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SYNTHESIS OF A HEPTASACCHARIDE HAPTEN RELATED TO A BI-ANTENNARY GLYCAN CHAIN OF HUMAN CHORIONIC GONADOTROPIN OF A CHORIOCARCINOMA PATIENT. A CONVERGENT APPROACH*

KHALID K. SADOZAI, TOMOO NUKADA, YUKISHIGE ITO, YOSHIAKI NAKAHARA, TOMOYA OGAWA**,
RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351 (Japan)

AND AKIRA KOBATA
Department of Biochemistry, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 180 (Japan)

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ABSTRACT

Synthesis of the heptasaccharide hapten 8-methoxycarbonyloctyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-[β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)]-*O*-α-D-mannopyranosyl-(1→3)-*O*-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranoside is described, by use of the known, protected 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, and the key glycopentaosyl donors *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*-[(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→4)]-3,6-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**5**) and the corresponding fluoride **7**, which, in turn, were prepared in 5 steps from allyl 3,6-di-*O*-benzyl-α-D-mannopyranoside in 35 and 22% overall yields, respectively. In model experiments, the key glycosyl donors **5** and **7** were also treated with the simple glycosyl acceptor 8-ethoxycarbonyloctanol, to give 8-methoxycarbonyloctyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-[β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)]-α (and β)-D-mannopyranoside.

INTRODUCTION

In 1983, human Chorionic Gonadotropin (hCG), isolated from the urine of a patient with choriocarcinoma, was found[2] to carry an anomalous, biantennary

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**To whom inquiries should be addressed.

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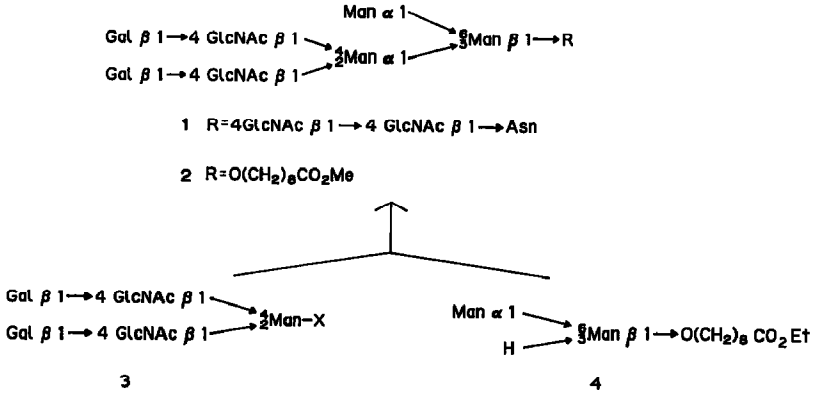
glycan chain (**1**). The unique, biantennary structure of **1** has not been detected[3] in the glycan of normal hCG. As part of a project on the synthesis of oligosaccharide haptens, we had described a stepwise approach[1] for the synthesis of heptasaccharide hapten **2**, designed after the molecular structure of **1**. We now describe a convergent approach for the stereoselective synthesis of **1**.

RESULTS AND DISCUSSION

The target structure **2** was retrosynthesized (see Scheme 1) into the glycosyl donor **3** and the glycosyl acceptor **4**, which carries a C_9 hydroxy ester as a spacer arm[4].

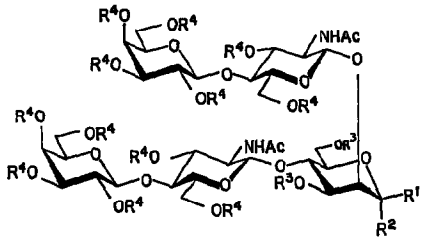
Because a synthesis of the glycosyl acceptor **9** (a synthetic equivalent of **4**) had been reported[5], we first describe synthesis of the glycosyl donors **5–8**, which are the synthetic equivalents of **3**, and then the glycosylation reaction of **9** with these donors.

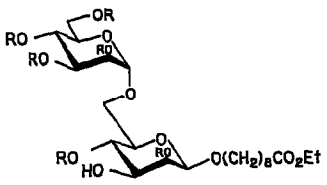
Allyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (**15**; ref. 6) was glycosylated



Scheme 1

with the lactosaminyl donor **11**, readily obtainable[7] from the acetate **10**, in the presence of silver triflate and *s*-collidine, to give the desired pentasaccharide **16** and the trisaccharide **25** in 57 and 42% yield, respectively. The monoglycosylated structure of **25** was determined by its transformation into the acetate **26**, the ^{1}H-n.m.r. spectrum of which contained a characteristic, deshielded signal for H-2a at δ 5.278 as a double doublet with J 1.8 and 3.4 Hz. This regioselectivity of glycosylation at the 4- rather than the 2-hydroxyl group was in agreement with previous observations[8]. The structure of the trisaccharide **25** was further confirmed by the conversion into the deblocked trisaccharide **27** in 5 steps (see scheme 2): (*i*) hydrogenolysis in the presence of 10% Pd–C in MeOH, (*ii*) deacetylation in 0.1M NaOMe–MeOH, (*iii*) *N*-dephthaloylation in $BuNH_2$–MeOH, (*iv*) acetylation in pyridine–Ac_2O, and finally, (*v*) deacetylation in 0.1M NaOMe–MeOH. The trisaccharide **27** was proved to be different from an authentic sample of Gal(β1→4)-





9 R = Bn

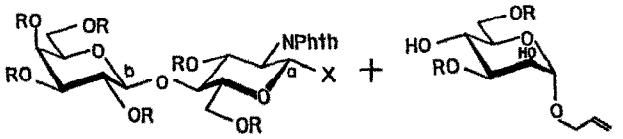
	R^1	R^2	R^3	R^4
5	H	$OCNHCCl_3$	Bn	Ac
6	H	$OCNHCCl_3$	Ac	Ac
7	H	F	Bn	Ac
8	F	H	Bn	Ac

GlcNAc(β1→2)-Man(α)-OPr[1], by comparison of ^{1}H- and ^{13}C-n.m.r. spectra. The ^{1}H-n.m.r. spectrum of **27** showed three signals for anomeric protons, at δ 4.851 (d, *J* 1.7 Hz), δ 4.569 (d, *J* 8.0 Hz), and δ 4.460 (d, *J* 7.8 Hz), for H-1a, H-1b, and H-1c, respectively, as well as a signal for the *N*-acetyl group of GlcNAc at δ2.057.

Besides the bromide **11**, trichloroacetimidate **13** (ref. 9) and methyl 1-thioglycoside **14** were also examined as lactosaminyl donors for preparation of the pentasaccharide **16**. Compound **13** was prepared by a route modified from that of Grundler and Schmidt[9]. The β-acetate **10** was transformed into an 18:1 mixture of β- and α-trichloroacetimidate **13** in 71% yield by successive treatment with (*i*) $H_2NNH_2 \cdot AcOH$ in DMF, and (*ii*) CCl_3CN–DBU. It is to be noted that the ^{1}H-n.m.r. data for the β anomer **13** were in agreement with those of Grundler and Schmidt[9], but their reported value of $[\alpha]_D$ +43° was almost identical with that of our α anomer **13** ($[\alpha]_D$ +41°). Upon treatment with methyl tributyltin sulfide in the presence of $SnCl_4$, the β-acetate **10** was converted into methyl 1-thioglycoside **14** (ref. 10) in 89% yield.

By use of β-trichloroacetidimidate **13** under the conditions of Schmidt and Michel[11], a high yield (73%) of pentasaccharide **16** was obtained. In this glycosylation, along with the major product **16**, a 1:1 mixture of trisaccharides **25** and **28** was isolated in 24% yield. Treatment of the acceptor **15** with methyl 1-thioglycoside **14** in the presence of methyl triflate according to Lönn[12] afforded only a 36% yield of the desired pentasaccharide **16**. Therefore, for preparation of pentasaccharide **16**, the most efficient lactosaminyl donor in our hands proved to be trichloroacetimidate **13**.

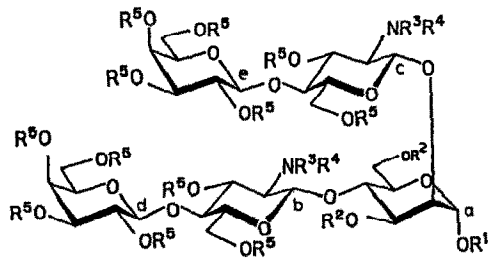
The structure of the pentasaccharide **16** was assigned from the ^{1}H-n.m.r. spectrum, which contained two signals for two H-4 of galactopyranosyl residues, at δ 5.326 and 5.301, and from the ^{13}C-n.m.r. data, which showed signals at δ 96.6 ($^1J_{CH}$ 165 Hz) for two β-linked, anomeric carbon atoms, C-1b and C-1c, δ 98.0



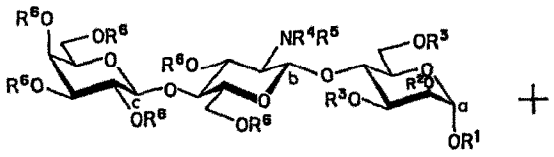
	R	X
10	Ac	Ac
11	Ac	Br
12	Ac	OH
13	Ac	NH O-C-CCl$_3$
14	Ac	SCH$_3$

Phth = phthaloyl

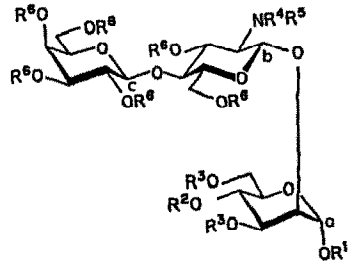
15 R=Bn



	R^1	R^2	R^3R^4	R^5
16	allyl	Bn	Phth	Ac
17	allyl	Bn	Phth	H
18	allyl	Bn	H,Ac	Ac
19	propyl	H	H,Ac	H
20	H	Bn	H,Ac	Ac
21	CH_2COCH_3	Bn	H,Ac	Ac
22	H	H	H,Ac	Ac
23	Ac	Ac	H,Ac	Ac
24	H	Ac	H,Ac	Ac



	R^1	R^2	R^3	R^4R^5	R^6
25	allyl	H	Bn	Phth	Ac
26	allyl	Ac	Bn	Phth	Ac
27	propyl	H	H	H,Ac	H



	R^1	R^2	R^3	R^4R^5	R^6
28	allyl	H	Bn	Phth	Ac
29	allyl	Ac	Bn	Phth	Ac

Scheme 2

($^1J_{CH}$ 167 Hz) for an α-linked anomeric carbon atom of mannose (C-1a), and δ 101.0 ($^1J_{CH}$ 162 Hz) for two β-linked, anomeric carbon atoms, C-1d and C-1e. The structure was further confirmed by transformation of **16** into the deblocked, propyl pentasaccharide **19** by the following sequence of reactions. *O*-Deacetylation of **16** in 0.5M NaOMe–MeOH afforded a 98% yield of benzylated pentasacharide **17**, which was *N*-dephthaloylated[13] with $BuNH_2$ in MeOH, and the product acetylated with Ac_2O–pyridine, to give the tetradecaacetylated product **18** in 64% yield. *O*-Deacetylation of **18**, and hydrogenolysis of the benzyl groups in the product afforded the propyl pentasaccharide **19** in 66% yield.

