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SYNTHESIS OF A HEPTASACCHARIDE HAPTEN RELATED TO AN ANOMALOUS BIANTENNARY GLYCAN-CHAIN OF HUMAN CHORIONIC GONADOTROPIN OF A PATIENT WITH CHORIOCARCINOMA. A STEPWISE APPROACH*†

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ABSTRACT

A stereocontrolled synthesis of a heptasaccharide hapten, 8-methoxycarbonyloctyl 3-*O*-[2,4-di-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranosyl]-6-*O*-α-D-mannopyranosyl-β-D-mannopyranoside (**2**), is described employing the lactosaminyl donor 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide and the mannotriosyl glycosyl acceptor 8-ethoxycarbonyloctyl 2,4-di-*O*-benzyl-3-*O*-(3,6-di-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside, the reaction of which gave the biantennary structure 8-ethoxycarbonyloctyl 2,4-di-*O*-benzyl-3-*O*-{3,6-di-*O*-benzyl-2,4-di-*O*-[3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**9**), as well as a monoglycosylated product, 8-ethoxycarbonyloctyl 2,4-di-*O*-benzyl-3-*O*-{3,6-di-*O*-benzyl-4-*O*-[3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside. The diglycosylated product **9** was transformed into **2**, and the structure was confirmed by ^{1}H-n.m.r. data.

INTRODUCTION

In 1983, an anomalous structure **1** was proposed by Kobata *et al.*[2] for the

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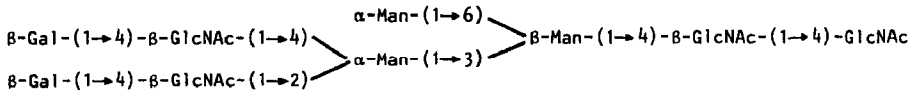
biantennary glycan-chain isolated from an α-subunit of the chorionic gonadotropin (hCG) of a choriocarcinoma patient. This glycan should be characteristic for choriocarcinoma patients since **1** was desialylated, even though the glycans of normal hCG are usually sialylated. The unique biantennary structure **1** could not be detected in the desialylated portion of the glycan chains of normal hCG.

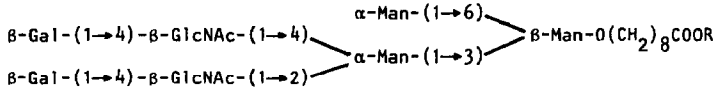
RESULTS AND DISCUSSION

As part of our project on the synthesis of artificial carbohydrate antigens, we now describe an approach to the synthesis of the heptasaccharide hapten **2**, which may be of use for the preparation of specific antibodies toward the glycan chain of hCG of choriocarcinoma patients. Retrosynthesis of the structure **2** gave the readily available glycosyl donor **3** and the mannotriosyl derivative **4**. A route to **4** was developed as follows.

The mannopyranose derivative **6** was readily prepared[3] (44% overall yield) from allyl α-D-mannopyranoside (**5**) with slight modifications. Glycosylation of the mannobiose derivative **7**[1] with **6** in the presence of silver triflate and powdered molecular sieves Type 4A in dichloroethane afforded 78% of the mannotriose derivative **8**, the stereochemistry of which was confirmed by the ^{13}C-n.m.r. data: signals for anomeric carbon atoms at δ ($CDCl_3$) 101.6 ($^1J_{CH}$ 154 Hz, C-1a), 99.7 ($^1J_{CH}$ 172 Hz, C-1c), and 98.6 ($^1J_{CH}$ 167 Hz, C-1b). Zemplén deacetylation of **8** gave a quantitative yield of the diol **4**.



1



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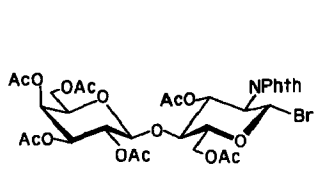

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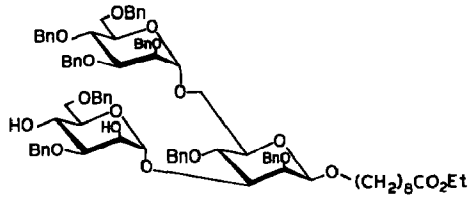

