Glycoconjugate Journal 17, 361–375, 2000

Synthesis of N-linked pentasaccharides with isomeric glycosidic linkage

Maki Takatani[1], Toshihiro Nakama[2], Kazusuke Kubo[2], Shino Manabe[1], Yuko Nakahara[1], Yukishige Ito[1] and Yoshiaki Nakahara[1,2*]

[1]*The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama, 351-0198, Japan*, [2]*Department of Industrial Chemistry, Tokai University, Kitakaname 1117, Hiratsuka-shi, Kanagawa, 259-1292, Japan CREST, Japan Science and Technology Corporation (JST)*

As part of a program to explore the structural requirement of N-glycans in the carbohydrate-mediated biological interactions, N-linked pentasaccharide core structure was stereochemically modified in terms of glycosidic linkage. Three isomers, α-D-Man-(1→3)-[α-D-Man-(1→6)]-α-D-Man-(1→4)-β-D-GlcNAc-(1→4)-β-D-GlcNAc-L-Asn, α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-α-D-GlcNAc-(1→4)-β-D-GlcNAc-L-Asn, and α-D-Man-(1→3)-[α-D-man-(1→6)]-α-D-Man-(1→4)-α-D-GlcNAc-(1→4)-β-D-GlcNAc-L-Asn, were synthesized. Synthesis of the pentasaccharide with natural linkage is also described.

Keywords: **N-glycan, synthesis, unnatural glycosidic linkage**

Abbreviations: **Man, D-mannose; GlcNAc, N-acetyl-D-glucosamine; Asn, L-asparagine; Gal, D-galactose; Fuc, L-fucose; Neu5Ac, N-acetyl-D-neuraminic acid; GNT-I, N-acetyl-D-glucosaminyltransferase-1; THF, tetrahydrofuran; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; DMF, N-N-dimethylformamide; $TMSN_3$, trimethylsilyl azide; TMSOTf, trimethylsilyl trifluoromethanesulfonate; HRMS, high resolution mass spectrometry; TLC, thin layer chromatography.**

Introduction

It has been recognized that the protein-bound oligosaccharides not only present necessary physical and chemical properties to the backbone protein (e.g., solubility, stability, or conformation) but also behave as the recognition signals in a variety of biological process [1]. The major N- and O-glycan structures have been elucidated extensively and grouped into the subclasses by the structural similarity. Among an enormous number of theoretically possible combinations in assembly of monosaccharide constituents and configurations, only limited numbers of them have been utilized in nature as a result of evolution of glycosylation enzymes. For instance, the N-glycans always start with (β)-D-GlcNAc, grow with β-D-GlcNAc, β- and α-D-Man, to form the common core pentasaccharide, and are further modified with β-D-GlcNAc, α-D-Man, β-D-Gal, α-D-Fuc, and α-D-Neu5Ac residues.

From a topological point of view, the peripheral sugar residues would be the most likely involved in the receptor-ligand interactions, although some lectins have been shown to recognize the interior residue of oligosaccharides. Thus the question has been raised whether the inner core structure is essential in the biological interactions.

In this context, we are interested in the construction of the glycans involving unnatural glycosidic linkages especially in the core region. Preliminary examination of the molecular model of N-glycan core pentasaccharide suggested that the two branching mannose residues seemed to change their spatial positions each other in the most probable conformation, when the neighboring Man-GlcNAc linkage is unnatural alpha. On the other hand, an alpha GlcNAc-GlcNAc linkage would allow the saccharide chain more distorted.

We describe herein the stereoselective syntheses of three asparagine-linked pentasaccharides possessing unnatural Man-GlcNAc and/or GlcNAc-GlcNAc linkages (compounds **2**, **3**, and **4**) as well as the natural one (**1**). With respect to the natural pentasaccharide and higher oligosaccharide derivatives, a number of synthetic efforts have been reported previously by several research groups [2,3].

*To whom correspondence should be addressed.

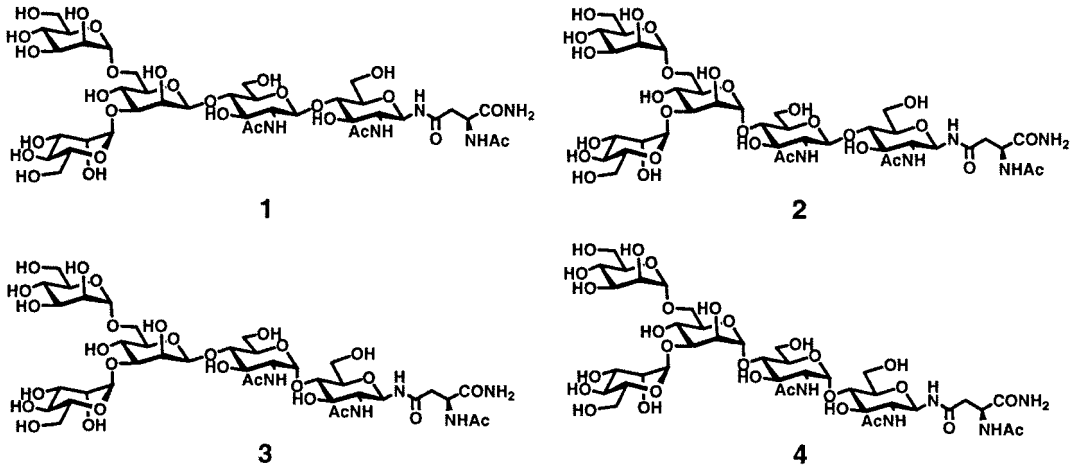


Figure 1. Structures of N-linked pentasaccharides with isomeric glycosidic linkage.

Materials and methods

General procedures

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 or 230–400 mesh). TLC and HPTLC were performed on Silica Gel 60 F_{254} (E Merck). ^1H and ^{13}C NMR spectra were recorded with a JEOL AL400 [^1H (400 MHz), ^{13}C (100 MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$, unless noted otherwise. Fab mass spectra (HRMS) were obtained with a JEOL JMS HX-110 spectrometer (3-nitrobenzyl alcohol was used as a matrix).

3,6-Di-O-allyl-2,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (**6**). A mixture of **5** (1.42 g, 1.4 mmol) and $Ce(NH_4)_2\cdot(NO_3)_6$ (CAN, 7.7 g, 14.0 mmol) in 4:3:2 toluene−CH_3CN−H_2O (40.5 ml) was stirred at room temperature for 2 h. The organic layer was separated, while the resulting aqueous layer was extracted with EtOAc.

The combined organic extracts were washed with water, aq. $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with 3:1 toluene–EtOAc to give **6** (0.82 g, 64.5%): Rf 0.21 (4:1 toluene–EtOAc); ^1H-NMR: δ 7.65–7.61 (m, 4H, Ar), 7.36–7.14 (m, 15H, Ar), 6.97–6.79 (m, 5H, Ar), 5.88 (m, 2H, −C*H*=CH_2), 5.31–5.22 (m, 3H, −CH=CH_2 and H-1b), 5.15–5.12 (m, 2H, −CH=CH_2). Anal. Calcd. for $C_{54}H_{57}NO_{12}\cdot H_2O$: C, 69.74; H, 6.39; N, 1.51. Found: C, 69.90; H, 6.20; N, 1.68.

3,6-Di-O-allyl-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl fluoride (*7*).

To a stirred solution of **6** (0.79 g, 0.87 mmol) in dry THF (10 ml), was added diethylaminosulfur trifluoride (DAST, 0.3 ml, 2.27 mmol) at 0°C. After stirring for 1 h, the reaction was quenched with MeOH (0.5 ml) and the mixture was concentrated *in vacuo*. The residue was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with 19:1 toluene–EtOAc to give **7** as an $\alpha/\beta = 1/3.9$ mixture (0.74 g, 93.5%); Rf 0.44 (9:1 toluene–EtOAc); ^1H-NMR: δ 7.69–7.64 (m, 4H, Ar), 7.37–7.15 (m, 15H, Ar), 6.95–6.78 (m, 5H, Ar), 5.88 (m, 2H, −C*H*=CH_2), 5.81 (dd, 0.8H, $J = 7.3$, 54.0 Hz, H-1a β-F), 5.55 (dd, 0.2H, $J = 2.9$, 53.7 Hz, H-1a α-F), 5.30–5.23 (m, 3H, −CH=CH_2 and H-1b), 5.15–5.12 (m, 2H, −CH=CH_2). Anal. Calcd. for $C_{54}H_{56}NO_{11}F\cdot H_2O$: C, 69.59; H, 6.27; N, 1.50; F, 2.04. Found: C, 69.83; H, 6.08; N, 1.53; F 2.15.

4–Methoxyphenyl 3,6-di-O-allyl-4,6-di-O-benzyl-α-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 (**9**).

A mixture of Cp_2ZrCl_2 (1.02 g, 3.5 mmol), $AgClO_4$ (1.45 g, 7 mmol), and dried molecular sieves 4A (3 g) in dry CH_2Cl_2 (20 ml) was stirred at room temperature for 1 h under Ar, and then cooled on an ice–MeOH bath. To the mixture was added a solution of **8** (1.25 g, 2.1 mmol) in dry CH_2Cl_2 (20 ml). After stirring for 30 min, a solution of **7** (1.6 g, 1.8 mmol) in dry CH_2Cl_2 (20 ml) was added to the mixture. The reaction mixture was stirred at –10°C for 1.5 h, before the reaction was quenched with aq. $NaHCO_3$. The mixture was filtered through Celite. The filtrate was extracted with $CHCl_3$, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with 9:1 toluene–EtOAc to give **9** (2.19 g, 84%): $[\alpha]_D$+41.2° (c = 1); Rf 0.55 (4:1 toluene–EtOAc); ^1H-NMR: δ 7.66–6.57 (m, 42H, Ar), 6.95–6.78 (m, 5H, Ar), 5.93–5.83 (m, 2H, −C*H*=CH_2), 5.42 (d, 1H, $J = 8.6$ Hz, H-1a), 5.30–5.21 (m, 4H, −CH=CH_2, H-1b, and H-1c), 5.15–5.09 (m, 2H, −CH=CH_2), 3.64 (s, 3H, Me). Anal. Calcd. for

$C_{89}H_{88}N_2O_{19} \cdot 0.5H_2O$: C, 71.33; H, 5.99; N, 1.87. Found: C, 71.31; H, 5.95; N, 1.57.

4–Methoxyphenyl 4,6-di-O-benzyl-α-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 red suspension of the Ir-complex {[Ir(COD)(P-MePh$_2$)]$_2$PF$_6$, 30 mg, 35.5 μmol} in freshly distilled THF (5 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 Ar. To the solution was added a carefully degassed solution of **9** (1.0 g, 0.67 mmol) in dry THF (10 ml). After stirring for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 90% aq acetone (45 ml) and stirred with $HgCl_2$ (790 mg, 1.7 mmol) and HgO (58 mg, 0.27 mmol) 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 **10** (825 mg, 87%): $[\alpha]_D$ + 62.5° (c = 1); Rf 0.38 (3:1 toluene–EtOAc); ^{1}H-NMR: δ 5.45 (d, 1H, J = 8.5 Hz, H-1a), 5.32 (d, 1H, J = 1.5 Hz, H-1c), 5.26 (d, 1H, 8.2 Hz, H-1b), 3.64 (s, 3H, Me); ^{13}C-NMR: δ 98.9 (C-1c), 97.5 (C-1b), 96.6 (C-1a). Anal. Calcd. for $C_{83}H_{80}N_2O_{19}$: C, 70.73; H, 5.72; N, 1.99. Found: C, 70.43; H, 5.74; N, 1.83.

4–Methoxyphenyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyransoyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyransoyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**).

A mixture of **10** (73 mg, 51 μmol), dried powdered molecular sieves 4A (480 mg), AgOTf (79 mg, 306 μmol) in dry CH_2Cl_2 (3 ml) was stirred at room temperature for 10 min under Ar, and cooled at –40°C. To the mixture was added a solution of **11** (104 mg, 203 μmol) and stirring was continued at –40°C – room temperature overnight. After the reaction was quenched with Et_3N, the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on Bio-beads S X 1 with 1:1 toluene–EtOAc and then on silica gel with 5:1 toluene–EtOAc to afford **12** (99 mg, 82%): $[\alpha]_D$+48.8° (c = 1.1); Rf 0.41 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.49 (s, 2H, H-2d and H-2e), 5.42 (d, 1H, J = 8.3 Hz, H-1a), 5.35 (s, 1H, H-1c), 5.24 (d, 1H, 8.2 Hz, H-1b), 5.23 and 4.94 (2s, 2H, H-1d and H-1e), 3.64 (s, 3H, Me), 2.09 and 2.05 (2s, 6H, Ac). Anal. Calcd. for $C_{141}H_{140}N_2O_{31}$: C, 71.80; H, 5.98; N, 1.19. Found: C, 71.69; H, 5.92; N, 1.30.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4, 6-di-O-benzyl-α-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-D-glucopyranose (**13**).

