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-a-d-glucopyranosyl bromide (9) and 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-b-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 Manß1→4GlcNAc (15). Benzoylation of 15, followed by deallylation, gave the Manß1→4GlcNAc derivative (18) having an unprotected hydroxyl group at the C-3' position. d-Mannosidase of 18 with acetobromomannose gave the protected Manα1→3Manß1→4GlcNAc (20) in 47.1% yield. Deprotection of 20, followed by acetylation, yielded 22.

2-O-acetyl-3,4,6-tri-O-benzyl-a-d-glucopyranosyl bromide (23) and 10 were coupled to give the protected Glcß1→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—acyetylated Manα1→3Manß1→4GlcNAc; acetylated Manα1→2Manß1→4GlcNAc; protected Glcß1→4GlcNAc; DMSO-Ac2O oxidation; protected Manß1→4GlcNAc; 1,6-anhydro sugar derivative; 1H-NMR; 13C-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→3Manß1→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→3Manß1→4GlcNAcβ1→4GlcNAc a-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 Gm1-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→2Manß1→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 (Manß1→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-β-glucopyranose prepared from 1,2: 5,6-di-O-isopropylidene-α-β-glucopyranose (3), was disopropylidenated 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-β-glucopyranose (4) as fine needles. In the proton nuclear magnetic resonance (1H-NMR) spectrum, the anomic 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-β-glucopyranose (5), according to a slight modification of the procedure described for the synthesis of the fully acetylated analog.8 Benzoylation 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-β-β-glucopyranose (6).

Benzoylation of 6 gave 3-O-allyl-1,2-di-O-benzoyl-4,6-di-O-benzyl-β-β-glucopyranose (7) as a syrup. In order to obtain crystalline di-β-acylates, anisoylation of 6 was carried out, but the resultant di-β-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-β-glucopyranosyl bromide (9), which was subjected to glucosidation.

![Chart 1](chart.png)

Condensation of one molar equivalent of 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-β-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-β-glucopyranosyl)-β-β-glucopyranose (12) in a yield of 63.7% with a small amount of 3-O-allyl-2-O-benzoyl-4,6-di-O-benzyl-β-β-glucopyranose.
pyranose (11). Treatment of 12 with alkali caused selective debenzylation 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 1H-NMR and carbon-13 nuclear magnetic resonance (13C-NMR) spectroscopies.

The unprotected hydroxyl group in 13 was then isomerized to α-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–Ac₂O) and, without purification, subsequent stereoselective 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, [α]D⁻³⁷ = -41.4°; 15, -48.5°) and different mobilities on thin-layer chromatography (TLC). In the 1H-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 13C-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 α-gluco to α-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 concomitant with tris(triphenylphosphine)rhodium chloride,12) and the resultant 1-propenyl ether was removed with acid10) to yield the deallylated product (18) in 43.2% yield. In the 1H-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 also be 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 protection of the prepared 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), 1H-NMR and 13C-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 acetylated 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 [α]D⁻¹⁰ = +20°. Jeanloz et al.8) reported the monohydrate of 22 to be an amorphous solid having mp 106—109°C and [α]D⁻¹⁰ = 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),13) was carried out by a modified Koenigs-Knorr reaction. After column chromatographic purification, the amino disaccharide derivative (24) was isolated with 39.2% yield. 13C-NMR spectroscopy confirmed the β-d-glucose 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–Ac₂O 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
13C-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 13C-NMR spectroscopy.
The final step is conversion of the protecting groups of 28 to acetyl groups. Hydrogenolytic debenzoylation followed by acetylation gave the fully acetylated 1,6-anhydro-β-trisaccharide (29). The 1,6-anhydro-β-linkage was then acetylated 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 13C-NMR spectroscopy.