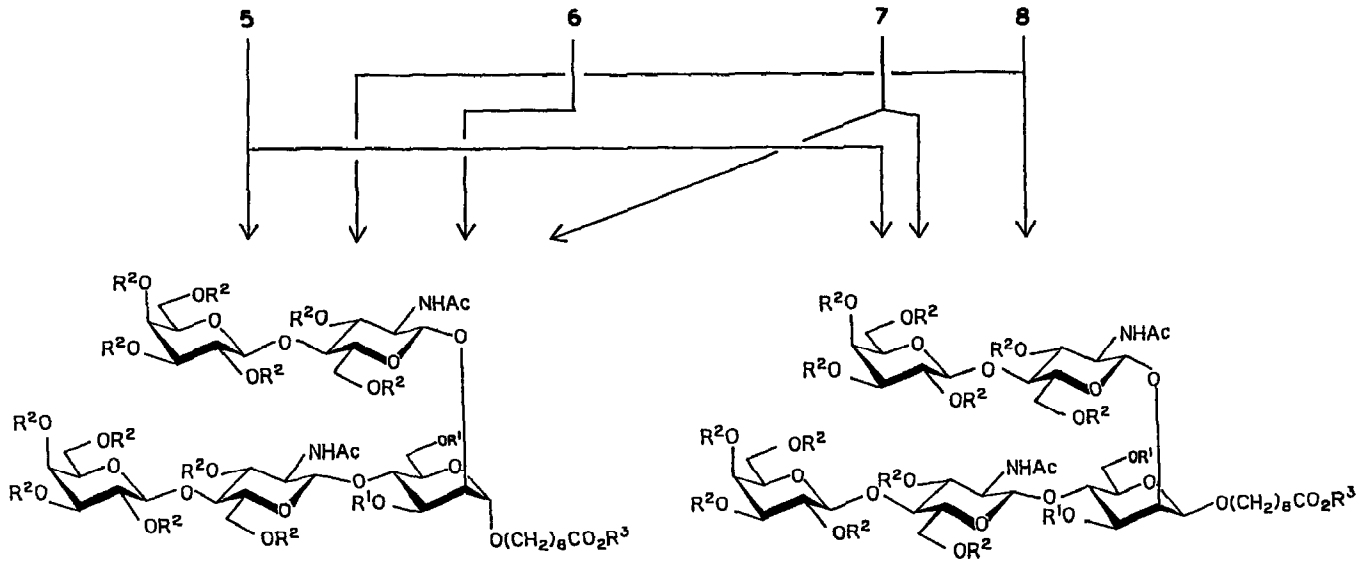
The ^{1}H-n.m.r. spectrum of **19** showed a singlet at δ 4.851 for H-1a, two doublets, at δ 4.523 (*J* 8.2 Hz) and 4.563 (*J* 7.6 Hz), for H-1b and H-1c, respectively, and a doublet for two protons, at δ 4.460 (*J* 7.6 Hz) for H-1d and H-1e. The two NAc signals appeared at δ 2.043 for a GlcNAc(β1→2)Man residue, and at δ 2.058 for a GlcNAc(β1→4)Man residue, respectively, thus confirming the configuration and the site of the glycosidic linkages of the diglycosylated product **16**. The ^{13}C-n.m.r. data of **19** were in accordance with the data[14] reported for a related pentasaccharide. Deallylation[15] of **18** with $PdCl_2$ and AcONa in aq. AcOH afforded a 50% yield of the desired hemiacetal **20**, as well as a 28% yield of the byproduct **21**. The structure of **21** was assignable from the ^{13}C-n.m.r. data, which showed a characteristic signal for CH_2COCH_3 at δ 25.9. The formation of **21** was avoided as follows. Treatment of **18** with tris(triphenylphosphine)rhodium(I) chloride and DABCO[16], followed by treatment[17] with I_2, gave an 82% yield of the hemiacetal **20**. Treatment of **20** with Cl_3CCN and sodium hydride in dichloromethane according to Schmidt and Michel[10] afforded a 92% yield of trichloroacetimidate **5**. The α-D configuration at C-1a of **5** was evident from the following ^{1}H- and ^{13}C-n.m.r. data. The signals for C=N*H* and H-1a appeared at δ 8.577 and 6.261, respectively, each as a singlet, and the signal for C-1a appeared at δ 95.8, in good agreement with the datum for a related trichloroacetimidate[1].

Because the reactivity of the donor is known to be influenced by the nature of the substituents on the hydroxyl groups[18], by replacement of the benzyl groups of **5** by acetyl groups, another glycosyl donor, namely, **6**, was designed.

For the preparation of trichloroacetimidate **6**, the hemiacetal **20** was transformed into peracetate **23** in 85% yield in two steps: (*i*) hydrogenolysis with 10% Pd–C in 10:1 methanol–acetic acid, and (*ii*) acetylation with acetic anhydride–pyridine. The α-D configuration of C-1a of **23** was assignable from ^{13}C-n.m.r. data, which contained a signal for C-1a at δ 90.8 ($^1J_{CH}$ 176 Hz). Site-selective deacetylation of **23** according to Excoffier *et al.*[19] afforded a 93% yield of hemiacetal **24**, which, upon treatment with DBU and trichloroacetonitrile, afforded trichloroacetimidate **6**. The configuration of C-1a of **6** was assigned from ^{13}C-n.m.r. data, which revealed a signal for C-1a at δ 95.4, as in case of **5**.

The anomeric fluorides **7** and **8** were, by treating **20** with diethylaminosulfur trifluoride (Et_2NSF_3) in dimethoxyethane, according to Rosenbrook *et al.*[20] and

Glycosylation with $HO(CH_2)_8CO_2Et$ (30)



31 $R^1=R^2=Ac$ $R^3=Et$

32 $R^1=R^2=H$ $R^3=Me$

33 $R^1=Bn$ $R^2=Ac$ $R^3=Et$

34 $R^1=Bn$ $R^2=Ac$ $R^3=Et$

35 $R^1=R^2=H$ $R^3=Me$

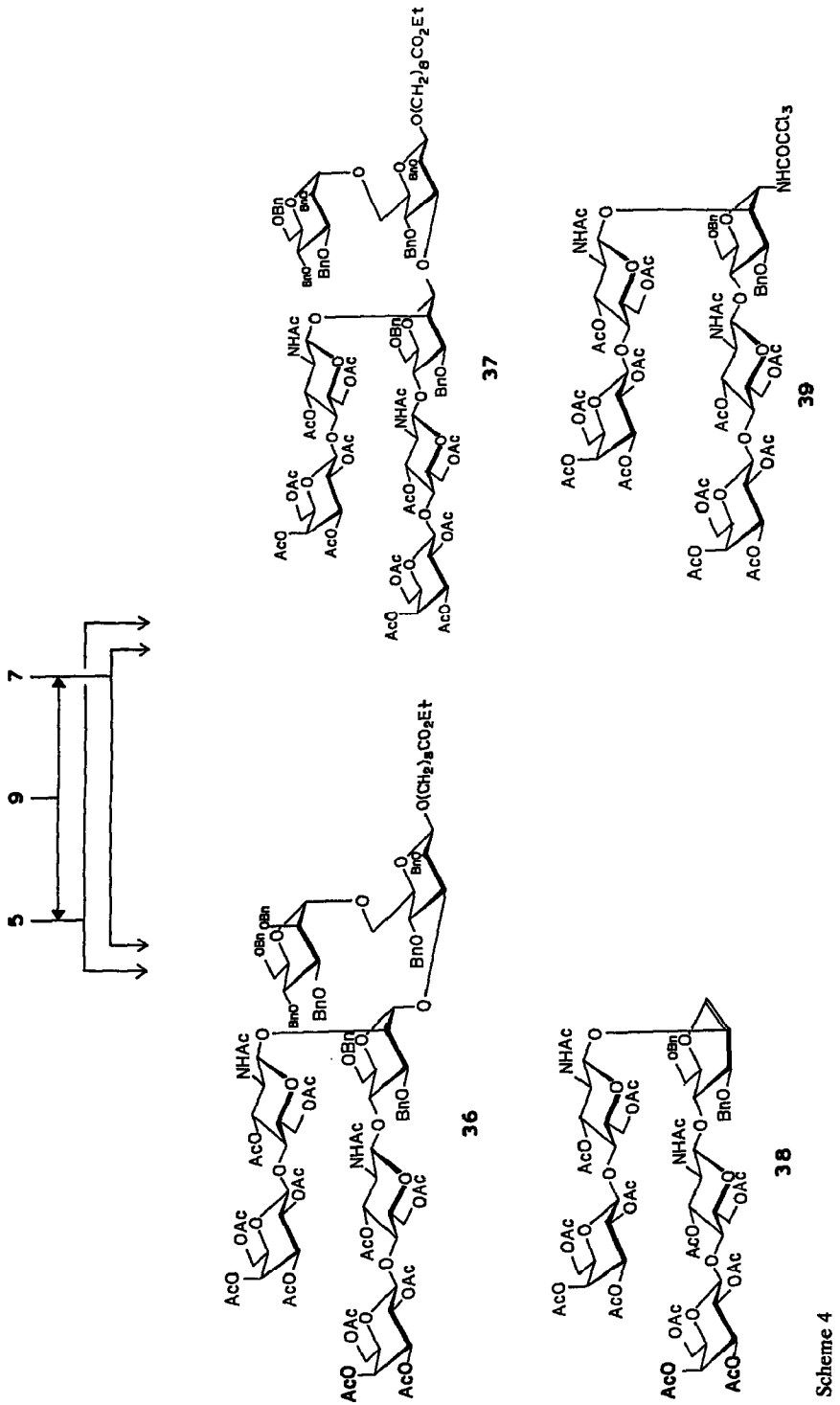
Scheme 3

Posner and Haines[21], prepared in 91% yield as a mixture of **7** and **8** in the ratio of 1.7:1. The configuration of C-1a of **7** and **8** was assigned from the following ^{1}H- and ^{19}F-n.m.r. data: the ^{1}H-n.m.r. spectrum of **7** showed a double doublet for H-1a at δ 5.538 ($^1J_{HF}$ 51.3 Hz, $^1J_{HH}$ 2.0 Hz), and a doublet in the ^{19}F-n.m.r. spectrum at δ_F 135.8 ($^1J_{HF}$ 51 Hz) in accordance with the data reported for α-mannosyl fluorides[22] and those for 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl fluoride, which was readily prepared from the corresponding hemiacetal by treatment with Et_2NSF_3 in THF, and which showed n.m.r. signals at δ_H 5.610 (H-1a, $^1J_{HF}$ 49.0 Hz) and δ_F 135.6 ($^1J_{HF}$ 49 Hz). The ^{19}F-n.m.r. spectrum of **8** showed a doublet, δ_F 139.6 ($^1J_{HF}$ 34.0 Hz). The observed smaller coupling constant[22] of 34 Hz in the case of **8** is in agreement with β-stereochemistry at the anomeric center of **8**.