Compound **12** (546 mg, 0.23 mmol) was treated with CAN (1.13 g, 2.07 mmol) in toluene−CH_3CN−H_2O as described above for **6**. After 4 h, the mixture was worked-up and the crude product was purified by column chromatography to give **12** (350 mg, 65%): Rf 0.31 (3:1 toluene–EtOAc); ^{1}H-NMR: δ 5.48–5.49 (m, 2H, H-2d and H-2e), 5.34 (s, 1H, H-1c), 5.23 (d, 1H, J = 7.8 Hz, H-1b), 5.23 (s, 1H) and 4.93 (d, 1H, J = 1.7 Hz) (H-1d and H-1e), 5.12 (br, 1H, H-1a), 2.09 and 2.05 (2s, 6H, Ac).

HRMS Calcd. for $C_{134}H_{135}N_2O_{30}$ $[M+H]^+$: 2251.9100. Found: 2251.9065.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4, 6-di-O-benzyl-α-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-glucopyranosyl fluoride (**14**).

Compound **13** (380 mg, 0.17 mmol) was treated with DAST (35 ml, 0.25 mmol) in dry CH_2Cl_2 as described above for **7**. Chromatography of the crude product afforded **14** (379 mg, quant.): Rf 0.39 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.67 (dd, 0.5H, J = 7.7, 53.8 Hz, H-1a), 5.49 (d, 2H, J = 1.8 Hz, H-2d and H-2e), 5.34 (s, 1H, H-1c), 5.24 (d, 1H, J = 8.1 Hz, H-1b), 5.23 (s, 1H) and 4.94 (d, 1H, J = 1.8 Hz) (H-1d and H-1e), 2.09 and 2.04 (2s, 6H, Ac); ^{13}C-NMR: δ 104.5 (d, J = 211.8 Hz, C-1a), 99.4 and 98.4 (C-1c, C-1d, and C-1e), 96.3 (C-1b). HRMS Calcd. for $C_{134}H_{134}N_2O_{30}F$ $[M+H]^+$: 2253.9056. Found: 2253.9004.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4, 6-di-O-benzyl-α-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-D-glucopyranosyl azide (**15**).

A mixture of Cp_2HfCl_2 (77 mg, 0.26 mmol), $AgClO_4$ (109 mg, 0.53 mmol), and dried powdered molecular sieves 4A (590 mg) in dry CH_2Cl_2 (10 ml) was stirred at room temperature for 30 min under Ar. To the mixture was added a solution of $TMSN_3$ (0.21 ml, 1.6 mmol) in dry CH_2Cl_2 (20 ml). After stirring for 30 min, the mixture was cooled on an ice–MeOH bath and a solution of **14** (400 mg, 0.18 mmol) in dry CH_2Cl_2 (20 ml) was added to the mixture. The stirring was continued at –10°C – room temperature overnight. 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 crude product was chromatographed on silica gel with 3:1 toluene–EtOAc to give **15** (376 mg, 93%): $[\alpha]_D$+34.3° (c = 1.1); Rf 0.41 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.49 and 5.48 (2s, 2H, H-2d and H-2e), 5.35 (s, 1H, H-1c), 5.25 (d, 1H, J = 8.8 Hz, H-1b), 5.23 (d, 1H, J = 2.2 Hz) and 4.94 (s, 1H) (H-1d and H-1e), 5.13 (d, 1H, J = 9.3 Hz, H-1a), 2.08 and 2.05 (2s, 6H, Ac). Anal. Calcd. for

$C_{134}H_{133}N_5O_{29}$: C, 70.67; H, 5.89; N, 3.08. Found: C, 70.32; H, 5.82; N, 2.80.

3,4,6-Tri-O-benzyl-α-D-mannopyranosyl-(1→3)- [3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-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-D-glucopyransoyl azide (**16**).

A mixture of **15** (34 mg, 15 μmol) and ethylenediamine (25 μl, 460 μmol) in EtOH (5 ml) was heated under reflux for 4 days and concentrated *in vacuo*. The residue was dissolved in MeOH (5 ml), stirred with Ac_2O (1 ml) at 0°C for 4 h, and concentrated *in vacuo*. To a solution of the product in MeOH (5 ml) was added 5.2 M NaOMe/MeOH (31 μl, 160 μmol). After stirring for 12 h, the reaction mixture was neutralized with Amberlist 15 and concentrated *in vacuo*. The crude product was purified by preparative TLC on a silica gel plate with 15:1 $CHCl_3$–MeOH to give **16** (20 mg, 67%): $[\alpha]_D$+7.3° (c = 0.9); Rf 0.46 (30:1 $CHCl_3$−MeOH); ^{1}H-NMR: δ 6.24 (d, 1H, J = 8.8 Hz, N*H*), 5.27 (s, 1H, H-1c), 5.26 and 5.01 (2s, 2H, H-1d, H-1e), 4.75 (d, 1H, J = 8.3 Hz, H-1a), 4.20 (d, 1H, J = 8.1 Hz, H-1b), 1.92 and 1.72 (2s, 6H, Ac). HRMS Calcd. for $C_{118}H_{130}N_5O_{25}$ $[M+H]^+$: 2016.9055. Found: 2016.9004.

N-Acetyl-L-aspartic α-tritylamide (**18**).

A stirred mixture of N-acetyl-L-aspartic α-amide **17** (247 mg, 1.4 mmol), triphenylmethanol (738 mg, 2.8 mmol), Ac_2O (267 μl, 2.8 mmol), and conc H_2SO_4 (7 μl, 13 μmol) in AcOH (4.2 ml) was heated at 50°C for 2 h. After cooling, the mixture was slowly poured into ice-water (42 ml). The resulting precipitate was collected by filtration and recrystallized from hexane–EtOAc to give **18** (472 mg, 80%): $[\alpha]_D$–58.6° (c = 1.2, MeOH); ^{1}H-NMR: δ 8.07 (s, 1H, N*H*Tr), 7.27–7.20 (m, 15H, Ar), 6.78 (d, 1H, J = 8.1 Hz, N*H*Ac), 4.89 (dt, 1H, J = 3.7, 8.1 Hz, α-H), 2.81 (dd, 1H, J = 3.7, 17.3 Hz, β-H), 2.62 (dd, 1H, J = 8.1, 17.3 Hz, β-H), 1.98 (s, 3H, Ac). HRMS Calcd. for $C_{25}H_{25}N_2O_4$ $[M+H]^+$: 417.1814. Found: 417.1817.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-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-glucopyranosyl}-L-asparagine α-tritylamide (**20**).

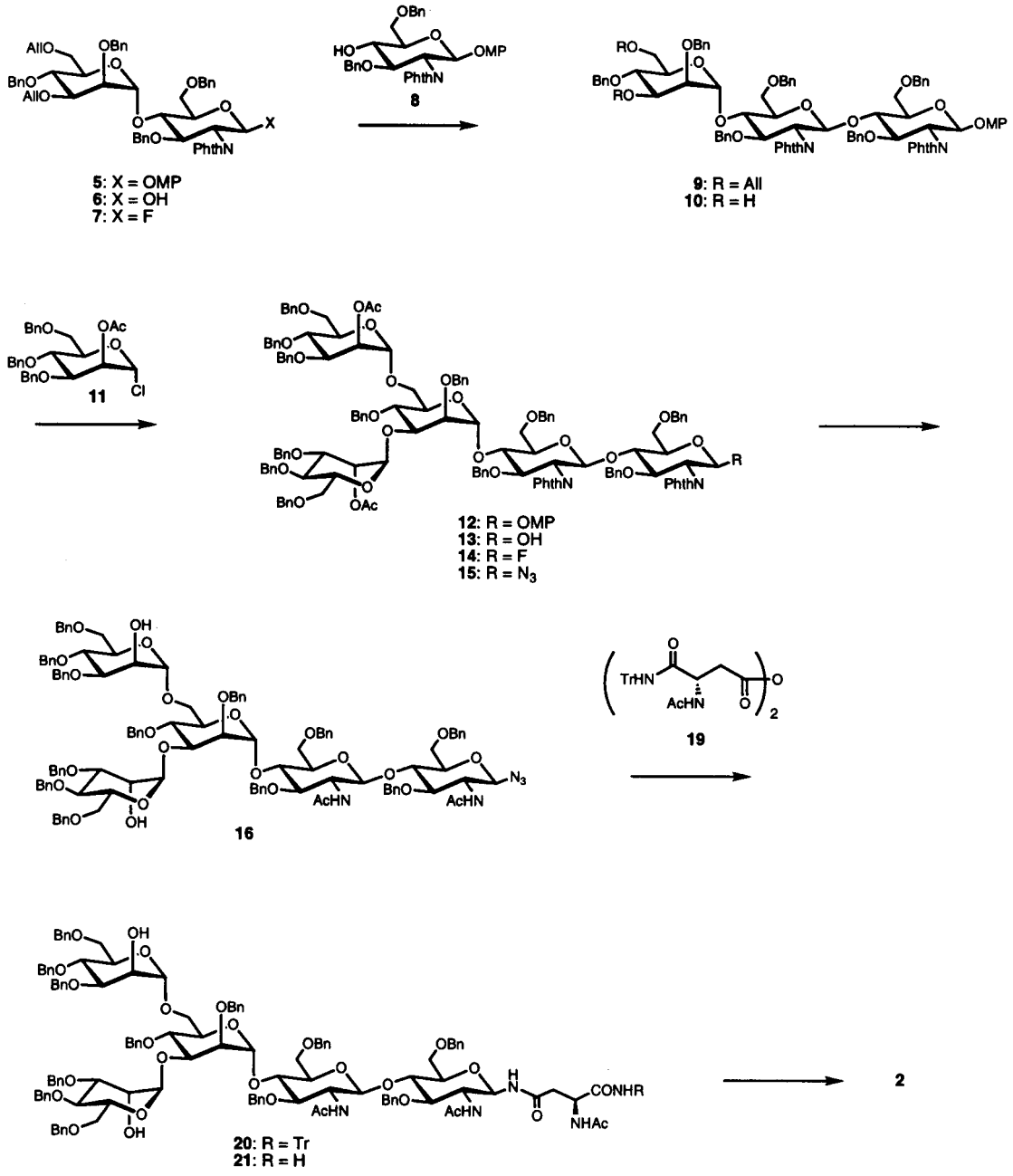
A mixture of **18** (10 mg, 24 μmol) and DCC (2.5 mg, 12 μmol) in dry CH_2Cl_2 (1 ml) was stirred at 0°C for 1 h and filtered through a membrane filter. The filtrate was concentrated *in vacuo* to give **19**, which was used for further reaction without any purification. To the residue (**19**), were added dried powdered molecular sieves 3A (85 mg), Lindler catalyst (15 mg), and then a solution of **16** (13 mg, 6.4 μmol) in dry CH_2Cl_2 (1.5 ml). After stirring for 10 min, the solvent was evaporated under vacuum and displaced with dry THF (4 ml). Then the mixture was stirred in an atmosphere of H_2 at room temperature for 8.5 h. The insoluble materials were filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by preparative TLC with 10:1 $CHCl_3$–MeOH to afford **20** (10.4 mg, 67%) and the α-isomer (2.6 mg, 17%). Compound **20**: $[\alpha]_D$+22.9° (c = 0.6); Rf 0.41 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 8.06 (s, 1H, N*H*Tr), 5.33 (d, 1H, J = 1.7 Hz, H-1c), 5.25 and 4.99 (2s, 2H, H-1d, H-1e), 4.78 (d, 1H, J = 8.1 Hz, H-1a), 4.38 (d, 1H, J = 6.1 Hz, H-1b), 4.01 (s, 2H, H-2d and H-2e), 2.68 (dd, 1H, J = 3.4, 15.9 Hz, Asn β-H), 2.36 (s, 1H, OH), 2.31 (dd, 1H, J = 6.1, 15.9 Hz, Asn β-H), 2.22 (s, 1H, OH), 1.95, 1.69 and 1.49 (3s, 9H, Ac). HRMS Calcd. for $C_{143}H_{154}N_5O_{28}$ $[M+H]^+$: 2389.0780. Found: 2389.0732. **α-isomer**: $[\alpha]_D$+11.3° (c = 0.8); Rf 0.42 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 8.52 (s, 1H, N*H*Tr), 6.98 (d, 1H, J = 6.8 Hz, Asn γ-CON*H*), 6.77 (d, 1H, J = 8.8 Hz, Asn N*H*Ac), 5.47 (d, 1H, J = 8.6 Hz, H-1a), 5.30 and 5.01 (2s, 2H, H-1d and H-le), 5.18 (s, 1H, H-1c), 4.01 (s, 2H, H-2d and H-2e), 2.88 (dd, 1H, J = 2.9, 16.4 Hz, Asn β-H), 2.46 (dd, 1H, J = 7.8, 16.4 Hz, and Asnβ-H), 2.37 (brs, 1H, OH), 2.25 (brs, 1H, OH), 2.14, 1.96 and 1.71 (3s, 9H, Ac). HRMS Calcd. for $C_{143}H_{154}N_5O_{28}$ $[M+H]^+$: 2389.0780. Found: 2389.0762.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-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-glucopyranosyl}-L-asparagine α-amide **(21)**.

