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RECOGNITION OF OLIGOSACCHARIDE SUBSTRATES BY *N*-ACETYLGLUCOSAMINYLTRANSFERASE-V*

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ABSTRACT

Six analogs of the trisaccharide 8-methoxycarbonyloctyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-mannopyranoside (**3**), a previously reported acceptor for *N*-acetylglucosaminyltransferase-V (GnT-V) have been chemically synthesized and evaluated as GnT-V acceptors. Replacement of the β-D-Man*p*-O$(CH_2)_8$COOMe "reducing end" of **3** by β-D-Glc*p*-O$(CH_2)_7CH_3$ gave octyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl)-β-D-glucopyranoside (**5**) whose activity was indistinguishable from that of **3**. Removal of the 4-OH group of the β-D-Glc residue in **5** had little effect on the activity, while the corresponding 4-*O*-methyl derivative was twice as active. Replacement of the C-6 *pro-R* hydrogen of the same residue by a methyl group gave the L-*glycero*-D-*gluco* derivative **8**, whereas replacement of the corresponding *pro-S* hydrogen gave the D-*glycero*-D-*gluco* compound **9**. Trisaccharide **8**, whose rotameric distribution about the C-5–C-6 bond is sterically biased towards the gg conformation was less than half as active as **5** as a GnT-V acceptor, whereas **9**, which is biased towards the gt conformation, was more than twice as active. These results provide evidence for the conformational control of oligosaccharide biosynthesis.

INTRODUCTION

The degree of branching, and accordingly the size, of the aspargine-linked (*N*-linked) oligosaccharides of plasma membrane glycoproteins is thought to be controlled by a series of *N*-acetylglucosaminyltransferases (GlcNAc-transferases, GnT's) which have been designated by the numerals I–VI (GnT-I to GnT-VI)[1]. All of these enzymes transfer 2-acetamido-2-deoxy-D-glucose (GlcNAc) from uridine 5-(2-acetamido-2-deoxy-D-glucosyl dihydrogen pyrophosphate) (UDP-GlcNAc) in

*Dedicated to Professor Bengt Lindberg
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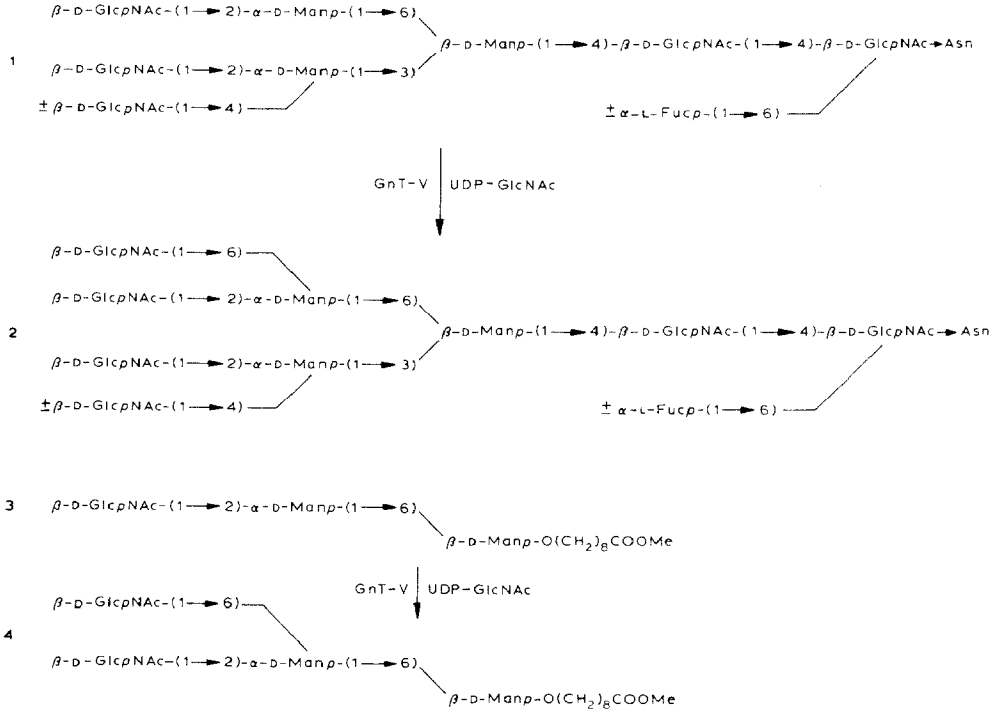
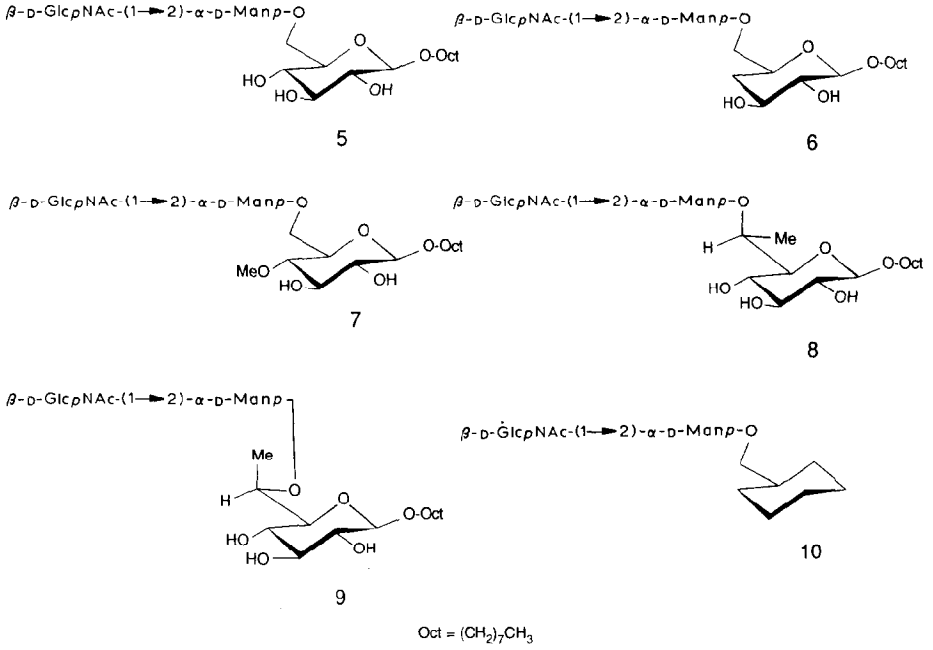
β-linkage to hydroxyl groups on D-mannose residues of growing *N*-linked carbohydrate chains. The enzymes are distinguished by their specificities for different acceptor oligosaccharides.

One of these enzymes, GnT-V[2], transfers a β-D-Glc*p*NAc residue to the 6-OH group of the core trimannosyl α-(1→6)-D-Man*p* unit of acceptors with structure **1**, giving rise to more highly branched oligosaccharides having structure **2**. The current interest in this enzyme follows observations that an increase in β-(1→6) branching accompanies both polyoma[3,4] and Rous-sarcoma-virus[5,6] transformation of baby-hamster kidney cells and that this increased branching correlates with the increased expression of GnT-V activity. Evidence has also been presented that such increased β-(1→6) branching is directly related to the metastatic potential of some transformed cell lines[7].

We have recently reported a simple assay[8] for GnT-V activity, which measures the transfer of radiolabelled GlcNAc from UDP-GlcNAc to the synthetic trisaccharide[9] β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*-O$(CH_2)_8$COOMe (**3**). The product of GnT-V action on **3** was isolated and shown, by ^{1}H-n.m.r. spectroscopy, to be identical to the synthetic tetrasaccharide β-D-Glc*p*NAc-(1→2)-[β-D-Glc*p*NAc-(1→6)]-α-D-Man*p*-(1→6)-β-D-Man*p*-O$(CH_2)_8$COOMe (**4**), thereby confirming the specificity of the acceptor in this assay. The finding[10] that GnT-V showed significant activity against the simple trisaccharide **3** provided an excellent opportunity to investigate, in more detail, the molecular specificity of this enzyme since oligo-



saccharides of this size can be readily prepared, in a reasonable time, by chemical synthesis. We now report the synthesis of the five trisaccharides **5–9**, and the "pseudo-trisaccharide" **10**, which are all analogs of **3**, designed to probe the molecular surfaces involved in the recognition of glycopeptides **1** by GnT-V.



Several considerations led to the selection of structures **5–10** as synthetic targets which might be useful in the study of GnT-V specificity. The decision to prepare these structures as glycosides of 1-octanol followed our finding that, in the assay of GnT-V in crude cell extracts, the hydrophobic 8-methoxycarbonyloctyl "linking-arm"[11,12] of **4** greatly facilitated its separation from unreacted sugar-nucleotide (and breakdown products) by reverse-phase chromatography[8]. No need was foreseen to derivatize **5–10** through a linking arm and the methyl ester was therefore omitted since this functional group can complicate several required synthetic manipulations, including the removal of *N*-phthalimido protecting groups by hydrazinolysis[13,14]. In an effort to simplify further the chemical syntheses, we first questioned whether the D-*manno* configuration was essential for the reducing-end sugar in **3** since the formation of the β-D-mannopyranosyl linkage is generally a problematic step in synthesis. The commercially available octyl β-D-glucopyranoside (**11**) was then a logical starting material for the preparation of **5**, which has the β-D-*gluco* configuration at the reducing end. When **5** was found to be equivalent to the D-*manno* epimer **3** as an acceptor for GnT-V (see Table IV), the decision was made to retain the D-*gluco* configuration in the remaining analogs. Glucoside **11** was

also used as the starting material for the preparation of **6** and **7**, whose activity against the enzyme would reflect the involvement of the 4-position of the reducing-end sugar.

The conformation about the α-(1→6) linkage in Asn-linked carbohydrates has been extensively studied[15–19] and complex oligosaccharides such as **1** are believed to exist, in aqueous solution, as a mixture of two rapidly equilibrating conformers which interconvert by rotation about the C-5–C-6 bond of the β-D-Man*p* residue. One of these conformers, termed[15] gt, has the so-called ω-angle (H-5–C-5–C-6–O-6) = −60° and the other, termed gg, has ω = 180°. One of the questions addressed in this work is whether GnT-V acts preferentially on one of these conformers and, if so, which one. Lemieux and co-workers[20,21] have demonstrated that replacing the pro-*S* hydrogen on C-6 of glycopyranosides by a methyl group gives a structure which is conformationally biased towards the gg rotamer, whereas replacement to the pro-*R* hydrogen by methyl provides a bias towards the gt conformation. Trisaccharides **8** and **9** might therefore serve as good models for **5** (and **1**) in the gg and gt conformations, respectively. Structure **10** was included in the series of analogs in order to probe the degree of involvement of the reducing-end sugar.