The present work further confirms9,16 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 prepared 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. 1H- and 13C-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. 1R spectra were recorded with a JASCO IRA-2 or an A-102 spectrometer. TLC, PTC, and preparative-layer chromatography (PLC) were performed on pre-coated plates of Silica Gel 60 F254 0.25 mm thick, 0.5 mm thick, and TLC plates 2 mm thick (E. Merck), respectively. The following solvent combinations were used for TLC: (A), CHCl3-acetone (6:1); (B), CHCl3-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-H2SO4-ETOH at 125°C-10°C (B), 1% KMnO4 in 2% Na2CO3 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-glucopyranose, prepared from 1,2,5,6-di-O-isopropylidene-α-D-glucopyranose (3, 12 g, 46.1 mmol) by the method of Corbett and McKay,7 in 1.2% (w/v)aq H2SO4 (180 ml) was heated for reflux for 3 h. The solution was neutralized with BaCO3, filtered, and the filtrate was extracted with CHCl3 to remove by-products. The aqueous phase was concentrated to dryness, and the residue was acetylated by heating for 1.5 h with Ac2O (100 ml) and anhyd. AcONa (5 g). The mixture was poured into ice-H2O and extracted with CHCl3. The combined extracts were successively washed with H2O, ice-cold aq. NaHCO3, and H2O, then dried (MgSO4), 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, [x]D+4.7° (c=1.3, CHCl3). 1H-NMR (CDCl3): 2.08, 2.09, 2.10, 2.11 (12H, each s, OAc×4), 5.62–5.67 (1H, m, CH2=C(CH3)2), 5.72 (1H, d, J1,2=8 Hz, H-1). 1R max cm−1: 1735 (OAc). TLC: Rf 0.78 (solvent A). Anal. Calcd for C20H34O10: C, 52.58; H, 6.23. Found: C, 52.27; H, 6.19.

4,6-Di-O-acetyl-3-O-allyl-1,2,5,6-di-O-(1-ethoxyethylidene)-α-D-glucopyranose (5)——A stirred solution of 4 (3 g, 7.72 mmol) in CH2Cl2 (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 CH2Cl2, successively washed with H2O, ice-cold aq. NaHCO3, and H2O, then dried (MgSO4), 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 CH2Cl2, the solution was successively washed with H2O, ice-cold 1N H2SO4 and aq. NaHCO3, and H2O, then dried (MgSO4), and concentrated to a syrup (2.56 g, 88.6%). For analysis, the syrup was chromatographed on a column with CHCl3-acetone (5:1) to yield pure 5, [x]D+42.8° (c=0.32, CHCl3). 1H-NMR (CDCl3): 1.20 (3H, t, J=7 Hz, CH3CH2O-), 1.70 (3H, s, C2OEt), 2.09 (6H, s, OAc×2), 3.60 (2H, q, J=7 Hz, CH2CH2O-), 5.63–6.20 (1H, m, CH2=C(CH3)2), 5.74 (1H, d, J1,2=5 Hz, H-1). TLC: Rf 0.44 (solvent A). Anal. Calcd for C19H30O7: C, 54.54; H, 7.00. Found: C, 54.17; H, 6.88.

