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Synthesis of a tetrasaccharide acceptor for use in the assay of UDP-Glc*p*NAc:*β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc (GlcNAc to Gal) *β*(1→3)-*N*-acetylglucosaminyltransferase activity and the pentasaccharide product that would be formed by its enzymic glycosylation

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ABSTRACT

The tetrasaccharide *β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc-(1→6)-*α*-D-Man*p*-(1→6)-*β*-D-Man*p*-OR (**2**) and the pentasaccharide *β*-D-Glc*p*NAc-(1→3)-*β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc-(1→6)-*α*-D-Man*p*-(1→6)-*β*-D-Man*p*-OR (**3**), where R = $(CH_2)_8COOMe$, have been prepared by using combined chemical and enzymic procedures. Structure **2** is a substrate for UDP-Glc*p*NAc:*β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc (GlcNAc to Gal) *β*(1→3)-*N*-acetylglucosaminyltransferase, and **3** would be the product of its action. Antibodies raised against **3** are intended for use in an ELISA assay that would quantitate the enzymic conversion of immobilized **2** into **3**.

INTRODUCTION

Polylactosamine chains, which consist of repeats of the disaccharide sequence -*β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc-(1→3)-, are found on animal cells attached to both *O*- and *N*-linked glycoproteins as well as on glycolipids[1,2]. These polymers, which can have more than 20 repeats, carry the human blood-group "i" antigenic determinant and can carry galactosyl, fucosyl, and sialyl residues. In many tumors, there is an increase in the content of polylactosamine structures on the cell surface, as well as their fucosylated poly-Le^x derivatives which contain *α*-L-Fuc*p*-(1→3) groups attached to the GlcNAc residues. These fucosylated oligosaccharides are important as tumor-associated antigens[1,2]. Equally important is the observation that increased polylactosamine biosynthesis accompanies the transition from benign to malignant phenotype in several human and rodent tumor cell lines[3]. In particular, polylactosamine chains in these latter cell lines appear to be synthesized preferentially on the *β*-(1→6)-linked Glc*p*NAc branches on both *O*- and *N*-linked glycoproteins[3,4].

An increase in the biosynthesis of polylactosamines may be the direct result of increased expression of the glycosyltransferases that control their biosynthesis[3–6]. For Asn-linked glycoproteins, polylactosamine chains are formed preferentially[7,8] attached

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to HO-6 of the (1→6)-linked α-D-Man*p*, as shown in Fig. 1. The specific formation of polylactosamines on this branch requires the sequential reaction of (*a*) UDP-Glc*p*NAc:α-D-Man*p*-(1→6)-β-D-Man*p*-R (GlcNAc to αMan) β(1→6)-*N*-acetyl-glucosaminyltransferase (GlcNAcT-V, EC 2.4.1.155), (*b*) UDP-Gal*p*:β-D-Glc*p*NAc-R β(1→4)-galactosyltransferase [β(1→4)GalT, EC 2.4.1.38], and (*c*) UDP-Glc*p*NAc:β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-R (GlcNAc to Gal) β(1→3)-*N*-acetylglucosaminyltransferase (GlcNAcT-"i", EC 2.4.1.149). The last enzyme is referred to as the "little-i" or "i" enzyme[9,10] because of its role in the synthesis of the antigenic determinant giving rise to this blood-group activity. We have developed radiochemical assays for GlcNAcT-V and β(1→4)GalT activities[11] that measure the rate of transfer of radiolabeled glycosyl residues from sugar nucleotides to synthetic acceptor oligosaccharides. The assays are simple when the acceptor oligosaccharides are attached to hydrophobic aglycons such as the octyl or 8-methoxycarbonyloctyl groups, since these have favorable retention characteristics on a reverse-phase C_{18} matrix[11]. An enzyme-linked immunosorbent assay (ELISA) was also developed for GlcNAcT-V activity, which used an antibody to detect and characterize the product formed by action of this enzyme on a synthetic substrate[12]. This ELISA also resulted in a 1000-fold increase in sensitivity over the radiochemical assay. An analogous ELISA has also been developed for β(1→4)GalT activity, using glycolipid substrates[13]. Similar techniques for assaying the GlcNAcT-"i" activity remain to be developed in order to allow quantitation of the entire panel of 3 glycosyltransferases that control the biosynthesis of the β-(1→6)-linked polylactosamine chains.

