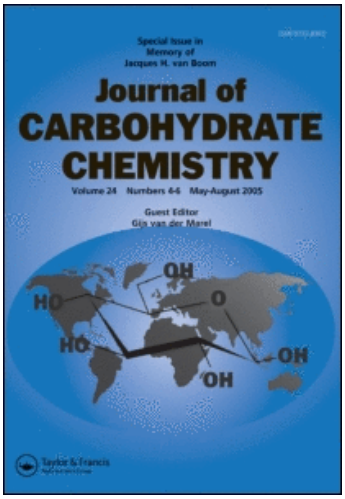


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# Synthesis of *O*-(2-Acetamido-2-Deoxy-β-D-Glucopyranosyl)-(2)-*O*-α-D-Mannopyranosyl-(6)-*O*-β-D-Glucopyranosyl-(1⟶4)-2-Acetamido-2-Deoxy-D-Glucopyranose. A Potential Acceptor-Substrate for *N*-Acetylglucosaminyltransferase-V (GnT-V)

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# SYNTHESIS OF *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→2)-*O*-α-D-MANNOPYRANOSYL-(1→6)-*O*-β-D-GLUCOPYRANOSYL-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE. A POTENTIAL ACCEPTOR-SUBSTRATE FOR *N*-ACETYLGLUCOSAMINYLTRANSFERASE-V (GnT-V).[1]

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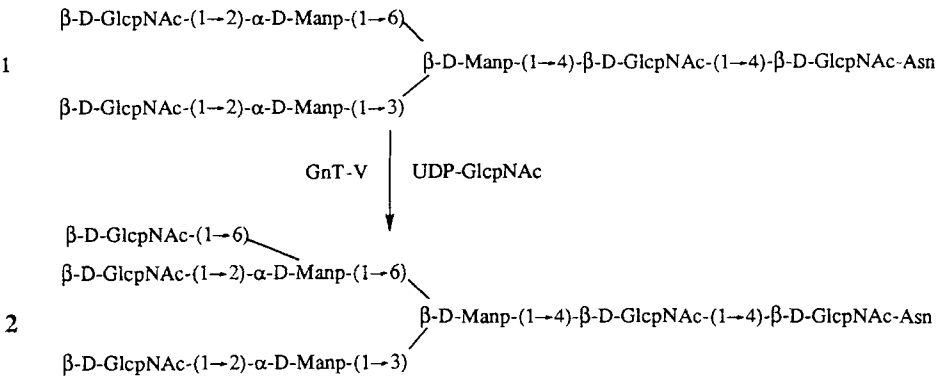
## ABSTRACT

The reaction of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside with methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside catalysed by iodonium ion (TfOH-NIS) followed by deacylation-acetylation afforded disaccharide **11**, which was readily converted (in four steps) to bromide **12**. A similar glycosylation with phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranoside of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside **16** followed by *O*-deacetylation of the resulting intermediate gave disaccharide **18**. The 4,6-*O*-benzylidene derivative of **18** was acetylated then deacetaled to give diol **21**. This diol acceptor was condensed with bromide **12** (promoted by mercuric cyanide) to give the partially protected tetrasaccharide derivative **22** which was *O*-deacetylated and then subjected to catalytic hydrogenation to furnish the title tetrasaccharide **6**. The structure assigned to **6** was supported by $^{1}H$ and $^{13}C$ NMR spectral data and FAB mass spectroscopy.

## INTRODUCTION

Previous papers from this group have described the synthesis of some oligosaccharides containing the *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl unit.[3,4] These oligosaccharides were required as a part of our project




1

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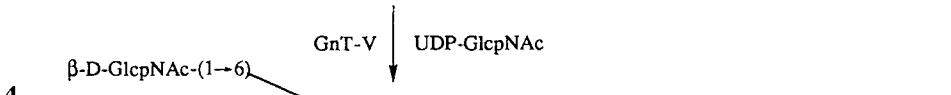
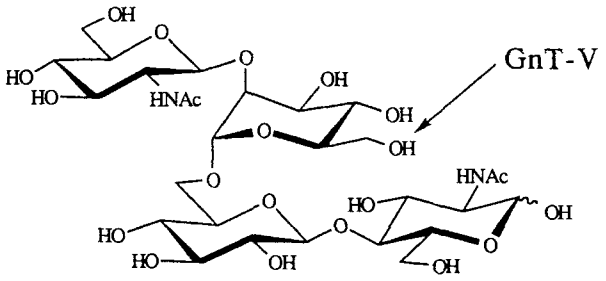
3 β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Manp-O-$(CH_2)_8CO_2CH_3$



