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Combined chemical–enzymic synthesis of deoxygenated oligosaccharide analogs: transfer of deoxygenated D-Glc*p*NAc residues from their UDP-Glc*p*NAc derivatives using *N*-acetylglucosaminyltransferase I

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ABSTRACT

The 3″-, 4″-, and 6″-deoxy analogs of UDP-Glc*p*NAc have been synthesized chemically and found to act as donor-substrates for *N*-acetylglucosaminyltransferase-I (GnT-I) from human milk. Incubation of UDP-Glc*p*NAc and these deoxy analogs with GnT-I in the presence of α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-β-D-Man*p*-O$(CH_2)_8$COOMe gave β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-β-D-Man*p*-O$(CH_2)_8$COOMe (**6**), and the deoxy analogs **12**–**14** where HO-3, HO-4, and HO-6, respectively, of the β-D-GlcNAc residue were replaced by hydrogen. The tetrasaccharide glycosides **6** and **12**–**14** were characterized by ^{1}H-n.m.r. spectroscopy and evaluated as acceptors for GnT-II, the next enzyme in the pathway of biosynthesis of Asn-linked oligosaccharides. Deoxygenation of the 3-position of the β-D-GlcNAc residue of **6** completely abolished its acceptor activity, whereas removal of HO-4 or HO-6 caused only modest decreases in activity.

INTRODUCTION

Many elegant methods for the chemical synthesis of complex carbohydrates have been developed during the last 15 years[1–5], largely in response to the need for well-characterized oligosaccharides in investigations on the biological function of glycosylation. Such structures have value for probing the molecular specificity of carbohydrate–protein interactions[6–13], as immunogens for the production of carbohydrate-specific antibodies[14–16], as inhibitors of bacterial and viral adhesion[17–20], and as substrates for assaying glycosyltransferases[21–28]. Oligosaccharides that contain 2–5 sugar residues are now considered to be "routine" targets for synthesis. Their chemical syntheses, however, are laborious and their efficient assembly requires experienced individuals. Thus, the oligosaccharides (2–5 sugar units) synthesized in our laboratory during recent years have required an average of 7 chemical steps per constituent monosaccharide residue with each step requiring approximately one week of work. The most difficult steps are the stereospecific formation of glycosidic linkages, particularly using deoxygenated analogs of the naturally occurring sugars.

Combined chemical–enzymic synthesis of oligosaccharides is an attractive al-

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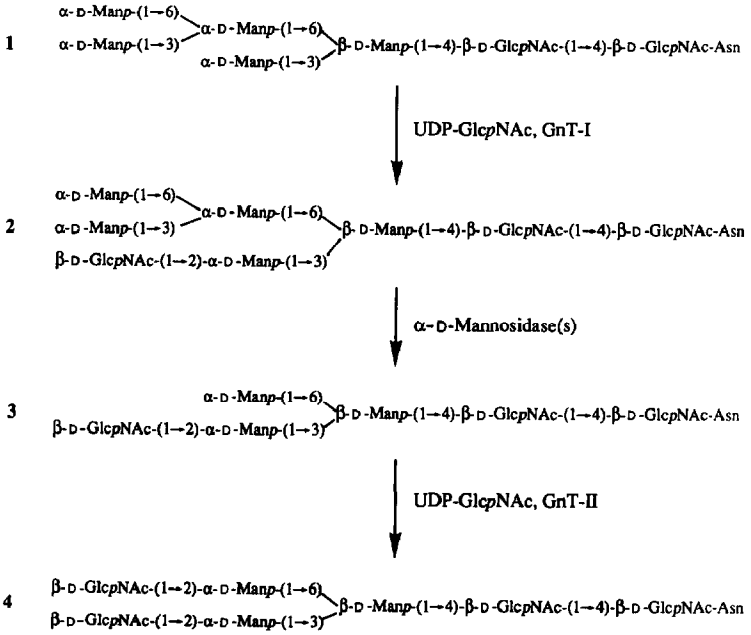
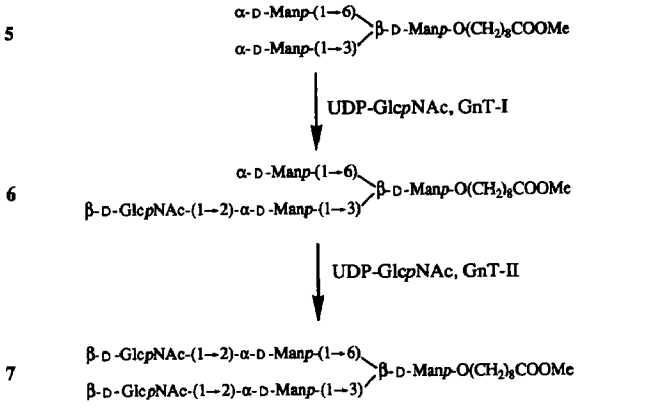
ternative to total chemical synthesis[29–34]. There have been several such approaches[35,36] with glycosidases and glycosyltransferases being used for the stereo- and regio-specific formation of glycosidic linkages. The breakthroughs being made in the cloning of the glycosyltransferases[37,38] suggest that these enzymes could become routine reagents for the preparation of biologically active oligosaccharides.

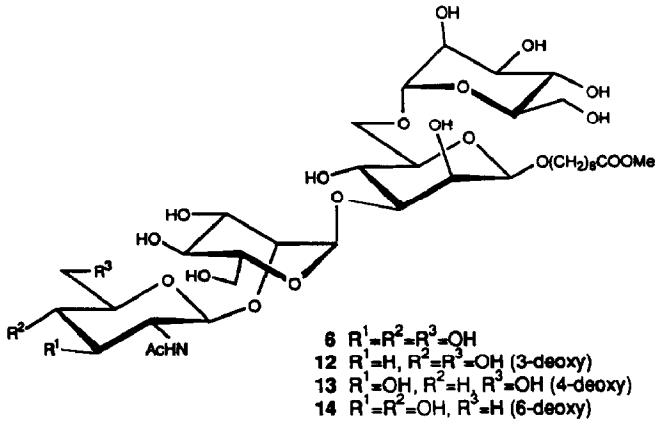
The glycosyltransferases involved in the biosynthesis of mammalian cell-surface carbohydrates generally transfer glycosyl residues from nucleotides to specific oligosaccharide acceptors[39]. A major potential impediment to the broad use of glycosyltransferases in the synthesis of oligosaccharides is their inability to provide analogs of the naturally occurring structures. For example, sugar-nucleotides with deoxygenated sugar moieties might not serve as donor-substrates for the enzymes. The utility of deoxygenated oligosaccharide analogs has been demonstrated by work from the groups of Lemieux[6–10] and Magnusson[18] in their studies of the origins of specificity of carbohydrate–protein interactions. The enzymically assisted preparation of such structures would greatly facilitate future studies of the molecular recognition of oligosaccharides. Few examples[40–43] have been described, however, where the transfer of deoxygenated glycosyl residues from nucleotides occurs. The scope of the combined chemical–enzymic synthesis of deoxygenated oligosaccharide analogs therefore remains undefined.