Compound **20** (15 mg, 6.3 μmol) was dissolved in 2:1 CH_2Cl_2–TFA (0.75 ml) and stirred at room temperature for 4 h before concentration *in vacuo*. The residue was chromatographed on Sephadex LH20 in MeOH and further purified by preprative TLC with 10:1 $CHCl_3$–MeOH to give **21** (8.2 mg, 63%): $[\alpha]_D$+29.7° (c = 1); Rf 0.24 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 7.71 (d, 1H, J = 7.3 Hz, Asn γ-CON*H*), 6.77 (d, 1H, J = 8.8 Hz, Asn N*H*Ac), 5.32 (s, 1H, H-1c), 5.24 and 4.99 (2s, 2H, H-1d, H-1e), 2.67 (dd, 1H, J = 3.7, 16.4 Hz, Asn β-H), 2.45 (dd, 1H, J = 6.6, 16.4 Hz, Asn β-H), 1.98, 1.79 and 1.69 (3s, 9H, Ac). HRMS Calcd. for $C_{124}H_{140}N_5O_{28}$ $[M+H]^+$: 2146.9685. Found: 2146.9663.

N^2-Acetyl-N^4-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucophyranosyl}-L-asparagine α-amide **(2)**.

Compound **21** (8.2 mg, 3.8 mmol) was hydrogenated over 10% Pd-C (10 mg) in 80% aq AcOH (1.5 ml) under atmospheric pressure for 1 day. The catalyst was filtered off through a membrane filter and the filtrate was concentrated *in vacuo* to give **2** (4.5 mg, quant.): $[\alpha]_D$+47.6° (c = 0.5, H_2O); Rf 0.33 (5:2:2:1 BuOH–EtOH–H_2O–AcOH); ^{1}H-NMR (D_2O, t-BuOH at δ 1.23): δ 5.23 (s, 1H, H-1c), 5.10 and 4.89 (2s, 2H, H-1d and H-1e), 5.04 (d, 1H, J = 9.8 Hz, H-1a), 4.58 (d, 1H, J = 7.6 Hz, H-1b), 4.18 (s, 1H, H-2c), 4.05 and 3.98 (2s, 2H, H-2d and H-2e), 2.79 (dd, 1H, J = 5.1, 16.1 Hz, Asn β-H), 2.73 (dd, 1H, J = 7.6, 16.1 Hz, Asn β-H), 2.05, 2.02 and 1.99



Scheme 1.

(3s, 9H, Ac). HRMS Calcd. for $C_{40}H_{68}N_5O_{28}$ $[M+H]^+$: 1066.4051. Found: 1066.4011.

t-Butyldiphenylsilyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-β-D-glucopyranoside **(23)**.

A mixture of 3,4,6-tri-O-acetyl-2-azido-2-deoxy-D-gluco- and manno-pyranose (3.2 g, 9.6 mmol) [7], t-$BuPh_2SiCl$ (4.5 g, 16.4 mmol) and imidazole (2.6 g, 38.2 mmol) in dry DMF (20 mL) was stirred at 60°C for 3 h. The mixture was diluted with water and extracted with ether. 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 then 3:2 hexane–EtOAc to give crystalline **23** (2.2 g, 40%) and syrupy manno isomer (2.0 g, 36%). Compound **23**: mp. 78–79°C (recrystallized from hexane); $[\alpha]_D$+6.8° (c = 1.3); Rf 0.38 (7:3 hexane–EtOAc); ^{1}H NMR: δ 7.71–7.68 (m, 4H, Ar), 7.45–7.36 (m, 6H, Ar), 4.96 (brt, 1H, J = 9.3 Hz, H-4), 4.90 (brt, 1H, J = 9.2 Hz, H-3), 4.46 (d, 1H, J = 7.8 Hz, H-1), 4.02 (dd, 1H, J = 5.3, 12.2 Hz, H-6), 3.84 (dd, 1H, J = 2.5, 12.2 Hz, H-6'), 3.57 (dd, 1H, J = 7.8, 10.0 Hz, H-2), 3.27 (m, 1H, H-5), 2.07, 1.96 and 1.95 (3s, 9 H, Ac), 1.12 (s, 9 H, t-Bu); Anal. Calcd. for $C_{28}H_{35}N_3O_8Si$: C, 59.03; H, 6.19; N, 7.38. Found: C, 59.03; H, 6.16; N, 7.44. **manno isomer** $[\alpha]_D$–52.5° (c = 1.1); Rf 0.31 (7:3 hexane–

EtOAc); ^{1}H NMR:δ 7.72 (dd, 2 H, $J = 1.5$, 8.0 Hz, Ar), 7.66 (dd, 2H, $J = 1.5$, 8.0 Hz, Ar), 7.48–7.35 (m, 6 H, Ar), 5.15 (t, 1 H, $J = 10.0$ Hz, H-4), 4.78 (dd, 1 H, $J = 3.6$, 10.0 Hz, H-3), 4.71 (d, 1 H, $J = 1.2$ Hz, H-1), 4.08 (dd, 1 H, $J = 5.8$, 11.9 Hz, H-6), 4.03 (dd, 1 H, $J = 1.2$, 3.6 Hz, H-2), 3.96 (dd, 1H, $J = 2.4$, 11.9 Hz, H-6'), 3.23 (m, 1H, H-5), 2.08, 1.99, and 1.97 (3s, 9H, Ac), 1.11 (s, 9H, t-Bu); Anal. Found: C, 58.79; H, 6.35; N, 7.28.

t-Butyldiphenylsilyl 2-azido-4,6-O-benzylindene-2-deoxy-β-D-glucopyranoside **(25)**.

To a stirred solution of **23** (7.3 g, 12.8 mmol) in MeOH (130 ml) was added 5.2 M NaOMe/MeOH (0.25 ml, 1.3 mmol) at 0°C. The reaction was continued for 3 h at 0°C – room temperature, before the mixture was neutralized with Amberlist 15 and then filtered. The filtrate was concentrated *in vacuo* to give **24**, which was used for the next reaction without further purification. A mixture of **24**, benzaldehyde dimethylacetal (2.9 ml, 19.2 mmol), and camphorsulfonic acid (297 mg) in dry CH_3CN (120 ml) was stirred at room temperature overnight. The reaction was quenched with Et_3N and diluted with EtOAc. The mixture was washed with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on silica gel with 5:1 hexane–EtOAc to give **25** (5.7 g, 88%): mp 158–158.5°C (recrystallized from hexane–EtOAc); $[\alpha]_D$-28.0° (c = 1.3); Rf 0.26 (5:1 hexane–EtOAc); ^{1}H-NMR: δ 7.72–7.33 (m, 15H, Ar), 5.47 [s, 1H, PhC*H*$(O)_2$], 4.52 (d, 1H, $J = 7.8$ Hz, H-1), 3.97 (dd, 1H, $J = 5.2$, 10.5 Hz, H-6), 3.60 (t, 1H, $J = 10.5$ Hz, H-6'), 3.57 (t, 1H, $J = 9.7$ Hz) and 3.50 (t, 1H, $J = 9.0$ Hz) (H-3 and H-4), 3.45 (dd, 1H, $J = 7.6$, 9.0 Hz, H-2), 3.01 (m, 1H, H-5), 1.12 (s, 9H, t-Bu). Anal. Calcd. for $C_{29}H_{33}N_3O_5Si$: C, 65.51; H, 6.26; N, 7.90. Found: C, 65.46; H, 6.24; N, 7.85.

t-Butyldiphenylsilyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**26**).

A mixture of **25** (5.96 g, 11.2 mmol), BnBr (2.1 ml, 18 mmol), and 60% NaH (672 mg, 17.9 mmol) in dry DMF (115 ml) was stirred at room temperature under Ar overnight. The reaction was quenched by careful addition of ice-water and the product was extracted with ether. The extract was washed with water, sat. aq $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on silica gel with 9:1 hexane–EtOAc to give **26** (6.96 g, quant.): $[\alpha]_D$–48.7° (c = 1); Rf 0.49 (5:1 hexane–EtOAc); ^{1}H-NMR: δ 7.71–7.12 (m, 20H, Ar), 5.50[s, 1H, PhC*H*$(O)_2$], 4.88 (d, 1H, $J = 11.3$ Hz, –CH_2Ph), 4.74 (d, 1H, $J = 11.3$ Hz, –CH_2Ph), 4.45 (d, 1H, $J = 7.3$ Hz, H-1), 3.99 (dd, 1H, $J = 5.0$, 10.4 Hz, H-6), 3.70–3.42 (m, 4H, H-2, H-3, H-4 and H-6'), 3.00 (m, 1H, H-5), 1.11 (s, 9H, t-Bu). Anal. Calcd. for $C_{36}H_{39}N_3O_5Si$: C, 69.56; H, 6.32; N, 6.76. Found: C, 69.69; H, 6.37; N, 6.83.

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-D-glucopyranose (**27**).

To a stirred mixture of **26** (6.96 g, 11.2 mmol) and AcOH (1.4 ml, 22 mmol) in THF (100 ml) was added 1 M Bu_4NF-THF (15 ml, 15 mmol) at 0°C. The mixture was stirred for 1 h and concentrated *in vacuo*. The residue was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The product was purified by column chromotography on silica gel with 2:1 hexane–EtOAc to afford **27** (4.29 g, quant.): Rf 0.27 (2:1 hexane–EtOAc). Anal. Calcd. for $C_{20}H_{21}N_3O_5$: C, 62.70; H, 5.52; N, 10.97. Found: C, 62.71; H, 5.52; N, 10.94.

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (**28**).

A mixture of **27** (3.6 g, 9.4 mmol), trichloroacetonitrile (6.6 ml, 66 mmol), and K_2CO_3 (5.2 g, 28 mmol) in dry CH_2Cl_2 (50 ml) was stirred at 0°C under Ar for 3 h. The mixture, without concentration, was submitted to flash chromatography on silica gel with 30:1 toluene–EtOAc to give **28** (4.56 g, 92%, α/β-imidate = 1/7). A part of the sample was further chromatographed to separate α- and β-imidates for analysis. ***β*-imidate**: Rf 0.42 (30:1 toluene–EtOAc); ^{1}H-NMR: δ 8.80 (s, 1H, N*H*), 7.50–7.25 (m, 10H, Ar), 5.71 (d, 1H, $J = 8.2$ Hz, H-1), 5.59 [s, 1H, PhC*H*$(O)_2$], 4.96 (d, 1H, $J = 11.2$ Hz, –CH_2Ph), 4.82 (d, 1H, $J = 11.2$ Hz, –CH_2Ph), 4.40 (dd, 1H, $J = 4.9$, 10.6 Hz, H-6), 3.86–3.48 (m, 5H, H-2, H-3, H-4, H-5, and H-6'). Anal. Calcd. for $C_{22}H_{21}N_4O_5Cl_3$: C, 49.78; H, 3.99; N, 10.56. Found: C, 50.00; H, 3.98; N, 10.59. **α-imidate**: Rf 0.30 (30:1 toluene–EtOAc); ^{1}H-NMR: δ 8.73 (s, 1H, N*H*), 7.51–7.15 (m, 10H, Ar), 6.37 (d, 1H, $J = 3.6$ Hz, H-1), 5.61[s, 1H, PhC*H*$(O)_2$], 5.03 (d, 1H, $J = 10.7$ Hz, –CH_2Ph), 4.84 (d, 1H, $J = 10.7$ Hz, –CH_2Ph), 4.36–3.69 (m, 6H, H-2, H-3, H-4, H-5, H-6, and H-6').

t-Butyldiphenylsilyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**31**).