3-O-Allyl-4,6-di-O-benzyl-α-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 CHCl3. After filtration, the filtrate was successively washed with H2O, ice-cold 10% H2SO4, aq. NaHCO3 and H2O, and then concentrated to a syrup. The resultant benzyl ether in 1,4-dioxane-1N H2SO4 [4:1 (v/v), 150 ml] was heated under reflux for 4 h to hydrolyze it. After neutralization with solid NaHCO3, the mixture was concentrated to a syrup, which was dissolved in CHCl3. The solution was washed with H2O, dried (MgSO4), 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, [x]D+72.5° (c=0.8, CHCl3). 1H-NMR (CDCl3): 5.80–6.20 (1H, m, CH2=C(CH3)2), 7.31 (10H, s, PhCH2×2), 3.19–5.50 (17H, 15H (unresolved ring protons) and 2H (exchangeable with D2O)), IR νmax cm−1: 3330 (OH). TLC:
3-O-Allyl-1, 2-di-O-benzoyl-4, 6-di-O-benzyl-β-D-glucopyranosone (7) — Benzoil 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 H₂O (1 ml) to decompose excess benzoil chloride, the mixture was concentrated to a syrup. This was dissolved in CH₃Cl₂, washed with ice-cold dil. HCl, H₂O, ice-cold aq. NaHCO₃ solution, and H₂O, then dried (MgSO₄), and concentrated to a syrup. On column chromatography with hexane–ether (3: 1), 7 (242.7 mg, 77.6%) was obtained as a syrup, [α]D₂⁰ = +143° (c = 0.66, CHCl₃). ¹H-NMR (CDCl₃): 5.87 (1H, d, J₁₋₂ = 8 Hz, H-1), 7.00—7.62 (16H, m, PhCH₂×2 and meta and para to C=O of benzoyl ×2), 7.03, 7.04 (4H, each d, J = 8 Hz, aromatic protons ortho to C=O of benzoyl ×2). IR νmax cm⁻¹: 1725 (O=O). TLC: Rf 0.64 (solvent C). Anal. Calc. for Cₒ₇H₄₄O₈: 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-glucopyranosone (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 CHCl₃ and affording 8 (283 mg, 80%) as a pale yellow syrup, [α]D₂⁰ = +53.1° (c = 1.3, CHCl₃). ¹H-NMR (CDCl₃): 3.77 (6H, s, OCH₃×2), 5.96 (1H, d, J₁₋₂ = 8 Hz, H-1), 6.84, 6.86 (4H, each d, J = 9 Hz, aromatic protons ortho to OCH₃ of anisoyl ×2), 7.28, 7.31 (10H, each s, PhCH₂×2), 7.96 (4H, d, J = 9 Hz, aromatic protons ortho to C=O of anisoyl). IR νmax cm⁻¹: 1722 (C=O). TLC: Rf 0.31 (solvent C). Anal. Calc. for C₃₃H₃₉O₈: C, 70.05; H, 6.83. Found: C, 70.06; H, 5.88.