The syntheses of **5–10** followed straightforward routes which encountered no particularly difficult steps. Preparation of the suitably protected reducing-end alcohols was followed by the addition of the common β-D-Glc*p*NAc-(1→2)-α-D-Man*p* terminii using, sequentially, the well known glycosyl-donors **38**[22,23] and **51**[24].

SYNTHESIS OF MONOSACCHARIDE ALCOHOLS

Reaction of octyl β-D-glucopyranoside (**11**) with chloro(4-methoxyphenyl)diphenylmethane gave **12**, which was directly benzylated to provide **13**. Treatment of **13** with aqueous acetic acid then gave alcohol **14**, which was isolated in 45% yield based on **11**.

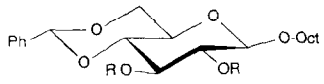
Reaction of **11** with α,α-dimethoxytoluene in *N*,*N*-dimethylformamide, catalyzed by tetrafluoroboric acid[25], gave the 4,6-*O*-benzylidene derivative **15** which was directly benzylated to produce **16** (67% from **11**). Acid-catalyzed hydrolysis of the benzylidene group in **16** gave diol **17** (86%) which was selectively 6-*O*-acetylated to yield **18** (72%). Acylation of alcohol **18** with phenyl chlorothionocarbonate[26] gave **19** (52%), which was reduced using tributylstannane and 2,2′-azobis(isobutyronitrile) to give the 4-deoxy derivative **20** (91%). Deacetylation of **20** then gave the D-*xylo* alcohol **21** (95%).

The 4-*O*-methyl alcohol **24** was prepared from diol **17** by sequential 6-*O*-methoxytritylation to produce **22** (78%), 4-*O*-methylation to give **23** (85%), and demethoxytritylation to give **24** (80%).

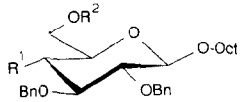
The route to the required 6-*C*-methyl alcohols **30** and **37** was based on the availability of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,5-dinitrobenzoyl)-7-deoxy-L-*glycero*-D-*gluco*-heptopyranoside (**25**), which had been synthesized by N.Le in the Lemieux laboratory where its structure was unequivocally established by a

11

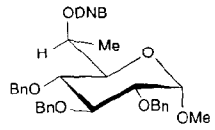
12 R^1 = H, R^2 = MMT
13 R^1 = Bn, R^2 = MMT
14 R^1 = Bn, R^2 = H



15 R = H
16 R = Bn



17 R^1 = OH, R^2 = H
18 R^1 = OH, R^2 = Ac
19 R^1 = OCSPh, R^2 = Ac
20 R^1 = H, R^2 = Ac
21 R^1 = R^2 = H
22 R^1 = OH, R^2 = MMT
23 R^1 = OMe, R^2 = MMT
24 R^1 = OMe, R^2 = H



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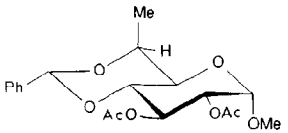


26

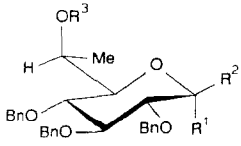
MMT = Mono-(*p*-methoxy)-trityl
DNB = 1,3-dinitrobenzoyl

^{1}H-n.m.r. study of its 4,6-*O*-benzylidene derivative **26**. Acetoylsis of **25** gave the 1-*O*-acetyl derivative **27** (73%) which was converted into the α-bromide **28**. Reaction of **28** with an excess of 1-octanol, in the presence of insoluble silver zeolite[27], gave the β-glycoside **29** in 73% yield; **29** was then deacylated to produce **30** (83%).

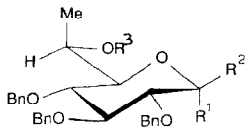
Deacylation of **25** gave alcohol **31** which was treated with methanesulfonyl chloride in pyridine to give the mesylate **32**. Reaction of **32** with sodium benzoate in *N*,*N*-dimethylformamide for 15 h at 120° then gave the D-*glycero*-D-*gluco* derivative **33** (53% from **25**). Acetolysis of **33** gave **34** (52%) which was converted into the octyl β-glycoside **36**, in 50% yield, as described for the preparation of **29**. Debenzoylation of **36** gave alcohol **37** (88%).

SYNTHESIS OF OLIGOSACCHARIDES

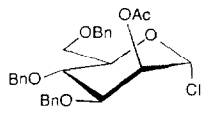
Glycosylation of alcohols **14**, **21**, **24**, **30**, **37**, and cyclohexylmethanol with the glycosyl-donor **38**, in the presence of silver trifluoromethanesulfonate and tetramethylurea[28], gave the protected disaccharides **39–44** in yields of 77, 76, 76, 80, 80, and 62%, respectively. Deacetylation of **39–44**, using sodium methoxide in methanol, then gave alcohols **45-50** (86, 82, 87, 82, 81, and 86%, respectively).



27 R^1, R^2 = OAc, R^3 = DNB
28 R^1 = Br, R^2 = H, R^3 = DNB
29 R^1 = H, R^2 = O-Oct, R^3 = DNB
30 R^1 = H, R^2 = O-Oct, R^3 = H
31 R^1 = OMe, R^2 = R^3 = H
32 R^1 = OMe, R^2 = H, R^3 = Ms



33 R^1 = OMe, R^2 = H, R^3 = Bz
34 R^1, R^2 = OAc, H, R^3 = Bz
35 R^1 = Br, R^2 = H, R^3 = Bz
36 R^1 = H, R^2 = O-Oct, R^3 = Bz
37 R^1 = H, R^2 = O-Oct, R^3 = H



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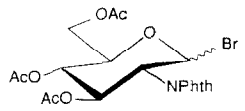

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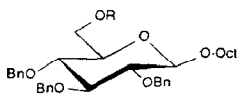
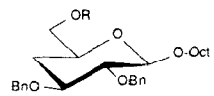
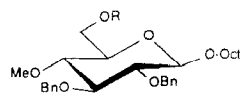


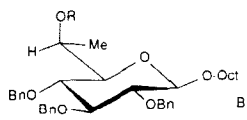
16 R=H
39 R=A
45 R=B
52 R=C
58 R=D



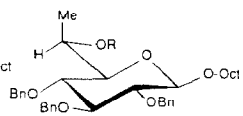
21 R=H
40 R=A
46 R=B
53 R=C
59 R=D



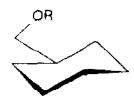
24 R=H
41 R=A
47 R=B
54 R=C
60 R=D



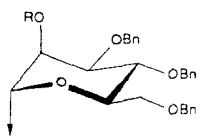
30 R=H
42 R=A
48 R=B
55 R=C
61 R=D



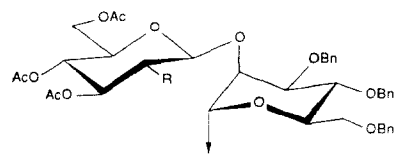
37 R=H
43 R=A
49 R=B
56 R=C
62 R=D



44 R=A
50 R=B
57 R=C
63 R=D



A R=Ac
B R=H



Phth= Phthalimido

C R=NPhth
D R=NHAc

Glycosylation of **45–50**, using the phthalimido bromide **51**[24] in dichloromethane in the presence of silver trifluoromethanesulfonate and *sym*-collidine, gave the trisaccharide derivatives **52–57** in yields of 72, 74, 72, 69, 67, and 74%, respectively. Treatment of **52–57** with hydrazine hydrate in refluxing methanol, followed by acetylation of the crude product using acetic anhydride in pyridine, gave **58–63** (71, 72, 68, 71, 68, and 69%, respectively).

The glycosylation reactions described above produced typically complex mix-

tures of products which required chromatographic purification on silica gel to produce the analytically pure materials described here. Iatrobeads were found to be a silica gel ideally suited to the chromatographic purification of such crude reaction mixtures since it provides resolution far superior to that observed on commercial analytical t.l.c. plates. The resolution approached that of 10 μm h.p.l.c. silica columns, and the relatively low cost renders it acceptable for single use.

Deprotection of **58–63** required the removal of only acetyl and benzyl groups When the acetyl groups were removed first, by transesterification with sodium methoxide in methanol, followed by neutralization with resin, resin removal, and solvent evaporation, the hydrogenoyltic cleavage of the benzyl ethers proved to be both difficult and irreproducible, requiring several days even at elevated (15 atm.) pressures of hydrogen. When the hydrogenolysis step preceded deacetylation, however, all the reactions were complete within 16 h at atmospheric pressure. Hydrogenation of **58–63** over 5% palladium-on-carbon, followed by deacetylation, chromatography on BioGel P-2, and lyophilization, then gave the final compounds **5–10** in yields of 82, 81, 77, 81, 80, and 76%, respectively.

Key ^{1}H- and ^{13}C-n.m.r. data for **5–10** are presented in Table III. In the ^{1}H-n.m.r. spectra of the 6-*C*-methyl trisaccharides **8** and **9**, the signal for H-6 could be readily assigned by its multiplicity and was found near 4.2 p.p.m., unobscured by other resonances. The small couplings of H-6 with H-5 (1.7 Hz for **8** and 2.3 Hz for **9**) are in complete agreement with the expectation[21] that the rotameric distribution about C-5–C-6 in **8** is in favor of the gg conformation while **9** is biased towards the gt rotamer.

