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Syntheses of Acetylated Trisaccharides, Manα1→3Manβ1→4GlcNAc and Manα1→2Manβ1→4GlcNAc, relating to Mannosidosis

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The title trisaccharides (**22** and **30**) were synthesized by stepwise condensation of suitably protected monosaccharide units.

3-*O*-Allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-glucopyranosyl bromide (**9**) and 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**10**) were coupled by a modified Koenigs–Knorr glycosidation to give the protected Glcβ1→4GlcNAc (**12**) in 63.7% yield. After removal of the benzoyl group of **12**, the C-2′ hydroxyl group was isomerized to the D-manno configuration by a sequence consisting of oxidation to ulose and stereoselective borohydride reduction to give the protected Manβ1→4GlcNAc (**15**). Benzylation of **15**, followed by deallylation, gave the Manβ1→4GlcNAc derivative (**18**) having an unprotected hydroxyl group at the C-3′ position. α-D-Mannosidation of **18** with acetobromomannose gave the protected Manα1→3Manβ1→4GlcNAc (**20**) in 47.1% yield. Deprotection of **20**, followed by acetylation, yielded **22**.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**23**) and **10** were coupled to give the protected Glcβ1→4GlcNAc (**24**) in 39.2% yield. Compound **30** was obtained from **24** *via* four steps using procedures analogous to those used to obtain **22** from **12**.

Keywords——acetylated Manα1→3Manβ1→4GlcNAc; acetylated Manα1→2Manβ1→4GlcNAc; protected Glcβ1→4GlcNAc; DMSO–Ac_2O oxidation; protected Manβ1→4GlcNAc; 1,6-anhydro sugar derivative; ^{1}H-NMR; ^{13}C-NMR

Mannosidosis has been shown to be an inherited lysosomal storage disease, in which oligosaccharides containing D-mannose (Man) and *N*-acetylglucosamine (GlcNAc) are the storage substances. Lundblad *et al.*[1] isolated a trisaccharide, Manα1→3Manβ1→4GlcNAc (**1**), from pooled urine of patients as the major storage material in mannosidosis, and suggested that the oligosaccharide is probably a degradation product derived from the inner core of glycoprotein chains. Recently, Jeanloz *et al.*[2] utilized **1** as a starting material for the synthesis of Manα1→3Manβ1→4GlcNAcβ1→4GlcNAc α-phosphate, the synthetic precursor of "lipid intermediate." In addition, **1** is also the common core structure in accumulating complex oligosaccharides isolated from the livers of patients suffering from a deficiency of β-D-galactosidase,[3] from G_{M1}-gangliosidosis, Type I,[4] and fucosidosis.[5]

In order to develop a reasonable approach toward the synthesis of sugar chains present in glycoproteins, in which a high-mannose or complex-type sugar chain is linked to protein by an *N*-glycosidic linkage, **1** and Manα1→2Manβ1→4GlcNAc (**2**), a structural isomer of **1**, were chosen as targets for our synthetic studies on oligosaccharides of biological interest. These compounds may provide useful substrates for studies of the glycosidases involved in the biosynthesis and catabolism of glycoproteins.

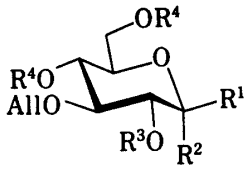
In this paper, fully acetylated **1** and **2** (**22** and **30**) were synthesized by stepwise condensation of suitably protected monosaccharide units. The key point of this work is the synthesis of an amino disaccharide bearing a β-D-mannopyranosyl linkage at the C-4 position of GlcNAc (Manβ1→4GlcNAc). This is because, despite several attempts,[6] stereoselective synthesis of β-D-mannopyranosides from D-mannopyranosyl halides has not yet been established, and the low reactivity of the hydroxyl group at the C-4 position of GlcNAc provides additional difficulties.

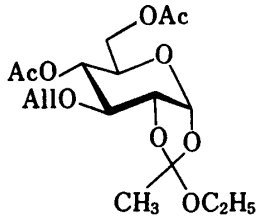
In order to overcome these barriers, the authors selected, as glucosyl donors, partially

etherated bromides bearing acyl groups at the C-2 position and, as a glucosyl acceptor, a 1,6-anhydro-β-GlcNAc derivative having an unprotected hydroxyl group at the C-4 position and a benzyl group at the C-3 position. We subsequently converted the Glcβ1→4GlcNAc thus obtained into a Manβ1→4GlcNAc derivative by a sequence of oxidation and stereoselective reduction, resulting in epimerization at C-2′. Therefore, synthesis of the glucosyl donor for amino disaccharide synthesis is first described.

3-*O*-Allyl-1,2: 5,6-di-*O*-isopropylidene-α-D-glucofuranose[7] prepared from 1,2: 5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**), was deisopropylidenated to give the 3-*O*-allyl ether. Without further purification, subsequent acetylation of the 3-*O*-allyl ether gave 1,2,4,6-tetra-*O*-acetyl-3-*O*-allyl-β-D-glucopyranose (**4**) as fine needles. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, the anomeric proton (H-1) of **4** appeared as a doublet with a reasonable coupling constant for the assigned configuration. The corresponding α-bromide was prepared from **4** by treatment with hydrogen bromide in acetic acid. Without purification, the bromide was converted to the corresponding orthoester, 4,6-di-*O*-acetyl-3-*O*-allyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose (**5**), according to a slight modification of the procedure described for the synthesis of the fully acetylated analog.[8] Benzylation of **5** with benzyl bromide and base, followed by removal of the ethoxyethylidene group by acid hydrolysis, gave 3-*O*-allyl-4,6-di-*O*-benzyl-D-glucopyranose (**6**).

Benzoylation of **6** gave 3-*O*-allyl-1,2-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-glucopyranose (**7**) as a syrup. In order to obtain crystalline di-*O*-acylates, anisoylation of **6** was carried out, but the resultant di-*O*-anisoyl ester (**8**) was also a syrup, and so this route was not further pursued. Treatment of **7** with hydrogen bromide in acetic acid gave 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-glucopyranosyl bromide (**9**), which was subjected to glucosidation.

