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Syntheses of Acetylated Tetrasaccharides, Manα1→2Manα1→3Manβ1→4GlcNAc and Manα1→3Manα1→6Manβ1→4GlcNAc[1)]

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As a preliminary experiment aimed ultimately at the total syntheses of high mannose-type oligosaccharides in glycoproteins, two fully acetylated tetrasaccharides, Manα1→2Manα1→3Manβ1→4GlcNAc (**32**) and Manα1→3Manα1→6Manβ1→4GlcNAc (**35**), were synthesized by block condensation of acetylated dimannopyranosyl bromides with suitably protected Manβ1→4GlcNAc derivatives by modified Koenigs–Knorr reactions, followed by acetylation of the deprotected tetrasaccharides. Proton and carbon-13 nuclear magnetic resonance spectral data for **32**, **35**, and synthetic intermediates are also presented.

The present work confirms that 1,6-anhydro-β-derivatives of GlcNAc and oligosaccharides are versatile starting materials or key intermediates for syntheses of complex oligosaccharides.

Keywords——acetylated Manα1→2Manα1→3Manβ1→4GlcNAc; acetylated Manα1→3Manα1→6Manβ1→4GlcNAc; protected Glcβ1→4GlcNAc; dimethylsulfoxide–dicyclohexylcarbodiimide oxidation; protected Manβ1→4GlcNAc; acetylated Manα1→2Man; acetylated Manα1→3Man; 1,6-anhydro oligosaccharide; Koenigs–Knorr reaction; NMR

The Asn-linked oligosaccharides of glycoproteins are of three major types: complex acidic, high mannose neutral, and hybrid types.[2)] They are considered to be derived from a common precusor oligosaccharide [$Glc_3Man_9GlcNAc_2$ (**1**)] that is pre-assembled on lipid and transferred to the Asn sites on polypeptides.[3)] The high mannose-type oligosaccharides have been characterized from many Asn-linked glycoproteins, and are also assumed to be important intermediates in the biosynthesis of glycoproteins.

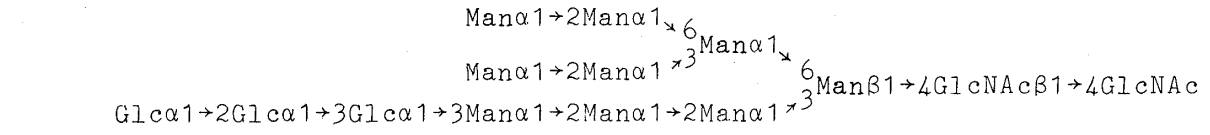


Chart 1. Structure of the Sugar Moiety [$Glc_3Man_9GlcNAc_2$ (**1**)] of Lipid-Linked Sugar Intermediate

Oligomannosyl GlcNAc derivatives have been isolated from pooled urine of patients with an inherited deficiency of lysosomal α-D-mannosidase (mannosidosis),[4)] and were also obtained after treatment of high mannose-type oligosaccharides with *endo*-β-*N*-acetylglucosaminidases from bacterial sources.[5)] They have a 3,6-branching mode at the β-D-mannopyranosyl residue attached to the non-reducing GlcNAc of the di-*N*-acetylchitobiose present in Asn-linked oligosaccharides.

In Part I,[1)] we reported the syntheses of acetylated Manα1→3Manβ1→4GlcNAc and Manα1→2Manβ1→4GlcNAc. The former of the de-*O*-acetylated trisaccharides exists in urine of mannosidosis patients and internal regions of high mannose-type oligosaccharides. As a preliminary experiment aimed ultimately at the total synthesis of high mannose-type

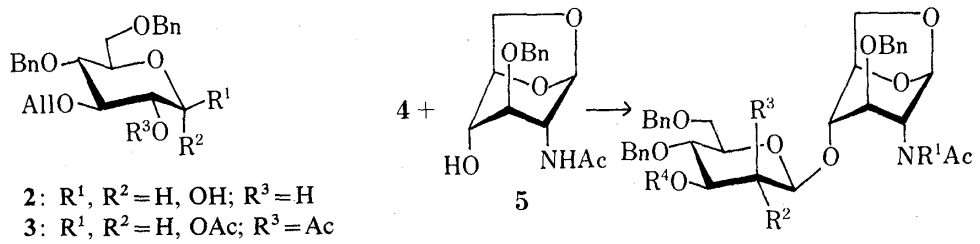
oligosaccharides, this paper reports syntheses of two fully acetylated tetrasaccharides, Manα1→2Manα1→3Manβ1→4GlcNAc (**32**) and Manα1→3Manα1→6Manβ1→4GlcNAc (**35**). This work was done because the corresponding de-*O*-acetylated tetrasaccharides are partial structures of high mannose-type oligosaccharides and, in addition, the former has been isolated from urine of mannosidosis patients.[6] The synthetic route is based on block condensation of acetylated dimannopyranosyl bromides with suitably protected Manβ1→4GlcNAc derivatives by modified Koenigs–Knorr reactions, followed by acetylation of the deprotected tetrasaccharides. We now report the details in the following three subsections.

Syntheses of Suitably Protected Manβ1→4GlcNAc Derivatives (11 and 21)

In order to synthesize the title tetrasaccharides by the route mentioned above, protected Manβ1→4GlcNAc derivatives having an unprotected hydroxyl group in the mannosyl residue are needed as acceptors of dimannopyranosyl residues. Namely, for **32**, the derivative having the C-3 hydroxyl free (**11**), while for **35**, that having the C-6 hydroxyl free (**21**) is required. Although the synthesis of the former, 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (**11**) was reported in Part I,[1] the yield was not satisfactory. Therefore, in order to increase the yield, a slight modification of the procedure was made.

3-*O*-Allyl-4,6-di-*O*-benzyl-D-glucopyranose (**2**)[1] was acetylated to give the anomeric 1,2-di-*O*-acetates (**3**). The corresponding bromide (**4**) was then coupled with 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**5**)[7] by a modified Koenigs–Knorr reaction. Subsequent removal of the acetyl group provided the Glcβ1→4GlcNAc derivative (**6**) having an unprotected hydroxyl group at the C-2 position of the Glc (C-2′). The hydroxyl group was then oxidized with dimethylsulfoxide–dicyclohexylcarbodiimide (DMSO–DCC) reagent[8] to yield the ulose (**7**), which was stereoselectively reduced with sodium borohydride to the Manβ1→4GlcNAc derivative (**8**). The product was indistinguishable from an authentic sample.[1]

Benzylation of the free hydroxyl group at C-2 of the Man in **8** was carried out with benzyl bromide and sodium hydride in *N,N*-dimethylformamide (DMF). When equimolar amounts of the hydride and **8** were used, the corresponding *O*-benzyl ether (**9**) was obtained in good yield with a small amount of unreacted **8**. However, when excess hydride (3.5 mol) was used, the benzylation resulted in the formation of almost the same amount of **9** together with the corresponding *N*-acetylbenzylamino derivative (**10**). The structure of **10** was confirmed by disappearance of the NH proton in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum and that of the amide II absorption in the infrared (IR) spectrum. The allyl group of