We now describe simple chemical syntheses of the 3″-deoxy- (**9**), 4″-deoxy- (**10**), and 6″-deoxy (**11**) analogs of UDP-Glc*p*NAc (**8**), and their behaviour as potential donor-substrates for a readily accessible, partially purified preparation of *N*-acetylglucosaminyltransferase I (GnT-I), a key enzyme involved in the branching of asparagine-linked oligosaccharides[44]. GnT-I can transfer[44] a D-Glc*p*NAc residue from UDP-

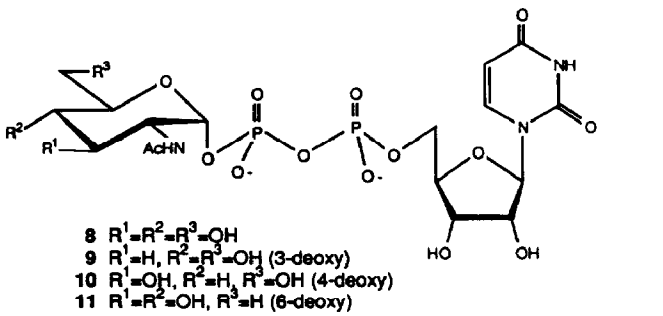


Glc*p*NAc to HO-2 of the α-D-Man*p*-(1→3) arm of the high-mannose glycopeptide **1**. The resulting glycopeptide **2** is then trimmed by α-D-mannosidases to produce **3** that serves as a substrate for further enzymes in the biosynthesis pathway, one of which is *N*-acetylglucosaminyltransferase II (GnT-II) that yields the ubiquitous biantennary structure **4** (ref. 44). The synthetic trisaccharide glycoside **5** is also a substrate for GnT-I, which catalyzes its conversion into **6** (refs. 28, 45). Furthermore, **6** is a substrate for GnT-II, which converts it into the pentasaccharide glycoside **7** (ref. 45).





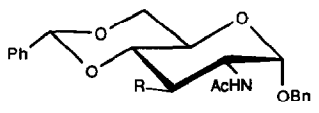
6 $R^1=R^2=R^3=OH$
12 $R^1=H$, $R^2=R^3=OH$ (3-deoxy)
13 $R^1=OH$, $R^2=H$, $R^3=OH$ (4-deoxy)
14 $R^1=R^2=OH$, $R^3=H$ (6-deoxy)



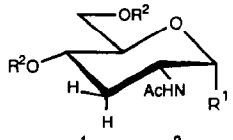
8 $R^1=R^2=R^3=OH$
9 $R^1=H$, $R^2=R^3=OH$ (3-deoxy)
10 $R^1=OH$, $R^2=H$, $R^3=OH$ (4-deoxy)
11 $R^1=R^2=OH$, $R^3=H$ (6-deoxy)

RESULTS AND DISCUSSION

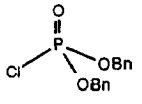
Chemical syntheses of UDP-GlcpNAc analogs. — At the start of this work, the oxazoline procedure[46,47] appeared to be the method of choice for the preparation of the required 3-, 4-, and 6-deoxygenated α-linked dibenzyl phosphates **22**, **27**, and **35**, respectively, which were to be deprotected to provide the free phosphates required for coupling to uridine-5'-phosphate (UMP) by the morpholidate procedure[48]. This strategy has been used[49] for the synthesis of the 4-deoxy-4-fluoro and 6-deoxy-6-fluoro derivatives of UDP-Glc*p*NAc. Preliminary studies showed that **35**, but not **27**, could be prepared readily by this method. Preparation of **22** by the oxazoline procedure was not attempted. It was reasoned that **27** (and, probably, **23**) was too labile under the acidic conditions of the oxazoline method and that preparation under basic conditions would be required. In fact, **27** so prepared was too labile to allow its purification even by chromatography on silica gel.



15 R=OH
16 R=OCSPh (92%)
17 R=H (79%)



18 R^1 = OBn, R^2=H (87%)
19 R^1 = OBn, R^2=Ac (78%)
20 R^1=α,β-OH, R^2=Ac (87%)



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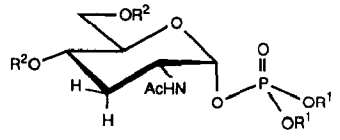


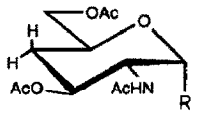
22 R^1=Bn, R^2=Ac (67%)
23 R^1=H, Et_3NH^+, R^2= Ac (78%)
24 R^1=Na^+, R^2=H (96%)

The general strategy for the synthesis of the target UDP-deoxy-Glc*p*NAc components involved preparation of the acetylated deoxy sugars **20**, **26**, and **34**, the 1-*O*-lithium salts of which were then phosphorylated[50] using dibenzyl phosphorochloridate (**21**). After deprotection, the α-phosphates could be coupled with UMP, using the morpholidate procedure[48].

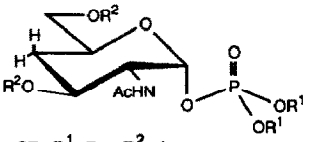
Reaction of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[51] (**15**) with phenyl chlorothionocarbonate[52] gave **16** (92%), which was reduced with tributylstannane to provide the 3-deoxyglycoside **17** (79%). Hydrolysis of the benzylidene group of **17** gave **18** (87%) which was *O*-acetylated to provide **19** (78%). Hydrogenolysis of **19** then gave the 3-deoxy sugar derivative **20** (87%). Reaction of **20**

with n-BuLi at −78°, followed by addition of **21** (ref. 50), gave the α-(dibenzyl phosphate) derivative **22** (67%) as the only detectable phosphorylated product. The benzyl groups in **22** were removed by hydrogenolysis to give **23** (78%), which was *O*-deacetylated to provide 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranosyl phosphate (**24**), isolated as its sodium salt (96%).

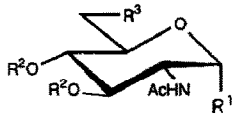
The preparation of **29** involved hydrogenolysis (Pd–C) of benzyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-α-D-*xylo*-hexopyranoside[53] (**25**) to provide the reducing sugar **26** (82%). Phosphorylation of **26** *via* its 1-*O*-lithium salt, using **21**, gave **27** which decomposed rapidly on attempted purification by column chromatography. Therefore, crude **27** was directly hydrogenolysed to provide the stable triethylammonium salt **28** (47% from **26**), *O*-deacetylation of which followed by ion-exchange provided 2-acetamido-2,4-dideoxy-α-D-*xylo*-hexopyranosyl phosphate (**29**, 89%).



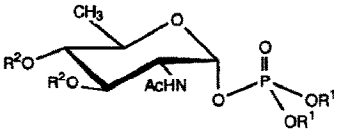
25 R=OBn
26 R=α,β-OH (82%)



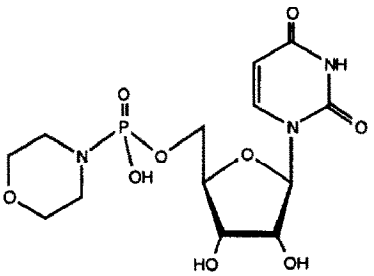
27 R^1=Bn, R^2=Ac
28 R^1=H, Et_3NH^+, R^2=Ac (46%)
29 R^1=Na^+, R^2=H (89%)



30 R^1 = OBn, R^2=H, R^3=OH
31 R^1 = OBn, R^2=H, R^3=I
32 R^1 = OBn, R^2=Ac, R^3=I (68%)
33 R^1 = OBn, R^2=Ac, R^3=H (86%)
34 R^1=α,β-OH, R^2=Ac, R^3=H (86%)



35 R^1=Bn, R^2=Ac (64%)
36 R^1=H, Et_3NH^+, R^2=Ac (86%)
37 R^1=Na^+, R^2=H (89%)



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The 6-deoxy-GlcNAc derivative **37** was prepared from benzyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside[51] (**30**) by iodination using Ph_3P–*N*-iodosuccinimide to give the iodo derivative **31**, which was not isolated but *O*-acetylated to provide **32** (68% from **30**). Reduction of **32** (Bu_3SnH) gave **33** (86%), which was hydrogenolysed to yield the reducing sugar **34** (86%). Phosphorylation of **34** gave **35** (64%), which was purified by chromatography and then hydrogenolysed to give **36** (86%); **36** was subsequently converted into the sodium salt (**37**, 89%) of 2-acetamido-2,6-dideoxy-α-D-glucopyranosyl phosphate.