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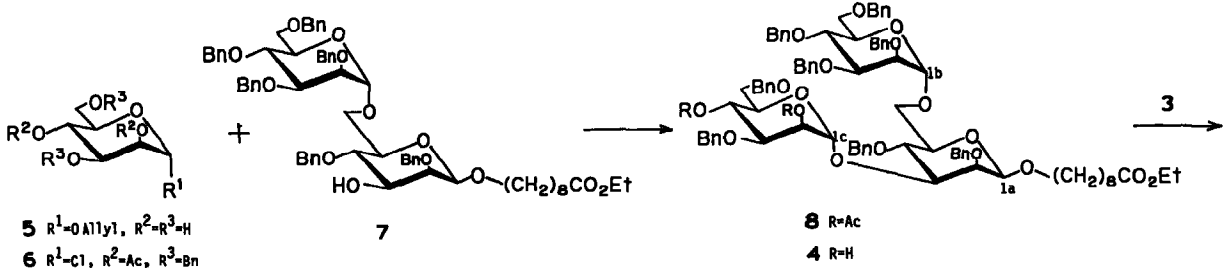
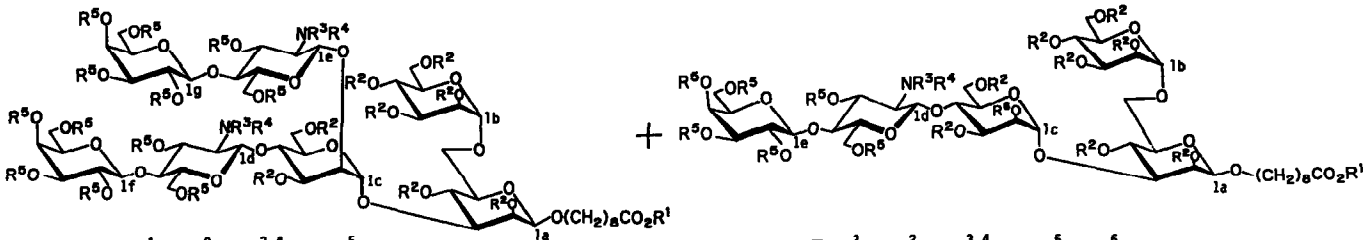


5 R^1=OAllyl, R^2=R^3=H
6 R^1=Cl, R^2=Ac, R^3=Bn

7

8 R=Ac
4 R=H



9 R^1=Et, R^2=Bn, R^3R^4=Phth, R^5=Ac
10 R^1=Et, R^2=Bn, R^3R^4=Phth, R^5=H
11 R^1=R^5=H, R^2=Bn, R^3R^4=Phth
12 R^1=R^3=R^4=R^5=H, R^2=Bn
13 R^1=R^3=R^5=H, R^2=Bn, R^4=Ac
14 R^1=R^2=R^3=R^5=H, R^4=Ac
15 R^1=R^4=H, R^2=R^3=R^5=Ac
16 R^1=Me, R^2=R^3=R^5=Ac, R^4=H
2 R^1=Me, R^2=R^4=R^5=H, R^3=Ac

17 R^1=Et, R^2=Bn, R^3R^4=Phth, R^5=Ac, R^6=H
18 R^1=Et, R^2=Bn, R^3R^4=Phth, R^5=R^6=Ac
19 R^1=R^5=R^6=H, R^2=Bn, R^3R^4=Phth
20 R^1=R^3=R^4=R^5=R^6=H, R^2=Bn
21 R^1-R^3=H, R^2=Bn, R^4-R^5-R^6=Ac
22 R^1=R^3=R^5=R^6=H, R^2=Bn, R^4=Ac
23 R^1=Me, R^2=Bn, R^3=R^5=R^6=H, R^4=Ac
24 R^1=Me, R^2=R^3=R^5=R^6=H, R^4=Ac

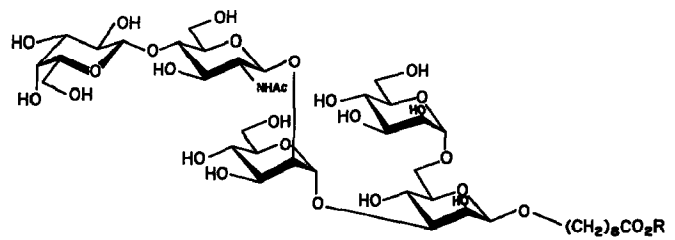
The glycosylation of **4** with the known[4] lactosaminyl donor **3** was performed in the presence of silver triflate and 2,4,6-trimethylpyridine[5] in dichloroethane. Chromatography of the products afforded 33% of a monoglycosylated product **17**, and also a mixture of two other products which were separated on Bio-Beads SX 8 to give 38% of the desired biantennary product **9** and also 1,3,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose contaminated with the bromide **3**.