2: R^1, R^2=H, OH; R^3=H
3: R^1, R^2=H, OAc; R^3=Ac
4: R^1=H; R^2=Br; R^3=Ac

Ac = acetyl
All = allyl
Bn = benzyl

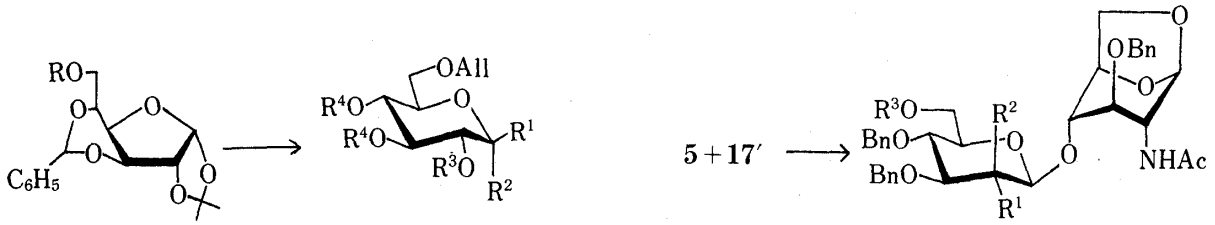
6: R^1=R^3=H; R^2=OH; R^4=All
7: R^1=H; R^2, R^3=O; R^4=All
8: R^1=R^2=H; R^3=OH; R^4=All
9: R^1=R^2=H; R^3=OBn; R^4=All
10: R^1=Bn; R^2=H; R^3=OBn; R^4=All
11: R^1=R^2=R^4=H; R^3=OBn

Chart 2

9 was then removed with 10% palladium on charcoal at 50 °C: the progress of the reaction must be monitored by thin-layer chromatography (TLC). The desired key intermediate (**11**) was thus obtained more smoothly and in better yield than before.[1)]

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (**21**), the key intermediate for synthesis of **35**, was prepared from **5** and 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (**12**)[9)] *via* procedures analogous to those used for the synthesis of **11**. Allylation of **12**, hydrolysis of the cycloacetal groups, and subsequent acetylation of the hydrolyzate gave 1,2,3,4-tetra-*O*-acetyl-6-*O*-allyl-α-D-glucopyranose (**14**), then 1,2-di-*O*-acetyl-6-*O*-allyl-3,4-di-*O*-benzyl-α- and -β-D-glucopyranoses (**16** and **17**) were obtained from **14** *via* four steps using procedures analogous to those used to obtain the corresponding 3-*O*-allyl isomers.[1)]

The anomeric mixture of the acetate (**16** and **17**) was converted into the α-bromide and coupled with **5**. After hydrolysis of the acetyl group at C-2 of the Glc of the resultant disaccharide (C-2′), the protected Glcβ1→4GlcNAc (**18**) having the C-2′ hydroxyl free was obtained in *ca.* 40% yield from **5**. Compound **18** was subsequently converted into the Manβ1→4GlcNAc derivative (**19**) by a sequence consisting of DMSO–acetic anhydride oxidation to ulose and stereospecific reduction, resulting in epimerization at the C-2′. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **18** and **19**, the chemical shifts and ^{1}J values due to the anomeric carbons of Glc and Man (C-1′) were consistent with those reported in Part I[1)] for the protected Glcβ1→4GlcNAc and Manβ1→4GlcNAc derivatives having a free hydroxyl group at the C-2′. Therefore, the occurence of isomerization from D-*gluco* to D-*manno* was confirmed.[10)] Benzylation of the C-2′ hydroxyl group of **19**, followed by deallylation, gave the desired key intermediate (**21**).



12: R=H
13: R=All

14: R^1=H; R^2=OAc; R^3=R^4=Ac
15: R^1, R^2=H, OH; R^3=H; R^4=Bn
16: R^1=H; R^2=OAc; R^3=Ac; R^4=Bn
17: R^1=OAc; R^2=H; R^3=Ac; R^4=Bn
17′: R^1=H; R^2=Br; R^3=Ac; R^4=Bn

18: R^1=OH; R^2=H; R^3=All
19: R^1=H; R^2=OH; R^3=All
20: R^1=H; R^2=OBn; R^3=All
21: R^1=R^3=H; R^2=OBn

Chart 3

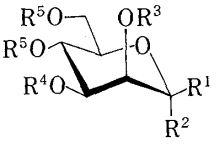
Syntheses of Acetylated Dimannopyranose Derivatives Bearing an α-D-Mannosidic Linkage (27 and 29)

As precursors of dimannopyranosyl donors for tetrasaccharides syntheses, the fully acetylated Manα1→2Man (**27**) and Manα1→3Man (**29**) were synthesized by a modified Koenigs–Knorr condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**25**) with a Man derivative bearing a free hydroxyl group at the C-2 or C-3 position.

Benzyl glycosidation of 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranose (**22**),[11)] followed by deacetylation gave benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**23**) in 30.4% yield. The low yield may be attributable to instability of the benzyl groups[12)] linked at the C-3, -4, and -6 positions in the formation of the bromide of **22**. A modified Koenigs–Knorr reaction of **23** and **25** gave the protected benzyl dimannopyranoside (**26**) bearing an α-D-mannosidic linkage in 84.5% yield. Catalytic debenzylation of **26**, followed by acetylation,

provided **27**.

Compound **29** was synthesized by condensation of 1,2,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**28**) with **25** in 44.6% yield according to a slight modification of the procedure reported by Ponpipom.[11] The product was crystallized as prisms from $CHCl_3$–ether–hexane. It is well known that in glycosidation with acylated α-D-mannosyl bromide, formation of α-D-mannosides proceeds predominantly because of the anchimeric effects on the acyl groups at the C-2 position. The α-D-configurations of the newly introduced mannosidic linkages of **27** and **29** were confirmed by ^{13}C-NMR spectroscopy.[1,10]



22: R^1, R^2=H, OAc; R^3=Ac; R^4=R^5=Bn
23: R^1=R^3=H; R^2=OBn; R^4=R^5=Bn
24: R^1=H; R^2=OBn; R^3=Ac; R^4=R^5=Bn
25: R^1=H; R^2=Br; R^3=R^4=R^5=Ac
26: R^1=H; R^2=OBn; R^3=AcMan; R^4=R^5=Bn
27: R^1=H; R^2=OAc; R^3=AcMan; R^4=R^5=Ac
27′: R^1=H; R^2=Br; R^3=AcMan; R^4=R^5=Ac
28: R^1=R^4=H; R^2=OAc; R^3=R^5=Ac
29: R^1=H; R^2=OAc; R^3=R^5=Ac; R^4=AcMan
29′: R^1=H; R^2=Br; R^3=R^5=Ac; R^4=AcMan

AcMan=2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl

Chart 4

Block Condensation of Disaccharide Units and Preparation of the Title Tetrasaccharides (32 and 35)

The fully acetylated Manα1→2Man (**22**) was converted into the corresponding bromide. Condensation of the excess bromide with the protected Manβ1→4GlcNAc (**11**) in benzene–nitromethane in the presence of mercuric cyanide and Drierite gave the protected tetrasaccharide contaminated with degradation products of the starting bromide. In order to isolate the desired tetrasaccharide derivative, the condensation product was de-*O*-acetylated and the resultant product was freed from the side products by preparative TLC (PTLC). Re-*O*-acetylation of the isolated tetrasaccharide fraction yielded the pure protected tetrasaccharide (**30**) in 40.2% yield from **11**. Debenzylation of **30**, followed by acetylation of the debenzylated product, gave the fully acetylated 1,6-anhydro-β-tetrasaccharide (**31**). The structures of **30** and **31** were characterized by ^{1}H- and ^{13}C-NMR spectroscopies, as well as IR spectroscopy and elemental analyses.

