively).

Finally compounds **9–14** were tested as substrates for the GlcNAcT-V reaction after ensuring galactosyltransferase-mediated elimination of the small fraction of **5** present in the acceptor preparations. Galactosyltransferase preincubations were performed as described above for radiolabelling experiments, using 10, 20 and 80 nmol of potential acceptors but only unlabelled UDP-Gal was used. The GlcNAcT-V reactions were then initiated by addition of GlcNAcT-V as well as 22 nmol [^{3}H]UDP-GlcNAc (25400 or 31800 dpm/nmol) as described for the 26 h incubations above. For comparison, the GlcNAcT-V reaction rate with the normal acceptor **5** was measured under similar conditions, except that galactosyltransferase was replaced with buffer only in the preincubation step, and reactions were only allowed to proceed for 7.5–10 min instead of 2 h. Reactions were quenched, and the radiolabeled product was isolated and quantitated as described above. Rate data at the three acceptor concentrations were fitted to the Michaelis–Menten equation using unweighted nonlinear regression with the SigmaPlot 4.0 program to estimate kinetic parameters shown in Table II.

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