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Note

Synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-β-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose. Acceptor–substrate recognition by *N*-acetylglucosaminyltransferase-V (GnT-V) ☆

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The enzyme UDP–Glc*p*NAc: α-D-mannopyranosyl-(1 → 6)-*N*-acetyl-β-D-glucosaminyltransferase (Glc*p*NAc–transferase V or GnT-V, EC 2.4.1.155) catalyzes the transfer of a β-D-Glc*p*NAc residue to O-6 of the (1 → 6)-linked α-D-mannosyl residue that forms the part of the trimannosyl core of oligosaccharides having structure **1** (Fig. 1) [2,3]. Recent years have seen a great surge of interest in GnT-V because of its increased activity in cells transformed by viruses [4,5] or by oncogenes [6] and its involvement in cancer metastasis [7–9]. More recently interest became enhanced when this enzyme was shown to be substantially elevated in malignant human breast cancer biopsies [10]. This enzyme has therefore become an attractive target for the synthesis of glycosyltransferase inhibitors which might have the potential to prevent metastasis [11–18].

☆ Synthetic studies in Carbohydrates, part 98. For part 97, see ref. [1].

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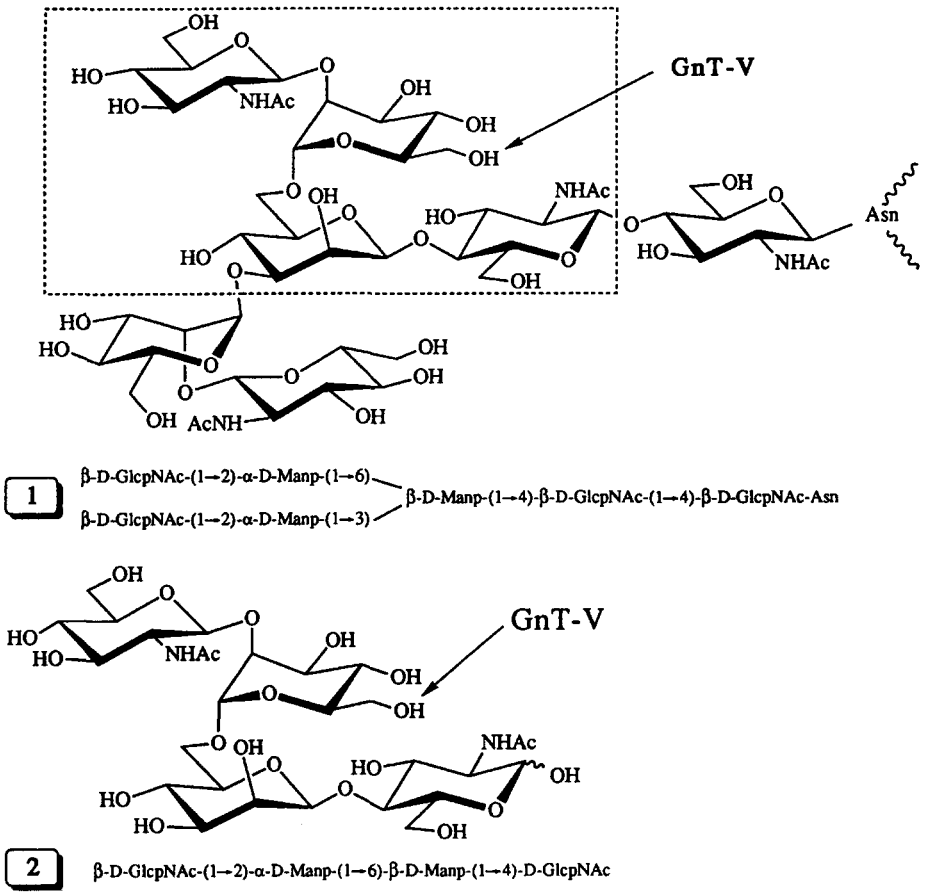
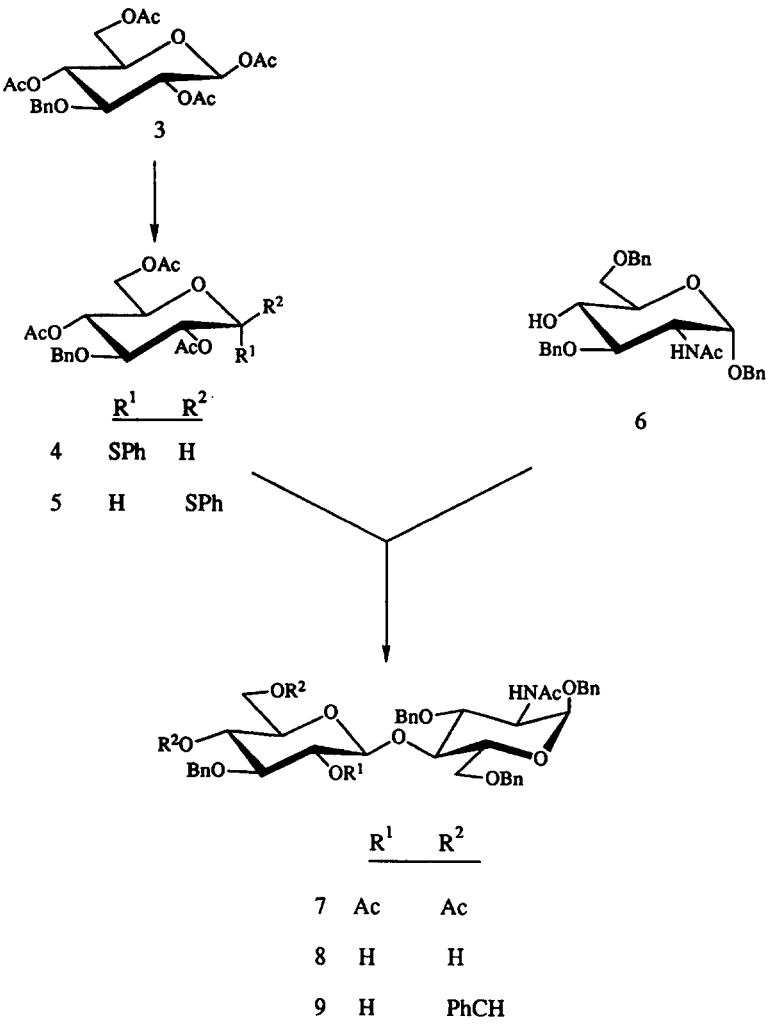