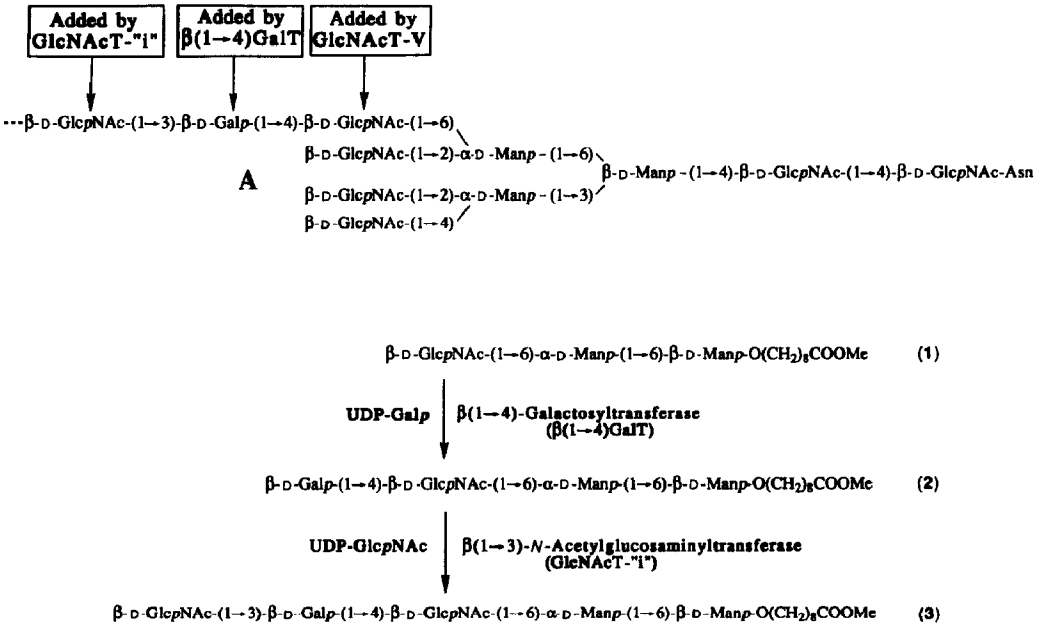


Fig. 1. Structure **A** shows a polylactosamine chain initiated on the preferred β(1→6) arm of Asn-linked oligosaccharide. The biosynthesis of this arm requires the sequential action of GlcNAcT-V, β(1→4)GalT, and GlcNAcT-"i". Structures **1–3** are synthetic oligosaccharide derivatives which are expected to also act as acceptors for these glycosyltransferases.

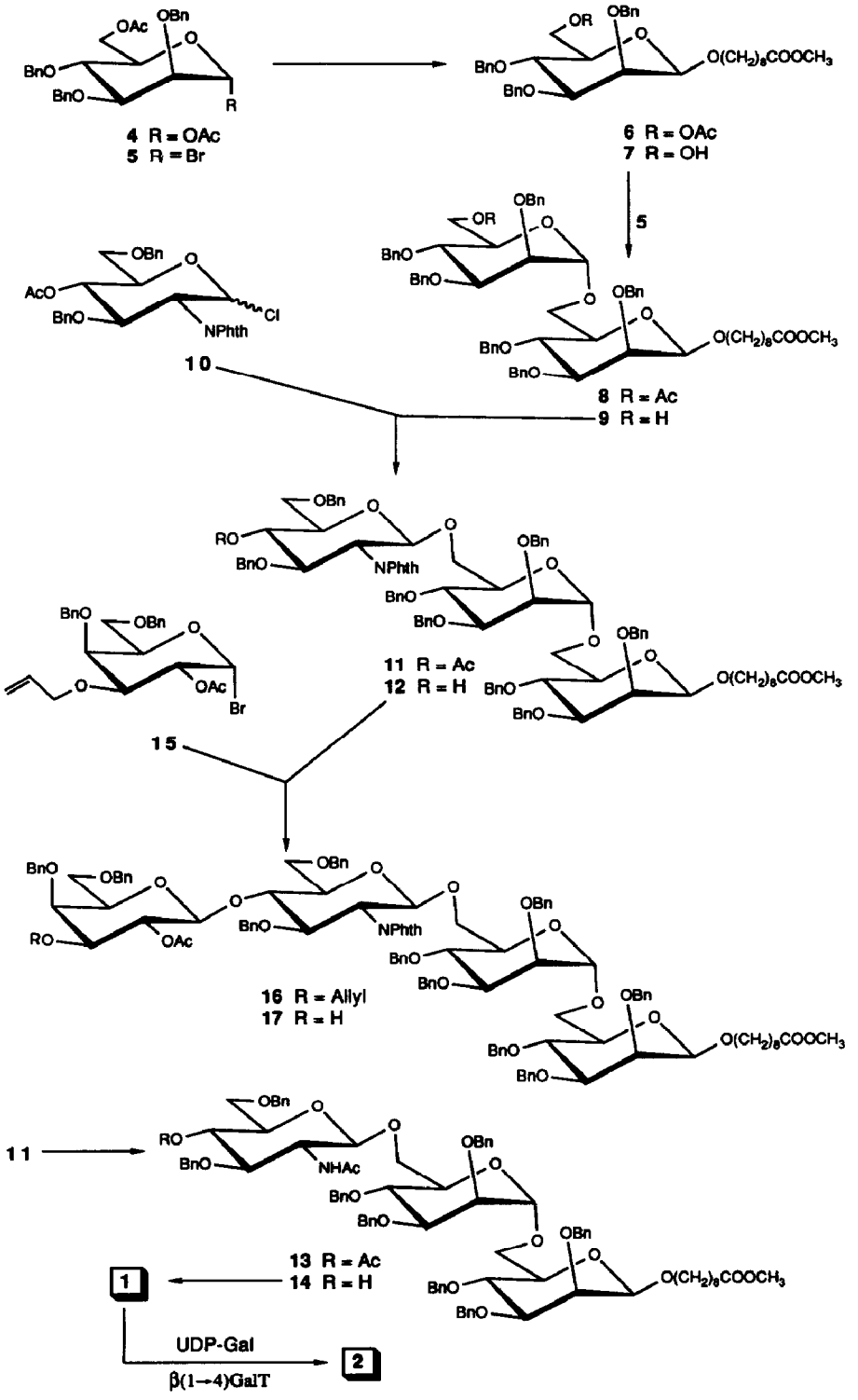
We now report the synthesis of tetrasaccharide **2** and the pentasaccharide product (**3**) which would be formed by the action of GlcNAcT-"i" on **2**. Compound **2** was expected to be the minimum structure required for good acceptor activity against GlcNAcT-"i" on the basis of two studies[7,8] which indicated that the chitobiose reducing end of Asn-linked acceptors was not necessary for good enzyme activity and that the simple *N*-acetyl-lactosamine sequence had only very low activity. The 8-methoxycarbonyloctyl glycosides **2** and **3** were prepared in order to allow for radioactive assays[11] as described above. In addition, immunization of rabbits or mice with a glycoprotein antigen prepared from **3** should produce polyclonal antibodies which recognize the structure of this product[12]. Antibodies that cross-react with **2** could then be removed from a polyvalent anti-serum on an affinity column prepared from **2** as in the development[12] of an ELISA assay for GlcNAcT-V. The synthetic manipulations required for the production of **2** and **3** were minimized by incorporating an enzymic step into the synthesis, namely the conversion of **1** into **2** using commercial $\beta(1\rightarrow4)$GalT.