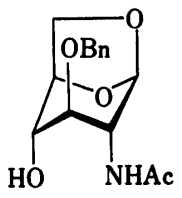




5

4: R^1=OAc, R^2=H, R^3=R^4=Ac
6: R^1, R^2=H, OH, R^3=H, R^4=Bn
7: R^1=OBz, R^2=H, R^3=Bz, R^4=Bn
8: R^1=OAnis, R^2=H, R^3=Anis, R^4=Bn
9: R^1=H, R^2=Br, R^3=Bz, R^4=Bn
11: R^1, R^2=H, OH, R^3=Bz, R^4=Bn

Ac=acetyl, All=allyl, Anis=anisoyl
Bn=benzyl, Bz=benzoyl



10

Chart 1

Condensation of one molar equivalent of 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**10**)[9] with about three molar equivalents of **9** in benzene–nitromethane in the presence of mercuric cyanide and Drierite gave 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (**12**) in a yield of 63.7% with a small amount of 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-D-gluco-

pyranose (**11**). Treatment of **12** with alkali caused selective debenzoylation to yield the disaccharide derivative (**13**) bearing only one unprotected hydroxyl group at the C-2 position of the D-glucose moiety. Acetylation of **13** gave the mono-*O*-acetate (**14**). The β-D-configuration of the newly introduced glucosidic linkage was confirmed by ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopies.

The unprotected hydroxyl group in **13** was then isomerized to D-manno configuration by reference to the method for isomerization of the amino disaccharide derivative.[10] Thus, oxidation of **13** with dimethyl sulfoxide–acetic anhydride (DMSO–Ac_2O) and, without purification, subsequent stereospecific reduction of the ulose with sodium borohydride yielded the β-D-mannopyranosyl disaccharide (**15**), which was acetylated to give the acetate (**16**).

Comparison of **13** with **15** showed differences in optical rotations (**13**, $[\alpha]_D^{20}$ −41.4°; **15**, −48.5°) and different mobilities on thin-layer chromatography (TLC). In the ^{1}H-NMR spectra of **13** and **15**, the one-proton singlet due to the hydroxyl at C-2′ was observed at 2.96 and 2.38 ppm, respectively. In the ^{13}C-NMR spectra of **13** and **15**, the resonances of C-1′ were observed at 102.1 and 99.3 ppm with ^1J values of 154.4 and 155.6 Hz, respectively. Therefore, the occurrence of isomerization from D-gluco to D-manno was confirmed.[11]

The unprotected hydroxyl group at C-2′ of **15** was benzylated to give the corresponding benzyl ether (**17**). In order to remove the allyl group at the C-3′ position, **17** was treated with tris(triphenylphosphine)rhodium chloride,[12] and the resultant 1-propenyl ether was removed with acid[13] to yield the deallylated product (**18**) in 43.2% yield. In the ^{1}H-NMR spectrum, the resonance of the hydroxyl proton at C-3′ was newly observed at 2.38 ppm as a one-proton singlet. Removal of the allyl group could be also effected by reaction of **17** with 10% palladium on charcoal. This method was recently recommended as a one-step deallylation procedure by Ogawa and Matsui.[14] However, the yield of **18** was not improved as much as expected.

Protected trisaccharide synthesis was then carried out by a conventional Koenigs–Knorr condensation as described for the preparation of the protected disaccharide (**12**). Namely, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**19**) was coupled with **18**. After a preliminary chromatographic purification, which did not separate the trisaccharide derivative (**20**) from **19**, the crude **20** was de-*O*-acetylated, and the product was separated by preparative TLC (PTLC). Re-acetylation gave **20** in 47.1% yield from **18**. It was characterized by elementary analysis, and infrared spectra (IR), ^{1}H-NMR and ^{13}C-NMR spectroscopies.

Hydrogenolytic removal of the benzyl groups of **20**, followed by acetylation, gave the fully acetylated 1,6-anhydro-β-trisaccharide (**21**) in 66.9% yield. The structure was characterized as described for **20**. Finally, the 1,6-anhydro-β-linkage of **21** was acetolyzed at 0°C with a mixture of boron trifluoride etherate and acetic anhydride to give the fully acetylated Manα1→3Manβ1→4GlcNAc (**22**) as an anomeric mixture in 70.1% yield. The product was separated as a white powder having $[\alpha]_D^{20}$ +20°. Jeanloz *et al.*[2] reported the monohydrate of **22** to be an amorphous solid having mp 106—109°C and $[\alpha]_D^{20}$ 0°.

The fully acetylated Manα1→2Manβ1→4GlcNAc (**30**), which is the second title sugar and a structural isomer of **22**, was synthesized *via* analogous procedures from **9** and **10**.

The first step, condensation of **10** and a glucosyl donor, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**23**),[15] was carried out by a modified Koenigs–Knorr reaction. After column chromatographic purification, the amino disaccharide derivative (**24**) was isolated in 39.2% yield. ^{13}C-NMR spectroscopy confirmed the β-D-gluco configuration of the newly introduced glucosidic linkage. Treatment of **24** with alkali gave the amino disaccharide derivative (**25**) bearing only one unprotected hydroxyl group at the C-2′ position.

The second step, isomerization of the β-D-glucopyranosyl moiety of **25** to β-D-manno configuration, was carried out by a sequence of oxidation with DMSO–Ac_2O and subsequent stereoselective reduction with sodium borohydride to give the β-D-mannosyl amino disaccharide (**26**). Acetylation of **26** yielded the mono-*O*-acetyl mannosyl amino disaccharide (**27**). The inversion from D-gluco to D-manno was confirmed by comparison of the *Rf* values on TLC and

