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Combined chemical–enzymic synthesis of a dideoxypentasaccharide for use in a study of the specificity of *N*-acetylglucosaminyltransferase-III

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ABSTRACT

The biantennary oligosaccharide glycoside β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-[β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)]-β-D-Man*p*-OR is a potential substrate for *N*-acetylglucosaminyltransferases (GlcNAcTs) III–V. The dideoxypentasaccharide glycoside β-D-Glc*p*NAc-(1→2)-4-deoxy-α-D-*lyxo*-Hex*p*-(1→3)-[β-DGlc*p*NAc-(1→2)-6-deoxy-α-D-Man*p*-(1→6)]-β-D-Man*p*-O$(CH_2)_7CH_3$ (**5**), where the hydroxyl groups that would be acted on by GlcNAcTs IV and V have been removed, was prepared as a possible specific acceptor for GlcNAcT-III. The strategy involved the chemical synthesis of β-D-Glc*p*NAc-(1→2)-4-deoxy-α-D-*lyxo*-Hex*p*-(1→3)-[6-deoxy-α-D-Man*p*-(1→6)]-β-D-Man*p*-O$(CH_2)_7CH_3$ and then addition of the last Glc*p*NAc residue using partially purified GlcNAcT-II from rabbit liver. Preliminary results, using detergent extracts from rat kidney, indicate that **5** is an acceptor for a GlcNAcT whose identity remains to be extablished.

INTRODUCTION

The *N*-acetylglucosaminyltransferases (GlcNAc-transferases, GlcNAcTs), which control the branching pattern of asparagine-linked (Asn-linked) oligosaccharides, each transfer a 2-acetamido-2-deoxy-β-D-glucopyranosyl group from uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl diphosphate) (UDP-GlcNAc) to a mannose residue[1]. GlcNAcTs I–VI have been identified to date[1,2] and their specificities have been characterized[2–10]. Interest in these GlcNAc-transferases follows demonstrations that there may be a correlation between the specific expression of some of these enzymes and altered cell-surface glycosylation[11–14].

Quantitation of GlcNAc-transferase activity generally involves measurement of the rate of transfer of GlcNAc from UDP-[^{3}H or ^{14}C]GlcNAc to acceptor oligosaccharides isolated from natural sources[1,15]. Such acceptors, however, are difficult to obtain in large amounts and in a pure form; moreover, most are acceptors for more than one GlcNAc-transferase[1]. When more than one enzyme can transfer to an acceptor, each product must be isolated and identified in order to quantitate the individual activities. This objective has been accomplished by reactions with lectins or specific enzymes[1,8–11], or by comigration of the products with authentic standards in chromatography[16,17]. The

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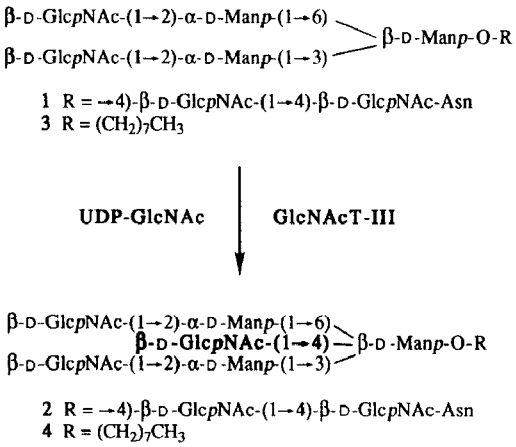
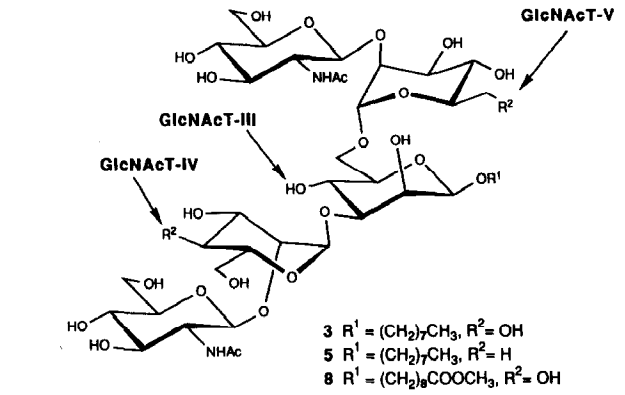
latter approach has also been used with acceptors labeled with fluorescent moieties and non-radioactive UDP-GlcNAc as the donor[18].

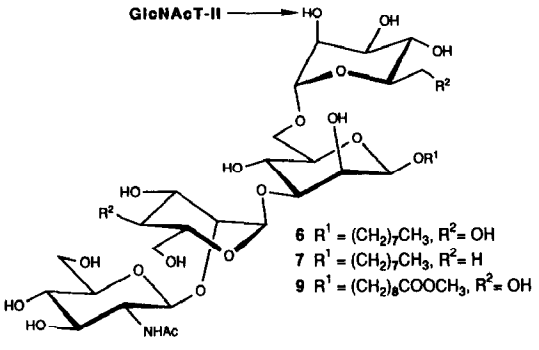
The above assays for the GlcNAc-transferases would be simplified by the availability of specific acceptors. Some success has been reported in this area with the preparation of fragments of the naturally occurring Asn-linked oligosaccharides where cross-reacting sugar residues have been removed[19,20]. Alternatively, the hydroxyl groups which are glycosylated by the interfering enzymes could be either masked[21] (by *O*-methylation) or removed[22]. The latter approach has been applied successfully in fucosyltransferase assays[21-24].

We now report the synthesis of the dideoxypentasaccharide glycoside **5** designed to be specific for *N*-acetylglucosaminyltransferase-III (GlcNAcT-III). GlcNAcT-III activity is elevated during liver carcinogenesis and a simple assay for this activity, which may be of potential clinical diagnostic value, is highly desirable. GlcNAcT-III transfers[1,8] a β-D-GlcNAc residue from UDP-GlcNAc to position 4 of the β-D-Man*p* residue of biantennary Asn-linked oligosaccharides such as **1** to produce **2**. The enzyme also acts on more complex hybrid structures. Current assays for GlcNAcT-III employ natural oligosaccharides and either lectin-affinity chromatography or h.p.l.c. of the radiolabeled products[8,16,17]. Acceptors labeled with a fluorescent moiety, in combination with h.p.l.c., have also been developed to assay GlcNAcT-III activity[26].