Because glycosyl donors of such molecular sizes as those of **5–8** have rarely been employed for glycosylation, their reaction with alcohol **30** was first studied. Glycosylation of **30** with the trichloroacetimidate donor **6** in the presence of $BF_3 \cdot Et_2O$ and molecular sieves AW-300 gave, stereoselectively, the α-glycoside **31** in 52% yield. The configuration of C-1a was assigned as α-D from the ^{1}H-n.m.r. spectrum of deblocked product **32**, which contained a singlet at δ 4.848 for H-1a, two doublets, at δ 4.566 (J 7.6 Hz) and δ 4.525 (J 8.0 Hz), for H-1c and H-1b, respectively, and a doublet at δ 4.60 (J 7.8 Hz) for H-1d and H-1e, in accordance with the data for related pentasaccharide **19**. In contrast to the peracetylated trichloroacetimidate **6**, the trichloroacetimidate **5** (carrying benzyl groups at both O-3a and O-6a) afforded under the same conditions a 1.2:1 mixture of compounds **33** and **34** in 66% yield. The yield of glycosylated product was higher, but low stereoselectivity was observed, in the case of donor **5**, indicating higher reactivity of **5** compared with **6**, in agreement with the trends observed for monosaccharide donors[18].

Upon treatment with alcohol **30** in the presence of silver perchlorate and stannous chloride[19] in diethyl ether, both the α- and the β-glycopentaosyl fluorides **7** and **8** afforded an ~70% yield of a 1:3 mixture of α-glycoside **33** and β anomer **34**, regardless of the stereochemistry of the starting glycosyl fluoride. This result strongly indicated that the α- and β-fluoride (**7** and **8**) react with silver perchlorate and stannous chloride to afford a common, reaction intermediate, most probably an ion-paired oxocarbenium ion. The structures of compounds **33** and **34** were assigned from the ^{1}H-n.m.r. data for a mixture of the deblocked products **32** and **35** in D_2O at 50°, which showed signals for H-1a of **32** and H-1a of **35** at δ 4.828 (0.25 H) and 4.618 (0.75 H), respectively, along with characteristic signals for H-1c and H-2a of β anomer **35** at δ 4.850, as a doublet with J 8.5 Hz, and at δ 4.202, as a doublet with J 3.2 Hz, respectively. The signal of H-1c of **35** is deshielded ~0.3 p.p.m. in comparison with that of **32**, due to the proximity of H-1c and the oxygen atom attached to C-1a of **35**.

Having demonstrated the suitable reactivity of the glycosyl donors **5–8**, the crucial glycosylation of acceptor **9** was next examined. Treatment of **9** with glycopentaosyl donor **5**, in the presence of $BF_3 \cdot Et_2O$ and molecular sieves AW-300,



Scheme 4

gave, after gel-permeation chromatography in benzene, a 2.3:1 mixture of the glycosylated products **36** and **37** in 11% yield based on **5**. The mixture of **36** and **37** was further separated by chromatography on SiO_2 in 2:1 $CHCl_3$–Me_2CO, to give pure **36** in 4.6% yield. The stereochemistry of the newly introduced glycosidic linkage in the major product **36** was proved to be Man(α1→3)Man by transformation into the deblocked heptasaccharide hapten **2** in two steps: (*i*) NaOMe–MeOH, and (*ii*) H_2–10% Pd–C. The ^{1}H-n.m.r. spectrum of **2** (obtained from **36**) was identical with that of authentic **2** prepared by a stepwise approach[1]. Even though the amount of the minor product **37** available was insufficient to permit its transformation into the deblocked hapten, it was tentatively assigned as the β anomer of **36** from ^{1}H-n.m.r. data which showed signals for 14 acetate methyl groups at δ 2.145–1.965, 40 aromatic protons, at δ 7.45–7.15, for 8 benzyl groups, and 15 methylene signals (for the spacer-arm portion) at δ 1.670–1.229.

The pentasaccharide fraction obtained from gel permeation chromatography consisted of two byproducts (**38** and **39**) which were separated by column chromatography on SiO_2, and their tentative structures were assigned, based on their ^{1}H-n.m.r. data. The ^{1}H-n.m.r. spectrum of **38** contained multiplet signals for 10 aromatic protons, at δ 7.45–7.15, and a deshielded singlet at δ 6.44 for H-1a. This chemical shift of the H-1 signal was in accord with the chemical shift of H-1 of a related glycal, namely, 1,5-anhydro-2,3,4,6-tetra-*O*-benzyl-D-*arabino*-hex-1-enitol, which appears at δ 6.29. Furthermore, the H-1 signal of 4-*O*-acetyl-1,6-anhydro-2,3-di-*O*-benzyl-β-D-mannopyranose appears at δ 5.48, which provides evidence that **38** is not a 1,6-anhydro compound[20]. The ^{1}H-n.m.r. spectrum of **39** showed, besides other signals, multiplet signals for 10 aromatic protons, at δ 7.45–7.20, and a signal for the amide proton of $NHCOCCl_3$ at δ 6.12 (d, J 10 Hz). On storage at room temperature, this product was slowly hydrolyzed, to give hemiacetal **20**. This chemical behaviour is in accord with the structure postulated.

Glycosylation of **9** with **7** in the presence of $AgClO_4$, $SnCl_2$ and molecular sieves 4A, followed by gel-permeation chromatography over Bio-Beads SX-3 in benzene, gave a 1:1 mixture of glycosylated products **36** and **37** in 26% yield (based on the donor **7**), as well as 68% of recovered donor **7**. In an attempt to determine the structure of **37**, the mixture of **36** and **37** was, without separation, transformed into the deblocked heptasaccharide hapten. Deacetylation in M NaOMe–MeOH, followed by hydrogenolysis with H_2 in the presence of 10% Pd–C, and purification of the product on Sephadex LH-20 in 1:1 THF–H_2O, gave the deblocked product in 36.3% yield. Surprisingly, the ^{1}H-n.m.r. spectrum was identical with that of authentic **2**, and did not show any signals for the β-glycosylated product. Because the isolated yield of the deblocked heptasaccharide hapten was only 36.3%, it is presumed that either the glycosylated product **37** is decomposed during the deblocking process, or the deblocked hapten is adsorbed on the gel.

Chloroacetimidate **6**, which showed exclusive α-stereoselectivity with alcohol **30**, was expected to give mainly α-glycosylated product on reaction with mannobiosyl acceptor **9**; the reaction of **6** and **9** was performed, but separation of the

resulting glycosylated products from the byproducts was found difficult by any chromatographic means. The mixture, without separation, was therefore deblocked to afford the heptasaccharide fraction, and its 400-MHz n.m.r. spectrum was recorded. However, owing to its very small amount and to contamination with other products, it was not possible to assign the correct stereochemistry of the products with confidence. The exact yield and stereochemical outcome of this glycosylation reaction was thus not clear.

In conclusion, the four pentasaccharide donors **5**–**8**, carrying two *N*-acetyllactosamine residues, were synthesized; they gave 50–70% yields of the glycosylated products with alcohol **30**. Change of the protective groups from benzyl to acetyl in case of the imidates **5** and **6** was found to change the stereochemical outcome of the glycosylation dramatically. The fluoride donors **7** and **8** gave higher yields of glycosylated products **33** and **34**, and the stereochemical outcome of the glycosylation was found to be independent of the anomeric stereochemistry of the glycosyl fluoride employed. A convergent type of synthesis of heptasaccharide hapten **2** in 4.2 and 9.4% overall yield, respectively, was successfully achieved by employing either imidate **5** or fluoride **7**, and starting from mannobiosyl acceptor **9**. These routes were found to be more efficient than the stepwise route[1], which gave heptasaccharide hapten **2** in only 2.6% overall yield, starting from the same mannobiosyl acceptor **9**.

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 high-performance t.l.c. were conducted on silica gel 60 F_{254} (Merck, Darmstadt). Molecular sieves were purchased from Nakari 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. downward from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise. 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) or Me_3COH (1.230), and 1,4-dioxane (67.4) or MeOH (49.8), respectively. Values of δ_F, expressed in p.p.m. upfield from trichlorofluoromethane, were measured against an internal standard of CF_3CO_2H (76.53). The letters a, b, ... g are used to designate the glycosyl residue in which a cited H or C atom is located.

O-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-D-*glucopyranose* (**12**). — A solution of **10** (8.0 g, 10.5 mmol) and H_2NNH_2–AcOH (0.96 g, 10.4 mmol) in DMF (20.0 mL) was stirred for 1 h at 25°, and diluted with EtOAc (200 mL). The organic layer was washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*, to give crude **12** in quantitative yield. A small portion was purified by chromatography over SiO_2 in 1:1 toluene–ethyl acetate, to give pure **12**; R_F 0.21 in 1:1 toluene–ethyl acetae; n.m.r. data: δ_H 7.906–7.728 (m, 4 H, aromatic), 5.775 (dd, 1 H, *J* 8.06 and 10.5 Hz, H-3a), 5.649 (dd, 1 H, *J* 6.0 and 8.0 Hz, H-1a), 5.338 (d, 1 H, *J* 2.6 Hz, H-4b), 5.129 (dd, 1 H, *J* 10.5 and 7.8 Hz, H-2b), 4.967 (dd, 1 H, *J* 10.5 and 3.4 Hz, H-3b), 4.559 (d, 1 H, *J* 7.8 Hz, H-1b), 4.161 (dd, 1 H, *J* 8.4 and 10.5 Hz, H-2a), 2.134, 2.068, 2.043, 1.967, and 1.911 (18 H, 6 Ac); δ_C 100.99 (C-1b) and 92.43 (C-1a).