The phosphates **24**, **29**, and **37** were converted into their UDP derivatives by coupling[48] with commercial UMP-morpholidate (**38**). The yields of **9**–**11** were modest (36–37%), but the nucleotides were isolated readily by sequential preparative t.l.c., chromatography on Bio-Gel P-2, and ion exchange. Sufficient material (13–25 mg) was obtained to allow their evaluation as potential donor-substrate analogs in the synthesis of oligosaccharides. The level of purity of **9**–**11** was 90–95% (^{1}H-n.m.r. data; Table I) with unreacted sugar phosphate as the contaminant. Further purification was not attempted because of the small amounts of material available.

Enzymic synthesis of **6** *and its deoxy analogs* **12**–**14**. — GnT-I has been purified both in soluble form, from bovine colostrum[22], and as the membrane-associated, detergent-solubilized form from rabbit liver[23,54]. For this study, a source of soluble GnT-I devoid of GnT-II activity was required. Soluble GnT-I, with an activity ~25-fold higher than that from bovine colostrum, was isolated from human milk. Soluble GnT-I from 450 mL of human milk was purified partially by defatting, precipitation with ammonium sulfate, ion-exchange on CM-Sephadex, and affinity chromatography on UDP-hexanolamine agarose. The final enzyme preparation was concentrated to 7 mL (which contained 3 munits of activity and 715 μg of protein) and stabilized by the addition of bovine serum albumin (1 mg.mL^{-1}). This preparation showed no GnT-II activity, when assayed using **6** as the acceptor, and was stable for at least one month at 4°.

The trimannoside **5** (2.4 mg) was incubated at 37° severally with **8**–**11** (2.4 mg of each) and 0.5 mL of the GnT-I preparation. After 24 h, more (2.4 mg) nucleotide was added and, after an additional 24 h, the oligosaccharides were isolated by adsorption on Sep-Pak C_{18} cartridges[28]. The extent of glycosylation of **5** was determined by integration of the resonances for H-1, NAc, and CH_2COO in the ^{1}H-n.m.r. spectra of the crude products. The conversions of **5** into the tetrasaccharide glycosides **6** and **12**–**14** were 100, 70, 10, and 37%, respectively. The products were separated from unreacted **5** by gel-permeation chromatography on Bio-Gel P-2 and quantitated by ^{1}H-n.m.r. spectroscopy. The quantities of pure tetrasaccharide glycosides thus isolated reflected the extent of enzymic conversion, with recoveries consistently being 72 ± 5%. The ^{1}H-n.m.r. data in support of the structural assignments of **6** and **12**–**14** are presented in Table II. GnT-I recognizes all the Glc*p*NAc hydroxyl groups in UDP-Glc*p*NAc, but they are not an absolute requirement for transfer to occur. Both 3″-deoxy (**9**) and 6″-deoxy (**11**) derivatives of UDP-Glc*p*NAc are reasonably reactive as donors for the enzyme, but the 4″-deoxy analog **10** is much less reactive. Nevertheless, sufficient quantities of all the

TABLE I

Selected ^{1}H- and ^{13}C-n.m.r. data for UDP-Glc*p*NAc (**8**) and its deoxy analogs **9**–**11**[a]

	8 (*UDP-Glc*p*NAc*)	**9** (*3″-deoxy*)	**10** (*4″-deoxy*)	**11** (*6″-deoxy*)
H-5 ($J_{5,6}$)[b]	5.94 (7.8)	5.93 (8.0)	5.95 (8.0)	5.93 (8.0)
H-6	7.94	7.96	7.95	7.94
H-1′ ($J_{1',2'}$)[c]	5.96 (4.0)	5.95 (4.0)	5.97 (4.0)	5.95 (4.0)
H-1″ ($J_{1'',2''}$, $J_{1'',P}$)	5.49 (3.5, 7.0)	5.45 (3.5, 7.0)	5.54 (3.5, 7.0)	5.45 (3.5, 7.0)
$COCH_3$	2.05	2.02	2.07	2.05
Other		2.07 (ddd, H-3″*e*, *J* 12.0, 5.0, 4.5) 1.77 (ddd, H-3″*a*, *J* 12.0, 11.5, 11.0)	2.05 (ddd, H-4″*e*, *J* 12.0, 4.5, 4.0) 1.58 (ddd, H-4″*a*, *J* 12.5, 12.0, 12.0)	1.28 (d, H-6″, *J* 6.5)
C-1′	89.4	89.5	89.3	89.3
C-4′ ($J_{4',P}$)	84.0 (8.5)	83.9 (9.0)	84.0 (9.0)	84.0 (9.0)
C-5′ ($J_{5',P}$)	65.8 (5.0)	65.7 (4.5)	66.0 (4.5)	65.7 (4.5)
C-1″ ($J_{1'',P}$)	95.3 (6.0)	94.0 (6.5)	96.1 (6.0)	95.1 (6.0)
C-2″ ($J_{2'',P}$)	54.5 (8.5)	48.7 (8.5)	55.8 (8.5)	54.7 (8.5)
COCH_3	175.6	174.7	175.7	175.5
CO*C*H_3	23.0	22.8	22.9	22.9
Deoxy-C″		32.5 (C-3″)	35.1 (C-4″)	17.6 (C-6″)

[a] ^{1}H-N.m.r. spectra were recorded at ambient temperatures at 300 or 360 MHz on solutions in D_2O with internal acetone (0.01%, δ 2.225), using 16k of computer memory. ^{13}C-N.m.r. spectra were recorded at 75 or 90 MHz on solutions in D_2O with external 1,4-dioxane (1%, δ 67.40). Only partial spectra were reported, but other spectral features were in accord with the assigned structures. [b] Coupling constants are reported as observed splittings, in Hz. [c] The atoms are designated as follows: uracil, unprimed; ribose, single primed; GlcNAc or deoxy analog, double primed.