The synthetic pentasaccharide glycoside **3** (or the 8-methoxycarbonyloctyl analogue **8**[27]) contains the β-D-Man*p* residue targeted by GlcNAcT-III and, therefore, may be an acceptor for this enzyme, which would convert it into **4**. However, **3** is also a potential substrate for GlcNAcT-IV [which would glycosylate HO-4 of the α-D-Man*p*-(1→3) residue] and GlcNAcT-V [which would glycosylate HO-6 of the α-D-Man*p*-(1→6) residue][1]. Therefore, the dideoxypentasaccharide **5** was synthesized, where the two potentially cross-reactive hydroxyl groups have been removed. The hydrophobic octyl aglycon in **5**, as well as those in **3–9**, permits their use in simple C_{18} Sep-Pak assays[28].



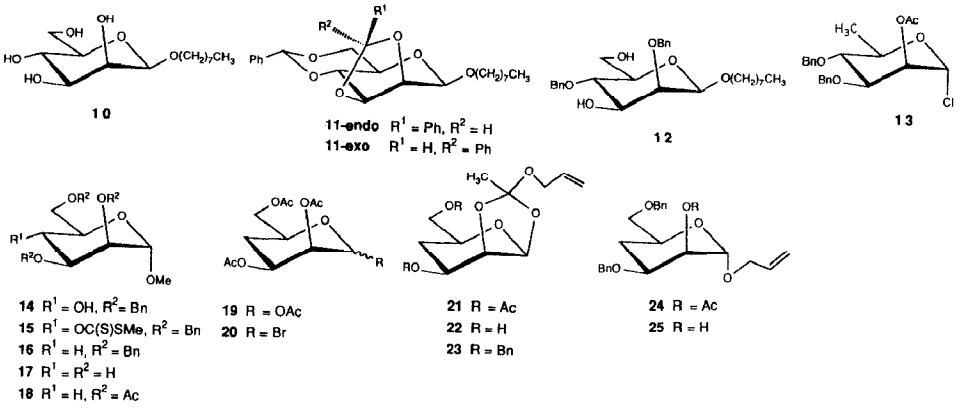


The strategy for the synthesis of **5** involved a chemical synthesis of the dideoxytetrasaccharide **7** followed by the use of GlcNAcT-II to add the last GlcNAc residue. GlcNAcT-II from rabbit liver[3] can be obtained in a highly active form and has been used[27] to convert the 8-methoxycarbonyloctyl glycoside **9** into the pentasaccharide glycoside **8**.

RESULTS AND DISCUSSION

The chemical synthesis of **7** began with di-*O*-benzylidenation of octyl *β*-D-mannopyranoside (**10**), which yielded 92% of a 1:1 mixture of the *exo* and *endo* isomers **11**. Treatment[30] of the presumed *endo* isomer with $LiAlH_4/AlCl_3$ reductively cleaved the benzylidene rings to give **12** (47%) with HO-3,6 unsubstituted.

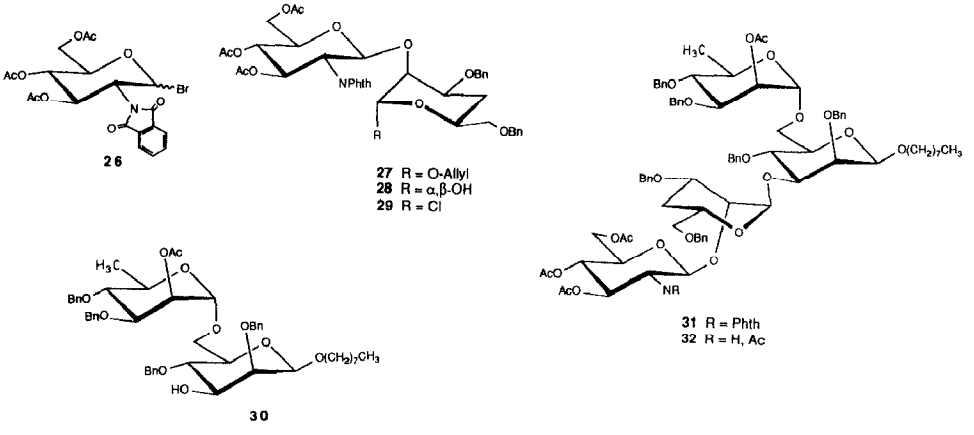
The 4-deoxydisaccharide donor **29** was prepared following established procedures[31,32]. The xanthate **15** was prepared (69%) from the known alcohol **14**[33] and reduced, using tributyltin hydride, to provide **16** (76%). Hydrogenolysis of the benzyl ethers in **16** gave **17**, which was acetylated to produce **18** (72% from **16**). Acetolysis of **18** then gave **19** (95%), which was converted into the glycosyl bromide **20** by reaction with hydrogen bromide. The orthoester **21** (74% from **19**) was produced on reaction of **20**



with allyl alcohol in the presence of 2,6-lutidine. *O*-Deacetylation of **21** gave the diol **22** which was benzylated to provide **23** (85%). Rearrangement of the orthoester **23** gave the allyl α-glycoside **24** (88%), which was *O*-deacetylated to provide **25** (85%), HO-2 of which was glycosylated using the phthalimido bromide **26**[34] to give the disaccharide derivative **27** (88%). *O*-Deallylation of **27** gave the reducing sugar **28** (80%) from which **29** was prepared by reaction with oxalyl chloride in the presence of *N,N*-dimethylformamide[35].

Glycosylation of the diol **12** with **13**, available from earlier work[20], resulted in preferential reaction of HO-6 to yield the disaccharide derivative **30** (60%) along with ~ 15% of diglycosylated material which was difficult to remove by chromatography. Glycosylation of **30** with **29** then gave **31** (68%). Conventional deprotection of **31** proceeded *via* **32** (71%) to produce the target tetrasaccharide glycoside **7** (87%).

The glycoside **7** was evaluated as an acceptor for GlcNAcT-II, which was partially purified[27] from rabbit liver by affinity chromatography on UDP–hexanolamine agarose. The K_m for the 8-methoxycarbonyloctyl tetrasaccharide **9**, a known[27] substrate for GlcNAcT-II, was 0.19 ± 0.02mM, whereas the dideoxy analogue **7** had K_m



0.31 ±0.02mM. The relative rate of glycoslation, V_{max} (**9**)/V_{max} (**7**), was 0.69. Clearly, removal of the two hydroxyl groups from **9** (→**7**) did not affect importantly the ability to act as an acceptor for GlcNAcT-II, and incubation of **7** with GlcNAcT-II and UDP-GlcNAc resulted in its complete conversion into **5**, which was isolated in 88% yield.

