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Rationally designed syntheses of high-mannose and complex type undecasaccharides ☆

Yuko Nakahara [a], Shohei Shibayama [a], Yoshiaki Nakahara [a], Tomoya Ogawa [a,b,*]

[a] *The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-01, Japan*
[b] *Graduate School for Agriculture and Life Sciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113, Japan*

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Abstract

Synthetic routes are described to a high-mannose type triantenary undecasaccharide **1** and a completely protected form **39** of the complex type biantenary undecasaccharide **2** carrying α-(2 → 3)-linked sialic acid residues. Starting from a previously reported trisaccharide **4**, the core pentasaccharides **15** and **37** were synthesized through regio- and/or stereo-selective mannosylation with a suitably protected mannosyl donor. Chain elongation of **15** by stepwise addition of a mannose residue afforded an undecasaccharide **20** that was eventually deprotected to give **1**. On the other hand, coupling of **38** and a trisaccharide glycosyl donor **36** afforded **39**.

Keywords: Undecasaccharide, high-mannose; Complex type; Glycosylation; Glycans, cell-surface

1. Introduction

Glycosylation of Asn residues of proteins and further processing of the glycans, which occurs in the endoplasmic reticulum and Golgi apparatus, are important processes that endow the proteins with a functional three-dimensional structure, stability against proteolysis, and capability in protein transport or secretion [2]. The most remarkable function of the structurally diverse oligosaccharides attachments is found in cellular recognition. However, details of the recognition mechanisms have only poorly been

☆ Part 102 in the series Synthetic Studies on Cell-surface Glycans. For Part 101, see ref. [1].

* Corresponding author.

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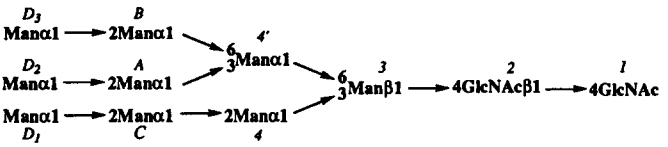

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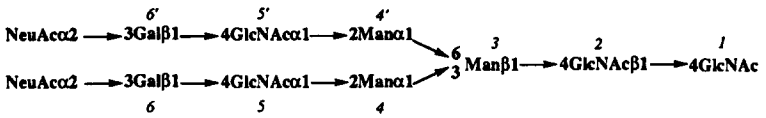

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understood. In order to clarify these molecular mechanisms, chemically or chemoenzymatically synthesized oligosaccharides are required because such homogeneous oligosaccharide samples are hardly obtainable from natural sources.

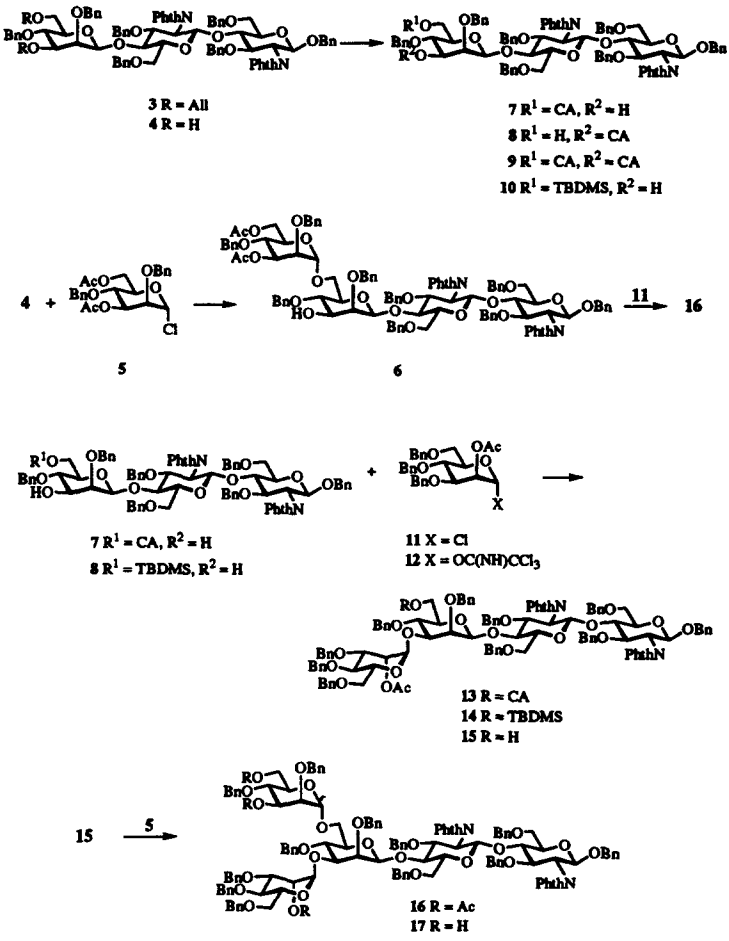
In the course of our synthetic studies on cell-surface glycans, we have reported syntheses of the N-linked oligosaccharides of glycoproteins and their fragments, where we have discussed several approaches in terms of strategies in blockwise synthesis as well as tactics in the glycosylation reaction [3]. Based on the knowledge obtained from these preliminary experiments, synthesis of larger oligosaccharides is now available.

We describe here a rationally designed synthetic route to the high mannose type and complex type undecasaccharides, both of which were synthesized through a common key intermediate trisaccharide **4** [3a,b].

2. Results and discussion

High-mannose type undecasaccharide.—The triantenary undecasaccharide **1** represents a basic structure of a high-mannose type N-linked glycan that was first isolated as the largest oligosaccharide of a unit A glycopeptide of calf thyroglobulin [4]. The same structure was identified in carbohydrates derived from various sources such as the urine of mannosidosis patients [5]. It is also notable that a series of high-mannose type glycans are expressed on the HIV glycoprotein gp 120 at high levels [6]. Synthesis was designed so that regioselective glycosylation of the trisaccharide **4**, either with a mannosyl donor **14** suitably substituted for 6-*O*-branching or with an another donor **9** for 3-*O*-branching would be expected on the basis of our previous findings [3d,e].

In 1983 we reported a properly protected glycotriosyl acceptor **4** corresponding to the core structure of N-linked glycan, which was synthesized via insoluble Ag silicate-mediated mannosylation [7], giving a precursor diallyl ether **3** in 40% yield, followed by subsequent $PdCl_2$-catalyzed deallylation (58%) [3a,b]. After more efficient deallylation

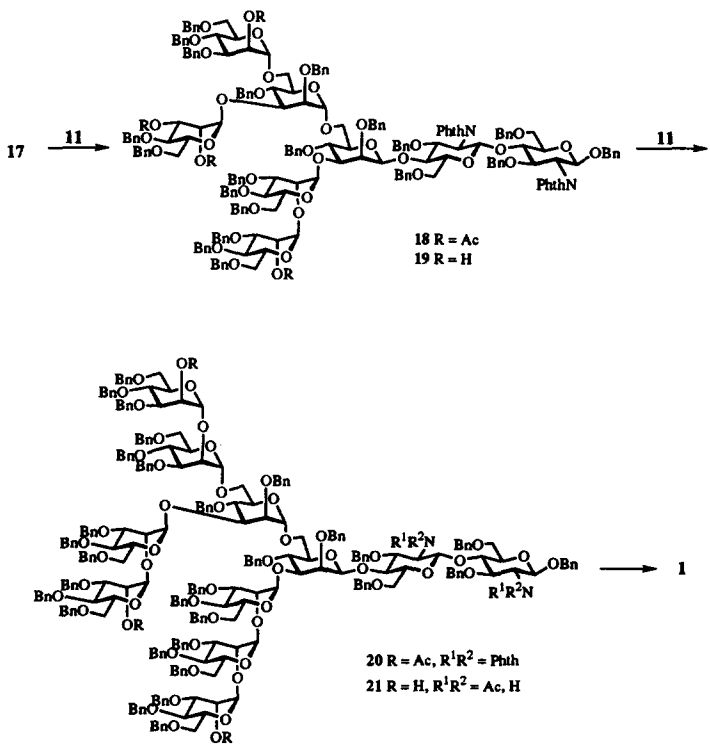


Scheme 1.

conditions were developed, we were able to obtain **4** quantitatively by Ir-complex catalyzed allyl–prop-1-enyl isomerization [8], followed by hydrolysis with Hg salts [9] (Scheme 1).

