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SYNTHESIS OF A PENTASACCHARIDE HAPTEN RELATED TO A MONOANTENNARY GLYCAN CHAIN OF HUMAN CHORIONIC GONADOTROPIN*

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ABSTRACT

A stereocontrolled synthesis of a pentasaccharide hapten, namely, 8-ethoxycarbonyloctyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranoside, is described employing a trihexosyl glycosyl-donor, *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate, and a mannobiosyl glycosyl-acceptor, 8-ethoxycarbonyloctyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,4-di-*O*-benzyl-β-D-mannopyranoside, as the key intermediates.

INTRODUCTION

The unique, monoantennary glycan chain **1** was found in the desialylated portion of the glycan chains isolated from an α-subunit of normal, human chorionic gonadotropin[2] (hCG). In 1983, hCG purified from the urine of a patient with choriocarcinoma was reported[3] to contain the anomalous, biantennary glycan chain **2**. The modification of **1** into **2** may be explained by the presence[4] of *N*-acetylglucosaminyltransferase IV in the choriocarcinoma tissue. In order to make an approach to the biological implication of such modification of the glycan chain of a glycoprotein, we initiated a project on the synthetic study of artificial, carbohydrate antigens. We describe here an unambiguous approach to the synthesis of pentasaccharide hapten **3**, closely related to the glycan chain **1**.

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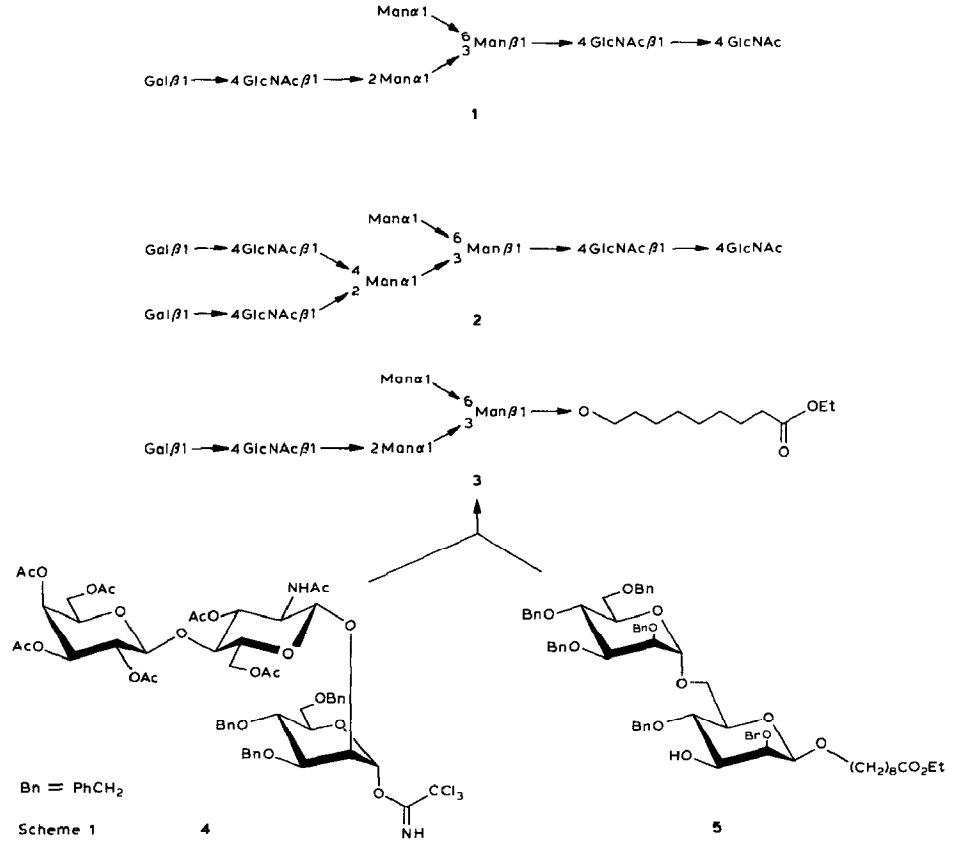
RESULTS AND DISCUSSION

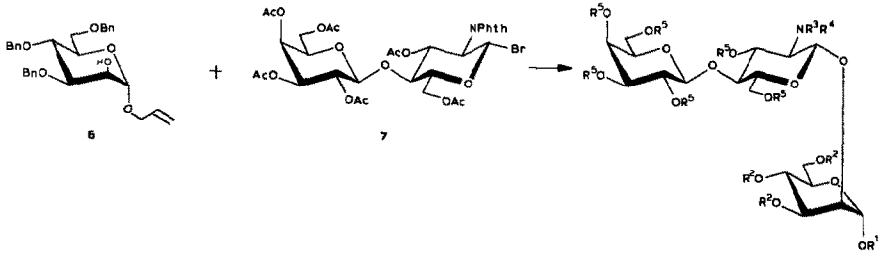
The target structure **3** was designed in order to contain the so-called signal-region of the glycan chain **1** and to have the β-D-mannopyranosyl residue directly linked to the spacer-arm, C_9 hydroxy ester[5].