Condensation of the fully acetylated Manα1→3Man (**29**) and **11** *via* the bromide of **29** was carried out according to the procedure mentioned above to provide the fully protected tetrasaccharide bearing Manα1→3Man (**33**) in 29.4% yield. Debenzylation of **33**, followed by acetylation of the debenzylated product yielded the fully acetylated 1,6-anhydro-β-tetrasaccharide (**34**).

The 1,6-anhydro-β-ring of **31** or **34** was finally cleaved by boron trifluoride etherate–Ac_2O treatment at 0 °C to give the title tetrasaccharide (**32** or **35**) in 79.2 or 66.9% yield as an anomeric mixture. The structures were characterized by ^{1}H- and ^{13}C-NMR spectroscopies.

Experimental[13]

1,2-Di-*O*-acetyl-3-*O*-allyl-4,6-di-*O*-benzyl-D-glucopyranose (3)——A solution of **2**[1] (10.47 g, 26.1 mmol) in Ac_2O (80 ml) and pyridine (120 ml) was stirred overnight at room temperature, and then concentrated to a syrup. This was column-chromatographed with hexane–ether (7:2) to yield **3** (10.91 g, 83.1%) as a syrupy anomeric mixture, $[\alpha]_D^{19}$

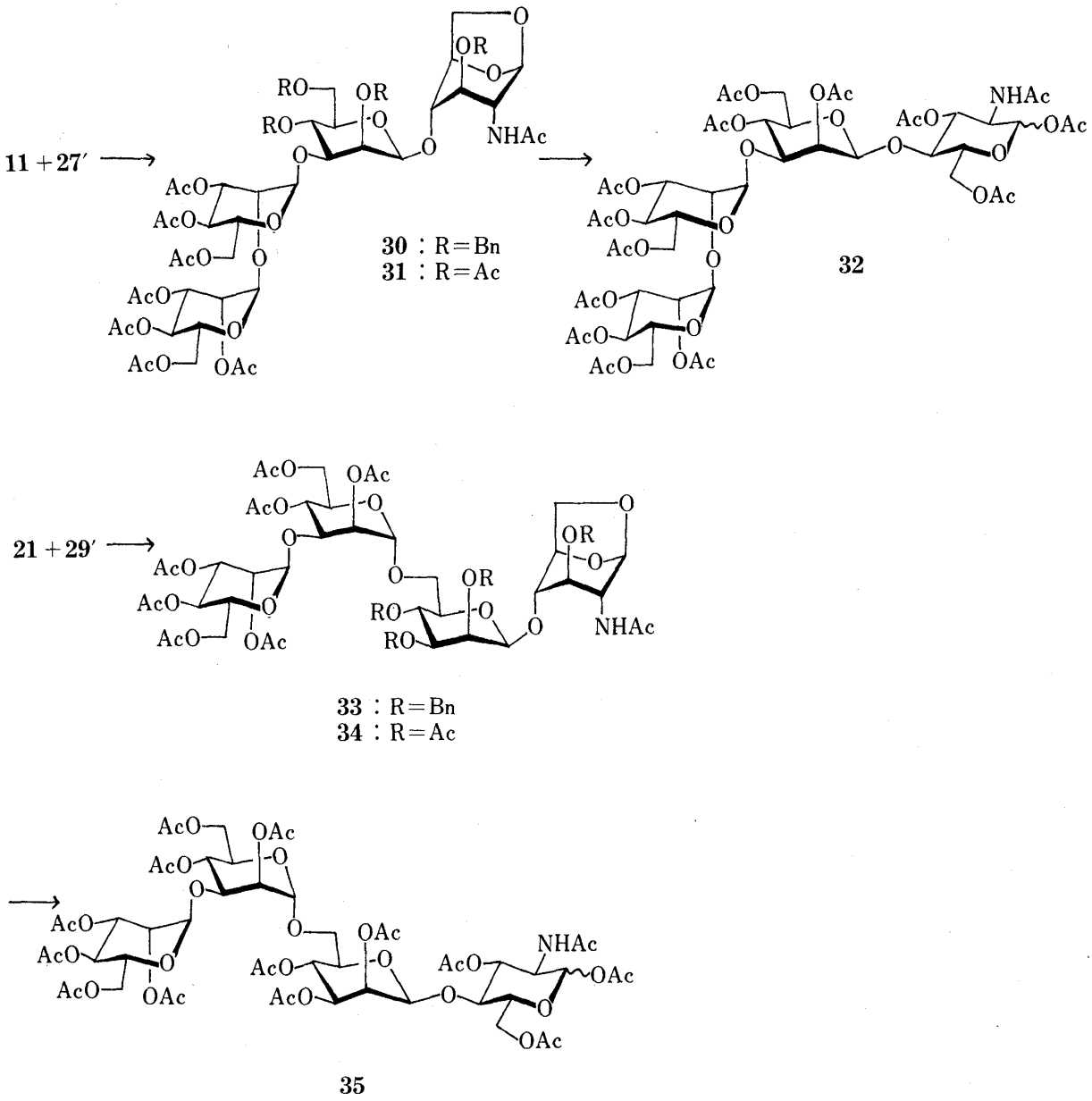


Chart 5

+61.8° (c=0.63, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.03, 2.07, 2.09 (6H, each s, OAc×2), 5.61 (d, $J_{1,2}$=8 Hz, H-1α), 6.28 (d, $J_{1,2}$=4 Hz, H-1β), 7.32 (10H, s, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 1754 (OAc). TLC: *Rf* 0.39 (solvent C). *Anal.* Calcd for $C_{27}H_{32}O_8 \cdot H_2O$: C, 64.53; H, 6.82. Found: C, 64.62; H, 6.83.

2-*O*-Acetyl-3-*O*-allyl-4,6-di-*O*-benzyl-α-D-glucopyranosyl Bromide (4)——A solution of **3** (6.91 g, 13.7 mmol) in dry CH_2Cl_2 (100 ml) with 30% (w/v) HBr–AcOH (30 ml) was stirred at 0 °C for 10 min. After being diluted with $CHCl_3$, the mixture was washed with H_2O, ice-cold aq. $NaHCO_3$ solution, and H_2O, then dried ($MgSO_4$) and filtered. The filtrate was concentrated to a syrup (6.92 g, 100%), which was used immediately.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-4,6-di-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (6)——A solution of **4** (6.92 g, 13.7 mmol) in benzene (20 ml) was added to a suspension of **5**[7] (1.91 g, 6.32 mmol), $Hg(CN)_2$ (2 g), $HgBr_2$ (2.8 g), and Drierite (2 g) in nitromethane (20 ml). The mixture was stirred for 48 h at room temperature, then filtered, and the filtrate was diluted with $CHCl_3$. The mixture was successively washed with H_2O, satd. KI, $Na_2S_2O_3$, and $NaHCO_3$ solutions, and H_2O, then dried ($MgSO_4$) and concentrated to a syrup. This was column-chromatographed with hexane–ether (2 : 7), and the fractions having *Rf* 0.63 (solvent A) were concentrated to dryness. A 0.5 N methanolic solution of MeONa (4 ml) was added to a solution of the residue in dry MeOH (40 ml). After being stirred overnight at room temperature, the solution was decationized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to a syrup, which was column-chromatographed with $CHCl_3$–MeOH (165 : 1) to obtain **6** (1.75 g, 39.4% from **5**) as a foamy solid, $[\alpha]_D^{17}$ −38.2° (c=0.33, $CHCl_3$). lit.[1] $[\alpha]_D^{22}$ −41.4° (c=0.46,

$CHCl_3$).