In order to install the dissymmetrical array of mannopentaosyl and mannotriosyl fragments onto the trisaccharide **4**, it was necessary to discriminate between the two hydroxyl groups of **4**. Selective mono-mannosylation of **4** was first examined using 1.4 molar equivalents of mannosyl donor **5** [3b] under Helferich conditions. However, conversion was poor and formation of the desired 6-*O*-mannosylated product **6** was only in 31% yield. Selective protection of the more reactive primary hydroxyl group with chloroacetyl chloride (1.6 equiv) in pyridine at a lowered temperature (−20°C) was carried out next. The reaction gave 6-*O*-monoacylated product **7** (60%), 3-*O*-isomer **8** (4%), and diacylated derivative **9** (22%). The latter two were readily hydrolyzed to

regenerate **4**. Glycosylation of **7** with **11** [3j] took place in the presence of AgOTf to give a tetrasaccharide **13** in a quantitative yield. Alternatively, selective protection of the 6-hydroxyl function was achieved by silylation with a bulky *tert*-butyldimethylsilyl group. Treatment of **4** with 2 molar equiv of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) and 2,6-lutidine exclusively afforded 6-*O*-silylated product **10**. An attempt to react **10** with **11** using AgOTf was unsuccessful due to the lability of the silyl protecting group under the conditions necessary for glycosylation at both the 6-*O*- and 3-*O*-positions. On the other hand, reaction of **10** with a trichloroacetimidate **12** produced the desired tetrasaccharide **14** in good yield by using $BF_3 \cdot Et_2O$ as a promoter [10]. Hydrolysis of chloroacetate **13** by heating with thiourea or by removal of the silyl group from **14** with aqueous hydrofluoric acid gave a tetrasaccharide acceptor **15** in 93% and quantitative yields, respectively. The coupling reaction of **5** with **15** using AgOTf afforded a pentasaccharide **16** in a quantitative yield, while glycosylation of **6** with **11** provided the same compound **16** in 68% yield. Having prepared the pentasaccharide **16**, α-mannoside chain-elongation was accomplished in a straightforward fashion by liberating the triol **17** under mildly basic conditions ($LiOH–H_2O_2$) without damaging the phthaloyl group in 80% yield, followed by glycosylation with 4.4 molar equiv of **11** to give an octasaccharide **18** (64%). The procedure for deacetylation and glycosylation was repeated for **18** to give a protected undecasaccharide **20** in 37% yield. The undecasac-



Scheme 2.

charide **20** was treated with hydrazine hydrate in ethanol at 90°C, then with acetic anhydride in methanol to convert phthalimide into acetamide and concomitantly hydrolyze the acetate, affording **21** in 65% yield. Finally, compound **21** was hydrogenated

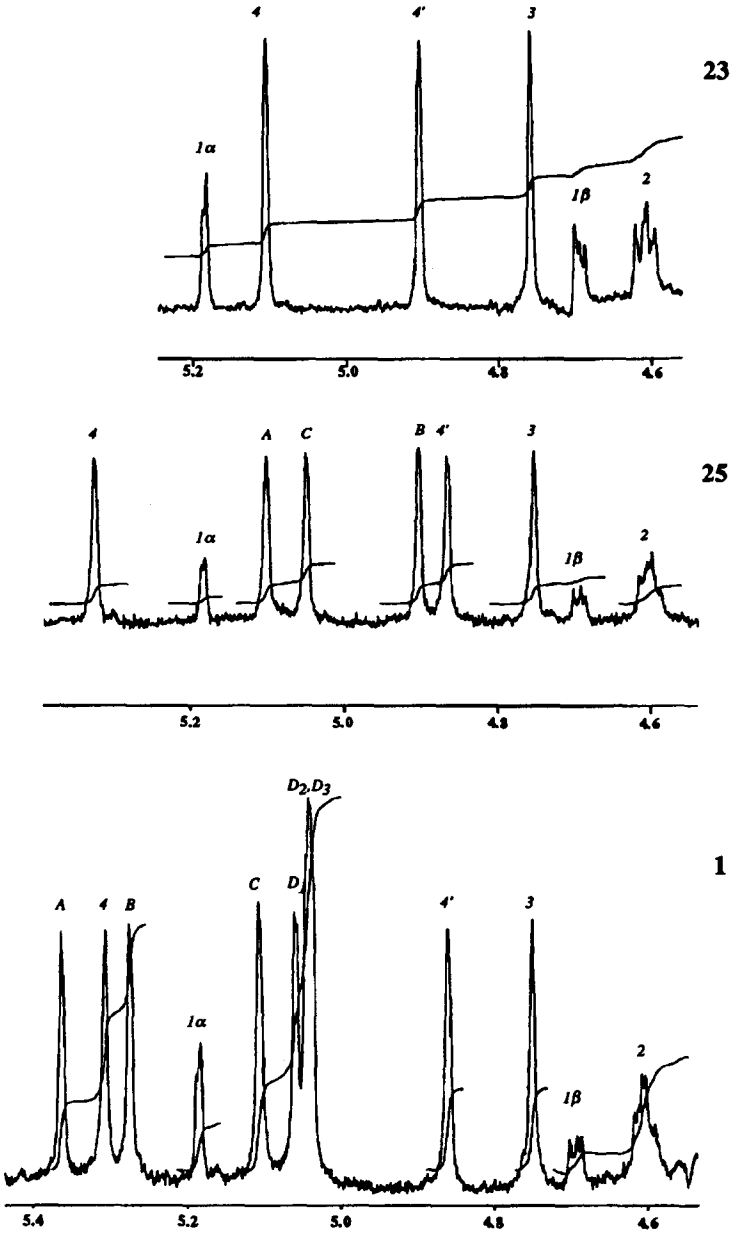
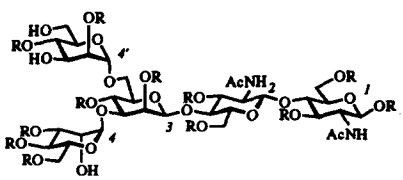


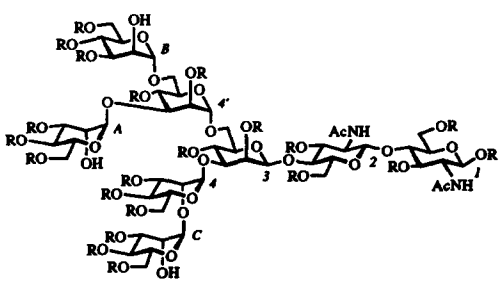
Fig. 1. Anomeric proton signal of **1**, **23**, and **25** in 500 MHz ^{1}H NMR spectra (D_2O, 60°C).

with 10% Pd–C in methanol, and the product was chromatographed by gel-permeation on Sephadex LH 20 to complete the synthesis of the fully deprotected undecasaccharide **1** (Scheme 2). ^{1}H NMR spectral data of the synthesized sample are in good agreement with those of the related natural decasaccharide derived from mannosidosis urine [5] (Fig. 1). The FAB mass spectrum of synthetic **1** indicated the desired molecular weight. The intermediary pentasaccharide **15** and octasaccharide **18** were also deprotected to free pentasaccharide **23** and octasaccharide **25**, respectively, and fully characterized (Fig. 1).

Complex-type undecasaccharide.—The undecasaccharide **2** is a member of a large family of N-glycans having their structures modified by multiple additions of *N*-acetyllactosamine units (complex type), and the peripheral branches are terminated with α-(2 → 3)-linked *N*-acetylneuraminic acid (sialic acid) residues. For example, the oligosaccharide **2** has been identified as an N-glycan attached to human chorionic gonadotropin (hCG) [11]. Although the asialo analog and some other partial structures have already been synthesized by several groups [3,12–14], this particular undecasaccharide **2** has not been constructed yet. In 1986, we reported a total synthesis of an α-D-Neu*p*5Ac-(2 → 6)-linked isomer of undecasaccharide, another typical complex type of glycan, which was accomplished by a convergent synthesis involving condensation of two molecules of nonreducing tetrasaccharide donor and the trisaccharide acceptor **4** in a stereocontrolled manner [3f]. For the synthesis of **2**, we now choose a different way of strategic bond disconnection, by taking into account the ready accessibility to the core