In order to develop a convergent type of approach, structure **3** was retro-synthesized into the glycotriosyl donor **4** and the mannobiosyl, C-9 ester **5** (see Scheme 1). The synthetic routes toward these two key intermediates, **4** and **5**, were developed as follows.



Allyl tri-*O*-benzyl-α-D-mannopyranoside **6** (ref. 6) was glycosylated with the *N,N*-phthaloyl-lactosaminyl donor **7** (ref. 7) in the presence of silver triflate and powdered molecular sieves 4A in 1,2-dichloroethane, to give an 88% yield of the desired trisaccharide **8**. *O*-Deacetylation of **8** with sodium methoxide in methanol, and subsequent *N,N*-dephthaloylation with butylamine in methanol[8], followed by treatment with acetic anhydride and pyridine, afforded a 75% yield of the *N*-acetyl-hexa-*O*-acetyl derivative **9**. The structure of **9** was confirmed by transformation

into deblocked trisaccharide **12**. The ^{1}H-n.m.r. spectrum of **12** contained three doublets, for H-1a, H-1b, and H-1c, at δ 4.866, 4.574, and 4.461, with $^3J_{1,2}$ 1.6, 7.8, and 7.8 Hz, respectively. *O*-Deallylation of **9** with palladium chloride[9] at 70° afforded a 63% yield of the hemiacetal **10**, as well as a 30% yield of by-product **11**. The structure of **11** was deduced from the n.m.r. data: the ^{1}H-n.m.r. spectrum contained 8 singlets for 8 $COCH_3$ groups, and the ^{13}C-n.m.r. spectrum showed both a signal for the carbonyl carbon atom of CH_2COCH_3 at 205.1 p.p.m., and a signal for the methyl group of CH_2COCH_3 at 26.0 p.p.m. Treatment of **10** with sodium hydride and trichloroacetonitrile in dichloromethane according to Schmidt and Michel[10] afforded a 95% yield of trichloroacetimidate **4** (see Scheme 2).