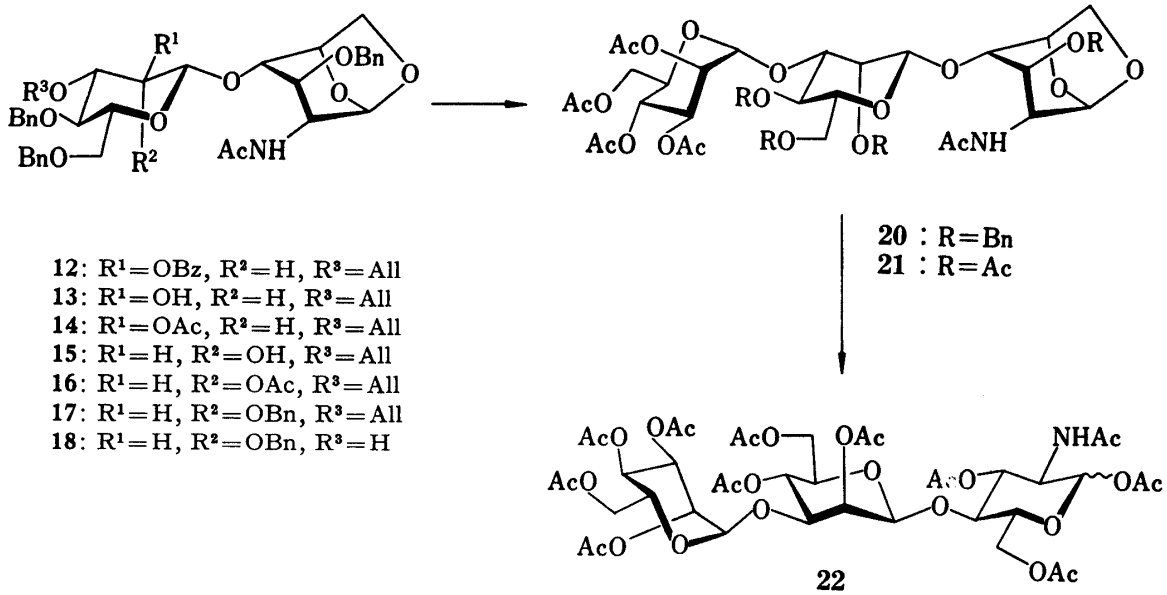


Chart 2

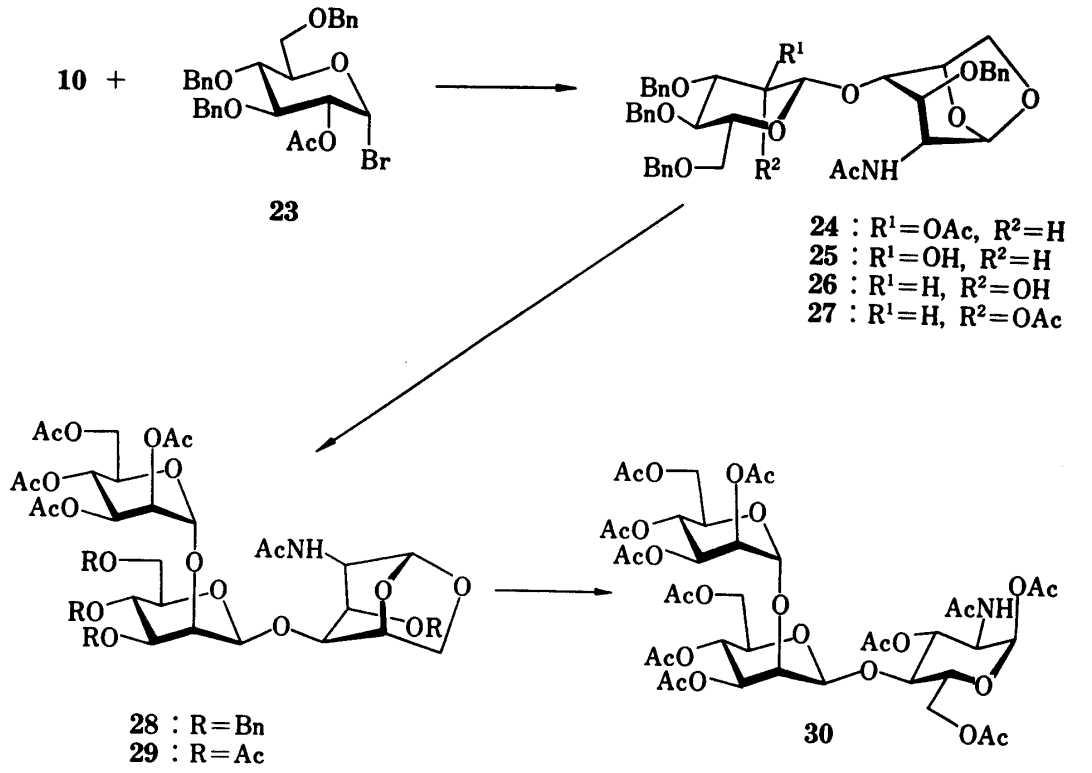


Chart 3

^{13}C-NMR spectral data for **25** and **26**, or **24** and **27**.

The third step is synthesis of the fully protected trisaccharide derivative (**28**). Condensation of acetobromomannose (**19**) with **26** was carried out by a modified Koenigs–Knorr reaction. The crude trisaccharide derivative was purified by a sequence of de-*O*-acetylation, chromatographic separation, re-acetylation, and PTLC. The yield was 40.7%. The α-D-configuration of the newly introduced mannosidic linkage was confirmed by ^{13}C-NMR spectroscopy.

The final step is conversion of the protecting groups of **28** to acetyl groups. Hydrogenolytic debenzylation followed by acetylation gave the fully acetylated 1,6-anhydro-β-trisaccharide (**29**). The 1,6-anhydro-β-linkage was then acetolyzed to give the fully acetylated Manα1→2Manβ1→4GlcNAc (**30**), which was separated as a white powder in 77.4% yield from **29**. The product was characterized as the α-acetate by ^{13}C-NMR spectroscopy.

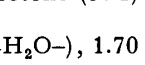
The present work further confirms[9,16] that 1,6-anhydro-β-derivatives of monosaccharides and oligosaccharides are extremely versatile starting materials or key intermediates for syntheses of complex oligosaccharides.

Experimental

Solutions were concentrated with a Büchi-Shibata Rotavapor EL-130 below 45°C under a vacuum. Melting points were determined with a Yanagimoto MP-S2 micro melting point apparatus, and are uncorrected. Optical rotations were measured with a Union Giken PM-201 automatic digital polarimeter in a 0.5 dm tube. ^{1}H- and ^{13}C-NMR spectra were recorded at 100 and 25 MHz, with JEOL JNM-MH-100 and -FX-100 spectrometers, respectively. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are given in ppm from TMS. IR spectra were recorded with a JASCO IRA-2 or an A-102 spectrometer. TLC, PTLC, and preparative-layer chromatography (PLC) were performed on pre-coated plates of Silica Gel 60 F_{254} 0.25 mm thick, 0.5 mm thick, and PLC plates 2 mm thick (E. Merck), respectively. The following solvent combinations were used for TLC: (A), $CHCl_3$–acetone (6: 1); (B), $CHCl_3$–acetone (3: 1); (C), hexane–ether (1: 1). Detection was effected by ultraviolet (UV) irradiation at 254 nm or with a spray reagent (A), anisaldehyde–H_2SO_4–EtOH at 125°C;[17] (B), 1% $KMnO_4$ in 2% Na_2CO_3 solution. Column chromatography was performed on Silica Gel 60 (70—230 mesh, E. Merck). All solvent compositions are given as v/v.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-allyl-β-D-glucopyranose (**4**)——3-*O*-Allyl-1,2: 5,6-di-*O*-isopropylidene-α-D-glucofuranose, prepared from 1,2: 5,6-di-*O*-isopropylidene-α-D-glucofuranose (3, 12 g, 46.1 mmol) by the method of Corbett and McKay,[7] in 1.2% (w/v) aq. H_2SO_4 (180 ml) was heated to reflux for 3 h. The solution was neutralized with $BaCO_3$, filtered, and the filtrate was extracted with $CHCl_3$ to remove by-products. The aqueous phase was concentrated to dryness, and the residue was acetylated by heating for 1.5 h with Ac_2O (100 ml) and anhyd. AcONa (5 g). The mixture was poured into ice-H_2O and extracted with $CHCl_3$. The combined extracts were successively washed with H_2O, ice-cold aq. $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and concentrated to a syrup, which was crystallized from EtOH–hexane as fine needles (10.06 g, 56.2% based on **3**), mp 119—120°C, $[\alpha]_D^{22}$ +4.7° (c=1.3, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.08, 2.09, 2.10, 2.11 (12H, each s, OAc×4), 5.62—6.08 (1H, m, CH_2=C<u>H</u>CH_2–), 5.72 (1H, d, $J_{1,2}$=8 Hz, H-1). IR ν_{max}^{KBr} cm^{-1}: 1735 (OAc). TLC: *Rf* 0.78 (solvent A). *Anal.* Calcd for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.27; H, 6.19.