The structure of **9** was deduced from the reaction sequence and the ^{1}H-n.m.r. data, which contained two signals for H-4f and H-4g of the two galactopyranosyl residues at δ 5.314 and 5.290, respectively, and confirmed by transformation into the heptasaccharide hapten **2**. A (1→4)-linked structure for the monoglycosylated product **17** was expected from previous observations[6] and assigned by the ^{1}H-n.m.r. data of **18**, the hexa-acetate of **17**, which revealed a deshielded signal for H-2c at δ 5.430 (dd, *J* 1.5 and 3.0 Hz). The structure of **17** was confirmed by its conversion into the pentasaccharide hapten **24** as follows.

Successive treatment of **17** with (*a*) lithium hydroxide and aqueous 31% hydrogen peroxide in tetrahydrofuran[7], (*b*) aqueous ethanolic hydrazine, and (*c*) acetic anhydride–pyridine afforded a 40% overall yield of **21**, *via* **19** and **20**. Zemplén deacetylation of **21** gave 89% of **22**, esterification of which with diazomethane in methanol–ether afforded 96% of the methyl ester **23**. Hydrogenolysis (Pd/C, methanol) of **23** and purification of the product on Sephadex G-25 afforded the pentasaccharide hapten **24**.

The ^{1}H-n.m.r. data (Fig. 1) for **24** were different from those of the authentic pentasaccharide hapten[1] **26**, which has a β-(1→2) linkage between the GlcNAc and Man residues, and which was readily obtainable from the reported **25**[8] by treatment with methanolic sodium methoxide. Hence, **24** contained a β-(1→4) linkage between the GlcNAc and Man residues.

The diglycosylated product **9** was transformed into the heptasaccharide hapten **2** by a similar route. Thus, treatment of **9** as in (*a*)–(*c*) above afforded a 71% overall yield of **13** *via* **10**–**12**. Attempted esterification of the acid **13** with diazomethane failed. Catalytic hydrogenolysis (Pd/C, methanol, acetic acid) of **13** and purification of the product on Sephadex G-25 afforded 44% of **14**. The structure of **14** was evident from the ^{1}H-n.m.r. data (Fig. 2) which was in good



