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Use of *N*-acetylglucosaminyltransferases I and II in the preparative synthesis of oligosaccharides

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ABSTRACT

8-Methoxycarbonyloctyl 3,6-di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside (**1**) has been synthesised chemically. Compound **1** is a substrate for *N*-acetylglucosaminyltransferase-I (GlcNAcT-I), which transfers a β-D-Glc*p*NAc residue from UDP-Glc*p*NAc to position 2 of the α-Man-(1→3) unit to produce **2**. In turn, the tetrasaccharide **2** is an acceptor for GlcNAcT-II which, in the presence of UDP-Glc*p*NAc, converts **2** into 8-methoxycarbonyloctyl 3,6-di-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-mannopyranoside (**3**). These conversions were carried out on a 50–100 mg scale using enzyme preparations obtained from rabbit liver in a single step by affinity chromatography.

INTRODUCTION

The combined use of chemical and enzymic synthesis in the preparation of oligosaccharides has the advantage over total chemical synthesis that relatively small and readily prepared synthetic "primers" can be rapidly homologated to an array of more complex structures[1–5]. The addition of sugar residues to the primer is carried out most easily using glycosyltransferases[1,6,7] that regio- and stereo-specifically transfer the sugar residue from a sugar nucleotide. All of the sugar-nucleotide donors required for the synthesis of the carbohydrate chains of both *O*- and *N*-linked glycoproteins are available commercially[1], and the major limitation of the combined chemical–enzymic strategy has been the availability of the required glycosyltransferases in amounts sufficient for preparative syntheses.

Almost all of the reported assays of glycosyltransferases measure the transfer of radiolabeled sugars from a sugar-nucleotide to an appropriate oligosaccharide. Synthetic oligosaccharides of well defined structure have been used widely[1,8–11] as acceptors for such assays, particularly where appropriate, naturally occurring acceptors have been difficult to isolate. The assays are simplified when the synthetic substrates are attached to hydrophobic aliphatic or aromatic aglycons. The radiolabeled hydrophobic product can then be quantitated after rapid separation using reverse-phase supports[12]. The amount of labeled product formed in such assays is typically of the order of a nanomol. In principle, scaling up such assays 10^4–10^6-fold, and using non-labeled

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sugar-nucleotide donors, would yield usable quantities of the product oligosaccharides which could be isolated by reverse-phase chromatography.

We have reported[12] that the trimannoside **1**, a partial structure of the core region of asparagine-linked oligosaccharides, can serve as such a hydrophopic substrate (K_m 0.59mM) for *N*-acetylglucosaminyltransferase-I (GlcNAcT-I), which catalyses the transfer of D-Glc*p*NAc from UDP-Glc*p*NAc to produce the tetrasaccharide **2**. Structure **2**, in turn, has been used[9,13] as an acceptor (K_m 0.19mM) to detect GlcNAcT-II activity which converts it into the pentasaccharide **3**. We now report that a Triton X-100 extract from rabbit liver[14], refined using a single affinity-chromatography step on UDP-hexanolamine Sepharose[15], yielded fractions that contained GlcNAcT-I and GlcNAcT-II activities sufficient to effect the conversion of **1** into either **2** or **3** on a preparative 50–100 mg scale. A chemical synthesis of **1** is also reported.