Anal. Calc. for $C_{32}H_{37}NO_{15}$: C, 53.11; H, 5.15; N, 1.93. Found: C, 53.08; H, 5.21; N, 1.86.

O-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β (and α)*-D-*glucopyranosyl trichloroacetimidate* (**13**). — A mixture of crude **12** (7.5 g, 10.4 mmol), Cl_3CCN (10.0 mL), and DBU (1.56 mL, 10.4 mmol) in $Cl(CH_2)_2Cl$ (40 mL) was stirred for 1 h at 20°, and the mixture directly chromatographed on SiO_2 in 1:1 toluene–ethyl acetate, to give **13** (β) (6.1 g, 67%); $[\alpha]_D$ +17.7° (*c* 1.4); R_F 0.44 in 1:1 toluene–ethyl acetate; n.m.r. data: δ_H 8.653 (s, 1 H, C=N*H*), 7.848–7.714 (m, 4 H, arom.), 6.612 (d, 1 H, *J* 8.8 Hz, H-1a), 5.872 (dd, 1 H, *J* 8.3 and 10.5 Hz, H-3a), 5.350 (d, 1 H, *J* 2.4 Hz, H-4b), 5.140 (dd, 1 H, *J* 7.8 and 10.5 Hz, H-2b), 4.965 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3b), 4.566–4.525 (m, 3 H, H-1b,2a,6b), 2.161, 2.149, 2.076, 2.074, 1.972, and 1.939 (6 s, 18 H, 6 OAc); δ_C 160.0 (OC=N), 100.7 (C-1b, J_{CH} 162 Hz), 93.2 (C-1a, J_{CH} 168 Hz), and 89.96 (CCl_3).

Further elution with the same solvent gave **13** (α) (342 mg, 3.7%); $[\alpha]_D$ +40.9° (*c* 1.5); R_F 0.34 in 1:1 toluene–ethyl acetate; δ_H 8.567 (s, 1 H, C=NH), 7.855–7.721 (m, 4 H, aromatic), 6.582 (dd, 1 H, *J* 9.3 and 11.2 Hz, H-3a), 6.360 (d, 1 H, *J* 3.9 Hz, H-1a), 5.359 (d, 1 H, *J* 3.2 Hz, H-4b), 5.147 (dd, 1 H, *J* 8.0 and 10.8 Hz, H-2b), 4.542 (d, 1 H, *J* 8.0 Hz, H-1b), 2.163, 2.136, 2.071, 2.065, and 1.973 (5 s, 18 H, 6 Ac).

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**14**). — To a solution of β-acetate **10** (236 mg, 0.3 mmol) and Bu_3SnSMe (104 mg, 0.41 mmol) in $Cl(CH_2)_2Cl$ (6 mL) was added $SnCl_4$ (36 μL, 0.31 mmol) at −15°. The mixture was stirred for 13 h at 20°, and poured into aq. $NaHCO_3$, and solid KF (500 mg) was added to precipitate the Sn derivatives. The insoluble material was filtered off through Celite, and the filtrate extracted with $Cl(CH_2)_2Cl$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated, to give an oily residue which was chromatographed on SiO_2 with 1:1 toluene–EtOAc, giving **14** as a glass (207 mg, 89.2%); $[\alpha]_D$ +20.6° (*c* 1.4); R_F 0.43 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 7.882–7.731 (m, 4 H, aromatic), 5.807 (dd, 1 H, *J* 8.3 and 10.2 Hz, H-3a), 5.384 (d,

1 H, *J* 10.7 Hz, H-1a), 5.342 (d, 1 H, *J* 2.7 Hz, H-4b), 5.132 (dd, 1 H, *J* 10.3 and 8.0 Hz, H-2b), 4.956 (dd, 1 H, *J* 10.3 and 3.4 Hz, H-3b), 4.536 (d, 1 H, *J* 7.81 Hz, H-1b), 4.311 (t, 1 H, *J* 10.2 Hz, H-2a), 2.147, 2.142, 2.135, 2.073, 2.048, 1.968, and 1.910 (7 s, 21 H, S-Me and 6 Ac); δ_C 100.9 (C-1b, $^1J_{CH}$ 163 Hz), 80.5 (C-1a), and 11.5 (S-CH_3).

Anal. Calc. for $C_{33}H_{39}NO_{17}S$: C, 52.58; H, 5.45; N, 1.86. Found: C, 52.84; H, 5.22; N, 1.88.

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)*-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→4)*]-*3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**16**), *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→4)-3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**25**), *and 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)-3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**28**). — *Method A.* To a solution of **15** (2.12 g, 5.3 mmol), *s*-collidine (2.6 mL, 20.1 mmol), and $AgOSO_2CF_3$ (4.99 g, 19.4 mmol) in $Cl(CH_2)_2Cl$ (15 mL) was added dropwise a solution of **11** (12.5 g, 15.9 mmol) at −20°. The mixture was stirred for 1 h at −20°, diluted with $Cl(CH_2)_2Cl$ (200 mL), and filtered through Celite. The filtrate was successively washed with M HCl, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 1:1 toluene–EtOAc, to give a fraction (R_F 0.40 in 1:1 toluene–EtOAc) which gave two spots on further examination by h.p.t.l.c. in 4:4:5 toluene–$CHCl_3$–EtOAc. Further purification of this fraction by gel-permeation chromatography on Bio-Beads SX-4 in benzene afforded **25** (2.44 g, 41.7%); $[\alpha]_D$ +29.0° (*c* 1.4); R_F 0.26 in 4:4:5 toluene–$CHCl_3$–EtOAc; n.m.r. data: δ_H 5.88–5.75 (m, 1 H, –C*H*=CH_2), 5.674 (d, 1 H, *J* 8.3 Hz, H-1b), 5.668 (dd, 1 H, *J* 8.9 and 10.8 Hz, H-3b), 5.312 (d, 1 H, *J* 2.4 Hz, H-4c), 5.076 (dd, 1 H, *J* 8.0 and 10.4 Hz, H-2c), 4.907 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3c), 4.448 (d, 1 H, *J* 8.0 Hz, H-1c), 2.130 (Ac), 2.031 (Ac), 2.027 (Ac), 1.983 (Ac), 1.958 (Ac), and 1.871 (Ac); δ_C 100.9 (C-1c, $^1J_{CH}$ 161 Hz), 98.1 (C-1a, $^1J_{CH}$ 167 Hz), and 97.6 (C-1b, $^1J_{CH}$ 165 Hz).

Anal. Calc. for $C_{55}H_{63}NO_{23}\cdot H_2O$: C, 58.76; H, 5.82; N, 1.25. Found: C, 58.93; H, 5.53; N, 1.51.

Further elution with 1:1 toluene–EtOAc afforded **16** (5.50 g, 57.3%); $[\alpha]_D$ +15.2° (*c* 1.8); R_F 0.25 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 5.673 (dd, 1 H, *J* 8.6 and 10.5 Hz, H-3c), 5.602 (q, 1 H, *J* 8.8 and 10.5 Hz, H-3b), 5.543 (d, 1 H, *J* 8.5 Hz, H-1b), 5.459 (d, 1 H, *J* 8.3 Hz, H-1c), 5.326 (d, 1 H, *J* 3.3 Hz, H-4e), 5.301 (d, 1 H, *J* 3.3 Hz, H-4d), 4.939 (dd, 1 H, *J* 3.3 and 10.5 Hz, H-3e), and 4.880 (dd, 1 H, *J* 3.3 and 10.5 Hz, H-3d); δ_C 101.0 (C-1e and C-1d, $^1J_{CH}$ 162 Hz), 98.0 (C-1a, $^1J_{CH}$ 167 Hz), 96.6 (C-1b and C-1c, $^1J_{CH}$ 165 Hz), 55.4 (C-2b or C-2c), and 54.7 (C-2c or C-2b).

Anal. Calc. for $C_{87}H_{98}N_2O_{40}$: C, 57.67; H, 5.45; N, 1.54. Found: C, 57.48; H, 5.52; N, 1.42.

Method B. To a stirred mixture of **15** (800 mg, 2.0 mmol), **13** (5.2 g, 6.0 mmol), and powdered molecular sieves AW-300 in $Cl(CH_2)_2Cl$ (20.0 mL) was added $BF_3 \cdot Et_2O$ at −15°. The mixture was stirred for 1 h at −15 to −20°, diluted with $Cl(CH_2)_2Cl$, and filtered through Celite. The filtrate 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 1:1 toluene–EtOAc, and further purification of the fraction having R_F 0.42 (in 1:1 toluene–EtOAc; broad spot) by gel-permeation chromatography on Bio-Beads SX-4 in benzene gave a 1:1 mixture of monoglycosylated products **25** and **28** (526 mg, 23.8%). N.m.r. data for **28**: δ_H 5.88–5.75 (m, –C*H*=CH_2), 5.777 (dd, 1 H, *J* 8.3 and 10.8 Hz, H-3b), 5.462 (d, 1 H, *J* 8.6 Hz, H-1b), 5.335 (d, 1 H, *J* 3.4 Hz, H-4c), 4.963 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3c), 4.543 (d, 1 H, *J* 8.0 Hz, H-1c), 2.129, 2.067, 2.027, 1.984, 1.968, and 1.909 (6 s, 18 H, 6 Ac).

Further elution with 1:1 toluene–EtOAc afforded **16** (2.65 g, 73.3%).

Method C. To a stirred mixture of **15** (31.0 mg, 0.08 mmol), $CF_3SO_3CH_3$ (32 μL, 0.28 mmol), and molecular sieves 4A in $Cl(CH_2)_2Cl$ (3.0 mL) was added a solution of **14** (145 mg, 0.19 mmol) in 2:3 Et_2O–$Cl(CH_2)_2Cl$ (10 mL) at −20°, and the mixture was allowed to attain room temperature. Three further additions of $CF_3SO_3CH_3$ (100 μL each) were needed in order to bring the reaction to completion. The mixture was diluted with $Cl(CH_2)_2Cl$, filtered through Celite, and the filtrate evaporated *in vacuo*. The residue was chromatographed on SiO_2 in toluene–EtOAc, to give **16** (51 mg, 36%).