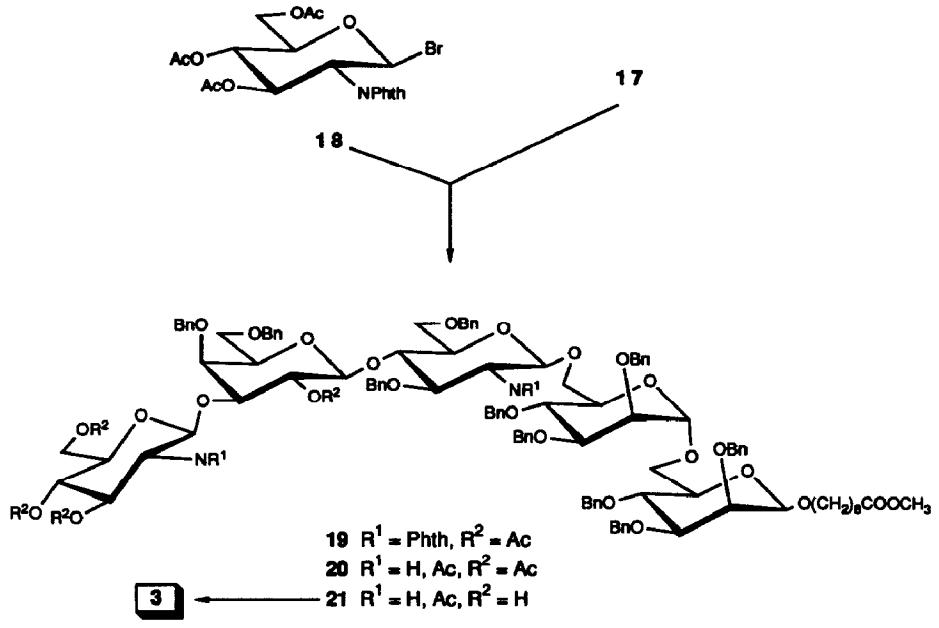
RESULTS AND DISCUSSION

The synthesis of the target oligosaccharides followed well-established procedures. Acetate **4** was converted into the glycosyl bromide **5**[14] which, on reaction with 8-methoxycarbonyloctanol in the presence of silver zeolite[15], gave the β-mannoside **6** (69%). *O*-Deacetylation of **6** gave the alcohol **7**, which was glycosylated, using **5** in the presence of silver triflate and *sym*-collidine, to produce the α-$(1\rightarrow6)$-linked disaccharide derivative **8** (48%) that was *O*-deacetylated to yield **9**. Reaction of **9** with the glycosyl donor **10**[16], promoted again by silver triflate and *sym*-collidine, gave the β-phthalimidoglycoside **11** (80%) which was *O*-deacetylated to provide the next glycosyl acceptor **12**. Reaction of **12** with the galactosyl bromide **15** (prepared in 8 steps from 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose[17]) produced the protected tetrasaccharide glycoside **16** (70%), from which the allyl group was removed by isomerization using Wilkinson's catalyst followed by hydrolysis[18]. Alcohol **17**, thus obtained in 73% yield, was converted into the protected pentasaccharide glycoside **19** (86%) by reaction with the phthalimido-glycosyl bromide **18**[19] under standard conditions. The target pentasaccharide **3** was obtained (80%) by deprotection of **19**, which involved exchanging the phthalimido groups for *N*-acetyl to yield **20** (68%), then *O*-deacetylation, and hydrogenation.