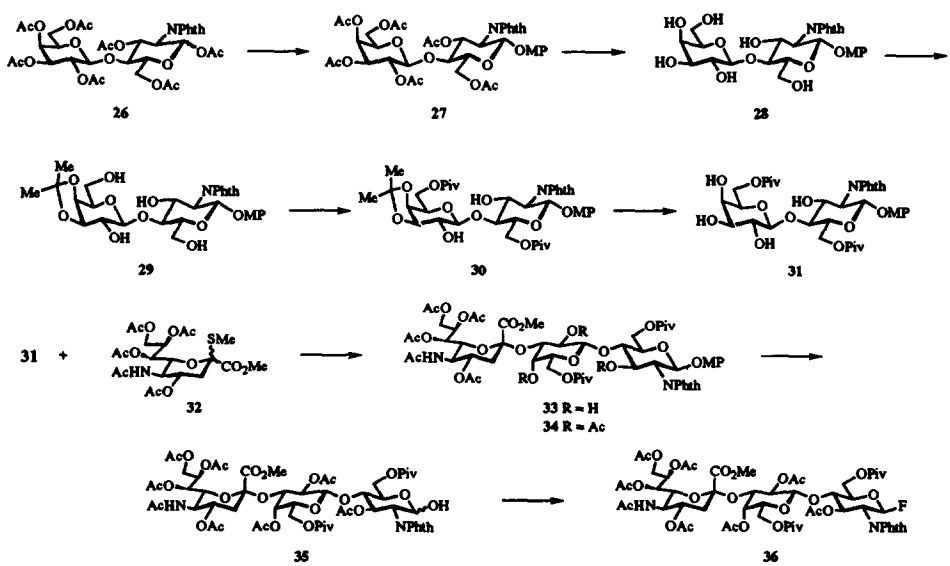


22 R = Bn
23 R = H

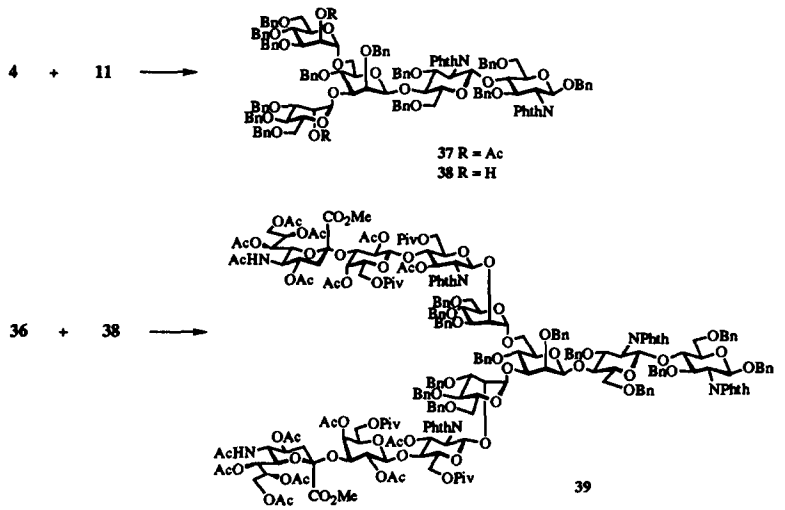


24 R = Bn
25 R = H

pentasaccharide as described above. The necessary trisaccharide donor **36** was prepared using recently developed procedures for α-selective sialylation [15]. Compound **26**, prepared by modifying the known procedure [16] through azidonitration of D-lactal hexaacetate, was treated with 4-methoxyphenol and trifluoromethanesulfonic acid (TfOH) to afford a 4-methoxyphenyl glycoside **27**, which on deacetylation gave **28** (Scheme 3). The methoxyphenyl glycoside **28** was regioselectively isopropylidenated to **29** (83%), which was then treated with pivaloyl chloride and DMAP in pyridine to give a dipivalate **30** in 83% yield. Upon hydrolysis of the acetonide of **30** with aqueous trifluoroacetic acid, a tetraol disaccharide **31** (78%) was obtained that was used as a glycosyl acceptor in the subsequent sialylation reaction. Reaction of **31** with methylthio glycoside **32** was promoted by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [17] in acetonitrile at $-40°C$ to produce trisaccharide **33** as a sole product, which was readily acetylated to give **34** in an isolated yield of 63%. The regio- and stereo-chemistry of the sialylation were assigned by the ^1H NMR spectrum of **34** by comparison with the reported data of the related compounds [15b,18]. The trisaccharide **34** was transformed into a glycosyl fluoride **36** in two steps by cleavage of the methoxyphenyl glycoside to generate hemiacetal **35**, and then by treatment with diethylaminosulfur trifluoride (DAST) in 67% overall yield. The acceptor pentasaccharide **38** was prepared by glycosylation of **4** with 4 molar equiv of **11**, followed by deacetylation in 72% overall yield (Scheme 4). With glycosyl donor **36** and acceptor **38** in hand, the coupling reaction was executed in the presence of a hafnocene-complex $[Cp_2Hf(ClO_4)_2]$ [19] in dichloromethane. The product was purified by gel-permeation chromatography with Bio-beads S, then by preparative thin-layer chromatography on silica gel, and finally by HPLC with a C_{18} reversed-phase column to give a 32% yield of the desired undecasaccharide **39**. The structure of **39** was assignable from the signals



Scheme 3.



Scheme 4.

in the 500 MHz ^{1}H NMR spectrum of four *tert*-butyl groups for pivalate and two methyl groups for the sialic acid ester. Electrospray ionization-mass spectral (ESIMS) data of **39** showed a dicationic fragment at 2320.7 for $M - CO_2CH_3$. The configuration of the newly formed glycosidic linkages was tentatively assigned based on the strongly β-selective nature of the glycosyl donor carrying a 2-phthalimido group [20].

Deprotection of the synthetic sample **39** remains to be accomplished. An attempted dephthaloylation of the Li-carboxylate derivative of **39** obtainable by treatment with LiI and pyridine by the conventional procedure with hydrazine afforded an unidentified product probably derived by cleavage of the acetamido group of the sialic acid residues.

In conclusion, we achieved a stereocontrolled synthesis of undecasaccharides with a typical high-mannose and a complex type N-linked glycoprotein glycan. We believe that the structurally diverse N-glycans of biological interest will become available through similar strategic bond disconnection approaches discussed here. Studies are underway to develop a practical technology for constructing complex oligosaccharide–peptide conjugates that will enhance the applicability and usefulness of synthetic samples as molecular probes.

3. Experimental

General.—Optical rotations were determined with a Jasco DIP-370 polarimeter for solutions in $CHCl_3$, unless noted otherwise. Column chromatography was performed on Silica Gel-60 (E. Merck, 70–230 mesh). Flash chromatography was performed on Wako Gel C-300 (200–300 mesh). TLC and HPTLC were performed on Silica Gel 60 F_{254} (E. Merck). ^{1}H, ^{13}C NMR spectra were recorded with either a JEOL GX500 [^{1}H (500MHz)]

or EX270 [^{1}H (270MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$.

Benzyl 2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**4**).—A red suspension of the Ir-complex {$[Ir(COD)(PMePh_2)]_2PF_6$, 21 mg} in freshly distilled THF (15 mL) was stirred in an atmosphere of H_2 at room temperature for 30 min to give a colorless solution of the activated catalyst, and then the atmosphere was replaced with N_2. To the solution was added a carefully degassed solution of **3** (1.02 g, 92 mmol) in dry THF (20 mL). After stirring for 30 min, the reaction mixture was concentrated in vacuo. The residue was dissolved in 90% aq acetone (9 mL) and stirred with $HgCl_2$ (690 mg, 2.54 mmol) and HgO (14 mg, 60 μmol) at room temperature for 1 h. The mixture was concentrated in vacuo to remove acetone, the residue was extracted with $CHCl_3$, the extract was washed with 10% aq KI and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by column chromatography on silica gel with 3:1 toluene–EtOAc to give **4** (940 mg, 98%): $[\alpha]_D$ −4.5° (*c* 1.0); R_f 0.39 (2:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.29 (d, 1 H, *J* 7.7 Hz, H-1*2*), 4.96 (d, 1 H, *J* 8.4 Hz, H-1*1*), 4.61 (bs, 1 H, H-1*3*); ^{13}C, δ 101.3 (C-1*3*), 97.1, 97.06 (C-1*1*, C-1*2*). Anal. Calcd for $C_{83}H_{80}N_2O_{18}$: C, 71.08; H, 5.82; N, 2.00. Found: C, 71.04; H, 5.78; N, 1.96.