GnT-V ACCEPTOR ASSAYS

The results of a preliminary evaluation of compounds **3** and **5–10** as GnT-V acceptors[8], using a crude hamster kidney homogenate as the source of the enzyme, are summarized in Table IV. The low degree of recognition of the reducing-end sugar residue by the enzyme is one of the striking conclusions drawn from this study. Neither inversion of C-2 of **3**, to provide the D-*gluco* derivative **5**, nor deoxygenation of C-4, to provide the D-*xylo*-trisaccharide **6**, adversely affected acceptor activity. With the added information that the glycopeptide-derived physiological substrates for the enzyme have structure **1**, where the β-D-Man residue is substituted at O-3 by at least a disaccharide, substrate recognition by GnT-V appears to involve only the first few atoms of the β-D-Man*p* residue in **3** (and the β-D-Glc*p* residue in **5**) and, perhaps, the hydrophobic octyl aglycons. Since the cyclohexylmethyl structure **10** retained only 25% of the activity of **5**, some recognition of the reducing end to the trisaccharide substrates **3** and **5–9** is evident. If this situation is indeed so, then the combining site of the enzyme, which acts on O-6 of the α-D-Man*p* residue, would be predicted to extend from this residue onto the β-D-Glc*p* unit and, perhaps, the octyl aglycon of **5** (which might mimic the hydrophobic face of the chitobiose unit in **1**). The combining site would then be predicted to recognize preferentially one of the

TABLE I

KEY ^{1}H-N.M.R. PARAMETERS FOR PROTECTED INTERMEDIATES **39–44** AND **52–63**[a,b]

Compound	*H-1* ($J_{1,2}$)	*H-1'* ($J_{1',2'}$)	*H-1"* ($J_{1'',2''}$)	CH_2CH_3 (*t, J 6.5 Hz*)	$OCOCH_3$	*Other*[c]
39	4.357 (8.0)	4.911 (1.8)		0.866	2.143	
40	4.287 (7.8)	4.855 (1.8)		0.864	2.147	H-4*e* 2.023 (ddd, *J* = 1.5, 5.2, 12.5), H-4*a* 1.447 (ddd, *J*'s = 12–13).
41	4.328 (7.8)	4.935 (1.9)		0.860	2.158	OCH_3 3.473
42	4.324 (7.9)	5.069 (1.8)		0.864	2.144	H-6 4.171 (dq, 2.0, 6.5) H-7 1.320
43	4.353 (7.8)	5.012 (1.8)		0.860	2.157	H-6 4.062 (dq, 1.0, 6.8) H-7 1.121
44		4.721 (1.9)			2.023	
52	4.323 (7.5)	4.960 (2)	5.592 (8.5)	0.857	2.043 2.004 1.850	H-3" 5.847 (dd, 9.0, 10.5)
53	4.263 (8.0)	4.606 (1.8)	5.527 (8.5)	0.867	2.059 2.042 2.187	H-3" 5.806 (dd, 9.0, 10.8) H-4*e* 1.936 (ddd, 1.0, 5.2, 12.5) H-4*a* 1.456 (ddd, 12.5)
54	4.349 (7.8)	4.728 (2.0)	5.558 (8.5)	0.860	2.042 2.024 1.840	H-3" 5.807 (dd, 9.0, 10.6) OCH_3 3.384
55	obsc.ND	4.896 (1.5)	5.531 (8.8)	0.862	2.076 2.047 1.869	H-3" 5.828 (dd, 9.0, 10.5) H-7 1.27
56	4.429 (8.0)	4.801 (1.8)	5.537 (8.5)	0.855	2.048 2.043 1.873	H-3" 5.817 (dd, 9.0, 10.5) H-7 1.060 (d, 6,8)
57		4.529 (obsc)	5.509 (8.5)		2.051 2.038 1.866	H-3" 5.821 (dd, 9.0, 10.5)
58	n.d.[d]	n.d.[d]	5.149 (8.2)	0.870	2.053 2.017 1.996	NH 5.390 (d, 7.5) $NCOCH_3$ 1.744

59	4.287 (7.8)	4.736 (obs)	5.186 (8.5)	0.868	2.037	NH 5.393 (d, 7.0)
					2.020	$NCOCH_3$ 1.747
					2.000	H-4*e* 1.975 (ddd, 1.0, 5.0, 12.0)
						H-4*a* 1.503 (ddd, all ~12 Hz)
60	4.337 (8.0)	4.828 (2)	5.139 (8.5)	0.867	2.057	NH 5.420 (d, 7.2)
					2.013	$NCOCH_3$ 1.762
					2.000	OCH_3 3.443
61	4.346 (8.0)	5.026 (2)	5.207 (8.2)	0.860	2.042	NH 5.337 (d, 7.0)
					2.006	$NCOCH_3$ 1.709
					1.982	H-7 1.3
62	4.379 (7.8)	4.753 (1.5)	5.113 (8.2)	0.867	2.019	NH 5.411 (d, 7.5)
					2.010	$NCOCH_3$ 1.742
					1.998	H-7 1.173 (d, 6,8)
63		4.677 (1.5)	5.167 (8.2)		2.037	NH 5.413 (d, 7.0)
					2.020	$NCOCH_3$ 1.750
					2.000	

[a]For solutions in $CDCl_3$, internal Me_4Si: standard coupling (*J*) in Hz. [b]Other ^{1}H-n.m.r. parameters were in accord with the assigned structures. [c]' refers to the α-D-Man unit, " refers to the β-D-GlcNAc unit. [d]Could not be determined due to spectral overlap.

rotamers about the C-5–C-6 bond in **5**, since the relative orientations of the α-D-Man*p* and the β-D-Glc*p* residues in the gg and gt rotamers are distinctly different.

As noted earlier, the 6-*S*-methyl trisaccharide **8** is expected to have a rotameric distribution about C-5–C-6 which is biased towards the gg conformation, wheras the 6-*R*-methyl compound **9** is expected to exist, in solution, primarily in the gt conformation. As seen in Table IV, the activity of **8** is less than half of that of the parent **9** while the gt structure **9** is over twice as active as **5**. The increased activity of the 4-*O*-methyl derivative **7** can similarly be explained in terms of a conformational bias. The increase in steric bulk at O-4, as a result of *O*-methylation, would be predicted to skew the conformational equilibrium in **7** away from the gg rotamer (where MeO-4 and O-6 are closer) towards the gt rotamer.

The results presented above represent the first direct experimental evidence in support of the proposal[1,29] that GnT-V, or any other GnT, may act preferentially on Asn-linked oligosaccharides in one of two readily accessible conformations which can interconvert rapidly by rotation about the C-5–C-6 bond of the core β-D-Man residue. In this instance, the inference is that GnT-V acts preferentially on glycopeptides, such as **1**, in their preferred[18,19] gt conformations. At present, this conclusion must be regarded as tentative since it is based on the study of a limited series of highly artificial synthetic substrates with a crude enzyme preparation. Efforts are underway to purify GnT-V with the objective of obtaining detailed kinetic parameters for the enzymic glycosylation of **3** and **5–10**, while n.m.r. studies are in progress to further define their solution conformations. Supplemented by the synthesis and evaluation of a new series of more rigid, conformationally well-defined substrates, these studies are expected to provide firm evidence for the conformational control of oligosaccharide biosynthesis.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin-Elmer 241 polarimeter at ambient temperatures (22 ±2°). T.l.c. was performed on precoated plates of silica gel (60-F_{254}, Merck) with detection by quenching of fluorescence, or by charring, or both, after spraying with 5% H_2SO_4 in ethanol. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (Merck, 40–63 μm). Iatrobead refers to a beaded silica gel manufactured by Iatron Laboratories, Tokyo (product No. 6RS-8060). For gel filtration, Bio-Gel P-2 (200–400 mesh) (Bio-Rad Laboratories) was used. ^{1}H-n.m.r. spectra were recorded at 360 MHz (Bruker WM-360) or 300 MHz (Bruker AM-300) with either tetramethylsilane (δ = 0 in $CDCl_3$) or acetone (δ = 2.225 in D_2O) as internal standard at ambient temperature. ^{13}C-N.m.r. spectra were recorded at 75.5 MHz (Bruker AM-300) with internal tetramethylsilane (δ = 0 in $CDCl_3$) or external 1% 1,4-dioxane (δ = 67.4 in D_2O) as reference standards. Only partial n.m.r. data are reported. Other spectral features were in accord with the proposed structures. The ^{1}H-n.m.r. chemical shifts and coupling constants are reported as though they were first order. Assignments of

TABLE II

KEY ^{13}C-N.M.R. PARAMETERS FOR PROTECTED INTERMEDIATES **39–44** AND **52–57**[a,b,c]

Compound	*C-1*	*C-1'*	*C-1"*	*C-2"*	*$COCH_3$*	*$COCH_3$*	*CH_2CH_3*	*Other*
39	103.54	97.83			170.33	21.12	14.09	
40	103.83	97.70			170.47	21.14	14.13	C-4 33.83
41	103.49	97.84			170.37	21.15	14.13	OCH_3 60.80
42	104.11	95.01			170.50	21.19	14.12	C-7 15.23
43	103.68	97.32			170.38	21.18	14.11	C-7 15.72
44		97.86			170.53	21.15		$(CH_2)_3CH$ 37.77
52	103.96	97.75	96.34	54.44	170.75	20.84	14.13	
					170.18	20.68		
					169.46	20.51		
53	103.94	97.18	96.71	54.48	170.70	20.77	14.10	C-4 33.47
					170.18	20.65		
					169.46	20.49		
54	103.79	97.61	96.27	54.43	170.71	20.78	14.09	OCH_3 60.77
					170.13	20.65		
					169.43	20.48		
55	104.40	96.61	92.68	54.36	170.74	20.82	14.11	C-7 14.17
					170.14	20.66		
					169.43	20.50		
56	103.71	97.43	96.67	55.42	170.68	20.76	14.08	C-7 14.97
					170.14	20.65		
					169.41	20.49		
57		96.99	96.84	54.53	170.67	20.74	14.21	$(CH_2)_3CH$ 37.75
					170.18	20.66		
					169.46	20.49		

[a]For solutions in $CDCl_3$, internal Me_4Si. [b]Other ^{13}C-n.m.r. parameters were in accord with the assigned structures. [c]' refers to the α-D-Man unit, " refers to the β-D-GlcNAc unit.