A mixture of **29** (10 g, 20 mmol) and hydrazine acetate (2.3 g, 0.3 mol) in dry DMF (50 ml) was heated at 50°C for 15 min, then cooled, and extracted with EtOAc. The extract was washed with sat. aq $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was crystallized from hexane to give **30** (7.6 g, 83%), which was dissolved in dry DMF (120 ml) and stirred with t-BuPh_2SiCl (8.2 g, 29.8 mmol), imidazole (2.0 g, 29.4 mmol), and 4-(dimethylamino)pyridine (0.28 g, 0.23 mmol) at room temperature overnight. After removal of most DMF *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 10:1 toluene–EtOAc to give **31** (10.4 g, 89% from **30**): $[\alpha]_D$+30.3° (c = 0.5); Rf 0.21 (3:1 toluene–EtOAc); ^{1}H-NMR: δ 7.79–7.04 (m, 14H, Ar), 5.71 (dd, 1H, $J = 9.2$, 10.2 Hz, H-3), 5.41 (d, 1H, $J = 8.0$ Hz, H-1), 5.12 (dd, 1H, $J = 9.1$, 10.2 Hz, H-4), 4.42 (dd, 1H, $J = 8.0$, 10.7 Hz, H-2), 4.13 (dd, 1H, $J = 5.4$, 12.0 Hz,

H-6), 4.00 (dd, 1H, $J = 2.4$, 12.0 Hz, H-6'), 3.55 (ddd, 1H, $J = 2.4$, 5.4, 10.2 Hz, H-5), 2.03, 1.98, and 1.84 (3s, 9H, Ac), 0.93 (s, 9H, t-Bu). Anal. Calcd. for $C_{36}H_{39}NO_{10}Si$: C, 64.17; H, 5.83; N, 2.08. Found: C, 64.19; H, 5.79; N, 2.08.

t-Butyldiphenylsilyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**33**).

According to the procedure as described for **25**, compound **31** (11.6 g, 17.2 mmol) was deacetylated to **32**: $[\alpha]_D$+0.2° (c = 1) (83%, after crystallization from hexane–ether) and then benzylidenated. Chromatography on silica gel with 6:1 toluene–EtOAc to afford **33** (7.7 g, 85% from **32**): $[\alpha]_D$–28.1°(C = 1); Rf 0.50 (4:1 toluene–EtOAc); ^{1}H-NMR: δ 7.73–7.06 (m, 19H, Ar), 5.53 [s, 1H, PhC*H*(O)$_2$], 5.36 (d, 1H, $J = 8.1$ Hz, H-1), 4.57 (m, 1H, H-3), 4.34 (dd, 1H, $J = 8.1$, 10.7 Hz, H-2), 4.15 (dd, 1H, $J = 5.1$, 10.5 Hz, H-6), 3.75 (brt, 1H, $J = 10.3$ Hz, H-6'), 3.59 (brt, 1H, $J = 9.3$ Hz, H-4), 3.35 (m, 1H, H-5), 0.94 (s, 9H, t-Bu). Anal. Calcd. for $C_{37}H_{37}NO_7Si$: C, 69.90; H, 5.87; N, 2.20. Found: C, 69.72; H, 5.88; N, 2.15.

t-Butyldiphenylsilyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**34**).

Compound **33** (3.3 g, 5.2 mmol) was benzylated as described above for **26**. The product **34** (2.8 g, 75%) was isolated by chromatography: $[\alpha]_D$+47.6° (C = 1); Rf 0.63 (4:1 toluene–EtOAc); ^{1}H-NMR: δ 7.71–6.83 (m, 24H, Ar), 5.57 [s, 1H, PhC*H*(O)$_2$], 5.28 (d, 1H, $J = 7.6$ Hz, H-1), 4.73 (d, 1H, $J = 12.2$ Hz, -C*H*$_2$Ph), 4.43 (d, 1H, $J = 12.2$ Hz, –C*H*$_2$Ph), 4.31 (m, 2H, H-2 and H-3), 4.15 (dd, 1H, $J = 4.9$, 10.3 Hz, H-6), 3.77 (m, 2H, H-4 and H-6'), 3.33 (m, 1H, H-5), 0.91 (s, 9H, t-Bu). Anal. Calcd. for $C_{44}H_{43}NO_7Si \cdot 0.5H_2O$: C, 71.91; H, 6.03; N, 1.91. Found: C, 71.90; H, 6.01; N, 2.03.

t-Butyldiphenylsilyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**35**).

To an ice-cooled mixture of **34** (10.6 g, 14.6 mmol), $NaBH_3CN$ (8.2 g, 130 mmol), and dried powdered molecular sieves 3A (25 g) in dry THF (200 ml), was added portionwise with stirring a solution of 1M HCl/ether (194 ml, 194 mmol). After stirring for 4 h at 0°C, the mixture was diluted with ether and filtered through Celite. The filtrate was washed with water, sat. aq $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromotographed on silica gel with 1:1 hexane–EtOAc to give **35** (9.5 g, 90%): $[\alpha]_D$+20.1° (c = 0.7); Rf 0.43 (1:1 hexane–EtOAc); ^{1}H-NMR: δ 7.68–6.93 (m, 24H, Ar), 5.22 (d, 1H, $J = 8.1$ Hz, H-1), 4.69 (d, 1H, $J = 12.2$ Hz, –C*H*$_2$Ph), 4.54 (d, 1H, $J = 12.0$ Hz, –C*H*$_2$Ph), 4.48 (d, 1H, $J = 12.2$ Hz, –C*H*$_2$Ph), 4.47 (d, 1H, $J = 12.0$ Hz, –C*H*$_2$Ph), 4.26 (dd, 1H, $J = 8.1$, 10.7 Hz, H-2), 4.16 (dd, 1H, $J = 8.3$, 10.7 Hz, H-3), 3.82 (m, 1H, H-4), 3.66 (dd, 1H, $J = 4.6$, 10.2 Hz, H-6), 3.60 (dd, 1H, $J = 4.9$, 10.2 Hz, H-6'), 3.29 (m, 1H, H-5), 2.77 (d, 1H, $J = 2.4$ Hz, O*H*), 0.89 (s, 9H, t-Bu).

Anal. Calcd. for $C_{44}H_{45}NO_7Si \cdot 0.2H_2O$: C, 72.24; H, 6.26; N, 1.91. Found: C, 72.19; H, 6.20; 1.95.

t-Butyldiphenylsilyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**36**).

A mixture of **35** (1.13 g, 1.55 mmol) and dried powdered molecular sieves 4A (4.2 g) in dry CH_2Cl_2 (50 ml) was stirred at room temperature under Ar for 30 min, then cooled at −40°C, and a solution of **28** (1.20 g, 2.26 mmol) was added to the mixture. To the stirred mixture was added TMSOTf (250 µl, 1.36 mmol) in four portions at the interval of 2 h and then the mixture was allowed to stir overnight at the temperature. Since consumption of **28** was incomplete, an additional amount of TMSOTf (87 µl, 0.45 mmol) was added and the temperature was raised to –20°C. After stirring for 6 h, the reaction was quenched with Et_3N, the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on Bio-beads SX8 with toluene and then on silica gel with 20:1 toluene–EtOAc to give **36** (0.94 g, 56%) and β-isomer (0.15 g, 9%). Compound **36**: $[\alpha]_D$+19.8° (c = 1.7); Rf 0.38 (15:1 toluene–EtOAc); ^{1}H-NMR: δ 7.73–6.94 (m, 34H, Ar), 5.57 (d, 1H, $J = 4.1$ Hz, H-1b), 5.54 [s, 1H, PhC*H*(O)$_2$], 5.21 (d, 1H, $J = 8.2$ Hz, H-1), 4.92, 4.78, 4.74, and 4.54 (4d, 4H, $J = 11.0$, 11.5, 11.0, and 12.5 Hz, –C*H*$_2$Ph), 4.49 (m, 1H, H-3a), 4.48 (d, 1H, $J = 12.5$ Hz, –C*H*$_2$Ph), 4.39 (dd, 1H, $J = 8.2$, 10.8 Hz, H-2a), 4.16 (m, 1H, H-4a), 4.12 (m, 1H, H-6b), 3.94 (t, 1H, $J = 9.6$ Hz, H-3b), 3.85 (dt, 1H, $J = 5.0$, 9.6 Hz, H-5b), 3.74 (dd, 1H, $J = 3.2$, 11.7 Hz, H-6a), 3.65 (t, 1H, $J = 9.6$ Hz, H-4b), 3.61 (t, 1H, $J = 9.6$ Hz, H-6'b), 3.44 (dd, 1H, $J = 1.6$, 11.7 Hz, H-6'a), 3.33 (dd, 1H, $J = 4.1$, 9.6 Hz, H-2b), 3.26 (m, 1H, H-5a), 0.89 (s, 9H, t-Bu). Anal. Calcd. for $C_{64}H_{64}N_4O_{11}Si \cdot 0.5H_2O$: C, 69.73; H, 5.94; N, 5.08. Found: C, 69.77; H, 5.93; N, 4.99. **β-isomer**: Rf 0.30 (15:1 toluene–EtOAc); ^{1}H-NMR: δ 7.73–6.94 (m, 34H, Ar), 5.46 [s, 1H, PhC*H*(O)$_2$], 5.20 (d, 1H, $J = 8.0$ Hz, H-1a), 4.49 (d, 1H, $J = 8.0$ Hz, H-1b), 0.89 (s, 9H, t-Bu).

*t-Butyldiphenylsilyl 2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**37**).*

Compound **36** (1.04 g, 0.95 mmol) was submitted to the reductive cleavage of benzylidene in a similar procedure as described for **35** to give **37** (0.93 g, 90%): $[\alpha]_D$+58.6° (c = 1.7); Rf 0.35 (15:1 toluene–EtOAc); ^{1}H-NMR: δ 7.68–7.01 (m, 34H, Ar), 5.58 (d, 1H, $J = 3.8$ Hz, H-1b), 5.22 (d, 1H, $J = 7.8$ Hz, H-1a), 4.83 (brs, 2H, –C*H*$_2$Ph), 4.79 (d, 1H, $J = 11$ Hz, –C*H*$_2$Ph), 4.51–4.30 (m, 7H, –C*H*$_2$Ph), 4.14 (t, 1H, $J = 9.1$ Hz, H-4a), 3.74–3.68 (m, 4H, H-6a, H-3b, H-4b, and H-5b), 3.56 (dd, 1H, $J = 3.1$, 7.5 Hz, H-6b), 3.46–3.37 (m, 2H, H-6a and H-6b), 3.30–3.23 (m, 2H, H-5a and H-2b), 2.67 (s, 1H, OH), 0.88 (s, 9H, t-Bu). Anal. Calcd. for $C_{64}H_{66}N_4O_{11}Si$: C, 70.18; H, 6.07. Found: C, 70.37; H, 6.20.

t-Butyldiphenylsilyl 3,6-di-O-allyl-2,4-di-O-benzyl-β-(**39**) *and -α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-*

deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**40**).