Benzyl 3,6-di-O-acetyl-2,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**).—To a mixture of **4** (76 mg, 54 μmol), $HgBr_2$ (79 mg, 0.22 mmol), $Hg(CN)_2$ (166 mg, 0.66 mmol) and powdered 4 Å molecular sieves (1 g) in dry dichloroethane (6 mL) was stirred at 0°C under N_2 for 15 min. Then a solution of **5** (37 mg, 77 μmol) in dry dichloroethane (6 mL) was added dropwise to the above mixture. The mixture was stirred at room temperature for 24 h, diluted with chloroform, and filtered through Celite. The filtrate was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 5:1 toluene–EtOAc to give **6** (31 mg, 31%) and unreacted **4** (53 mg, 69%): $[\alpha]_D$ −4.3° (*c* 1.7); R_f 0.29 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.24 (dd, 1 H, *J* 9.5 and 3.3 Hz, H-3*4'*), 5.23 (d, 1 H, *J* 7.7 Hz, H-1*2*), 4.99 (d, 1 H, *J* 1.8 Hz, H-1*4'*), 4.93 (d, 1 H, *J* 8.4 Hz, H-1*1*), 1.90 (s, 3 H, Ac), 1.86 (s, 3 H, Ac). Anal. Calcd for $C_{107}H_{106}N_2O_{26} \cdot 0.5H_2O$: C, 70.27; H, 5.90; N, 1.53. Found: C, 70.15; H, 5.90; N, 1.58.

Benzyl 2,4-di-O-benzyl-6-O-chloroacetyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**).—A mixture of **4** (438 mg, 0.314 mmol) and chloroacetyl chloride (55 μL, 0.691 mmol) in dry pyridine (10 mL) was stirred at −20°C for 1 h. The mixture was diluted with a 1:1 mixture of ether and EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 2:1 toluene–EtOAc to give **7** (275 mg, 59.6%): $[\alpha]_D$ + 0.4° (*c* 0.5); R_f 0.39 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.27 (d, 1 H, *J* 8.4 Hz, H-1*2*), 4.94 (d, 1 H, *J* 8.1 Hz, H-1*1*), 4.61 (bs, 1 H, H-1*3*). Anal. Calcd for $C_{85}H_{81}ClN_2O_{19}$: C, 69.45; H, 5.55; N, 1.91. Found: C, 69.25; H, 5.59; N, 1.86.

Benzyl 2,4-di-O-benzyl-6-O-tert-butyldimethylsilyl-β-D-mannopyranosyl-(1 → 4)-3,6-

di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**10**).—A mixture of **4** (100 mg, 0.07 mmol), TBDMSOTf (35 μL, 0.15 mmol) and 2,6-lutidine (26 μL, 0.22 mmol) in dry CH_2Cl_2 (2 mL) was stirred at 0°C for 2 h, then diluted with EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 2:1 toluene–EtOAc to give **10** (98 mg, 91%): $[\alpha]_D$ −4.7° (*c* 1.0); R_f 0.44 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.22 (d, 1 H, *J* 8.25 Hz, H-1*2*), 4.93 (d, 1 H, *J* 8.25 Hz, H-1*1*), 0.76 (s, 9 H, *t*-Bu), −0.03 (s, 3 H), −0.10 (s, 3 H). Anal. Calcd for $C_{89}H_{94}N_2O_{18}Si \cdot 0.5H_2O$: C, 70.47; H, 6.31; N, 1.85. Found: C, 70.48; H, 6.27; N, 1.82.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)-2,4-di-O-benzyl-6-chloroacetyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**13**).—A mixture of **10** (189 mg, 0.13 mmol), AgOTf (360 mg, 1.40 mmol) and dried powdered 4 Å molecular sieves (5 g) in dry dichloroethane (7 mL) was stirred at 0°C under N_2 for 30 min. Then a solution of **11** (89 mg, 0.17 mmol) in dry dichloroethane (7 mL) was added dropwise to the above mixture. The mixture was stirred at room temperature for 2 h, diluted with chloroform, and filtered through Celite. The filtrate was washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 5:1 toluene–EtOAc to give **13** (250 mg, quantitative): $[\alpha]_D$ +1.8° (*c* 2.8); R_f 0.38 (4:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.43 (dd, 1 H, *J* 3.3 and 1.8 Hz, H-2*4'*), 5.25 (d, 1 H, *J* 8.1 Hz, H-1*2*), 5.12 (d, 1 H, *J* 1.5 Hz, H-1*4'*), 4.94 (d, 1 H, *J* 8.2 Hz, H-1*1*), 2.11 (s, 3 H, Ac); ^{13}C, δ 101.4 (C-1*3*), 99.7 (C-1*4'*), 97.7 and 97.1 (C-1*1* and C-1*2*). Anal. Calcd for $C_{114}H_{111}ClN_2O_{25}$: C, 70.41; H, 5.75; N, 1.44. Found: C, 70.22; H, 5.71; N, 1.37.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)-2,4-di-O-benzyl-6-O-tert-butyldimethylsilyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**).—A mixture of **10** (60 mg, 40 μmol), **12** (75 mg, 0.12 mmol) and dried powdered AW-300 molecular sieves (200 mg) in dry dichloroethane (2 mL) was stirred at −78°C under N_2. Then a solution of $BF_3 \cdot Et_2O$ (2.5 μL, 0.02 mmol) in dry dichloroethane (25 μL) was added to the above mixture. The mixture was stirred at −78°C for 2 h, quenched with satd aq $NaHCO_3$, diluted with EtOAc, and filtered through Celite. The filtrate was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on Bio-beads S ×1 with 3:1 toluene–EtOAc, then on silica gel with 3:1 toluene–EtOAc to give **14** (57 mg, 72%) and unreacted **10** (7 mg, 12%): $[\alpha]_D$ −2.3° (*c* 1.8); R_f 0.61 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.46 (dd, 1 H, H-2*4'*), 5.21 (d, 1 H, *J* 8.2 Hz, H-1*2*), 5.16 (d, 1 H, H-1*4'*), 4.92 (d, 1 H, *J* 8.2 Hz, H-1*1*), 2.09 (s, 3 H, Ac), 0.75 (s, 9 H, *t*-Bu), −0.05 (s, 3 H, Me), −0.11 (s, 3 H, Me); ^{13}C, δ 107.1, 105.0, 102.5, 102.4. Anal. Calcd for $C_{118}H_{124}N_2O_{24}Si \cdot 1.33H_2O$: C, 70.64; H, 6.36; N, 1.40. Found: C, 70.68; H, 6.24; N, 1.41.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**15**).—*(a) By dechloroacetylation of* **13**. A mixture of **13** (244 mg, 0.126 mmol) and thiourea (20 mg,

0.263 mmol) in dry DMF (2 mL) was heated at 90°C for 20 h. The mixture was diluted with chloroform, washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 6:5 hexane–EtOAc to give **15** (219 mg, 93%).

(b) By desilylation of **14**. A mixture of **14** (14 mg, 7 μmol) and 1:19 50% hydrofluoric acid–CH_3CN (2 mL) was stirred at room temperature for 15 min. The mixture was diluted with chloroform, washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 3:1 toluene–EtOAc to give **15** (13 mg, quantitative): $[\alpha]_D$ −2.0° (*c* 1.9); R_f 0.48 (2:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.46 (dd, 1 H, *J* 2.9 and 1.8 Hz, H-2*4'*), 5.28 (d, 1 H, *J* 8.1 Hz, H-1*2*), 5.16 (s, 1 H, H-1*4'*), 4.95 (d, 1 H, *J* 8.4 Hz, H-1*1*), 2.09 (s, 3 H, Ac); ^{13}C, δ 100.8 (C-1*3*), 99.6 (C-1*4'*), 97.1 (C-1*1* and C-1*2*). Anal. Calcd for $C_{112}H_{110}N_2O_{24}$: C, 72.01; H, 5.94; N, 1.50. Found: C, 71.93; H, 5.92; N, 1.52.

*Benzyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-β-*D*-mannopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**16**).—*(a) By coupling of* **5** *and* **15**. Coupling reaction of **5** (71 mg, 0.164 mmol) and **15** (190 mg, 0.102 mmol) was performed with AgOTf (85 mg, 0.331 mmol) and dried powdered 4 Å molecular sieves (1.5 g) in dry dichloroethane (8 mL) at room temperature for 24 h, and worked up as described for **13** to give **16** (233 mg, quant).