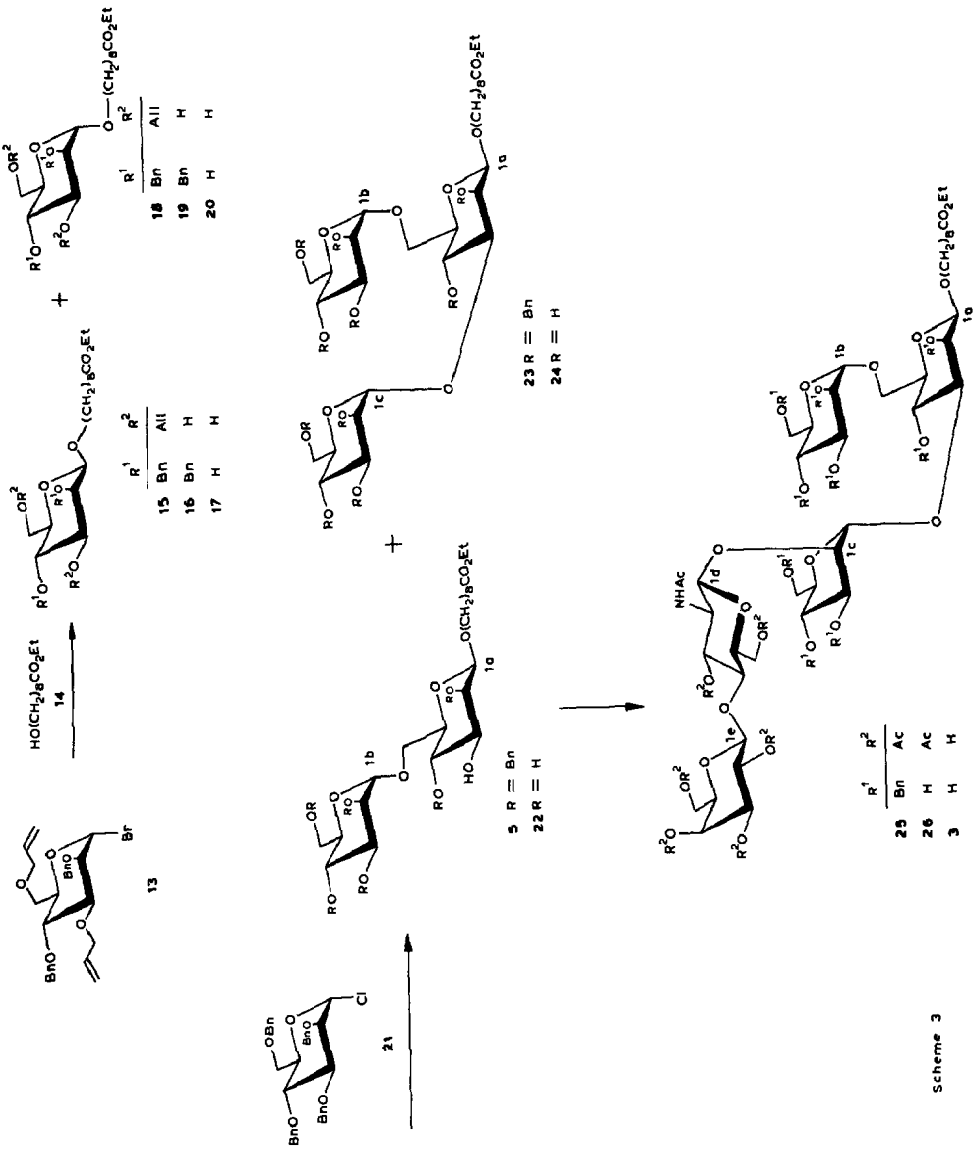
Scheme 2

	R^1	R^2	R^3	R^4	R^5
4	C(=NH)CCl$_3$	Bn	Ac	H	Ac
8	All	Bn	Phth		Ac
9	All	Bn	Ac	H	Ac
10	H	Bn	Ac	H	Ac
11	CH_2COCH_3	Bn	Ac	H	Ac
12	Pr	H	Ac	H	H

All = allyl Phth = phthaloyl

Mannobiosyl C-9 ester **5** was prepared starting from the hydroxy ester **14** (ref. 11). Glycosylation of **14** with the readily available D-mannosyl donor **13** (ref. 12) in the presence of silver silicate[13] and powdered molecular sieves 4A afforded a 68% yield of the β-D-mannopyranosyl C-9 ester **15**, δ_C 101.6 (C-1a, $^1J_{CH}$ 154 Hz), together with a 17% yield of the α-D-mannopyranosyl isomer **18**, δ 97.9 (C-1a, $^1J_{CH}$ 168 Hz). *O*-Deallylation of **15** with palladium chloride[9] afforded a 74% yield of the diol **16**. Glycosylation of **16** with tetra-*O*-benzyl-α-D-mannopyranosyl chloride (**21**) in the presence of tetrabutylammonium chloride in 1,2-dichloroethane afforded a 78% yield of the desired product **5**, as well as an 11% yield of the di-*O*-mannosylated product **23**. The structures of **5** and **23** were established by their ^{1}H- and ^{13}C-n.m.r. data, and confirmed by their transformation into free mannobioside **22** and mannotrioside **24** (see Scheme 3).

Having prepared the two key intermediates **4** and **5**, the crucial glycosylation of **5** with **4** was performed in the presence of boron trifluoride etherate[14] and powdered molecular sieves AW-300, to give a 45% yield of the protected pentasaccharide derivative **25**. Hydrogenolysis of **25** in the presence of 10% palladium-on-carbon in acetic acid gave **26**, which was *O*-deacetylated with sodium ethoxide



Scheme 3

in ethanol, to afford an 85% yield of the target, the spacer-arm glycopentaoside **3**, $[\alpha]_D$ +4.9° (*c* 0.425, H_2O), R_F 0.44 in 2:1:1 1-BuOH–EtOH–H_2O. The structure of **3** was evident from the synthetic sequence, and the newly introduced, anomeric configuration in compound **25** was assigned as α-D through the observation of both a characteristic signal[15] for the anomeric proton (H-1c) at δ 5.146 as a singlet, and a signal for C-1c at δ 100.2 with a $^1J_{CH}$ value of 171 Hz in the spectrum of deblocked pentasaccharide **3**.

In conclusion, pentasaccharide hapten **3**, carrying a unique, monoantennary glycan chain of the hCG α-subunit was synthesized in a stereocontrolled manner.

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 conducted on columns of Wakogel C-300 (200–300 mesh). T.l.c. and high-performance t.l.c. were performed on Silica Gel 60 F_{254} (Merck, Darmstadt). Molecular sieves were purchased from Nakarai Chemicals, Ltd. I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, using KBr pellets for the crystalline samples and films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with either a JNM-GX400 or a JNM-FX90Q n.m.r. spectrometer. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downwards from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise.

Allyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**8**). — To a stirred mixture of **6** (ref. 6; 1.0 g, 2.0 mmol), $AgOSO_2CF_3$ (2.3 g, 8.9 mmol), and powdered molecular sieves 4A (12 g) in $Cl(CH_2)_2Cl$ (30 mL) was added dropwise to a solution of **7** (3.2 g, 4.1 mmol) in $Cl(CH_2)_2Cl$ (40 mL) at 0°. The mixture was stirred for 22 h at 10–20°, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*; chromatography on SiO_2 in 3:1 toluene–EtOAc afforded **8** (2.1 g, 88%); $[\alpha]_D$ −2.7° (*c* 1.3); R_F 0.38 in 2:1 toluene–EtOAc; n.m.r. data: δ_H 5.507 (d, 1 H, *J* 8.6 Hz, H-1b), 4.643 (d, 1 H, *J* 1.8 Hz, H-1a), and 4.550 (d, 1 H, *J* 8.0 Hz, H-1c); δ_C 101.1 ($^1J_{CH}$ 159 Hz, C-1c), 96.7 ($^1J_{CH}$ 164 Hz, C-1b), and 96.2 ($^1J_{CH}$ 167 Hz, C-1a).