The tetrasaccharide **2** could have been produced by deprotection of **17**. However, it was more convenient, and less wasteful of valuable **17**, to deprotect the more accessible precursor trisaccharide derivative **13** to provide **1** (58%). Addition of the terminal β-D-Gal*p*$(1\rightarrow4)$ group could then be accomplished using a commercial galactosyltransferase and UDP-Gal, to produce tetrasaccharide **2** (83%).

The tetrasaccharide **2**, which has been used in radioactive assays to measure the change in GlcNAcT-"i" activity that accompanies differentiating granulocytes[6], is also an acceptor for an *N*-acetylglucosaminyltransferase activity present in human serum ($K_m \sim$ 2.0mM, data not reported). It is hoped that specific antibodies produced against **3**


4 R = OAc
5 R = Br
6 R = OAc
7 R = OH
5
10
8 R = Ac
9 R = H
11 R = Ac
12 R = H
15
16 R = Allyl
17 R = H
11
13 R = Ac
14 R = H
1
UDP-Gal
β(1→4)GalT
2



can be used to confirm the structure of the product formed in the above glycosyltransferase assays which employ a crude source of enzyme.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2°. T.l.c. was performed on silica gel (Merck, 60-F_{254}) 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). 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 mm) filter units were from Millipore and C_{18} Sep-Pak cartridges were from Waters Associates. UDP-galactose and $\beta(1\rightarrow4)$galactosyltransferase were from Sigma. 8-Methoxycarbonyloctanol was a gift from Chembiomed Ltd. (Edmonton). ^{1}H-N.m.r. spectra were recorded at 300 or 360 MHz [with 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. 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 $\leq 40°$ (bath)/12 mmHg.

8-Methoxycarbonyloctyl 6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**6**). — A mixture of 8-methoxycarbonyloctanol (2 g, 10.6 mmol) and silver zeolite (2.4 g) in dry dichloromethane (6 mL) was stirred at room temperature for 1 h, then cooled to −78°, and a solution of **5**, freshly prepared from 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**4**; 1.17 g, 2.19 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, dried, and concentrated to a syrup which was purified by chromatography on Iatrobeads (toluene–ethyl acetate, 20:1) to give **6** (1.0 g, 69%), isolated as a syrup, $[\alpha]_D$ −12° (*c* 2.5, chloroform), R_F 0.61 (9:1 toluene–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 4.36 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 3.66 (s, 3 H, OMe), 3.90 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.52 (dd, $J_{2,3}$ 3.0 Hz, H-3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.00 (s, 3 H, Ac); ^{13}C, δ 174.3 ($COOCH_3$), 171.0 ($COCH_3$), 138.7, 138.2, and 138.1 (quaternary C), 101.9 ($J_{C\text{-}1,H\text{-}1}$ 153.4 Hz, C-1), 63.9 (C-6), 51.5 (OCH_3), 34.1 (CH_2COO), 21.0 ($COCH_3$).

Anal. Calc. for $C_{39}H_{50}O_9$: C, 70.67; H, 7.60. Found: C, 70.74; H, 7.83.

8-Methoxycarbonyloctyl 2,3,4-tri-O-benzyl-β-D-mannopyranoside (**7**). — A solution of **6** (996 mg, 1.5 mmol) in dry methanol (20 mL) containing a trace of sodium methoxide (~ 0.01M) was kept for 4 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin, and filtered, and the solvent was evaporated to leave **7** as a white foam (895 mg, 95%), $[\alpha]_D$ −35° (*c* 1.5, chloroform), R_F 0.45 (9:1 toluene–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 3.91 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.66 (s, 3 H, OMe), 3.52 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-3), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.15 (br, 1 H, HO-6, D_2O exchangeable); ^{13}C, δ 174.3 ($COOCH_3$), 138.7, 138.3, and 138.2 (quaternary C), 101.8 (C-1), 62.7 (C-6), 51.5 (OCH_3), 34.1 (CH_2COO).

Anal. Calc. for $C_{37}H_{48}O_8$: C, 71.58; H, 7.79. Found: C, 71.35; H, 7.77.