(b) By coupling of **6** *and* **11**. Coupling reaction of **6** (43 mg, 24 μmol) and **11** (61 mg, 0.119 mmol) was performed with AgOTf (70 mg, 0.272 mmol) and dried powdered 4 Å molecular sieves (1.0 g) in dry dichloroethane (4 mL) at room temperature for 2 h, and worked up as described for **13** to give **16** (37 mg, 68%): $[\alpha]_D$ +4.1° (*c* 1.6); R_f 0.41 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.50 (dd, 1 H, *J* 3.3 and 1.8 Hz, H-2*B*), 5.23 (dd, 1 H, *J* 3.3 and 9.2 Hz, H-3*4'*), 5.20 (d, 1 H, *J* 9.1 Hz, H-1*2*), 5.18 (d, 1 H, *J* 1.5 Hz, H-1*B*), 5.12 (d, 1 H, *J* 1.8 Hz, H-1*4'*), 4.92 (d, 1 H, *J* 8.4 Hz, H-1*1*), 2.08 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.86 (s, 3 H, Ac); ^{13}C, δ 102.0 (C-1*3*), 99.7 (C-1*4'*), 97.2 (C-1*B*), 97.1, 97.0 (C-1*1* and C-1*2*). Anal. Calcd for $C_{136}H_{136}N_2O_{31}$: C, 71.19; H, 5.97; N, 1.22. Found: C, 71.23; H, 6.00; N, 1.22.

*Benzyl 2,4-di-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 6)-[3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-β-*D*-mannopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**17**).—To a stirred solution of **16** (107 mg, 47 μmol) in THF (30 mL) was added at 0°C a mixture of H_2O_2 (6 mL) and N LiOH (6 mL). After stirring at 0°C for 3 days, the mixture was diluted with EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 4:6 hexane–EtOAc gave **17** (86 mg, 80%): $[\alpha]_D$ +8.7° (*c* 0.7); R_f 0.37 (1:1 hexane–EtOAc). NMR data: ^{1}H, δ 5.22 (d, 1 H, *J* 7.7 Hz, H-1*2*), 5.19 (bs, 1 H, H-1*B*), 5.12 (bs, 1 H, H-1*4'*), 4.92 (d, 1 H, *J* 8.4 Hz, H-1*1*). Anal. Calcd for $C_{130}H_{130}N_2O_{28} \cdot 0.5H_2O$: C, 71.71; H, 6.02; N, 1.29. Found: C, 71.60; H, 6.02; N, 1.44.

*Benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-α-*D*-mannopyranosyl-(1 →*

6)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**18**).—Glycosylation of **17** (88 mg, 41 μmol) with **11** (93 mg, 0.182 mmol) was performed with AgOTf (70 mg, 0.272 mmol) and dried powdered 4 Å molecular sieves (2 g) in dichloroethane (5 mL) at room temperature for 24 h as described for **13**. The crude product was chromatographed on Bio-beads S ×2 in 1:4 toluene–EtOAc, on silica gel with 1:1 hexane–EtOAc and then on a preparative TLC plate with 6:1 toluene–EtOAc to give **18** (94 mg, 64%): $[\alpha]_D$ +23.0° (*c* 1.0); R_f 0.56 (3:1 toluene–EtOAc). NMR data: ^{1}H, δ 5.52 (dd, 1 H, *J* 2.9 and 1.8 Hz), 5.46 (m, 1 H), and 5.35 (m, 1 H), (H-2*A*, H-2*B*, and H-2*C*), 5.17 (bs, 1 H), 5.13 (bs, 1 H) and 5.04 (d, 1 H, *J* 1.8 Hz), (Man H-1 ×3), 4.92 (d, 1 H, *J* 8.1 Hz, H-1*I*), 2.09 (s, 3 H), 2.07 (s, 3 H) and 2.00 (s, 3 H), (Ac ×3). Anal. Calcd for $C_{217}H_{220}N_2O_{46}$: C, 72.56; H, 6.17; N, 0.78. Found: C, 72.47; H, 6.19; N, 0.73.

Benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 3)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**).—Compound **18** (61 mg, 17 μmol) was deacetylated with a mixture of H_2O_2 (1 mL) and N LiOH (2 mL). After stirring at 0°C for 24 h, the mixture was worked up. The crude product was chromatographed on a preparative TLC plate with 3:1:1 hexane–EtOAc–acetone to give **19** (48 mg, 82%): $[\alpha]_D$ +31.1° (*c* 2.0); R_f 0.36 (3:2 toluene–EtOAc). NMR data: ^{1}H, δ 5.18 (d, 1 H, *J* 7.7 Hz, H-1*2*), 5.18 (bs, 1 H), 5.13 (bs, 1 H), 5.09 (bs, 1 H), 4.96 (bs, 1 H), and 4.87 (bs, 1 H), (Man H-1 ×5), 4.92 (d, 1 H, *J* 8.4 Hz, H-1*I*). Anal. Calcd for $C_{211}H_{214}N_2O_{43} \cdot H_2O$: C, 72.74; H, 6.25; N, 0.80. Found: C, 72.74; H, 6.12; N, 0.71.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**20**).—Glycosylation of **19** (50 mg, 14.4 μmol) with **11** (44 mg, 86 μmol) was carried out with AgOTf (33 mg, 0.144 mmol) and dried powdered 4 Å molecular sieves (200 mg) in dry dichloroethane (5 mL) at room temperature for 18 h, as described for **13**. The crude product was chromatographed on Bio-beads S ×2 in 1:4 toluene–EtOAc and then on a preparative TLC plate with 6:1 toluene–EtOAc to give **20** (32 mg, 46%): $[\alpha]_D$ +20.3° (*c* 1.6); R_f 0.27 (5:4 hexane–EtOAc). NMR data: ^{1}H, δ 5.54 (m, 1 H) and 5.49 (m, 2 H), (H-2D_1, H-2D_2, and H-D_3), 5.19 (bs, 2 H, Man H-1), 5.16 (d, 1 H, *J* 7.9 Hz, H-1*2*), 5.12 (bs, 2 H, Man H-1), 5.10 (bs, 2 H, Man H-1), 4.99 (bs, 2 H, Man H-1), 4.91 (d, 1 H, *J* 8.5 Hz, H-1*I*), 2.09 (s, 6 H, Ac ×2), 2.06 (s, 3 H, Ac). Anal. Calcd for $C_{298}H_{304}N_2O_{61}$: C, 73.20; H, 6.27; N, 0.57. Found: C, 73.18; H, 6.26; N, 0.67.

Benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**21**).—A mixture of **20** (31 mg, 6.3 μmol) and $NH_2NH_2 \cdot H_2O$ (100 μL) in EtOH (1 mL) was heated at 90°C for 24 h and then concentrated in vacuo. The oily residue was stirred with Ac_2O (100 μL) in MeOH (1 mL) for 3 days at room temperature. The mixture was concentrated in vacuo. The crude product was purified by column chromatography on Sephadex LH-20 in 1:1 $CHCl_3$–MeOH and preparative TLC with 20:2:1 toluene–EtOAc–EtOH to give **21** (19 mg, 65%): $[\alpha]_D$ +21.4° (*c* 1.0); R_f 0.18 (40:4:1 toluene–EtOAc–EtOH). NMR data: ^{1}H, δ 6.99 (d, 1 H, *J* 7.7 Hz, NH), 5.23, 5.20, 5.18, 5.16, 5.14, 5.05, 5.00 (bs, 8 H, Man H-1), 1.50 (s, 3 H, Ac), 1.89 (s, 3 H, Ac). Anal. Calcd for $C_{280}H_{292}N_2O_{56}$: C, 73.31; H, 6.55; N, 0.61. Found: C, 73.13; H, 6.56; N, 0.69.

α-D-Mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)]-α-D-mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)]-β-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose (**1**).—A mixture of **21** (31 mg, 6.34 μmol) and 10% Pd–C (17 mg) in MeOH (4 mL) was stirred under H_2 for 20 h at 35°C, then filtered through Celite, and concentrated in vacuo. The residue was chromatographed on Sephadex LH-20 in 9:1 MeOH–H_2O to give **1** (8 mg, quant): $[\alpha]_D$ +20.8° (*c* 0.4, H_2O); R_f 0.20 (1:1:1:1 *n*-BuOH–EtOH–AcOH–H_2O). NMR data: ^{1}H, (D_2O, 60°C) δ 5.36 (s, 1 H, H-1*B*), 5.31 (s, 1 H, H-1*4*), 5.28 (s, 1 H, H-1*C*), 5.18 (d, 1 H, *J* 2.7 Hz, H-1*1α*), 5.11 (s, 1 H, H-1*B*), 5.06 (d, 1 H, *J* 1.5 Hz, H-1D_3), 5.05 (s, 1 H, H-1D_1), 5.04 (s, 1 H, H-1D_2), 4.86 (s, 1 H, H-1*4'*), 4.75 (s, 1 H, H-1*3*), 4.69 (m, 1 H, H-1*1B*), 4.60 (m, 1 H, H-1*2*), 2.07 (s, 3 H, Ac), 2.03 (s, 3 H, Ac); ^{13}C, (D_2O), δ 103.1, 103.0, and 103.0 (C-1D_1, C-1D_2, and C-1D_3), 102.2 (C-1*2*), 101.6, 101.4, and 101.4 (C-1*4*, C-1*A*, and C-1*C*), 101.0 (C-1*3*), 100.5 (C-1*4'*), 98.8 (C-1*B*). FABMS; *m/z* = 1883.64 (M + 1), 1905.61 (M + Na).