1 α-D-Man*p*-(1→6)\
α-D-Man*p*-(1→3) > β-D-Man*p*-O(CH$_2$)$_8$COOMe

↓ UDP-Glc*p*NAc, GlcNAcT-I

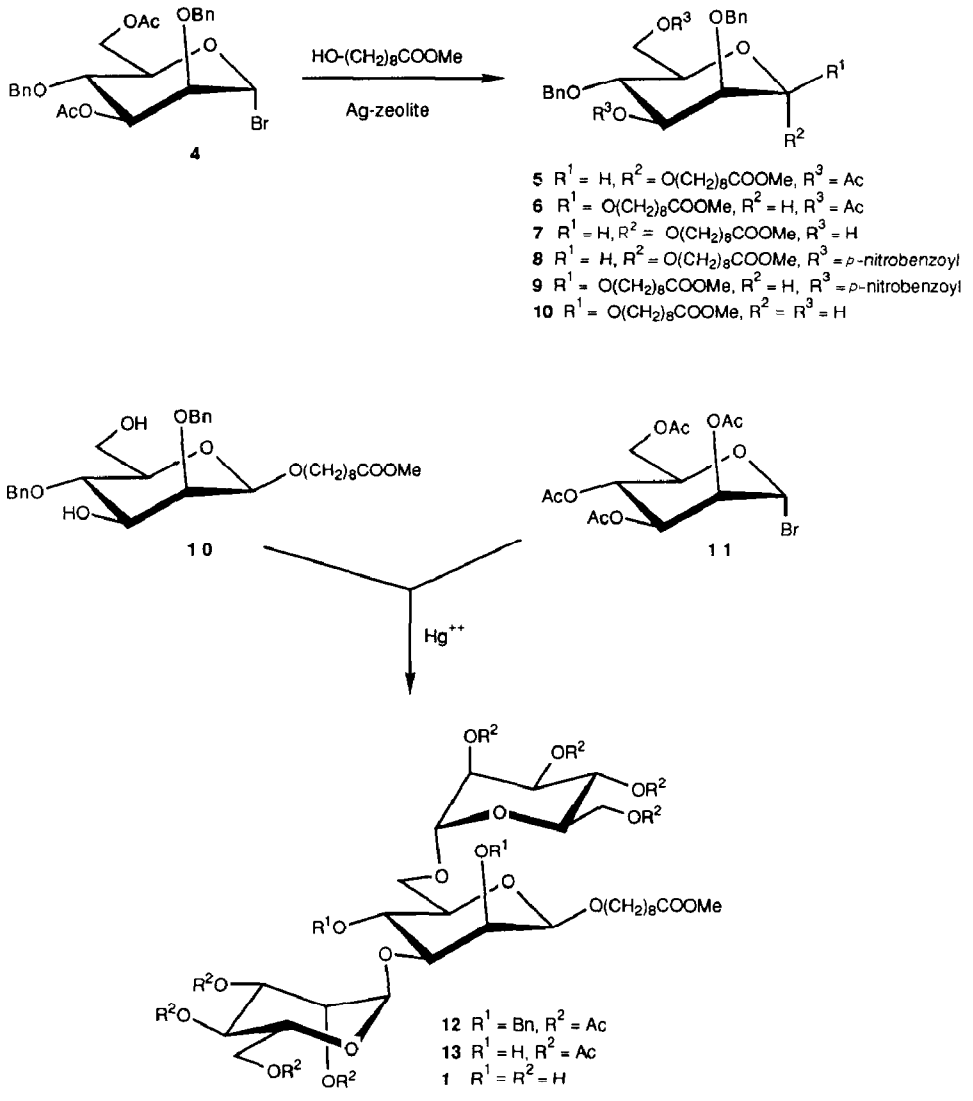
2 α-D-Man*p*-(1→6)\
β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3) > β-D-Man*p*-O(CH$_2$)$_8$COOMe

↓ UDP-Glc*p*NAc, GlcNAcT-II

3 e c a\
β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)\
β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3) > β-D-Man*p*-O(CH$_2$)$_8$COOMe\
d b

RESULTS AND DISCUSSION

The synthesis of **1** followed well-established procedures[16–20] for the assembly of oligomannosides related to the core region of asparagine-linked carbohydrate chains. Glycosylation of 8-methoxycarbonyloctanol, using the glycosyl donor **4** and silver zeolite[21] as promoter, gave a mixture (71%) of the α- (**5**) and β-glycoside **6**. These anomers could not be separated by silica gel chromatography but were directly *O*-deacetylated (→**7**) and then *p*-nitrobenzoylated to give the α- (**8**) and β-di-*p*-nitrobenzoate (**9**), which could be separated to give the required β anomer (21% from **4**). *O*-Deacylation of **9** then gave diol **10** (78%), glycosylation of which, using acetobromo-



mannose (**11**) in the presence of mercuric bromide–mercuric cyanide, yielded the trisaccharide **12**. Debenzylation of **12** furnished **13** (70% from **10** after purification by column chromatography), *O*-deacetylation of which then gave **1** (84%).

Rabbit liver was a convenient source of GlcNAcT-I and -II. Enzyme fractions suitable for preparative conversions of **1** into the tetrasaccharide **2**, or the pentasaccharide **3**, were obtained from a Triton X-100 extract of rabbit liver acetone powder following slight modifications of reported procedures[14]. The enzymes were separated partially by elution from a UDP-hexanolamine Sepharose affinity column[15], using a linear salt gradient. Earlier fractions eluted between 1.2 and 2.1M NaCl (fraction **D**, Table II, see Experimental) contained substantial GlcNAcT-I and -II activities, whereas fraction **E**, eluted between 2.1 and 3.0M NaCl, was enriched ~30 fold in GlcNAcT-I