Fig. 1. Natural heptasaccharide acceptor **1** for GnT-V compared with the potential synthetic acceptor **2**.

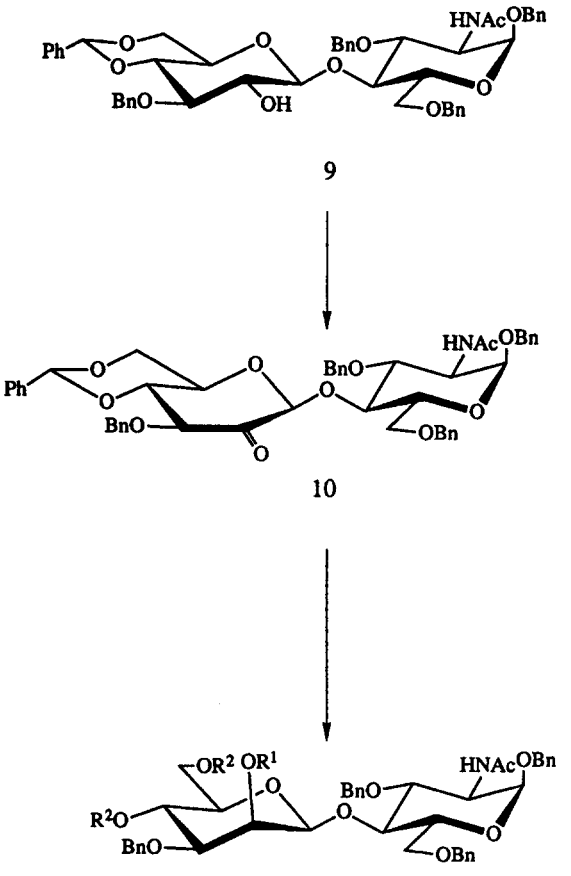
Pioneering work by Tahir and Hindsgaul [19] has established that oligosaccharides much smaller than **1** can be effectively used to assay the activity of this enzyme [20,21]. Since then, this enzyme has been the subject of extensive investigations [22–24], and several laboratories including our own have reported the synthesis of various acceptor analogs in order to probe the enzyme's acceptor binding site [25–33]. Thus, in a continuing effort to shed more light on the substrate specificity of GnT-V, we describe herein the synthesis of title tetrasaccharide **2**, which represents a part of the natural heptasaccharide acceptor (see structure **1**, Fig. 1). It should be pointed out that compound **2** also proved useful [34] in specificity studies of β-(1 → 4)-*N*-acetylglucosaminyltransferase (GnT-VI′) acting on the α-3 and α-6 arms of *N*-linked oligosaccharides.