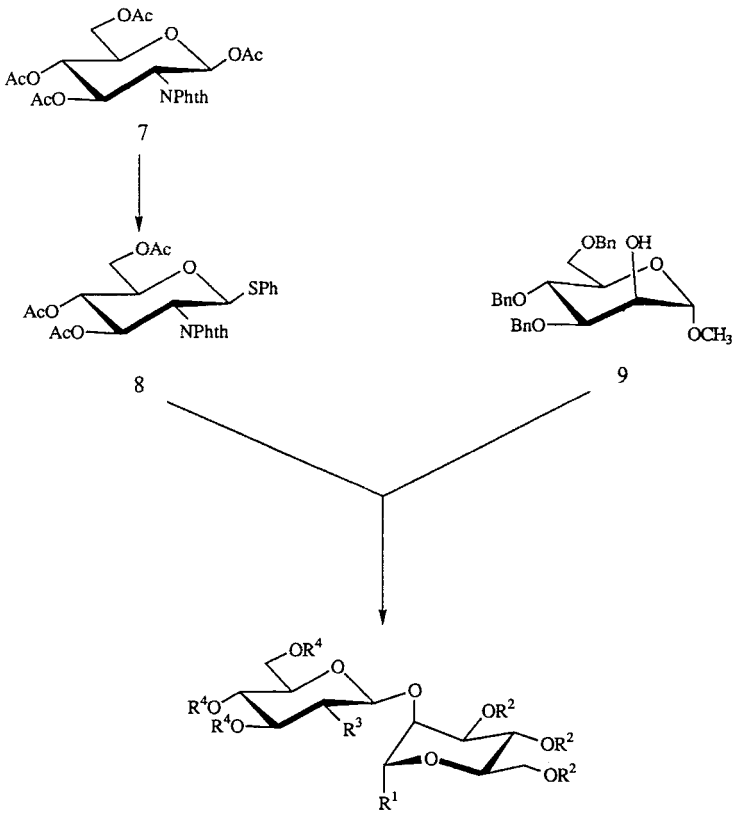

4 β-D-GlcpNAc-(1→6)
β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Manp-O-$(CH_2)_8CO_2CH_3$

5 β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Glcp-O-$(CH_2)_7CH_3$

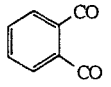



6 β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Glcp-(1→4)-D-GlcpNAc

on the substrate specificity of the enzyme UDP-GlcpNAc:α-D-mannopyranosyl-(1→6)-*N*-acetyl-β-D-glucosaminyltransferase (GlcpNAc-transferase V or GnT-V, EC 2.4.1.155). This enzyme has attracted a great deal of interest as a potential tumor marker because of its increased expression in cells transformed by tumor viruses[5,6] or oncogenes.[7] Furthermore, Dennis and coworkers[8,9] have suggested that an increase in intracellular activity of GnT-V is directly related to metastatic potential of certain tumor cell lines. In

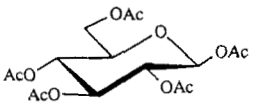



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 10: | $OCH_3$ | Bn | NPhth | Ac |
| 11: | $OCH_3$ | Bn | HNAc | Ac |
| 12: | Br | Ac | HNAc | Ac |

Phth = 


addition, these authors[10] have also reported that an increased expression of GnT-V activity results in cell surface structures which are implicated in a number of human breast carcinomas.