8-Methoxycarbonyloctyl 6-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**8**). — A solution of the glycosyl bromide **5**, freshly prepared from acetate **4** (843 mg, 1.58 mmol), in dichloromethane (30 mL) was added dropwise during 0.5 h to a stirred mixture of **7** (490 mg, 0.79 mmol), *sym*-collidine (208 μL, 1.58 mmol), silver trifluoromethanesulfonate (405 mg, 1.58 mmol), and pulverized 4 A molecular sieves (3 g) in dichloromethane (30 mL) at 0°. After 15 h, dichloromethane (30 mL) was added, the molecular sieves were collected and washed with dichloromethane (30 mL), and the combined filtrate and washings were concentrated. Column chromatography twice (hexane–ethyl acetate, 6:1) of the residue on Iatrobeads gave **8** (418 mg, 48%), isolated as a syrup, $[\alpha]_D$ −13° (*c* 2.7, chloroform), R_F 0.60 (3:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.13 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.33 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 4.02 (dd, 1 H, $J_{2',3'}$ 3.0 Hz), 3.66 (s, 3 H, OMe), 3.52 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3), 2.29 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.00 (s, 3 H, Ac); ^{13}C; δ 174.3 ($COOCH_3$), 170.9 ($COCH_3$), 101.9 ($J_{C\text{-}1,H\text{-}1}$ 153.2 Hz, C-1), 98.6 ($J_{C\text{-}1',H\text{-}1'}$ 170.3 Hz, C-1'), 66.7 (C-6), 63.6 (C-6'), 51.5 (OCH_3), 34.0 (CH_2COO), 20.9 ($COOCH_3$).

Anal. Calc. for $C_{66}H_{78}O_{14}$: C, 72.37; H, 7.18. Found: C, 72.61; H, 7.09.

8-Methoxycarbonyloctyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**9**). — Treatment of **8** (350 mg, 0.32 mmol) with methanolic sodium methoxide, as described for the preparation of **7**, provided **9** (310 mg, 93%) as a syrup, $[\alpha]_D$ −14° (*c* 3.1, chloroform), R_F 0.35 (2:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.08 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.33 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 3.98 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.68 (s, 3 H, OMe), 3.52 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3), 2.29 (t, 2 H, *J* 7.5 Hz, CH_2COO), 1.90 (br, 1 H, HO-6', D_2O exchangeable); ^{13}C, δ 174.3 ($COOCH_3$), 101.8 (C-1), 98.7 (C-1'), 66.5 (C-6), 62.3 (C-6'), 51.4 (OCH_3), 34.1 (CH_2COO).

Anal. Calc. for $C_{64}H_{76}O_{13}$: C, 72.97; H, 7.27. Found: C, 72.63; H, 7.23.

8-Methoxycarbonyloctyl 6-O-[6-O-(4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**11**). — A mixture of **9** (250 mg, 0.24 mmol) and silver trifluoromethanesulfonate (344 mg, 1.34 mmol) was dried *in vacuo* over P_2O_5 for 1 h at 25° and dissolved in dichloromethane (2 mL) under nitrogen. To this stirred mixture was added *sym*-collidine (177 μL, 1.34 mmol) and pulverized molecular sieves 4 A (500 mg), and the mixture was stirred at −30° for 10 min. A solution of the glycosyl chloride **10** (0.80 mmol) in dichloromethane (2 mL) was added and the mixture was allowed to warm to room temperature. After 24 h, dichloromethane (15 mL) was added, the molecular sieves were collected and washed with dichloromethane (15 mL), and the combined filtrate and washings were concentrated. Column chromatography (hexane–ethyl acetate, 5:1) of the residue on Iatrobeads gave **11** (300 mg, 80%), isolated as a syrup, $[\alpha]_D$ +1.5° (*c* 5.0, chloroform), R_F 0.60 (hexane–ethyl acetate, ~1:1). N.m.r. data ($CDCl_3$): ^1H, δ 5.25 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1''), 5.11 (dd, 1 H, $J_{3'',4''}$ 9.0, $J_{4'',5''}$ 10.0 Hz, H-4''), 4.80 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.28 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 2.30 (d, 1 H, *J* 7.5 Hz, CH_2COO), 1.92 (s, 3 H, Ac); ^{13}C, δ 174.3 ($COOCH_3$), 169.7 ($COCH_3$), 101.8 ($J_{C\text{-}1,H\text{-}1}$ 153.1 Hz, C-1), 98.6 ($J_{C\text{-}1'',H\text{-}1''}$) 164.0 Hz, C-1''), 98.4 ($J_{C\text{-}1',H\text{-}1'}$ 170.2 Hz, C-1'), 51.5 (OCH_3), 34.1 (CH_2COO), 20.9 (CH_3).

Anal. Calc. for $C_{94}H_{103}NO_{20}$: C, 72.06; H, 6.63; N, 0.89. Found: C, 71.97; H, 6.55; N, 0.89.

8-Methoxycarbonyloctyl 2,3,4-tri-O-benzyl-6-O-[2,3,4-tri-O-benzyl-6-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-mannopyranoside (**12**). — Compound **11** (130 mg, 0.09 mmol) was *O*-deacetylated as described for the conversion of **6** into **7**. Column chromatography (hexane–ethyl acetate, 1:2) of the product on Iatrobeads yielded **12** (110 mg, 87%) as a foam, R_F 0.41 (1:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.23 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.85 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 3.67 (s, 3 H, OMe), 3.03 (br, 1 H, OH), 2.30 (t, 3 H, CH_2COO).