A retrosynthetic analysis of the target molecule **2** suggested, as the key intermediates, the disaccharide donor **14** and disaccharide acceptor **13**. (See Schemes 1–3.) The synthesis of donor **14** has already been reported [29,31]. Therefore, a synthetic route



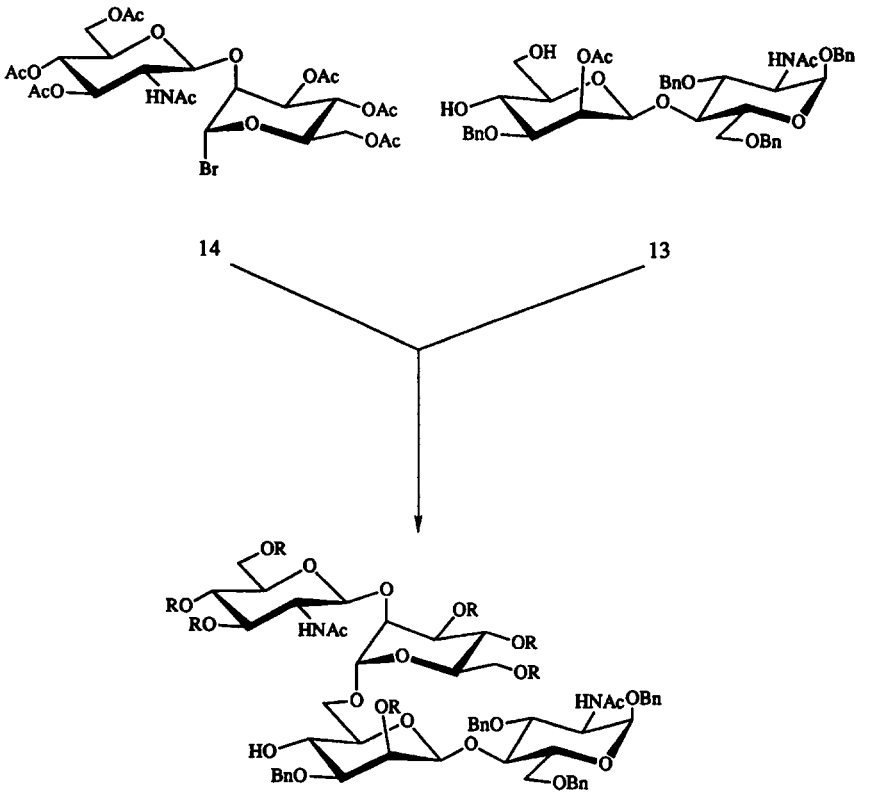
Scheme 1.

towards **13** was sought. The synthesis of **13** entailed two key problems; (*i*) the glycosylation of **6** at the C-4 hydroxyl which is known [35,36] to be a very unreactive position, and (*ii*) the formation of a β-D-mannopyranosyl linkage. The problem of synthesizing a β-(1 → 4)-linkage was circumvented by the use of methods developed by Fraser-Reid et al. [37] and van Boom et al. [38] which give respectable yields of the (1 → 4)-*trans* disaccharide. For the synthesis of a β-D-Man residue we employed a route that involves an oxidation–reduction sequence at O-2 of the corresponding β-D-Glc*p* derivative resulting in epimerization at C-2 [39]. Thus, the preparation of **12** was achieved as follows. The condensation of an α,β mixture of thioglycoside **4/5** with alcohol **6** [40] in the presence of iodonium ion (NIS–TfOH) gave the fully protected



Scheme 2.

disaccharide **7** (53%), and subsequent *O*-deacetylation of **7** gave **8** (93%). Thioglycoside **4/5** was readily prepared in 76% yield by treating its precursor 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose (**3**) [41] with phenyl thiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate according to a literature procedure [42]. Benzylidenation of **8** with α,α-dimethoxytoluene in *N,N*-dimethylformamide and in the presence of 4-toluenesulfonic acid afforded the 4,6-*O*-benzylidene derivative **9** (84%)



Scheme 3.

which was oxidized by the procedure of Albright and Goldman [43] to give benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-*arabino*-hexopyranosyl-2-ulose-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**10**, 97%). Stereoselective reduction of **10** by the action of sodium borohydride gave **11** (66%), together with a small proportion of the D-glucose derivative **9** (11%), which was removed by column chromatography and recycled. Although various excellent methods for the synthesis of β-D-mannopyranosides have been reported [44–54] over the years, the method we applied here still remains one of the few practical methods of choice [39,55–61].

Acetylation of **11** (2:1 Py–Ac_2O) into the corresponding *O*-acetate **12** (98%), followed by cleavage of the benzylidine acetal group with hot, 60% aq acetic acid gave benzyl 2-*O*-acetyl-3-*O*-benzyl-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**13**) (82%). Regioselective glycosylation of **13** with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl bromide (**14**) [26,28] under Helferich-type conditions afforded the partially protected tetrasaccharide **15** (56%). Zemplén transesterification of **15** gave **16** (63%) which was subjected to hydrogenolysis in glacial acetic acid and in the presence of 10% Pd–C to afford the title tetrasaccharide **2** (70%) as a white amorphous powder.