TABLE III

SELECTED CHEMICAL SHIFTS δ AND COUPLING CONSTANTS (Hz)[a] FOR COMPOUNDS 5–10[b,c]

Nucleus	**5**	**6**	**7**	**8**	**9**	**10**
H-1 ($J_{1,2}$)	4.476 (8.1)	4.376 (8.1)	4.428 (8.0)	4.430 (8.0)	4.435 (8.0)	
H-1′ ($J_{1',2'}$)	4.895 (1.7)	4.884 (1.8)	4.904 (1.7)	4.991 (1.8)	5.018 (1.7)	4.838 (2.0)
H-1″ ($J_{1'',2''}$)	4.567 (8.5)	4.559 (8.3)	4.557 (8.5)	4.573 (8.4)	4.560 (8.5)	4.544 (8.1)
H-2 ($J_{2,3}$)	4.117 (3.4)	4.100 (3.4)	4.147 (3.5)	4.070 (3.3)	4.026 (3.5)	4.053 (3.5)
H-2′ ($J_{2',3'}$)	3.249 (9.5)	3.154 (9.2)	3.256 (9.4)	3.239 (9.5)	3.243 (9.2)	
$COCH_3$	2.056	2.053	2.060	2.062	2.056	2.051
CH_2CH_3	0.863	0.862	0.862	0.863	0.863	
Other H		2.001 (ddd, 1.8, 5.2, 12.8, H-4*e*) 1.528 (ddd, 11.5, 11.5, 12.8, H-4*a*)	3.580 (OCH_3)	4.244 (dq, 1.7, 6.6, H-6) 1.310 (d, H-7)	4.189 (dq, 2.3, 6.6, H-6) 1.284 (d, H-7)	
C-1	103.19	103.45	103.08	103.58	103.46	
C-1′	100.39	100.39	100.37	100.71	100.34	100.53
C-1″	97.68	97.54	98.33	94.20	96.66	97.71
C-2″	56.24	56.23	56.24	56.36	56.30	56.25
$COCH_3$	175.75	175.64	175.60	175.47	175.67	175.64
$COCH_3$	23.18	23.16	23.20	23.26	23.22	23.18
CH_2CH_3	14.27	14.27	14.31	14.37	14.27	
Other C		35.13 (C-4)	61.05 (OCH_3)	14.62 (C-7)	15.44 (C-7)	37.96 [$(CH_2)_3CH$]

[a] In parentheses. [b] For solutions in D_2O; experimental conditions and reference standards are described in the Experimental Section. [c] ′ refers to the α-D-Man unit, ″ refers to the β-D-GlcNAc unit.

TABLE IV

SUBSTRATE SPECIFICITY OF GnT-V[a]

Acceptor substrate	*GnT-V activity[b,c] (GlcNAc transferred, nmol/mg of protein/h*
3	0.18 ±0.13
5	0.22 ±0.01
6	0.21 ±0.11
7	0.41 ±0.11
8	0.14 ±0.03
9	0.52 ±0.02
10	0.05 ±0.02

[a]Hamster kidney homogenate was used as the source of the enzyme at 37°. [b]Acceptor concentration in the assay mixture (20 μL) was 1mM and UDP-[^{3}H]-GlcNAc (10 c.p.m./pmol) was 5mM. [c]The values are the average from 3 incubations. For detailed experimental procedures, see ref. 8.

^{13}C resonances are tentative. Unless otherwise noted, all reactions were carried out at ambient temperature, and, in the processing of reaction mixtures, solutions of organic solvents were washed with equal volumes of aqueous solutions. Organic solutions were dried over sodium sulfate prior to solvent removal on a rotary evaporator under the vacuum of a water aspirator with bath temperature of 40° or lower. The microanalyses were carried out by the Analytical Services Laboratory of this department.

The following solvent systems have been designated by letters: *A*, ethyl acetate–hexane (1:5 v/v); *B*, ethyl acetate–hexane (1:2); *C*, ethyl acetate–hexane (1:1); *D*, ethyl acetate-hexane (2:1); *E*, ethyl acetate-hexane (1:8); *F*, ethyl acetate-hexane (1:4); *G*, ethyl acetate–hexane (1:9); *H*, ethyl acetate–hexane (1:3), *I*, chloroform–methanol–water (60:35:6); *J*, toluene–acetone (4:1).

*Octyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**14**). — Octyl β-D-glucopyranoside (**11**; 519 mg, 1.77 mmol) was dissolved in anhydrous pyridine (5 mL) and added to chloro-4-methoxyphenyldiphenylmethane (822 mg, 2.66 mmol). After 24 h, the mixture was evaporated to dryness, a solution of the residue in 10% methanol in dichloromethane (10 mL) was filtered through a column of silica gel, and the eluate containing **12** (R_F 0.44 in 1:9 methanol–dichloromethane) was taken to dryness. The residue was not characterized, but was directly benzylated with sodium hydride (270 mg of a 60% dispersion in oil) and benzyl bromide (0.80 mL) in *N*,*N*-dimethylformamide (20 mL) at room temperature for 16 h. Solvent evaporation and purification of the major product by chromatography (solvent *E*) gave chromatographically homogeneous **13**, R_F 0.55 (solvent *H*). This material was dissolved in 80% aqueous acetic acid (75 mL) and kept for 20 h at room temperature. Solvent evaporation followed by chromatographic purification (solvent *F*) of the residue gave **14** (451 mg, 45%) as a syrup, $[\alpha]_D$ +15° (*c* 0.86, chloroform); R_F 0.52 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.433 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 1.927 (dd, 1 H, $J_{OH,6} = J_{OH,6'}$

= 6.5 Hz, OH, D_2O-exchangeable), 0.873 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.57, 138.42, and 138.01 [quaternary aromatic (quat. arom.)], 103.70 (C-1), 61.99 (C-6), 14.08 (CH_3).

Anal. Calc. for $C_{35}H_{46}O_6$: C, 74.70; H, 8.24. Found: C, 74.62; H, 8.33.

Octyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (**16**). — Compound **11** (339 mg, 1.16 mmol) was dissolved in *N*,*N*-dimethylformamide (10 mL), and α,α-dimethoxytoluene (0.19 mL, 1.28 mmol) and tetrafluorboric acid (0.21 mL of a 54% solution in diethyl ether, 1.28 mmol) were added. After 1 h, triethylamine (0.2 mL) was added and the solvent was evaporated. The residue was dissolved in dichloromethane (10 mL), and the mixture was filtered through a short column of silica gel and evaporated to dryness. The residue (**15**) (R_F 0.20, solvent *C*) was not characterized at this stage but benzylated directly with sodium hydride (107 mg of a 60% dispersion in oil) and benzyl bromide (0.32 mL) in *N*,*N*-dimethylformamide (15 mL) for 20 h at room temperature. After solvent evaporation, the residue was purified by chromatography (solvent *E*) to provide **16** (435 mg, 67%) as a syrup, $[\alpha]_D$ −31° (*c* 0.37, chloroform); R_F 0.56 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.563 (s, 1 H, $C_6H_5CHO_2$), 4.504 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 0.878 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 139.59, 139.44, and 138.40 (quat. arom.), 105.18 (C-1), 102.11 ($C_6H_5CHO_2$), 15.11 (CH_3).

Anal. Calc. for $C_{35}H_{44}O_6$: C, 74.97; H, 7.91. Found: C, 74.84; H, 7.98.

Octyl 2,3-di-O-benzyl-β-D-glucopyranoside (**17**). — Compound **16** (386 mg, 0.69 mmol) was dissolved in dichloromethane (4 mL), and aqueous 80% acetic acid (50 mL) was added. After heating the mixture for 2 h at 80°, the solvent was evaporated and the residue was purified by chromatography (solvent *C*). Pure **8** (280 mg, 86%) was obtained as a white powder, $[\alpha]_D$ +35.4° (*c* 0.18, chloroform); R_F 0.11 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.452 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 2.200 (d, 1 H, $J_{OH,4}$ 2.1 Hz, OH-4, D_2O-exchangeable), 1.998 (dd, 1 H, $J_{6,OH} = J_{6',OH}$ = 6.6 Hz, OH-6, D_2O-exchangeable), 8.873 (t, 3 H, J 6.6 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.82 and 138.63 (quat. arom.), 104.05 (C-1), 62.75 (C-6), 14.33 (CH_3).

Anal. Calc. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 71.14; H, 8.26.

Octyl 6-O-acetyl-2,3-di-O-benzyl-β-D-glucopyranoside (**18**). — To a solution of **17** (250 mg, 0.53 mmol) in dichloromethane (3 mL) and pyridine (128 μL, 1.6 mmol) was added dropwise acetyl chloride (117 μL, 1.6 mmol) at −78°, and the mixture was stirred for 3 h at −78°, then 1 h at 0°. After dilution with dichloromethane (50 mL), the mixture was poured into ice–water and washed with ice-cold water (3 × 50 mL) before concentration to a syrup which was purified by chromatography (solvent *B*). Pure **9** (195 mg, 71.6%) was obtained as a syrup, $[\alpha]_D$ −25.3° (*c* 0.34, chloroform); R_F 0.38 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.414–4.240 (m, 3 H, H-1 at 4.403, $J_{1,2}$ 7 Hz and H-6,6'), 2.602 (d, 1 H, $J_{OH,4}$ 1.8 Hz, HO-4, D_2O-exchangeable), 2.080 (s, 3 H, $COCH_3$), 0.880 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 171.43 ($COCH_3$), 138.52 and 138.38 (quat. arom.), 103.84 (C-1), 63.38 (C-6), 20.86 ($COCH_3$), 14.09 (CH_3).

Anal. Calc. for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 70.05; H, 8.33.

Octyl 6-O-acetyl-2,3-di-O-benzyl-4-O-phenoxythiocarbonyl-β-D-glucopyranoside (**19**). — A mixture of **18** (152 mg, 0.30 mmol), 4-dimethylaminopyridine (1.26 g, 10.3 mmol), and phenyl chlorothionocarbonate (0.82 mL, 5.9 mmol) in dry acetonitrile (10 mL) was heated under reflux for 5 h, and then left for 16 h at room temperature. The mixture was diluted with dichloromethane (50 mL), and washed sequentially with ice-cold 0.5M hydrochloric acid (25 mL) and water (25 mL). Evaporation of the solvent and chromatographic purification of the residue (solvent *E*) gave **10** as a solid (100 mg, 52%), $[\alpha]_D$ +27.1° (*c* 0.29, chloroform); R_F 0.39 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.680 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.477 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.363 (dd, 1 H, $J_{5,6}$ 5.0, $J_{6,6'}$ −12.0 Hz, H-6), 4.224 (dd, 1 H, $J_{5,6'}$ 2.8 Hz, H-6'), 2.080 (s, 3 H, $COCH_3$), 0.877 (s, 3 H, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 170.79 ($COCH_3$), 103.76 (C-1), 62.47 (C-6), 21.09 ($COCH_3$), 14.14 (CH_3).