Benzyl 2,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**22**).—Compound **16** (24 mg, 10.4 μmol) was dephthaloylated and *N*-acetylated as described for **21**. The product was purified by column chromatography on Sephadex LH-20 in 1:1 $CHCl_3$–MeOH and by preparative TLC with 10:2:1 toluene–EtOAc–EtOH to give **22** (12 mg, 55%): $[\alpha]_D$ −9.0° (*c* 0.7); R_f 0.19 (20:4:1 toluene–EtOAc–EtOH). NMR data: ^{1}H, δ 6.30 (d, 1 H, *J* 8.8 Hz, NH), 5.98 and 5.11 (2 bs, 2 H, Man H-1), 1.85 (s, 3 H, Ac), 1.46 (s, 3 H, Ac).

α-D-Mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 3)]-β-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose (**23**).—Compound **22** (11 mg, 5.5 μmol) was hydrogenated as described for

1 to give **23** (4 mg, 84%): R_f 0.37 (1:1:1:1 *n*-BuOH–EtOH–AcOH–H_2O). NMR data: ^1H, (D_2O, 60°C) δ 5.18 (bs, 1 H, H-1*1*α), 5.10 (s, 1 H, H-1*4*), 4.90 (s, 1 H, H-1*4'*), 4.76 (s, 1 H, H-1*3*), 4.69 (m, 1 H, H-1*1*β), 4.61 (m, 1 H, H-1*2*).

*Benzyl 3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 6)-[3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 6)-[3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 3)]2,4-di-*O*-benzyl-*β*-*D*-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranoside* (**24**).—Compound **18** (15 mg, 4.1 μmol) was dephthaloylated and *N*-acetylated as described for **21**. The product was purified by column chromatography on Sephadex LH-20 in 1:1 $CHCl_3$–MeOH and by preparative TLC with 15:2:1 toluene–EtOAc–EtOH to gave **24** (7 mg, 52%): $[\alpha]_D$ +17.1° (*c* 0.5); R_f 0.42 (20:4:1 toluene–EtOAc–EtOH). NMR data: ^1H, δ 5.17, 5.10, 5.08, 5.04, and 4.89, (5 bs, 5 H, Man H-1), 6.33 (d, 1 H, *J* 8.4 Hz, NH), 1.89 (s, 3 H, Ac), 1.52 (s, 3 H, Ac).

α-*D*-Mannopyranosyl-(1 → 6)-[α-*D*-mannopyranosyl-(1 → 3)]-α-*D*-mannopyranosyl-(1 → 6)-[α-*D*-mannopyranosyl-(1 → 2)-α-*D*-mannopyranosyl-(1 → 3)]-β-*D*-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-*D*-glucopyranose* (**25**).—Compound **24** (7 mg, 2.1 μmol) was hydrogenated as described for **1** to give **25** (4 mg, quant). R_f 0.30 (1:1:1:1 *n*-BuOH–EtOH–AcOH–H_2O). NMR data: ^1H, (D_2O, 60°C) δ 5.33 (s, 1 H, H-1*4*), 5.18 (d, 1 H, *J* 2.2 Hz, H-1*1*α), 5.10 (s, 1 H, H-1*A*), 5.05 (d, 1 H, H-1*C*), 4.90 (s, 1 H, H-1*B*), 4.87 (s, 1 H, H-1*4'*), 4.75 (s, 1 H, H-1*3*), 4.70 (m, 1 H, H-1*1*β), 4.60 (m, 1 H, H-1*2*), 2.05 (s, 3 H, Ac), 2.01 (s, 3 H, Ac).

*4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-*β*-*D*-galactopyranosyl-(1 → 4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranoside* (**27**).—To a stirred mixture of **26** (8.9 g, 11.7 mmol) and 4-methoxyphenol (2.9 g, 23.5 mmol) in dry dichloroethane (400 mL), was added TfOH (200 μL) at 0°C under Ar. The mixture was allowed to stir at 0°C for 1.5 h then at room temperature for 1.5 h and quenched with satd aq $NaHCO_3$ (3 mL). The mixture was diluted with EtOAc (1 L), washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was chromatographed on silica gel with (3:1 → 2:1) toluene–EtOAc to afford **27** (8.8 g, 91%): $[\alpha]_D$ +28.0 (*c* 1.0); R_f 0.69 (4:1 $CHCl_3$–EtOH). NMR data: ^1H, δ 5.83 (d, 1 H, *J* 8.4 Hz, H-1a), 5.81 (dd, 1 H, *J* 10.6 and 8.1 Hz, H-3a), 5.35 (d, 1 H, *J* 2.9 Hz, H-4b), 5.14 (dd, 1 H, *J* 10.6 and 7.7 Hz, H-2*2*), 4.97 (dd, 1 H, *J* 10.6 and 3.3 Hz, H-3*2*), 4.56 (d, 1 H, *J* 7.7 Hz, H-1b), 4.46 (dd, 1 H, *J* 8.4 and 10.6 Hz, H-2a), 3.94 (t, 1 H, *J* 8.1 Hz, H-4*1*), 3.72 (s, 3 H, OMe), 2.14 (s, 3 H, Ac), 2.13 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), 1.93 (s, 3 H, Ac). Anal. Calcd for $C_{39}H_{43}NO_{19}$: C, 56.45; H, 5.22; N, 1.69; Found: C, 56.18; H, 5.18; N, 1.69.

*4-Methoxyphenyl β-*D*-galactopyranosyl-(1 → 4)-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**28**).—To a solution of **27** (8.8 g, 10.6 mmol) in 1:1 THF–MeOH (88 mL) was added 0.1 M methanolic NaOMe (21 mL, 2.1 mmol). After stirring for 1 h at room temperature, the resulting precipitate was collected by filtration and washed thoroughly with MeOH and ether to afford **28** (5.7 g, 84%), which was used for the next reaction without further purification: $[\alpha]_D$ +3.0 (*c* 1.0, Me_2SO); mp > 300°C, R_f 0.07 (5:1 $CHCl_3$–EtOH). NMR data: ^1H, δ 5.59 (d, 1 H, H-1*1*), 5.02 (d, 1 H, H-4*2*), 4.32 (d,

1 H, H-12), 3.67 (s, 3 H, OMe). Anal. Calcd for $C_{27}H_{31}NO_{13}$: C, 56.15; H, 5.41; N, 2.43; Found: C, 55.74; H, 5.35; N, 2.33.

4-Methoxyphenyl 3,4-O-isopropylidene-β-D-galactopyranosyl-(1 → 4)-2-O-deoxy-2-phthalimido-β-D-glucopyranoside (**29**).—A mixture of **28** (1.65 g, 2.86 mmol), 2,2-dimethoxypropane (25 mL), and *p*-TsOH (100 mg) in dry acetone (25 mL) was stirred at room temperature for 19 h. The reaction was quenched with Et_3N (0.5 mL) and the mixture was concentrated in vacuo. The residue was extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was chromatographed on silica gel with 4:1 $CHCl_3$–EtOH to give **29** (1.46 g, 83%): $[\alpha]_D$ +38.3° (*c* 1.7); R_f 0.31 (5:1 toluene–EtOH). NMR data: ^{1}H, δ 3.61 (s, 3 H, OMe), 1.40 (s, 3 H, Me), 1.21 (s, 3 H, Me). Anal. Calcd for $C_{30}H_{35}NO_{13} \cdot 0.75H_2O$: C, 57.09; H, 5.83; N, 2.22; Found: C, 57.12; H, 5.62; N, 2.20.