A mixture of **37** (27.9 mg, 26 μmol), Ag-silica-alumina (280 mg), molecular sieves 4A (100 mg) in dry CH_2Cl_2 (1.5 ml) was stirred at room temperature under Ar for 30 min, and then cooled to 0°C. To the stirred mixture was added a solution of **38** (67 mg, 0.13 mmol). The mixture was allowed to stir at 0°C – room temperature for 20 h before dilution with EtOAc and filtration through Celite. The filtrate was washed with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromatographed on Bio-beads SX3 with 1:1 toluene–EtOAc and then on silica gel with 10:1 toluene–EtOAc to give **39** (13.1 mg, 34%) and **40** (11.3 mg, 29%). Compound **39**: $[\alpha]_D$+29.1° (c = 0.6); Rf 0.28 (10:1 toluene–EtOAc); ^{1}H-NMR:δ 5.58 (d, 1H, J = 3.9 Hz, H-1b), 5.23 (d, 1H, J = 8.3 Hz, H-1a), 4.33 (s, 1H, H-1c), 0.88 (s, 9H, t-Bu); ^{13}C-NMR: δ 100.7 (C-1c, $^1J_{C\text{-}H}$ = 156.4 Hz), 97.0 (C-1b), 93.4 (C-1a). Anal. Calcd. for $C_{90}H_{96}N_4O_{16}Si$: C, 71.22; H, 6.38; N, 3.69. Found: C, 71.11; H, 6.44; N, 3.32. Compound **40**: $[\alpha]_D$+88.7° (c = 1); Rf 0.47 (10:1 toluene–EtOAc); ^{1}H-NMR: δ 5.58 (d, 1H, J = 3.9 Hz, H-1b), 5.21 (d, 1H, J = 8.3 Hz, H-1a), 5.24 (d, 1H, J = 1.5 Hz, H-1c), 0.88 (s, 9H, t-Bu); ^{13}C-NMR: δ 100.4 (C-1c, $^1J_{C\text{-}H}$ = 170.6 Hz), 97.0 (C-1b), 93.4 (C-1a). Anal. Found: C,71.08; H, 6.33; N, 3.65.

t-Butyldiphenylsilyl 2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**41**).

Compound **39** (161 mg, 0.11 mmol) was deallylated according to the procedure as described for **10**. The crude product was chromatographed on silica gel with 5:1 toluene–EtOAc to afford **41** (133 mg, 87%): $[\alpha]_D$+20.8° (c = 0.9); Rf 0.38 (3:1 toluene–EtOAc); ^{1}H-NMR: δ 5.60 (d, 1H, J = 3.9 Hz, H-1b), 5.23 (d, 1H, J = 7.9 Hz, H-1a), 4.33 (s, 1H, H-1c), 0.88 (s, 9H, t-Bu); ^{13}C-NMR: δ 101.0 (C-1c), 96.8 (C-1b), 93.5 (C-1a). Anal. Calcd. for $C_{84}H_{88}N_4O_{16}Si \cdot 1.5H_2O$: C, 68.88; H, 6.26; N, 3.83. Found: C, 68.13; H, 6.09; N, 3.76.

t-Butyldiphenylsilyl 2,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyransosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**42**).

Compound **40** (78 mg, 51 μmol) was deallylated according to the procedure as described for **10**. The crude product was chromatographed on silica gel with 5:1 toluene–EtOAc to afford **42** (63 mg, 85%): $[\alpha]_D$+53.3°(c = 0.7); Rf 0.40 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.62 (d, 1H, J = 3.7 Hz, H-1b), 5.23 (d, 1H, J = 7.8 Hz, H-1a), 5.19 (d, 1H, J = 1.7 Hz, H-1c), 0.89 (s, 9H, t-Bu); ^{13}C-NMR: δ 99.0 (C-1c), 96.8 (C-1b), 93.4 (C-1a). Anal. Calcd. for $C_{84}H_{88}N_4O_{16}Si$: C, 70.17; H, 6.17; N, 3.90. Found: C, 70.26; H, 6.06; N, 3.97.

t-Butyldiphenylsilyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**43**).

Compound **41** (50 mg, 35 μmol) was glycosylated with **11** (86 mg, 170 μmol) according to the procedure as described for **12**. The product was purified by chromatography on Bio-beads SX3 with 1:1 toluene–EtOAc to give **43** (73 mg, 88%): $[\alpha]_D$+28.1° (c = 1.1); Rf 0.43 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.46 (d, 1H, J = 1.7 Hz) and 5.30 (s, 1H) (H-2d and H-2e), 5.22 (d, 1H, J = 3.9 Hz, H-1b), 5.17 (d, 1H, J = 7.8 Hz, H-1a), 5.12 and 4.72 (2d, 2H, J = 1.5 and 1.7 Hz, H-1d and H-1e), 2.09 and 2.01 (2s, 6H, Ac), 0.86 (s, 9H, t-Bu); ^{13}C-NMR: δ 100.2(C-1c), 99.4 and 97.4 (C-1d and C-1e), 97.0 (C-1b), 93.3 (C-1a), Anal. Calcd. for $C_{142}H_{148}N_4O_{28}Si$: C, 71.46; H, 6.25; N, 2.35. Found: C, 71.58; H, 6.17; N, 2.20.

t-Butyldiphenylsilyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**44**).

To a stirred mixture of **43** (70 mg, 29 μmol) and AcOH (150 μl) in CH_2Cl_2 (5 ml), was added powdered Zn (400 mg). The mixture was stirred at room temperature for 2 h before filtration through Celite. The mixture was concentrated with toluene and the residue was stirred with a mixture of Ac_2O (0.3 ml), CH_2Cl_2 (5 ml), and MeOH (5 ml) at room temperature for 2 h. The mixture was diluted EtOAc, washed with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with 1:1 toluene–EtOAc to give **44** (63 mg, 88%): $[\alpha]_D$+38.8°(c = 0.7); Rf 0.54 (1:1 toluene–EtOAc); ^{1}H-NMR: δ 6.09 (d, 1H, J = 9.8 Hz, N*H*), 5.48 and 5.36 (2s, 2H, H-2d and H-2e), 5.13 and 4.81 (2s, 2H, H-1d and H-1e), 5.12 (d, 1H, J = 3.2 Hz, H-1b), 5.08 (d, 1H, J = 7.3 Hz, H-1a), 2.08 and 1.91 (2s, 6H, AcO-), 1.59 (s, 3H, AcNH-), 0.85 (s, 9H, t-Bu); ^{13}C-NMR: δ 102.0 (C-1c), 99.5 and 98.2 (C-1d and C-1e), 98.9 (C-1b), 93.3 (C-1a). Anal. Calcd. for $C_{144}H_{152}N_2O_{29}Si \cdot H_2O$: C, 71.44; H, 6.41; N, 1.16. Found: C, 71.48; H, 6.36; N, 0.98.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (**45**).

Compound **44** (63 mg, 26 μmol) was desilylated in a similar manner as described for **26**. Chromatography of the crude product on silica gel with 1:1 toluene–EtOAc to give **45** (42 mg, 74%): Rf 0.38 (1:1 toluene–EtOAc); ^{1}H-NMR: δ 6.12 (d, 1H, J = 9.3 Hz, N*H*), 5.49 and 5.37 (2s, 2H, H-2d and H-2e), 5.21 (brd, 1H, J = 4.4 Hz, H-1aα), 5.14 and 4.83 (2s, 2H, H-1d and H-1e), 5.11 (d, 1H, J = 3.4 Hz, H-1b), 2.08

and 1.93 (2s, 6H, AcO-), 1.59 (s, 3H, AcNH-); ^{13}C-NMR: δ 101.9 (C-1c), 99.5 and 98.3 (C-1d and C-1e), 99.1 (C-1b), 92.8 (C-1a). Anal. Calcd. for $C_{128}H_{134}N_2O_{29} \cdot H_2O$: C, 70.44; H, 6.28; N, 1.28. Found: C, 70.58; H, 5.98; N, 1.50.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-α- and β-D-glucopyranosyl fluoride (**46**).

In a similar procedure described for **7**, a solution of hemiacetal **45** (40 mg, 18 μmol) in CH_2Cl_2 (3 ml) was treated with DAST. The product was chromatographed on silica gel with 1:1 toluene–EtOAc to give **46** (40 mg, quant) as an $\alpha/\beta = 2/3$ mixture: Rf 0.42 (3:2 toluene–EtOAc); ^{1}H-NMR: δ 5.90 (d, 0.6H, $J = 9.0$ Hz, N*H*), 5.76 (dd, 0.6H, $J = 7.6$, 54.0 Hz, H-1aβ), 5.50 and 5.38 (2s, 1.2H, H-2dβ and H-2eβ), 5.48 (dd, 0.4H, $J = 2.7$, 53.5 Hz, H-1aα) Anal. Calcd. for $C_{128}H_{133}N_2O_{28}F$: C, 70.96; H, 6.19; N, 1.29. Found: C, 71.19; H, 6.30; N, 1.24.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (**47**).

According to the procedure described for **15**, compound **46** (165 mg, 75 μmol) was converted to azide **47** (150 mg, 90%): $[\alpha]_D$+44.0° (c = 1); Rf 0.42 (3:2 toluene–EtOAc); ^{1}H-NMR: δ 5.98 (d, 1H, $J = 9.3$ Hz, N*H*), 5.49 and 5.37 (2s, 2H, H-2d and H-2e), 5.23 (d, 1H, $J = 9.5$ Hz, H-1a), 5.16 (d, 1H, $J = 3.3$ Hz, H-1b), 5.15 and 4.84 (2s, 2H, H-1d and H-1e), 2.09 and 1.92 (2s, 6H, AcO-), 1.61 (s, 3H, AcNH-). ^{13}C-NMR: δ 101.9 (C-1c), 99.5 and 98.3 (C-1d and C-1e), 98.9 (C-1b), 85.4 (C-1a). Anal. Calcd. for $C_{128}H_{133}N_5O_{28}$: C, 70.22; H, 6.12; N, 3.20. Found: C, 70.37;H, 6.40; N, 3.10.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl azide (**48**).

Compound **47** (51 mg, 22.8 μmol) was submitted sequentially to dephthaloylation, acetylation, and O-deacetylation according to the procedure as described for **16**. The crude product was chromatographed on silica gel with 15:1 $CHCl_3$–MeOH to give **48** (20 mg, 67%): $[\alpha]_D$+15.8° (c = 0.8); Rf 0.47 (25:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 6.30 and 5.96 (2d, 2H, $J = 9.6$ and 8.6 Hz, N*H*), 5.18 and 5.01 (2s, 2H, H-1d and H-1e), 5.05 (d, 1H, $J = 3.2$ Hz, H-1b), 4.83 (d, 1H, $J = 7.1$ Hz, H-1a), 4.68 (s,1H, H-1c), 1.71 and 1.35 (2s, 6H, Ac). HRMS: Calcd. for $C_{118}H_{130}N_5O_{25}$ $[M+H]^+$: 2016.9055. Found: 2016.9137.

t-Butyldiphenylsilyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**49**).