The evaluation of the dideoxypentasaccharide glycoside **5** as a potential acceptor for GlcNAcT-III is in progress. Nishikawa *et al.*[26] reported a fluorescence-based h.p.l.c. assay for this enzyme, using the isolated reducing biantennary heptasaccharide structure present in **1**, which had been reductively linked to 2-aminopyridine, as the acceptor. Thus, the crude Triton X-100 extracts of rat kidney were shown[26] to contain GlcNAcT-III with a specific activity of 6.4 μmol of GlcNAc transferred from UDP-GlcNAc/mg of protein/h. In a similar assay with **5** as the acceptor, a specific activity of 3.0 μmol of transfer/mg of protein/h was detected and abolished when EDTA was added to the incubation mixture. This property is characteristic[8,26] of GlcNAcT-III. Since the hydroxyl groups that are the target for GlcNAcT-I and -II are glycosylated in **5**, and since hydroxyl groups that are targets in GlcNAcT-IV and -V have been removed, the activity detected by **5** may be that of GlcNAcT-III. GlcNAcT-VI is not found in mammalian tissues[2]. The structure of the product obtained by the transfer of GlcNAc to **5** is being investigated.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ±2°. 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. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (Merck, 40–63 μm). Iatrobead refers to a beaded Silica Gel (6RS-8060) manufactured by Iatron Laboratories (Tokyo). For gel filtration, Bio-Gel P-2 (200–400 mesh) (Bio-Rad Laboratories) was used. C_{18} Sep-Pak sample-preparation cartridges were obtained from Waters Associates. UDP-6-[^{3}H]GlcNAc (specific activity, 18.9 Ci/mmol) was obtained from New England Nuclear and liquid scintillation counting was performed with a Beckman LS-5000 instrument, using quench correction as described[28]. ^{1}H-N.m.r. spectra were recorded at 300 or 360 MHz (Bruker spectrometers) on solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal acetone, δ 2.225). ^{13}C-N.m.r. spectra were recorded at 75.5 MHz on solutions in $CDCl_3$ (internal Me_4Si) or D_2O (external 1% 1,4-dioxane in D_2O, δ 67.4). Only partial n.m.r. data are reported; the other data were in accord with the proposed structures. The chemical shifts and coupling constants (as observed splittings) for ^{1}H resonances are reported as though they were first order. The assignments of ^{13}C resonances are tentative. F.a.b.-mass spectra were obtained using an AEI MS-9 instrument with Xe as the bombarding gas with 5:1 1,4-dithiothreitol–1,4-dithioerythritol as the matrix. Unless otherwise noted, all reactions were carried out at ambient temperatures and, in the work-up, 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)/12 mmHg. The microanalyses were carried out by the Analytical Services

Laboratory of this Department. The following solvent systems were used: EtOAc–hexane mixtures *A*, 1:8; *B*, 1:7; *C*, 1:4; *D*, 2:7; *E*, 1:3; *F*, 2:5; *G*, 1:1; *H*, 2:3; *I*, 1:1; *J*, 3:2; *K*, 2:1; CH_2Cl_2–MeOH mixtures *L*, 49:1; *M*, 19:1; *N*, 9:1; *O*, 60:35:6 CH_2Cl_2–MeOH–water.

Octyl endo- *and* exo-*2,3;4,6-di*-O-*benzylidene-β*-D-*mannopyranoside* (**11**). — A mixture of **10** (747 mg, 2.56 mmol), benzaldehyde dimethyl acetal (0.96 mL, 6.39 mmol), and tetrafluoroboric acid–ether complex (0.69 mL, 5.11 mmol) in *N,N*-dimethylformamide was stirred at room temperature for 48 h. Triethylamine (0.71 mL, 5.11 mmol) was added and the solvent was evaporated. Column chromatography (solvent *A*) of the residue gave **11**, R_F 0.17 (solvent *B*), presumably the *endo* isomer, as a white solid (550 mg, 46%), $[\alpha]_D$ −112° (*c* 4.5, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.94 (s, 1 H, PhC*H*), 5.56 (s, 1 H, benzylic H), 4.95 (d, 1 H, $J_{1,2}$ 2.8 Hz, H-1), 0.89 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C, δ 137.16, 136.54 (quaternary arom.), 105.32, 101.56 (2 Ph*C*H), 98.27 (C-1), 14.12 (CH_3).

Anal. Calc. for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 72.18; H, 7.78.

The presumed *exo* isomer was also obtained (550 mg, 46%), R_F 0.22. ^1H-N.m.r. data ($CDCl_3$): δ 6.31, 5.61 (2 s, each 1 H, PhCH_2), 4.91 (d, 1 H, $J_{1,2}$ 2.8 Hz, H-1), 0.88 (t, 3 H, *J* 6.5 Hz, CH_3).

Octyl 2,4-di-O-benzyl-β-D-*mannopyranoside* (**12**). — To a stirred solution of **11** (*endo* isomer, 481 mg, 1.03 mmol) in 1:1 ether–CH_2Cl_2 (25 mL) was added, portionwise, $LiAlH_4$ (234 mg, 6.16 mmol), and the mixture was slowly heated to boiling. To the solution, boiling under reflux, was added a solution of $AlCl_3$ (820 mg, 6.16 mmol) in ether (12 mL) during 30 min when t.l.c. indicated the absence of **11**. The mixture was cooled, excess of $LiAlH_4$ was decomposed by the addition of EtOAc (10 mL), and the aluminum salts were precipitated by the addition of water (10 mL). The mixture was diluted with ether (50 mL), the aqueous layer was extracted with CH_2Cl_2, and the combined organic solutions were washed with water (3 × 50 mL), dried, and concentrated. Column chromatography (solvent *G*) of the residue gave **12** as a white solid (228 mg, 47%), R_F 0.49 (solvent *I*), $[\alpha]_D$ −66.5° (*c* 2, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.06, 4.64 (2 d, each 1 H, *J* 12.0 Hz, PhCH_2), 4.91, 4.62 (2 d, each 1 H, *J* 11.0 Hz, PhCH_2), 4.51 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 3.82 (dd, 1 H, $J_{2,3}$ 4.0 Hz, H-2), 2.39 (d, 1 H, $J_{3,HO-3}$ 10.0 Hz, HO-3), 2.10 (t, 1 H, $J_{6,HO-6}$ 7.0 Hz, HO-6), 0.88 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C, δ 101.92 (C-1), 75.07, 74.88 (2 PhCH_2), 70.39 (OCH_2CH_2), 62.63 (C-6), 14.12 (CH_3).

Anal. Calc. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 70.75; H, 8.57.