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→4)-2*-O-*acetyl-3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**26**). — A solution of **25** (3.5 mg) in 1:1 Ac_2O–pyridine (0.5 mL) was stirred for 16 h at 25°, and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 1:1 toluene–EtOAc, to give **26** (3.0 mg, 83%); $[\alpha]_D$ +41.1° (*c* 0.2); R_F 0.53 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 5.667 (d, 1 H, *J* 8.6 Hz, H-1b), 5.630 (dd, 1 H, *J* 8.7 and 10.7 Hz, H-3b), 5.304 (d, 1 H, *J* 2.4 Hz, H-4c), 5.278 (dd, 1 H, *J* 1.8 and 3.4 Hz, H-2a), 4.760 (s, 1 H, H-1a), 4.414 (d, 1 H, *J* 8.0 Hz, H-1c), 2.121 (Ac), 2.079 (Ac), 2.045 (Ac), 2.027 (Ac), 1.951 (2 Ac), and 1.852 (Ac).

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)-4*-O-*acetyl-3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**29**). — A mixture of the monoglycosylated products **25** and **28** (43 mg) was treated with 1:1 Ac_2O–C_5H_5N (4.0 mL) for 16 h at 20°. Evaporation of the solvent *in vacuo*, and separation of the residue by chromatography on SiO_2 in 2:1 toluene–ethyl acetate gave **26** (13.2 mg, 29.6%), R_F 0.53 in 1:1 toluene–EtOAc. Further elution gave **29** (10.2 mg, 23%); R_F 0.47 in 1:1 toluene–EtOAc; $[\alpha]_D$ +2.64° (*c* 0.51); n.m.r. data: δ_H 7.772–7.118 (aromatic), 5.835–5.734 (m, 1 H, –C*H*=CH_2), 5.758 (dd, 1 H, *J* 8.5 and 10.5 Hz, H-3b), 5.498 (d, 1 H, *J* 8.5 Hz, H-1b), 5.333 (d, 1 H, *J* 2.9 Hz, H-4c), 4.959 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3c), 4.922 (t, 1 H, *J* 9.3 Hz, H-4a), 4.650 (d, 1 H, *J* 1.9 Hz, H-1a), 4.536 (d,

1 H, *J* 7.8 Hz, H-1c), 4.349 (dd, 1 H, *J* 8.5 and 10.5 Hz, H-2b), 2.138, 2.064, 2.055, 2.028, 1.967, 1.911, and 1.877 (7 s, 21 H, 7 Ac).

Propyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*-*α*-D-*mannopyranoside* (**27**). — A mixture of **25** (80 mg, 0.72 mmol) and 10% Pd–C (50 mg) in MeOH (6 mL) was stirred under hydrogen for 17 h at 29°; the usual work-up afforded debenzylated product (64 mg, 95%; R_F 0.59 in 9:1 $CHCl_3$–MeOH), which was dissolved in 0.1M NaOMe–MeOH (4 mL). The mixture was stirred for 16 h at 29°, treated with Amberlyst A-15, and filtered. Evaporation of the filtrate *in vacuo* afforded crude deacetylated product (38 mg, 83%; R_F 0.52 in 2:1:1 BuOH–EtOH–H_2O). The product in MeOH (5 mL)–$BuNH_2$ (1 mL) was refluxed for 20 h to give the dephthaloylated product (R_F 0.28 in 2:1:1 BuOH–EtOH–H_2O), which was mixed with pyridine (2 mL)–Ac_2O (2 mL). The mixture was stirred for 16 h at 25°, and evaporated *in vacuo*. The residue was purified by chromatography on SiO_2 in 24:1 $CHCl_3$–MeOH, to give peracetylated product (52 mg, 96%; R_F 0.28 in 24:1 $CHCl_3$–MeOH). This product was deacetylated in 0.1M NaOMe–MeOH (3 mL), to give crude **27**, which was purified by chromatography on Sephadex G-25 in H_2O, to give pure **27** (20 mg, 63%); $[\alpha]_D$ +24.5° (*c* 0.45, H_2O);R_F 0.53 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°) 4.851 (d, 1 H, *J* 1.8 Hz, H-1a), 4.586 (d, 1 H, *J* 7.8 Hz, H-1b), 3.986 (bs, 1 H, H-2a), 2.057 (NAc), 1.597 (m, 2 H, CH_2CH_3), and 0.899 (t, 3 H, *J* 7.3 Hz, CH_2CH_3); δ_H (D_2O, 27°) 4.570 (d, 1 H, *J* 8.1 Hz, H-1b), 4.460 (d, 1 H, *J* 7.8 Hz, H-1c), 3.984 (bs, 1 H, H-2a), 2.057 (NAc), 1.600 (m, 2 H, CH_2CH_3), and 0.901 (t, 3 H, *J* 7.3 Hz, CH_2CH_3); δ_C (D_2O) 103.9 (C-1c, $^1J_{CH}$ 157 Hz), 102.3 (C-1b, $^1J_{CH}$ 164 Hz), 100.3 (C-1a, $^1J_{CH}$ 173 Hz), 23.0 (CH_2CH_3), 22.8 (CH_3CONH), and 10.8 (CH_3).

Anal. Calc. for $C_{23}H_{42}NO_{16}$: C, 47.01; H, 7.03; N, 2.38. Found: C, 47.06; H, 6.89; N, 2.61.

Allyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→2)*-O-[*β*-D-*galctopyranosyl-(1→4)*-O-*(2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)*]-*3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**17**). — A solution of **16** (5.3 g, 2.9 mmol) in 0.05M NaOMe–MeOH (120 mL) was stirred for 2 h at 25°, made neutral with Amberlyst A-15, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo*, to give crude **17** (3.75 g, 98%), a small portion of which was purified by chromatography on Sephadex LH-20 in MeOH to give pure **17**; $[\alpha]_D$ −10.1° (*c* 0.80, MeOH); R_F 0.61 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (CD_3OD) 5.75–5.60 (m, 1 H, $-CH=CH_2$), 5.311 (d, 1 H, *J* 8.6 Hz, H-1b or H-1c), 5.268 (d, 1 H, *J* 8.3 Hz, H-1c or H-1b), and 5.05–4.95 (m, 2 H, $CH=CH_2$); δ_C (CD_3OD) 105.1 (C-1d and C-1e, $^1J_{CH}$ 161 Hz), 99.2 (C-1b or C-1c), 98.4 (C-1c or C-1b), 97.8 (C-1a, $^1J_{CH}$ 167 Hz), 58.4 (C-2b or C-2c), and 57.7 (C-2c or C-2b).

Anal. Calc. for $C_{63}H_{74}N_2O_{28}\cdot H_2O$: C, 57.09; H, 5.78; N, 2.11. Found: C, 57.12; H, 5.71; N, 2.07.

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)-O-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)]-3,6-di-O-benzyl-α-D-mannopyranoside (**18**). — A solution of **17** (3.5 g, 2.7 mmol) in 1:1 $BuNH_2$–MeOH (150 mL) was stirred under reflux for 24 h, and evaporated *in vacuo*. The residue was homogeneous in t.l.c. (R_F 0.33 in 2:1:1 BuOH–EtOH–H_2O), and was dissolved in pyridine (150 mL) and Ac_2O (30 mL). The mixture was stirred for 16 h at 25°, and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 3:2 $CHCl_3$–Me_2CO, to give **18** (2.8 g, 64%); $[\alpha]_D$ −5.5° (*c* 1.1); R_F 0.58 in 1:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 5.95–5.8 (m, 1 H, *–CH*=CH_2), 5.357 (d, 1 H, *J* 3.2 Hz, H-4d), 5.327 (d, 1 H, *J* 3.2 Hz, H-4d), 4.968 (dd, 1 H, *J* 3.2 and 10.5 Hz, H-3e), and 4.909 (dd, 1 H, *J* 3.2 and 10.5 Hz, H-3d); δ_C 101.0 (C-1c*, C-1d, and C-1e, $^1J_{CH}$ 160 Hz), 99.4 (C-1b*, $^1J_{CH}$ 159 Hz), 96.2 (C-1a, $^1J_{CH}$ 169 Hz), 54.3 (C-2b and C-2c), and 23.1 (2 *C*H_3CONH).

Anal. Calc. for $C_{75}H_{98}N_2O_{38}\cdot H_2O$: C, 5.46; H, 6.09; N, 1.69. Found: C, 54.50; H, 6.09; N, 1.69.

Propyl O-β-D-galactopyranosyl-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-[β-D-galactopyranosyl-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)]-α-D-mannopyranoside (**19**). — A solution of **18** (168 mg, 0.1 mmol) in 0.1M NaOMe–MeOH (10 mL) was stirred for 2 h at 20°, treated with Amberlyst A-15, and the suspension filtered through Celite. Evaporation of the filtrate *in vacuo* afforded deacetylated product (77 mg; R_F 0.48 in 2:1:1 BuOH–EtOH–H_2O). A mixture of this product and 10% Pd–C (50 mg) in AcOH (3 mL) was stirred under H_2 for 3 h at 60°. The usual work-up, and purification by Sephadex G-25 in H_2O afforded **19** (29 mg, 66%); $[\alpha]_D$ 0° (*c* 0.3, H_2O); R_F 0.27 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°) 4.838 (d, 1 H, *J* 2 Hz, H-1a), 4.568 (d, 1 H, *J* 8.0 Hz, H-1c), 4.548 (d, 1 H, *J* 8.0 Hz, H-1b), 4.465 (d, 2 H, *J* 8.2 Hz, H-1d and H-1e), 4.052 (dd, 1 H, *J* 2.0 and 4.4 Hz, H-2a), 2.057 (NAc), 2.044 (NAc), 1.597 (m, 2 H, CH_2CH$_3$), and 0.901 (t, 3 H, *J* 7.7 Hz, CH_2CH_3); δ_H (D_2O, 20°) 4.564 (d, 1 H, *J* 7.6 Hz, H-1c), 4.523 (d, 1 H, *J* 8.2 Hz, H-1b), 4.460 (d, 2 H, *J* 7.6 Hz, H-1d and H-1e), 4.079 (bs, 1 H, H-2a), 2.058 (NAc), 2.043 (NAc), 1.598 (m, 2 H, CH_2CH$_3$), and 0.902 (t, 3 H, *J* 7.6 Hz, CH_2CH_3); δ_C (D_2O, 20°) 103.8 (C-1d and C-1e, $^1J_{CH}$ 157 Hz), 102.2 (C-1b, $^1J_{CH}$ 162 Hz), 100.4 (C-1c, $^1J_{CH}$ 163 Hz), 97.4 (C-1a, $^1J_{CH}$ 166 Hz), 23.2 (CH_2CH_3), 22.9 (CH_3CONH), 22.8 (CH_3CONH), and 10.7 (CH_2CH_3).