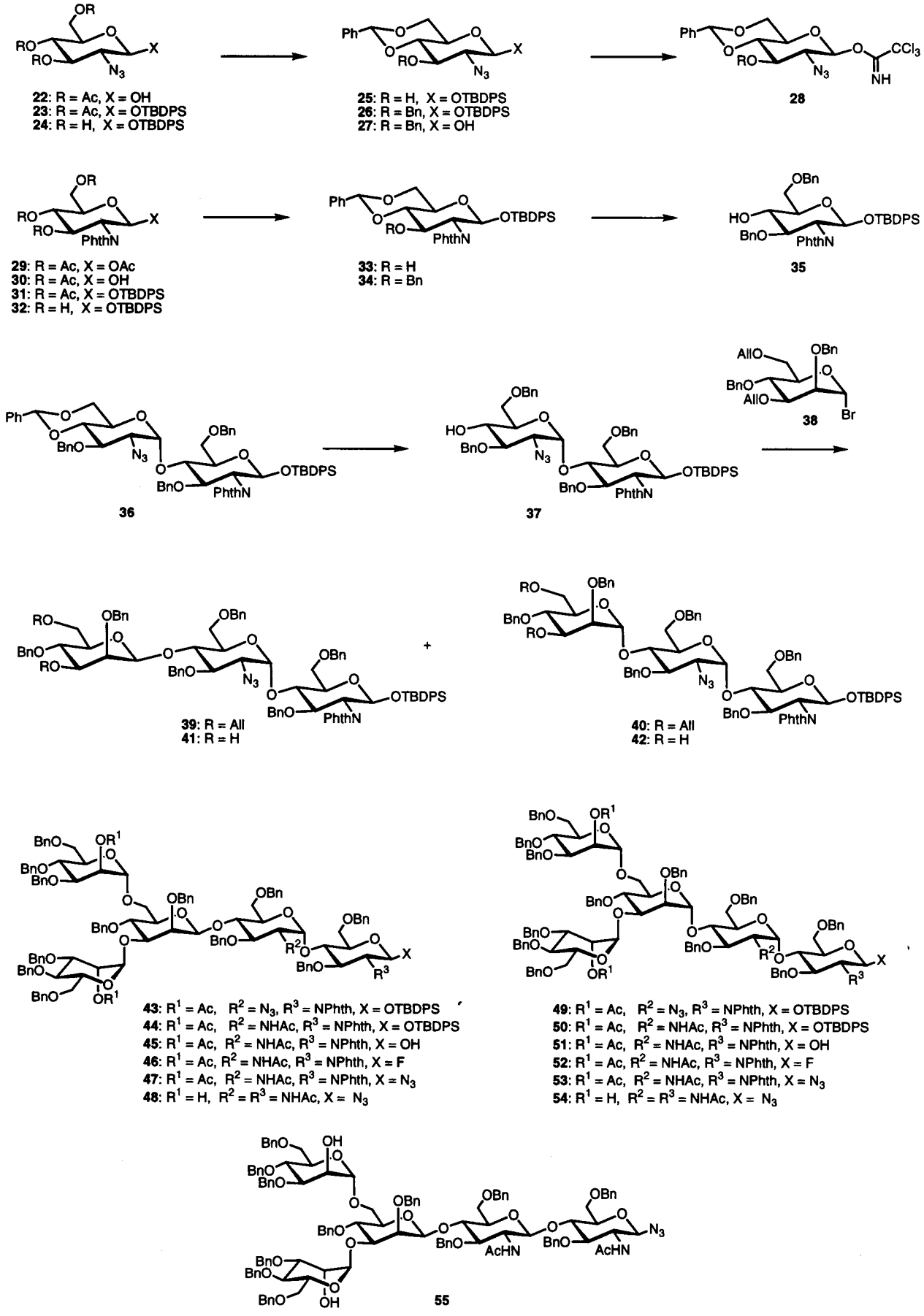
Compound **42** (95 mg, 66 μmol) was glycosylated with **11** (169 mg, 330 μmol) according to the procedure as described for **12**. The product was purified by chromatography on Biobeads SX3 with 3:1 toluene–EtOAc and then on silica gel with 5:1 toluene–EtOAc to give **49** (114 mg, 72%): $[\alpha]_D$+45.0° (c = 0.7); Rf 0.67 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 5.58 (d, 1H, $J = 3.7$ Hz, H-1b), 5.47 and 5.42 (2s, 1H, H-2d and H-2e), 5.39 (s, 1H, H-1c), 5.22 (d, 1H, $J = 7.6$ Hz, H-1a), 5.21 and 4.79 (2s, 2H, H-1d and H-1e), 2.08 (s, 6H, Ac), 0.88 (s, 9H, t-Bu); ^{13}C-NMR: δ 99.5 and 98.1 (C-1d and C-1e), 98.3 (C-1c), 97.1 (C-1b), 93.3 (C-1a). Anal. Calcd. for $C_{142}H_{148}N_4O_{28}Si$: C, 71.46; H, 6.25; N, 2.35. Found: C, 71.71; H, 6.32; N, 2.48.

t-Butyldiphenylsilyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**50**).

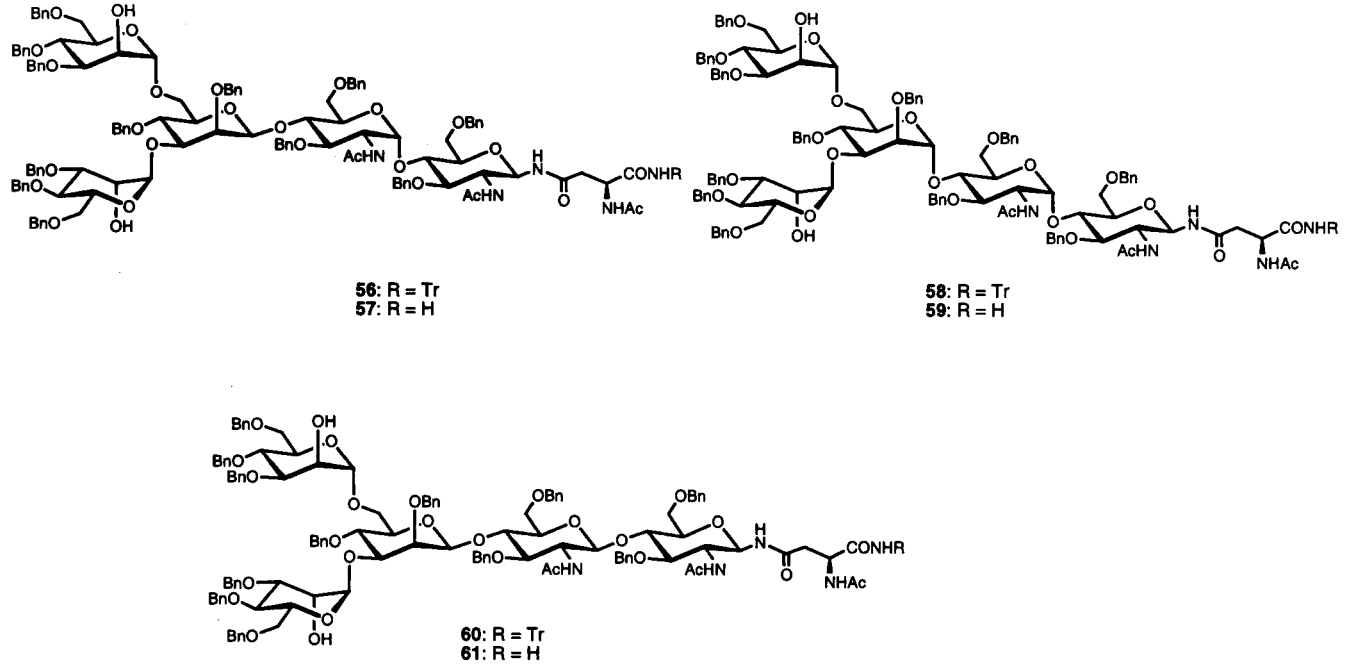
Compound **49** (113 mg, 47 μmol) was reduced with Zn and acetylated in the same procedure as described for **44**. The product was chromatographed on silica gel with 3:1 toluene–EtOAc to afford **50** (85 mg, 75%): $[\alpha]_D$+41.1° (c = 0.3); Rf 0.22 (5:1 toluene–EtOAc); ^{1}H-NMR: δ 6.39 (d, 1H, $J = 9.8$ Hz, N*H*), 5.47 (s, 1H) and 5.36 (t, 1H, $J = 2.0$ Hz) (H-2d and H-2e), 5.36 (s, 1H, H-c), 5.22 (s, 1H) and 4.90 (d, 1H, $J = 2.0$ Hz) (H-1d and H-1e), 5.15 (d, 1H, $J = 7.3$ Hz, H-1a), 5.12 (d, 1H, $J = 3.4$ Hz, H-1b), 2.09 and 2.06 (2s, 6H, AcO-), 1.48 (s, 3H, AcNH-), 0.88 (s, 9H, t-Bu); ^{13}C-NMR: δ 99.5 and 98.4 (C-1d and C-1e), 99.5 (C-1b), 98.3 (C-1c), 93.3 (C-1a). Anal. Calcd. for $C_{144}H_{152}N_2O_{29}Si$: C, 71.98; H, 6.38; N, 1.17. Found: C, 71.84; H, 6.41; N, 1.17.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannnopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (**51**).

Compound **50** (56 mg, 24 μmol) was desilylated in a similar manner as described for **27**. Chromatography of the crude product on silica gel with 2:1 toluene–EtOAC to give **51** (44 mg, 87%): Rf 0.62 (1:1 toluene–EtOAC); ^{1}H-NMR: δ 6.45 (d, 1H, $J = 9.3$ Hz, N*H*), 5.47 (s, 1H) and 5.46 (t, 1H, $J = 2.0$ Hz) (H-2d and H-2e), 5.31 (s, 1H, H-1c), 5.26 (br, 1H, H-1a), 5.24 (s, 1H) and 4.91 (d, 1H, $J = 2.0$ Hz) (H-1d and H-1e), 5.07 (d, 1H, $J = 3.2$ Hz, H-1b), 2.10 and 2.07 (2s, 6H, AcO-), 1.46 (s, 3H, AcNH-); ^{13}C-NMR: δ 99.8 (C-1b), 99.4 and 98.4 (C-1d and C-1e), 98.4 (C-1c), 92.9 (C-1a). HRMS:


22: R = Ac, X = OH
23: R = Ac, X = OTBDPS
24: R = H, X = OTBDPS
25: R = H, X = OTBDPS
26: R = Bn, X = OTBDPS
27: R = Bn, X = OH
28
29: R = Ac, X = OAc
30: R = Ac, X = OH
31: R = Ac, X = OTBDPS
32: R = H, X = OTBDPS
33: R = H
34: R = Bn
35
36
37
38
39: R = All
41: R = H
40: R = All
42: R = H
43: R1 = Ac, R2 = N3, R3 = NPhth, X = OTBDPS
44: R1 = Ac, R2 = NHAc, R3 = NPhth, X = OTBDPS
45: R1 = Ac, R2 = NHAc, R3 = NPhth, X = OH
46: R1 = Ac, R2 = NHAc, R3 = NPhth, X = F
47: R1 = Ac, R2 = NHAc, R3 = NPhth, X = N3
48: R1 = H, R2 = R3 = NHAc, X = N3
49: R1 = Ac, R2 = N3, R3 = NPhth, X = OTBDPS
50: R1 = Ac, R2 = NHAc, R3 = NPhth, X = OTBDPS
51: R1 = Ac, R2 = NHAc, R3 = NPhth, X = OH
52: R1 = Ac, R2 = NHAc, R3 = NPhth, X = F
53: R1 = Ac, R2 = NHAc, R3 = NPhth, X = N3
54: R1 = H, R2 = R3 = NHAc, X = N3
55

Scheme 2.



Scheme 3.

Calcd. for $C_{128}H_{135}N_2O_{29}$ $[M+H]^+$: 2163.9151. Found: 2163.9131.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-α- and β-D-glucopyranosyl fluoride (**52**).

In a similar procedure described for **7**, a solution of hemiacetal **51** (45 mg, 21 μmol) in CH_2Cl_2 (4 ml) was treated with DAST. The product was chromatographed on silica gel with 3:1 toluene–EtOAc to give **52** (45 mg, quant) as an $\alpha/\beta = 1/3$ mixture: Rf 0.82 (1:1 toluene–EtOAc); ^{1}H-NMR: δ 6.21 (d, 0.6H, $J = 9.8$ Hz, NH), 5.81 (dd, 0.75H, $J = 7.3$, 53.7 Hz, H-1aβ), 5.47 and 5.46 (2s, 1.5H, H-2dβ and H-2eβ), 5.35 (s, 0.75H, H-1cβ), 5.24 and 4.92 (2s, 1.5H, H-1dβ and H-1eβ), 5.12 (d, 0.75H, $J = 3.2$ Hz, H-1bβ), 2.10 and 2.07 (2s, 6H, AcO-), 1.50 (s, 3H, AcNH–). HRMS. Calcd. for $C_{128}H_{134}N_2O_{28}F$ $[M+H]^+$: 2165.9107. Found: 2165.9182.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (**53**).

According to the procedure described for **15**, compound **52** (35 mg, 16 μmol) was reacted with $TMSN_3$. The product was chromatographed on silica gel with 3:1 toulene–EtOAc to give **53** (32 mg, 91%) $[\alpha]_D$+44.0° (c = 1); Rf 0.42 (3:2 toluene–EtOAc); ^{1}H-NMR: δ 6.30 (d, 1H, $J = 9.8$ Hz, N*H*), 5.47 and 5.46 (2s, 2H, H-2d and H-2e), 5.35 (s, 1H, H-1c), 5.28 (d, 1H, $J = 9.3$Hz, H-1a), 5.23 and 4.91 (2s, 2H, H-1d and H-1e), 5.13 (d, 1H, $J = 3.2$ Hz, H-1b), 2.11 and 2.07 (2s, 6H, AcO–), 1.49 (s, 3H, AcNH-); ^{13}C-NMR: δ 99.7 (C-1b), 98.4 (C-1c), 99.7 and 98.4 (C-1d and C-le), 85.5 (C-1a). HRMS: Calcd. for $C_{128}H_{134}N_5O_{28}$ $[M+H]^+$: 2188.9215. Found: 2188.9275.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyransoyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyransoyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyransoyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl azide (**54**).