Biosynthetically, this enzyme catalyses the transfer of a β-D-GlcpNAc residue to oligosaccharide acceptors having structure **1**, resulting in the synthesis of octasaccharide **2**.[11,12] Hindsgaul's group[13-15] has reported the synthesis of trisaccharide **3** (partial structure of **1**) and showed that it is an effective acceptor for GnT-V which transformed it



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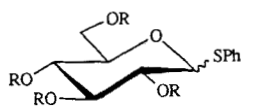
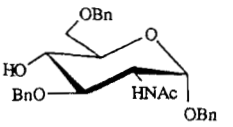


14: R = H

15: R = Ac



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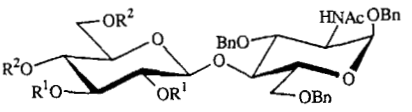
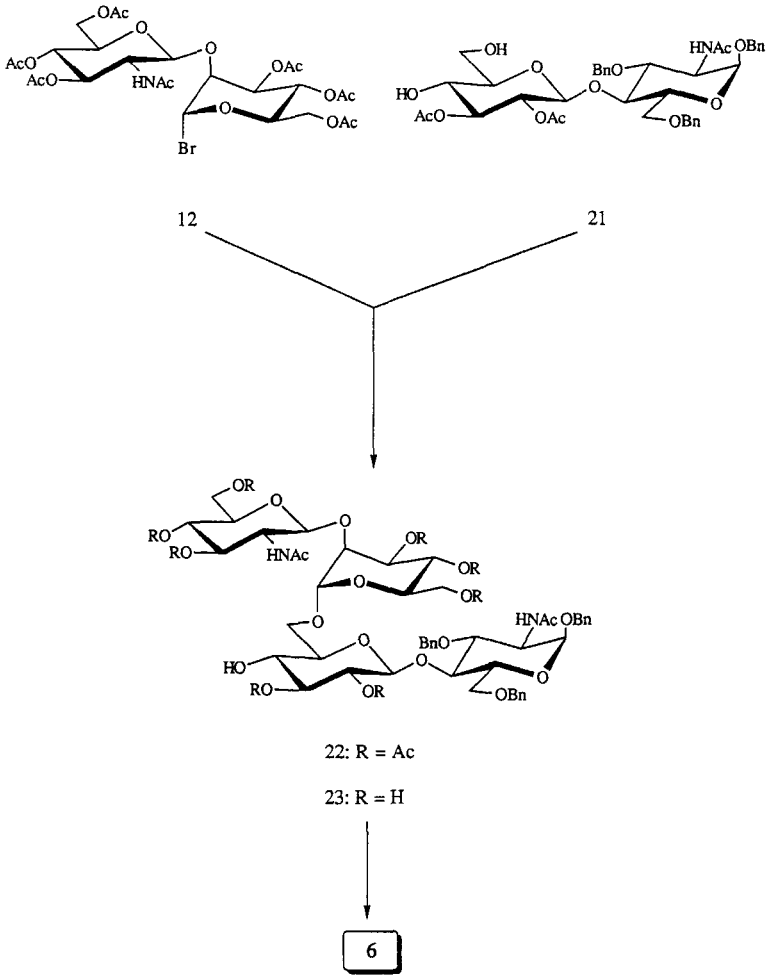


| | $R^1$ | $R^2$ |
|---|---|---|
| 17: | Ac | Ac |
| 18: | H | H |
| 19: | H | PhCH |
| 20: | Ac | PhCH |
| 21: | Ac | H |

to the expected tetrasaccharide **4**. We have postulated earlier[3] that the HO-2 of β-D-Man residue may not be a stringent requirement in recognition by this enzyme which was later supported by Hindsgaul *et al.*[16] who showed that the trisaccharide **5** was also an excellent acceptor substrate.

Thus, in order to extend the earlier studies and gain added insights on the substrate specificity of GnT-V, we describe herein the synthesis of title tetrasaccharide **6**, because of its similarity to part of the heptasaccharide acceptor (see structure **1**), with the exception that the β-D-Manp-(1→4) residue has been replaced by a β-D-Glcp-(1→4) residue. This was intended as a further test for the relevance (or lack of it) of HO-2 of the β-D-Manp residue in recognition by GnT-V.