4-Methoxyphenyl 3,4-isopropylidene-6-O-pivaloyl-β-D-galactopyranosyl-(1 → 4)-2-deoxy-2-phthalimido-6-O-pivaloyl-β-D-glucopyranoside (**30**).—A mixture of **29** (1.46 g, 2.36 mmol), pivaloyl chloride (1.14 mL, 4.26 mmol), pyridine (1 mL, 2.36 mmol), and 4-(dimethylamino)pyridine (50 mg) in CH_2Cl_2 (20 mL) was stirred at room temperature for 4.5 h. The mixture was washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 7:3 toluene–EtOAc to give **30** (1.53 g, 83%): $[\alpha]_D$ +37.7° (*c* 1.0); R_f 0.29 (19:1 toluene–EtOH). NMR data: ^{1}H, δ 5.74 (d, 1 H, *J* 8.3 Hz, H-1*1*), 3.73 (s, 3 H, OMe), 1.51 (s, 3 H, Me), 1.34 (s, 3 H, Me), 1.22 (s, 9 H, *t*-Bu), 1.00 (s, 9 H, *t*-Bu). Anal. Calcd for $C_{42}H_{51}NO_{15} \cdot 0.75H_2O$: C, 60.10; H, 6.62; N, 1.75; Found: C, 60.08; H, 6.47; N, 1.69.

4-Methoxyphenyl 6-O-pivaloyl-β-D-galactopyranosyl-(1 → 4)-2-deoxy-2-phthalimido-6-O-pivaloyl-β-D-glucopyranoside (**31**).—To a stirred solution of **30** (1.53 g, 1.95 mmol) in CH_2Cl_2 (20 mL) was added 80% aq trifluoroacetic acid (5 mL). The mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was chromatographed on silica gel with 4:1 toluene–EtOH to give **31** (1.17 g, 78%): $[\alpha]_D$ +27.6° (*c* 1.0); R_f 0.42 (5:1 toluene–EtOH). NMR data: ^{1}H, δ 5.66 (d, 1 H, *J* 7.9 Hz, H-1a), 3.64 (s, 3 H, OMe), 1.14 (s, 9 H, *t*-Bu), 0.95 (s, 9 H, *t*-Bu). Anal. Calcd for $C_{37}H_{47}NO_{15} \cdot 0.5H_2O$: C, 58.88; H, 6.41; N, 1.86. Found: C, 59.01; H, 6.35; N, 1.80.

4-Methoxyphenyl [methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)oate]-(2 → 3)-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl-(1 → 4)-3-O-acetyl-2-deoxy-2-phthalimido-6-O-pivaloyl-β-D-glucopyranoside (**34**).—A mixture of **31** (978 mg, 1.31 mmol), **32** (1.51 g, 2.90 mmol), and dried powdered 3 Å molecular sieves (9.5 g) in dry CH_3CN (80 mL) was stirred at −40°C under N_2. To the above mixture was added a mixture of NIS (655 mg, 2.90 mmol) and TfOH (23 μL, 2.60 mmol) in dry CH_3CN (5 mL). After stirring at −40°C for 2 h, the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was stirred with acetic anhydride (2 mL), pyridine (2 mL) and DMAP (200 mg) in CH_2Cl_2 (40 mL) for 1 h at room temperature. The reaction mixture was concentrated and then purified on silica gel with 9:1 toluene–EtOH to give **34** (1.1 g, 63%): R_f 0.44 (5:1 toluene–EtOH). NMR data: ^{1}H, δ 5.86 (d, 1 H, *J* 8.6 Hz, H-1*1*), 5.62 (m, 1 H, H-8*3*), 5.39 (dd, 1 H, $J_{7,8}$ 9.5, $J_{6,7}$ 2.6 Hz, H-7*3*), 5.15 (d, 1 H, *J* 10.3 Hz, NH), 4.78 (d, 1 H,

J 8.1 Hz, H-1*2*), 3.83 (s, 3 H, OMe), 3.72 (s, 3 H, OMe), 2.59 (dd, 1 H, *J* 12.5 and 4.6 Hz, H-3*3*β), 2.27 (s, 3 H, Ac), 2.14 (s, 3 H, Ac), 2.10 (s, 6 H, Ac), 2.08 (s, 3 H, Ac), 2.01 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.68 (t, 1 H, *J* 12.5 Hz, H-3c α), 1.21 (s, 9 H, *t*-Bu), 1.18 (s, 9 H, *t*-Bu); ^{13}C, δ 101.3, 97.1, 96.7.

*[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-*α*-*D*-galacto-2-nonulopyranosyl)oate]-(2 → 3)-(2,4-di-*O*-acetyl-6-*O*-pivaloyl-*β*-*D*-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-pivaloyl-*β*-*D*-glucopyranosyl fluoride* (**36**).—A mixture of **34** (611 mg, 0.45 mmol) and ceric ammonium nitrate (5.0 g, 9.1 mmol) in toluene–CH_3CN–H_2O (8 mL, 6 mL, 4 mL) was vigorously stirred at room temperature for 2 h, and then extracted with EtOAc. The extract was washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 93:7 toluene–EtOH to give **35** (410 mg). To a stirred mixture of **35** (410 mg) in dry CH_2Cl_2 (10 mL) was added diethylaminosulfur trifluoride (DAST, 260 μL) at 0°C. After stirring for 1 h, the reaction was quenched with a few drops of MeOH. The mixture was diluted with EtOAc, washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 3:1 toluene–EtOAc to give **36** (378 mg, 67% from **34**): $[\alpha]_D$ +15.2° (*c* 0.6); R_f 0.36 (9:1 toluene–EtOH). NMR data: ^{1}H, δ 6.11 (dd, 1 H, *J* 52.8 and 7.9 Hz, H-1*1*), 5.12 (d, 1 H, *J* 10.2 Hz, NH), 4.75 (d, 1 H, *J* 7.9 Hz, H-1*2*), 4.37 (dd, 1 H, *J* 12.5 and 2.6 Hz), 3.83 (s, 3 H, OMe), 3.61 (dd, 1 H, *J* 10.6 and 2.6 Hz, H-2*1*), 2.69 (dd, 1 H, *J* 12.5 and 4.6 Hz, H-3*3*β), 2.26 (s, 3 H, Ac), 2.14 (s, 3 H, Ac), 2.09 (s, 6 H, Ac), 2.08 (s, 3 H, Ac), 1.95 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.25 (s, 9 H, *t*-Bu), 1.19 (s, 9 H, *t*-Bu); ^{13}C, δ 104.1 (d, $J_{C,F}$ 214.8 Hz, C-1*1*), 100.9 (C-1*2*), 96.6 (C-1*3*). Anal. Calcd for $C_{56}H_{73}FN_2O_{28} \cdot 0.5H_2O$: C, 53.80; H, 5.97; N, 2.24; F, 1.52. Found: C, 53.81; H, 5.92; N, 2.26; F, 1.34.

*Benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-*β*-*D*-mannopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranoside* (**37**).—Glycosylation of **4** (230 mg, 0.17 mmol) with **11** (340 mg, 0.67 mmol) was performed with AgOTf (170 mg, 0.66 mmol) and powdered 4 Å molecular sieves (500 mg) in CH_2Cl_2 (10 mL) at room temperature under N_2 overnight as desctibed for **13**. The product was chromatographed on Bio-beads S ×1 to give **36** (340 mg, 88%): $[\alpha]_D$ +13.4° (*c* 1.3); R_f 0.27 (3:2 hexane–EtOAc). NMR data: ^{1}H, δ 5.49 (dd, 1 H, *J* 3.3 and 1.8 Hz) and 5.31 (dd, 1 H, *J* 2.9 and 1.8 Hz, H-2d and H-2*B*), 5.20 (d, 1 H, *J* 8.1 Hz, H-1*2*), 5.14 (d, 1 H, *J* 1.5 Hz, Man H-1), 4.92 (d, 1 H, *J* 8.4 Hz, H-1*1*), 2.08 (s, 3 H, Ac), 1.79 (s, 3 H, Ac); ^{13}C, δ 101.9, 99.6, 98.2, 97.0, 96.9 (C-1). Anal. Calcd for $C_{141}H_{140}N_2O_{30} \cdot H_2O$: C, 71.74; H, 6.06; N, 1.19. Found: C, 71.67; H, 6.05; N, 0.99.