4,6-Di-*O*-acetyl-3-*O*-allyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose (**5**)——A stirred solution of **4** (3 g, 7.72 mmol) in CH_2Cl_2 (20 ml) was treated dropwise with 30% (w/v) HBr–AcOH (12 ml) at 0°C. After being stirred for 1 h at 0°C, the mixture was diluted with CH_2Cl_2, successively washed with H_2O, ice-cold aq. $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and concentrated to yield a syrupy bromide.

A mixture of the bromide, EtOH (1.5 ml), 2,6-lutidine (1.8 ml), and nitromethane (20 ml) was stirred at 40°C for 16 h. After being diluted with CH_2Cl_2, the solution was successively washed with H_2O, ice-cold 1 N H_2SO_4 and aq. $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and concentrated to a syrup (2.56 g, 88.6%). For analysis, the syrup was chromatographed on a column with $CHCl_3$–acetone (5: 1) to yield pure **5**, $[\alpha]_D^{23}$ +42.8° (c=0.32, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.20 (3H, t, J=7 Hz, CH_3CH_2O–), 1.70 (3H, s, ), 2.09 (6H, s, OAc×2), 3.60 (2H, q, J=7 Hz, $CH_3C\underline{H}_2O$–), 5.63—6.20 (1H, m, CH_2=C<u>H</u>CH_2–), 5.74 (1H, d, $J_{1,2}$=5 Hz, H-1). TLC: *Rf* 0.44 (solvent A). *Anal.* Calcd for $C_{17}H_{26}O_9$: C, 54.54; H, 7.00. Found: C, 54.17; H, 6.88.

3-*O*-Allyl-4,6-di-*O*-benzyl-D-glucopyranose (**6**)——Benzyl bromide (20 ml) and powdered KOH (20 g) were added to a solution of **5** (10.8 g, 29 mmol) in 1,4-dioxane (100 ml). The mixture was stirred at 70°C for 4 h, cooled, and diluted with $CHCl_3$. After filtration, the filtrate was successively washed with H_2O, ice-cold 10% H_2SO_4, aq. $NaHCO_3$ and H_2O, and then concentrated to a syrup.

The resultant benzyl ether in 1,4-dioxane–1 M H_2SO_4 [4: 1 (v/v), 150 ml] was heated under reflux for 4 h to hydrolyze it. After neutralization with solid $NaHCO_3$, the mixture was concentrated to a syrup, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried ($MgSO_4$), and concentrated to a syrup, which was column-chromatographed with hexane–AcOEt (1: 1) to yield **6** as a white solid (4.67 g, 40.2%). For analysis, the solid was crystallized from ether–pentane as white silky needles, mp 84—85°C, $[\alpha]_D^{20}$ +72.5° (c=0.8, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 5.80—6.20 (1H, m, CH_2=C<u>H</u>CH_2–), 7.31 (10H, s, <u>Ph</u>CH_2×2), 3.19—5.50 [17H, 15H (unresolved ring protons) and 2H (exchangeable with D_2O)], IR ν_{max}^{KBr} cm^{-1}: 3330 (OH). TLC:

Rf 0.60 (solvent B). *Anal.* Calcd for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 69.01; H, 7.19.

3-*O*-Allyl-1,2-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-glucopyranose (7)——Benzoyl chloride (0.4 ml) was added to a chilled solution of **6** (206 mg, 5.14×10^{-4} mol) in dry pyridine (5 ml), and the mixture was stirred for 18 h at room temperature. After dropwise addition of H_2O (1 ml) to decompose excess benzoyl chloride, the mixture was concentrated to a syrup. This was dissolved in CH_2Cl_2, washed with ice-cold dil. HCl, H_2O, ice-cold aq. $NaHCO_3$ solution, and H_2O, then dried ($MgSO_4$), and concentrated to a syrup. On column chromatography with hexane–ether (3: 1), **7** (242.7 mg, 77.6%) was obtained as a syrup, $[\alpha]_D^{21}$ +143° ($c=0.66$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 5.87 (1H, d, $J_{1,2}=8$ Hz, H-1), 7.00—7.62 (16H, m, PhCH$_2\times2$ and *meta* and *para* to C=O of benzoyl$\times2$), 7.03, 7.04 (4H, each d, $J=8$ Hz, aromatic protons *ortho* to C=O of benzoyl$\times2$). IR ν_{max}^{neat} cm^{-1}: 1725 (OBz). TLC: *Rf* 0.64 (solvent C). *Anal.* Calcd for $C_{37}H_{36}O_8$: C, 73.01; H, 5.96. Found: C, 72.81; H, 5.89.