Anal. Calc. for $C_{62}H_{69}NO_{23} \cdot 0.5\ C_7H_8$: C, 63.33; H, 5.92; N, 1.13. Found: C, 63.32; H, 5.95; N, 1.16.

Allyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*manno-pyranoside* (**9**). — A solution of **8** (3.4 g, 2.8 mmol) in 0.03M NaOMe–MeOH (77

mL) was stirred for 17 h at 20°, the base neutralized with Amberlyst 15, and the suspension filtered. The filtrate was evaporated to an oil which was refluxed in 1:5 1-$BuNH_2$–MeOH (180 mL) for 60 h. After evaporation of the solution, the residue was stirred with Ac_2O (75 mL) and pyridine (75 mL) for 18 h at 20°. Evaporation *in vacuo*, and chromatography on SiO_2 in 1:1 toluene–EtOAc afforded **9** (2.54 g, 81%); $[\alpha]_D$ +2.8° (*c* 0.5); R_F 0.50 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 2.156 (s, 3 H, Ac), 2.072 (s, 3 H, Ac), 2.050 (s, 6 H, Ac), 2.016 (s, 3 H, Ac), 1.971 (s, 3 H, Ac), and 1.799 (s, 3 H, Ac); δ_C 100.6 ($^1J_{CH}$ 162 Hz, C-1c), 98.5 ($^1J_{CH}$ 162 Hz, C-1b), and 96.4 ($^1J_{CH}$ 168 Hz, C-1a).

Anal. Calc. for $C_{56}H_{69}NO_{22}$: C, 60.69; H, 6.28; N, 1.26. Found: C, 60.56; H, 6.30; N, 1.38.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-D-mannopyranose (**10**) *and 2-oxopropyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**11**). — A mixture of **9** (300 mg, 0.3 mmol), $PdCl_2$ (53 mg, 0.3 mmol), and NaOAc (49 mg, 0.6 mmol) in 19:1 AcOH–H_2O (7.0 mL) was stirred for 1 h at 70°, filtered through Celite, and the filtrate evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 3:1 $CHCl_3$–Me_2CO, to afford **11** (90 mg, 30%); $[\alpha]_D$ +9.3° (*c* 0.9); R_F 0.51 in 3:2 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 2.155, 2.085, 2.073, 2.069, 2.054, 2.027, 1.970, and 1.840 (8 s, 24 H, Ac); δ_C 205.1 (CH_2COCH_3), 100.7 ($^1J_{CH}$ 164 Hz, C-1c), 99.0 ($^1J_{CH}$ 160 Hz, C-1b), 97.8 ($^1J_{CH}$ 171 Hz, C-1a), 26.0 (CH_2COCH_3), 22.9 ($HNCOCH_3$), and 20.6–20.4 ($OCOCH_3$ × 6).

Anal. Calc. for $C_{56}H_{69}NO_{23}$: C, 59.83; H, 6.18; N, 1.24. Found: C, 59.70; H, 6.19; N, 1.28.

Further elution with the same solvent afforded **10** (182 mg, 63%); $[\alpha]_D$ −10.6° (*c* 1.4); R_F 0.31 in 3:2 $CHCl_3$–Me_2CO; n.m.r. data: δ_C 100.8 ($^1J_{CH}$ 162 Hz, C-1c), 99.0 ($^1J_{CH}$ 164 Hz, C-1b), 92.1 ($^1J_{CH}$ 170 Hz, C-1a), 22.9 ($NHCOCH_3$), and 20.7–20.4 ($OCOCH_3$ × 6).

Anal. Calc. for $C_{53}H_{65}NO_{22}$: C, 59.59; H, 6.13; N, 1.31. Found: C, 59.60; H, 6.19; N, 1.22.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloroacetimidate (**4**). — To a solution of **10** (500 mg, 0.47 mmol) and Cl_3CCN (1.8 mL) in CH_2Cl_2 (18.0 mL) was added NaH (50%; 22.4 mg, 0.47 mmol) at 0°, and the mixture was stirred for 3 h at 0–20°. Filtration of the mixture through Celite, and evaporation of the filtrate *in vacuo*, followed by chromatography on SiO_2 in 1:3 toluene–EtOAc, yielded **4** (540 mg, 95%); $[\alpha]_D$ +9.1° (*c* 1.1); R_F 0.54 in 1:3 toluene–EtOAc; n.m.r. data: δ_H 8.555 (s, 1 H, C=N*H*), 6.214 (d, 1 H, *J* 1.7 Hz, H-1a), and 5.360 (d, 1 H, *J* 2.4 Hz, H-4c); δ_C 159.8 (O*C*=N), 100.5 ($^1J_{CH}$ 160 Hz, C-1c), 98.7 ($^1J_{CH}$ 162 Hz, C-1b), 95.3 ($^1J_{CH}$ 177 Hz, C-1a), 90.5 (CCl_3), and 22.7 ($NHCOCH_3$).