Anal. Calc. for $C_{37}H_{46}O_8S$: C, 68.28; H, 7.13; S, 4.93. Found: C, 68.05; H, 7.06; S, 5.06.

Octyl 6-O-acetyl-2,3-di-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside (**20**). — A solution of **19** (85.7 mg, 0.13 mmol) in dry toluene (5 mL) was heated to 80° under nitrogen, then 2,2'-azobis(isobutyronitrile) (40.6 mg, 0.15 mmol) was added followed by tributylstannane (531 μL, 2.0 mmol). After 2 h at 80°, the mixture was allowed to cool to room temperature and the solvent was evaporated. Column chromatography (solvent *A*) of the residue provided **20** (60 mg, 91%) as a syrup, $[\alpha]_D$ −7.5° (*c* 0.22, chloroform), R_F 0.28 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.327 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.170 (dd, 1 H, $J_{5,6}$ 6.0, $J_{6,6'}$ 11.5 Hz, H-6), 4.073 (dd, 1 H, $J_{5,6'}$ 4.0 Hz, H-6'), 2.080 (s, 3 H, $COCH_3$), 2.030 (ddd, 1 H, $J_{3,4e}$ 5.0, $J_{4e,5}$ 1.9, $J_{4e,4a}$ 12.5 Hz, H-4*e*), 1.502 (ddd, 1 H, $J_{3,4a} = J_{4a,5} = 11.8$ Hz, H-4*a*), 0.872 (s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 170.83 ($COCH_3$), 103.88 (C-1), 66.02 (C-6), 33.44 (C-4), 20.87 ($COCH_3$), 14.11 (CH_3).

Anal. Calc. for $C_{30}H_{42}O_6$: C, 72.26; H, 8.49. Found: C, 72.16; H, 8.29.

Octyl 2,3-di-O-benzyl-4-deoxy-β-D-xylo-hexopyranoside (**21**). — Treatment of **20** (52.5 mg, 0.11 mmol) with sodium methoxide in methanol for 5 h at room temperature, followed by neutralization with IRC-150 (H^+) resin, subsequent removal of the resin, and solvent evaporation, provided **21** (45.8 mg, 95%) as a syrup, $[\alpha]_D$ −9.9° (*c* 0.42, chloroform), R_F 0.24 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.364 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 2.033 (br, 1 H, HO-6, D_2O-exchangeable), 1.958 (ddd, 1 H, $J_{3,4e}$ 5.2, $J_{4e,5}$ 1.9, $J_{4a,4e}$ 12.5 Hz, H-4*e*), 1.488 (ddd, 1 H, $J_{3,4a} = J_{4a,5} = 11.8$ Hz, H-4*a*), 0.876 (t, 3 H, *J* 6.2 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 103.97 (C-1), 65.33 (C-6), 32.83 (C-4), 14.14 (CH_3).

Anal. Calc. for $C_{28}H_{40}O_5$: C, 73.30; H, 8.79. Found: C, 73.65; H, 8.83.

Octyl 2,3-di-O-benzyl-6-O-(4-methoxyphenyldiphenylmethyl)-β-D-glucopyranoside (**22**). — Compound **17** (450 mg, 0.95 mmol) was dissolved in anhydrous pyridine (5 mL) and added to chloro-4-methoxyphenyldiphenylmethane (*p*-anisylchlorodiphenylmethane) (441 mg, 1.43 mmol). After stirring for 15 h at room temperature, methanol (5 mL) was added and the solvent was evaporated. A solution of the residual syrup in dichloromethane (50 mL) was washed sequentially with water

(50 mL), 5% HCl (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and finally twice with water (2 × 50 mL) before concentration to a syrup which was purified by chromatography (solvent *A*) to provide **22** (550.6 mg, 77.6%) as a syrup, $[\alpha]_D$ −19° (*c* 0.19, chloroform), R_F 0.33 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.020 (m, 1 H, $J_{1,2}$ 7.5 Hz, virtual coupling to H-2 and H-3, H-1), 3.787 (s, 3 H, OCH_3), 2.524 (d, 1 H, $J_{OH,4}$ 2.2 Hz, H-4, D_2O-exchangeable), 0.865 (t, 3 H, *J* 6.0 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 158.71 (quat. arom.), 113.27 (tert. arom.), 103.79 (C-1), 86.72 (Ph_3C), 64.31 (C-6), 55.29 (OCH_3), 14.20 (CH_3).

Anal. Calc. for $C_{48}H_{56}O_7$: C, 77.39; H, 7.58. Found: C, 77.22; H, 7.44.

*Octyl 2,3-di-O-benzyl-6-O-(4-methoxyphenyldiphenylmethyl)-4-O-methyl-β-*D-glucopyronoside (**23**). — To a suspension of sodium hydride (70 mg of a 50% dispersion in oil) in *N*,*N*-dimethylformamide (20 mL) was added, dropwise, a solution of **22** (435 mg, 0.58 mmol) in *N*,*N*-dimethylformamide (20 mL), and the mixture was stirred for 1 h at 20%. Methyl iodide (109 μL, 1.75 mmol) was then added dropwise at 0–5°, and the mixture was stirred for 3 h at room temperature and then taken to dryness. A solution of the residue in dichloromethane (50 mL) was washed with water (3 × 50 mL) before evaporation to an oil which was purified by chromatography (solvent *G*) to provide **23** (380 mg, 84.7%) as a clear syrup, $[\alpha]_D$ +21.8° (*c* 0.20, chloroform), R_F 0.50 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.405 (m, 1 H, $J_{1,2}$ 7.5 Hz, virtual coupling to H-2 and H-3, H-1), 3.783 (s, 3 H, $ArOCH_3$), 3.320 (s, 3 H, $CHOCH_3$), 0.870 (t, 3 H, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 158.41 (quat. arom.), 112.94 (tert. arom.), 103.54 (C-1), 85.68 (Ar_3C), 62.25 (C-6), 60.49 ($CHOCH_3$), 55.90 ($ArOCH_3$), 14.01 (CH_3).

Anal. Calc. for $C_{49}H_{58}O_7$: C, 77.54; H, 7.70. Found: C, 77.19; H, 7.53.

*Octyl 2,3-di-O-benzyl-4-O-methyl-β-*D-*glucopyranoside* (**24**). — Compound **23** (350 mg, 0.46 mmol) was dissolved in dichloromethane (3 mL), and aqueous 80% acetic acid (60 mL) was added. After 15 h, the solvents were evaporated and the residue was chromatographed (solvent *B*) to provide **24** (180 mg, 80.2%) as a syrup; $[\alpha]_D$ +17.3° (*c* 0.37, chloroform); R_F 0.27 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.411 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.553 (s, 3 H, OCH_3), 1.957 (dd, 1 H, $J_{6,OH}$ 5.5, $J_{6',OH}$ 8.0 Hz, D_2O-exchangeable, OH), 0.870 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.45 and 138.28 (quat. arom.), 103.50 (C-1), 61.92 (C-6), 60.60 (OCH_3), 13.91 (CH_3).

Anal. Calc. for $C_{29}H_{42}O_6$: C, 71.57; H, 8.70. Found: C, 71.48; H, 8.57.

*1-O-Acetyl-2,3,4-tri-O-benzyl-7-deoxy-6-O-(3,5-dinitrobenzoyl)-α,β-*L-glycero-D-gluco-*heptopyranose* (**27**). — A solution of conc. sulfuric acid (13 μL) in acetic anhydride (1.0 mL) was added dropwise, over a 5-minute period, to a solution of **25** (R_F 0.18, solvent *A*) (852 mg,. 1.27 mmol) in acetic anhdyride (4.0 mL) at 0°. The mixture was stirred for 30 min at room temperature, then poured into a stirred mixture of dichloromethane (50 mL) and ice-cold, saturated, aqueous sodium hydrogencarbonate, and the resulting mixture was stirred for 30 min at room temperature. The organic and aqueous layers were separated, and the aqueous layer was extracted with dichloromethane (50 mL). The dichloromethane solutions were

combined and washed with saturated aqueous sodium hydrogencarbonate and water, and then concentrated. The residual syrup was purified by chromatography on Iatrobeads (solvent *F*) to yield **27** (650 mg, 73.2%) as a white powder; R_F 0.19 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 9.30–9.05 (m, 3 H, arom.), 6.400 (d, 0.8 H, $J_{1,2}$ 3.6 Hz, H-1β), 5.622 (m, 1.2 H, H-1α and dq, $J_{5,6}$ 1.5, $J_{6,7}$ 6.5 Hz, H-6), 2.184 (s, 2.4 H, $COCH_3\beta$), 2.122 (s, 0.6 H, $COCH_3\alpha$), 1.476 (d, 0.6 H, H-7α), 1.404 (d, 2.4 Hz, H-7β); ^{13}C-n.m.r. ($CDCl_3$): δ 169.29 ($COCH_3$), 161.63 ($COCH_3$), 94.48 (C-1α), 89.59 (C-1β), 21.03 ($COCH_3\beta$), 16.47 (C-7β).

Anal. Calc. for $C_{37}H_{37}N_2O_{12}$: C, 63.33; H, 5.32; N, 3.99. Found: C, 63.17; H, 5.14; N, 3.89.