Methyl 2,3,6-tri-O-*benzyl-4*-O-(methylthio)thiocarbonyl-α-D-*mannopyranoside* (**15**). — A mixture of **14** (2.02 g, 4.35 mmol), sodium hydride (296 mg of a 60% dispersion in oil), and imidazole (0.7 mg) in tetrahydrofuran (20 mL) was boiled under reflux for 3 h. Carbon disulphide (1.75 mL, 29.2 mmol) was added and, after 1 h, methyl iodide (1.76 mL, 28.3 mmol), and boiling was continued for 1 h. Acetic acid was then added until the mixture cleared. The solvent was evaporated, and a solution of the residue in dichloromethane was washed with water, then concentrated. Column chromatography (solvent *C*) of the residue gave **15** (1.665 g, 69%), isolated as a syrup, R_F 0.44 (solvent *G*), $[\alpha]_D$ +30° (*c* 1, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 6.32 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.75 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.80 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.35 (s, 3 H, OMe), 2.51 (s, 3 H, SMe); ^{13}C, δ 215.49 (CS), 99.35 (C-1), 54.95 (OCH_3), 19.22 (SCH_3).

Anal. Calc. for $C_{30}H_{34}O_6S_2$: C, 64.96; H, 6.18; S, 11.56. Found: C, 65.13; H, 6.44; S, 11.81.

*Methyl 2,3,6-tri-O-benzyl-4-deoxy-α-*D-lyxo-*hexopyranoside* (**16**). — A solution of **15** (1.57 g, 2.83 mmol) in dry toluene (35 mL) was heated to 80° under nitrogen, then α,α′-azobisisobutyronitrile (534 mg, 3.26 mmol) was added followed by tributylstannane (11.7 mL, 43.6 mmol). After 2 h at 80°, the mixture was allowed to cool to room temperature, and the solvent was evaporated. A solution of the residue in acetonitrile was washed with light petroleum, then concentrated. Column chromatography (solvent *C*) of the residue gave **16** (964 mg, 76%), isolated as a syrup, R_F 0.44 (solvent *G*), $[\alpha]_D$ +20° (*c* 1.2, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 4.79 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 3.33 (s, 3 H, OMe), 1.95 (ddd, 1 H, $J_{3,4ax} = J_{4,5ax} = J_{4ax,4eq}$ 12.0 Hz, H-4*ax*), 1.81 (m, 1 H, H-4*eq*); ^{13}C, δ 100.17 (C-1), 54.72 (OCH_3), 29.28 (C-4).

Anal. Calc. for $C_{28}H_{32}O_5$: C, 74.97; H, 7.19. Found: C, 74.65; H, 7.13.

*Methyl 2,3,6-tri-*O*-acetyl-4-deoxy-α-*D-lyxo-*hexopyranoside* (**18**). — A solution of **16** (800 mg, 1.79 mmol) in aq. 95% EtOH (70 mL) was stirred under hydrogen (1 atm.) for 19 h in the presence of 5% Pd/C (400 mg), then filtered, and the solvent was evaporated. A solution of the residue (**17**, 316 mg), R_F 0.14 (solvent *N*), in dry pyridine (10 mL) and acetic anhydride (5 mL) was stirred overnight at room temperature, then concentrated. Column chromatography (solvent *E*) of the residue gave **18**, R_F 0.53 (solvent *G*), isolated as a syrup (388 mg, 72%), $[\alpha]_D$ +52° (*c* 0.6, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.27 (m, 1 H, H-3), 5.08 (ddd, 1 H, $J_{2,4eq}$ 1.0, $J_{2,3}$ 3.5, $J_{1,2}$ 1.9 Hz, H-2), 4.74 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 3.38 (s, 3 H, OMe), 2.14, 2.11, 2.02 (3 s, each 3 H, 3 OAc), 1.90–1.76 (m, 2 H, H-4*ax*,4*eq*); ^{13}C, δ 170.62, 170.04, 169.79 (*C*OCH_3), 99.16 (C-1), 54.91 (OCH_3), 27.92 (C-4), 20.83 (2 CO*C*H_3), 20.69 (CO*C*H_3).

Anal. Calc. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.63. Found: C, 51.34; H, 6.64.

*1,2,3,6-Tetra-*O*-acetyl-4-deoxy-α,β-*D-lyxo-*hexopyranose* (**19**). — A solution of 1% conc. H_2SO_4 in acetic anhydride (0.6 mL) was added dropwise to a solution of **18** (380 mg, 1.25 mmol) in acetic anhydride (4.0 mL) at 0°. The mixture was stirred at room temperature for 1 h, then poured into a stirred mixture of CH_2Cl_2 (25 mL) and ice-cold sat. aq. $NaHCO_3$, and the mixture was stirred at room temperature for 30 min. The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), and the combined CH_2Cl_2 solutions were washed with sat. aq. $NaHCO_3$ and water, dried, and concentrated. Column chromatography (solvent *H*) of the residue yielded **19**, R_F 0.38 (solvent *I*), isolated as a syrup (395 mg, 95%), α:β-ratio 15:1. N.m.r. data ($CDCl_3$): ^1H, δ 6.11 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1α), 5.76 (d, 1 H, $J_{1,2}$ 1 Hz, H-1β), 2.17, 2.15, 2.10, 2.04 (4 s, each 3 H, 4 OAc), 1.94–1.82 (m, 2 H, H-4,4); ^{13}C, δ 170.65, 169.93, 169.75, 168.28 (*C*OCH_3), 91.56 (C-1), 27.76 (C-4).

Anal. Calc. for $C_{14}H_{20}O_9$: C, 50.59; H, 6.07. Found: C, 50.77; H, 6.13.

*3,6-Di-*O*-acetyl-1,2-*O*-(1-allyloxyethylidene)-4-deoxy-β-*D-lyxo-*hexopyranose* (**21**). — To a solution of **19** (394 mg, 1.19 mmol) in dry CH_2Cl_2 (8 mL) was added with stirring at 0° 33% HBr in acetic acid (1.5 mL) containing 3% of acetic anhydride. The mixture was stirred at 0° for 30 min, then diluted with CH_2Cl_2 (50 mL), washed with ice–water, sat. aq. $NaHCO_3$, and ice–water, and dried (Na_2SO_4), and the solvent was

evaporated. Toluene (3 × 1 mL) was distilled from the residue to leave the syrupy bromide **20**, R_F 0.48 (solvent *I*). N.m.r. data ($CDCl_3$): ^1H, δ 6.37 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1).