Preliminary evaluation [62] of tetrasaccharide **2** as acceptor for GlcNAcT-V shows that it is very poorly recognized by the enzyme.

1. Experimental

General methods.—Optical rotations were measured at 22 ± 2° with a Perkin–Elmer 241 polarimeter. TLC was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-254 (E. Merck); the compounds were located by UV light and/or by charring with 5% sulfuric acid. Column chromatography was performed on silica gel (Baker Analyzed, 60–200 mesh). The following solvent systems (v/v) were used for chromatography: A, 2:1 hexane–ethyl acetate; B, 1:1 hexane–ethyl acetate; C, 24:1 chloroform–methanol; D, 2:1 toluene–ethyl acetate; E, 97:3 dichloromethane–methanol; F, 19:1 dichloromethane–methanol; G, 9:1 chloroform–methanol; H, 26:12:1 chloroform–methanol–water; I, 13:6:1 chloroform–methanol–water; J, 10:9:1 chloroform–methanol–water; K, 5:4:1 chloroform–methanol–water. IR spectra were recorded with a Nicolet 20 SX FTIR spectrometer using thin film on NaCl plates. ^1H NMR spectra were recorded at 300 MHz (Bruker AM 300) for solutions in $CDCl_3$ or CD_3OD (internal Me_4Si, δ 0) or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM 300) for solutions in $CDCl_3$ or CD_3OD (internal Me_4Si, δ 0) or D_2O (external 1% 1,4-dioxane in D_2O, δ 67.4). Only partial NMR data are reported, and the assignments of ^{13}C chemical shifts are tentative. Fast atom bombardment mass spectra (FABMS) were obtained using an AEI MS-9 instrument with xenon as the bombarding gas and 5:1 1,4-dithiothreitol–1,4-dithioerythritol as matrix. Unless otherwise indicated, all reactions were carried out at ambient temperatures. Solutions were dried with Na_2SO_4 and concentrated at 40–50°C/2 kPa. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, NJ 08940.

Phenyl 2,4,6-tri-O-acetyl-3-O-benzyl-1-thio-α- (**4**) *and -β-D-glucopyranoside* (**5**).—To a cold (0°C bath), stirred solution of **3** (5 g, 11.4 mmol) in dry dichloroethane (25 mL) were added trimethylsilyl trifluoromethanesulfonate (5.29 mL, 27.35 mmol) and phenyl thiomethylsilane (6.48 mL, 34.2 mmol). After stirring at 0°C for 4 h, the mixture was allowed to warm to room temperature, and stirring was continued overnight. The mixture was then diluted with dichloromethane (100 mL), successively washed with water, satd $NaHCO_3$, and water, dried and concentrated to a syrup that was chromatographed (5 → 20% ethyl acetate in hexane). Evaporations of earlier fractions gave a solid residue that was dissolved in ethyl acetate. Addition of ether–petroleum ether

caused the precipitation of **4** (0.56 g, 10%) as an amorphous solid: $[\alpha]_D$ +173.5° (*c* 1.4, chloroform); TLC (solvent A): R_f 0.44; ^{1}H NMR ($CDCl_3$): δ 7.48–7.24 (m, 10 H, Ar), 5.92 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1), 5.14–5.05 (m, 2 H, H-2, H-4), 4.77, 4.65 (2d, 1 H each, J_{gem} 11.5 Hz, PhCH_2), 4.5–4.41 (m, 1 H, H-5), 4.22 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12 Hz, H-6a), 4.02 (dd, 1 H, $J_{5,6a}$ 2.25, $J_{6a,6b}$ 12 Hz, H-6b), 3.92 (t, 1 H, $J_{2,3} = J_{4,5} = 9.5$ Hz, H-3), 2.1, 2.02, and 1.96 (s, 3 H each, 3 OAc); ^{13}C NMR ($CDCl_3$): δ 170.64, 169.77, 169.47 (*C*OCH$_3$), 85.24 (C-1), 77.83 (C-3), 75.06 (PhCH$_2$O), 73.26, 69.73, and 69.69 (C-2,4,5), 62.23 (C-6), 20.89, 20.74, and 20.70 (CO*C*H$_3$). Anal. Calcd for $C_{25}H_{28}O_8S$ (488.56): C, 61.46; H, 5.78. Found: C, 61.55; H, 5.86.