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-4,6-di-*O*-benzyl-β-D-*arabino*-2-hexulopyranosyl)-β-D-glucopyranose (7)——DCC (0.13 g, 0.63 mmol) was added under cooling to a solution of **6** (0.27 g, 3.84×10^{-4} mol) in a mixture of DMSO (1 ml) and dry benzene (10 ml) containing pyridine (0.1 ml) and trifluoroacetic acid (0.05 ml). The mixture was stirred for 46 h at room temperature, diluted with ether (80 ml), and then filtered. The filtrate was washed with H_2O, dried ($MgSO_4$), and concentrated to a white foamy solid (0.25 g), which contained crystalline dicyclohexylurea. This product was used without purification. For analysis, this crude ulose was purified by PTLC with $CHCl_3$–acetone (3 : 1). From the band having *Rf* 0.23, pure **7** was obtained as a hygroscopic foamy solid, $[\alpha]_D^{20}$ −52.6° (*c* = 0.43, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.98 (3H, s, NAc), 5.68—6.16 (1H, m, $CH_2=CH-CH_2O-$), 6.46 (1H, d, $J_{NH,2}$ = 10 Hz, NH), 7.26, 7.29 (15H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.9 ($^1J_{C\text{-}1-H\text{-}1}$ = 175.8 Hz, C-1), 97.2 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 161.9 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH, OH), 1754 (C=O), 1666 (amide I), 1528 (amide II). TLC: *Rf* 0.23 (solvent B). *Anal.* Calcd for $C_{38}H_{43}NO_{10} \cdot 1.5H_2O$: C, 65.13; H, 6.62; N, 2.00. Found: C, 64.86; H, 6.53; N, 1.94.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-4,6-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (8)——$NaBH_4$ (0.2 g) was added under stirring at 0 °C to a solution of crude **7** (prepared from 0.27 g of **6** as described in the preceding section) in CH_2Cl_2–MeOH (1 : 1, 6 ml). The mixture was stirred overnight at room temperature and then diluted with $CHCl_3$. The whole was successively washed with ice-cold 10% citric acid and satd. $NaHCO_3$ solutions, and H_2O, then dried ($MgSO_4$) and concentrated to a foamy solid. This was purified by PLC with $CHCl_3$–acetone (3 : 1). The band having *Rf* 0.31 was scraped from the plates and extracted with $CHCl_3$–MeOH (4 : 1) to yield **8** (0.18 g, 68% from **6**) as a foamy solid, $[\alpha]_D^{20}$ −50.5° (*c* = 0.38, $CHCl_3$). lit.[1] $[\alpha]_D^{19}$ 48.5° (*c* = 0.41, $CHCl_3$).

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (9) and 2-(*N*-Acetylbenzylamino)-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (10)——1) Benzylation with Equimolar Sodium Hydride: NaH (12 mg, 62% oil suspension, 3.13×10^{-4} mol) was added to a chilled solution of **8** (196.1 mg, 2.79×10^{-4} mol) and benzyl bromide (0.4 ml) in DMF (3 ml). The mixture was stirred overnight at room temperature, and the excess bromide was decomposed by addition of MeOH. After being diluted with $CHCl_3$, the whole was washed with H_2O, dried ($MgSO_4$), and concentrated to a syrup, which showed two spots on TLC (solvent A). This material was column-chromatographed with hexane–ether (1 : 4). From the faster-moving fractions, **9** (180.8 mg, 84.6%) was obtained as a glass, $[\alpha]_D^{17}$ −75.9° (*c* = 1.43, $CHCl_3$). lit.[1] $[\alpha]_D^{18}$ −87° (*c* = 0.94, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.61 (3H, s, NAc), 5.67—6.11 (1H, m, $CH_2=CH-CH_2O-$), 6.09 (1H, d, $J_{NH,2}$ = 9 Hz, NH), 7.27 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.6 ($^1J_{C\text{-}1-H\text{-}1}$ = 177.0 Hz, C-1; $^1J_{C\text{-}1'-H\text{-}1'}$ = 158.7 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1675 (amide I), 1513 (amide II). TLC: *Rf* 0.57 (solvent A). *Anal.* Calcd for $C_{45}H_{51}NO_{10}$: C, 70.57; H, 6.71; N, 1.83. Found: C, 70.10; H, 6.25; N, 1.35. After **9** had emerged, **8** (25.3 mg, 12.9%) was recovered.

2) Benzylation with Excess Sodium Hydride: Benzylation of **8** (153 mg, 2.18×10^{-4} mol) in DMF (2 ml) with benzyl bromide (0.4 ml) and NaH (30 mg, 62% oil suspension, 7.75×10^{-4} mol) was carried out as described in 1). From the faster-moving fractions upon column chromatography, **10** (66.6 mg, 35.7%) was isolated as a glass, $[\alpha]_D^{16}$ −37.1° (*c* = 0.36, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.86 (3H, s, NAc), 5.63—6.15 (1H, m, $CH_2=CH-CH_2O-$), 7.27 (25H, s, aromatic protons). IR ν_{max}^{KBr} cm^{-1}: 1647 (amide I). TLC: *Rf* 0.69 (solvent A). *Anal.* Calcd for $C_{52}H_{57}NO_{10} \cdot 1.5H_2O$: C, 72.20; H, 6.76; N, 1.62. Found: C, 72.46; H, 6.83; N, 1.49. After **10** had emerged, **9** (54.6 mg, 32.7%) was obtained as a glass, $[\alpha]_D^{17}$ −75.9° (*c* = 1.43, $CHCl_3$).

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (11)——A mixture of **9** (180.8 mg, 2.36×10^{-4} mol) and 10% Pd on charcoal (150 mg) suspended in AcOH–H_2O (10 : 1, 8.8 ml) was stirred at 50 °C for 6 h, and then filtered. The filtrate was concentrated to dryness by repeated co-distillation with toluene. The residue was purified by PTLC with $CHCl_3$–acetone (3 : 1). The band having *Rf* 0.63 was scraped from the plates and extracted with $CHCl_3$–MeOH (4 : 1) to provide **11** (109.3 mg, 64%) as a foamy solid, $[\alpha]_D^{20}$ −86° (*c* = 0.29, $CHCl_3$). lit.[1] $[\alpha]_D^{21}$ −85.9° (*c* = 0.17, $CHCl_3$).

6-*O*-Allyl-3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (13)——A mixture of **12**[9] (10 g, 32.4 mmol), KOH (10 g), and allyl bromide (50 ml) in dioxane (50 ml) was stirred at 110 °C for 2 h with exclusion of moisture. After being diluted with $CHCl_3$, the organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to a syrup, which was used without purification. For analysis, a portion of this syrup was purified by PTLC with hexane–ether (1 : 1). The band having *Rf* 0.55 was extracted with $CHCl_3$–MeOH (9 : 1) to provide **13**. Pure **13**, mp 74 °C, $[\alpha]_D^{23}$ +3° (*c* = 1.83, $CHCl_3$), was crystallized as fine needles from hexane. ^{1}H-NMR ($CDCl_3$): 1.31, 1.51 (6H, each s, $CH_3 \times 2$), 3.61—6.17 [14H: 7H (unresolved ring protons), 5H (allyl residue), 2H (CH_2 in $PhCH_2$)], 7.21—7.65 (5H, m, aromatic protons). TLC: *Rf* 0.55 (solvent C). *Anal.* Calcd for $C_{19}H_{24}O_6$: C, 65.50; H, 6.94. Found: C, 65.11; H, 6.95.