3-*O*-Allyl-1,2-di-*O*-anisoyl-4,6-di-*O*-benzyl-β-D-glucopyranose (8)——Anisoyl chloride (0.7 g) was added to a solution of **6** (212 mg, 5.29×10^{-4} mol) in dry pyridine (5 ml). After being stirred for 42 h at room temperature, the mixture was treated as described for the procedure employing **7**. Two column chromatographies with CH_2Cl_2 afforded **8** (283 mg, 80%) as a pale yellow syrup, $[\alpha]_D^{21}$ +53.1° ($c=1.3$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 3.77 (6H, s, OCH$_3\times2$), 5.96 (1H, d, $J_{1,2}=8$ Hz, H-1), 6.84, 6.86 (4H, each d, $J=9$ Hz, aromatic protons *ortho* to OCH_3 of anisoyl$\times2$), 7.28, 7.31 (10H, each s, PhCH$_2\times2$), 7.96 (4H, d, $J=9$ Hz, aromatic protons *ortho* to C=O of anisoyl). IR ν_{max}^{neat} cm^{-1}: 1722 (C=O). TLC: *Rf* 0.31 (solvent C). *Anal.* Calcd for $C_{39}H_{40}O_{10}$: C, 70.05; H, 6.03. Found: C, 70.06; H, 5.88.

3-*O*-Allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-glucopyranosyl Bromide (9)——A mixture of **7** (3.5 g, 5.75 mmol) in dry CH_2Cl_2 (70 ml) with 30% (w/v) HBr–AcOH (18 ml) was stirred at 0°C for 40 min. After dilution with CH_2Cl_2, 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 dryness by repeated co-distillation with dry toluene to yield **9** (2.94 g, 90.1%), which was immediately used.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (12)——A solution of **9** (2.94 g, 5.18 mmol) in benzene–nitromethane (1: 1, 10 ml) was added to a suspension of **10**[9] (542 mg, 1.79 mmol), $Hg(CN)_2$ (2 g), and Drierite (0.6 g) in the same solvent (4 ml). The mixture was stirred for 25 h at room temperature, then filtered, and the filtrate was diluted with $CHCl_3$. This solution was successively washed with H_2O, satd. KI and $NaHCO_3$ solutions, and H_2O, then dried ($MgSO_4$), and concentrated to a syrup, which was column-chromatographed with hexane–ether (1: 4). From the faster moving fractions having *Rf* 0.76 (solvent A), a trace of 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-D-glucopyranose (**11**) was isolated after removal of the solvent. Compound **11** was crystallized from $CHCl_3$–hexane as white needles, mp 139—140°C, $[\alpha]_D^{19}$ +123.7° ($c=0.35$, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{32}O_7$: C, 71.41; H, 6.39. Found: C, 71.64; H, 6.40.

After **11** had emerged, **12** was eluted with the same solvent, and isolated as a foamy solid (903 mg, 63.7%), $[\alpha]_D^{22}$ −32.9° ($c=0.47$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.12 (3H, s, NAc), 6.44 (1H, d, $J_{NH,2}=10$ Hz, NH), 7.06—8.22 (20H, m, aromatic protons). IR ν_{max}^{KBr} cm^{-1}: 3360 (NH), 1716 (OBz), 1669 (amide I), 1509 (amide II). TLC: *Rf* 0.71 (solvent A). *Anal.* Calcd for $C_{45}H_{49}NO_{11}\cdot1/2H_2O$: C, 68.51; H, 6.39; N, 1.78. Found: C, 68.27; H, 6.44; N, 1.89.

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 (13)——A 0.5 N methanolic solution of MeONa (10 ml) was added to a solution of **12** (2.07 g, 2.62 mmol) in dry MeOH (50 ml). After being stirred overnight at room temperature, the mixture was decationated with Amberlite IR-120 (H^+) resin, filtered, and concentrated to a syrup, which was chromatographed on a column with $CHCl_3$–MeOH (100: 1). Removal of the solvent from the major fractions provided **13** as a foamy solid (1.4 g, 76%), $[\alpha]_D^{22}$ −41.4° ($c=0.46$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.98 (3H, s, NAc), 2.92 (1H, s, OH), 6.30 (1H, d, $J_{NH,2}=8$ Hz, NH), 7.26, 7.29 (15H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 102.1 ($^1J_{C\text{-}1'\text{-}H\text{-}1'}=154.4$ Hz, C-1'), 100.5 ($^1J_{C\text{-}1\text{-}H\text{-}1}=173.3$ Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH, NH), 1650 (amide I), 1522 (amide II). TLC: *Rf* 0.46 (solvent B). *Anal.* Calcd for $C_{38}H_{45}NO_{10}\cdot1.5H_2O$: C, 64.94; H, 6.88; N, 1.99. Found: C, 65.05; H, 6.67; N, 2.13.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-allyl-4,6-di-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (14)——A mixture of **13** (36.9 mg, 5.25×10^{-5} mol) in Ac_2O (0.5 ml) and pyridine (1 ml) was stirred overnight at room temperature, then concentrated to a syrup. This was column-chromatographed with toluene–acetone (4: 1) to isolate **14** (37.5 mg, 99.5%) as a syrup, $[\alpha]_D^{24}$ −101.5° ($c=0.13$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.09, 2.16 (6H, each s, OAc, NAc), 6.33 (1H, d, $J_{NH,2}=10$ Hz, NH), 7.12—7.48 (15H, m, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 3375 (NH), 1738 (OAc). TLC: *Rf* 0.63 (solvent A). *Anal.* Calcd for $C_{40}H_{47}NO_{11}$: C, 66.93; H, 6.60; N, 1.95. Found: C, 66.83; H, 6.88; N, 2.11.

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 (15)——A solution of **13** (233.7 mg, 3.33×10^{-4} mol) in DMSO–Ac_2O (2: 1, v/v, 6 ml) was stirred for 48 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.

A mixture of this syrup and $NaBH_4$ (200 mg) in CH_2Cl_2–MeOH (1: 1, 6 ml) was stirred for 4 h at room temperature, and then diluted with $CHCl_3$. The whole was successively washed with H_2O, ice-cold 10%

citric acid and satd. $NaHCO_3$ solutions, and H_2O, dried ($MgSO_4$), and concentrated to a syrup. This was chromatographed on a column with $CHCl_3$–MeOH (100: 1) to provide **15** (137.1 mg, 58.6%) as a foamy solid, $[\alpha]_D^{19}$ −48.5° ($c=0.41$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.99 (3H, s, NAc), 2.38 (1H, s, OH), 5.48 (1H, s, H-1), 6.46 (1H, d, $J_{NH,2}=10$ Hz, NH), 7.36, 7.41 (15H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.6 ($^1J_{C-1-H-1}=175.2$ Hz, C-1), 99.3 ($^1J_{C-1'-H-1'}=155.6$ Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH, OH), 1652 (amide I), 1520 (amide II). TLC: *Rf* 0.31 (solvent B). *Anal.* Calcd for $C_{38}H_{45}NO_{10}\cdot 1.5H_2O$: C, 64.94; H, 6.88; N, 1.99. Found: C, 64.89; H, 6.95; N, 2.24.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-allyl-4,6-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (16)——Acetylation of **15** (30.4 mg, 4.33×10^{-5} mol) with Ac_2O (0.5 ml) and pyridine (1 ml) as described for **14** provided **16** (31 mg, 99.5%) as a syrup, $[\alpha]_D^{17}$ −100° ($c=0.12$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.07, 2.17 (6H, each s, OAc, NAc), 6.18 (1H, d, $J_{NH,2}=10$ Hz, NH), 7.26, 7.31 (15H, each s, aromatic protons). IR ν_{max}^{neat} cm^{-1}: 3370 (NH), 1730 (OAc). TLC: *Rf* 0.49 (solvent A). *Anal.* Calcd for $C_{40}H_{47}NO_{11}$: C, 66.93; H, 6.60; N, 1.95. Found: C, 66.80; H, 6.43; N, 1.75.