TABLE II

Selected ^{1}H-n.m.r. data for **5**, **6**, and **12**–**14**[a,b]

	5	**6**	**12** (*3-deoxy*)	**13** (*4-deoxy*)	**14** (*6-deoxy*)
H-1 ($J_{1,2}$)	4.675 (<1)	4.672 (<1)	4.674 (<1)	4.673 (<1)	4.673 (<1)
H-1′ ($J_{1',2'}$)	5.104 (1.6)	5.127 (1.7)	5.142 (1.5)	5.127 (1.7)	5.131 (1.6)
H-1″ ($J_{1'',2''}$	4.908 (1.7)	4.910 (1.7)	4.911 (1.8)	4.910 (1.7)	4.910 (1.8)
H-1‴ ($J_{1''',2'''}$)		4.550 (8.3)	4.491 (8.3)	4.455 (8.3)	4.537 (8.4)
H-2 ($J_{2,3}$)	4.137 (3.1)	4.131 (3.3)	4.130 (3.0)	4.132 (3.0)	4.130 (3.0)
H-2′ ($J_{2',3'}$)	4.066 (3.4)	4.189 (3.4)	4.194 (3.4)	4.185 (3.4)	4.158 (3.4)
H-2″ ($J_{2'',3''}$)	3.992 (3.5)	3.990 (3.4)	3.992 (3.5)	3.991 (3.4)	3.992 (3.5)
OCMe	3.688	3.687	3.687	3.687	3.687
CH_2COO	2.390	2.389	2.388	2.389	2.388
COMe		2.051	2.002	2.052	2.099
Other			2.265 (ddd, H-3″*e*, *J* 12.2, 2.020 (m, H-4″*e*) 4.7, 4.7) 1.60 (H-3″a)		1.318 (d, H-6″, *J* 6.2)

[a] Recorded at 360 MHz at 22 ± 1°, using 32k of computer memory, an acquisition time of 4 s, and a relaxation delay of 10 s. Other conditions are as in Table I. [b] Protons are designated as follows: β-Man, primed; (1→3)-α-Man, single primed; (1→6)-α-Man, double primed; (1→2)-β-GlcNAc or deoxyhexose analog, triple primed.

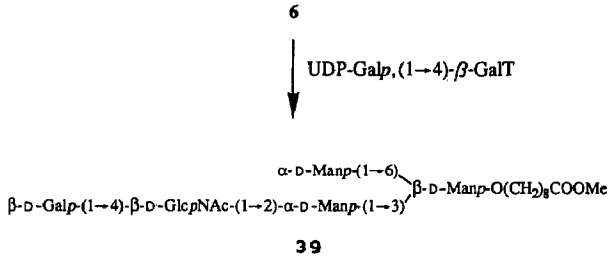
TABLE III

Evaluation of synthetic oligosaccharides as acceptors for GnT-II

Acceptor structure	*Activity (pmol.min^{-1})*	*Relative activity (%)*
6	2.95	100
12 (3‴-deoxy)	0	0
13 (4‴-deoxy)	1.75	59
14 (5‴-deoxy)	1.85	63
5	0.10	3
7	0.13	4
39	0.01	0

tetrasaccharide glycosides were obtained for evaluation as potential acceptor substrates for GnT-II. The kinetic characteristics for the modified donors **9–11** were not investigated.

Evaluation of GnT-II acceptor analogs. — The trisaccharide glycoside **5**, tetrasaccharide glycoside **6** and its deoxy analogs **12–14**, and the pentasaccharide glycosides **7** and **39** (produced by enzymic galactosylation of **6**) were evaluated as acceptors for GnT-II, partially purified from rat liver[55]. Each compound was evaluated at 0.2mM, the K_m for **6** (data not shown). This K_m is indistinguishable from that[55] of the natural hexasaccharide acceptor **3**. The results are summarized in Table III.



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The acceptor characteristics of **5–7** parallel those of the more complex natural structures **1–4** (refs. 44, 45) and confirm the tetrasaccharide moiety in **6** to be the minimum structure required for recognition by GnT-II. Galactosylation of HO-4 of the β-GlcNAc residue of **6**, to give **39**, abolishes the acceptor activity as has been observed previously for the corresponding natural structures[44,55]. The results in Table III suggest that this loss of activity is due to the bulk of an additional sugar residue and not to a requirement for hydrogen bonding to HO-4, since the 4‴-deoxy compound **13** is an acceptor. Evidence that the β-GlcNAc residue of **6** is recognized specifically by the enzyme comes from the observation that removal of HO-3‴ from **6**, to provide **12**, abolishes acceptor activity. The conclusion is that HO-3‴ is involved in a critical hydrogen-bonding interaction with the combining-site of GnT-II. On the other hand, HO-6‴ of **6** is not important since **14** is a good acceptor. Taken together, these results

demonstrate the limited but specific recognition of the β-GlcNAc residue of **6** by GnT-II. A salient feature of this work is that, once the sugar-nucleotide analogs and enzyme were in hand, conversion of the trisaccharide glycoside **5** into the four target tetrasaccharide glycosides **6** and **12–14** required less than one week of work for a single researcher.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ±2°. Analytical t.l.c. was performed on Silica Gel 60-F_{254} (Merck) with detection by quenching of fluorescence and/or by charring with H_2SO_4. Preparative t.l.c. was performed on Whatman PLK5F plates. 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. Millex-GV (0.22 μm) filter units were from Millipore, C_{18} Sep-Pak sample-preparation cartridges were from Waters Associates, Scintiverse E was from Fisher, and UMP-morpholidate was from Sigma. UDP-[6^{3}H]GlcNAc (specific activity, 18.9 Ci/mmol) was from New England Nuclear. ^{1}H-N.m.r. spectra were recorded at 360 (Bruker WM-360) or 300 MHz (Bruker AM-300) with either internal Me_4Si (δ 0, $CDCl_3$) or acetone (δ 2.225, D_2O). ^{13}C-N.m.r. spectra were recorded at 75.5 MHz (Bruker AM-300) with internal Me_4Si (δ 0, $CDCl_3$) or external 1% 1,4-dioxane (δ 67.4, D_2O). Only partial n.m.r. data are reported. Other spectral features were in accord with the proposed structures. The ^{1}H-n.m.r. data are reported as though they were first order. Assignments of ^{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 (Na_2SO_4) prior to concentration at <40° (bath). Microanalyses were carried out by the Analytical Services Laboratory of this department.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-phenoxythiocarbonyl-α-D-glucopyranoside (**16**). — A mixture of **15** (2.5 g, 6.26 mmol), 4-dimethylaminopyridine (3.44 g, 28.2 mmol), and phenyl chlorothionocarbonate (3.0 mL, 21.9 mmol) in dry acetonitrile (60 mL) was stirred for 15 h at room temperature, then diluted with dichloromethane (250 mL), and washed sequentially with ice-cold 0.5M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water. Evaporation of the solvent followed by column chromatography of the residue (dichloromethane–methanol, 49:1) gave **16** (3.0 g, 92%), $[\alpha]_D$ +21° (*c* 1.9, chloroform), R_F 0.71 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.54–7.00 (m, 15 H, 3 Ph), 5.98 (dd, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.90 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 5.56 (s, 1 H, PhC*H*O_2), 4.97 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.47 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.61 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 10.0 Hz, H-6), 4.26 (dd, 1 H, $J_{5,6'}$ 4.5 Hz, H-6'), 4.02 (m, 1 H, H-5), 3.89 (dd, 1 H, H-2), 3.81 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4); ^{13}C, δ 196.1 (C–S), 169.9 (CO*C*H_3), 101.6 (Ph*C*HO_2), 97.4 (C-1), 52.7 (C-2), 23.4 (*C*OCH_3).

Benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-ribo-hexopyranoside (**17**). — A solution of **16** (3.17 g, 6.10 mmol) in dry toluene (30 mL) was heated to 80° under nitrogen, and α,α′-azobisisobutyronitrile (753 mg, 4.52 mmol) was added followed by tributylstannane (9.9 mL, 36.6 mmol). After 2 h at 80°, the mixture was allowed to cool to room temperature and the solvent was evaporated. Column chromatography of the residue (dichloromethane–methanol, 49:1) provided **17** (1.85 g, 79%) as a white solid, $[\alpha]_D$ +86° (*c* 0.25, 1:1 chloroform–methanol), R_F 0.47 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^1H, δ 5.57 (s, 1 H, PhC*H*O_2), 4.83 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.77 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.52 (d, 1 H, PhC*H*H), 4.22 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 10.0 Hz, H-6), 2.12 (ddd, 1 H, $J_{3a,3e}$ 12.0, $J_{3e,2}$ 5.0, $J_{3e,4}$ 4.5 Hz, H-3*e*), 1.93 (s, 3 H, Ac); ^{13}C, δ 170.5 (*C*OCH_3), 101.5 (Ph*C*HO_2), 95.8 (C-1), 47.3 (C-2), 29.9 (C-3), 22.2 (CO*C*H_3).

Anal. Calc. for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57; N, 3.65. Found: C, 68.84; H, 6.65; N, 3.67.

Benzyl 2-acetamido-2,3-dideoxy-α-D-ribo-hexopyranoside (**18**). — A solution of **17** (1.5 g, 3.9 mmol) in acetic acid–water (9:1, 100 mL) was kept for 15 h at 40°, then concentrated. Column chromatography (dichloromethane–methanol, 17:3) of the residue gave **18** (1.0 g, 87%), $[\alpha]_D$ +31° (*c* 1.2, methanol), R_F 0.46. N.m.r. data (CD_3OD): ^1H, δ 7.43–7.23 (m, 5 H, Ph), 4.79 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.78 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.52 (d, 1 H, PhCH*H*), 4.00 (m, 1 H, H-2), 1.90 (s, 3 H, Ac); ^{13}C, δ 172.7 (*C*OCH_3), 96.2 (C-1), 74.9 (Ph*C*H_2), 62.8 (C-6), 49.1 (C-2), 34.0 (C-3), 22.4 (CO*C*H_3).

Benzyl 2-acetamido-4,6-di-O-acetyl-2,3-dideoxy-α-D-ribo-hexopyranoside (**19**). — A solution of **18** (1.0 g, 3.39 mmol) in pyridine (5 mL) and acetic anhydride (5 mL) was stirred overnight at room temperature, then concentrated, and toluene was evaporated from the residue. Column chromatography (dichloromethane–methanol, 49:1) then gave **19** (1.0 g, 78%), as a white solid, $[\alpha]_D$ +120° (*c* 0.7, chloroform), R_F 0.52 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^1H, δ 7.45–7.30 (m, 5 H, Ph), 5.65 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 4.84 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.76 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.52 (d, 1 H, PhCH*H*), 4.23 (d, 1 H, $J_{5,6}$ 5.0, $J_{6,6'}$ 12.0 Hz, H-6), 4.09 (d, 1 H, $J_{5,6'}$ 2.5 Hz, H-6′), 3.91 (m, 1 H, H-5), 2.24 (ddd, 1 H, $J_{3a,3e}$ 12.0, $J_{3e,2}$ 5.0, $J_{3e,4}$ 4.5 Hz, H-3*e*), 2.09, 2.05, 2.03 (3 s, each 3 H, 3 Ac), 1.72 (ddd, 1 H, *J* 11.5 and 11.0 Hz, H-3*a*); ^{13}C, δ 170.8, 169.4, 169.2 (3 *C*OCH_3), 95.8 (C-1), 62.5 (C-6), 46.6 (C-2), 30.6 (C-3), 23.2, 20.9, 20.8 (3 CO*C*H_3).

Anal. Calc. for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.22; H, 6.53; N, 3.45.

2-Acetamido-4,6-di-O-acetyl-2,3-dideoxy-α,β-D-ribo-hexopyranose (**20**). — A solution of **5** (950 mg, 2.5 mmol) in aqueous 95% ethanol (5 mL) containing 5% Pd–C (950 mg) was stirred under hydrogen (1 atm.) for 2 days, then filtered, and concentrated to give a ~6:1 α,β-mixture as a white solid (630 mg, 87%), R_F 0.29 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): δ 5.03 (d, $J_{1,2}$ 3.5 Hz, H-1α), 4.60 (d, $J_{1,2}$ 8.0 Hz, H-1β).

2-Acetamido-4,6-di-O-acetyl-2,3-dideoxy-α-D-ribo-hexopyranosyl dibenzyl phosphate (**22**). — To a solution of **20** (80 mg, 0.28 mmol) in dry tetrahydrofuran (1 mL) was added 1.6M butyl-lithium in hexane (173 μL, 0.28 mmol) at −70°. After 2 min, a solution

of dibenzyl phosphorochloridate[56] (**21**; 0.4 mL, 1.38 mmol) in tetrahydrofuran (2 mL) was added slowly at the same temperature, and the mixture was stirred for a further 10 min at −60°. Triethylamine was added to pH 8, the solvent was evaporated, and the residue was purified by chromatography (dichloromethane–methanol, 49:1) on Iatrobeads to give **22** (102 mg, 67%), as a white solid, $[\alpha]_D$ +64° (*c* 1, chloroform), R_F 0.50 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): δ 5.62–5.55 (2 H, H-1 and NH), 5.18–5.01 (m, 5 H, 2 PhCH_2 and H-3), 4.84 (ddd, 1 H, $J_{3e,4}$ 5.0, $J_{3a,4}$ 11.0, $J_{4,5}$ 10.0 Hz, H-4), 4.30 (m, 1 H, H-2), 3.92 (m, 1 H, H-5), 2.20 (ddd, 1 H, $J_{3a,3e}$ 12.0, $J_{2,3e}$ 4.5 Hz, H-3*e*), 2.05, 1.99, 1.75 (3 s, each 3 H, 3 Ac), 1.60 (ddd, 1 H, *J* 11.5 and 11.0 Hz, H-3*a*); ^{13}C, δ 170.7, 169.5, 169.3 (3 $COCH_3$), 95.9 (d, $J_{C,P}$ 6.4 Hz, C-1), 46.6 (d, $J_{C,P}$ 7.5 Hz, C-2), 29.5 (C-3), 22.9, 20.9, 20.7 (3 $COCH_3$).

Anal. Calc. for $C_{26}H_{32}NO_{10}P$: C, 56.83; H, 5.87; N, 2.55. Found: C, 56.87; H, 5.96; N, 2.42.

*2-Acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-*α*-*D*-*ribo*-hexopyranosyl phosphate.* — (*a*) *Triethylammonium salt* (**23**). A solution of **22** (82 mg, 0.15 mmol) in methanol (5 mL) and triethylamine (50 μL) was stirred under hydrogen (1 atm.) in the presence of 5% Pd–C (41 mg) for 1 h at room temperature, then filtered, and concentrated to give **23** (55 mg, 78%), R_F 0.38 (60:35:6) chloroform–methanol–water). ^1H-N.m.r. data (CD_3OD): δ 5.43 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.0 Hz, H-1), 4.85 (ddd, 1 H, $J_{3e,4}$ 5.0, $J_{3a,4}$ 10.5, $J_{4,5}$ 10.0 Hz, H-4), 4.25 (dd, 1 H, $J_{5,6}$ 4.5, $J_{6,6'}$ 12.0 Hz, H-6), 3.20 (q, 6 H, 3 NCH_2CH_3), 2.12 (ddd, 1 H, $J_{3a,3e}$ 12.0, $J_{3e,2}$ 5.0, $J_{3e,4}$ 4.5 Hz, H-3*e*), 2.03, 2.01, and 1.95 (3 s, each 3 H, 3 Ac), 1.85 (ddd, 1 H, $J_{3a,2}$ 11.5, $J_{3a,4}$ 11.0 Hz, H-3*a*), 1.31 (t, 9 H, 3 NCH_2CH_3).