Continued elution of the column and evaporation of solvent gave mixed fractions (**4** and **5**, 1.43 g, 25.7%), followed by a pure solid residue that was dissolved in ethyl acetate. Addition of ether–petroleum ether caused the precipitation of **5** (2.23 g, 40%) as an amorphous solid: $[\alpha]_D$ −18.2° (*c* 1.3, chloroform); TLC (solvent A): R_f 0.33; ^{1}H NMR ($CDCl_3$): δ 7.54–7.17 (m, 10 H, Ar), 5.12–5.01 (m, 2 H, H-2, H-4), 4.64 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.6, 4.57 (2d, 1 H each, J_{gem} 11 Hz, PhCH_2), 4.16 (m, 2 H, H-6a, H-6b), 3.72 (t, 1 H, $J_{2,3} = J_{4,5} = 9.5$ Hz, H-3), 3.68–3.59 (m, 1 H, H-5), 2.08, 2.03, and 1.96 (s, 3 H each, 3 OAc); ^{13}C NMR ($CDCl_3$): δ 170.67, 169.33, 169.22 (*C*OCH$_3$), 86.26 (C-1), 81.56 (C-3), 74.24 (PhCH$_2$O), 76.14, 71.39, and 69.67 (C-2,4,5), 62.59 (C-6), 20.97, and 20.77 (2C) (CO*C*H$_3$). Anal. Calcd for $C_{25}H_{28}O_8S$ (488.56): C, 61.46; H, 5.78. Found: C, 61.66; H, 5.84.

*Benzyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**7**).—A mixture of thioglycosides **4** and **5** (2.98 g, 6.1 mmol) and alcohol **6** (2 g, 4.1 mmol) was dissolved in dry dichloromethane (21 mL). Pulverized activated molecular sieves (4 Å, 1.5 g) and *N*-iodosuccinimide (2.27 g, 10.1 mmol) were added, and the mixture was stirred for 30 min in the dark in an atmosphere of argon. The mixture was then cooled (0°C bath) and a solution of trifluoromethanesulfonic acid (42 μL) in dichloromethane (42 mL) was added dropwise, and the stirring was continued for 1.5 h. It was then diluted with dichloromethane (200 mL), and the solids were filtered off (Celite bed) and washed with dichloromethane. The filtrate and washings were combined, successively washed with water, aq $NaHCO_3$, aq $Na_2S_2O_3$, and water, dried and concentrated to dryness. Evaporation of the solvent and purification of the residue by chromatography (15 → 50% ethyl acetate in hexane) gave first unchanged **6** (0.52 g). Continued elution of the column and concentration of the fractions corresponding to the product gave a solid that was dissolved in a little methanol. Addition of ether–hexane caused the precipitation of **7** (1.85 g, 52.3%) as an amorphous solid: $[\alpha]_D$ +64° (*c* 1.7, chloroform); TLC (solvent B): R_f 0.1; ^{1}H NMR ($CDCl_3$): δ 7.43–7.19 (m, 20 H, Ar), 4.92 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.97, 1.94, 1.92 (s, 3 H each, 3 OAc), and 1.73 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 170.74, 169.73, 169.29, 168.97 (*C*OCH$_3$), 100.45. (C-1′), 97.08 (C-1), 80.43 (C-4), 74.06, 73.86, 73.66, 69.93 (PhCH$_2$O), 67.61 (C-6), 61.98 (C-6′), 52.26 (C-2), and 23.25, 20.91, 20.77, 20.67 (CO*C*H$_3$). Anal. Calcd for $C_{48}H_{55}NO_{14}$ (869.97): C, 66.27; H, 6.37; N, 1.61. Found: C, 66.55; H, 6.76; N, 1.91.

*Benzyl 3-*O*-benzyl-β-*D*-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**8**).—Compound **7** (1.51 g, 1.74 mmol) was taken up in 0.1 M methanolic sodium methoxide (33 mL) and stirred overnight at room temperature.

The base was neutralized by Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite bed), thoroughly washed with methanol, and the filtrate and washings were combined and concentrated to give **8** (1.2 g, 93%) as an amorphous solid: $[\alpha]_D$ +80° (*c* 0.7, chloroform); TLC (solvent C): R_f 0.56; ^{1}H NMR ($CDCl_3$): δ 7.40–7.20 (m, 20 H, Ar), 5.32 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.90 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.28 (dt, $J_{2,3}$ 10.0 Hz, H-2), and 1.80 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 169.80 ($COCH_3$), 102.64 (C-1′), 97.20 (C-1), 74.72, 74.23, 73.79, 69.92 ($PhCH_2O$), 68.43 (C-6), 62.41 (C-6′), 52.38 (C-2), and 23.36 ($COCH_3$). Anal. Calcd for $C_{42}H_{49}NO_{11}$ (743.86): C, 67.82; H, 6.64; N, 1.88. Found: C, 67.85; H, 6.76; N, 1.91.