*Octyl 2,3,4-tri-*O*-benzyl-7-deoxy-6-*O*-(3,5-dinitrobenzoyl)-*α*-*L*-glycero-*D*-gluco-heptopyranoside* (**29**). — The acetate mixture **27** (525 mg, 0.75 mmol) was dissolved in dichloromethane (1.0 mL) at 0° and a saturated solution of hydrogen bromide in dichloromethane (5 mL) at 0° was added. The resulting solution was stirred for 0.5 h at 0°, and then for 1 h at room temperature, by which time t.l.c. indicated the complete conversion of **27** (R_F 0.24, solvent *H*) to a less polar product (R_F 0.36, solvent *H*), presumably the glycosyl bromide **28**. Solvent was evaporated at 20° and toluene (3 × 10 mL) was added to, and evaporated from, the pale-yellow syrupy residue which was dissolved in dry dichloromethane (2.0 mL). This solution was added dropwise over 10 min to a stirred mixture of 1-octanol (2.4 mL, 15 mmol), silver zeolite (2.4 g), and dichloromethane (1.0 mL) at −78°. After 1 h at −78° and 16 h at room temperature, the mixture was diluted with dichloromethane (25 mL) and filtered through Celite. The filtrate was washed with water (25 mL) and concentrated to a syrup which was purified by chromatography (solvent *G*) to provide **29** (416 mg, 73%) as a syrup, $[\alpha]_D$ +33.3° (*c* 0.23, chloroform); R_F 0.42 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 9.25–9.05 (m, 3 H, arom.), 5.604 (dq, 1 H, $J_{5,6}$ 1.8, $J_{6,7}$ 6.5 Hz, H-6), 4.330 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.380 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 1.517 (d, 3 H, H-7), 0.867 (t, 3 H, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 161.98 (Ar*C*O), 104.22 (C-1), 85.16, 82.24, 76.78, 75.67, and 70.93 (all methine, C-2,3,4,5,6), 75.75, 74.85, and 74.68 (ArCH_2), 70.33 (OCH_2CH_2), 31.84, 29.79, 29.41, 29.26, 26.19, and 22.67 (aliphatic), 16.31 (C-7), 14.09 (CH_3).

Anal. Calc. for $C_{43}H_{50}N_2O_{11}$: C, 66.99; H, 6.54; N, 3.63. Found: C, 66.81; H, 6.41; N, 3.52.

*Octyl 2,3,4-tri-*O*-benzyl-7-deoxy-α-*L*-glycero-*D*-gluco-heptopyranoside* (**30**). — Compound **29** (350 mg, 0.46 mmol) was dissolved in 1,4-dioxane (3 mL), M NaOH (1 mL) was added, and, after stirring for 1 h at room temperature, the solvent was evaporated. A solution of the residue in dichloromethane (50 mL) was washed with water (3 × 50 mL). The organic layer was concentrated to a syrup which was purified by chromatography (solvent *F*). Pure **30** (220 mg, 83%) was obtained as a syrup, $[\alpha]_D$ −10.4° (*c* 0.24, chloroform), R_F 0.53 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.408 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.050 (ddq, $J_{5,6}$ 1.5, $J_{6,OH}$ 10.5, $J_{6,7}$ 6.5 Hz, H-6), 3.100 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-5), 1.780 (d, 1 H, D_2O-exchangeable, OH), 1.300 (d, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.70, 138.51, 138.27 (ArCH_2), 103.94 (C-1),

65.41 (C-6), 20.33 (C-7), 14.14 (CH_3).

Anal. Calc. for $C_{36}H_{48}O_6$: C, 74.97; H, 8.39. Found: C, 75.05; H, 8.22.

*Methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-*D-glycero-D-gluco-*heptopyranoside* (**33**). — Compound **25** (800 mg, 1.24 mmol, R_F 0.33, solvent *H*) was deacylated as described for the preparation of **30**. After dilution of the reaction mixture with dichloromethane and washing with water, t.l.c. indicated the presence of a single compound (**31**), with R_F 0.20 (solvent *H*), which was not further characterized. The dichloromethane was evaporated, the residue was dissolved in anhydrous pyridine (5 mL), and methanesulfonyl chloride (1 mL) was added. After stirring for 5 h at room temperature, t.l.c. indicated almost quantitative conversion into a new product (R_F 0.65, solvent *J*), presumably the mesylate **32**. The pyridine was removed by evaporation, and the residue was dissolved in dichloromethane (50 mL) and washed sequentially with water, aqueous 5% hydrochloric acid, water, saturated aqueous sodium hydrogencarbonate, and finally water (50 mL of each). After drying and evaporation, the residue was dissolved in *N*,*N*-dimethylformamide (15 mL), anhydrous sodium benzoate (811 mg, 5.63 mmol) was added, and the mixture was heated for 15 h at 120°. After cooling and dilution with dichloromethane (100 mL), solids were filtered off and the organic solution was washed five times with ice-water. Concentration and chromatography on Iatrobeads (solvent *E*) provided **33** (350 mg, 53%) as a syrup, $[\alpha]_D$ +8.6° (*c* 0.38, chloroform), R_F 0.34 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.470 (dq, 1 H, $J_{5,6}$ 1.8, $J_{6,7}$ 6.5 Hz, H-6), 4.638 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.047 (dd, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.920 (dd, 1 H, $J_{4,5}$ 10.2 Hz, H-5), 3.523 (dd, 1 H, H-2), 3.423 (dd, 1 H, H-4), 3.377 (s, 3 H, OCH_3), 1.187 (d, 3 H, H-7); ^{13}C-n.m.r. ($CDCl_3$): δ 165.74 (Ar*C*O), 97.78 (C-1), 82.42, 80.13, 77.79, 71.36, and 70.10 (methine, C-2,3,4,5,6), 75.90, 74.59, and 73.38 (ArCH_2), 54.97 (OCH_3), 13.54 (C-7).

Anal. Calc. for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 74.50; H, 6.37.

*1-O-Acetyl-6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α,β-*D-glycero-D-gluco-*heptopyranose* (**34**). — Acetolysis of **33** (311 mg, 0.53 mmol), as described for the preparation of **27**, gave, after chromatographic purification (solvent *A*), the α,β-mixture **34** (170 mg, 52%) as a syrup, R_F 0.31 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 6.317 (d, 0.8 H, $J_{1,2}$ 3.6 Hz, H-1α), 5.643 (d, 0.2 H, $J_{1,2}$ 8.0 Hz, H-1β), 5.453 (m, 1 H, H-6αβ), 2.086 (s, 2.4 H, $COCH_3\alpha$), 2.067 (s, 0.6 H, $COCH_3\beta$), 1.213 (d, $J_{6,7}$ 6.5 Hz, H-7β), 1.204 (d, $J_{6,7}$ 6.5 Hz, H-7α).

Anal. Calc. for $C_{37}H_{38}O_8$: C, 72.77; H, 6.27. Found: C, 72.61; H, 6.23.

*Octyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-β-*D-glycero-D-gluco-*heptopyranoside* (**36**). — Reaction of **34** (150 mg, 0.25 mmol) with hydrogen bromide, as described for the preparation of **28**, effected its conversion into a less polar product (**35**; R_F 0.48, solvent *A*), which was not characterized. After solvent evaporation and co-evaporations with toluene, the residue was dissolved in dichloromethane (2 mL) and added to a stirred mixture of 1-octanol (4.9 mmol) and silver zeolite (770 mg) in dichloromethane (1 mL) at −78°. After 1 h at −78° and 15 h at room temperature, the reaction mixture was processed as described for the preparation of **29**. Com-

pound **36** (83 mg, 50%) was isolated as a syrup after chromatographic purification (solvent *G*); $[\alpha]_D$ 0° (*c* 0.42, chloroform); R_F 0.61 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.468 (dq, 1 H, $J_{5,6}$ 1.8, $J_{6,7}$ 6.5 Hz, H-6), 4.407 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 0.865 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 165.76 (Ar*C*O), 103.78 (C-1), 85.13, 82.40, 77.83, 75.86, and 70.19 (methine, C-2,3,4,5,6), 14.13 and 13.72 (C-7 and CH_2CH_3).

Anal. Calc. for $C_{43}H_{52}O_7$: C, 75.85; H, 7.70. Found: C, 75.73; H, 7.57.

*Octyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-*D*-glycero-*D*-gluco-heptopyranoside* (**37**). — Compound **36** (75 mg, 0.11 mmol) was dissolved in 1,4-dioxane (3.0 mL) and M NaOH (1.0 mL) was added. After 15 h at 70°, the solution was diluted with dichloromethane (50 mL) and washed three times with water (50 mL) before solvent evaporation and purification of the residue by chromatography (solvent *H*). The product (56 mg, 88%) was obtained as a syrup, $[\alpha]_D$ +11.2° (*c* 0.25, chloroform); R_F 0.46 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.411 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.987 (ddq, 1 H, $J_{5,6}$ 4.5, $J_{6,OH}$ 5.0, $J_{6,7}$ 6.5 Hz, H-6), 3.267 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 2.710 (d, 1 H, D_2O-exchangeable, O*H*), 1.162 (d, 3 H, H-7), 0.873 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.46, 138.42, and 137.63 (quat. arom.), 103.91 (C-1), 85.00, 82.56, 79.92, 76.98, and 68.55 (methine, C-2,3,4,5,6), 17.96 (C-7), 14.12 (CH_3).

Anal. Calc. for $C_{36}H_{48}O_6$: C, 74.97; H, 8.39. Found: C, 74.84; H, 8.36.

*Octyl 6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**39**). — A solution of **38** (252 mg, 0.45 mmol) in dichloromethane (2 mL) was added dropwise to a stirred mixture of alcohol **16** (170 mg, 0.30 mmol), silver trifluoromethanesulfonate (116 mg, 0.45 mmol), and tetramethylurea (72 μL, 0.60 mmol) in the same solvent (2 mL). After 2 h, the mixture was diluted with dichloromethane (25 mL), and *sym*-collidine (60 μL) followed by silver trifluoromethanesulfonate (115 mg) were added to destroy excess of **38**. After 0.5 h, tetraethylammonium bromide (95 mg) was added to precipitate excess of silver, and solids were removed by filtration and washed with dichloromethane (25 mL). The filtrate was washed twice with saturated aqueous sodium hydrogencarbonate (50 mL) and twice with water (50 μL) before evaporation and purification by chromatography on Iatrobeads (solvent *H*). Disaccharide **39** (243 mg, 77%) was obtained as a clear syryp, $[\alpha]_D$ +18.7° (*c* 0.54, chloroform); R_F 0.52 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{64}H_{76}O_{12}$: C, 74.10; H, 7.39. Found: C, 73.92; H, 7.43.