TABLE I

Selected ^{1}H- and ^{13}C-n.m.r. data for **1–3**[a]

Nucleus	**1**	**2**	**3**
H-1a ($J_{1,2}$)	4.676 (< 1)	4.674 (< 1)	4.571 (< 1)
H-1b ($J_{1,2}$)	5.103 (1.7)	5.131 (~ 1)	5.128 (~ 1)
H-1c ($J_{1,2}$)	4.908 (1.7)	4.911 (1.5)	4.918 (1.5)
H-1d ($J_{1,2}$)	—	4.553 (8.3)	4.552 (8.3)
H-1e ($J_{1,2}$)	—	—	4.582 (8.3)
H-2a ($J_{2,3}$)	4.138 (2.7)	4.134 (2.5)	4.143 (2.4)
H-2b ($J_{2,3}$)	4.066 (3.4)	4.191 (3.4)	4.188 (3.4)
H-2c ($J_{2,3}$)	3.991 (3.4)	3.993 (3.5)	4.135 (3.5)
COOMe	3.688 (s)	3.688 (s)	3.688 (s)
CH_3COO	2.391 (t, 7.6)	2.391 (t, 7.5)	2.389 (t, 7.5)
Ac	—	2.053	2.052, 2.059
C-1a	103.2	100.6	100.6
C-1b	100.6	100.5	100.2
C-1c	100.2	100.2	97.6
C-1d	—	100.1	100.4
C-1e	—	—	100.3
C-2d	—	52.9	52.9
C-2e	—	—	52.9
$COCH_3$	—	175.6	175.6 (2 C)
$COCH_3$	—	23.1	23.1 (2 C)
$COOCH_3$	178.7	178.7	178.7
$COOCH_3$	52.9	56.2	56.1
CH_3COO	34.5	34.5	34.5

[a] See formula **3** for designation of the sugar residues.

TABLE II

Characterisation of GlcNAcT-I and -II activity in fractions eluted from UDP-hexanolamine Sepharose

Fraction	*Vol. (mL)*	*GlcNAcT-I activity*		*GlcNAcT-II activity*	
		mUnits	*%*	*mUnits*	*%*
A Crude ectract	200	7.4	100	12.6	100
B 0.2M NaCl wash	120	0.4	3	16.1	77
C 0.2–1.2M NaCl	80	1.3	7	7.5	24
D 1.2–2.1M NaCl	60	27.1	110	7.0	17
E 2.1–3.0M NaCl	60	8.4	34	0.3	< 1

[a] One unit (U) is defined as the quantity of enzyme that produces 1 μmol/min of product under the assay conditions (see Experimental).

activity. Incubation of **1** (80 mg) with fraction **D** (35 mL) and UDP-Glc*p*NAc for 6 days at room temperature gave **3** (102 mg, 80%), which was isolated by reverse-phase chromatography. Similarly, incubation of **1** (98 mg) with fraction **E** for 5 days yielded **2** (52 mg, 41%). This latter reaction was stopped before the undesired conversion **2**→**3** could be detected by t.l.c., and unreacted **1** was recovered. Both **2** and **3** were purified by gel-permeation chromatography on Bio-Gel P-2, which resolved completely mixtures of oligosaccharides **1**–**3**, and were obtained with a purity of >98% (^{1}H-n.m.r. data). Key ^{1}H- and ^{13}C-n.m.r. data for **1**–**3** are reported in Table I and are in good agreement with those reported[22,23] for analogs that contain the core trimannoside. A NOESY spectrum of **3** (not shown) showed cross-peaks between H-1 of the β-D-Glc*p*NAc residues and H-2 of the α-D-Man*p* residues as assigned in Table I, thereby confirming that the products expected had been formed.

The choice of rabbit liver as the source of GlcNAcT-I and -II was prompted by the availability of a simple and reproducible protocol[14] for their partial purification. GlcNAcT's III–V[7], however, were potentially present in the fractions used for the syntheses described, but their activities could not be detected using **3** as a substrate in radioactive assays[12]. If any of these enzymes acted on **3**, the product would be a larger oligosaccharide that ought to be separated during the gel-permeation chromatography, which is why this additional purification step was included. No larger oligosaccharides could be detected in either **2** or **3** by ^{1}H-n.m.r. spectroscopy. Oligosaccharides **2** and **3** may be of use in the assay of other glycosyltransferases acting in the biosynthetic pathway of asparagine-linked oligosaccharides.