3-O-Allyl-2-O-benzoyl-4, 6-di-O-benzyl-α-L-naphthopyranosyl Bromide (9) — A mixture of 7 (3.5 g, 5.75 mmol) in dry CH₂Cl₂ (70 ml) with 30% (w/v) HBr-AcOH (18 ml) was stirred at 60°C for 40 min. After dilution with CH₂Cl₂, the mixture was washed with H₂O, ice-cold aq. NaHCO₃ solution, and H₂O, then dried (MgSO₄), 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-glucopyranosyl)-β-D-glucopyranosyl) (12) — A solution of 9 (2.94 g, 5.18 mmol) in benzene–nitrromethane (1: 1, 10 ml) was added to a suspension of 10⁻⁰ (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 CH₂Cl₂. This solution was successively washed with H₂O, satd. KI and NaHCO₃ solutions, and H₂O, then dried (MgSO₄), 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-β-n-glucopyranosyl (11) was isolated after removal of the solvent. Compound 11 was crystallized from CH₂Cl₂–hexane as white needles, mp 139—140°C, [α]D₂⁰ = +123.7° (c = 0.35, CHCl₃). Anal. Calc. for C₃₅H₃₅O₁₅: 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%), [α]D₂⁰ = -32.9° (c = 0.47, CHCl₃). ¹H-NMR (CDCl₃): 2.12 (3H, s, NAc), 6.44 (1H, d, J₉₋₁₀ = 10 Hz, NH), 7.06—8.22 (20H, m, aromatic protons). IR νmax cm⁻¹: 3360 (NH), 1716 (O=O), 1669 (amide I), 1509 (amide II). TLC: Rf 0.71 (solvent A). Anal. Calc. for C₅₃H₂₄NO₁₉·1/2H₂O: 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-glucopyranosyl) (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 decanted with Amberlite IR-120 (H⁺) resin, filtered, and concentrated to a syrup, which was chromatographed on a column with CH₂Cl₂–MeOH (100: 1). Removal of the solution from the major fractions provided 13 as a foamy solid (1.4 g, 76%), [α]D₂⁰ = -41.4° (c = 0.46, CHCl₃). ¹H-NMR (CDCl₃): 1.98 (3H, s, NAc), 2.92 (1H, s, OPh), 6.30 (1H, d, J₉₋₁₀ = 8 Hz, NH), 7.26, 7.29 (15H, each s, aromatic protons). ¹³C-NMR (CDCl₃): 102.1 (JC₁₋₁₋H₁ = 154.4 Hz, C-1'), 100.5 (JC₁₋₁₋H₁ = 173.3 Hz, C-1). IR νmax cm⁻¹: 3380 (OH, NH), 1650 (amide I), 1522 (amide II). TLC: Rf 0.46 (solvent B). Anal. Calc. for C₃₅H₂₄NO₁₉·1/2H₂O: 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-glucopyranosyl) (14) — A mixture of 13 (38.9 mg, 5.25×10⁻⁴ mol) in Ac₂O (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, [α]D₂⁰ = -101.5° (c = 0.13, CHCl₃). ¹H-NMR (CDCl₃): 2.09, 2.16 (6H, each s, OAc, NAc), 6.33 (1H, d, J₉₋₁₀ = 10 Hz, NH), 7.12—7.48 (15H, m, aromatic protons). IR νmax cm⁻¹: 3375 (NH), 1738 (OAc). TLC: Rf 0.63 (solvent A). Anal. Calc. for C₅₃H₂₄NO₁₉·1/2H₂O: 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-glucopyranosyl) (15) — A solution of 13 (233.7 mg, 3.33×10⁻⁴ mol) in DMSO–Ac₂O (2: 1, v/v, 6 ml) was stirred for 48 h at room temperature. After dilution with CHCl₃, the whole was washed with H₂O, dried (MgSO₄), and concentrated to a syrup by repeated co-distillation with toluene.