To a solution of this syrup in dry $CHCl_3$ (5 mL) were added allyl alcohol (4.0 mL, 59.4 mmol) and 2,6-lutidine (0.35 mL, 2.97 mmol) at 0°. The mixture was stirred at room temperature for 2 h, then diluted with CH_2Cl_2 (50 mL), washed twice with ice–water, dried, and concentrated. Column chromatography (solvent *G*) of the residue gave **21**, R_F 0.46 (solvent *I*), as a white powder (292 mg, 74%), $[\alpha]_D$ −7.7° (*c* 2, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.90 (m, 1 H, allyl), 5.45 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.44 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 2.14, 2.08 (2 s, each 3 H, 2 OAc), 1.92–1.78 (m, 2 H, H-4,4), 1.76 (s, 3 H, Me); ^{13}C, δ 170.62, 170.26 (2 *C*OCH$_3$), 134.15 (H_2C = *C*H), 124.09 (H_3CCO), 116.57 (*C*H_2 = CH), 97.78 (C-1), 26.85 (C-4), 24.88 (CH_3), 20.97, 20.73 (2 CO*C*H_3).

Anal. Calc. for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.45; H, 6.70.

*1,2-O-(1-Allyloxyethylidene)-3,6-di-O-benzyl-4-deoxy-β-*D-lyxo-*hexopyranose* (**23**). — A solution of **21** (288 mg, 0.095 mmol) in dry MeOH (5.0 mL) containing a trace (0.01M) of sodium methoxide was kept for 1.5 h at room temperature, then concentrated to leave syrupy **22**, R_F 0.14 (solvent *M*). Sodium hydride (105 mg of 60% dispersion in oil) was added to *N,N*-dimethylformamide (2 mL), and a solution of **22** (215 mg, 0.88 mmol) in *N,N*-dimethylformamide (5 mL) was added dropwise at 0°. Benzyl bromide (0.31 mL, 2.62 mmol) was then slowly added, maintaining the temperature of the mixture at 0–5°. The mixture was stirred at room temperature for 17 h, the excess of NaH was destroyed by the addition of MeOH, and the mixture was diluted with CH_2Cl_2, washed with water, dried, and concentrated. Column chromatography (solvent *E*) of the residue gave **2**, isolated as a syrup (315 mg, 85%), R_F 0.59 (solvent, *I*), $[\alpha]_D$ −14° (*c* 0.5, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 7.40–7.26 (m, 10 H, 2 Ph), 5.35 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.70, 4.63 (2 d, each 1 H, *J* 12.5 Hz, PhCH_2), 4.59, 4.53 (d, 1 H each, *J* 12.0 Hz, PhCH_2); ^{13}C, δ 137.94, 137.73 (2 quaternary arom. C), 134.46 (CH_2 = *C*H), 123.77 (CH_3*C*), 116.42 (*C*H_2 = CH), 97.95 (C-1), 28.03 (C-4), 24.84 (CH_3).

Anal. Calc. for $C_{25}H_{30}O_6$: C, 70.40; H, 7.09. Found: C, 70.46; H, 6.95.

*Allyl 2-O-acetyl-3,6-di-O-benzyl-4-deoxy-α-*D-lyxo-*hexopyranoside* (**24**). — A mixture of **23** (315.2 mg, 0.739 mmol), powdered molecular sieves 4 Å (150 mg), trimethylsilyl triflate (47.7 μL, 0.247 mmol), and 1,2-dichloroethane (5 mL) was stirred at room temperature for 3 h under nitrogen, then filtered, washed with sat. aq. $NaHCO_3$ and water, dried, and concentrated. Column chromatography (solvent *E*) of the residue gave **24** (278 mg, 88%), R_F 0.47 (solvent *G*), $[\alpha]_D$ +14° (*c* 0.8, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.89 (m, 1 H, allyl), 5.29 (H-2), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 2.11 (s, 3 H, OAc), 1.92–1.79 (m, 2 H, H-4,4); ^{13}C, δ 170.46 (*C*OCH$_3$), 133.70 (CH_2 = *C*H), 117.43 (*C*H_2 = CH), 97.60 (C-1), 29.64 (C-4), 21.05 (CO*C*H_4).

Anal. Calc. for $C_{25}H_{30}O_6$: C, 70.40; H, 7.09. Found: C, 70.35; H, 6.97.

*Allyl 3,6-di-O-benzyl-4-deoxy-α-*D-lyxo-*hexopyranoside* (**25**). — Compound **24** (277 mg, 0.65 mmol; R_F 0.67, solvent *I*) was *O*-deacetylated as described for the preparation of **22**. Column chromatography (solvent *G*) of the product gave **25** (213 mg, 85%), isolated as a syrup, R_F 0.46 (solvent *I*). N.m.r. data ($CDCl_3$): ^1H, δ 4.95 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 2.55 (bs, 1 H, OH); ^{13}C, δ 133.94 (CH_2 = *C*H), 117.21 (*C*H_2 = CH), 99.15 (C-1), 28.37 (C-4).

*Allyl 3,6-di-*O*-benzyl-4-deoxy-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-lyxo-hexopyranoside* (**27**). — To a solution of **25** (212 mg, 0.55 mmol) in dry CH_2Cl_2 (5 mL) was added silver trifluoromethanesulfonate (1.42 g, 5.53 mmol), *sym*-collidine (0.73 mL, 5.53 mmol), and 4 Å molecular sieves (1.0 g), and the mixture was cooled to −50°. A solution of the bromide **26** (275 mg, 0.55 mmol), R_F 0.39 (solvent *I*), in dry CH_2Cl_2 was then added and, after 15 min at −50°, the mixture was warmed to room temperature during 1 h. T.l.c. then showed the presence of ~ 25% of unreacted **25**. The mixture was cooled to −50°, more **26** (275 mg) in dry CH_2Cl_2 (5.0 mL) was added, and, after 15 min, the mixture was warmed to room temperature and kept thereat for 1 h. The mixture was diluted with CH_2Cl_2 (50 mL), filtered through Celite, washed with ice–water, sat. aq. $NaHCO_3$, and water (100 mL each), then concentrated. Column chromatography (solvent *F*) of the residue gave amorphous **27** (389 mg, 88%), R_F 0.39 (solvent *I*), $[\alpha]_D$ −1.3° (*c* 0.9, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.86 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 9.0 Hz, H-3'), 5.81 (m, 1 H, allyl), 5.50 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.65 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.47 (dd, 1 H, H-2'), 4.04 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 2.99 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 10.0 Hz, H-6a), 2.89 (dd, 1 H, $J_{5,6b}$ 6.5 Hz, H-6b), 2.04, 2.03, 1.87 (3 s, each 3 H, 3 OAc), 1.66–1.56 (m, 2 H, H-4,4); ^{13}C, δ 170.64, 170.14, 169.43 ($COCH_3$), 117.33 (CH_2=CH), 96.65 (C-1), 96.54 (C-1'), 54.47 (C-2'), 20.67, 20.63, 20.48 ($CHCH_3$).