8-Methoxycarbonyloctyl 6-O-[6-O-(2-acetamido-4-O-acetyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**13**). — A mixture of **11** (215 mg, 0.14 mmol) and hydrazine acetate (379 mg, 4.11 mmol) in dry methanol (5 mL) was boiled under reflux for 3 h, more hydrazine acetate (379 mg, 4.11 mmol) was added, the refluxing was continued for

20 h, and the solvent was then evaporated. A solution of the residue in pyridine (2 mL) and acetic anhydride (2 mL) was kept for 16 h at room temperature, ethanol (2 mL) was added at 0°, the solvent was evaporated, and toluene (2 × 10 mL) was evaporated from the residue. A solution of the residue in dichloromethane (20 mL) was washed with aqueous 5% HCl, saturated aqueous $NaHCO_3$, and cold water, then concentrated. Column chromatography (hexane–ethyl acetate, 2:1) of the residue on Iatrobeads gave **13** (150 mg, 73.9%), isolated as a syrup, $[\alpha]_D$ −3.1° (*c* 2.9, chloroform), R_F 0.56 (1:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.78 (d, 1 H, $J_{2,NH}$ 7.5 Hz, NH), 5.16 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 5.07 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.91 (dd, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 10.0 Hz, H-4''), 4.07 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.5 Hz, H-3'), 4.01 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.07 (m, 1 H, H-2''), 2.28 (t, 2 H, *J* 7.5 Hz, CH_2COO), 1.82 (s, 1 H, Ac); ^{13}C, δ 174.3 ($COOCH_3$), 171.2 and 169.9 ($COCH_3$), 101.8 ($J_{C-1,H-1}$ 153.9 Hz, C-1), 98.8 ($J_{C-1'',H-1''}$ 163.5 Hz, C-1''), 98.5 ($J_{C-1',H-1'}$ 170.3 Hz, C-1'), 51.4 (OCH_3), 34.1 (CH_2COO), 20.9 ($COCH_3$).

Anal. Calc. for $C_{88}H_{103}NO_{19}$: C, 71.47; H, 7.02; N, 0.95. Found: C, 71.97; H, 6.55; N, 0.89.

8-Methoxycarbonyloctyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→6)-α-*D-*mannopyranosyl-(1→6)-β-*D-*mannopyranoside* (**1**). — *O*-Deacetylation of **13** (110 mg, 0.074 mmol), as described for the preparation of **7**, gave **14** (R_F 0.41, 1:1 hexane–ethyl acetate), a solution of which in dry methanol (5 mL) containing 5% Pd/C (50 mg) was stirred under 1 atm. of hydrogen for 15 h, then filtered. The solvent was evaporated to provide **1** as a white powder (41.5 mg, 78%), $[\alpha]_D$ −5.8° (*c* 0.3, water), R_F 0.40 (60:35:6 dichloromethane–methanol–water). N.m.r. data (D_2O): ^1H, δ 4.87 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.65 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1''), 3.68 (s, 3 H, OMe), 2.38 (t, 2 H, *J* 7.5 Hz, CH_2COO); ^{13}C, δ 178.7 ($COOCH_3$), 175.3 ($NCOCH_3$), 102.3 (C-1), 100.8 (C-1''), 100.4 (C-1'), 52.9 (OCH_3), 34.5 (CH_2COO), 23.1 ($NCOCH_3$).

Anal. Calc. for $C_{30}H_{53}NO_{18}\cdot 2H_2O$: C, 47.99; H, 7.51; N, 1.89. Found: C, 48.06; H, 6.96; N, 1.91.

*8-Methoxycarbonyloctyl 6-*O-*{6-*O-*[4-*O-*(2-*O-*acetyl-3-*O-*allyl-4,6-di-*O-*benzyl-β-*D-*galactopyranosyl)-3,6-di-*O-*benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl]-2,3,4-tri-*O-*benzyl-α-*D-*mannopyranosyl}-2,3,4-tri-*O-*benzyl-β-*D-*mannopyranoside* (**16**). — A solution of silver trifluoromethanesulfonate (32.2 mg, 0.13 mmol) and *sym*-collidine (13 μL, 0.1 mmol) in dichloromethane–toluene (3:2, 1 mL) was added dropwise to a stirred mixture of **12** (90 mg, 0.06 mmol), 2-*O*-acetyl-3-*O*-allyl-4,6-di-*O*-benzyl-α-D-galactopyranosyl bromide (**15**; 32 mg, 0.13 mmol), and ground 4 A molecular sieves (180 mg) in toluene (1 mL) at −20° under dry nitrogen. The mixture was stirred for 30 min, diluted with dichloromethane (25 mL), filtered through Celite, washed with water, and concentrated. Column chromatography (hexane–ethyl acetate, 1:1) of the residue on Iatrobeads gave **16**, isolated as a syrup (85.4 mg, 69.9%), $[\alpha]_D$ −0.59° (*c* 8.5 chloroform), R_F 0.68 (1:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.84 (m, 1 H, $CH_2CH=CH_2$), 5.29 (dd, 1 H, $J_{1''',2'''}$ 8.0, $J_{2''',3'''}$ 10.0 Hz, H-2'''), 5.18 (d, 1 H, H-1'''), 3.65 (s, 3 H, OMe), 2.29 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.04 (s, 3 H, Ac); ^{13}C, δ 174.2 ($COOCH_3$), 169.3 ($COCH_3$), 101.8 ($J_{C-1,H-1}$ 155 Hz, C-1), 100.8 ($J_{C-1''',H-1'''}$ 157 Hz,