1,2,3,4-Tetra-*O*-acetyl-6-*O*-allyl-α-D-glucopyranose (14)——Crude **13** (prepared from 10 g of **12**) was dissolved in trifluoroacetic acid–H_2O (17 : 2, 19 ml). The solution was stirred for 2 h at room temperature, then diluted with H_2O (100 ml), and extracted with $CHCl_3$ to remove by-products. The aqueous layer was concentrated to dryness and the resultant solid was acetylated with anhyd. AcONa (5 g) and Ac_2O (70 ml) at 100 °C for 1.5 h under stirring. After addition of crushed ice, the whole was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with H_2O,

satd. $NaHCO_3$ solution and H_2O, then dried ($MgSO_4$) and evaporated to dryness. The resultant syrup was column-chromatographed with hexane–ether (1 : 1). Fractions having *Rf* 0.18 (solvent C) were concentrated to a syrup, which was crystallized from ether–hexane as fine needles (6.70 g, 53.4% from **12**), mp 74—75 °C, $[\alpha]_D^{22}$ +96.4° (*c* = 1.4, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.94—2.16 (12H, m, OAc × 4). IR ν_{max}^{KBr} cm^{-1}: 1744 (OAc). TLC: *Rf* 0.18 (solvent C). *Anal.* Calcd for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.41; H, 6.44.

6-*O*-Allyl-3,4-di-*O*-benzyl-D-glucopyranose (15)——A solution of 30% (w/v) HBr in AcOH (24 ml) was added dropwise at 0 °C to a stirred solution of **14** (6 g, 15.4 mmol) in CH_2Cl_2 (36 ml), and stirring was continued for 30 min at 0 °C. The mixture was treated as described for the preparation of **4** to provide the corresponding bromide.

A mixture of the bromide, MeOH (3 ml), 2,6-lutidine (4 ml), and nitromethane (36 ml) was stirred at 45 °C for 16 h, then diluted with $CHCl_3$. The whole was washed with H_2O, dried ($MgSO_4$), and concentrated to yield the syrupy orthoester.

The orthoester was then benzylated with benzyl chloride (12 ml) and KOH (12 g) in dioxane (46 ml) by heating to reflux for 5 h under stirring. The mixture was diluted with $CHCl_3$, and filtered. The resultant benzyl ether in dioxane (60 ml) and 1 M H_2SO_4 (18 ml) was stirred at 110 °C for 5 h to hydrolyze the orthoester. After being diluted with $CHCl_3$, the whole was washed with H_2O, satd. $NaHCO_3$ solution, and H_2O, then dried ($MgSO_4$), and concentrated to a syrup. This was column-chromatographed with hexane–AcOEt (1 : 2) to yield **15** (1.65 g, 26.8%), which was crystallized from hexane–ether as silky needles, mp 86.5—88 °C, $[\alpha]_D^{26}$ +49.1° (*c* = 1.37, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.85 (1H, d, *J* = 8 Hz, OH), 5.67—6.11 (1H, m, CH_2=C<u>H</u>–CH_2O–), 7.29 (10H, s, aromatic protons). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH). TLC: *Rf* 0.41 (solvent B). *Anal.* Calcd for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 68.71; H, 6.76.

1,2-Di-*O*-acetyl-6-*O*-allyl-3,4-di-*O*-benzyl-α- and β-D-glucopyranoses (16 and 17)——Compound **15** (79.2 mg, 1.98×10^{-4} mol) in Ac_2O (1 ml) and pyridine (2 ml) was acetylated as described for the preparation of **3**. The resultant crude acetates were column-chromatographed with hexane–ether (3 : 1). From the faster-moving fractions, the α-acetate (**16**, 32.1 mg, 33.4%) was isolated as a syrup, $[\alpha]_D^{22}$ +81.6° (*c* = 0.3, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.95, 2.10 (6H, each s, OAc × 2), 5.68—6.16 (1H, m, CH_2=C<u>H</u>–CH_2O–), 6.28 (1H, d, $J_{1,2}$ = 4 Hz, H-1α), 7.31 (10H, s, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 1754 (OAc). TLC: *Rf* 0.39 (solvent C). *Anal.* Calcd for $C_{27}H_{32}O_8$: C, 66.93; H, 6.66. Found: C, 67.01; H, 6.68.

From the subsequent fractions, the anomeric mixture (**16** and **17**) was obtained as a syrup (25.4 mg, 26.5%). After these fractions had emerged, the β-acetate (**17**, 28.9 mg, 30.1%) was isolated as a syrup, which crystallized as fine needles, mp 49—52 °C, $[\alpha]_D^{22}$ +33.7° (*c* = 0.19, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.92, 2.07 (6H, each s, OAc × 2), 5.60 (1H, d, $J_{1,2}$ = 8 Hz, H-1β), 5.69—6.13 (1H, m, CH_2=C<u>H</u>–CH_2O–), 7.31 (10H, s, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 1758 (OAc). TLC: *Rf* 0.34 (solvent C). *Anal.* Calcd for $C_{27}H_{32}O_8$: C, 66.93; H, 6.66. Found: C, 67.00; H, 6.86.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-allyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (18)——The anomeric mixture of the acetates (**16** and **17**, 1.10 g, 2.27 mmol) in CH_2Cl_2 (20 ml) was treated with 30% (w/v) HBr–AcOH (5 ml) at 0 °C for 30 min as described for the preparation of **4** to yield the corresponding syrupy bromide (1.14 g, 100%), which was used immediately.