Anal. Calc. for $C_{55}H_{65}Cl_3O_{22}N_2 \cdot H_2O$: C, 53.60; H, 5.57; Cl, 8.64; N, 2.27. Found: C, 53.60; H, 5.26; Cl, 8.97; N, 2.47.

Propyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*gluco-pyranosyl)-(1→2)-α*-D-*mannopyranoside* (**12**). — A solution of **9** (65 mg, 0.06 mmol) in 0.02M NaOMe–MeOH (2 mL) was stirred for 18 h at 20°. Neutralization of the base with Amberlyst 15, and filtration of the mixture, followed by evaporation of the filtrate *in vacuo*, gave a residue (43 mg; R_F 0.41 in 3:1 $CHCl_3$–MeOH). A mixture of the residue and 10% Pd–C (50 mg) in MeOH (3 mL) was stirred under H_2 for 20 h at 20°. The usual processing afforded an oil which was purified by passage through a column (2.5 × 40 cm) of Sephadex LH-20 in MeOH, to afford **12** (29 mg, 86%); $[\alpha]_D$ −4.5° (*c* 1.0, MeOH); R_F 0.46 in 2:1:1 1-BuOH–MeOH–H_2O; n.m.r. data*: δ_H (D_2O, 20°): 4.866 (d, 1 H, *J* 1.6 Hz, H-1a), 4.574 (d, 1 H, *J* 7.8 Hz, H-1b), 4.461 (d, 1 H, *J* 7.8 Hz, H-1c), 4.054 (dd, 1 H, *J* 1.6 and 3.3 Hz, H-2a), and 2.045 (s, 3 H, NCOCH_3); δ_C (D_2O, 20°): 103.8 ($^1J_{CH}$ 161 Hz, C-1c), 100.3 ($^1J_{CH}$ 161 Hz, C-1b), and 97.6 ($^1J_{CH}$ 169 Hz, C-1a).

Anal. Calc. for $C_{23}H_{41}NO_{16} \cdot 0.5\ H_2O$: C, 46.15; H, 7.07; N, 2.34. Found: C, 46.26; H, 7.06; N, 2.34.

*8-Ethoxycarbonyloctyl 3,6-di-*O-*allyl-2,4-di-*O-*benzyl-β*-D-*mannopyranoside* (**15**) *and its α anomer* (**18**). — To a stirred mixture of **14** (1.01 g, 5.0 mmol), powdered molecular sieves 4A (5.0 g), and silver silicate (5.0 g) in $Cl(CH_2)_2Cl$ (20 mL) was added dropwise a solution of **13** (2.5 g, 4.4 mmol) in $Cl(CH_2)_2Cl$ (10 mL) at 0°. The mixture was stirred for 2.5 h at 0–20°, when t.l.c. examination in 10:1 toluene–EtOAc showed the disappearance of **13** (R_F 0.64) and the formation of a major product **15** (R_F 0.41) and a minor product **18** (R_F 0.52). The mixture was diluted with $Cl(CH_2)_2Cl$ (250 mL), filtered through Celite, and the filtrate successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 10:1 toluene–EtOAc, to give **18** (0.53 g, 17%); $[\alpha]_D$ +16.3° (*c* 1.0); R_F 0.52 in 10:1 toluene–EtOAc; n.m.r. data: δ_H 6.0–5.8 (m, 2 H, -C*H*=CH_2 × 2), 5.35–5.10 (m, 4 H, CH=CH_2 × 2), and 4.840 (d, 1 H, *J* 1.5 Hz, H-1a); δ_C 97.9 ($^1J_{CH}$ 167 Hz, C-1a).

Anal. Calc. for $C_{37}H_{52}O_8$: C, 71.12; H, 8.39. Found: C, 70.89; H, 8.31.

Further elution with the same eluant gave **15** (2.12 g, 68%); $[\alpha]_D$ −44.3° (*c* 1.0); R_F 0.41 in 10:1 toluene–EtOAc; n.m.r. data: δ_H 6.0–5.8 (m, 2 H, -C*H*=CH_2 × 2), 5.3–5.1 (m, 4 H, CH=CH_2 × 2), and 4.353 (s, 1 H, H-1a); δ_C 101.6 ($^1J_{CH}$ 154 Hz, C-1a).

Anal. Calc. for $C_{37}H_{52}O_8$: C, 71.12; H, 8.39. Found: C, 70.87; H, 8.34.

*8-Ethoxycarbonyloctyl 2,4-di-*O-*benzyl-β*-D-*mannopyranoside* (**16**). — A mixture of **15** (5.25 g, 8.4 mmol), $PdCl_2$ (3.28 g, 18.4 mmol), and NaOAc (3.32 g, 40.4 mmol) in 19:1 AcOH–H_2O (30 mL) was stirred for 1 h at 80°, cooled, filtered through Celite, the filtrate evaporated *in vacuo*, and the residue diluted with

*Values of δ_H (D_2O) and δ_C (D_2O) are expressed in p.p.m. downward from Me_4Si, by reference to internal standards of Me_2CO (2.225) and 1,4-dioxane (67.4), respectively.