C-1‴), 98.8 ($J_{C\text{-}1'',H\text{-}1''}$ 161.0 Hz, C-1″), 98.4 ($J_{C\text{-}1',H\text{-}1'}$ 170.0 Hz, C-1′), 51.4 (OCH_3), 34.1 (CH_2COO), 21.1 ($COOCH_3$).

Anal. Calc. for $C_{117}H_{129}NO_{25}$: C, 72.09; H, 6.69; N, 0.72. Found: C, 71.99; H, 6.67; N, 0.68.

8-Methoxycarbonyloctyl 6-O-{6-O-[4-O-(2-O-acetyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2,3,4-tri-O-benzyl-α-D-mannopyranosyl}-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**17**). — A solution of **16** (72.5 mg, 0.037 mmol), tris(triphenylphosphine)rhodium(I) chloride (4.8 mg, 0.005 mmol), and 1,8-diazabicyclo[2.2.2]octane (1.8 mg, 0.015 mmol) in ethanol–benzene–water (7:3:1, 2 mL) was boiled under reflux for 24 h. The solvent was evaporated and to a solution of the residue in acetone (5 mL) containing mercuric oxide (1 mg) was added a solution of mercuric chloride (50 mg) in acetone–water (9:1, 5 mL). The mixture was stirred for 45 min, the solvent was evaporated, and a solution of the residue in dichloromethane (25 mL) was washed with aqueous 30% KBr and then water, and concentrated. Column chromatography (hexane–ethyl acetate, 1:1) of the residue on Iatrobeads gave **17**, isolated as a syrup (52 mg, 73.2%), $[\alpha]_D$ −8.7° (*c* 0.3, chloroform), R_F 0.36 (1:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.22 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 3.66 (s, 3 H, OMe), 2.30 (t, 2 H, CH_2COO), 2.15 (d, 1 H, *J* 10 Hz, OH), 2.06 (s, 3 H, Ac).

8-Methoxycarbonyloctyl 6-O-(6-O-{4-O-[2-O-acetyl-4,6-di-O-benzyl-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl}-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-β-D-mannopyranoside (**19**). — To a mixture of **17** (66.9 mg, 0.035 mmol) in dry dichloromethane (1 mL) were added silver trifluoromethanesulfonate (45 mg, 0.18 mmol), *sym*-collidine (23.2 μL, 0.18 mmol), and 4 A molecular sieves at −50°, followed dropwise by a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (52.4 mg, 0.11 mmol) in dry dichloromethane (2 mL). The mixture was stirred at −50° for 1 h, then allowed to warm to room temperature, and stirring was continued for 15 h. The mixture was diluted with dichloromethane (25 mL), filtered through Celite, washed with ice–water, ice-cold M HCl, and saturated aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and filtered, and the solvent was evaporated. Column chromatography (1:1 hexane–ethyl acetate) of the residue on Iatrobeads gave **19**, isolated as a syrup (70 mg, 85.9%), $[\alpha]_D$ −2.8° (*c* 3.4, chloroform), R_F 0.29 (1:1 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.84 (dd, 1 H, $J_{3''',4'''}$ 9.0, $J_{2''',3'''}$ 11.0 Hz, H-3‴), 5.40 (d, 1 H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 5.14 (dd, 1 H, $J_{4''',5'''}$ 10.0 Hz, H-4‴), 5.12 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 5.03 (dd, 1 H, $J_{2'',3''}$ 9.0 Hz, H-2″), 3.66 (s, 3 H, OMe), 2.29 (t, 2 H, CH_2COO), 2.05, 2.01, 1.85, and 1.69 (each s, 3 H, 3 Ac); ^{13}C, δ 174.2 ($COOCH_3$), 170.5, 170.0, 169.5, and 168.8 (4 $COCH_3$), 101.8 ($J_{C\text{-}1,H\text{-}1}$) 153 Hz, C-1), 100.4 ($J_{C\text{-}1''',H\text{-}1'''}$ 152.5 Hz, C-1‴), 98.7 ($J_{C\text{-}1'',H\text{-}1''}$ 164.4 Hz, C-1″), 98.3 ($J_{C\text{-}1',H\text{-}1'}$ 170.2 Hz, C-1′), 51.4 (OCH_3), 34.1 (CH_2COO), 20.7, 20.6, and 20.4 (4 $COOCH_3$).

Anal. Calc. for $C_{134}H_{144}N_2O_{34}$: C, 69.18; H, 6.23; N, 1.20. Found: C, 69.27; H, 6.27; N, 1.31.