25 R = Et

26 R = Me

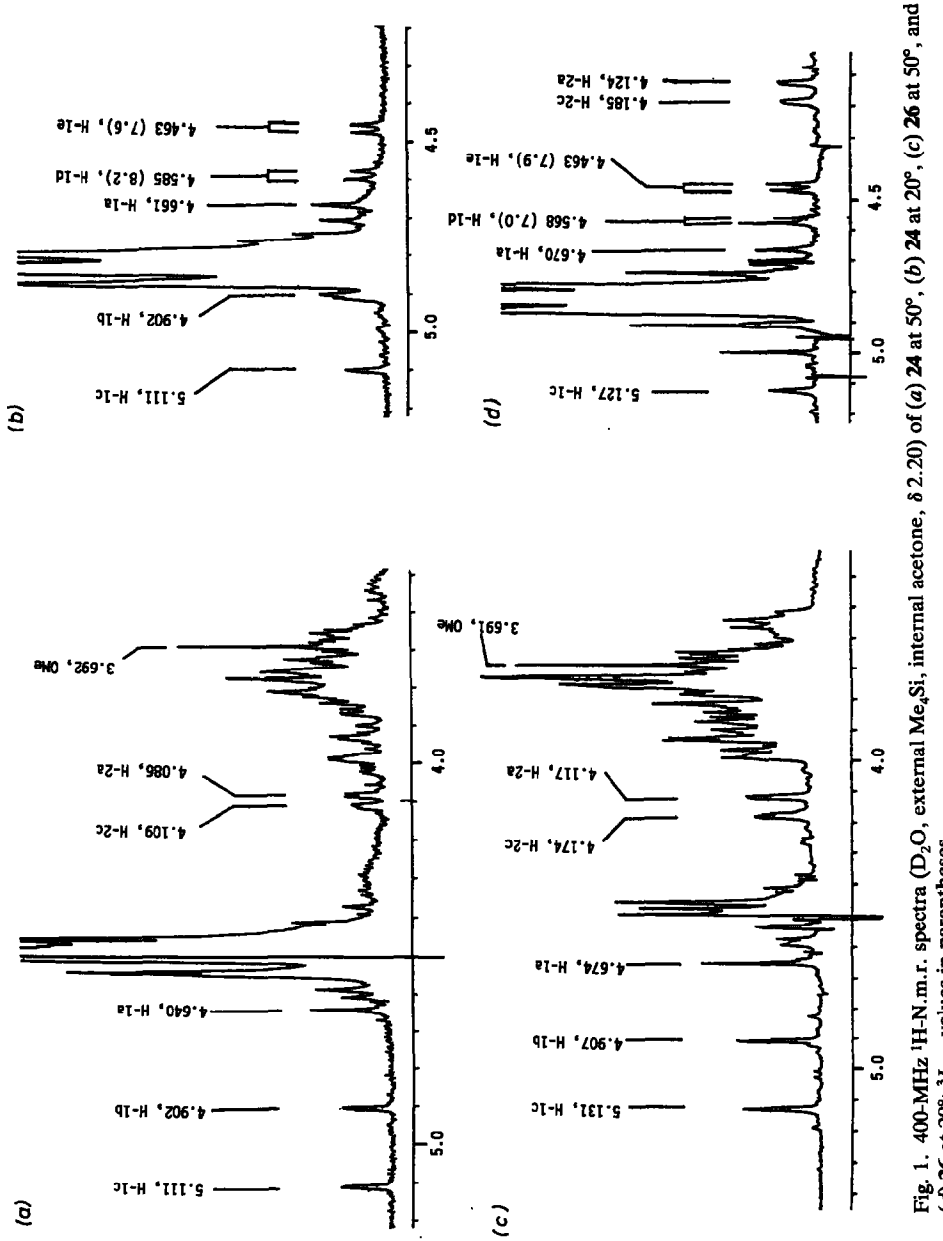


Fig. 1. 400-MHz ^{1}H-N.m.r. spectra (D_2O, external Me_4Si, internal acetone, δ 2.20) of (*a*) **24** at 50°, (*b*) **24** at 20°, (*c*) **26** at 50°, and (*d*) **26** at 20°; $^3J_{H,H}$ values in parentheses.

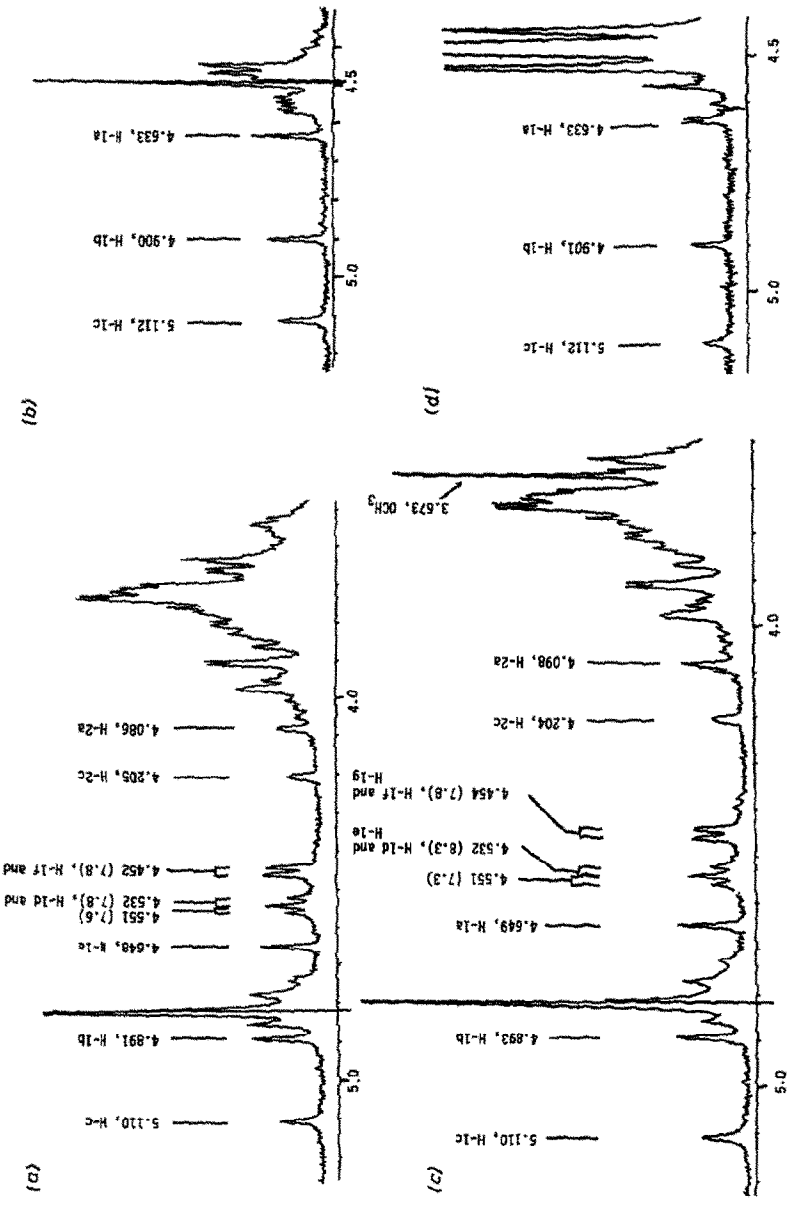


Fig. 2. 400-MHz ^{1}H-N.m.r. spectra (D_2O, external Me_4Si, internal ^{t}BuOH, δ 1.230) of (*a*) **14** at 20°, (*b*) **14** at 50°, (*c*) **2** at 20°, and (*d*) **2** at 50°.