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 sulfuric acid. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (Merck, 40–63 μm). Reverse-phase chromatography was performed on an octadecylsilane support (55–105 μm irregular, Waters Associates). 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. Millex-GV (0.22 μm) filter units were from Millipore, C_{18} Sep-Pak sample-preparation cartridges were from Waters Associates, Sepharose-4B was from Pharmacia, Scintiverse E was from Fisher, and UMP-morpholidate was from Sigma. 8-Methoxycarbonyloctanol was a gift from Chembiomed Ltd. (Edmonton). UDP-6-[^{3}H]GlcNAc (specific activity, 18.9 Ci/mmol) was from New England Nuclear and liquid scintillation counting was performed with a Beckman LS-5000 instrument, using quench correction[12]. ^{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 67.48). 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 ^1H 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 xenon as the bombarding gas, and with 1,4-dithiothreitol–1,4-dithioerythritol (5:1) 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 ≤40P (bath)/12 mmHg. The microanalyses were carried out by the Analytical Services Laboratory of this Department. The following solvent systems were used: *A*, ethyl acetate–hexane (1:4), *B*, ethyl acetate–hexane (1:3); *C*, ethyl acetate–hexane (1:2), *D* ethyl acetate–hexane (2:3); *E*, ethyl acetate–hexane (1:1); *F*, ethyl acetate–hexane (2:1); *G*, dichloromethane–methanol–water (60:35:6).

*8-Methoxycarbonyloctyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-* (**5**) *and -β-*D*-mannopyranoside* (**6**). A mixture of 8-methoxycarbonyloctanol (1.28 g, 6.83 mmol) (R_f 0.29, solvent *D*) and silver zeolite (5.0 g) in dry dichloromethane (5 mL) was stirred at room temperature for 1 h, then cooled to −78°, and a solution of **4** (0.69 g, 1.36 mmol) in dry dichloromethane (5 mL) was added dropwise. Stirring was continued for 2 h at −78°, then for 16 h at room temperature. The mixture was diluted with dichloromethane (25 mL), filtered through Celite, washed with water, and dried. Column chromatography (solvent *B*) of the residue gave a syrupy mixture of **5** and **6**, R_f 0.46 (solvent *D*). The α:β ratio was estimated by ^1H-n.m.r. spectroscopy to be 8:5. ^1H-N.m.r. data ($CDCl_3$): δ 5.24 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.0 Hz, H-3α), 4.82 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3β), 4.80 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1α), 3.67 (s, OMe), 3.55–3.48 (m, 1 H, H-5β), 2.29 (t, *J* 7.5 Hz, CH_2COO), 2.07 and 1.98 (s, Acα), 2.06 and 1.87 (s, Acβ).

*8-Methoxycarbonyloctyl 2,4-di-*O*-benzyl-3,6-di-*O*-p-nitrobenzoyl-α-* (**8**) *and -β-*D*-mannopyranoside* (**9**). A solution of the above mixture of **5** and **6** (595 mg, 0.97 mmol) in dry methanol (15 mL) containing sodium methoxide (~0.01M) was stirred for 2 h at room temperature, then neutralised with Amberlite IR-120(H^+) resin, filtered, and concentrated. A solution of the resulting syrupy diol (**7**) in anhydrous pyridine (10 mL) containing *p*-nitrobenzoyl chloride (526 mg, 2.83 mmol) was stirred for 15 h at room temperature, then concentrated. A solution of the residual syrup in dichloromethane (50 mL) was washed sequentially with water, aqueous 5% HCl, saturated aqueous sodium hydrogen carbonate, and water, then concentrated to a syrup which was purified by chromatography on Iatrobeads (solvent *A*) to provide **8** (300 mg, 38%), isolated as a syrup, R_f 0.47 (solvent *C*). ^1H-N.m.r. data ($CDCl_3$): δ 5.64 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3), 4.98 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.67 (s, 3 H, OMe), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2COO). The β anomer **9** (235 mg, 30%) was also obtained as a clear syrup, $[\alpha]_D$ −33° (*c* 1.5, chloroform), R_f 0.41 (solvent *C*). N.m.r. data ($CDCl_3$): ^1H, δ 8.30–8.00 (m, 8 H, $PhNO_2$), 7.40–7.10 (m, 10 H, 2 Ph), 5.18 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3), 4.68 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 4.20 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.14 (d, 1 H, H-2), 3.65 (s, 3 H, OMe), 2.28 (t, 2 H, *J* 7.5 Hz, CH_2COO); ^{13}C, δ 174.3 ($COOCH_3$), 164.4 and 163.8 (OCO), 101.3 (C-1), 51.5 (OCH_3), 34.1 (CH_2COO).