Anal. Calc. for $C_{43}H_{47}H_{14}NO_{14}$: C, 64.41; H, 5.91; N, 1.75. Found: C, 64.39; H, 5.87; N, 1.70.

*3,6-Di-*O*-benzyl-4-deoxy-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α,β-*D*-lyxo-hexopyranose* (**28**). — A solution of **27** (177 mg, 0.221 mmol), tristriphenylphospinerhodium(I) chloride (28 mg, 0.03 mmol), and 1,6-diazabicyclo[2.2.2]octane (10 mg, 0.09 mmol) in 7:3:1 EtOH–benzene–water (10 mL) was boiled under reflux for 15 h, then concentrated. To a solution of the residue in acetone (5 mL) containing mercuric oxide (~ 1 mg) was added a solution of mercuric chloride (300 mg) in 9:1 acetone–water (5 mL), and the mixture was stirred at room temperature for 22 h, then concentrated. A solution of the residue in CH_2Cl_2 (50 mL) was washed with aq. 30% KBr and water, then dried, and the solvent was evaporated. Column chromatography (solvent *I*) of the residue gave **28**, isolated as a glass (134 mg, 80%), R_F 0.18 (solvent *I*), α:β-ratio ~ 13:2. N.m.r. data ($CDCl_3$): ^1H, δ 5.95 (dd, 1 H, $J_{2',3'}$ 9.0, $J_{3',4'}$ 10.0 Hz, H-3'β), 5.94 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 10.0 Hz, H-3'α), 5.83 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1α), 5.73 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1β), 3.58 (d, 1 H, *J* 3.5 Hz, HO-1α), 2.88 (dd, 1 H, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 10.0 Hz, H-6a), 2.49 (dd, 1 H, $J_{5,6b}$ 8.0 Hz, H-6b); ^{13}C, δ 170.75, 170.01, 169.48 (3 $COCH_3$), 95.53 (C-1'), 91.77 (C-1), 54.62 (C-2'), 28.82 (C-4), 20.72, 20.64, 20.52 (3 $COCH_3$).

*3,6-Di-*O*-benzyl-4-deoxy-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glycopyranosyl)-α,β-*D*-lyxo-hexopyranosyl chloride* (**29**). — A solution of oxalyl chloride (23 μL, 0.27 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to a stirred solution of **28** (75 mg, 0.1 mmol) and *N,N*-dimethylformamide (2.3 μL, 0.03 mmol) in CH_2Cl_2 (1.0 mL) at 0°. The mixture was stirred for 2 h at room temperature, then diluted with CH_2Cl_2 (10 mL), and poured into ice–water. The organic layer was washed with cold water (3 × 50 μL), dried, and concentrated to a syrup that was shown by t.l.c. (solvent *I*) to contain both anomeric chlorides (**29**), R_F 0.43 and 0.38.

Octyl 6-O-(2-O-acetyl-3,4-di-O-benzyl-6-deoxy-α-D-mannopyranosyl)-2,4-di-O-benzyl-β-D-mannopyranoside (**30**). — A solution of **13** (107 mg, 0.263 mmol), R_F 0.68 (solvent *G*), in dry CH_2Cl_2 (1.5 mL) was added to a solution of **12** (83 mg, 0.175 mmol), R_F 0.38 (solvent *G*), tetramethylurea (42 μL, 0.351 mmol), and silver trifluoromethanesulfonate (68 mg, 0.263 mmol) in the same solvent (1 mL) at 0°. After 1.5 h, the mixture was warmed to room temperature, then diluted with CH_2Cl_2 (15 mL), and *sym*-collidine followed by silver trifluoromethanesulfonate (65 mg) were added to destroy excess of **13.** After 0.5 h, tetraethylammonium bromide (60 mg) was added and the mixture was filtered through Celite that was then washed with CH_2Cl_2 (25 mL). The combined filtrate and washings were washed with aq. sat. $NaHCO_3$ (50 mL) and water (2 × 50 mL), then concentrated. Column chromatography on Iatrobeads (solvent *D*) of the residue gave, first, a mixture (108 mg) of what appeared to be octyl 3,6-di-*O*-(2-*O*-acetyl-3,4-di-*O*-benzyl-6-deoxy-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-β-D-mannopyranoside, R_F 0.58 (solvent *G*), and **30**, R_F 0.56, in the ratio 2:7 (^{1}H-n.m.r. data). Eluted later was **30** (13.1 mg), isolated as a syrup, $[\alpha]_D$ − 13° (*c* 0.9, $CHCl_3$). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.457 (dd, 1 H, $J_{1',2'}$ 1.7, $J_{2',3'}$ 3.0 Hz, H-2'), 4.792 (d, 1 H, H-1'), 4.459 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 3.805 (dd, 1 H $J_{2,3}$ 3.5 Hz, H-2), 2.502 (d, 1 H, $J_{3,HO-3}$ 10.00 Hz, HO-3), 2.149 (s, 3 H, OAc), 1.262 (d, 3 H, $J_{5',6'}$ 6.0 Hz, H-6',6',6'), 0.867 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C δ 170.28 ($COCH_3$), 101.68, 97.71 (C-1,1'), 66.85 (C-6), 21.17 ($COCH_3$), 18.04 (C-6'), 14.14 (CH_3).

Anal. Calc. for $C_{50}H_{64}O_{11}$: C, 71.40; H, 7.67. Found: C, 71.30; H, 7.52.

Octyl 6 O-(2-O-acetyl-3,4-di-O-benzyl-6-deoxy-α-D-mannopyranosyl)-2,4-di-O-benzyl-3-O-[3,6-di-O-benzyl-4-deoxy-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-lyxo-hexopyranosyl]-β-D-mannopyranoside (**31**). — To a solution of the above 7:2 mixture (70 mg) of di- and tri-saccharides in dry CH_2Cl_2 (1 mL) was added silver trifluoromethanesulfonate (214 mg, 0.83 mmol), *sym*-collidine (104 μL, 0.83 mmol), and powdered molecular sieves 4 Å (350 mg). The mixture was cooled to − 20°, a solution of chloride **29** (78 mg, 0.1 mmol), R_F 0.42 (solvent *I*), in dry CH_2Cl_2 (1 mL) was added and, after 0.5 h at − 20°, the mixture was stirred for 1.5 h at room temperature, then diluted with CH_2Cl_2 (50 mL), and filtered through Celite. The filtrate was washed with ice–water, cold sat. aq. $NaCHO_3$, and water (50 mL each), and the solvent was evaporated. Column chromatography (solvent *E*) of the residue gave **31** (64 mg, 68% yield based on **30** present initially), $[\alpha]_D$ − 5.7° (*c* 1.4, $CHCl_3$), R_F 0.47 (solvent *I*). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.608 (dd, 1 H, $J_{2''',3'''}$ 10.5, $J_{3''',4'''}$ 10.0 Hz, H-3'''), 5.110 (d, *J* 8.5 Hz, H-1'''), 4.996 (d, 1 H, *J* 1.5 Hz, H-1'), 4.759 (d, 1 H, *J* 1.5 Hz, H-1''), 4.309 (s, 1 H, H-1), 2.136, 2.029, 1.929, and 1.845 (4 s, each 3 H, 4 OAc), 1.248 (d, 3 H, $J_{5'',6''}$ 6.0 Hz, H-6'',6'',6''), 0.857 (t, 3 H, *J* 6.5 Hz, CH_2CH_3); ^{13}C, 170.63, 170.20, 170.14, and 169.33 (4 $COCH_3$), 101.85, 99.54, 97.91, and 95.69 (C-1,1',1'',1'''), 54.39 (C-2'''), 28.87 (C-4'), 21.13 20.67, 20.60, and 20.54 (4 $COCH_3$), 18.01 (C-6''), 14.13 (CH_2CH_3).