agreement with that reported[9] for related complex-type glycan chains. In order to obtain a heptasaccharide hapten with an ester function at the end of the spacer arm, the acidic hapten **14** was transformed into methyl ester **2** by treatment in sequence with (*a*) acetic anhydride–pyridine, (*b*) diazomethane in ether–tetrahydrofuran, and (*c*) methanolic sodium methoxide. The structure of **2** was confirmed by the ^{1}H-n.m.r. data (Fig. 2).

Thus, the heptasaccharide hapten **2**, carrying an anomalous biantennary glycan-chain of hCG of a patient with choriocarcinoma, was synthesised in a stereo- and regio-controlled manner by employing the key intermediates **3** and **4**.

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on columns of silica gel (Merck, 70–230 mesh). Flash chromatography was performed on columns of Wako gel C-300 (200–300 mesh). T.l.c. and h.p.t.l.c. were performed on Silica Gel 60 F_{254} (Merck). I.r. spectra (KBr pellets or liquid films) were recorded with an EPI-G2 Hitachi spectrophotometer. ^{1}H-N.m.r. spectra ($CDCl_3$, internal Me_4Si) were recorded with a JNM-GX400 or FX90Q spectrometer. ^{13}C-N.m.r. spectra (25.05 MHz) were recorded with a JNM-FX 100FT n.m.r. spectrometer.

*8-Ethoxycarbonyloctyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-*D*-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**8**). — To a mixture of 8-ethoxycarbonyloctyl 2,4-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside[1] (**7**; 1.06 g, 1 mmol), $AgOSO_2CF_3$ (385 mg, 1.5 mmol), and powdered molecular sieves Type 4A (2.0 g) in $Cl(CH_2)_2Cl$ (10 mL) was added dropwise a solution of 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl chloride[3] (**6**; 0.64 g, 1.4 mmol) in $Cl(CH_2)_2Cl$ (6 mL) at 0–5°. After stirring for 16 h at 20°, the mixture was diluted with $Cl(CH_2)_2Cl$ (20 mL), filtered through Celite, washed with aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (3:1 EtOAc–hexane) of the residue gave **8** (818 mg, 77.5%), $[\alpha]_D$ −3° (*c* 0.58), R_F 0.38 (2:1 EtOAc–hexane). N.m.r. data: ^{1}H, δ 5.524 (dd, *J* 1.5 and 4 Hz, H-2c), 5.196 (d, *J* 1.5 Hz, H-1c), 5.146 (d, *J* 1.5 Hz, H-1b), 4.294 (s, H-1a), 2.251 (t, *J* 7.3 Hz, CH_2COOEt), 2.054 (s, OAc), 1.890 (s, OAc), 1.7–1.4 (m, 6 H), and 1.35–1.15 (m, 9 H); ^{13}C, δ 101.6 ($^1J_{CH}$ 154 Hz, C-1a), 99.7 ($^1J_{CH}$ 172 Hz, C-1c), and 98.6 ($^1J_{CH}$ 167 Hz, C-1b).

Anal. Calc. for $C_{89}H_{104}O_{20} \cdot H_2O$: C, 70.71; H, 7.06. Found: C, 70.81; H, 6.95.

*8-Ethoxycarbonyloctyl 2,4-di-*O*-benzyl-3-*O*-(3,6-di-*O*-benzyl-α-*D*-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**4**). — A solution of **8** (720 mg) in 0.1M NaOEt in EtOH (15 mL) was stirred

for 16 h at 20°, then neutralised with Amberlyst A-15 (H^+) resin, filtered, and concentrated *in vacuo*. Column chromatography (1:1 EtOAc–hexane) of the residue gave **4** (672 mg, 99%), $[\alpha]_D$ +0.8° (*c* 1.1), R_F 0.49. N.m.r. data: ^1H, δ 5.226 (d, *J* 1.5 Hz, H-1c), 5.141 (d, *J* 1.5 Hz, H-1b), 4.341 (s, H-1a), 4.116 (q, *J* 7.1 Hz, OCH_2CH_3), 2.253 (t, *J* 7.6 Hz, CH_2CO), 1.65–1.45 (m, 4 H), and 1.35–1.15 (m, 11 H); ^{13}C, δ 101.6 ($^1J_{CH}$ 154 Hz, C-1a, and $^1J_{CH}$ 169 Hz, C-1c), 98.6 ($^1J_{CH}$ 170 Hz, C-1b), and 60.0 (OCH_2CH_3).