Compound **53** (29 mg, 13 μmol) was submitted sequentially to dephthaloylation, acetylation, and O-deacetylation according to the procedure as described for **16**. The crude product was chromatographed on silica gel with 15:1 $CHCl_3$–MeOH to give **54** (17 mg, 63%): $[\alpha]_D$ +38.8° (c = 1.1); Rf 0.44 (1:3 toluene–EtOAc); ^{1}H-NMR: δ 6.26 and 5.46 (2d, 2H, $J = 9.5$ and 8.3 Hz, N*H*), 5.35 (d, 1H, $J = 1.7$ Hz, H-1c), 5.25 (s, 1H) and 4.99 (d, 1H, $J = 1.5$ Hz) (H-1d and H-1e), 5.09 (d, 1H, $J = 3.4$ Hz, H-1b), 4.94 (d, 1H, $J = 9.0$ Hz, H-1a), 1.85 and 1.42 (2s, 6H, Ac). ^{13}C-NMR: δ 100.5 and 99.9 (C-1d and C-1e), 99.4 (C-1b), 98.4 (C-1c), 87.3 (C-1a). HRMS: Calcd. for $C_{118}H_{130}N_5O_{25}$ $[M+H]^+$: 2016.9155. Found: 2019.9137.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,

6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-tritylamide (**56**).

Compound **48** (19 mg, 9.4 μmol) was hydrogenated with Lindlar catalyst in the presence of **19** as described for **20**. The product was purified by preparative TLC with 10:1 $CHCL_3$–MeOH to afford **56** (20 mg, 87%): $[\alpha]_D$+19.5° (c = 1); Rf 0.43 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 8.21 (s, 1H, N*H*Tr), 7.42, 7.04, 6.32, and 5.55 (4d, 4H, J = 7.1, 7.8, 9.3, and 8.6 Hz, Asn α-N*H*, Asn γ-CON*H*, GlcNAcN*H*, and GlcNAcN*H*), 5.18 and 5.04 (2s, 2H, H-1d and H-1e), 5.08 (d, 1H, J = 3.2 Hz, H-1b), 4.88-4.85 (m, 1H, Asn α-H), 4.69 (s, 1H, H-1c), 2.64 (dd, 1H, J = 3.2, 16.1 Hz, Asn β-H), 2.34 (dd, 1H, J = 7.1, 16.1 Hz, Asn β-H), 1.94, 1.40 and 1.38 (3s, 9H, Ac). HRMS Calcd. for $C_{143}H_{154}N_5O_{28}$ $[M+H]^+$: 2389.0780. Found: 2389.0732.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-amide (**57**).

Compound **56** (11 mg, 4.6 μmol) was detritylated as described for **21**. The product was purified by gel-permeation chromatography on Sephadex LH20 in MeOH and then by preparative TLC with 10:1 $CHCl_3$–MeOH to give **57** (9 mg, 91%): $[\alpha]_D$+39.6° (c = 1); Rf 0.29 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 7.40 (d, 1H, J = 7.4 Hz, Asn γ-CON*H*), 6.74 and 5.41 (2brs, 2H, –CONH_2), 6.24 and 6.15 (2d, J = 8.8 and 8.6 Hz, GlcNAc AcN*H*), 5.18 (s, 1H) and 5.07 (s, 2H) (H-1d, H-1e, and H-1b), 4.98 (t, 1H, J = 8.6 Hz, H-1a), 4.81 (s, 1H, H-1c), 2.68 (dd, 1H, J = 3.4, 16.4 Hz, Asn β-H), 2.45 (dd, 1H, J = 6.4, 16.4 Hz, Asn β-H), 1.95, 1.69 and 1.37 (3s, 9H, Ac). HRMS Calcd. for $C_{124}H_{139}N_5O_{28}Na$ $[M+Na]^+$: 2168.9504. Found: 2168.9507.

N^2-Acetyl-N^4-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-amide (**3**).

Compound **57** (18 mg, 8 μmol) was hydrogenated for 3 days under the same condition as described for **2**. The catalyst was filtered off with a membrane filter and the filtrate was concentrated *in vacuo* to give **3** (9 mg, quant): $[\alpha]_D$+71.2° (c = 1.1, H_2O); ^{1}H-NMR: (D_2O, t-BuOH at δ 1.23): δ 5.40 (d, 1H, J = 2.4 Hz, H-1b), 5.10 and 4.90 (2s, 2H, H-1d and H-le), 5.06 (d, 1H, J = 8.6 Hz, H-1a), 4.25 (s, 1H, H-2c), 2.81 (dd, 1H, J = 4.8, 16.2 Hz, Asn β-H), 2.73 (dd, 1H, J = 7.3, 16.2 Hz, Asn β-H), 2.04, 2.02 and 2.00 (3s, 9H, Ac). HRMS Calcd. for $C_{40}H_{68}N_5O_{28}$ $[M+H]^+$: 1066.4051. Found: 1066.4047.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-tritylamide (**58**).

Compound **54** (16 mg, 7.9 μmol) was hydrogenated with Lindlar catalyst in the presence of **19** as dsescribed for **20**. The product was purified by preparative TLC with 10:1 $CHCl_3$–MeOH to afford **56** (15 mg, 80%): $[\alpha]_D$+32.3° (c = 1); Rf 0.38 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 8.13 (s, 1H, N*H*Tr), 7.58, 7.05, 6.37, and 5.12 (4d, 4H, J = 7.6, 7.3, 9.7, and 8.1 Hz, Asn α-N*H*, Asn γ-CON*H*, GlcNAcN*H*, and GlcNAcN*H*), 5.36 (s, 1H, H-1c), 5.26 and 5.01 (2s, 2H, H-1d and H-1e), 5.03 (d, 1H, J = 3.2 Hz, H-1b), 4.89-4.83 (m, 1H, Asn α-H), 4.02 (s, 2H, H-2d and H-2e), 2.61 (dd, 1H, J = 3.4, 15.9 Hz, Asn β-H), 2.35 (dd, 1H, J = 5.9, 15.9 Hz, Asn β-H), 1.98, 1.45 and 1.37 (3s, 9H, Ac). HRMS Calcd. for $C_{143}H_{154}N_5O_{28}$ $[M+H]^+$: 2389.0780. Found: 2389.0732.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-amide (**59**).

Compound **58** (15 mg, 6.3 μmol) was detritylated as described for **21**. The product was purified by gel-permeation chromatography on Sephadex LH20 in MeOH and then by preparative TLC with 10:1 $CHCl_3$–MeOH to give **59** (12 mg, 91%): $[\alpha]_D$+42.8° (c = 1); Rf 0.25 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 7.42 (d, 1H, J = 8.1 Hz, Asn α-N*H*), 7.38 (d, 1H, J = 8.8 Hz, Asn γ-CON*H*), 6.76 and 5.62 (2brs, 2H, −CONH_2), 6.35 and 5.77 (2d, J = 9.3 and 8.1 Hz, GlcNAc AcN*H*), 5.34 (s, 1H, H-1c), 5.24 and 5.01 (2s, 2H, H-1d and H-1e), 5.06 (d, 1H, J = 2.9 Hz, H-1b), 4.90 (t, 1H, J = 8.8 Hz, H-1a), 4.74-4.69 (m, 1H, Asn α-H), 4.01 (s, 2H, H-2d and H-2e), 2.67 (dd, 1H, J = 3.7, 16.1 Hz, Asn β-H), 2.48 (dd, 1H, J = 5.4, 16.1 Hz, Asn β-H), 1.99, 1.79 and 1.44 (3s, 9H, Ac). HRMS Calcd. for $C_{124}H_{140}N_5O_{28}$ $[M+H]^+$: 2146.9685. Found: 2146.9697.

N^2-Acetyl-N^4-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranosyl}-L-asparagine α-amide (**4**).

Compound **59** (12 mg, 5.6 μmol) was hydrogenated for 1 day under the same condition as described for **2**. The catalyst was filtered off with a membrane filter and the filtrate was concentrated *in vacuo* to give **4** (7 mg, quant): $[\alpha]_D$+91.7° (c = 0.7, H_2O); ^{1}H-NMR (D_2O, t-BuOH at δ 1.23): δ 5.38 (d, 1H, J = 3.7 Hz, H-1b), 5.24 (s, 1H, H-1c), 5.11 and 4.89 (2s, 2H, H-1d and H-le), 5.07 (d, 1H, J = 9.3 Hz, H-1a), 4.19 (s, 1H, H-2c), 4.05 and 3.99 (2s, 2H, H-2d and H-2e), 2.80 (dd, 1H, J = 5.1, 16.1 Hz, Asn β-H), 2.74 (dd, 1H, J = 7.6, 16.1 Hz, Asn β-H), 2.03 (s, 6H, Ac), 2.00 (s, 3H, Ac). HRMS Calcd. for $C_{40}H_{67}N_5O_{28}Na$ $[M+Na]^+$: 1088.3870. Found: 1088.3861.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-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-glucopyranosyl}-L-asparagine α-tritylamide (**60**).

Compound **55** (41 mg, 20 μmol) was hydrogenated with Lindlar catalyst in the presence of **19** as described for **20**. The product was purified by preparative TLC with 10:1 $CHCl_3$–MeOH to afford **60** (38 mg, 78%) as a mixture of inseparable anomers of N-glycoside ($\alpha/\beta = 1/3$); Rf 0.43 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR (β-anomer): δ 8.04 (s, 1H, N*H*Tr), 7.54 (d, 1H, $J = 7.1$ Hz, Asn γ-CON*H*), 7.41 (d, 1H, $J = 6.1$ Hz, Asn α-N*H*), 5.14 and 4.93 (2s, 2H, H-1d and H-1e), 2.64 (dd, 1H, $J = 3.4$, 15.9 Hz, Asn β-H), 2.31 (dd, 1H, $J = 5.9$, 15.9 Hz, Asn β-H); (α-anomer): 8.57 (s, 1H, N*H*Tr), 6.98 (d, 1H, $J = 6.6$ Hz, Asn α-N*H*), 6.88 (d, 1H, $J = 8.1$ Hz, Asn γ-CON*H*), 5.48 (d, 1H, $J = 8.8$ Hz, H-la), 5.16 and 4.97 (2s, 2H, H-1d and H-1e), 2.84 (dd, 1H, $J = 3.2$, 16.4 Hz, Asn β-H), 2.45 (dd, 1H, $J = 7.6$, 16.4 Hz, Asn β-H). HRMS Calcd. for $C_{143}H_{154}N_5O_{28}$ $[M+H]^+$: 2389.0780. Found: 2389.0762.

N^2-Acetyl-N^4-{3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-4,6-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-glucopyranosyl}-L-asparagine α-amide (**61**).

Compound **60** (28 mg, 11.7 μmol) was detritylated as described for **21**. The product was purified by gel-permeation chromatography on Sephadex LH20 in MeOH and then by preparative TLC with 10:1 $CHCl_3$–MeOH to give **61** (15 mg, 60%) and α-anomer (5 mg, 20%). Compound **61**: $[\alpha]_D$ +9.2° (c = 1); Rf 0.20 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 7.70 (d, 1H, $J = 8.8$ Hz, Asn γ−CON*H*), 6.76 and 5.48 (2brs, 2H, –CONH_2), 5.14 and 4.92 (2s, 2H, H-1d and H-1e), 2.66 (dd, 1H, $J = 3.2$, 16.4 Hz, Asn β-H), 2.44 (dd, 1H, $J = 6.1$, 16.4 Hz, Asn β-H), 1.96, 1.78 and 1.60 (3s, 9H, Ac). HRMS Calcd. for $C_{124}H_{140}N_5O_{28}$ $[M+H]^+$: 2146.9685. Found: 2146.9651. **α-anomer**: $[\alpha]_D$-2.6° (c = 0.6); Rf 0.24 (10:1 $CHCl_3$–MeOH); ^{1}H-NMR: δ 6.95 (brs, 1H, Ans γ-CON*H*), 5.55 (d, 1H, $J = 8.8$ Hz, H-1a), 5.16 and 4.99 (2s, 2H, H-1d and H-1e), 2.87 (dd, 1H, $J = 3.4$, 1.60 Hz, Asn β-H), 2.48 (dd, 1H, $J = 7.3$, 16.0 Hz, Asn β-H), 2.09, 1.99 and 172 (3s, 9H, Ac). HRMS Calcd. for $C_{124}H_{140}N_5O_{28}$ $[M+H]^+$: 2146.9685. Found: 2146.9663.

N^2-Acetyl-N^4-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl}-L-asparagine α-amide (**1**).