Octyl 3-O-[2-O-(acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-4-deoxy-α-D-lyxo-hexopyranosyl]-6-O-(2-O-acetyl-3,4-di-O-benzyl-6-deoxy-α-D-mannopyranosyl)-2,4-di-O-benzyl-β-D-mannopyranoside (**32**). — A mixture of **31** (48 mg, 0.03 mmol), aq. 85% hydrazine hydrate (146 μL, 3.01 mmol) and dry MeOH (2.0 mL) was boiled under reflux for 50 h, then concentrated. To a solution of the

residue in pyridine (3 mL) was added acetic anhydride (2 mL), the mixture was stirred for 38 h, EtOH (2 mL) was then added at 0°, and the solvent was evaporated. A solution of the residue in CH_2Cl_2 (50 mL) was washed with water and then concentrated. Column chromatography (solvent *J*) of the residue gave **32** (32 mg, 71%), R_F 0.36 (solvent *K*), as a white solid, $[\alpha]_D$ −13° (*c* 0.82, $CHCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.055 (d, 1 H, *J* 8.0 Hz, H-1‴), 5.008 (d, 1 H, *J* 9.0 Hz, NH), 2.137, 2.027, 1.995, 1.918, and 1.814 (5 s, each 3 H, 5 Ac), 1.231 (d, 3 H, *J* 6.0 Hz, H-6″,6‴,6″), 0.867 (t, 3 H, *J* 6.5 Hz, CH_2CH_3); ^{13}C, δ 170.72, 170.62, 170.24, 170.11, and 169.34 (5 $COCH_3$), 101.98, 100.09, 99.15, and 97.98 (C-1,1′,1″,1‴), 54.41 (C-2‴), 28.82 (C-4′), 18.01 (C-6′), 14.13 (CH_2CH_3).

Octyl 3-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-deoxy-α-D-lyxo-hexopyranosyl]-6-O-(6-deoxy-α-D-mannopyranosyl)-β-D-mannopyranoside (**7**). — Compound **32** (27.5 mg, 0.018 mmol) was *O*-deacetylated as described for the preparation of **20**. A solution of the product, R_F 0.31 (solvent *N*), in MeOH (5 mL) was stirred with 5% Pd/C (30 mg) under 1 atm. of hydrogen for 20 h, then filtered, neutralized with IR-120 (H^+) resin, filtered, and concentrated. The residue, which contained partially hydrogenolyzed products, was dissolved in aq. 95% EtOH and hydrogenolyzed (1 atm.) over 5% Pd/C (30 mg) for 44 h to give **7**, R_F 0.69 (solvent *O*), as a white solid which was eluted from a column (1.5 × 60 cm) of Bio-Gel P-2 (200–400 mesh) with aq. 10% EtOH. The appropriate fractions were combined, concentrated, and lyophilized to provide **2** as a white powder (12.6 mg, 87%), $[\alpha]_D$ +10° (*c* 0.3, H_2O). F.a.b.-mass spectrum: *m/z* 810 (25%, M + Na^+). N.m.r. data (D_2O): ^1H, δ 5.158 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.841 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.678 (s, 1 H, H-1), 4.587 (d, 1 H, $J_{1''',2'''}$ 8.5 Hz, H-1‴), 4.122 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.038 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, H-2′), 3.991 (dd, 1 H, $J_{2'',3''}$ 3.5 Hz, H-2″), 2.056 (s, 3 H, Ac), 1.611 (m, 2 H, H-4′,4′), 1.299 (d, 3 H, *J* 6.0 Hz, H-6″,6‴,6″), 0.863 (t, 3 H, *J* 6.5 Hz, CH_2CH_3); ^{13}C, δ 175.55 ($COCH_3$), 100.99, 100.56, 100.44, and 100.24 (C-1,1′,1″,1‴), 56.21 (C-2″), 30.47 (C-4′), 23.13 ($COCH_3$), 17.41 (C-6″), 14.19 (CH_2CH_3).

Evaluation of the tetrasaccharide glycosides **7** *and* **9** *as acceptors for GlcNAcT-II.* — Incubation mixtures in 500-μL plastic microfuge tubes contained: UDP-GlcNAc (252 nmol), UDP-[^{3}H]GlcNAc (260 000 d.p.m.), 1–5 nmol of **7** or **9**, and GlcNAcT-II (16.5 μU, prepared as described below) in 50 μL of buffer consisting of 10mM $MnCl_2$ and 50mM sodium cacodylate containing 0.75% of Triton X-100 at pH 6.5. After reaction at 37° for 1 h, each mixture was diluted with water (5 mL) and added to a Sep-Pak C_{18} cartridge, which was washed with water (20 mL). Radiolabeled product was eluted with MeOH (2 × 5 mL) directly into scintillation vials for counting. The parameters K_m and V_{max}, evaluated using a computer program based on the statistical method of Wilkinson[36], were K_m 0.31 ±0.02mM and V_{max} 58 ±1.7 pmol/min for **7**, and K_m 0.19 ±0.02mM and V_{max} 40 ±1.6 pmol/min for **9**.

Preparative enzymic glycosylation: — GlcNAcT-II was partially purified[27] from a Triton X-100 (0.75%) extract of rabbit-liver acetone powder by adsorption onto UDP–hexanolamine Sepharose and elution with 0.2M NaCl at 4°. The eluate was dialyzed against buffer consisting of 50mM sodium cacodylate and 10mM $MnCl_2$ containing 0.75% of Triton X-100 at pH 6.5, then concentrated to 1.4 mL which contained 21 mU of activity (1 mU is the quantity of enzyme that catalyses the conversion of **9** into **8** at 1 nmol/min under saturating conditions).