## RESULTS AND DISCUSSION

The synthesis of tetrasaccharide **6** proceeded by way of the suitably protected reducing glycosyl acceptor **21** to which disaccharide terminal unit β-D-GlcpNAc-(1→2)-α-D-Manp was added, by using the glycosyl donor **12**, following well-precedented procedures.[3,4] Bromide **12** was obtained from **11** in four steps as described earlier.[3] Disaccharide **11** was prepared by a modification[17,18] of Lönn's thioglycoside method[19] and involved the condensation of thioglycoside donor **8**[20] with HO-2 mannose acceptor **9**[21] in the presence of iodonium ion generated by trifluoromethane-sulfonic acid and *N*-iodosuccinimide, followed by deacylation-reacetylation reaction sequence.

**TABLE I.**

PROPOSED $^{13}C$ NMR ASSIGNMENTS.[a]

| Residue | Compd. | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | $\underline{C}OCH_3$ | $CO\underline{C}H_3$ |
|---|---|---|---|---|---|---|---|---|---|
| β-D-GlcpNAc-(1→2) | **24** | 101.36 | 55.80 | 74.12 | 70.12 | 77.20 | 61.06 | - | 23.10 |
| α-D-Manp-OMe | | 98.73 | 78.84 | 70.66 | 67.40 | 74.12 | 61.42 | - | - |
| β-D-Glcp-(1→4) | **25** | 103.43 | 73.41 | 76.96 | 69.97 | 76.44 | 60.53 | - | - |
| α-D-GlcpNAc | | 90.43 | 53.88 | 69.97 | 81.73 | 68.58 | 60.93 | 169.22 | 22.52 |
| β-D-GlcpNAc-(1→2) | **6**[b] | 101.51 | 55.63 | 74.13 | 70.27 | 77.12 | 61.13 | 170.51 | 23.18 |
| α-D-Manp-(1→6) | | 97.47 | 78.82 | 70.66 | 67.58 | 74.13 | 61.55 | - | - |
| β-D-Glcp-(1→4) | | 103.68 | 73.39 | 76.59 | 69.32 | 74.56 | 65.98 | - | - |
| α-D-GlcpNAc | | 90.51 | 53.83 | 69.98 | 82.21 | 68.93 | 60.69 | 170.07 | 22.66 |

a. For solutions in DMSO-$d_6$ at 50.3 MHz for **25** and at 25.2 MHz for **6** with $Me_4Si$ as the internal standard. The chemical shifts for compound **24** and **25** are included for comparison purposes. $^{13}C$ NMR values of compound **24** are taken from ref. 4.

b. Some additional resonances with substantially reduced intensities, apparently due to the portion of the compound having the β-D-configuration at the 2-acetamido-2-deoxy-D-glucopyranose residue were also present.

The disaccharide benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside **17** (a precursor of **21**) was prepared next. The general method[22] used to prepare **17** involved the condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with suitably protected HO-4 acceptor **16** catalysed by mercuric bromide. Preparation of **17** under these conditions did not result in appreciable yield. We, therefore, explored the use of thioglycoside **15** as a glycosyl donor, under conditions developed by Fraser-Reid *et al.*[17] and van Boom *et al.*[18] Thus the condensation of **15** with acceptor **16**[23] in the presence of iodonium ion, as described above, followed by *O*-deacetylation (to facilitate chromatographic purification) gave the desired disaccharide **18** (58%). This glycosylation procedure offers a milder and efficient way to glycosylate unreactive acceptor **16**. Thioglycoside **15** was readily prepared in 85% yield by treating its commercially available precursor 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose **13** with phenyl thiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate according to a recent literature procedure.[24] Deacetylation-reacetylation (15→14→15) was necessary to facilitate the purification of **15**. Benzylidenation of **18** with α,α-dimethoxytoluene in *N,N*-dimethylformamide containing 4-toluenesulfonic acid afforded the 4,6-*O*-benzylidene derivative **19** (91%) which was converted (2:1 Py-$Ac_2O$) into the corresponding di-*O*-acetate **20** (91%). Cleavage of the benzylidene acetal group with hot, 60% aqueous acetic acid gave the desired diol acceptor **21** (78%). Regioselective glycosylation of **21** with glycosyl bromide **12**, catalysed by mercuric cyanide, gave the partially protected tetrasaccharide **22** (64%). Zemplén transesterification of **22** gave **23** (74%) which was subjected to hydrogenolysis in glacial acetic acid and in the presence of 10% Pd-C to afford the title tetrasaccharide **6** (68%) as white amorphous powder.