Benzyl 3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**9**).—To a stirred solution of **8** (1.5 g, 2.02 mmol) in *N,N*-dimethylformamide (35 mL) were added 4-toluenesulfonic acid (50 mg, 0.26 mmol) and α,α-dimethoxytoluene (1.52 mL, 10.12 mmol), and the stirring was continued overnight at room temperature. The acid was then neutralized with a little triethylamine, and the solution was concentrated to a syrup that was dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of **9** (1.4 g, 83.5%): $[\alpha]_D$ +74° (*c* 1, chloroform); TLC (solvent D): R_f 0.2; IR (neat): ν_{max} 3465 (OH), 3320 (NH), 1648, 1550 (NCO), 733, and 696 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$): δ 7.50–7.3 (m, 25 H, Ar), 5.46 (s, 1 H, PhC*H*), 5.30 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.91 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.27 (dt, $J_{2,3}$ 10.0 Hz, H-2), and 1.79 (s, 3 H, NAc); ^{13}C NMR: ($CDCl_3$): δ 169.72 ($COCH_3$), 103.42 ($PhCH_2O_2$), 101.25 (C-1′), 97.25 (C-1), 74.59 (C-2′), 74.21, 73.64, 70.89, 69.89 ($C_6H_5CH_2O$), 68.70, 68.22 (C-6,6′), 52.40 (C-2), and 23.38 ($COCH_3$). Anal. Calcd for $C_{49}H_{53}NO_{11}$ (831.97): C, 70.74; H, 6.42; N, 1.68. Found: C, 70.89; H, 6.55; N, 1.71.

Benzyl 3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**11**).—A solution of **9** (1.35 g, 1.62 mmol) in 1:2 acetic anhydride–dimethyl sulfoxide (45 mL) was stirred overnight at room temperature. The solvents were evaporated and the residue was chromatographed (solvent E) to give **10** as a white solid (1.3 g, 96.5%): $[\alpha]_D$ +69° (*c* 1.8, chloroform); TLC (solvent E): R_f 0.33; IR (neat): ν_{max} 3308 (NH), 1745 (C=O), 1648, 1545 (NCO), 735, and 696 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$): δ 7.52–7.15 (m, 25 H, Ar), 5.45 (s, 1 H, PhC*H*), 5.31 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.91 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.27 (dt, $J_{2,3}$ 10.0 Hz, H-2), and 1.79 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 197.22 (C-2′ as carbonyl), 169.71 ($COCH_3$), 101.95 ($PhCH_2O_2$), 101.06 (C-1′), 97.26 (C-1), 74.73, 73.47, 73.31, 69.97 ($PhCH_2O$), 68.46, 68.11 (C-6,6′), 52.46 (C-2′), 23.36 ($COCH_3$).

A solution of **10** (1.2 g, 1.45 mmol) in 1:1 dichloromethane–methanol (94 mL) was treated with sodium borohydride (0.62 g, 16.4 mmol) for 4 h at room temperature. The mixture was diluted with chloroform (150 mL), and successively washed with water, 5% citric acid solution, aq $KHCO_3$, and water. Evaporation of the solvent and purification of the residue by chromatography (solvent B) first gave **9** (0.13 g, 10.8%), followed by **11** as a white solid (0.79 g, 65.7%): $[\alpha]_D$ +62° (*c* 1, chloroform); TLC (solvent D): R_f 0.13; IR (neat): ν_{max} 3468 (OH), 3298 (NH), 1651, 1553 (NCO), 731, and 695 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$): δ 7.52–7.18 (m, 25 H, Ar), 5.51 (s, 1 H, PhC*H*), 5.35 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.95 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.59 (br s, 1 H, H-1′), 4.29 (dt, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 3.96 (br t, 1 H, $J_{2',3'}$ 2.8 Hz, H-2′), 3.42 (dd, $J_{3',4'}$ 9.5 Hz, H-3′), and

1.84 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 169.74 ($COCH_3$), 101.53 ($C_6H_5CH_2O_2$), 100.77 (C-1′), 97.31 (C-1), 69.54 (C-2′), 74.39, 73.65, 72.46, 69.95 ($C_6H_5CH_2O$), 68.55, 68.40 (C-6,6′), 52.38 (C-2), and 23.38 ($COCH_3$). Anal. Calcd for $C_{49}H_{53}NO_{11}$ (831.97): C, 70.74; H, 6.42; N, 1.68. Found: C, 70.80; H, 6.39; N, 1.70.