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 (17)——Benzyl bromide (2 ml) was added to a mixture of **15** (0.64 g, 9.11×10^{-4} mol), powdered BaO (1.7 g), and $Ba(OH)_2\cdot 8H_2O$ (0.7 g), suspended in dry DMF (30 ml). The mixture was stirred at 50°C for 48 h, cooled, diluted with $CHCl_3$, and filtered. The filtrate was successively washed with ice-cold dil. HCl, H_2O, ice-cold $NaHCO_3$ solution, and H_2O, dried ($MgSO_4$), and concentrated to a syrup, which was column-chromatographed with hexane–ether (1: 4). Fractions having *Rf* 0.58 (solvent A) were further purified by PLC with toluene–acetone (4: 1). A zone having *Rf* 0.38 was excluded from the plates and extracted with $CHCl_3$–MeOH (9: 1) to isolate **17** (0.48 g, 69.5%) as a syrup, $[\alpha]_D^{18}$ −87° ($c=0.94$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.61 (3H, s, NAc), 6.05 (1H, d, $J_{NH,2}=9$ Hz, NH), 7.22 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.6 (C-1, C-1′). IR ν_{max}^{neat} cm^{-1}: 3380 (NH), 1665 (amide I), 1505 (amide II). TLC: *Rf* 0.58 (solvent A).

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-(2,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (18)——A) Deallylation with Pd-catalyst: A mixture of **17** (56.4 mg, 7.36×10^{-5} mol) and 10% Pd-on-charcoal (60 mg) suspended in EtOH–AcOH–H_2O (2: 1: 1, 5 ml) was stirred at 75°C for 5 h. then filtered. The filtrate was concentrated to dryness by repeated co-distillation with EtOH and toluene. The residue was purified by PTLC with solvent B. The band having *Rf* 0.63 was excluded from the plates and extracted with $CHCl_3$–MeOH (9: 1) to isolate **18** (19.1 mg, 35.7%) as a foamy solid, $[\alpha]_D^{21}$ −85.9° ($c=0.17$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.70 (3H, s, NAc), 2.38 (1H, s, OH), 6.07 (1H, d, $J_{NH,2}=10$ Hz, NH), 7.33 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.7 ($^1J_{C-1'-H-1'}=158.1$ Hz, C-1′), 100.6 ($^1J_{C-1-H-1}=171.5$Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH, OH), 1664 (amide I), 1515 (amide II). TLC: *Rf* 0.63 (solvent B). *Anal.* Calcd for $C_{42}H_{47}NO_{10}$: C, 69.50; H, 6.53; N, 1.93. Found: C, 69.61; H, 6.68; N, 1.96.

B) Deallylation with Rhodium Complex: A mixture of **17** (252.3 mg, 3.29×10^{-4} mol), tris(triphenylphosphine) rhodium chloride (22 mg), and 1,4-diazabicyclo[2.2.2]octane (33 mg) dissolved in EtOH–benzene–H_2O (7: 3: 1, 8 ml) was boiled under reflux for 4 h under stirring, then concentrated to a syrup. This was dissolved in 80% (v/v) aq. AcOH (13 ml). The solution was stirred at 80°C for 2 h, cooled, and concentrated to a syrup by repeated co-distillation with toluene. The resultant syrup was purified by PTLC as described in method A) to isolate the deallylation product (102.8 mg, 43.2%), which was indistinguishable from **18** by TLC, IR and NMR comparisons.

***O*-(2,3,4,6-Tetra-*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 (20)**——A solution of **19** (250mg, 6.08×10^{-4} mol) in dry benzene (1.5 ml) was added to a suspension of **18** (43.3 mg, 5.97×10^{-5} mol), $Hg(CN)_2$ (230 mg), and Drierite (80 mg) in dry nitromethane (1.5 ml). After being stirred at 50°C for 3 d, the mixture was diluted with $CHCl_3$, and filtered, and the filtrate was treated as described in the case of **12** to separate the coupling product as a syrup. The resultant syrup in dry MeOH (4 ml) was de-*O*-acetylated with a 0.5 N methanolic solution of MeONa (0.2 ml), and the crude deacetylated product was purified by PTLC with $CHCl_3$–acetone (1: 2). The band having *Rf* 0.17 was removed from the plates, extracted with $CHCl_3$–MeOH (1: 1), and concentrated to a syrup.

The syrup was re-acetylated with Ac_2O (1 ml) and pyridine (2 ml) for 24 h at room temperature, then concentrated to dryness. The residue was purified by PTLC with $CHCl_3$–acetone (6: 1). From the band having *Rf* 0.44, **20** (29.7 mg, 47.1%) was isolated as a foamy solid, $[\alpha]_D^{21}$ −27.8° ($c=0.22$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.68 (3H, s, NAc), 2.02, 2.04, 2.07, 2.08 (12H, each s, OAc×4), 6.09 (1H, d, $J_{NH,2}=9$ Hz, NH), 7.31 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.0 ($^1J_{C-1'-H-1'}=156.3$ Hz, C-1′), 100.7 ($^1J_{C-1-H-1}=170.9$ Hz, C-1), 99.6 ($^1J_{C-1''-H-1''}=175.8$ Hz, C-1″). IR ν_{max}^{KBr} cm^{-1}: 3410 (NH), 1750 (OAc), 1674 (amide I), 1511 (amide II). TLC: *Rf* 0.44 (solvent A). *Anal.* Calcd for $C_{56}H_{65}NO_{19}$: C, 63.69; H, 6.20; N, 1.33. Found: C, 63.50; H, 6.35; N, 1.35.