Anal. Calc. for $C_{37}H_{64}N_2O_{26}\cdot H_2O$: C, 45.77; H, 6.64; N, 2.88. Found: C, 45.52; H, 6.53; N, 2.90.

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-[(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→4)]-3,6-di-O-benzyl-α-D-mannopyranose (**20**) *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)-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→4)]-*3,6-di*-O-*benzyl*-α-D-*mannopyranoside* (**21**). — *Method A.* A mixture of **18** (2.8 g, 1.7 mmol), NaOAc (304 mg), and $PdCl_2$ (329 mg, 1.85 mmol) in AcOH (58 mL)–H_2O (2 mL) was stirred for 16 h at 20–25° and then for 1 h at 60°. Filtration of the mixture through Celite, and evaporation of the filtrate *in vacuo* afforded a residue which was dissolved in EtOAc (100 mL) and the solution successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated. Chromatography of the residue on SiO_2 in 3:2 $CHCl_3$–Me_2CO afforded **20** (1.36 g, 50%); $[\alpha]_D$ −14.8° (*c* 1.1); R_F 0.24 in 1:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 5.374 (d, 1 H, *J* 3.3 Hz, H-4e) and 5.328 (d, 1 H, *J* 3.4 Hz, H-4d); δ_C 100.6 (C-1b, C-1c, C-1d, and C-1e), 91.8 (C-1a), 54.0 (C-2b and C-2c), and 22.9 (2 CH_3CONH).

Anal. Calc. for $C_{72}H_{94}N_2O_{38}\cdot H_2O$: C, 53.59; H, 5.87; N, 1.73. Found: C, 53.77; H, 5.96; N, 1.53.

From the less-polar fractions, the major by-product **21** (790 mg, 28%) was isolated; R_F 0.38 in 1:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 7.5–7.3 (m, 10 H, aromatic), 5.355 (d, 1 H, *J* 3.5 Hz, H-4e), and 5.339 (d, 1 H, *J* 3.5 Hz, H-4d); δ_C 100.7 (C-1b*, C-1d and C-1e, $^1J_{CH}$ 161 Hz), 99.5 (C-1c*, $^1J_{CH}$ 164 Hz), 98.3 (C-1a, $^1J_{CH}$ 171 Hz), 54.0 (C-2b and C-2c), 25.9 (CH_2COCH_3), 22.9 (2 CH_3CONH), and 20.6 and 20.4 (12 CH_3COO).

Anal. Calc. for $C_{75}H_{98}N_2O_{39}\cdot H_2O$: C, 53.95; H, 6.03; N, 1.67. Found: C, 53.77; H, 5.93; N, 1.58.

Method B. A mixture of **18** (440 mg, 0.27 mmol), tris(triphenylphosphine)rhodium(I) chloride (25 mg), and 1,4-diazabicyclo(2.2.2)octane (DABCO) (175 mg) in 8:3:1 EtOH–benzene–H_2O (20 mL) was refluxed for 4 h. The mixture was evaporated to dryness *in vacuo,* and a solution of the residue in $CHCl_3$ was washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo,* to afford a residue (400 mg) (R_F 0.62 in 1:1 $CHCl_3$–acetone), which was dissolved in 8:2 THF–H_2O (10 mL). Iodine (97.0 mg) was added, and the solution was stirred for 80 min at 20°. The mixture was diluted with $CHCl_3$, successively washed with a saturated solution of $NaHSO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo,* to give a residue which was chromatographed on SiO_2 in 1:1 $CHCl_3$–acetone, to afford the desired hemiacetal **20** (355 mg, 82.7%).

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-[*(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→4)*]-*3,6-di*-O-*benzyl*-α-D-*mannopyranosyl trichloroacetimidate* (**5**). — To a stirred solution of **20** (206 mg, 124 μmol) in CH_2Cl_2 (5 mL) was added NaH (50%; 5.9 mg, 0.13 mmol) and Cl_3CCN (1.0 mL) at 0°. The mixture was stirred for 1 h at 0–20°, and filtered through Celite. The filtrate was evaporated *in vacuo,* and the residue was chromatographed on SiO_2 in EtOAc, to give **5** (206 mg, 92%); R_F 0.31 in EtOAc; n.m.r. data: δ_H 8.577 (s, 1 H, C=N*H*) and 6.261 (s, 1 H, H-1a); δ_C 160.2 (O-*C*=N), 100.7 (C-1b*, C-1d, and C-1e), 99.4 (C-1c*), 95.8 (C-1a), 90.6 (CCl_3), 54.0 (C-2b and C-2c), 22.9 (2 CH_3CONH), and 20.5 (12 CH_3CO_2).

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-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-β-D-glucopyranosyl)-(1→4)]-D-mannopyranose (**22**). — A mixture of **20** (500 mg, 0.31 mmol) and 10% Pd–C (100 mg) in 10:1 MeOH–AcOH (30 mL) was stirred under H_2 for 24 h at 20°. The usual work-up, and purification by chromatography on SiO_2 in 9:1 $CHCl_3$–MeOH, afforded **22** (348 mg, 90%); $[\alpha]_D$ −5.0° (*c* 0.2); R_F 0.24 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.358 (bs, 2 H, H-4d and H-4e) and 2.16–1.95 (14 Ac); δ_C 101.9 (C-1b, C-1c, C-1d, and C-1e, bs), 92.6 (C-1a), 23.6 (CH_3CONH), and 23.1 (CH_3CONH).

Anal. Calc. for $C_{56}H_{82}N_2O_{38}\cdot H_2O$: C, 48.60; H, 5.90; N, 1.95. Found: C, 48.51; H, 5.74; N, 1.90.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-β-D-glucopyranosyl)-(1→2)-O-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-β-D-glucopyranosyl)-(1→4)]-1,3,6-tri-O-acetyl-α-D-mannopyranose (**23**). — A solution of **22** (440 mg, 0.31 mmol) in 1:1 pyridine–Ac_2O (30 mL) was stirred for 19 h at 20°, and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 30:1 $CHCl_3$–MeOH, to give **23** (447 mg, 94%); $[\alpha]_D$ −9.8° (*c* 1.1); R_F 0.45 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 6.076 (d, 1 H, *J* 9.8 Hz, N*H*), 5.914 (d, 1 H, *J* 2.0 Hz, H-1a), 5.761 (d, 1 H, *J* 9.5 Hz, N*H*), 5.353 (d, 2 H, *J* 3.4 Hz, H-4d and H-4c), 4.482 (d, 2 H, *J* 7.8 Hz, H-1d and H-1e), and 2.184–1.938 (17 Ac); δ_C 101.4 (C-1b, C-1c, C-1d, and C-1e, $^1J_{CH}$ 165 Hz), 90.8 (C-1a, $^1J_{CH}$ 176 Hz), 53.6 (C-2b*), 53.2 (C-2c*), 22.7 (2 CH_3CONH), and 20.3 (15 CH_3COO).

Anal. Calc. for $C_{64}H_{88}N_2O_{41}\cdot 2\,H_2O$: C, 48.73; H, 5.87; N, 1.77. Found: C, 48.69; H, 5.57; N, 1.72.

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-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)]-3,6-di-O-acetyl-D-mannopyranose (**24**). — A solution of **23** (197 mg, 121 μmol) and $H_2NNH_2\cdot$AcOH (11.7 mg, 127 μmol) in DMF (3.0 mL) was stirred for 1 h at 25°, and diluted with EtOAc (50 mL). The organic layer was washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*, to give crude **24** (178 mg, 93%). A small portion was purified by chromatography on SiO_2 in 9:1 $CHCl_3$–MeOH, to give pure **24**; $[\alpha]_D$ −18.4° (*c* 1.5); R_F 0.32 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.355 (d, 2 H, *J* 3.2 Hz, H-4d and H-4e), and 2.156–1.906 (16 Ac).

Anal. Calc. for $C_{62}H_{86}N_2O_{46}\cdot H_2O$: C, 49.08; H, 5.84; N, 1.85. Found: C, 49.08; H, 5.66; N, 2.08.

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-[(2,3,4,6-tetra-O-acetyl-β-D-galctopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)]-3,6-di-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**6**). — A mixture of crude **24** (167 mg, 0.11 mmol), Cl_3CCN (223 μL, 2.22 mmol), and DBU (186 μL, 124 μmol) in $Cl(CH_2)_2Cl$ (3 mL) was stirred for 1 h at 0–20°, and then

directly applied to a chromatographic column of SiO_2 in 9:1 EtOAc–THF, to give **6** (182 mg, 99%). A small portion of **6** was further purified by chromatography on SiO_2 in 20:1 $CHCl_3$–MeOH; $[\alpha]_D$ −4.5° (*c* 1.1); R_F 0.46 in 9:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 8.636 (s, 1 H, C=N*H*), 6.245 (d, 1 H, *J* 9.5 Hz, N*H*), 6.086 (d, 1 H, *J* 2.0 Hz, H-1a), 5.840 (d, 1 H, *J* 9.5 Hz, N*H*), 5.359 (d, 1 H, *J* 3.0 Hz, H-4d*), 5.352 (d, 1 H, *J* 3.0 Hz, H-4e*), 4.483 (d, 2 H, *J* 8.1 Hz, H-1d and H-1e), and 2.162–1.956 (16 Ac); δ_C 160.4 (O–*C*=N), 100.8 (C-1b, C-1c, C-1d, and C-1e), 95.4 (C-1a), 90.5 (*C*Cl_3), 22.9 (2 *C*H_3CONH), 20.6, and 20.4 (14 *C*H_3COO).

Anal. Calc. for $C_{64}H_{86}Cl_3N_3O_{40}\cdot 1/6\ CHCl_3$: C, 46.32; H, 5.21; Cl, 7.45; N, 2.52. Found: C, 45.99; H, 5.21; Cl, 7.31; N, 2.47.