8-Methoxycarbonyloctyl 6-O-(6-O-{2-acetamido-4-O-[3-O-(2-acetamido-3,4,6-

tri-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*acetyl*-4,6-*di*-O-*benzyl*-β-D-*galactopyranosyl*]-3,6-*di*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*}-2,3,4-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-2,3,4-*tri*-O-*benzyl*-β-D-*mannopyranoside* (**20**). — The phthalimido groups in **19** (50 mg, 0.022 mmol) were removed by reaction with hydrazine as described for the preparation of **13**. The re-acetylated product was purified by column chromatography (ethyl acetate–hexane, 2:1) on Iatrobeads to give **20**, isolated as a syrup (31.5 mg, 68.2%), $[\alpha]_D$ −23° (*c* 0.43, chloroform), R_F 0.28 (1:4 hexane–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 5.92 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 3.65 (s, 3 H, OMe), 2.28 (t, 2 H, CH_2COO), 2.05, 2.03, 2.01, 1.97, 1.89, and 1.76 (6 s, each 3 H, 6 Ac); ^{13}C, δ 177.3 ($COOCH_3$), 171.6, 170.7, 170.6, 170.4, 170.1, and 169.6 (6 $COCH_3$), 101.9 (C-1), 100.4 (C-1‴), 100.1 (C-1″), 99.9 (C-1⁗), 98.5 (C-1′), 51.5 (OCH_3), 34.1 (CH_2COO).

Anal. Calc. for $C_{122}H_{144}N_2O_{32}$: C, 68.14; H, 6.75; N, 1.30. Found: C, 68.50; H, 6.28; N, 1.16.

8-Methoxycarbonyloctyl O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→3)-O-β-D-*galactopyranosyl*-(1→4)-O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→6)-O-α-D-*mannopyranosyl*-(1→6)-O-D-*mannopyranoside* (**3**). — Compound **20** (30 mg, 0.014 mmol) was *O*-deacetylated as described for the preparation of **7**, to provide **21** (25 mg, 90%), a solution of which in 98% ethanol (2 mL) was hydrogenated over 5% Pd/C (15 mg) at 1 atm. pressure for 15 h. The mixture was processed as described for the preparation of **1** to give amorphous **3** (9.6 mg, 70%), $[\alpha]_D$ −7.8° (*c* 0.36, water), R_F 0.13 (60:35:6 dichloromethane–methanol–water). N.m.r. data (D_2O): ^1H, δ 4.86 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.65 (d, 1 H, $J_{1''',2'''}$ 8.0 Hz, H-1⁗)), 4.64 (d, 1 H, $J_{1,2}$ < 1 Hz, H-1), 4.55 (d, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.44 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1‴), 3.67 (s, 3 H, OMe), 2.36 (t, 2 H, *J* 7.5 Hz, CH_2COO), 2.02, 2.01 (2 s, each 3 H, 2 NAc); ^{13}C, δ 178.7 ($COOCH_3$), 175.8 and 175.3 ($COCH_3$), 103.7 (C-1), 103.6 (C-1″), 102.2 (C-1), 100.7 (C-1‴), 100.4 (C-1⁗), 52.9 (OCH_3), 34.5 (CH_2COO), 23.1 and 23.0 (2 $NCOCH_3$).

8-Methoxycarbonyloctyl O-β-D-*galactopyranosyl*-(1→4)-O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*-(1→6)-O-α-D-*mannopyranosyl*-(1→6)-O-D-*mannopyranoside* (**2**). — A solution of **1** (10.3 mg, 0.014 mmol), galactosyltransferase (5 units), and UDP-galactose (20 mg, 0.035 mmol) in 100mM sodium cacodylate (200 μL, pH 7.0) containing 10mM $MnCl_2$ was kept at ambient temperature for 15 h. T.l.c. (60:35:6 chloroform–methanol–water) of the mixture showed complete conversion of **1** (R_F 0.40) into a new product (R_F 0.22). The mixture was diluted with water (10 mL) and passed directly onto a Sep-Pak C_{18} reverse-phase cartridge which had been prewashed with 20 mL each of methanol, 1:1 chloroform–methanol, 1:1 methanol–water, and water. The cartridge was washed with water (30 mL) and the product was eluted with methanol (20 mL). The methanol was evaporated and a solution of the residue in water (6 mL) was passed through a Millipore 0.2-μm filter, then lyophilized to yield **2** as a white powder (10.5 mg, 83%), $[\alpha]_D$ −11° (*c* 0.6, water), R_F 0.22 (60:35:6 dichloromethane–methanol–water). N.m.r. data (D_2O): ^1H, δ 4.88 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.66 (d, $J_{1,2}$ < 1 Hz, H-1), 4.58 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.47 (d, 1 H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 2.05 (s, NAc); ^{13}C, δ 178.7 and 175.3 ($COCH_3$), 103.7 (C-1), 102.2 (C-1′), 100.7 (C-1″), 100.4 (C-1‴), 52.8 (OCH_3), 34.5 (CH_2COO), 23.1 ($COCH_3$).

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