EtOAc. The organic layer was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 2:1 hexane–EtOAc afforded **16** (3.32 g, 72.6%); $[\alpha]_D$ +4.6° (*c* 1.0); R_F 0.52 in 1:1 hexane–EtOAc; n.m.r. data: δ_H 4.504 (d, 1 H, *J* 1.0 Hz, H-1a); δ_C 101.6 ($^1J_{CH}$ 156 Hz, C-1a) and 62.3 (C-6a).

Anal. Calc. for $C_{31}H_{44}O_8$: C, 68.36; H, 8.14. Found: C, 68.00; H, 8.11.

*8-Ethoxycarbonyloctyl 2,4-di-*O*-benzyl-α-*D*-mannopyranoside* (**19**). — Compound **18** (550 mg, 0.88 mmol) was transformed into **19** (287 mg, 60%) as for **15**; $[\alpha]_D$ +13.1° (*c* 2.1); R_F 0.48 in 3:2 hexane–EtOAc; n.m.r. data: δ_C 97.1 ($^1J_{CH}$ 165 Hz, C-1a).

Anal. Calc. for $C_{31}H_{44}O_8$: C, 68.36; H, 8.14. Found: C, 67.96; H, 7.89.

*8-Ethoxycarbonyloctyl β-*D*-mannopyranoside* (**17**). — A solution of **16** (100 mg, 0.18 mmol) in MeOH (4 mL) was stirred under H_2 in the presence of added 10% Pd–C (70 mg) for 16 h at 20°. The usual processing, and purification by means of Sephadex G-25 in H_2O, afforded **17** (47 mg, 70%); $[\alpha]_D$ −30.0° (*c* 1.5, MeOH); R_F 0.15 in 2:1:1 1-BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°): 4.631 (s, 1 H, H-1a); δ_C (D_2O): 100.8 ($^1J_{CH}$ 159 Hz, C-1a).

Anal. Calc. for $C_{17}H_{32}O_8 \cdot 2/3\ H_2O$: C, 54.24; H, 8.92. Found: C, 53.92; H, 8.46.

*8-Ethoxycarbonyloctyl α-*D*-mannopyranoside* (**20**). — Compound **19** (225 mg) was transformed into **20** (80 mg, 54%) as already described; $[\alpha]_D$ +56.6° (*c* 1.5, MeOH); R_F 0.19 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H (D_2O, 50°) 4.818 (d, 1 H, *J* 1.0 Hz, H-1a); δ_C (CD_3OD) 101.7 ($^1J_{CH}$ 168 Hz, C-1a).

Anal. Calc. for $C_{17}H_{32}O_8 \cdot 2/3\ H_2O$: C, 54.24; H, 8.92. Found: C, 53.87; H, 8.40.

8-Ethoxycarbonyloctyl O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-mannopyranosyl)-(1→6)-2,4-di-*O*-benzyl-β-*D*-mannopyranoside* (**5**) *and 8-ethoxylcarbonyloctyl* O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-*D*-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-β-*D*-mannopyranoside* (**23**). — To a mixture of **16** (4.1 g, 7.5 mmol), powdered molecular sieves 4A (18.0 g), and Bu_4NCl (4.6 g, 20 mmol) in $Cl(CH_2)_2Cl$ (90 mL) was injected dropwise a solution of **21** (6.0 g, 10.7 mmol) in $Cl(CH_2)_2Cl$ (30 mL) at 20° under Ar. After stirring the mixture for 3 h at 90–95°, further **21** (6.0 g) in $Cl(CH_2)_2Cl$ (30 mL) was added. The mixture was stirred for 48 h at 90°, cooled, filtered through Celite, and the filtrate successively washed with aq. $NaHCO_3$ and H_2O dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 2:1 hexane–EtOAc gave **23** (777 mg, 10.2%); $[\alpha]_D$ +9.8° (*c* 1.6); R_F 0.45 in 2:1 hexane–EtOAc; n.m.r. data: δ_H 5.267 (d, 1 H, *J* 1.5 Hz, H-1c) and 5.102 (d, 1 H, *J* 1.5 Hz, H-1b); δ_C 101.7 ($^1J_{CH}$ 155 Hz, C-1a), 100.1 ($^1J_{CH}$ 176 Hz, C-1c), 98.6 ($^1J_{CH}$ 173 Hz, C-1b), and 66.4 (C-6a).

Anal. Calc. for $C_{99}H_{112}O_{18}$: C, 74.79; H, 7.10. Found: C, 74.63; H, 7.26.

Further elution with the same eluant afforded **5** (3.927 g, 77.2% based on the recovered **16**, *i.e.*, 1.5 g, R_F 0.24); $[\alpha]_D$ −4.5° (*c* 1.8); R_F 0.38 in 2:1 hexane–EtOAc;

n.m.r. data: δ_H 5.091 (d, 1 H, *J* 1.5 Hz, H-1b); δ_C 101.7 ($^1J_{CH}$ 156 Hz, C-1a), 98.3 ($^1J_{CH}$ 167 Hz, C-1b), and 66.5 (C-6a).