*Benzyl 3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 6)-[3,4,6-tri-*O*-benzyl-*α*-*D*-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzyl-*β*-*D*-mannopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranoside* (**38**).—To a stirred solution of **37** (340 mg, 0.15 mmol) in CH_2Cl_2 (30 mL) was added at 0°C a mixture of H_2O_2 (4 mL) and N LiOH (4 mL). After stirring at 4°C for 20 h, the mixture was diluted with EtOAc, washed with

water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel with 2:1 toluene–EtOAc to give **38** (268 mg, 82%): $[\alpha]_D$ +11.4° (*c* 0.7); R_f 0.26 (1:1 hexane–EtOAc). NMR data: ^1H, δ 5.23 (d, 1 H, *J* 8.1 Hz, H-12), 5.15 (bs, 1 H, Man H-1), 4.93 (d, *J* 8.4 Hz, H-11), 4.91 (bs, 1 H, Man H-1); ^{13}C, δ 101.7, 101.4, 99.8, 97.0 (C-1). Anal. Calcd for $C_{137}H_{136}N_2O_{28} \cdot 2H_2O$: C, 71.71; H, 6.15; N, 1.22. Found: C, 71.66; H, 5.97; N, 1.24.

Benzyl [methyl (5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)oate]-(2 → 3)-2,4-di*-O-*acetyl-6*-O-*pivaloyl*-β-D-galacto-*pyranosyl-(1 → 4)-3*-O-*acetyl-2-deoxy-2-phthalimido-6*-O-*pivaloyl*-β-D-*glucopyranosyl-(1 → 2)-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1 → 6)-{[methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)oate]-(2 → 3)-2, 4-di*-O-*acetyl-6*-O-*pivaloyl*-β-D-*galactopyranosyl-(1 → 4)-3*-O-*acetyl-2-deoxy-2-phthalimido-6*-O-*pivaloyl*-β-D-*glucopyranosyl-(1 → 2)-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1 → 3)}-2,4-di*-O-*benzyl*-β-D-*mannopyranosyl-(1 → 4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1 → 4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**39**).—A mixture of **38** (30 mg, 13 μmol), Cp_2HfCl_2 (96 mg, 25 μmol), $AgClO_4$ (138 mg, 67 μmol), and dried powdered 4 Å molecular sieves (600 mg) in dry CH_2Cl_2 (6 mL) was stirred under N_2 at room temperature for 30 min and cooled to −20°C. Then a solution of **36** (70 mg, 56 μmol) in dry CH_2Cl_2 (4.5 mL) was added dropwise to the above mixture, which was allowed to stir overnight at −20°C gradually warming to room temperature. The reaction was quenched with pyridine (0.5 mL). The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was chromatographed on Bio-beads S × 1 in toluene, then on a preparative TLC plate with 9:1 toluene–EtOH. The product was further purified by HPLC on a C_{18}-reversed phase column with 99:1 CH_3CN–H_2O to give **39** (20 mg, 32%): $[\alpha]_D$ +23.9° (*c* 0.5); R_f 0.30 (9:1 toluene–EtOH). HPLC: [ODS (20 × 250 mm)], 99% CH_3CN; t_R 13.6 min; [prep-sil (20 × 250 mm)], 1:19 EtOH–$CHCl_3$; t_R 16.3 min. NMR data: ^1H, δ 3.838 (s, 3 H, OMe), 3.826 (s, 3 H, OMe), 2.200 (s, 3 H, Ac), 2.184 (s, 3 H, Ac), 2.146 (s, 3 H, Ac), 2.143 (s, 3 H, Ac), 2.126 (s, 3 H, Ac), 2.098 (s, 3 H, Ac), 2.089 (s, 6 H, Ac), 2.077 (s, 3 H, Ac), 2.075 (s, 3 H, Ac), 2.071 (s, 3 H, Ac), 2.020 (s, 3 H, Ac), 2.006 (s, 3 H, Ac), 1.863 (s, 3 H, Ac), 1.859 (s, 3 H, Ac), 1.154 (s, 9 H, *t*-Bu), 1.149 (s, 9 H, *t*-Bu), 1.105 (s, 9 H, *t*-Bu), 1.089 (s, 9 H, *t*-Bu); ^{13}C, δ 103.43, 103.35, 102.34, 102.18, 101.02, 97.42, 97.28, 96.9 (NeuAc C-2 ×2), 96.08, 95.69. ESIMS: 2320.7($^{+2}$) (M − CO_2CH_3; 4639).

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References

[1] J. Tamura, K. Neumann, and T. Ogawa, *Bioorg. Med. Chem. Lett.*, 5 (1995) 1351–1354.
[2] J. Montreuil, *Comprehensive Biochemistry*, 19 BII (1982) 1–188.
[3] (a) T. Ogawa, H. Yamamoto, T. Nukada, T. Kitajima, and M. Sugimoto, *Pure Appl. Chem.*, 56 (1984) 779–795, and references cited therein; (b) T. Ogawa, T. Kitajima, and T. Nukada, *Carbohydr. Res.*, 123 (1983) C5–C7; (c) T. Ogawa and T. Nukada, *Carbohydr. Res.*, 136 (1985) 135–152; (d) K.K. Sadozai, Y. Ito, T. Nukada, and T. Ogawa, *Carbohydr. Res.*, 150 (1986) 91–101; (e) K.K. Sadozai, T. Kitajima, Y. Nakahara, T. Ogawa, and A. Kobata, *Carbohydr. Res.*, 152 (1986) 173–182; (f) T. Ogawa, M, Sugimoto, T. Kitajima, K.K. Sadozai, and T. Nukada, *Tetrahedron Lett.*, 27 (1986) 5739–5742; (g) F. Yamazaki, T. Kitajima, T. Nukada, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 15–30; (h) F. Yamazaki, S. Sato, T. Nukada, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 31–50; F. Yamazaki, T. Nukada, Y. Ito, S. Sato, and T. Ogawa, *Tetrahedron Lett.*, 30 (1989) 4417–4420; (i) T. Nukada, T. Kitajima, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 228 (1992) 157–170; (j) T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, 64 (1978) C3–C9.
[4] S. Ito, K. Yamashita, R.G. Spiro, and A. Kobata, *J. Biochem.*, 81 (1977) 1621–1631.
[5] H. van Halbeek, L. Dorland, G.A. Veldink, J.F.G. Vliegenthart, G. Strecker, J.-C. Michalski, J. Montreuil, and W.E. Hull, *FEBS Lett.*, 121 (1980) 71–77.
[6] T. Mizuochi, T.J. Mattews, M. Kato, J. Hamako, K. Titani, J. Solomon, and T. Feizi, *J. Biol, Chem.*, 265 (1990) 8519–8524.
[7] H. Paulsen and O. Lockhoff, *Chem. Ber.*, 114 (1981) 3102–3114.
[8] J.J. Oltvoort, C.A.A. van Boeckel, J.H. de Koning, and J.H. van Boom, *Synthesis*, (1981) 305–308.
[9] R. Gigg and C.D. Warren, *J. Chem. Soc. C*, (1968) 1903–1911.
[10] R.R. Schmit, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.
[11] Y. Endo, K. Yamashita, Y. Tachibana, S. Tojo, and A. Kobata, *J. Biochem.*, 85 (1979) 669–679.
[12] H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 29 (1990) 823–839.
[13] J. Arnarp, and J. Lönngren, *J. Chem., Soc., Perkin Trans. 1*, (1981) 2070–2074.
[14] C. Unverzagt, *Angew. Chem., Int. Ed. Engl.*, 33 (1994) 1102–1104.
[15] (a) O. Kanie, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 7 (1988) 501–506; A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 10 (1991) 493–498; (b) Y. Ito, T. Ogawa, M. Numata, and M. Sugimoto, *Carbohydr. Res.*, 202 (1990) 165–175; (c) T. Kondo, H. Abe, and T. Goto, *Chem. Lett.*, (1988) 1657; (d) A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 195 (1990) 303–308; (e) H. Lönn and K. Stenvall, *Tetrahedron Lett.*, 33 (1992) 115–116; (f) K. Kondo, Y. Ichikawa, and C.H. Wong, *J. Am. Chem. Soc.*, 114 (1992) 8748–8750; (g) T.J. Martin and R.R. Schmidt, *Tetrahedron Lett.*, 33 (1992) 6123–6126.
[16] R.U. Lemieux, S.Z. Abbas, M.H. Burynska, and R.M. Ratcliffe, *Can. J. Chem.*, 60 (1982) 63–67.
[17] (a) P. Konradsson, D.R. Mootoo, R.E. McDevitt, and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, 1990, 270–272; (b) G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334; (c) A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 10 (1991) 493–498.
[18] K. Okamoto, T. Kondo, and T. Goto, *Tetrahedron*, 43 (1987) 5919–5928.
[19] K. Suzuki, H. Maeta, and T. Matsumoto, *Tetrahedron Lett.*, 30 (1989) 4853–4856.
[20] Y. Matsuzaki, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, 33 (1992) 6343–6346.