Benzyl 2-O-acetyl-3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**12**).—Compound **11** (0.6 g, 0.72 mmol) was dissolved in a mixture of acetic anhydride (5 mL) and pyridine (10 mL) and stirred overnight at room temperature. Acetic anhydride and pyridine were evaporated under diminished pressure, and the residue was chromatographed (solvent B) to afford **11** (0.62 g, 98%) as an amorphous solid: $[\alpha]_D$ +42° (*c* 1, chloroform); TLC (solvent D): R_f 0.32; ^{1}H NMR ($CDCl_3$): δ 7.54–7.14 (m, 25 H, Ar), 5.52 (s, 1 H, PhC*H*), 5.42 (dd, 1 H, $J_{1',2'}$ 1, $J_{2',3'}$ 3.5 Hz, H-2′), 5.28 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.94 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.57 (br s, 1 H, H-1′), 3.43 (dd, 1 H, $J_{3',4'}$ 9.8 Hz, H-3′), 2.18 (s, 3 H, OAc) and 1.82 (s, 3 H, NAc); ^{13}C NMR: ($CDCl_3$): δ 170.31, 169.63 ($COCH_3$), 101.48 ($PhCH_2O_2$), 99.42 (C-1′), 97.16 (C-1), 74.25, 73.52, 71.64, 69.92 ($PhCH_2O$), 68.44, 68.28 (C-6,6′), 52.32 (C-2), and 23.32, 21.11 ($COCH_3$). Anal. Calcd for $C_{51}H_{55}NO_{12}$ (874.01): C, 70.09; H, 6.34; N, 1.60. Found: C, 70.00; H, 6.30; N, 1.56.

Benzyl 2-O-acetyl-3-O-benzyl-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**13**).—Compound **12** (0.65 g, 0.74 mmol) was taken up in 60% aq acetic acid (40 mL) and heated for 2 h at 70°C. The acetic acid was evaporated under reduced pressure to leave a residue that was chromatographed (solvent E) to give **13** (0.48 g, 82%): $[\alpha]_D$ +34° (*c* 1.6, chloroform); TLC (solvent F): R_f 0.26; ^{1}H NMR ($CDCl_3$): δ 7.44–7.20 (m, 20 H, Ar), 5.36 (br d, 1 H, $J_{2',3'}$ 3.5 Hz, H-2′), 5.27 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 4.92 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.56 (s, 1 H, H-1′), 2.12 (s, 3 H, OAc) and 1.80 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 170.32, 169.72 ($COCH_3$), 98.52 (C-1′), 97.13 (C-1), 74.40, 73.58, 71.35, 69.94 ($PhCH_2O$), 68.36 (C-6), 62.34 (C-6′), 52.31 (C-2), and 23.33, 21.07 ($COCH_3$). Anal. Calcd for $C_{44}H_{51}NO_{12}$ (785.90): C, 67.25; H, 6.54; N, 1.78. Found: C, 67.14; H, 6.34; N, 1.72.

Benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 6)-2-O-acetyl-3-O-benzyl-β-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**15**).—A stirred mixture of diol acceptor **13** (0.5 g, 0.64 mmol) powdered $Hg(CN)_2$ (0.17 g, 0.67 mmol) and 4 Å molecular sieves (0.5 g) in 1:1 benzene–nitromethane (80 mL) was boiled until 20 mL of the solvent had distilled off. After cooling to room temperature bromide **14** (0.57 g, 0.82 mmol) in 1:1 benzene–nitromethane (9 mL) was added and stirring was continued overnight at 40–45°C. The mixture was filtered (Celite), the solids were thoroughly washed with benzene, and the filtrate and washings were combined and diluted with benzene to a total volume of 200 mL. This solution was successively washed with water, M KI, aq $NaHCO_3$, and water, dried, and concentrated to give a solid residue. TLC (solvent G) of the crude mixture showed the presence of a major product, slightly faster migrating than **13**. Small proportions of some faster and some slower migrating contaminants, as well as of **13**, were also revealed in TLC. The crude product was chromatographed (0 → 2% methanol in chloroform), and concentration of the fractions corresponding to the product gave a solid that was dissolved in dichloromethane and precipitated by the addition of ether–hexane to furnish **15** (0.5 g,