*Octyl 6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-2,3-di-*O*-benzyl-4-deoxy-β-*D*-xylo-hexopyranoside* (**40**). — Reaction of alcohol **21** (95 mg, 0.21 mmol) with 1.5 equiv. of **38**, as described for the preparation of **39**, gave, after chromatographic purification on Iatrobeads (solvent *H*), disaccharide **40** (148 mg, 76%) as a syrup, $[\alpha]_D$ + 10.2° (*c* 0.22, chloroform); R_F 0.40 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{57}H_{70}O_{11}$: C, 73.52; H, 7.58. Found: C, 73.17; H, 7.36.

*Octyl 6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-2,3-di-*O*-benzyl-4-*O*-methyl-β-*D*-glucopyranoside* (**41**). — Reaction of alcohol **24** (110 mg, 0.23

mmol) with **38** (1.5 equiv.), as described for the preparation of **39**, gave, after chromatographic purification on Iatrobeads (solvent *H*), disaccharide **41** (165 mg, 76%) as a syrup, $[\alpha]_D$ +24.7° (*c* 0.91, chloroform), R_F 0.45 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{58}H_{72}O_{12}$: C, 72.47; H, 7.55. Found: C, 72.35; H, 7.40.

Octyl 6-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-di-O-benzyl-7-deoxy-α-L-glycero-D-gluco-heptopyranoside (**42**). — Reaction of alcohol **30** (85 mg, 0.15 mmol) with **38** (1.5 equiv.), as described for the preparation of **39**, gave, after chromatographic purification on Iatrobeads (solvent *A*), disaccharide **42** (125 mg, 80%) as a syrup, $[\alpha]_D$ +19.9° (*c* 0.25, chloroform), R_F 0.62 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{65}H_{78}O_{12}$: C, 74.26; H, 7.48. Found: C, 74.02; H, 7.35.

Octyl 6-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-di-O-benzyl-7-deoxy-β-D-glycero-D-gluco-heptopyranoside (**43**). — Reaction of alcohol **37** (75 mg, 0.13 mmol) with **38** (1.5 equiv.), as described for the preparation of **39**, gave, after chromatographic purification on Iatrobeads (solvent *A*), disaccharide **43** (110 mg, 80%) as a syrup, $[\alpha]_D$ +21.5° (*c* 0.23, chloroform): R_F 0.64 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{65}H_{78}O_{12}$: C, 74.26; H, 7.48. Found: C, 74.12; H, 7.35.

Cyclohexylmethyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**44**). — Reaction of cyclohexylmethanol (150 mg, 1.31 mmol) with **38** (1.5 equiv.), as described for the preparation of **39**, gave, after chromatographic purification on Iatrobeads (solvent *H*), glycoside **44** (480 mg, 62%) as a syrup, $[\alpha]_D$ +24° (*c* 1.07, chloroform); R_F 0.63 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{36}H_{44}O_7$: C, 73.44; H, 7.53. Found: C, 73.09; H, 7.41.

*Octyl 2,3,4-tri-O-benzyl-6-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-*acetyl-2-*deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside* (**52**). — Treatment of acetate **39** (180 mg, 0.17 mmol; R_F 0.52 in solvent *B*) with methanolic sodium methoxide for 5 h at room temperature resulted in its complete conversion into the alcohol **45** (R_F 0.30, solvent *B*) which was not further characterized. Neutralization of the mixture with IRC-50 (H^+) resin, subsequent removal of the resin by filtration, and evaporation left **45** (148 mg, 86%) as an oil which was dried under high vacuum overnight. This oil (102 mg, 0.10 mmol) was dissolved in dry dichloromethane (2.0 mL), silver trifluoromethanesulfonate (264 mg, 1.0 mmol), *sym*-collidine (136 μL, 1.0 mmol), and molecular sieve (4 Å, 0.5 g) were added, and the mixture was cooled to −50°. A solution of bromide **51** (50 mg, 0.1 mmol) in dichloromethane (2.0 mL) was then added and, after 15 min at −50°, the mixture was warmed to room temperature during 1 h, when t.l.c. indicated the presence of ~25% of **45**. The mixture was cooled to −50°, more **51** (50 mg, 0.1 mmol) in dry dichloromethane (2.0 mL) was added, and, after 15 min, the reaction was warmed to room temperature where it was kept for an additional 1 h. The mixture was then diluted with dichloromethane (50 mL) and filtered through Celite. The filtrate was washed sequentially with ice-water, cold M HCl, saturated

aqueous sodium hydrogencarbonate, and finally water (50 mL of each). Solvent was evaporated and the residue was purified by chromatography on Iatrobeads (solvent *B*) to provide trisaccharide **52** (106 mg, 72%) as a syrup, $[\alpha]_D$ +7.6° (*c* 0.37, chloroform); R_F 0.59 (solvent *C*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{82}H_{93}NO_{20}$: C, 69.72; H, 6.64; N, 0.99. Found: C, 70.04; H, 6.58; N, 0.74.

*Octyl 2,3-di-*O*-benzyl-4-deoxy-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-xylo-hexopyranoside* (**53**). — Deacetylation of **40** (120 mg, 0.13 mmol, R_F 0.40 in solvent *B*), as described for the preparation of **45**, gave alcohol **46** (R_F 0.14, solvent *B*) as an oil (94 mg, 82%), which was not characterized. Reaction of **46** (60 mg, 0.70 mmol), as described for the preparation of **52**, provided, after chromatographic purification on Iatrobeads (solvent *C*), trisaccharide **53** (65 mg, 74%) as a syrup, $[\alpha]_D$ −1.3° (*c* 0.41, chloroform); R_F 0.19 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc for $C_{75}H_{87}NO_{19}$: C, 68.95; H, 6.71; N, 1.07. Found: C, 68.91; H, 6.69; N, 0.98.

*Octyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-glucopyranoside* (**54**). — Deacetylation of **41** (102 mg, 0.11 mmol, R_F 0.45 in solvent *B*), as described for the preparation of **45**, gave alcohol **47** (R_F 0.15, solvent *B*) as a syrup (85 mg, 87%) which was not characterized. Reaction of **47** (62.5 mg, 0.07 mmol), as described for the preparation of **52**, provided, after chromatographic purification on Iatrobeads (solvent *B*), trisaccharide **54** (65 mg, 72%) as a syrup, $[\alpha]_D$ +10.0° (*c* 0.34, chloroform); R_F 0.55 (solvent *C*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{76}H_{89}NO_{20}$: C, 68.30; H, 6.71; N, 1.05. Found: C, 68.04; H, 6.99; N, 0.92.

*Octyl 2,3-di-*O*-benzyl-7-deoxy-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-β-*D*-mannopyranosyl]-α-*L*-glycero-*D*-gluco-heptopyranoside* (**55**). — Deacetylation of **42** (101.5 mg, 0.10 mmol, R_F 0.62 in solvent *B*), as described for the preparation of **45**, gave alcohol **48** (R_F 0.30, solvent *B*) as a syrup (80 mg, 82%) which was not characterized. Reaction of **48** (50 mg, 0.05 mmol) with **51**, as described for the preparation of **52**, gave, after chromatographic purification on Iatrobeads (solvent *B*), trisaccharide **55** (48 mg, 69%) as a syrup; $[\alpha]_D$ 0° (*c* 0.23, chloroform); R_F 0.34 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{83}H_{95}NO_{20}$: C, 69.88; H, 6.71; N, 0.98. Found: C, 70.24; H, 6.85; N, 0.75.

*Octyl 2,3-*di-O-*benzyl-7-deoxy-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl]-α-*D*-mannopyranosyl]-β-*D*-glycero-*D*-gluco-*heptopyranoside* (**56**). — Deacetylation of **43** (96 mg, 0.091 mmol; R_F 0.64 in solvent *B*), as decribed for the preparation of **45**, gave alcohol **49** (R_F 0.31, solvent *B*) as a syrup (75 mg, 81%) which was not characterized. Reaction of **49** (53 mg,

0.053 mmol) with **51**, as described for the preparation of **52**, gave, after chromatographic purifiction on Iatrobeads (solvent *B*), trisaccharide **56** (50 mg, 67%) as a syrup, $[\alpha]_D$ +11.1° (*c* 0.23, chloroform); R_F 0.33 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{83}H_{95}NO_{20}$: C, 69.88; H, 6.71; N, 0.98. Found: C, 70.13; H, 6.68; N, 0.86.

*Cyclohexylmethyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranoside* (**57**). — Deacetylation of **44** (350 mg, 0.60 mmol; R_F 0.63 in solvent *B*), as described for the preparation of **45**, gave alcohol **50** (R_F 0.27, solvent *B*) as an oil (280 mg, 86%) which was not characterized. Reaction of **50** (160 mg, 0.29 mmol) with **51**, as described for the preparation of **52**, gave, after chromatographic purification on Iatrobeads (solvent *B*), trisaccharide **57** (210 mg, 74%) as a syrup, $[\alpha]_D$ +1.3° (*c* 1.0, chloroform); R_F 0.27 (solvent *B*). N.m.r. data are presented in Tables I and II.

Anal. Calc. for $C_{54}H_{61}NO_{15}$: C, 67.27; H, 6.38; N, 1.45. Found: C, 67.04; H, 6.25; N, 1.25.

*Octyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**58**). — A mixture of **52** (53 mg, 0.038 mmol) and hydrazine hydrate (182 μL, 3.8 mmol) in dry methanol (2 mL) was boiled under reflux for 5 h and then taken to dryness, the residue was dissolved in pyridine (1 mL), and acetic anhydride (1 mL) was added. After stirring for 16 h, excess of acetic anhydride was decomposed by dropwise addition of ethanol (5 mL) at 0°. Solvent was evaporated, and a solution of the residue in dichloromethane (25 mL) was washed with 5% HCl, saturated aqueous sodium hydrogencarbonate, and water. Evaporation left a white solid which was purified by chromatography on Iatrobeads (solvent *C*). The title compound (35.5 mg, 71%) was obtained as a clear syrup; R_F 0.19 (solvent *C*). N.m.r. data are presented in Tables I and II.

*Octyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,3-di-*O*-benzyl-4-deoxy-β-*D*-xylo-hexopyranoside* (**59**). — Compound **53** (45 mg, 0.034 mmol) was treated with hydrazine as described for the conversion of **52** into **58**. Chromatographic purification on Iatrobeads (solvent *D*) gave **59** as a syrup (30 mg, 72%); R_F 0.27 (solvent *D*). N.m.r. data are presented in Tables I and II.

*Octyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,3-di-*O*-benzyl-4-*O*-methyl-β-*D*-glucopyranoside* (**60**). — Compound **54** (50 mg, 0.037 mmol) was treated with hydrazine as described for the conversion of **52** into **58**. Chromatographic purification on Iatrobeads (solvent *D*) gave **60** as a foam (32 mg, 68%); R_F 0.29 (solvent *D*). N.m.r. data are presented in Tables I and II.

*Octyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,3,4-tri-*O*-benzyl-7-deoxy-α-*L*-glycero-*D*-gluco-heptopyranoside* (**61**). — Compound **55** (42 mg, 0.030 mmol) was treated

with hydrazine as described for the conversion of **52** into **58**. Chromatographic purification on Iatrobeads (solvent *C*) gave **61** as a syrup (28 mg, 71%); R_F 0.28 (solvent *C*). N.m.r. data are presented in Tables I and II.

*Octyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,3,4-tri-*O*-benzyl-7-deoxy-β-*D*-glycero-*D*-gluco-heptopyranoside* (**62**). — Compound **56** (44 mg, 0.031 mmol) was reacted with hydrazine as described for the conversion of **52** into **58**. Chromatographic purification on Iatrobeads (solvent *C*) gave **62** as a syrup (28 mg, 68%); R_F 0.31 (solvent *C*). N.m.r. data are given in Tables I and II.

*Cyclohexylmethyl 6-*O*-[2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**63**). — Compound **57** (83 mg, 0.086 mmol) was treated with hydrazine as described for the conversion of **52** into **58**. Chromatographic purification on Iatrobeads (solvent *D*) gave **63** as a foam (52 mg, 69%); R_F 0.31 (solvent *D*). N.m.r. data are presented in Tables I and II.

*Octyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-glucopyranoside* (**5**). — Compound **58** (32 mg, 0.024 mmol) was dissolved in 95% ethanol (4 mL) containing 5% palladium-on-carbon (32 mg) and stirred under one atmosphere of hydrogen for 15 h, by which time t.l.c. showed the complete disappearance of **58** to give one major spot, R_F 0.71 (solvent *I*), which was devoid of u.v. absorption in t.l.c. Removal of the catalyst by filtration, followed by evaporation and drying for 15 h over phosphorus pentaoxide, left a glass which was dissolved in dry methanol containing a trace of sodium methoxide and kept at 0° until all the material was converted into a single compound (R_F 0.30, solvent *I*). After neutralization with IRC-50 (H^+), removal of the resin, and evaporation, the residue was passed through a column of BioGel P-2 (200–400 mesh) (50 × 2.5 cm) using aqueous 10% ethanol as eluent. The carbohydrate-containing fractions were combined, concentrated, and lyophilized, to provide **5** as a white powder (13 mg, 82%), $[\alpha]_D$ −11.5° (*c* 0.29, water). ^{1}H-n.m.r. data are presented in Table III. ^{13}C-n.m.r. (D_2O): δ 175.75 ($COCH_3$), 103.19 (C-1), 100.39 (C-1′), 97.68 (C-1″), 77.11, 76.89, 76.70, 74.93, 74.20, 73.95, 73.70, 71.89, 70.75, 70.31, and 68.08 (all methine, C-2,3,4,5,2′,3′,4′,5′,3″,4″,5″), 71.65 (OCH_2CH_2), 66.61 (C-6), 62.38 and 61.46 (C-6′,6″), 56.24 (C-2″), 31.93, 29.61, 29.27, 29.21, 25.89, and 22.87 (octyl CH_2), 23.18 ($COCH_3$), 14.27 (CH_2CH_3).

Anal. Calc. for $C_{28}H_{51}NO_{16}\cdot 2H_2O$: C, 48.47; H, 7.99; N, 2.02. Found: C, 48.58; H, 7.63; N, 2.08.

*Octyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-4-deoxy-β-*D*-*xylo*-hexopyranoside* (**6**). — Compound **59** (28 mg, 0.023 mmol) was deprotected as described for the preparation of **5**. After deacetylation at 0°, Bio-gel P-2 chromatography, and lyophilization, **6** was obtained as a white powder (12 mg, 81%), $[\alpha]_D$ −12.6° (*c* 0.18, water), R_F 0.37 (solvent *I*). The ^{1}H-N.m.r. data are presented in Table III. ^{13}C-N.m.r. (D_2O): δ 175.64 ($COCH_3$), 103.45 (C-1), 100.39 (C-1′), 97.54 (C-1″), 77.15, 76.70, 75.70, 74.19, 73.78, 71.31 (2 × C), 70.75, 70.43, 68.08 (all methine, C-2,3,5,2′,3′,4′,5′,3″,4″,5″), 71.54 (OCH_2CH_3), 69.60

(C-6), 62.38 and 61.46 (C-6′,6″), 56.23 (C-2″), 35.13 (C-4), 31.93, 29.69, 29.29, 29.21, 25.92, and 22.86 (octyl CH_2), 23.16 ($COCH_3$), 14.27 (CH_2CH_3).

Anal. Calc. for $C_{28}H_{51}NO_{15}\cdot 3H_2O$: C, 48.33; H, 8.26; N, 2.01. Found: C, 48.03; H, 8.01; N, 2.18.

Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-4-O-methyl-β-D-glucopyranoside (**7**). — Compound **60** (31 mg, 0.025 mmol) was deprotected as described for the preparation of **5**. The product was obtained as a white powder (13 mg, 77%), $[\alpha]_D$ −9.8° (*c* 0.27, water); R_F 0.39 (solvent *I*). The ^{1}H-n.m.r. data are presented in Table III. ^{13}C-N.m.r. (D_2O): δ 175.64 ($COCH_3$), 103.08 (C-1), 100.37 (C-1′), 98.33 (C-1″), 80.06, 77.07, 76.69, 76.46, 74.19, 74.01 (3 × C), 70.75, 70.52, 68.03 (all methine, C-2,3,4,5,2′,3′,4′,5′,3″,4″,5″), 71.66 (OCH_2CH_3), 67.11 (C-6), 62.38 and 61.46 (C-6′,6″), 61.05 (OCH_3), 56.24 (C-2″), 31.95, 29.60, 29.30, 29.24, 25.90, and 22.88 (octyl CH_2), 23.20 ($COCH_3$), 14.31 (CH_2CH_3).

Anal. Calc. for $C_{29}H_{53}NO_{16}\cdot 2H_2O$: C, 49.21; H, 8.12; N, 1.98. Found: C, 49.07; H, 7.58; N, 2.07.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-7-deoxy-α-L-*glycero-D-gluco-*heptopyranoside* (**8**). — Compound **61** (27 mg, 0.020 mmol) was deprotected as described for the preparation of **5**. The product was obtained as a white powder (11 mg, 81%), $[\alpha]_D$ −4.9° (*c* 0.35, water); R_F 0.29 (solvent *I*). The ^{1}H-n.m.r. data are presented in Table III. ^{13}C-N.m.r. (D_2O): δ 175.67 ($COCH_3$), 103.58 (C-1), 100.71 (C-1′), 92.40 (C-1″), 78.14, 77.45, 76.74, 74.22, 74.18, 74.06, 70.75, 70.67, 70.21, 68.22, 68.09 (all methine, C-2,3,4,5,2′,3′, 4′,5′,3″,4″,5″), 71.60 (OCH_2CH_3), 62.37 and 61.48 (C-6′,6″), 56.36 (C-2″), 31.99, 29.68, 29.30, 29.25, 25.92, and 22.90 (octyl CH_2), 23.26 ($COCH_3$), 14.62 (C-7), 14.37 (CH_2CH_3).

Anal. Calc. for $C_{29}H_{53}NO_{16}\cdot 2H_2O$: C, 49.21; H, 8.12; N, 1.98. Found: C, 49.11; H, 7.48; N, 1.92.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-7-deoxy-β-D-*glycero-D-gluco-*heptopyranoside* (**9**). — Compound **62** (25 mg, 0.019 mmol) was deprotected as described for the preparation of **5**. The product was obtained as a white powder (10 mg, 80%), $[\alpha]_D$ +9.7° (*c* 0.20, water); R_F 0.31 (solvent *I*). The ^{1}H-n.m.r. data are presented in Table III. ^{13}C-N.m.r. (D_2O): δ 175.67 ($COCH_3$), 103.46 (C-1), 100.34 (C-1′), 96.66 (C-1″), 77.54, 76.96, 76.79, 76.36, 74.16, 74.04, 73.78, 71.29, 70.72, 70.41, 68.23 (all methine, C-2,3,4,5,2′,3′,4′,5′, 3″,4″,5″), 71.87 (OCH_2CH_2), 62.48 and 61.45 (C-6′,6″), 56.30 (C-2″), 31.96, 29.74, 29.28 (2 × C), 25.95 and 22.88 (octyl CH_2), 23.22 ($COCH_3$), 15.44 (C-7), 14.27 (CH_2CH_3).

Cyclohexylmethyl 6-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**10**). — Compound **10** (55 mg, 0.063 mmol) was deprotected as described for the preparation of **5**. The product was obtained as a syrup (23 mg, 76%), $[\alpha]_D$ +3.6° (*c* 0.17, water), R_F 0.45 (solvent *I*). The ^{1}H-n.m.r. data are presented in Table III. ^{13}C-N.m.r. data (D_2O) (′ refers to the α-D-Man unit, ″ refers

to the β-D-GlcNAc unit): δ 175.64 ($COCH_3$), 100.53 (C-1′), 97.71 (C-1″), 77.47, 76.70, 74.18, 73.70, 70.77, 70.60, and 68.18 (all methine, C-2′,3′,4′,5′,3″,4″,5″), 74.26 (OCH_2-CH), 62.38 and 61.48 (C-6′,6″), 56.25 (C-2″), 37.96 (OCH_2CH), 30.48, 30.13, 26.93, 26.18, and 26.09 (cyclohexyl CH_2), 23.18 ($COCH_3$).

Anal. Calc. for $C_{21}H_{37}NO_{11}\cdot H_2O$: C, 50.69; H, 7.90; N, 2.81. Found: C, 50.73; H, 7.79; N, 2.75.

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