A solution of the bromide (1.14 g, 2.26 mmol) in benzene (3 ml) was added to a mixture of **5** (250 mg, 8.27×10^{-4} mol), $Hg(CN)_2$ (0.9 g), and Drierite (0.3 g) in nitromethane (3 ml). This was treated as described for the preparation of **6**. The condensation product was then column-chromatographed with hexane–ether (1 : 4). Fractions having *Rf* 0.66 (solvent A) were concentrated to a syrup, and this was de-*O*-acetylated as described for **6** to provide **18** as a hygroscopic foamy solid (227.5 mg, 40.1% from **5**), $[\alpha]_D^{24}$ −48.3° (*c* = 0.71, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.92 (3H, s, NAc), 5.41 (1H, s, H-1), 5.62—6.06 (1H, m, CH_2=C<u>H</u>–CH_2O–), 6.39 (1H, d, $J_{NH,2}$ = 9 Hz, NH), 7.34 (15H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 102.1 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 156.3 Hz, C-1′), 100.5 ($^1J_{C\text{-}1-H\text{-}1}$ = 175.8 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH, OH), 1656 (amide I), 1530 (amide II). TLC: *Rf* 0.51 (solvent B). *Anal.* Calcd for $C_{38}H_{45}NO_{10} \cdot 0.5H_2O$: C, 66.65; H, 6.77; N, 2.05. Found: C, 66.61; H, 6.39; N, 1.99.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-allyl-3,4-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (19)——A solution of **18** (622.4 mg, 9.09×10^{-4} mol) in DMSO–Ac_2O (2 : 1, 18 ml) was stirred for 72 h at room temperature. After dilution with $CHCl_3$, the whole was washed with H_2O, dried ($MgSO_4$), and concentrated to a syrup by repeated co-distillation with toluene to provide the ulose. A mixture of this syrup and $NaBH_4$ (1 g) in CH_2Cl_2–MeOH (1 : 1, 20 ml) was stirred overnight at room temperature, and then diluted with $CHCl_3$. The mixture was washed with H_2O, ice-cold 10% citric acid and satd. $NaHCO_3$ solutions, and H_2O, then dried ($MgSO_4$) and concentrated to a syrup, which was column-chromatographed with $CHCl_3$–MeOH (100 : 1) to provide **19** (323.8 mg, 52%) as a syrup, $[\alpha]_D^{23}$ −45.9° (*c* = 0.64, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.98 (3H, s, NAc), 2.65 (1H, br s, OH), 5.41 (1H, s, H-1), 5.63—6.07 (1H, m, CH_2=C<u>H</u>–CH_2O–), 6.40 (1H, d, $J_{NH,2}$ = 9 Hz, NH), 7.27, 7.31, 7.34 (15H, all s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.6 ($^1J_{C\text{-}1-H\text{-}1}$ = 179.4 Hz, C-1), 99.3 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 153.8 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH, OH), 1654 (amide I), 1533 (amide II). TLC: *Rf* 0.36 (solvent B). *Anal.* Calcd for $C_{38}H_{45}NO_{10} \cdot 0.5H_2O$: C, 66.65; H, 6.77; N, 2.05. Found: C, 66.95; H, 6.32; N, 1.98.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (20)——Benzyl bromide (0.5 ml) was added to a mixture of **19** (323.8 mg, 4.73×10^{-4} mol), powdered BaO (0.8 g), and $Ba(OH)_2 \cdot 8H_2O$ (0.35 g) suspended in DMF (15 ml). The mixture was stirred at 50 °C for 4 d, and then filtered. The filtrate was concentrated: traces of DMF were completely removed by co-distillation

with toluene. The residue was column-chromatographed with hexane–ether (1 : 4) to yield **20** (129.9 mg, 35.9%) as a syrup, $[\alpha]_D^{19}$ −75.9° (c=0.65, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.64 (3H, s, NAc), 5.41 (1H, s, H-1), 5.61—6.02 (1H, m, CH_2=CH–CH_2O–), 6.07 (1H, d, $J_{NH,2}$=9 Hz, NH), 7.31 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.7 ($^1J_{C\text{-}1-H\text{-}1}$=175.8 Hz, C-1; $^1J_{C\text{-}1'-H\text{-}1'}$=157.5 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1667 (amide I), 1519 (amide II). TLC: *Rf* 0.58 (solvent A). *Anal.* Calcd for $C_{45}H_{51}NO_{10}\cdot H_2O$: C, 68.95; H, 6.81; N, 1.79. Found: C, 68.78; H, 6.68; N, 1.87.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (21)——A mixture of **20** (129.9 mg, 1.70×10^{-4} mol) and 10% Pd on charcoal (120 mg), suspended in AcOH–H_2O (10 : 1, 11 ml), was stirred at 50 °C for 7 h. After filtration, the filtrate was concentrated to dryness by repeated co-distillation with toluene. The residue was purified by PTLC with $CHCl_3$–acetone (6 : 1). The band having *Rf* 0.27 was extracted with $CHCl_3$–MeOH (9 : 1) to yield **21** (68 mg, 53.8%) as a foamy solid. $[\alpha]_D^{20}$ −100° (c=0.11, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.68 (3H, s, NAc), 2.59 (1H, br s, OH), 5.40 (1H, s, H-1), 5.89 (1H, d, $J_{NH,2}$=9 Hz, NH), 7.28 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.3 ($^1J_{C\text{-}1'-H\text{-}1'}$=151.4 Hz, C-1′), 100.6 ($^1J_{C\text{-}1-H\text{-}1}$=170.9 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3410 (NH, OH), 1664 (amide I), 1517 (amide II). TLC: *Rf* 0.27 (solvent A). *Anal.* Calcd for $C_{42}H_{47}NO_{10}$: C, 67.82; H, 6.64; N, 1.88. Found: C, 67.74; H, 6.30; N, 2.17.

Benzyl 3,4,6-Tri-*O*-benzyl-α-D-mannopyranoside (23)——A solution of **22**[11] (15.95 g, 29.8 mmol) in CH_2Cl_2 (200 ml) with 30% (w/v) HBr–AcOH (60 ml) was stirred at 0 °C for 30 min. The mixture was treated as described for the preparation of **4** to yield the corresponding crude bromide.

Benzyl alcohol (8 ml) and $Hg(CN)_2$ (12 g) were added to a solution of the bromide in benzene–nitromethane (1 : 1, 60 ml). The mixture was stirred overnight at room temperature, and then treated as described for the preparation of **6** to yield the crude glycoside, which was column-chromatographed with hexane–ether (4 : 1). The syrup isolated from the fractions having *Rf* 0.48 (solvent C) was deacetylated with a 0.5 N methanolic solution of MeONa (8 ml) in dry MeOH (80 ml) as described for **6**. The deacetylated product was column-chromatographed with hexane–ether (1 : 1) to provide **23** (4.9 g, 30.4%) as a syrup, $[\alpha]_D^{22}$ +48° (c=1.85, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.94 (1H, s, OH), 7.21, 7.28 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 98.5 ($^1J_{C\text{-}1-H\text{-}1}$=170.9 Hz, C-1). IR ν_{max}^{neat} cm^{-1}: 3440 (OH). TLC: *Rf* 0.15 (solvent C). *Anal.* Calcd for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.26; H, 6.59.

Benzyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (24)——Acetylation of **23** (37.4 mg, 6.92×10^{-5} mol) with Ac_2O (0.5 ml) and pyridine (1 ml) was carried out as described for **2**. The crude acetate was purified by PTLC with hexane–ether (1 : 1). From the band having *Rf* 0.48, **24** (34.9 mg, 86.6%) was isolated as a syrup, $[\alpha]_D^{23}$ +43.6° (c=0.33, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.12 (3H, s, OAc), 7.26, 7.32 (20H, each s, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 1740 (OAc). TLC: *Rf* 0.48 (solvent C). *Anal.* Calcd for $C_{36}H_{38}O_7$: C, 74.21; H, 6.57. Found: C, 73.93; H, 6.55.