(*b*) *Disodium salt* (**24**). — Compound **23** (50 mg, 0.11 mmol) was *O*-deacetylated conventionally with methanol in sodium methoxide at room temperature. The solution was neutralized with Dowex 50 (pyridinium) resin, filtered, and concentrated, and the residue was passed through a column of Dowex 50-X8 (Na^+) resin (5 mL) to give **24** as a gum (33.4 mg, 96%), $[\alpha]_D$ +13.9° (*c* 2.3, water), R_F 0.23 (7:3:1 2-propanol–water–ammonium hydroxide). N.m.r. data (D_2O): ^1H, δ 5.34 (dd, 1 H, $J_{1,2}$ 3, $J_{1,P}$ 7.0 Hz, H-1), 2.07 (ddd, 1 H, $J_{3a,3e}$ 12.0, $J_{3,4}$ 5.0, $J_{3e,4}$ 4.5 Hz, H-3*e*), 1.99 (s, 3 H, Ac), 1.80 (ddd, 1 H, *J* 11.5 and 11.0 Hz, H-3); ^{13}C, δ 174.6 ($COCH_3$), 92.0 (d, $J_{C,P}$ 5.0 Hz, C-1), 49.2 (d, $J_{2,P}$ 7.0 Hz, C-2), 32.6 (C-3), 23.1 ($COCH_3$).

*2-Acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-α,β-D-xylo-hexopyranose* (**26**). — Compound **25** (350 mg, 0.92 mmol) was hydrogenolysed, as described for the preparation of **20**, to yield **26** (220 mg, 82%), which was mainly (>90%) the α anomer, R_F 0.23 (19:1 dichloromethane–methanol). ^1H.N.m.r. data (CD_3OD): δ 5.17 (ddd, $J_{3,4e}$ 5.0, $J_{3,4}$ 12.0, $J_{2,3}$ 10.0 Hz, H-3), 3.80 (dd, $J_{5,6}$ 3.5, $J_{6,6'}$ 12.0 Hz, H-6), 2.10 (m, 1 H, H-4*e*), 2.05, 2.00, 1.93 (3 s each 3 H, 3 Ac), 1.52 (ddd, 0.9 H, $J_{3,4} = J_{4,5} = 12.0$, $J_{4a,4e}$ 12.5 Hz, H-4).

*2-Acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-α-D-xylo-hexopyranosyl phosphate.* (*a*) *Triethylammonium salt* (**28**). — Compound **26** (80 mg, 0.28 mmol) was phosphorylated as described for the preparation of **21**. The crude product **27** decomposed on attempted column chromatography and was therefore hydrogenolysed in the presence of triethylamine (50 μL) and methanol (5 mL), using 5% Pd–C (80 mg). Removal of the catalyst and evaporation of the solvent gave a syrup which was purified by chromatography

(dichloromethane–methanol–water, 60:35:6) on Iatrobeads to give **28** (61.5 mg, 47%) as a clear syrup, R_F 0.38 (60:35:6 dichloromethane–methanol–water). ^{1}H-N.m.r. data (CD_3OD): δ 5.51 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.0 Hz, H-1), 5.18 (ddd, 1 H, $J_{4e,3}$ 5.0 Hz, H-3), 3.20 (q, 6 H, NCH_2CH_3), 2.04, 2.00, 1.95 (3 s, each 3 H, 3 Ac), 1.31 (t, 9 H, 3 NCH_2CH_3).

(*b*) *Disodium salt* (**29**). — Compound **28** (56 mg, 0.12 mmol) was *O*-deacetylated, as described for the preparation of **24**, to give **29** (35 mg, 89%) as a gum, $[\alpha]_D$ +26° (*c* 0.6, water), R_F 0.21 (7:3:1 2-propanol–water–ammonium hydroxide). N.m.r. data (D_2O): ^{1}H, δ 5.42 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.0 Hz, H-1), 4.05 (ddd, 1 H, $J_{3,4e}$ 5.0, $J_{3,4}$ 12.0, $J_{2,3}$ 10.0 Hz, H-3), 2.05 (s, 3 H, Ac); ^{13}C, δ 175.7 ($COCH_3$), 94.5 (d, $J_{C,P}$ 5.7 Hz, C-1), 56.2 (d, $J_{2,P}$ 8.3 Hz, C-2), 35.3 (C-4), 22.9 ($COCH_3$).

Benzyl 2-acetamido-3,4-di-O-*acetyl-2,6-dideoxy-6-iodo*-α-D-*glucopyranoside* (**32**). — To a solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[51] (**30**; 1.20 g, 3.85 mmol) in dry *N,N*-dimethylformamide (60 mL) was added *N*-iodosuccinimide (1.73 g, 7.71 mmol). The mixture was cooled to 0°, triphenylphosphine (2.02 g, 7.71 mmol) was added slowly in portions, the mixture was heated for 20 min at 50°, and then the solvent was evaporated. Column chromatography (dichloromethane–methanol, 17:3) of the residue gave crude **31**, which was treated conventionally with acetic anhydride (5 mL) and pyridine (5 mL) for 15 h. Column chromatography (dichloromethane–methanol, 49:1) of the product gave **32** (1.33 g, 68%) as white powder, $[\alpha]_D$ +83° (*c* 0.3, chloroform), R_F 0.82 (17:3 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.42–7.32 (m, 5 H, Ph), 6.02 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 5.22 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4), 4.93 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.92 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 4.87 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.54 (d, 1 H, PhCH*H*), 4.38 (m, 1 H, H-2), 3.87 (m, 1 H, H-5), 3.29 (dd, 1 H, $J_{5,6}$ 2.5, $J_{6,6'}$ 11.0 Hz, H-6), 3.15 (dd, 1 H, $J_{5,6'}$ 9.0 Hz, H-6'), 2.05, 2.00, 1.90 (3 s, each 3 H, 3 Ac); ^{13}C, δ 171.2, 169.8, 169.4 (3 $COCH_3$), 96.1 (C-1), 51.9 (C-2), 23.2 ($COCH_3$), 20.7 (2 $COCH_3$), 3.78 (C-6).

Anal. Calc. for $C_{19}H_{24}INO_7$: C, 45.16; H, 4.79; N, 2.77. Found: C, 45.45; H, 4.92; N, 2.70.

Benzyl 2-acetamido-3,4-di-O-*acetyl-2,6-dideoxy*-α-D-*glucopyranoside* (**33**). — Compound **32** (850 mg, 1.68 mmol) was deoxygenated by treatment with tributylstannane, as described for the preparation of **17**. Column chromatography (dichloromethane–methanol, 49:1) of the product provided **33** (550 mg, 86%), $[\alpha]_D$ +131° (*c* 0.5, chloroform), R_F 0.41 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.43–7.30 (m, 5 H, Ph), 5.66 (d, 1 H, $J_{2,NH}$ 9.5 Hz, N*H*), 5.19 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 10.0 Hz, H-3), 4.87 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.86 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-4), 4.72 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*H), 4.50 (d, 1 H, PhCH*H*), 4.32 (m, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 3.89 (dq, 1 H, $J_{5,6}$ 6.5 Hz), 2.04, 2.01, 1.89 (3 s, each 3 H, 3 Ac), 1.17 (d, 3 H, H-6,6,6); ^{13}C, δ 171.3, 169.6, 169.5 (3 $COCH_3$), 96.4 (C-1), 52.1 (C-2), 23.1, 20.7, 20.6 (3 $COCH_3$), 17.3 (C-6).