Anal. Calc. for $C_{65}H_{78}O_{13}$: C, 72.60; H, 7.36. Found: C, 73.10; H, 7.36.

8-Ethoxycarbonyloctyl O-*α*-D-*mannopyranosyl-(1→6)*-*β*-D-*mannopyranoside* (**22**). — A solution of **5** (60 mg, 56 μmol) in MeOH (3.0 mL) was stirred under H_2 in the presence of added 10% Pd–C (40 mg) for 18 h at 27°. The usual work-up, and purification by use of Sephadex LH-20 in MeOH, afforded **22** (19 mg, 64%); $[\alpha]_D$ +7.7° (*c* 0.5, MeOH); R_F 0.70 in 2:1:1 1-BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 27°) 4.906 (d, 1 H, *J* 1.5 Hz, H-1b), 4.658 (s, 1 H, H-1a), 4.147 (q, 2 H, *J* 7.3 Hz, OCH_2CH_3), 2.367 (t, 2 H, *J* 7.3 Hz, $-CH_2CO$), 1.599 (t, 4 H, *J* 6.9 Hz, CH_2 × 2), and 1.4–1.2 (m, 11 H); δ_C (D_2O, 20°) 100.9 ($^1J_{CH}$ 155 Hz, C-1a) and 100.4 ($^1J_{CH}$ 173 Hz, C-1b).

Anal. Calc. for $C_{23}H_{42}O_{13}$: C, 52.46; H, 8.03. Found: C, 52.33; H, 7.85.

8-Ethoxycarbonyloctyl O-*α*-D-*mannopyranosyl-(1→3)*-O-[*α*-D-*mannopyranosyl-(1→6)*]-*β*-D-*mannopyranoside* (**24**). — Compound **23** (600 mg, 0.38 mmol) was hydrogenolyzed as for **5** to give **24** (196 mg, 76%); $[\alpha]_D$ +24.3° (*c* 0.43, H_2O); R_F 0.62 in 2:1:1 1-BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°) 5.108 (d, 1 H, *J* 1.5 Hz, H-1c), 4.905 (d, 1 H, *J* 1.5 Hz, H-1b), and 4.651 (s, 1 H, H-1a); δ_C (D_2O) 103.2 ($^1J_{CH}$, 172 Hz, C-1c), 100.6 ($^1J_{CH}$ 158 Hz, C-1a), 100.3 ($^1J_{CH}$ 172 Hz, C-1b), and 81.7 (C-3a).

Anal. Calc. for $C_{29}H_{52}O_{18} \cdot 1.5\ H_2O$: C, 48.66; H, 7.32. Found: C, 48.85; H, 7.27.

8-Ethoxycarbonyloctyl O-*(2,3,4,6-tetra*-O-*acetyl*-*β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-*β*-D-*glucopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*benzyl*-*α*-D-*mannopyranosyl)-(1→3)*-O-[*2,3,4,6-tetra*-O-*benzyl*-*α*-D-*mannopyranosyl-(1→6)*]-*2,4-di*-O-*benzyl*-*α*-D-*mannopyranoside* (**25**). — To a stirred mixture of **4** (131 mg, 0.11 mmol), **5** (131 mg, 0.12 mmol), and powdered molecular sieves AW-300 (350 mg) in $Cl(CH_2)_2Cl$ (3 mL) was added $BF_3 \cdot Et_2O$ (132 μL, 0.12 mmol) at 0°. The mixture was stirred under Ar for 1 h at 0–20°, filtered through Celite, and the filtrate successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 1:1 toluene–EtOAc afforded **25** (102 mg, 45%); $[\alpha]_D$ −8.7° (*c* 0.36); R_F 0.26 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 5.536 (d, 1 H, *J* 3.1 Hz, H-4e) and 1.8–1.2 (m, 15 H, for spacer arm); δ_C 101.9 ($^1J_{CH}$ 154 Hz, C-1a), 101.1 ($^1J_{CH}$ 159 Hz, C-1e), 99.4 ($^1J_{CH}$ 156 Hz, C-1d), 99.0 ($^1J_{CH}$ 170 Hz, C-1c), and 98.8 ($^1J_{CH}$ 170 Hz, C-1b).

Anal. Calc. for $C_{118}H_{141}NO_{34} \cdot 4\ H_2O$: C, 64.73; H, 6.49; N, 0.64. found: C, 64.62; H, 6.42; N, 0.85.

8-Ethoxycarbonyloctyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy*-*β*-D-*glucopyranosyl)-(1→2)*-O-*α*-D-*mannopyranosyl-(1→3)*-O-[*α*-D-*mannopyranosyl-(1→6)*]-*α*-D-*mannopyranoside* (**3**). — A mixture of **25** (124 mg, 0.06 mmol) and 10% Pd–C (70 mg) in AcOH (6 mL) was stirred under H_2 for 1 h at 60° and filtered through Celite. The filtrate was evaporated *in vacuo*, to give crude **26** (R_F 0.69 in 2:1:1 1-BuOH–EtOH–H_2O).