Anal. Calc. for $C_{44}H_{48}N_2O_{14}$: C, 63.76; H, 5.84; N, 3.38. Found: C, 63.50; H, 6.02; N, 3.20.

8-Methoxycarbonyloctyl 2,4-di-O-benzyl-β-D-mannopyranoside (**10**). — To a solution of **9** (180.5 mg, 0.217 mmol) in methanol–dichloromethane (3:1) was added sodium methoxide (60 mg). After 15 h, the solution was neutralised with acetic acid, washed with water, then concentrated. Purification of the residue by chromatography on Iatrobeads (solvent *C*) gave **10** (99 mg, 87%) as a white solid, $[\alpha]_D$ −53° (*c* 0.8, chloroform), R_F 0.41 (solvent *E*). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.51 (d, 1 H, $J_{1,2}$ 0.80 Hz, H-1), 3.66 (s, 3 H, OMe), 2.39 (d, 1 H, $J_{3,OH}$ 9.9 Hz, OH), 2.29 (t, 2 H, *J* 7.4 Hz, CH_2COO), 2.11 (dd, 1 H, $J_{6,OH} = J_{6',OH}$ = 7.0 Hz, OH).

Anal. Calc. for $C_{30}H_{42}O_8$: C, 67.89; H, 7.98. Found: C, 68.10; H, 7.80.

8-Methoxycarbonyloctyl 2,4-di-O-benzyl-3,6-di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**12**) *and 8-methoxycarbonyloctyl 3,6-di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside* (**13**). — To a solution of **10** (71.2 mg, 0.134 mmol) in dry acetonitrile (1.5 mL) containing powdered 4 Å molecular sieves (0.50 g) were added, in sequence, mercuric bromide (0.290 g, 0.806 mmol) and mercuric cyanide (0.204 g, 0.806 mmol) followed by a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**11**; 0.331 g, 0.806 mmol) in dry acetonitrile (1.5 mL) at 0°. After storage for 30 min at 0°, the mixture was stirred for 4 h at room temperature, then filtered through Celite, and concentrated. The solid residue was extracted with dichloromethane, and the extract was washed with saturated aqueous potassium chloride, saturated aqueous sodium hydrogen carbonate, and water, dried, and concentrated. The resulting syrup was purified by chromatography on Iatrobeads (solvent *E*) to provide **12** (440 mg), a solution of which in aqueous 95% ethanol (10 mL) containing 5% Pd–C (100 mg) was stirred under 1 atm. of hydrogen for 14 h, then filtered, and concentrated. The residue was purified by chromatography on Iatrobeads (solvent *F*) to give **13** (95 mg, 70% from **10**), isolated as a clear syrup, $[\alpha]_D$ +19° (*c* 1.4, chloroform), R_F 0.31 (solvent *F*). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.47–5.22 (6 H, H-2′,3′,4′,2″,3″,4″), 5.06 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1′), 4.95 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.47 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 3.67 (s, 3 H, OMe), 3.23 (b, 1 H, D_2O-exchangeable, OH), 2.56 (d, 1 H, *J* 2.7 Hz, D_2O-exchangeable, OH), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.16, 2.15, 2.12, 2.10, 2.06, 2.04 (6 s, each 3 H, 6 Ac), 1.99 (s, 6 H, 2 Ac); ^{13}C, δ 174.20 ($COOCH_3$), 170.8, 170.6, 170.1, 170.0, 169.9, 169.8, 169.7 ($COCH_3$), 99.8, 99.4, 97.6 (C-1, C-1′, and C-1″), 51.4 (OCH_3), 34.1 (CH_2COO).

Anal. Calc. for $C_{44}H_{66}O_{26}$: C, 52.27; H, 6.58. Found: C, 51.88; H, 6.60.

8-Methoxycarbonyloctyl 3,6-di-O-(α-D-mannopyranosyl)-β-D-mannopyranoside (**1**). — Compound **13** (87.2 mg; 0.086 mmol) was *O*-deacetylated as described above for **5** and **6**, to provide a white foamy residue which was eluted from a column (50 × 2.5 cm) of Bio-Gel P-2 (200–400 mesh) with aqueous 10% ethanol. The carbohydrate-containing fractions were combined, concentrated, and lyophilised, to give **1** as a white powder (48.6 mg, 84%), R_F 0.4 (solvent *G*), $[\alpha]_D$ +21° (*c* 0.2, water). F.a.b.-mass spectrum: *m/z* 697 [100%, $(M + Na)^+$]. The ^{1}H- and ^{13}C-n.m.r. parameters are reported in Table I.