Anal. Calc. for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 60.40; H, 6.46; N, 3.61.

2-Acetamido-3,4-di-O-*acetyl-2,6-dideoxy*-α,β-D-*glucopyranose* (**34**). — Compound **33** (350 mg, 0.92 mmol) was hydrogenolysed as described for the preparation of **20**, to provide **34** (230 mg, 86%) as a ~7:1 α,β-mixture, R_F 0.35 (19:1 dichloromethane–

methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.88 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 5.15 (dd, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 4.86 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 4.27 (m, 1 H, H-2), 4.11 (dq, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 1.97, 2.03, 2.08 (3 s, each 3 H, 3 Ac), 1.18 (d, 3 H, H-6,6,6).

*2-Acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-*α*-*D*-glucopyranosyl dibenzyl phosphate* (**35**). — Compound **34** (80 mg, 0.28 mmol) was phosphorylated as described for the preparation of **21**, to give **35** (98 mg, 64%) as a white powder after chromatography (dichloromethane–methanol, 49:1) on Iatrobeads; $[\alpha]_D$ +68° (*c* 0.5, chloroform), R_F 0.46 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.45–7.30 (10 H, 2 Ph), 5.65 (d, 1 H, $J_{1,2}$ 3.5 Hz), 5.61 (d, 1 H, NH), 5.15–5.00 (m, 5 H, 2 PhCH_2 and H-3), 4.87 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 4.33 (m, 1 H, H-2), 3.94 (dq, *J* 6.5 Hz, H-5), 2.05, 2.02, 1.70 (3 s, each 3 H, 3 Ac), 1.10 (dd, 3 H, *J* 6.5 Hz, H-6,6,6); ^{13}C, δ 171.3, 170.1, 169.5 (3 *C*OCH$_3$), 96.4 (d, $J_{C,P}$ 6.6 Hz, C-1), 52.2 (d, $J_{2,P}$ 7.8 Hz, C-2), 22.8 (2 *C*OCH$_3$), 20.7 (*C*OCH$_3$), 17.2 (C-6).

Anal. Calc. for $C_{26}H_{32}NO_{10}P$: C, 56.83; H, 5.87; N, 2.55. Found: C, 57.07; H, 5.92; N, 2.36.

*2-Acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-*α*-*D*-glucopyranosyl phosphate.* — (*a*) *Triethylammonium salt* (**36**). — Compound **35** (54 mg, 0.10 mmol) was hydrogenolysed, as described for the preparation of **23**, to give **36** (40 mg, 86%), R_F 0.38 (60:35:6 dichloromethane–methanol–water). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.43 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.0 Hz, H-1), 5.24 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 4.77 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 4.23 (m, 1 H, H-2), 4.17 (dq, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 3.18 (q, 6 H, 3 NCH_2CH$_3$), 2.03, 1.96, 1.93 (3 s, each 3 H, 3 Ac), 1.31 (t, 9 H, 3 NCH$_2$CH_3), 1.15 (d, 3 H, H-6,6,6).

(*b*) *Disodium salt* (**37**). — Compound **36** (40 mg, 0.09 mmol) was *O*-deacetylated, as described for the preparation of **24**. After neutralization with Dowex 50-X8 (pyridinium) resin, the mixture was filtered and concentrated to give a syrup that was passed through Dowex 50-X8 (Na^+) resin to give **37** (25 mg, 89%), $[\alpha]_D$ +50° (*c* 0.6, water), R_F 0.31 (7:3:1 2-propanol–water–ammonium hydroxide). N.m.r. data (D_2O): ^{1}H, δ 5.31 (1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.0 Hz, H-1), 4.01–3.87 (m, 2 H, H-2,5), 3.71 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.21 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 2.05 (s, 3 H, Ac), 1.25 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6,6,6); ^{13}C, δ 175.5 (*C*OCH$_3$), 93.6 (d, $J_{C,P}$ 5.7 Hz, C-1), 55.2 (d, $J_{2,P}$ 7.6 Hz, C-2), 23.0 (COCH_3), 17.7 (C-6).

*8-Methoxycarbonyloctyl [*O*-β-*D*-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→2)-*O*-α-*D*-mannopyranosyl-(1→3)]-*O*-α-*D*-mannopyranosyl-(1→6)-β-*D*-mannopyranoside* (**39**). — A solution of **6** (5.25 mg, 7.8 μmol) and UDP-galactose (13.6 mg, 19.3 μmol) in 1.3 mL of buffer containing 22mM $MnCl_2$, 100mM sodium cacodylate (pH 7.5), and 65 mU of bovine galactosyltransferase was kept for 24 h at 37°, then diluted with water (5 mL), and passed through a C_{18} Sep-Pak cartridge which was washed with water (5 × 5 mL). The product was eluted with methanol (2 × 5 mL) and the eluate was concentrated. Elution of the residue from a column (2.6 × 50 cm) of Bio-Gel P-2 with aqueous 10% ethanol at 0.2 mL.min^{-1} gave **39**, isolated as a white powder (7.3 mg, 90%) after lyophilization. ^{1}H-N.m.r. data (D_2O): δ 5.131 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.909 (d, 1 H, $J_{1'',2''}$ 1.7 Hz, H-1''), 4.674 (s, 1 H, H-1), 4.577 (d, 1 H, $J_{1''',2'''}$ 7.8 Hz, H-1'''), 4.468 (d, 1 H, $J_{1'''',2''''}$ 7.8 Hz, H-1''''), 4.193 (dd, 1 H, $J_{2',3'}$

3.4 Hz, H-2'), 4.132 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.991 (dd, 1 H, $J_{2'',3''}$ 34 Hz, H-2''), 3.687 (s, OMe), 2.389 (t, 2 H, CH_2COO), 2.050 (s, 3 H, Ac).

Preparation of sugar nucleotides. — A solution of 4-morpholine *N,N'*-dicyclohexylcarboxamidinium uridine 5'-phosphoromorpholidate (**38**, 1.0 mmol) in anhydrous pyridine (10–15 mL) was concentrated to dryness *in vacuo*. The process of dissolution and concentration was repeated twice, dry nitrogen being admitted into the flask after each concentration. A solution of each disodium salt (**24**, **29**, or **37**; 1 equiv. relative to the morpholidate) in water was passed slowly through a column of Dowex 50-X8 (pyridinium) resin and the column was thoroughly washed with water. The eluate was basified with pyridine and concentrated *in vacuo* to ~5 mL, and a solution of trioctylamine (2 equiv. relative to the amount of the sugar phosphate) in pyridine was added. The solution was then concentrated to dryness and the residue was rendered anhydrous by four times dissolving it in dry pyridine and evaporating the solvent *in vacuo*. A solution of the residue in pyridine was added to the dry phosphoromorpholidate described above. The solution was concentrated *in vacuo*, and a solution of the residue in anhydrous pyridine (2 mL) was kept at room temperature for 5 days, then concentrated *in vacuo* to ~0.5 mL. Preparative t.l.c. (2-propanol–water–ammonium hydroxide, 7:3:1), visualization by u.v. light, and extraction of the appropriate zone with 2-propanol–water (4:1) gave a product that was eluted from a column (2.0 × 65.0 cm) of Bio-Gel P-2 with water–ethanol (10:1) to remove traces of silica gel. Concentration of the eluate gave the sugar nucleotides, which were converted into the disodium salts by passage through the Dowex 50-X8 (Na^+) resin.