Benzyl 3,4,6-Tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (26)——A solution of **25** (9.5 g, 23.1 mmol) in dry benzene (40 ml) was added to a suspension of **23** (1.08 g, 2 mmol), $Hg(CN)_2$ (8.5 g), and Drierite (3 g) in nitromethane (40 ml). After being stirred overnight at room temperature, the mixture was treated as described for the preparation of **6** to yield a crude disaccharide derivative. This was column-chromatographed with hexane–ether (1 : 1). Fractions having *Rf* 0.13 (solvent C) were further purified by PLC with toluene–acetone (4 : 1). A zone having *Rf* 0.70 was scraped from the plates and extracted with $CHCl_3$–MeOH (9 : 1) to yield **26** (1.47 g, 84.5%) as a syrup, $[\alpha]_D^{23}$ +52.2° (c=2.53, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.96, 1.98, 2.03, 2.08 (12H, all s, OAc×4), 7.23, 7.31 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 99.2 ($^1J_{C\text{-}1'-H\text{-}1'}$=175.8 Hz, C-1′), 97.7 ($^1J_{C\text{-}1-H\text{-}1}$=170.9 Hz, C-1). IR ν_{max}^{neat} cm^{-1}: 1747 (OAc). TLC: *Rf* 0.13 (solvent C). *Anal.* Calcd for $C_{48}H_{54}O_{15}$: C, 66.20; H, 6.25. Found: C, 66.00; H, 6.07.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranose (27)——A mixture of **26** (1.32 g, 1.52 mmol) and 10% Pd on charcoal (1 g) suspended in glacial AcOH (60 ml) was hydrogenated for 4 d at room temperature under atmospheric pressure, then filtered, and concentrated to dryness to give a white powder, which was acetylated wih Ac_2O (20 ml) and pyridine (20 ml) as described for **2**. After column chromatography with hexane–ether (1 : 4), **27** was obtained from the fraction having *Rf* 0.55 (solvent A). The product was crystallized from hexane–ether as fine needles (0.79 g, 76.3%), mp 135—137 °C, $[\alpha]_D^{23}$ +42.1° (c=0.47, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.01, 2.05, 2.09, 2.11, 2.14, 2.16 (24H, all s, OAc×8), 4.94 (1H, d, $J_{1',2'}$=2 Hz, H-1′), 6.24 (1H, d, $J_{1,2}$=2 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 99.3 ($^1J_{C\text{-}1'-H\text{-}1'}$=170.9 Hz, C-1′), 91.5 ($^1J_{C\text{-}1-H\text{-}1}$=178.2 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 1745 (OAc). TLC: *Rf* 0.55 (solvent A). *Anal.* Calcd for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.45; H, 5.29.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranose (29)——A solution of **25** (2.63 g, 6.40 mmol) in CH_2Cl_2 (8 ml) was added to a mixture of **28**[11] (2 g, 5.74 mmol), $Hg(CN)_2$ (0.7 g), and $HgBr_2$ (1 g) in acetonitrile (16 ml). The mixture was treated as described for the preparation of **26** to provide crude **29**. Pure **29** was isolated by column chromatography as a syrup from the fractions eluted with $CHCl_3$–AcOEt (12 : 1), *Rf* 0.59 (solvent A). The product (1.74 g, 44.6%) was crystallized from $CHCl_3$–ether–hexane as prisms, mp 170—172 °C, $[\alpha]_D^{22}$ +37.6° (c=0.51, $CHCl_3$). lit.[11] foam, $[\alpha]_D^{27}$ +35.9° (c=1.52, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.99, 2.05, 2.08, 2.13, 2.22 (24H, all s, OAc×8), 5.02 (1H, s, H-1′), 6.08 (1H, d, $J_{1,2}$=2 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 99.1 ($^1J_{C\text{-}1'-H\text{-}1'}$= 173.3 Hz, C-1′), 90.6 ($^1J_{C\text{-}1-H\text{-}1}$=178.2 Hz, C-1). TLC: *Rf* 0.59 (solvent A).

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (30)**——A solution of **27** (0.66 g, 9.37×10^{-4} mol) in dry CH_2Cl_2 (15 ml) with 30% (w/v) HBr–AcOH (3 ml) was stirred at room temperature for 3 h. The mixture was treated as described for the preparation of **4** to yield the corresponding bromide (0.67 g, 98.5%) as a foamy solid, which was used immediately.

A solution of the bromide (650 mg, 9.29×10^{-4} mol) in benzene (2 ml) was added to a suspension of **11** (109.3 mg, 1.51×10^{-4} mol), $Hg(CN)_2$ (270 mg), and Drierite (250 mg) in nitromethane (2 ml). After being stirred at 50 °C for 72 h, the mixture was treated as described for the preparation of **6**. The resultant syrup was de-*O*-acetylated with a 0.5 N methanolic solution of MeONa (2 ml) in dry MeOH (10 ml). From the band having *Rf* 0.40 on PLC with $CHCl_3$–MeOH (3 : 1), the de-*O*-acetylated tetrasaccharide was isolated. The product was then re-*O*-acetylated with Ac_2O (1 ml) and pyridine (1 ml), and the crude syrup was purified by PTLC with $CHCl_3$–acetone (6 : 1). From the band having *Rf* 0.26, **30** (81.6 mg, 40.2% from **11**) was obtained as a foamy solid, $[\alpha]_D^{24}$ −21.8° (*c* = 0.11, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.63 (3H, s, NAc), 1.98, 2.01, 2.08, 2.12 (21H, all s, OAc × 7), 6.00 (1H, d, $J_{NH,2}$ = 10 Hz, NH), 7.26, 7.28 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.1 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 153.8 Hz, C-1′), 100.7 ($^1J_{C\text{-}1-H\text{-}1}$ = 175.8 Hz, C-1; $^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}$ = 175.8 Hz, C-1″ or C-1‴), 99.1 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}$ = 173.3 Hz, C-1‴ or C-1″). IR ν_{max}^{KBr} cm^{-1}: 3410 (NH), 1750 (OAc), 1675 (amide I), 1508 (amide II). TLC: *Rf* 0.26 (solvent A). *Anal.* Calcd for $C_{68}H_{81}NO_{27}$: C, 60.75; H, 6.07; N, 1.04. Found: C, 60.44; H, 5.99; N, 1.10.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (31)**——Debenzylation of **30** (59.1 mg, 4.40×10^{-5} mol) was carried out as described for **26**. The resultant product was then acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) as described for **2**. The crude acetate was purified by PTLC with $CHCl_3$–acetone (3 : 1). From the band having *Rf* 0.17, **31** was isolated as a glass. For analysis, the product was precipitated from $CHCl_3$–hexane as a white powder (40.9 mg, 79.5%). $[\alpha]_D^{22}$ −4° (*c* = 0.1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.00, 2.04, 2.06, 2.11, 2.15, 2.25 (36H, all s, NAc, OAc × 11), 6.15 (1H, d, $J_{NH,2}$ = 9 Hz, NH). ^{13}C-NMR ($CDCl_3$): 100.4 ($^1J_{C\text{-}1-H\text{-}1}$ = 175.8 Hz, C-1), 99.9 ($^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}$ = 168.5 Hz, C-1″ or C-1‴), 99.5 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}$ = 170.9 Hz, C-1‴ or C-1″), 96.4 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 158.7 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3420 (NH), 1750 (OAc), 1678 (amide I), 1516 (amide II). TLC: *Rf* 0.17 (solvent B). *Anal.* Calcd for $C_{48}H_{65}NO_{31} \cdot H_2O$: C, 49.27; H, 5.77; N, 1.20. Found: C, 49.23; H, 5.68; N, 1.09.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (32)**——A solution of **31** (31 mg, 2.65×10^{-5} mol) in ice-cold acetolysis reagent [boron trifluoride etherate–Ac_2O (1 : 30, v/v) 1 ml] was stirred for 2 h at 0 °C. A piece of ice was added, and the mixture was stirred for 2 h at room temperature to decompose the excess acetolysis reagent, then diluted with $CHCl_3$. The whole was neutralized with solid $NaHCO_3$. The separated organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to dryness. The residue was purified by PTLC with $CHCl_3$–acetone (1 : 1). The band having *Rf* 0.50—0.60 was excluded from the plates and extracted with $CHCl_3$–MeOH (9 : 1) to isolate **32** as a glassy mass, which was obtained as a white powder (27.1 mg, 79.2%) from $CHCl_3$–hexane. $[\alpha]_D^{17}$ +18° (*c* = 0.26, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.93, 1.99, 2.03, 2.05, 2.09, 2.14, 2.15, 2.18 (42H, all s, NAc, OAc × 13), 6.10 (*ca.* 0.7H, d, $J_{1,2}$ = 4 Hz, H-1α). ^{13}C-NMR ($CDCl_3$): 99.6 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}$ = 173.3 Hz, C-1‴ or C-1″), 99.2 ($^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}$ = 178.2 Hz, C-1″ or C-1‴), 98.4 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 163.6 Hz, C-1′), 92.6 ($^1J_{C\text{-}1-H\text{-}1}$ = 168.5 Hz, C-1β), 90.6 ($^1J_{C\text{-}1-H\text{-}1}$ = 178.2 Hz, C-1α). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH), 1747 (OAc), 1683 (amide I), 1530 (amide II). TLC: *Rf* 0.59 and 0.54 [$CHCl_3$–acetone (1 : 1), α- and β-anomers]. *Anal.* Calcd for $C_{52}H_{71}NO_{34} \cdot 2H_2O$: C, 48.41; H, 5.86; N, 1.09. Found: C, 48.41; H, 5.56; N, 0.97.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (33)**——The bromide of **29** was prepared by treatment of a solution of **29** (640 mg, 9.43×10^{-4} mol) in CH_2Cl_2 (10 ml) with 30% (w/v) HBr–AcOH (3 ml) as described for the preparation of **4** to yield the corresponding bromide as a foamy solid (660 mg, 100%), which was used immediately.