A mixture of this syrup and NaBH₄ (200 mg) in CH₂Cl₂–MeOH (1: 1, 6 ml) was stirred for 4 h at room temperature, and then diluted with CH₂Cl₂, which was successively washed with H₂O, ice-cold 10%
citric acid and satd. NaHCO₃ solutions, and H₂O, dried (MgSO₄), and concentrated to a syrup. This was chromatographed on a column with CHCl₃-MeOH (100: 1) to provide 15 (157.1 mg, 58.6% ν) as a foamy solid, [α]D₂0 = 48.5° (c = 0.41, CHCl₃). ¹H-NMR (CDCl₃): 1.99 (3H, s, NAc), 2.38 (1H, s, OQ), 5.48 (1H, s, H-1), 6.48 (1H, d, J₉H₈ = 10 Hz, NH), 7.36, 7.41 (15H, each s, aromatic protons). ¹³C-NMR (CDCl₃): 100.6 (J₁⁻C₃-H₃ = 157.5 Hz, C-1), 99.3 (J₁⁻C₃-H₃ = 156.6 Hz, C-1'). IR νmax cm⁻¹: 3380 (NH, OH), 1552 (amide II). TLC: Rf 0.31 (solvent B). Anal. Calcd for C₇₆H₅₂N₀₁₉: 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-0-benzyl-p-n-mannopyranosyl)-β-D-glucopyranose (16)—Acetylation of 15 (30.4 mg, 4.33 × 10⁻² mol) with Ac₂O (0.5 ml) and pyridine (1 ml) as described for 14 provided 16 (31 mg, 99.5% ν) as a syrup, [α]D₂0 = 100° (c = 0.12, CHCl₃). ¹H-NMR (CDCl₃): 2.07, 2.17 (6H, each s, OAc, NAc), 6.18 (1H, d, J₉H₈ = 10 Hz, NH), 7.26, 7.31 (15H, each s, aromatic protons). IR νmax cm⁻¹: 3370 (NH), 1730 (OAc). TLC: Rf 0.49 (solvent A). Anal. Calcd for C₈₄H₆₄NO₃₄: 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-p-D-mannopyranosyl)-β-D-gluco- pyranose (17)—Benzyl bromide (2 ml) was added to a mixture of 15 (0.64 g, 9.11 × 10⁻⁴ mol), powdered Ba(OH)₂ (1.7 g), and Ba(OH)₂·8H₂O (0.7 g), suspended in dry DMSO (30 ml). The mixture was stirred at 50°C for 48 h, cooled, diluted with CHCl₃, and filtered. The filtrate was successively washed with ice-cold dil. HCl, H₂O, ice-cold NaHCO₃ solution, and H₂O, dried (MgSO₄), 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 TLC with toluene-acetone (4: 1). A zone having Rf 0.38 was excluded from the plates and extracted with CHCl₃-MeOH (9: 1) to isolate 17 (10.4 g, 65.9% ν) as a syrup, [α]D₂0 = 87° (c = 0.04, CHCl₃). ¹H-NMR (CDCl₃): 1.61 (3H, s, NAc), 6.05 (1H, d, J₉H₆ = 9 Hz, NH), 7.22 (20H, s, aromatic protons). ¹³C-NMR (CDCl₃): 100.6 (C-1, C-1'). IR νmax cm⁻¹: 3380 (NH, OH), 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-p-D-mannopyranosyl)-β-D-glucopyranose (18)—A Dealllylation with Pd-catalyst: A mixture of 17 (56.4 mg, 7.36 × 10⁻⁴ mol) and 10% Pd-on-charcoal (60 mg) suspended in EtOH-AcOH-H₂O (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 CHCl₃-MeOH (9: 1) to isolate 18 (19.1 mg, 35.7% ν) as a foamy solid, [α]D₂0 = -85.9° (c = 0.17, CHCl₃). ¹H-NMR (CDCl₃): 1.70 (3H, s, NAc), 2.28 (1H, s, OQ), 6.07 (1H, d, J₉H₈ = 10 Hz, NH), 7.33 (20H, s, aromatic protons). ¹³C-NMR (CDCl₃): 100.7 (J₁⁻C₃-H₃ = 151.8 Hz, C-1'), 100.6 (J₁⁻C₃-H₃ = 171.5Hz, C-1). IR νmax cm⁻¹: 3400 (NH, OH), 1664 (amide I), 1515 (amide II). TLC: Rf 0.63 (solvent B). Anal. Calcd for C₉₆H₇₂N₀₁₉: 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 × 10⁻³ mol), tris(triphenylphosphine) rhodium chloride (22 mg), and 1,4-diazabicyclo[2.2.2]octane (33 mg) dissolved in EtOH–benzene–H₂O (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% ν), which was indistinguishable from 18 by TLC, IR, and NMR comparisons.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(2,4,6-tri-O-benzyl-p-D-mannopyranosyl)-(1-3)-O-(2,4,6-tri-O-benzyl-p-D-mannopyranosyl)-(1-4)-2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(2-O-acetyl-3-O-allyl-4,6-di-0-benzyl-p-D-mannopyranosyl)-β-D-gluco- pyranose (20)—A solution of 19 (250 mg, 6.08 × 10⁻³ mol) in dry benzene (1.5 ml) was added to a suspension of 18 (43.3 mg, 5.97 × 10⁻⁴ mol), Hg(CH₃CN)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 CHCl₃, and filtered, and the filtrate was treated as described in the case of 12 to separate the component to a syrup. The resultant syrup in dry MeOH (4 ml) was de-O-acetylated with a 0.5% methanolic solution of MeONa (0.2 ml), and the crude deacylated product was purified by PTLC with CHCl₃-acetone (1: 2). The band having Rf 0.17 was removed from the plates, extracted with CHCl₃-MeOH (1: 1), and concentrated to a syrup.