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-[(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→4)]-3,6-di-O-acetyl-α-D-mannopyranosyl fluoride (**7**) *and its β anomer* (**8**). — To a cooled solution of **20** (104 mg, 65 μmol) in DME (2.0 mL) was added diethylaminosulfur trifluoride Et_2NSF_3 (17.4 μL, 0.14 mmol) under Ar at 0°. The mixture was stirred for 1 h at 0°, and diluted with CH_2Cl_2 (20 mL). The organic layer was washed with ice–water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 1:1 $CHCl_3$–Me_2CO, to give **7** (58 mg, 57%); $[\alpha]_D$ −14.9° (*c* 2.2); R_F 0.61 in 1:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 5.717 (d, 1 H, *J* 9.5 Hz, N*H*), 6.628 (d, 1 H, *J* 9.8 Hz, N*H*), 5.538 (dd, 1 H, $^2J_{HF}$ 51.3, $^3J_{HH}$ 2.0 Hz, H-1a), 5.355 (d, 1 H, *J* 3.4 Hz, H-4e), 5.355 (d, 1 H, *J* 2.4 Hz, H-4d), 4.965 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3e), 4.923 (dd, 1 H, *J* 3.4 and 10.5 Hz, H-3d), and 2.148–1.764 (14 Ac); δ_C 100.8 (C-1b, C-1c, C-1d, and C-1e, $^1J_{CH}$ 162 Hz), 99.7 (one of the pair of doublets for C-1a, $^1J_{CH}$ 167 Hz); δ_F 135.8 (d, J_{HF} 51 Hz).

Anal. Calc. for $C_{72}H_{93}FN_2O_{37}$: C, 54.13; H, 5.87; F, 1.75; N, 1.75. Found: C, 54.06; H, 5.75; F, 1.77; N, 1.81.

Further elution afforded **8** (34 mg, 34%); $[\alpha]_D$ +22.3° (*c* 0.33); R_F 0.46 in 1:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 5.775 (d, 1 H, *J* 8.0 Hz, N*H*), 5.547 (d, 1 H, *J* 7.8 Hz, N*H*), 5.355 (d, 1 H, *J* 3.5 Hz, H-4e), and 5.322 (d, 1 H, *J* 3.5 Hz, H-4d); δ_F 139.6 (d, J_{HF} 34 Hz).

Anal. Calc. for $C_{72}H_{97}FN_2O_{37}\cdot H_2O$: C, 53.53; H, 5.92; N, 1.73. Found: C, 53.53; H, 5.85; N, 1.77.

2,4-Di-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranosyl fluoride. — To a cooled solution of 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranose (102 mg, 0.23 mmol) in dry THF (2.0 mL) was added diethylaminosulfur trifluoride Et_2NSF_3 (61.5 μL, 0.5 mmol), and the mixture was stirred for 1 h. The usual work-up, as described for **7** and **8**, gave crude product, which was chromatographed on SiO_2 in 5:1 hexane–ethyl acetate, to give a 4:1 mixture of α- and β-fluoride (82 mg, 50%); R_F 0.49 in 5:1 hexane–EtOAc; n.m.r. data: δ_H 5.610 (dd, 0.8 H, *J* 1.7 Hz, $^1J_{HF}$ 49.0 Hz, H-1α), 5.579 (dd, 0.2 H, *J* 2.2 Hz, $^1J_{HF}$ 48.3 Hz, H-1β), 5.459 (m, 1 H, H-2a), and 5.314 (t, 1 H, *J* 10 Hz, H-4); δ_C 105.3 (C-1α, $^1J_{CF}$ 222, $^1J_{CH}$ 183 Hz); δ_F 135.6 (d, $^1J_{HF}$ 49.0 Hz).

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-[*(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→4)-3,6-di*-O-*acetyl-α*-D-*mannopyranoside* (**31**). — To a mixture of **30** (10.5 mg, 51 μmol), **6** (35.6 mg, 22 μmol) and powdered molecular sieves AW-300 (250 mg) in $Cl(CH_2)_2Cl$ (1.6 mL) was added $BF_3 \cdot Et_2O$ (2.9 μL, 23.7 mmol) at −15°. The mixture was stirred for 13 h at −15 to 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 24:1 $CHCl_3$–MeOH afforded pure **31** (19.0 mg, 52% based on **6**);$[\alpha]_D$ −8.02° (*c* 0.93); R_F 0.5 in 9:1 $CHCl_3$–MeOH; ^{1}H-n.m.r. data: δ_H 6.451 (d, 1 H, *J* 9.7 Hz, N*H*), 5.900 (d, 1 H, *J* 9.5 Hz, N*H*), 5.360 (d, 1 H, *J* 3.9 Hz, H-4e), 5.350 (d, 1 H, *J* 4.1 Hz, H-4d), 4.957 (dd, 1 H, *J* 3.4 and 9.0 Hz, H-3e), 4.948 (dd, 1 H, *J* 3.4 and 9.0 Hz, H-3d), 4.619 (d, 1 H, *J* 1.5 Hz, H-1α), 4.506 (d, 1 H, *J* 8.3 Hz, H-1d*), 4.486 (d, 1 H, *J* 8.0 Hz, H-1e*), 2.158–1.943 (48 H, 16 Ac), 1.636–1.305 (12 H, spacer arm), and 1.256 (t, 2 H, *J* 7.2 Hz, $-CH_2CH_3$).

Anal. Calc. for $C_{73}H_{106}N_2O_{42} \cdot CHCl_3$: C, 49.28; H, 5.98; N, 1.55. Found: C, 49.48; H, 6.01; N, 1.67.

8-Methoxycarbonyloctyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→2)*-O-[*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*]-*α*-D-*mannopyranoside* (**32**). — A solution of **31** (17.3 mg, 10.2 μmol) in 0.1M NaOMe–MeOH (3.0 mL) was stirred for 20 h at 20°, treated with Amberlyst A-15, and the suspension filtered through Celite. Evaporation of the filtrate *in vacuo* afforded crude **32** (8.0 mg, 71.5%), which was purified on Sephadex LH-20 in 1:1 THF–H_2O, to give pure **32** (6.0 mg, 54%); $[\alpha]_D$ +1° (*c* 0.23, H_2O); R_F 0.41 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 50°) 4.831 (s, 1 H, H-1a), 4.053 (m, 1 H, H-2a); δ_H (D_2O, 20°) 4.848 (s, 1 H, H-1a), 4.566 (d, 1 H, *J* 7.6 Hz, H-1c), 4.525 (d, 1 H, *J* 8.0 Hz, H-1b), 4.460 (d, 2 H, *J* 7.8 Hz, H-1d, H-1e), 4.067 (m, 1 H, H-2a), 3.683 (s, 3 H, OCH_3), 2.387 (t, 2 H, *J* 7.4 Hz, $-CH_2CO$), 2.057 (NAc), 2.044 (NAc), and 1.595–1.303 (12 H, spacer arm).

Anal. Calc. for $C_{44}H_{76}N_2O_{28} \cdot 4\ H_2O$: C, 45.83; H, 2.43. Found: C, 45.86; H, 2.74.

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-[*(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→4)]-3,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**33**) *and β anomer* (**34**). — *Method A.* To a mixture of **30** (14 mg, 69 μmol), **5** (39 mg, 22 μmol), and powdered molecular sieves AW-300 (200 mg) in $Cl(CH_2)_2Cl$ (1.6 mL) was added $BF_3 \cdot Et_2O$ (2.7 μL, 22 μmol) at −15°. The mixture was stirred for 2 h at −15°, then for 16 h at 25°, and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 3:2 $CHCl_3$–Me_2CO, to give

a mixture of **33** and **34** (26 mg, 66% based on **5**). This mixture was further separated by preparative t.l.c. in 24:1 $CHCl_3$–MeOH, to give **33** (9.0 mg), and **34** (7.7 mg).

Physical data for **33**: $[\alpha]_D$ −12.5° (*c* 0.16); R_F 0.21 in 24:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 7.40–7.25 (m, 10 H, 2 C_6H_5), 5.358 (d, 1 H, *J* 3.5 Hz, H-4e), 5.330 (d, 1 H, *J* 3.5 Hz, H-4d), 2.294 (t, 2 H, *J* 7.6 Hz, CH_2CO), 2.20–1.90 (14 Ac), and 1.35–1.20 (11 H, m, spacer arm).

Physical data for **34**: $[\alpha]_D$ −22.1° (*c* 0.28); R_F 0.24 in 24:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 7.40–7.25 (m, 10 H, 2 C_6H_5), 5.350 (d, 1 H, *J* 3.5 Hz, H-4e), 5.328 (d, 1 H, *J* 3.5 Hz, H-4d), 2.288 (t, 2 H, *J* 7.6 Hz, CH_2CO), 2.18–1.94 (14 Ac), and 1.38–1.20 (m, 11 H).

Anal. of a mixture of **33** and **34**. Calc. for $C_{83}H_{114}N_2O_{40}\cdot H_2O$: C, 55.45; H, 6.50; N, 1.55. Found: C, 55.21; H, 6.42; N, 1.52.

Method B. To a mixture of **30** (10.0 mg, 0.05 mmol), $AgClO_4$ (6.0 mg, 0.03 mmol), $SnCl_2$ (6.0 mg, 0.03 mmol), and powdered molecular sieves 4Å in dry Et_2O (6.0 mL), was added a solution of **7** (39.0 mg, 24 μmol) in 5:3 Et_2O–$Cl(CH_2)_2Cl$ (1.5 mL) at −15°. The mixture was stirred for 13 h at −15 to 20°, filtered through Celite, and the filtrate evaporated *in vacuo,* to afford a residue which was chromatographed on SiO_2 in 30:1 $CHCl_3$–MeOH, to give a 1:3 mixture (t.l.c. evidence) of **33** and **34** (31.0 mg, 71% based on **7**).

Method C. To a mixture of **30** (4.7 mg, 23 μmol), $AgClO_4$ (3.75 mg, 18 μmol), $SnCl_2$ (4.0 mg, 21 μmol), and powdered molecular sieves 4Å (200 mg) in dry Et_2O (3.0 mL) was added a solution of **8** (26.7 mg, 17 μmol) at −15°, and the mixture was stirred for 13 h at −15 to 20°. The usual work-up, and chromatography on SiO_2 in 30:1 $CHCl_3$–MeOH, gave a 1:3 mixture of **33** and **34** (19.0 mg, 64%).