*Disodium uridine 5'-(2-acetamido-2,3-dideoxy-α-*D*-ribo-hexopyranosyl diphosphate)* (**9**). — Morpholidate **38** (75.4 mg, 0.11 mmol) and the trioctylammonium salt of **24** (69.9 mg, 0.11 mmol) were condensed by the general procedure described above, to afford **9** as a white solid (25 mg, 36%), R_F 0.60 (7:3:1 2-propanol–water–ammonium hydroxide).

*Disodium uridine 5'-(2-acetamido-2,4-dideoxy-α-*D*-xylo-hexopyranosyl diphosphate)* (**10**). — Morpholidate **38** (37.6 mg, 0.055 mmol) was condensed with the trioctylammonium salt of **29** (34.9 mg, 0.055 mmol) to provide **10** (13 mg, 37%), as a white solid, R_F 0.48 (7:3:1 2-propanol–water–ammonium hydroxide).

*Disodium uridine 5'-(2-acetamido-2,6-dideoxy-α-*D*-glucopyranosyl diphosphate)* (**11**). — Morpholidate **38** (52.7 mg, 0.077 mmol) was condensed with the trioctylammonium salt of **37** (48.9 mg, 0.077 mmol) to provide **11** (17.4 mg, 36%) as a white solid, R_F 0.47 (7:3:1 2-propanol–water–ammonia).

Partial purification of human milk GnT-I. — The partial purification of GnT-I from human milk was carried out at 4° by a modified procedure[33]. Thawed milk (450 mL) was defatted by centrifugation, and protein that precipitated at 65% $(NH_4)_2SO_4$ saturation was dialyzed against 25mM sodium cacodylate (pH 6.5, 3 × 4 L) containing 5mM $MnCl_2$ (buffer A). After dialysis, precipitated protein was removed by centrifugation, and the supernatant solution was loaded onto a column (2.5 × 35 cm) of CM-Sephadex C50 equilibrated in buffer A. The column was washed with buffer A until no more protein was eluted and then stepwise using buffer A containing 0.1, 0.2, and

0.3M NaCl (30 mL of each). The 0.3mM eluate was dialyzed against 25mM sodium cacodylate (pH 6.8) that contained 10mM $MnCl_2$ (500 mL, buffer B). The retentate was then applied to a 2-mL column of UDP-hexanolamine agarose[57] (13 μmol of ligand.mL^{-1} gel) equilibrated in buffer B. The column was washed with buffer B (50 mL), then eluted with 30mM uridine 5'-phosphate (UMP) in buffer B. The eluate was dialyzed (PM-10 membrane) against buffer B (2 L) and concentrated to 7 mL by ultrafiltration on an Amicon PM-10 membrane. This preparation of GnT-I contained 3 mU of activity and 715 μg of protein, and corresponded to 13% recovery of the enzyme activity and 1200-fold purification. Bovine serum albumin (BSA) was added to provide a 1 mg.mL^{-1} solution which was used in the preparative syntheses without further purification. One unit is defined here as the quantity of enzyme that forms 1 μmol of product.min^{-1} with 0.15mM UDP-Glc*p*NAc and 0.5mM **5** at 37° in buffer B.

Enzymic synthesis of tetrasaccharide glycosides **6** *and* **12–14**. — The GnT-I preparation described above (0.5 mL) was added to 1.5-mL plastic microfuge tubes each containing **5** (2.4 mg) and one of **8–11** (2.4 mg). After 24 h at 37°, more **8–11** (2.4 mg) was added to the appropriate tube. After an additional 24 h at 37°, each sample was diluted with water (5 mL) and passed through a C_{18} Sep-Pak as described[27]. After washing with water (3 × 5 mL), the oligosaccharide glycosides were eluted with methanol (3 × 5 mL) and the methanol was evaporated. A solution of each residue in water (5 mL) was filtered through a 0.22-μm Millex filter and then lyophilized. D_2O (1.0 mL) was added to the residual white powder and the resulting solutions were lyophilized. Each residue was dissolved in 100% D_2O and the ^{1}H-n.m.r. spectra were recorded using a 45° pulse with 10-s delays between pulses. Increases in the relaxation delays did not alter the relative intensities of the signals. The percent conversion of **5** into **6** and **12–14**, estimated from the integrated intensities of the signals for H-1, NAc, and CH_2COO, were 100 (**6**), 70 (**12**), 10 (**13**), and 37 (**14**).

Each sample was then recovered by lyophilization, unreacted **5** was removed by elution from a column (1.5 × 50 cm) of Bio-Gel P-2 with aqueous 10% ethanol at 0.25 mL.min^{-1}, and fractions of 1.25 mL were collected. The mannose content of the eluate was monitored using the phenol–sulfuric acid assay[58] on aliquots (50 μL) of individual fractions. Tetrasaccharide glycosides were eluted in fractions 28–32, and **5** in fractions 34–39. Fractions containing each of **6** and **12–14**, uncontaminated by **5**, were combined and concentrated, and the residues were lyophilized from D_2O. Each residue was dissolved in 100% D_2O (450 μL) that contained 0.61 μmol of acetone (0.01%) as internal standard for both chemical shifts and oligosaccharide concentration. The ^{1}H-n.m.r. spectra of **6** and **12–14** were recorded as described in Table II. The concentration (1.36mM) of internal acetone in the D_2O used for the preparation of n.m.r. samples was established in a separate experiment by integration *vs.* a weighed quantity of sodium benzoate. Thus, the quantities of purified tetrasaccharide glycosides, isolated after Bio-Gel Chromatography, were estimated to be 2.13 (**6**, 68%), 1.58 (**12**, 52%), 0.22 (**13**, 7%), and 0.88 mg (**14**, 29%) (errors estimated to be < 10%).

Assay for GnT-II activity. — GnT-II from rat liver (5 mU.mL^{-1}) was obtained as an ~1000-fold purified preparation that contained 25mM MES (pH 6.85), 0.05M NaCl,

5mM $MnCl_2$, 0.1% of Triton X-100, 20% (w/v) of glycerol, and 0.02% of NaN_3 (this preparation consists of the activity that eluted between 40 and 50 mL in Fig. 5 of ref. 55). In the assays for GnT-II incubation, mixtures contained, in 20 μL, 0.35mM UDP-GlcNAc, 250 000 d.p.m. of UDP-^{3}H-GlcNAc, 0.2mM acceptor oligosaccharide (**5**, **6**, **7**, **12–14**, and **39**), GnT-II (3 μU), 1% of Triton X-100, 10mM $MnCl_2$, and 50mM sodium cacodylate (pH 6.5). After reaction for 1.5 h at 37°, each mixture was diluted with water (5.0 mL) and passed through C_{18} Sep-Pak cartridges as described previously. After washing with water (5 × 5 mL), radiolabelled products were eluted with methanol (2 × 5 mL) and quantitated as d.p.m. in 10 mL of Scintiverse E liquid scintillation cocktail, using a Beckman LS5000TD Scintillation Counter. The results are presented in Table III.

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