The syrup was re-acetylated with Ac₂O (1 ml) and pyridine (2 ml) for 24 h at room temperature, then concentrated to dryness. The residue was purified by PTLC with CHCl₃-acetone (6: 1). From the band having Rf 0.44, 20 (29.7 mg, 47.1% ν) was isolated as a foamy solid, [α]D₂0 = 27.8° (c = 0.22, CHCl₃). ¹H-NMR (CDCl₃): 1.68 (3H, s, NAc), 2.02, 2.04, 2.07, 2.08 (12H, each s, OAc × 4), 6.09 (1H, d, J₉H₈ = 9 Hz, NH), 7.31 (20H, s, aromatic protons). ¹³C-NMR (CDCl₃): 101.0 (J₁⁻C₃-H₃ = 156.3 Hz, C-1'), 100.7 (J₁⁻C₃-H₃ = 170.9 Hz, C-1), 99.6 (J₁⁻C₃-H₃ = 157.8 Hz, C-1'). IR νmax cm⁻¹: 3410 (NH), 1750 (OAc), 1674 (amide I), 1511 (amide II). TLC: Rf 0.44 (solvent A). Anal. Calcd for C₉₈H₇₄N₀₂₁: C, 63.69; H, 6.20; N, 1.33. Found: C, 63.50; H, 6.35; N, 1.35.
temperature. The mixture was concentrated to dryness, and the residue was purified by PTLC with CHCl₃-acetone (3:1). From the band having Rf 0.25, 21 was isolated as a foamy solid. For analysis, the product was recrystallized from CHCl₃-hexane as a white powder (23.4 mg, 66.9%, \(\text{x}_{290} = 10^2\) c = 0.1, CHCl₃). ²H-NMR (CDCl₃): 1.99, 2.02, 2.06, 2.10, 2.14, 2.27 (27H, each s, OAc × 8, NAc); 6.14 (1H, d, \(J_{\text{H-N}} = 9\) Hz, NH). ³C-NMR (CDCl₃): 100.4 (\(\text{J}_\text{C=H} = 157.8\) Hz, C-1), 90.2 (\(\text{J}_\text{C-O} = 175.8\) Hz, C-1'), 86.5 (\(\text{J}_\text{C-O} = 158.7\) Hz, C-1'). IR: 3410 cm⁻¹ (NH), 1746 (OAc), 1676 (amide I), 1516 (amide II). TLC: Rf 0.25 (solvent B). Anal. Calc. for C₇₅H₅₈NO₃₂: 1/2H₂O: 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-a-d-mannopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-d-mannopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-O-acetyl-β-d-p-glucopyranosyl-(2→)-D-glucose (22) — A solution of 21 (26 mg, 2.98 × 10⁻³ mol) in an ice-cooled acetylation reagent [boron trifluoride etherate-Ac₂O (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 acetylation reagent. The solution was then diluted with CHCl₃, and neutralized with solid NaHCO₃. The separated organic layer was washed with H₂O, dried (MgSO₄), and concentrated to dryness. The resultant syrup was treated with CHCl₃-hexane to yield 22 (20.2 mg, 70.1%, as a white powder, \(\text{x}_{290} = 20^2\) c = 0.14, CHCl₃). ²H-NMR (CDCl₃): 1.93, 1.98, 2.05, 2.09, 2.11, 2.19 (33H, each s, OAc × 10, NAc); 6.12 (d, \(J_{\text{H,N}} = 4\) Hz, H-1). ³C-NMR (CDCl₃): 97.9 (C-1, β), 90.6 (C-1', β). IR: 3380 cm⁻¹ (NH), 1746 (OAc), 1684 (amide I), 1526 (amide II). TLC: Rf 0.29 and 0.22 (amonic mixture, solvent B). Anal. Calc. for C₁₉₂H₁₀₈NO₃₈: 1/2H₂O: C, 49.28; H, 5.79; N, 1.44. Found: C, 49.25; H, 5.82; N, 1.44. lit. amorphous (monohydrate), mp 109—110°C (c = 2.3, 5:1 CHCl₃-MeOH), 1H-NMR (CDCl₃): 6.1 (d, 8 H, H-1β).
charide was isolated.