Anal. Calc. for $C_{85}H_{100}O_{18}$: C, 72.42; H, 7.15. Found: C, 72.36; H, 7.14.

8-Ethoxycarbonyloctyl 2,4-di-O-benzyl-3-O-{3,6-di-O-benzyl-2,4-di-O-[3,6-di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-6-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**9**) *and 8-ethoxycarbonyloctyl 2,4-di-O-benzyl-3-O-{3,6-di-O-benzyl-4-O-[3,6-di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-6-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside* (**17**). — To a mixture of **4** (235 mg, 0.17 mmol), 2,4,6-trimethylpyridine (97.0 μL, 0.73 mmol), and $AgOSO_2CF_3$ (188 mg, 0.73 mmol) in $Cl(CH_2)_2Cl$ (6 mL) was added dropwise a solution of 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide[4] (**3**; 524 mg, 0.66 mmol) in $Cl(CH_2)_2Cl$ (6.0 mL) at −15°. The mixture was stirred for 1 h at −15°, diluted with $Cl(CH_2)_2Cl$ (20 mL), filtered through Celite, washed with water, aqueous HCl, aqueous $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (3:2 toluene–EtOAc) of the residue gave, first, **17** (115 mg, 32.6%), $[\alpha]_D$ +14° (*c* 1), R_F 0.44 (1:1 toluene–EtOAc). N.m.r. data: ^1H, δ 5.648 (dd, *J* 7.5 and 8.9 Hz, H-3d), 5.647 (d, *J* 8.5 Hz, H-1d), 5.315 (d, *J* 3.0 Hz, H-4e), 5.124 (d, *J* 1.5 Hz, H-1b or H-1c), 5.116 (d, *J* 1.5 Hz, H-1c or H-1b), 5.076 (dd, *J* 8.0 and 10.4 Hz, H-2e), 4.918 (dd, *J* 3.4 and 10.4 Hz, H-3e), 4.468 (d, *J* 7.7 Hz, H-1e), 4.218 (s, H-1a), 2.131 (Ac), 2.027 (Ac), 1.973 (Ac), 1.970 (Ac), 1.959 (Ac), 1.876 (Ac), 1.7–1.4 (m, 4 H), and 1.3–1.1 (m, 11 H).

Anal. Calc. for $C_{117}H_{135}NO_{35}$: C, 66.43; H, 6.43; N, 0.66. Found: C, 66.23; H, 6.39; N, 0.64.

Eluted second was a fraction containing **9** (367 mg), R_F 0.34 (1:1 toluene–EtOAc), and R_F 0.29 and 0.16 (1:2:1 hexane–EtOAc–$CHCl_3$) in the ratio of ~1:1. Elution of this fraction from a column (200 × 2 cm) of Bio-Beads SX 8 with benzene afforded **9** (174 mg, 37%) and 1,3,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose (170 mg, R_F 0.29), which had been present in **3**. Compound **9** had $[\alpha]_D$ +2.5° (*c* 1.1), R_F 0.16 (1:2:1 hexane–EtOAc–$CHCl_3$). N.m.r. data: ^1H, δ 5.577 (d, *J* 8.5 Hz, H-1f), 5.561 (dd, *J* 8.5 and 10.0 Hz, H-3d), 5.400 (dd, *J* 8.5 and 9.0 Hz, H-3e), 5.314 (d, *J* 4.0 Hz, H-4f), 5.290 (d, *J* 4.0 Hz, H-4g), 5.092 (dd, *J* 8.0 and 10.0 Hz, H-2f), 5.057 (d, *J* 1.5 Hz, H-1b and H-1c), 5.035 (dd, *J* 8.0 and 10.4 Hz, H-2g), 2.136 (Ac), 2.119 (Ac), 2.060 (Ac), 2.058 (Ac), 2.021 (Ac), 1.973 (Ac), 1.950 (Ac × 3), 1.844 (Ac), 1.827 (Ac), and 1.685 (Ac).

Anal. Calc. for $C_{149}H_{170}N_2O_{52}$: C, 63.44; H, 6.07; N, 0.99. Found: C, 62.90; H, 6.03; N, 0.99.

8-Ethoxycarbonyloctyl 3-O-{2-O-acetyl-3,6-di-O-benzyl-4-O-[3,6-di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-2,4-di-O-benzyl-6-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**18**). — A solution of **17** (3.0 mg) in 1:1 pyridine–Ac_2O (0.5 mL) was stirred for 18 h at 25° and then concentrated *in vacuo*. Column chromatography (1:1 toluene–EtOAc) of the residue gave **18**, R_F 0.64 (1:1 toluene–EtOAc). N.m.r. data: ^1H, δ 5.430 (dd, *J* 1.5 and 3 Hz, H-2c), 2.121 (Ac), 2.027 (Ac), 2.000 (Ac), 1.951 (Ac), 1.944 (Ac), 1.928 (Ac), and 1.859 (Ac).