***O*-(2,3,4,6-Tetra-*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 (21)**——A solution of **20** (42.8 mg, 4.05×10^{-5} mol) in glacial AcOH (3 ml) was mixed with 10% Pd-on-charcoal (40 mg), and hydrogenated at room temperature under atmospheric pressure for 48 h. The catalyst was filtered off, and the filtrate was concentrated to give a glassy mass, which was acetylated with Ac_2O (1 ml) and pyridine (2 ml) for 24 h at room

temperature. The mixture was concentrated to dryness, and the residue was purified by PTLC with $CHCl_3$–acetone (3: 1). From the band having *Rf* 0.25, **21** was isolated as a foamy solid. For analysis, the product was precipitated from $CHCl_3$–hexane as a white powder (23.4 mg, 66.9%), $[\alpha]_D^{21}$ −10° (*c*=0.1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.99, 2.02, 2.06, 2.10, 2.14, 2.27 (27H, each s, OAc×8, NAc), 6.14 (1H, d, $J_{NH,2}$=9 Hz, NH). ^{13}C-NMR ($CDCl_3$): 100.4 ($^1J_{C-1-H-1}$=175.8 Hz, C-1), 99.2 ($^1J_{C-1''-H-1''}$=175.8 Hz, C-1″), 96.5 ($^1J_{C-1'-H-1'}$=158.7 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3410 (NH), 1746 (OAc), 1676 (amide I), 1516 (amide II). TLC: *Rf* 0.25 (solvent B). *Anal.* Calcd for $C_{36}H_{49}NO_{23}\cdot 1/2H_2O$: C, 49.54; H, 5.77; N, 1.60. Found: C, 49.35; H, 5.65; N, 1.69.

O-(2,3,4,6-Tetra-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 (22)——A solution of **21** (26 mg, 2.98×10^{-5} mol) in an ice-cold acetolysis reagent [boron trifluoride etherate–Ac_2O (1: 25, v/v) 0.65 ml] was stirred for 2 h at 0°C. A piece of ice was added, and the mixture was stirred overnight at room temperature to decompose excess acetolysis reagent. The solution was then diluted with $CHCl_3$, and neutralized with solid $NaHCO_3$. The separated organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to dryness. The resultant syrup was treated with $CHCl_3$–hexane to yield **22** (20.2 mg, 70.1%) as a white powder, $[\alpha]_D^{22}$ +20° (*c*=0.14, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.93, 1.98, 2.05, 2.09, 2.11, 2.19 (33H, each s, OAc×10, NAc), 6.12 (d, $J_{1,2}$=4 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 97.9 (C-1, β), 90.6 (C-1, α). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1746 (OAc), 1684 (amide I), 1526 (amide II). TLC: *Rf* 0.29 and 0.22 (anomeric mixture, solvent B). *Anal.* Calcd for $C_{40}H_{55}NO_{26}\cdot 1/2H_2O$: C, 49.28; H, 5.79; N, 1.44. Found: C, 49.25; H, 5.82; N, 1.44. lit.[2] amorphous (monohydrate), mp 106—109°C, $[\alpha]_D^{20}$ 0° (*c*=2.3, 5: 1 $CHCl_3$–MeOH), ^{1}H-NMR ($CDCl_3$): 6.1 (d, 8 Hz, H-1β).

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (24)——A solution of **23**[15] (2.69 g, 4.84 mmol) in dry benzene (8 ml) was added to a suspension of **10**[9] (514.9 mg, 1.7 mmol), $Hg(CN)_2$ (2 g), and Drierite (1 g) in nitromethane (8 ml). After being stirred at 55°C for 24 h, the mixture was treated as described for **12**, yielding crude **24**, which was column-chromatographed with hexane–ether (1: 4). Removal of the solvent from the fractions having *Rf* 0.62 (solvent A) provided pure **24** (511.2 mg, 39.2%), $[\alpha]_D^{21}$ −59.7° (*c*=0.58, $CHCl_3$), as a foamy solid. ^{1}H-NMR ($CDCl_3$): 2.01, 2.05 (6H, each s, OAc, NAc), 5.23 (1H, s, H-1), 6.28 (1H, d, $J_{NH,2}$=10 Hz, NH), 7.25 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.2 ($^1J_{C-1-H-1}$=174.6 Hz, C-1), 98.9 ($^1J_{C-1'-H-1'}$=158.7 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3360 (NH), 1729 (OAc), 1667 (amide I), 1505 (amide II). TLC: *Rf* 0.62 (solvent A). *Anal.* Calcd for $C_{44}H_{49}NO_{11}$: C, 68.82; H, 6.43; N, 1.82. Found: C, 68.61; H, 6.48; N, 1.82.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (25)——Deacetylation of **24** (148.7 mg, 1.94×10^{-4} mol) in dry MeOH (4 ml) with a 0.5 N methanolic solution of MeONa (0.2 ml) was carried out as for **13** to yield crude **25**, which was purified by PTLC with $CHCl_3$–acetone (3: 1). The band having *Rf* 0.44 was removed from the plates and extracted with $CHCl_3$–MeOH (3: 1). Removal of the solvent provided **25** as a foamy solid (133.5 mg, 94.8%), $[\alpha]_D^{23}$ −41.9° (*c*=0.27, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.89 (3H, s, NAc), 5.39 (1H, s, H-1), 6.36 (1H, d, $J_{NH,2}$=8 Hz, NH), 7.27 (20H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 102.1 ($^1J_{C-1'-H-1'}$=155.0 Hz, C-1′), 100.5 ($^1J_{C-1-H-1}$=172.3 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH, NH), 1642 (amide I), 1505 (amide II). TLC: *Rf* 0.44 (solvent B). *Anal.* Calcd for $C_{42}H_{47}NO_{10}$: C, 69.50; H, 6.53; N, 1.93. Found: C, 69.42; H, 6.49; N, 2.12.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (26)——Oxidation of **25** (378 mg, 5.21×10^{-4} mol) in DMSO–Ac_2O (2: 1, v/v, 13.5 ml) to the corresponding ulose and subsequent reduction of the ulose to crude **26** with $NaBH_4$ (420 mg) was carried out as described for **15** (from **13**). Pure **26** was obtained by column chromatography of the crude product with $CHCl_3$–acetone (20: 1) as a foamy solid (227.3 mg, 58.7%), $[\alpha]_D^{19}$ −54.2° (*c*=0.21, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.92 (3H, s, NAc), 2.82 (1H, br s, OH), 5.40 (1H, s, H-1), 6.40 (1H, d, $J_{NH,2}$=9 Hz, NH), 7.25, 7.32 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.6 ($^1J_{C-1-H-1}$=175.8 Hz, C-1), 99.3 ($^1J_{C-1'-H-1'}$=156.3 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH, NH), 1658 (amide I), 1530 (amide II). TLC: *Rf* 0.31 (solvent B). *Anal.* Calcd for $C_{42}H_{47}NO_{10}\cdot H_2O$: C, 67.82; H, 6.64; N, 1.88. Found: C, 67.84; H, 6.41; N, 2.16.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (27)——Acetylation of **26** (30.5 mg, 4.1×10^{-5} mol) with Ac_2O (0.5 ml) and pyridine (1 ml) was carried out as described for **14**. After purification of the crude acetate by PTLC with $CHCl_3$–acetone (6: 1, pure **27** was isolated as a syrup (27.2 mg, 85.4%), $[\alpha]_D^{20}$ −56.5° (*c*=0.37, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.02, 2.15 (6H, each s, OAc, NAc), 5.36 (1H, s, H-1), 6.10 (1H, d, $J_{NH,2}$=10 Hz, NH), 7.27, 7.29 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.1 ($^1J_{C-1-H-1}$=173.3 Hz, C-1), 95.2 ($^1J_{C-1'-H-1'}$=153.8 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH), 1738 (OAc), 1672 (amide I), 1509 (amide II). TLC: *Rf* 0.43 (solvent A). *Anal.* Calcd for $C_{44}H_{49}NO_{11}\cdot 1/2H_2O$: C, 68.03; H, 6.49; N, 1.80. Found: C, 68.07; H, 6.77; N, 1.91.