Anal. Calc. for $C_{28}H_{50}O_{18}\cdot H_2O$: C, 48.55; H, 7.28. Found: C, 48.62; H, 7.46.

Enzymic conversion of **1** *into* **2** *and* **3**. — GlcNAcT-I and -II were partially purified from rabbit liver, using slight modifications of a published procedure[14]. A Triton X-100 extract (200 mL) of rabbit liver acetone powder (150 g) was applied to a column (2.5 × 12 cm) of UDP-hexanolamine Sepharose (6.1 μmol/mL). Buffer *A* (50mM sodium cacodylate, 10mM $MnCl_2$, 1% of Triton X-100) (55 mL) was passed through the column at 0.4 mL/min, followed by buffer *A* containing 0.2M NaCl (120 mL). A linear gradient (200 mL) from 0.2→3.0M NaCl in buffer *A* was then passed through the column and 3 fractions (C–E) were collected that were eluted at 0.2–1.2 (C), 1.2–2.1 (D), and 2.1–3.0M NaCl (E). Each fraction was dialysed against buffer *A* to provide enzyme preparations which were stable for at least one month at 4°.

The GlcNAcT-I and -II activities of these solutions were measured using a previously described assay[12] where each incubation mixture of 100 μL contained 25.1 nmol of **1** or **2**, 7.4 nmol of UDP-GlcNAc, 112,000 d.p.m. UDP-[^{3}H]GlcNAc in 50mM sodium cacodylate, 10mM $MnCl_2$, 1% of Triton X-100, and 50 μL of enzyme preparation. After storage for 1 h at 37°, the mixture was diluted to 5 mL with water, and applied to a C_{18} Sep-Pak cartridge (Millipore-Waters), which was washed with water (5 × 5 mL). The radiolabelled product was eluted with methanol (2 × 5 mL) and quantitated (d.p.m. by liquid scintillation counting). The enzyme assay results are summarised in Table II where one unit of activity is defined as the amount of enzyme that catalyses the formation of 1 μmol of product per min under the assay conditions.

*8-Methoxycarbonyloctyl 3-O-[2-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-6-O-α-*D*-mannopyranosyl-β-*D*-mannopyranoside* (**2**). — A mixture of **1** (98 mg, 0.145 mmol), UDP-Glc*p*NAc (0.5 g, 0.71 mmol), and enzyme fraction **E** (35 mL) was kept at room temperature for 5 days, then applied directly to a C_{18} column (2.5 × 60 cm), and eluted with methanol–water (35:65, 3.6 L) at 2 mL/min. Fractions (7 mL) were analysed by t.l.c. Fractions that contained pure **2** were combined and concentrated, whereas fractions that contained a mixture of **2** and **1** were concentrated and applied to a column (2.5 × 60 cm) of Bio-Gel P-2 which was eluted with aqueous 10% ethanol. The earlier fractions from this column, which contained **2** (t.l.c.), were combined and concentrated. Compound **2** (total yield, 52 mg, 41%) was obtained as a white powder after lyophilisation; $[\alpha]_D$ +6.9° (*c* 0.6, water). R_f 0.26 (solvent *G*). F.a.b.-mass spectrum: *m/z* 900 [9%, (M + Na)$^+$]. The ^{1}H- and ^{13}C-n.m.r. parameters are reported in Table I.

*8-Methoxycarbonyloctyl 3,6-di-O-[2-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-β-*D*-mannopyranoside* (**3**). — A mixture of **1** (80 mg, 0.12 mmol), UDP-Glc*p*NAc (0.71 g, 1.0 mmol), and enzyme fraction **D** (35 mL) was kept at room temperature for 3 days. More UDP-Glc*p*NAc (0.32 g) was added and, after 3 more days, the mixture was applied to a C_{18} column (3.5 × 25 cm) and eluted with methanol–water (35:65, 5 L) at 1 mL/min. Fractions (25 mL) were examined by t.l.c. The appropriate fractions were combined and **3** was obtained as a white powder (102 mg, 80%) after concentration and lyophilisation; $[\alpha]_D$ −3.5° (*c* 0.6, water). R_f 0.14 (solvent *G*). F.a.b.-mass spectrum: *m/z* 1103 [19%, (M + Na)$^+$]. The ^{1}H- and ^{13}C-n.m.r. parameters are reported in Table I.

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