8-Methoxycarbonyloctyl 3-O-[4-O-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranosyl]-6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**24**). — To a solution of **17** (31.0 mg, 0.015 mmol) in tetrahydrofuran (1 mL) was added aqueous 31% H_2O_2 (0.24 mL) at 0°, and the mixture was stirred for 5 min at 0°. 1.25M LiOH (89 μL, 0.11 mmol) was then added and stirring was continued for 1 h at 0–20°. The mixture was neutralised with Amberlyst A-15 (H^+) resin, diluted with EtOAc (10 mL), filtered through Celite, washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to give crude **19** (14.5 mg, 56%), R_F 0.36 (6:1 $CHCl_3$–MeOH).

A solution of **19** (14.0 mg, 0.008 mmol) in EtOH (4 mL) containing $NH_2NH_2 \cdot H_2O$ (20 μL) was boiled under reflux for 18 h and then concentrated *in vacuo*. The residual oil **20**, R_F 0.23 (6:1 $CHCl_3$–MeOH), was stirred with 2:1 pyridine–Ac_2O (3 mL) for 20 h at 20°. The solution was then concentrated *in vacuo* and column chromatography (1:1 toluene–EtOAc) of the residue gave **21** (11.0 mg, 70%), R_F 0.26 (1:1 toluene–EtOAc). ^1H-N.m.r. data: δ 1.90 (Ac), 1.96 (Ac), 1.97 (2 Ac), 2.02 (Ac), 2.04 (Ac), 2.14 (Ac), and 1.69 (AcN).

A solution of **21** (17 mg) in 0.1M NaOMe–MeOH (4 mL) was stirred for 18 h at 20°, then neutralised with Amberlyst A-15 (H^+) resin, and concentrated *in vacuo* to give **22** (13 mg, 90%), R_F 0.21 (6:1 $CHCl_3$–MeOH). ^1H-N.m.r. data (CD_3OD): δ 1.82 (AcN).

A solution of **22** (12.5 mg) in MeOH (4 mL) was treated with ethereal diazomethane for 2.5 h at 20°. Excess of diazomethane was decomposed with AcOH in MeOH, and the mixture was concentrated. Column chromatography (6:1 $CHCl_3$–MeOH) of the residue gave **23** (12 mg, 95%), R_F 0.21 (6:1 $CHCl_3$–MeOH). ^1H-N.m.r. data (CD_3OD): δ 3.63 (OMe) and 1.82 (AcN).

A mixture of **23** (12 mg) and 10% Pd/C (10 mg) in MeOH) (4 mL) was stirred under H_2 for 4 h at 20°, then filtered through Celite, and concentrated *in vacuo*. The foamy residue was eluted from Sephadex G-25 with H_2O to give **24** (2 mg, 28%), R_F 0.44 (2:1:1 *n*-BuOH–EtOH–H_2O). N.m.r. data: ^1H, δ (D_2O, 50°) 5.111 (bs, H-1c), 4.902 (d, *J* 1.5 Hz, H-1b), 4.640 (s, H-1a), 3.692 (s, OMe), and 2.064 (AcN); δ (D_2O, 20°) 5.111 (bs, H-1c), 4.902 (bs, H-1b), 4.661 (s, H-1a), 4.585 (d, *J* 8.2 Hz, H-1d), 4.463 (d, *J* 7.6 Hz, H-1e), 3.683 (s, OMe), and 2.050 (AcN).

8-Ethoxycarbonyloctyl 3-O-[2,4-di-O-(2-acetamido-2-deoxy-4-O-β-D-galacto-pyranosyl-β-D-glucopyranosyl)-α-D-mannopyranosyl]-6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**14**). — A solution of **9** (130 mg, 0.05 mmol) in tetrahydrofuran (5 mL) and aqueous 31% H_2O_2 (1.2 mL) was stirred for 5 min at 0°. To this solution was added dropwise 1.25M LiOH (0.525 mL, 0.66 mmol), and the mixture was stirred for 3 h at 0–25°, then neutralised with Amberlyst A-15 (H^+) resin, filtered, treated with excess of Me_2S, and concentrated *in vacuo*. Elution of the residue from Sephadex LH-20 with MeOH gave a mixture (87 mg, 82%) of **10** and its monoacetate, R_F 0.57 and 0.65 (2:1:1 *n*-BuOH–EtOH–H_2O). N.m.r. data (CD_3OD): ^{13}C, δ 14.9 (OCH_2CH_3); ^1H, δ 1.95 (AcO).