8-Methoxycarbonyloctyl O-β-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)*-O-[β-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→4)*]-α-D-*mannopyranoside* (**32**) *and its β anomer* (**35**). — The 1:3 mixture of **33** and **34** (49.0 mg, 27.4 μmol) and 10% Pd–C in MeOH (5 mL) was stirred under H_2 for 20 h at 20°. The usual work-up gave the crude, debenzylated product (29.6 mg, 67%), which was dissolved in MeOH (2.0 mL) and the solution treated with 0.1M NaOMe (0.2 mL) for 6 h at 20°. Neutralization of the base with Amberlyst A-15, followed by filtration, and evaporation of the filtrate *in vacuo,* afforded a crude mixture of **32** and **35** which was purified on Sephadex LH-20 in 1:1 THF–H_2O, to give a mixture of **32** and **35** (14.1 mg, 70.4%); R_F 0.41 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data for **32**: δ_H (D_2O, 50°) 4.828 (s, 0.25 H, H-1a), 4.465 (d, 0.5 H, *J* 7.8 Hz, H-1d and H-1e), 4.040 (m, 0.25 H, H-2a), 3.692 (s, 0.75 H, OCH_3), 2.282 (t, 0.5 H, *J* 7.3 Hz, CH_2CO), 2.058 (NAc), and 2.044 (NAc); δ_H (D_2O, 27°) 4.565 (d, 0.25 H, *J* 7.6 Hz, H-1c), 4.525 (d, 0.25 H, *J* 8.0 Hz, H-1b), 4.460 (d, 0.5 H, *J* 7.8 Hz), and 4.066 (m, 0.25 H, H-2a); n.m.r. data for **35**: δ_H (D_2O, 50°) 4.850 (d, 0.75 H, *J* 8.5 Hz, H-1c), 4.618 (s, 0.75 H, H-1a), 4.465 (d, 1.5 H, *J* 7.8 Hz, H-1d and H-1e), 4.202 (d, 0.75 H, *J* 3.2 Hz, H-2a), 3.692 (s, 2.25 H, OCH_3), 2.282 (t, 1.5 H, *J* 7.3 Hz, CH_2CO), 2.058 (NAc), and 2.044 (NAc); δ_H (D_2O, 27°) 4.850 (bd, 0.75 H, *J* 8.5 Hz, H-1c), 4.630 (s, 0.75 H, H-1a),

4.525 (d, 0.75 H, *J* 8.0 Hz, H-1b), 4.460 (d, 1.5 H, *J* 7.8 Hz, H-1d and H-1e), and 4.212 (d, 0.75 H, *J* 2.9 Hz, H-2a).

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-*[(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→4)*]-O-*(3,6-di*-O*-benzyl-α-and -β-*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* (**36**) *and* (**37**). — *Method A.* To a mixture of **9** (139 mg, 0.13 mmol), powdered molecular sieves AW 300 (500 mg), and **5** (195 mg, 113 μmol) in $Cl(CH_2)_2Cl$ (3.0 mL) was aded $BF_3 \cdot Et_2O$ (13.8 μL, 113 μmol) at 0°. The mixture was stirred 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*. The residue was chromatographed on Bio-Beads SX-3 (120 cm × 3 cm) in benzene, to give a heptasaccharide fraction (32 mg, 11.0% based on **5**; 45% based on **9**), which was a 1:2 mixture giving two spots, R_F 0.45 and 0.35 in 2:1 $CHCl_3$–Me_2CO. Purification by chromatography on SiO_2 in 2:1 $CHCl_3$–Me_2CO afforded **37** (6.0 mg); $[\alpha]_D$ −10.0° (*c* 0.19); R_F 0.45 in 2:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 7.45–7.15 (m, 40 H, 8 C_6H_5), 3.175 (m, 2 H, H-4f, H-4g), 2.250 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.145–1.965 (14 Ac), and 1.670–1.229 (m, 15 H, spacer arm).

Anal. Calc. for $C_{137}H_{170}N_2O_{50} \cdot CHCl_3$: C, 59.96; H, 6.23; N, 1.01. Found: C, 60.07; H, 6.38; N, 0.88%

Further elution with the same eluant afforded **36** (13.5 mg, 4.6%); $[\alpha]_D$ −6.6° (*c* 0.2); R_F 0.35 in 2:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 7.4–7.2 (m, 40 H, 8 C_6H_5), 5.355 (d, 1 H, *J* 3.5 Hz, H-4g), 5.320 (d, 1 H, *J* 3.5 Hz, H-4f), 2.261 (t, 2 H, *J* 7.5 Hz, CH_2CO), 2.181–1.758 (14 Ac), 1.62–1.50 (m, 4 H, 2 CH_2), and 1.35–1.20 (m, 11 H).

Anal. Calc. for $C_{137}H_{170}N_2O_{50} \cdot CHCl_3$: C, 59.96; H, 6.23; N, 1.01. Found: C, 60.21; H, 6.15; N, 1.18.

The second fraction obtained from the gel-permeation chromatography on Bio-Beads SX-3 consisted of a mixture of byproducts (140 mg) arising from the pentasaccharide donor. Purification of the mixture by column chromatography in 1.5:1 $CHCl_3$–Me_2CO gave **38** (40 mg), **39** (55 mg), and the hydrolyzed donor **20** (20 mg). The structures of **38** and **39** were tentatively assigned by their n.m.r. data.

Physical data for **38**: R_F 0.52 in 1.5:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 7.45–7.15 (m, 10 H, 2 C_6H_5), 6.442 (d, 1 H, *J* 2 Hz, H-1a), 5.669 (d, 1 H, *J* 8.5 Hz, N*H*), 5.359 (d, 1 H, *J* 4.2 Hz, H-4e), 5.348 (d, 1 H, *J* 4.2 Hz, H-4c), and 2.2–1.7 (14 Ac).

Physical data for **39**: R_F 0.39 in 1.5:1 $CHCl_3$–Me_2CO; n.m.r. data: δ_H 7.45–7.20 (m, 10 H, 2 C_6H_5), 6.120 (d, 1 H, *J* 10 Hz, N*H*$COCCl_3$), 5.920 (d, 1 H, *J* 9.8 Hz, N*H*), 5.670 (d, 1 H, *J* 9.0 Hz, N*H*), 5.357 (d, 1 H, *J* 3.4 Hz, H-4e), 5.330 (d, 1 H, *J* 3.4 Hz, H-4d), and 2.2–1.7 (14 Ac).

Method B. To a mixture of **9** (88.0 mg, 82 μmol), $AgClO_4$ (13.7 mg, 66 μmol), $SnCl_2$ (13.5 mg, 72 μmol) and powdered molecular sieves 4A (750 mg) in diethyl ether (3.0 mL) was added a solution of **7** (88.0 mg, 55 μmol) in 8:3 Et_2O–

$Cl(CH_2)_2Cl$ (11 mL) at −15°. The mixture was stirred for 16 h at −15 to 20°, and filtered through Celite. The filtrate was evaporated *in vacuo*, the residue dissolved in $CHCl_3$, and the solution washed successively with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*, to afford a residue which was separated by gel-permeation chromatography on Bio-Beads SX-3 (120 × 3 cm) in benzene, to give a heptasaccharide fraction (12.0 mg, 26% based on the donor) and a pentasaccharide fraction (62 mg). T.l.c. examination of the heptasaccharide fraction in 2:1 $CHCl_3$–Me_2CO indicated a 1:1 mixture of **36** and **37**. The mixture was converted into the free heptasaccharide haptens without separation. The pentasaccharide fraction was purified by chromatography on SiO_2 in 2:1 $CHCl_3$–Me_2CO, to give unreacted donor **7** (60 mg, 65%).

8-Methoxycarbonyloctyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→2)*-O-[*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*]-O-*α*-D-*mannopyranosyl-(1→3)*-O-[*α*-D-*mannopyranosyl-(1→6)*]-*β*-D-*mannopyranoside* (**2**). — *Method A.* A solution of **36** (4.9 mg, 1.8 μmol) in 0.1M NaOMe–MeOH (1 mL) was stirred for 5 h at 18°, made neutral with Amberlyst A-15, and the suspension filtered. The filtrate was evaporated *in vacuo*, to give deacetylated product (2.5 mg, 64%); R_F 0.59 in 2:1:1 BuOH–EtOH–H_2O. A mixture of the deacetylation product (2.5 mg) and 10% Pd–C (10 mg) in 9:1 MeOH–AcOH (1 mL) was stirred under H_2 for 36 h at 20°, and then filtered through Celite. The Celite was washed with 1:1 THF–H_2O. The filtrates were combined, and evaporated *in vacuo*, to give crude **2**, which was purified by chromatography on Sephadex LH-20 in 1:1 THF–H_2O, to give **2** (1.5 mg, 54%); R_F 0.23 in 2:1:1 BuOH–EtOH–H_2O, which was identified by comparing the ^{1}H-n.m.r. data with those of authentic **2** previously prepared by a stepwise approach.

Method B. A 1:1 mixture of **36** and **37** (12.0 mg, 44 μmol) was deacetylated in 0.1M NaOMe–MeOH (2.0 mL) for 14 h at 18° made neutral with Amberlyst A-15, the suspension filtered, and the filtrate evaporated *in vacuo*, to afford the deacetylation product; R_F 0.59 in 2:1:1 BuOH–EtOH–H_2O. Hydrogenolysis of this product in 9:1 MeOH–AcOH for 16 h at 20°, and the usual work-up afforded crude heptasaccharide hapten, which was purified on Sephadex LH-20 in 1:1 THF–H_2O, to give **2** (2.5 mg, 36.3%); n.m.r. data: δ_H (D_2O, 20°) 5.119 (s, 1 H, H-1c), 4.920 (s, 1 H, H-1b), 4.766 (s, 1 H, H-1a), 4.561 (d, 1 H, *J* 7.5 Hz, H-1d or H-1e), 4.541 (d, 1 H, *J* 7.8 Hz, H-1e or H-1d), 4.462 (d, 2 H, *J* 7.6 Hz, H-1f and H-1g), 4.214 (bs, 1 H, H-2c), 4.094 (bs, 1 H, H-2a), 3.683 (OCH_3), 2.385 (t, 2 H, *J* 7.3 Hz, CH_2CO), 2.068 (NAc), 2.042 (NAc), 1.595 (m, 4 H), and 1.4–1.2 (m, 11 H, spacer arm).

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