The product was then re-acetylated with Ac₂O (1 ml) and pyridine (2 ml), and the resultant acetate was purified by PTLC with CHCl₃-acetone (6:1). From the band having Rf 0.36, pure 28 was isolated as a foamy solid (65.8 mg, 40.7%, [α]D⁰ -29 (c = 0.2, CHCl₃). ¹H-NMR (CDCl₃): 1.90, 2.07, 2.09 (15H, all s, OAc×4, NAc), 6.16 (1H, d, J=18Hz, H₄), 7.25, 7.27 (2OH, each s, arom protons). ¹³C-NMR (CDCl₃): 100.9 (J₆,₇-H=155.8Hz, C-1′), 100.5 (J₆,₇-H=175.8Hz, C-1), 98.1 (J₆,₇-H=173.3Hz, C-1′). IR νmax cm⁻¹: 3410 (NH), 1750 (OAc), 1673 (amide I), 1511 (amide II). TLC: Rf 0.36 (solvent A). Anal. Calcd for C₂₉H₄₈NO₂₅: 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-glucopyranosyl)-(1→4)-2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (29) — Catalytic debenzylation of 28 (57.4 mg, 5.43×10⁻³ mol) in glacial AcOH (3 ml) with 10% Pd-on-charcoal (50 mg) and subsequent acetylation of the resultant debenzylated product with Ac₂O (1 ml) and pyridine (2 ml) were carried out as described for 14. The crude acetate was column-chromatographed with CHCl₃-ether-MeOH (30:5:1). From the fractions having Rf 0.21 with CHCl₃-acetone (3:1), 29 was isolated as a syrup. For analysis, the syrup was treated with CH₂Cl₂-hexane to yield a white powder (35.3 mg, 75.3%, [α]D⁰ -39.8° (c = 0.22, CHCl₃). ¹H-NMR (CDCl₃): 2.02, 2.05, 2.09, 2.16 (27H, each s, OAc×8, NAc), 6.12 (1H, d, J=18Hz, H₄), 100.2 (J₆,₇-H=173.3Hz, C-1), 97.6 (J₆,₇-H=173.3Hz, C-1′). IR νmax cm⁻¹: 3420 (NH), 1745 (OAc), 1679 (amide I), 1516 (amide II). TLC: Rf 0.21 (solvent B). Anal. Calcd for C₅₀H₇₆NO₃₂: C, 50.06; H, 5.72; N, 1.62. Found: C, 49.89; H, 5.63; 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-glucopyranosyl)-(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, 3.28×10⁻³ mol) with acetic acid mixture (0.7 ml) was carried out as described for 22. The resultant syrup was treated with CH₂Cl₂-hexane to give 30 as a white powder (24.5 mg, 72.4%, [α]D⁰ +32.7° (c = 0.11, CHCl₃). ¹H-NMR (CDCl₃): 1.95, 2.02, 2.09, 2.11, 2.15, 2.19 (33H, each s, OAc×10, NAc), 5.60 (1H, d, J=18Hz, H₄), 6.11 (1H, d, J=4Hz, H-1). ¹³C-NMR (CDCl₃): 99.3 (J₆,₇-H=161.1Hz, C-1), 98.9 (J₆,₇-H=170.9Hz, C-1′), 90.5 (J₆,₇-H=178.2Hz, C-1). IR νmax cm⁻¹: 3360 (NH), 1744 (OAc), 1682 (amide I), 1528 (amide II). TLC: Rf 0.20 (solvent B). Anal. Calcd for C₄₀H₆₄NO₃₂: C, 49.74; H, 5.74; N, 1.45. Found: C, 49.88; H, 6.24; N, 1.43.

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References and Notes