Anal. Calc. for $C_{55}H_{87}O_{34} \cdot 3\ H_2O$: C, 47.31; H, 6.71; N, 1.01. Found: C, 47.55; H, 6.29; N, 1.43.

A solution of **26** in 0.02M NaOEt–EtOH (5 mL) was stirred for 18 h at 20°. Neutralization of the base with Amberlyst A-15, filtration, and evaporation of the filtrate *in vacuo*, afforded crude **3**, which was purified by use of Sephadex G-25 in H_2O, to give **3** (48 mg, 85%); $[\alpha]_D$ +4.9° (*c* 0.43, H_2O); R_F 0.44 in 2:1:1 1-BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°) 5.146 (s, 1 H, H-1c), 4.923 (s, 1 H, H-1b), 4.667 (s, 1 H, H-1a), 4.580 (d, 1 H, *J* 7.6 Hz, H-1d), 4.482 (d, 1 H, *J* 7.6 Hz, H-1e), 2.368 (t, 2 H, *J* 7.6 Hz, CH_2CH_2CO), 2.044 (s, 3 H, $NHCOCH_3$), and 1.7–1.2 (m, 15 H, spacer arm); δ_C (D_2O) 103.8 ($^1J_{CH}$ 164 Hz, C-1d and C-1e), 100.6 ($^1J_{CH}$ 159 Hz, C-1a), and 100.2 ($^1J_{CH}$ 171 Hz, C-1b and C-1c).

Anal. Calc. for $C_{43}H_{75}NO_{28} \cdot 6\ H_2O$: C, 44.44; H, 6.50; N, 1.20. Found: C, 44.54; H, 6.52; N, 1.56.

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REFERENCES

1 K. KOIKE, M. SUGIMOTO, Y. NAKHARA, AND T. OGAWA, *Glycoconjugate J.*, 2 (1985) 105–108.
2 Y. ENDO, K. YAMASHITA, Y. TACHIBANA, S. TOJO, AND A. KOBATA, *J. Biochem. (Tokyo)*, 85 (1979) 669–679; T. MIZUOCHI AND A. KOBATA, *Biochem. Biophys. Res. Commun.*, 97 (1980) 772–778.
3 T. MIZUOCHI, R. NISHIMURA, C. DERAPPE, T. TANIGUCHI, T. HAMAMOTO, M. MOCHIZUKI, AND A. KOBATA, *J. Biol. Chem.*, 258 (1983) 14,126–14,129.
4 P. GLEESON, G. VELLA, S. NARASIMHAN, AND H. SCHACHTER, *Fed. Proc.*, 41 (1982) 1147.
5 R. U. LEMIEUX, D. R. BUNDLE, AND D. A. BAKER, *Can. J. Biochem.*, 55 (1977) 507–512.
6 T. OGAWA AND T. NUKADA, *Carbohydr. Res.*, 136 (1985) 135–152; T. OGAWA, K. BEPPU, AND S. NAKABAYASHI, *ibid.*, 93 (1983) c6–c9.
7 M. M. PONPIPOM, R. L. BUGIANESI, AND T. Y. SHEN, *Tetrahedron Lett.*, (1978) 1717–1720; J. ARNARP AND J. LÖNNGREN, *J. Chem. Soc., Chem. Commun.*, (1980) 1000–1002; *J. Chem. Soc., Perkin Trans. 1*, (1981) 2070–2074; T. OGAWA AND S. NAKABAYASHI, *Carbohydr. Res.*, 97 (1981) 81–86; R. U. LEMIEUX, S. Z. ABBAS, AND B. Y. CHUNG, *Can. J. Chem.*, 60 (1982) 58–62.
8 P. L. DURETTE, E. P. MEITZNER, AND T. Y. SHEN, *Tetrahedron Lett.*, (1979) 4013–4016; *Carbohydr. Res.*, 77 (1979) c1–c4.
9 R. BOSS AND R. SCHEFFOLD, *Angew. Chem.*, 88 (1976) 578–579; T. OGAWA AND S. NAKABAYASHI, *Carbohydr. Res.*, 93 (1981) c1–c5.
10 R. R. SCHMIDT AND J. MICHEL, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 731–732.
11 R. U. LEMIEUX, D. R. BUNDLE, AND D. A. BAKER, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
12 T. OGAWA, T. KITAJIMA, AND T. NUKADA, *Carbohydr. Res.*, 123 (1983) c5–c7.
13 H. PAULSEN AND O. LOCKHOFF, *Chem. Ber.*, 114 (1981) 3102–3114; H. PAULSEN, R. LEBUHN, AND O. LOCKHOFF, *Carbohydr. Res.*, 103 (1982) c7–c11.
14 R. R. SCHMIDT AND J. MICHEL, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 72–73.
15 T. OGAWA, H. YAMAMOTO, T. NUKADA, T. KITAJIMA, AND M. SUGIMOTO, *Pure Appl. Chem.*, 56 (1984) 779–795; T. OGAWA AND S. NAKABAYASHI, *Agric. Biol. Chem.*, 45 (1981) 2329–2335; T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.