A solution of crude **10** (60 mg, 0.026 mmol) in MeOH (6 mL)–0.1M NaOH (0.4 mL) was stirred for 16 h at 20°, then diluted with 1:1 tetrahydrofuran–H_2O (6 mL), neutralised with Amberlyst A-15 (H^+) resin, filtered, and concentrated to give crude **11** (55 mg), R_F 0.55 (2:1:1 *n*-BuOH–EtOH–H_2O). A mixture of **11** (55 mg) in $NH_2NH_2 \cdot H_2O$ (0.5 mL) and EtOH (12 mL) was boiled under reflux for 48 h. More $NH_2NH_2 \cdot H_2O$ (0.5 mL) was then added and boiling was continued for 60 h. The mixture was then concentrated *in vacuo*, and the residue was eluted from Sephadex LH-20 with MeOH to give **12** (44 mg, 90%), R_F 0.43 (2:1:1 *n*-BuOH–EtOH–H_2O). A solution of **12** in MeOH (5 mL) and Ac_2O (1 mL) was stirred for 16 h at 20° and then concentrated *in vacuo*, and the residue was eluted from Sephadex LH-20 with MeOH to give **13** (44 mg, 96%), R_F 0.58 (2:1:1 *n*-BuOH–EtOH–H_2O) and 0.22 (40:20:3 $CHCl_3$–MeOH–H_2O). A mixture of **13** (43 mg) and 10% Pd/C (50 mg) in MeOH (4 mL) and AcOH (0.4 mL) was stirred under H_2 for 3 h at 50°, then filtered through Celite, and concentrated, and the residue was eluted from Sephadex G-25 with H_2O to give **14** as an amorphous powder (13 mg, 43%), $[\alpha]_D$ +4° (*c* 0.5, water), R_F 0.17 (2:1:1 *n*-BuOH–EtOH–H_2O). N.m.r. data: ^1H, δ (D_2O, 20°) 5.110 (s, H-1c), 4.891 (H-1b), 4.648 (s, H-1a), 4.551 (d, *J* 7.6 Hz, H-1d or H-1e), 4.532 (d, *J* 7.8 Hz, H-1e or H-1d), 4.452 (d, *J* 7.8 Hz, H-1f and H-1g), 4.205 (bs, H-2c), 4.086 (bs, H-2a), 2.060 (AcN), and 2.034 (AcN).

Anal. Calc. for $C_{55}H_{94}N_2O_{38} \cdot H_2O$: C, 46.87; H, 6.86. Found: C, 46.56; H, 6.78.

8-Methoxycarbonyloctyl 3-O-[2,4-di-O-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranosyl]-6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**2**). — A solution of **14** (6.5 mg, 4.6 μmol) in pyridine (2 mL)–Ac_2O (1 mL) was stirred for 16 h at 25° and then concentrated *in vacuo*, and the residue was eluted from Sephadex LH-20 with MeOH to give **15** (10.2 mg, 98%), R_F 0.23 (24:1 $CHCl_3$–MeOH). To a solution of **15** (10.2 mg) in tetrahydrofuran (2 mL) was added excess of diazomethane in ether at 0°, and the mixture was stored for 16 h at 0°. T.l.c. then revealed a product with an R_F value identical to that of **15**. The usual work-up afforded **16**, a solution of which in 0.1M NaOMe–MeOH (3 mL) was stirred for 3 h at 20°, then neutralised with Amberlyst A-15 (H^+) resin, filtered, and concentrated *in vacuo* to give a mixture (5.3 mg) of **2** and **14**, R_F 0.23 and 0.17 (2:1:1 *n*-BuOH–EtOH–H_2O). Preparative t.l.c. (2:1:1 *n*-

BuOH–EtOH–H_2O) afforded **2** (1.8 mg, 27%) and **14** (2.2 mg). N.m.r. data (D_2O, 20°) for **2**: ^1H, δ 5.110 (s, H-1c), 4.893 (s, H-1b), 4.649 (s, H-1a), 4.551 (d, J 7.3 Hz, H-1d or H-1e), 4.532 (d, J 8.3 Hz, H-1e or H-1d), 4.454 (d, J 7.8 Hz, H-1f and H-1g), 4.204 (bs, H-2c), 4.098 (bs, H-2a), 3.673 (OMe), 2.376 (t, J 7.3 Hz, CH_2CO), 2.059 (AcN), 2.033 (AcN), 1.584 (m, 4 H), and 1.4–1.2 (m, 11 H).

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