A solution of the bromide (660 mg, 9.43×10^{-4} mol) in benzene (2 ml) was added to a suspension of **21** (68 mg, 9.14×10^{-5} mol), $Hg(CN)_2$ (400 mg), and Drierite (200 mg) in nitromethane (2 ml). After being stirred for 72 h at 50 °C, the mixture was treated as described for the preparation of **6** to yield the crude condensation product. This was then de-*O*-acetylated with a 0.5 N methanolic solution of MeONa (0.5 ml) in dry MeOH (5 ml) as described for **30**. The resultant foamy solid was purified by PTLC with $CHCl_3$–MeOH (3 : 1). The band having *Rf* 0.44 was extracted with $CHCl_3$–MeOH (1 : 3) to yield the de-*O*-acetylated tetrasaccharide.

The product was re-*O*-acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) as described for **2**. The resultant syrup was purified by PTLC with $CHCl_3$–acetone (6 : 1) to give **33** (36.1 mg, 29.4% from **21**) as a foamy solid, $[\alpha]_D^{21}$ −13.3° (*c* = 0.15, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.72 (3H, s, NAc), 1.98, 2.01, 2.03, 2.12 (21H, all s, OAc × 7), 5.89 (1H, d, $J_{NH,2}$ = 9 Hz, NH), 7.31 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.8 ($^1J_{C\text{-}1-H\text{-}1}$ = 175.8 Hz, C-1), 99.0 ($^1J_{C\text{-}1'-H\text{-}1'}$ = 156.3 Hz, C-1′), 98.9 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}$ = 173.3 Hz, C-1‴ or C-1″), 97.7 ($^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}$ = 173.3 Hz, C-1″ or C-1‴). IR ν_{max}^{KBr} cm^{-1}: 3420 (NH), 1750 (OAc), 1676 (amide I), 1510 (amide II). TLC: *Rf* 0.30 (solvent A). *Anal.* Calcd for $C_{68}H_{81}NO_{27}$: C, 60.75; H, 6.07; N, 1.04. Found: C, 60.55; H, 6.29; N, 1.09.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (34)**——A mixture of **33** (28.3 mg, 2.11×10^{-5} mol) and 10% Pd on charcoal (28 mg) in glacial AcOH (2 ml) was hydrogenated for 72 h at room temperature under atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was acetylated with Ac_2O (1 ml) and pyridine (2 ml) as described for **2** to yield the crude acetate, which was purified by PTLC with $CHCl_3$–acetone (3 : 1). Pure **34** was isolated as a glass from the band having *Rf* 0.26. For analysis, the product was precipitated from $CHCl_3$–hexane as a white powder (18.3 mg, 75.4%). $[\alpha]_D^{24}$ +1.4° ($c=0.14$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.98, 2.03, 2.06, 2.09, 2.10, 2.12, 2.18, 2.19 (36H, all s, NAc, OAc × 11), 6.08 (1H, d, $J_{NH,2}=10$ Hz, NH). ^{13}C-NMR ($CDCl_3$): 100.7 ($^1J_{C\text{-}1-H\text{-}1}=178.2$ Hz, C-1), 98.9 ($^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}=173.3$ Hz, C-1″ or C-1‴), 97.4 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}=173.3$ Hz, C-1‴ or C-1″), 95.7 ($^1J_{C\text{-}1'-H\text{-}1'}=158.7$ Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1750 (OAc), 1678 (amide I), 1517 (amide II). TLC: *Rf* 0.26 (solvent B). *Anal.* Calcd for $C_{48}H_{65}NO_{31}\cdot H_2O$: C, 49.27; H, 5.77; N, 1.20. Found: C, 49.22; H, 5.36; N, 1.28.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (35)**——The 1,6-anhydro-β-ring of **34** (14.6 mg, 1.25×10^{-5} mol) was cleaved with acetolysis reagent (0.5 ml) as described for **31**. The resultant crude **35** was purified by PTLC as described for the preparation of **32** to provide **35** as a glass. For analysis, the product was precipitated from $CHCl_3$–hexane as a white powder (10.7 mg, 66.9%). $[\alpha]_D^{21}$ +51° ($c=0.1$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.91, 2.00, 2.04, 2.06, 2.11, 2.18, 2.20 (42H, all s, NAc, OAc × 13), 6.09 (d, $J_{1,2}=4$ Hz, H-1α). ^{13}C-NMR ($CDCl_3$): 98.9 ($^1J_{C\text{-}1''-H\text{-}1''\text{ or }C\text{-}1'''-H\text{-}1'''}=168.5$ Hz, C-1″ or C-1‴), 98.1 ($^1J_{C\text{-}1'-H\text{-}1'}=156.3$ Hz, C-1′), 97.7 ($^1J_{C\text{-}1'''-H\text{-}1'''\text{ or }C\text{-}1''-H\text{-}1''}=175.8$ Hz, C-1‴ or C-1″), 90.6 ($^1J_{C\text{-}1-H\text{-}1}=173.3$ Hz, C-1α). IR ν_{max}^{KBr} cm^{-1}: 3370 (NH), 1750 (OAc), 1684 (amide I), 1528 (amide II). TLC: *Rf* 0.70 and 0.64 [$CHCl_3$–acetone (1 : 1); α and β anomers). *Anal.* Calcd for $C_{52}H_{71}NO_{34}\cdot 1.5H_2O$: C, 48.79; H, 5.75; N, 1.09. Found: C, 48.52; H, 5.54; N, 1.13.

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References and Notes

1) This paper constitutes Part II of the series entitled "Partial Syntheses of Oligosaccharides in the High Mannose Type Glycoproteins." Part I: Y. Itoh and S. Tejima, *Chem. Pharm. Bull.*, **31**, 1632 (1983).
Abbreviations: Glc, D-glucopyranose; Man, D-mannopyranose; GlcNAc, *N*-acetyl-D-glucosamine; Asn, L-asparagine.
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13) Instruments used and conditions for chromatography were the same as in Part I unless otherwise indicated.
Abbreviation: PLC, preparative-layer chromatography.