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose (28)——Glycosidation of **26** (113.7 mg, 1.53×10^{-4} mol) with **19** (668.7 mg, 1.63 mmol) in benzene (3 ml) in the presence of a suspension of $Hg(CN)_2$ (600 mg) and Drierite (200 mg) in dry nitromethane (3 ml) was carried out as described for **20**. Because the crude trisaccharide was contaminated with a side product having the same *Rf* value on TLC, it was de-*O*-acetylated with a 0.5 N methanolic solution of MeONa (0.5 ml) in dry MeOH (10 ml) as described for **13**. From the band having *Rf* 0.17 on PTLC with $CHCl_3$–acetone (1: 2), the pure de-*O*-acetylated trisac-

charide was isolated.

The product was then re-acetylated with Ac_2O (1 ml) and pyridine (2 ml), and the resultant acetate was purified by PTLC with $CHCl_3$–acetone (6: 1). From the band having *Rf* 0.36, pure **28** was isolated as a foamy solid (65.8 mg, 40.7%), $[\alpha]_D^{19}$ −29° (c=0.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.99, 2.07, 2.09 (15H, all s, OAc×4, NAc), 6.16 (1H, d, $J_{NH,2}$=10 Hz, NH), 7.25, 7.29 (20H, each s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 100.9 ($^1J_{C-1-C-H}$=153.8 Hz, C-1′), 100.5 ($^1J_{C-1-H-1}$=175.8 Hz, C-1), 98.1 ($^1J_{C-1''-H-1''}$=173.3 Hz, C-1″). IR ν_{max}^{KBr} cm^{-1}: 3410 (NH), 1750 (OAc), 1673 (amide I), 1511 (amide II). TLC: *Rf* 0.36 (solvent A). *Anal.* Calcd for $C_{56}H_{65}NO_{19}$: C, 63.69; H, 6.20; N, 1.33. Found: C, 63.73; H, 6.23; N, 1.60.

***O*-(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-*β*-D-mannopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-1,6-anhydro-2-deoxy-*β*-D-glucopyranose (29)**——Catalytic debenzylation of **28** (57.4 mg, 5.43×10^{-5} mol) in glacial AcOH (3 ml) with 10% Pd-on-charcoal (50 mg) and subsequent acetylation of the resultant debenzylated product with Ac_2O (1 ml) and pyridine (2 ml) were carried out as described for **14**. The crude acetate was column-chromatographed with $CHCl_3$–ether–MeOH (30: 5: 1). From the fractions having *Rf* 0.21 with $CHCl_3$–acetone (3: 1), **29** was isolated as a syrup. For analysis, the syrup was treated with CH_2Cl_2–hexane to yield a white powder (35.3 mg, 75.3%), $[\alpha]_D^{21}$ −39.8° (c=0.22, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.02, 2.05, 2.09, 2.16 (27H, each s, OAc×8, NAc), 6.12 (1H, d, $J_{NH,2}$=10 Hz, NH). ^{13}C-NMR ($CDCl_3$): 100.6 ($^1J_{C-1'-H-1'}$=156.3 Hz, C-1′), 100.2 ($^1J_{C-1-H-1}$=178.2 Hz, C-1), 97.6 ($^1J_{C-1''-H-1''}$=170.9 Hz, C-1″). IR ν_{max}^{KBr} cm^{-1}: 3420 (NH), 1745 (OAc), 1679 (amide I), 1516 (amide II). TLC: *Rf* 0.21 (solvent B). *Anal.* Calcd for $C_{36}H_{49}NO_{23}$: C, 50.06; H, 5.72; N, 1.62. Found: C, 49.89; H, 5.83; N, 1.58.

***O*-(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-*β*-D-mannopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-*α*-D-glucopyranose (30)**——Cleavage of the 1,6-anhydro-ring of **29** (28.3 mg, 3.28×10^{-5} mol) with acetolysis mixture (0.7 ml) was carried out as described for **22**. The resultant syrup was treated with CH_2Cl_2–hexane to give **30** as a white powder (24.5 mg, 77.4%), $[\alpha]_D^{19}$ +32.7° (c=0.11, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.95, 2.02, 2.09, 2.11, 2.15, 2.19 (33H, each s, OAc×10, NAc), 5.66 (1H, d, $J_{NH,2}$=9 Hz, NH), 6.11 (1H, d, $J_{1,2}$=4 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 99.3 ($^1J_{C-1'-H-1'}$=161.1 Hz, C-1′), 98.9 ($^1J_{C-1''-H-1''}$=170.9 Hz, C-1″), 90.5 ($^1J_{C-1-H-1}$=178.2 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3360 (NH), 1744 (OAc), 1682 (amide I), 1528 (amide II). TLC: *Rf* 0.20 (solvent B). *Anal.* Calcd for $C_{40}H_{55}NO_{26}$: C, 49.74; H, 5.74; N, 1.45. Found: C, 49.88; H, 6.24; N, 1.43.

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