56%) as an amorphous solid: $[\alpha]_D$ +1.8° (*c* 1, chloroform); TLC (solvent G): R_f 0.71; ^{1}H NMR ($CDCl_3$): δ 7.43–7.20 (m, 20 H, Ar), 5.75 (d, 1 H, $J_{NH,2}$ 8.5 Hz, NH), 2.15 (s, 3 H, OAc), 2.04, 2.03, (s, 6 H each, 4 OAc), 2.0, 1.96 (s, 3 H each, 2 OAc), 1.89, and 1.80 (s, 3 H each, 2 NAc); ^{13}C NMR ($CDCl_3$): δ 170.82, 170.65, 170.58, 170.05, 169.82, 169.62, 169.44 ($COCH_3$), 99.50 (C-1‴), 98.96 (C-1′), 97.85 (C-1″), 97.17 (C-1), 80.03 (C-4), 78.91 (C-2″), 73.79, 73.23, 71.33, 69.66 ($PhCH_2$), 68.45 (C-6), 66.41 (C-6′), 62.59 (C-6″), 61.88 (C-6‴), 55.28 (C-2‴), 52.34 (C-2), 23.19, 23.12, 20.94, 20.69, 20.67, 20.63, and 20.55 ($COCH_3$). Anal. Calcd for $C_{70}H_{86}N_2O_{28}$ (1403.46): C, 59.91; H, 6.18; N, 2.00. Found: C, 59.64; H, 6.10; N, 2.11.

*Benzyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-α-*D*-mannopyranosyl-(1 → 6)-3-*O*-benzyl-β-*D*-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**16**).—Compound **15** (0.38 g, 0.27 mmol) was *O*-deacetylated in 20 mM methanolic sodium methoxide (50 mL) exactly as described for the preparation of **8** to give after chromatography (10 → 20% methanol in chloroform) a solid residue that was dissolved in a small amount of methanol. Addition of ether caused the precipitation of **16** (0.19 g, 63%) as an amorphous solid: $[\alpha]_D$ +2.4° (*c* 8.7, H_2O); TLC (solvent H): R_f 0.54; ^{1}H NMR (CD_3OD): δ 7.46–7.20 (m, 20 H, Ar), 4.78 (d, 1 H, $J_{1'',2''}$ 1.8 Hz, H-1″), 4.55 (d, 1 H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.42.(s, 1 H, H-1′), 1.90 (s, 6 H, 2 NAc); ^{13}C NMR (CD_3OD): δ 174.38, 173.32 ($COCH_3$), 102.13, 101.95 (C-1′, C-1‴), 98.59 (C-1″), 97.94 (C-1′), 70.66 (C-6), 69.84 (C-6′), 63.15 (C-6″), 62.43 (C-6‴), 57.22 (C-2‴), 54.21 (C-2), 23.91, and 22.65 ($COCH_3$). Anal. Calcd for $C_{56}H_{72}N_2O_{21}$ (1109.20): C, 60.64; H, 6.54; N, 2.53. Found: C, 60.80; H, 6.56; N, 2.50.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-α-*D*-mannopyranosyl-(1 → 6)-β-*D*-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-*D*-glucoyranose* (**2**).—A mixture of **16** (0.1 g, 90 μmol) and 10% Pd–C (0.12 g) in glacial acetic acid (10 mL) was shaken under H_2 at 345 kPa for 2 days at room temperature. The suspension was filtered (Celite), the solid was thoroughly washed with glacial acetic acid and methanol (Caution! Fire hazard.), and the filtrate and washings were combined and concentrated. The residue was chromatographed (solvent I → J → K) to give a solid that was dissolved in a small volume of water and lyophilized to give **2** (47 mg, 69.6%): $[\alpha]_D$ +5.4° (initial) → +6.4° (12 h, *c* 0.3, water); TLC (solvent K): R_f 0.1. FABMS: 749 $(M + 1)^+$ and 771 $(M + Na)^+$. ^{1}H NMR (D_2O): δ 5.19 (d, $J_{1,2}$ 2.5 Hz, H-1α), 4.90 (d, $J_{1'',2''}$ 1 Hz, H-1″), 4.71 (s, H-1′), 4.54 (d, $J_{1''',2'''}$ 8.5 Hz, H-1‴), 4.50 (d, $J_{1,2}$ 8.5 Hz, H-1β), 4.02 (br t, $J_{2'',3''}$ 3.30 Hz, H-2″), 2.05 (NAc, C-2α), 2.04 (NAc, C-2″) and 2.03 (NAc, C-2β); ^{13}C NMR (D_2O): δ 175.63 ($COCH_3$), 101.39 (C-1‴), 100.46 (C-1′), 99.96 (C-1″), 91.35 (C-1α), 67.00 (C-6′), 62.46 (C-6″), 61.47 (C-6‴), 60.97 (C-6), 56.19 (C-2‴), 54.44 (C-2), 23.18 and 22.67 ($COCH_3$). Anal. Calcd for $C_{28}H_{48}N_2O_{21} \cdot 2H_2O$ (784.73): C, 42.86; H, 6.68; N, 3.57. Found: C, 42.61; H, 6.78; N, 3.48.

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