Compound **61** (14 mg, 6.5 μmol) was hydrogenated for 1 day under the same condition as described for **2**. The catalyst was filtered off with a membrane filter and the filtrate was concentrated *in vacuo* to give **1** (7 mg, quant): $[\alpha]_D$+22.0° (c = 0.9, H_2O); ^{1}H-NMR (D_2O, t-BuOH at δ 1.23): δ 5.09 and 4.90 (2brs, 2H, H-1d and H-1e), 5.04 (d, 1H, $J = 9.5$ Hz, H-1a), 4.69 (dd, 1H, $J = 5.1$, 7.3 Hz, Asn α-H), 4.60 (d, 1H, $J = 7.8$ Hz, H-1b), 2.80 (dd, 1H, $J = 5.1$, 16.1 Hz, Asn β-H), 2.73 (dd, 1H, $J = 7.3$, 16.1 Hz, Asn β-H), 2.06, 2.02 and 1.99 (3s, 9H, Ac). HRMS Calcd. for $C_{40}H_{68}N_5O_{28}$ $[M+H]^+$: 1066.4051. Found: 1066.4087.

Results and discussion

The syntheses of the compounds (**1**–**4**) were designed essentially based on the procedure for the natural pentasaccharide reported previously by our group [2]. The typical synthetic route is first described for the compound **2**.

The synthesis was commenced with the known disaccharide **5**, which had been obtained as a major side-product in the insoluble Ag-mediated mannosylation reaction [2c]. Oxidative cleavage of *p*-methoxyphenyl glycoside with $Ce(NH_4)_2 \cdot (NO_3)_6$ afforded hemiacetal **6** (65%), which was treated with Et_2NSF_3 in THF to give a mixture of α-and β-fluoride **7** (94%, $\alpha/\beta = 1/4$). Coupling of **7** and **8** [4] was promoted by Cp_2ZrCl_2-$AgClO_4$ [5] to stereoselectively produce trisaccharide **9** in 84% yield. Then **9** was deallylated in two steps (1. Ir-catalyzed isomerization of olefin [6], 2. Hg^{2+}-mediated hydrolysis) to furnish **10** in 87% yield. Glycosylation of **10** with 4 equivalents of mannosyl donor **11** was promoted by AgOTf to give pentasaccharide **12** in 82% yield. Before introduction of anomeric azide group *p*-methoxyphenyl group was removed and resulting hemiacetal **13** was convered into fluoride **14** (65% in 2 steps). The reaction of **14** with $TMSN_3$ under the glycodidation conditions afforded **15** exclusively. Dephthaloylation of **15** with ethylenediamine [7] was followed by acetylation furnished **16** in 67% yield.

In order to form N^4-asparagine glycoside, acylating agents derived from properly protected aspartic acid were prepared as follows. Commercial N-acetyl-L-aspartic α-amide **17** was tritylated with triphenylmethanol, acetic anhydride, and sulfuric acid in AcOH at 50 °C to produce carboxylic acid **18** in 80% yield. For coupling with pentasaccharide moiety, the compound **18** was transformed into acid anhydride. Hydrogenation of azide **16** with Lindlar catalyst in the presence of **19** in THF was accompanied by partial isomerization of 1-amino group to produce N-glycosyl asparagine as a mixture of α/β (1:3–1:4) isomers in 84% yield. The desired β product **20** was separated from the α by preparative TLC and further purification was made after N-detritylation with TFA. The isolated **20** was N-detritylated and finally hydrogenated with Pd-C in 80% AcOH to give compound **2**, quantitatively.

Construction of the α-D-GlcNAc-(1→4)-D-GlcNAc linkage present in the compounds **3** and **4** was next investigated. On the basis of the widely accepted strategy to access to the α-glycoside of 2-acetamido sugar, the glycosyl donor possessing non-participating 2-azido group was prepared. The known 2-azido-glucose derivative **22** [8] was silylated to give **23**, which was converted into 3-O-benzyl-4,6-O-benzylidene deri-

vative **25** in 71% yield via 3 steps. Desilylation of **25** followed by treatment with trichloroacetonitrile in the presence of K_2CO_3 provided a potential glycosyl donor **28** (92%, $\alpha/\beta = 1/7$). On the other hand, an acceptor monosaccharide protected as a t-butyldiphenylsilyl glycoside, which was cleaved easier than p-methoxyphenyl glycoside, was prepared. The known tetraacetate **29** was regioselectively hydrolyzed [9] and silylated to **31**. In a similar route as described for the conversion of **23** into **26**, **34** was synthesized from **31** in 62% overall yield. Reductive cleavage of the acetal ring afforded 6-O-benzyl derivative **35** exclusively. Coupling reaction of **28** and **35** was successful, when TMSOTf was used as the promoter in ether. A mixture of desired disaccharides **36** and the β-isomer was produced in 52–76% with the stereoselectivity of $\alpha/\beta = 4$–$6/1$. The structure of **36** was readily assigned by ^{13}C-NMR, where characteristic $^1J_{C\text{-}H}$ (170.6 Hz) for the anomeric carbon of α-glycoside was exhibited. Compound **36** was then submitted to the reductive acetal ring opening and resulting **37** was allowed to react with **38** in the presence of Ag-silica-alumina.

The reaction proceeded with loss of stereoselectivity to give a 1:1 mixture of **39** and **40** in 63% yield. The structure of each isomer was established by NMR. Subsequently, each product was transformed into pentasaccharide, independently. By deallylation and glycosylation with **38**, compound **39** gave **43** in 77% overall yield. Before generation of 1-azide glycoside, the azide group present in **43** was converted into acetamide group by reduction with Zn-AcOH and acetylation with Ac_2O. The compound **44** was further transformed into **48** in a similar manner as above. Analogously, the α,α-trisaccharide **40** was led to **54** through 7 steps. The compounds **48** and **54** were coupled with **19** under the reductive conditions as described for **20** to produce **56** (87%) and **58** (80%), respectively, with little epimerization ($<5\%$) of 1-amino group. Trityl group was then removed and resulting **57** and **58** were hydrogenerated to **3** and **4** respectively.

The known pentasaccharide of natural configuration **55** [2c] was also reduced and condensed with **19**. In this transformation, the intermediary amino glycoside was prone to isomerize as observed for **20** and the condensed product **60** was obtained as an $\alpha/\beta = 1/3$ mixture. Isolation of the desired isomer was successful after removing trityl group. The compound **61** was submitted to hydrogenation to afford **1**. All the stereoisomers **1, 2, 3** and **4** were characterized by NMR and high resolution mass spectra.

In summary, four stereoisomers of N-glycan core pentasaccharide were synthesized through the unambiguous procedures. ^{1}H-NMR spectra of these isomers are very similar each other except for the signals of isomeric anomeric protones. Further detailed studies are necessary to clarify their stable conformations. The samples presented here would also be the useful tools for biological studies, for example, substrate-structural requirement of glycosyltransferases in the process of further glycosylative modifications. Relating to this study, synthesis of oligopeptides carrying unnatural oligosaccharide attachment has currently been under investigation.

Acknowledgements

We thank Dr. T. Chihara and his staff for elemental analyses, and Ms. A. Takahashi for technical assistance.

References

1 Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J, ed. *Essentials in Glycobiology*, (Cold Spring Harbor Laboratory Press, New York 1999).

2 (a) Ogawa T, Kitajima T, Nukada T, Synthesis of a nonahexosyl unit of a complex type of glycan chain of a glycoprotein, *Carbohydr Res* **123**, C8–C11 (1983). (b) Ogawa T, Sugimoto M, Kitajima T, Sadozai KK, Nukada T, Total synthesis of a undecasaccharide: a typical carbohydrate sequence for the complex type of glycan chains of a glycoprotein, *Tetrahedron Lett* **27**, 5739–42 (1986). (c) Matsuo I, Nakahara Y, Ito Y, Nukada T, Nakahara Y, Ogawa T, Synthesis of a glycopeptide carrying a N-linked core pentasaccharide, *Bioorg Med Chem* **3**, 1455–63 (1995). (d) Nakahara Y, Shibayama S, Nakahara Y, Ogawa T, Rationally designed syntheses of high-mannose and complex type undecasaccharides, *Carbohydr Res* **280**, 67–84 (1996). (e) Dan A, Ito Y, Ogawa T, An efficient synthetic route to bisecting N-acetylglucosamine containing asparagine-linked glycan, *Carbohydr Lett* **1**, 469–74 (1996). (f) Unverzagt C, Chemoenzymatic synthesis of a siaylated undecasaccharide-asparagine conjugate, *Angew Chem Int Ed* **35**, 2350–3 (1996). (g) Guo Z-W, Nakahara Y, Ogawa T, A practical and efficient synthesis of complex-type biantennary heptasaccharide-asparagine conjugate, a key building block for the synthesis of complex N-linked glycopeptides, *Tetrahedron Lett* **38**, 4799–802 (1997). (h) Guo Z-W, Nakahara Y, Nakahara Y, Ogawa T, Solid-phase synthesis of CD52 glycopeptide and an efficient route to Asn-core pentasaccharide conjugate, *Bioorg Med Chem* **5**, 1917–24 (1997). (i) Seifert J, Unverzagt C, Synthesis of three biantennary N-glycans containing the α-1, 6 core-fucosyl motif, *Tetrahedron Lett* **38**, 7857–60 (1997). (j) Dan A, Lergenmüller M, Amano M, Nakahara Y, Ogawa T, Ito Y, p-Methoxybenzylidene-tethered b-mannosylation for stereoselective synthesis of asparagine-linked glycan chains, *Chem Eur J* **4**, 2182–90 (1998). (k) Seifert J, Lergenmüller M, Ito Y, Synthesis of an α-(2,3)-sialylated, complex-type undecasaccharide, *Angew Chem Int Ed* **39**, 531–4 (2000). (l) Unverzagt C, Seifert J, Chemoenzymatic synthesis of deca and dodecasaccharide N-glycans of the 'bisecting' type, *Tetrahedron Lett* **41**, 4549–53 (2000).

3 For reviews on the earlier works in this field, see: Paulsen H, Syntheses, conformations and X-ray structure analyses of the saccharide chains from the core regions of glycoproteins. *Angew Chem Int Ed Engl* **29**, 823–39 (1990).

4 Nakano T, Ito Y, Ogawa T, Synthesis of sulfated glucuronyl glycosphingolipids; carbohydrate epitopes of neural cell-adhesion molecules, *Carbohydr Res* **243**, 43–69 (1993).

5 (a) Suzuki K, Maeta H, Matsumoto T, Tsuchihashi G, New glycosidation reaction 1. Combinational use of Cp_2ZrCl_2-$AgClO_4$ for activation of glycosyl flurides and application to highly β-selective glycosidation of D-mycinose, *Tetra-*

hedron Lett **29**, 3567–70 (1988). (b) Suzuki K, Maeta H, Matsumoto T, Tsuchihashi G, New glycosidation reaction 2. Preparation of 1-fluoro-D-desosamine derivative and its efficient glycosidation by the use of Cp_2HfCl_2-$AgClO_4$ as the activator, *Tetrahedron Lett* **29**, 3571–4 (1988). (c) Suzuki K, Maeta H, Matsumoto T, An improved procedure for metallocene-promoted glycosidation. Enchanced reactivity by employing 1:2-ratio of Cp_2HfCl_2-$AgClO_4$, *Tetrahedron Lett* **30**, 4853–6 (1989).

6 Oltvoort JJ, van Boeckel CAA, de Koning JH, van Boom JH, Use of cationic iridium complex 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate in carbohydrate chemistry: Smooth isomerization of allyl ethers to 1-propenyl ethers, *Synthesis* 305–8 (1981).

7 Kanie O, Crawley SC, Palcic MM, Hindsgaul O, Acceptor-substrate recognition by *N*-acetylglucosaminyltransferase-V: critical role of the 4"-hydroxyl group in β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*(1 → 6)-β-D-Glc*p*-OR, *Carbohydr Res* **243**, 139–64 (1993).

8 Grundler G, Schmidt RR, Anwendung des Trichoracetimidat-Verfahrens auf 2-Azidoglucose- und 2-Azidogalactose-Derivative, *Liebigs Ann Chem* 1826–147 (1984).

9 Excoffier G, Gagnaire D, Utille J-P, Coupure sélective par l'hydrazine des groupements acétyles anomères de résidus glycosyles acétylés, *Carbohydr Res* **39**, 368–73 (1975).

Received and revised 4 July 2000; accepted 11 August 2000