Octyl 3-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-deoxy-α-D-lyxo-hexopyranosyl]-6-O-[2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-deoxy-α-D-mannopyranosyl)]-β-D-mannopyranoside (**5**). — A mixture of **7** (3.98 mg, 5.05 μmol), UDP-GlcNAc (6.5 mg, 10 μmol), and GlcNAcT-II (15 mU, see above) was kept at 37° for 8 h, then diluted with water to 30 mL, and passed onto a Sep-Pak C_{18} cartridge which was washed with water (25 mL). The product was eluted with MeOH (25 mL), the solvent was evaporated, and the residue was applied to a column (1.5 × 60 cm) of Bio-Gel P2 which was eluted with aq. 10% EtOH. The fractions containing **5** were combined, the solvent was evaporated, and the residue was lyophilized from water to yield a white powder (4.4 mg, 88%). F.a.b.-mass spectrum: *m/z* 1013 (50%, M + Na^+). N.m.r. data (D_2O): ^1H, δ 5.156 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.869 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1''), 4.674 (d, 1 H, $J_{1,2}$ ~ 0.8 Hz, H-1), 4.588 and 4.571 (2 d each 1 H, *J* 8.5 Hz, H-1''',1''''), 4.156 (dd, 1 H, $J_{2'',3''}$ 3.5 Hz, H-2''), 4.123 (dd, $J_{2,3}$ 3.0 Hz, H-2), 4.036 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, H-2'), 2.055 (s, 6 H, 2 Ac), 1.607 (m, 2 H, H-4',4'), 1.268 (d, 3 H, *J* 6.0 Hz, H-6'',6'',6''), 0.863 (t, 3 H, *J* 6.5 Hz, CH_2CH_3).

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REFERENCES

1 H. Schachter, *Biochem. Cell Biol.*, 64 (1986) 163–181.
2 I. Brockhausen, E. Hull, O. Hindsgaul, H. Schachter, R. N. Shah, S. W. Michnick, and P. J. Carver, *J. Biol. Chem.*, 264 (1989) 11211–11221.
3 C. L. Oppenheimer and R. L. Hill, *J. Biol. Chem.*, 256 (1981) 799–804.
4 G. Vella, H. Paulsen, and H. Schachter, *Can. J. Biochem. Cell. Biol.*, 62 (1984) 409–417.
5 Y. Nishikawa, W. Pegg, H. Paulsen, and H. Schachter, *J. Biol. Chem.*, 263 (1988) 8270–8281.
6 C. L. Oppenheimer, A. E. Eckhardt, and R. L. Hill, *J. Biol. Chem.*, 256 (1981).
7 B. Bendiak and H. Schachter, *J. Biol. Chem.*, 262 (1987) 5784-5790.
8 S. Narasimhan, *J. Biol. Chem.*, 257 (1982) 10235–10242.
9 P. A. Gleeson and H. Schachter, *J. Biol. Chem.*, 258 (1983) 6162-6173.
10 R. D. Cummings, I. S. Trowbridge, and S. Kornfeld, *J. Biol. Chem.*, 257 (1982) 13421–13427.
11 K. Yamashita, Y. Tachibana, T. Ohkura, and A. Kobata, *J. Biol. Chem.*, 260 (1985) 3963-3969.
12 J. Arango and M. Pierce, *J. Cell. Biochem.*, 37 (1988) 225–231.
13 A. Kobata, *Biochimie*, 70 (1988) 1575–1585.
14 J. W. Dennis, *Cancer Surv.*, 7 (1988) 573–594.
15 T. A. Beyer, J. E. Sadler, J. C. Paulson, and R. L. Hill, *Adv. Enzymol.*, 52 (1981) 23–175.
16 I. Brockhausen, J. P. Carver, and H. Schachter, *Biochem. Cell Biol.*, 66 (1988) 1134–1151.
17 A. H. L. Koenderman, P. W. Wijermans, and D. H. van den Eijnden, *FEBS Lett.*, 222 (1987) 42–46.
18 S. Hase, T. Ibuki, and T. Ikenaga, *J. Biol. Chem.*, 95 (1984) 197–203.
19 M. Pierce, J. Arango, S. H. Tahir, and O. Hindsgaul, *Biochem. Biophys. Res. Commun.*, 146 (1987) 679–684.
20 M. M. Palcic, J. Ripka, K. J. Kaur, M. Shoreibah, O. Hindsgaul, and M. Pierce, *J. Biol. Chem.*, 265 (1990) 6759–6769.
21 R. Madiyalakan, S. Yazawa, S. A. Abbas, J. Barlow, and K. L. Matta, *Anal. Biochem.*, 152 (1986) 22–28.
22 D. P. Khare, O. Hindsgaul, and R. U. Lemieux, *Carbohydr. Res.*, 136 (1985) 285–308.
23 J. Le Pendu, J. P. Cartron, R. U. Lemieux, and R. Oriol, *Am. J. Hum. Genet.*, 37 (1985) 749–760.

24 H. Muramatsu, Y. Kamada, and T. Muramatsu, *J. Biochem. (Tokyo)*, 157 (1986) 71–75.
25 S. Narasimhan, H. Schachter, and S. Rajalakshmi, *J. Biol. Chem.*, 263 (1988) 1273–1281.
26 A. Nishikawa, S. Fujii, T. Sugiyama, and N. Taniguchi, *Anal. Biochem.*, 170 (1988) 349–354.
27 K. J. Kaur, G. Alton, and O. Hindsgaul, *Carbohydr. Res.*, 210 (1991) 145–153.
28 M. M. Palcic, L. D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.
29 K. J. Kaur and O. Hindsgaul, *Glycoconj. J.*, 8 (1991) 90–94.
30 A. Liptak, P. Fugedi, and P. Nanasi, *Carbohydr. Res.*, 51 (1976) c19-c21.
31 T. Takeda, S.-I. Fujisawa, Y. Ogihara, and T. Hori, *Chem. Pharm. Bull.*, 33 (1985) 540–543.
32 K. Sadozai, T. Kitajima, T. Ogawa, and A. Kobata, *Carbohydr. Res.*, 152 (1986) 173–182.
33 R. Madiyalakan, M. S. Chowdhary, S. S. Rana, and K. L. Matta, *Carbohydr. Res.*, 152 (1986) 183–194.
34 R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–114.
35 T. Iversen and D. R. Bundle, *Carbohydr. Res.*, 103 (1982) 29–40.
36 G. N. Wilkinson, *Biochem. J.*, 80 (1961) 324–332.