Assignments of the $^{13}C$ NMR spectrum of tetrasaccharide **6** were based on comparison with the spectrum of methyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-D-mannopyranoside **24**[4] and *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-α-D-glucopyranose **25**.[22] In the $^{13}C$ NMR spectrum of **6**, the presence of four anomeric resonances at δ 90.51, 97.47, 101.51, and 103.68 were indicative of two α-D- and two β-D-configurations at interglycosidic linkages. The signal for C-6' was shifted downfield, resonating at δ 65.98, a clear indication that this carbon atom was glycosylated. That C-4 and C-2" were sites of glycosylation could readily be seen by the occurrence of their respective signals at large frequencies (δ 82.21 and 78.82, respectively).

## EXPERIMENTAL

**General methods.** Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured at 22±2 °C with a Perkin-

Elmer 241 polarimeter. TLC was conducted on aluminum sheets, precoated with 0.2-mm layers of silica Gel 60F-254 (Merck); the compounds were located by quenching of fluorescence and/or by charring with 5% sulfuric acid. Column chromatography was performed on silica gel (Baker Analyzed, 60-200 mesh). Generally 25 mL fractions were collected and the flow rate was maintained at 5 mL/min. $^1$H NMR spectra were recorded at 90 (Varian EM-390), 300 (Bruker AM-300), or at 500 MHz ( Bruker AM-500). The chemical shift reference in organic solvents was internal $Me_4Si$ (δ 0) and in $D_2O$ was internal acetone (δ 2.225). $^{13}$C NMR spectra were recorded either at 25.2 (Varian XL-100), 50.3 (Bruker WP-200) or at 75.5 MHz (Bruker AM-300) on solutions in $CDCl_3$, $CD_3OD$, or DMSO-$d_6$ (internal $Me_4Si$) or $D_2O$ (external 1% 1,4 dioxane in $D_2O$, δ 67.4). Only partial NMR data are reported, the other data were in accord with the overall proposed structures. The assignments of $^{13}$C NMR chemical shifts are tentative. FAB mass spectrum was obtained using an AEI MS-9 instrument with xenon as the bombarding gas with 1,4-dithiothreitol-1,4-dithioerythritol (5:1) as matrix. Unless otherwise indicated, all reactions were carried out at ambient temperatures, and in the work-up, solutions in organic solvents were washed with equal volumes of aqueous solutions. Organic solutions were generally dried (anhydrous $Na_2SO_4$) prior to concentration (at a bath temperature of 40-50 °C) on a rotary evaporator under the reduced pressure obtained from a water aspirator. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 08940 (U.S.A.). The following solvent systems (v/v) were used for chromatography: **A**, hexane-ethyl acetate (2:1); **B**, chloroform-methanol (9:1); **C**, chloroform-acetone (4:1); **D**, chloroform-methanol (4:1); **E**, chloroform-methanol-water (13:6:1); **F**, chloroform-methanol-water (10:9:1); **G**, chloroform-methanol-water (5:4:1).



**Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8).** Compound **8** was obtained from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**) as described by Matta *et al.*[20]: $^1$H NMR (300 MHz in $CDCl_3$) δ 7.91-7.73 (m, 4H, Phth), 7.44-7.24 (m, 5H, SPh), 5.80 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{2,3}$ = 10 Hz, H-3), 5.72 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.14 (dd, 1H, $J_{4,5}$ = 10 Hz, H-4), 4.36 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 10 Hz, H-2), 4.32-4.19 (m, 2H, H-6,6'), 3.94-3.88 (m, 1H, H-5), 2.10, 2.02, and 1.85 (s, 3H each, 3 OAc); $^{13}$C NMR (75.5 MHz in $CDCl_3$) δ 170.64, 170.12, 169.48 (3 $\underline{C}OCH_3$), 166.99, 166.61 ($\underline{C}O$, Phth), 134.46, 133.32, 131.02, 128.93, 128.45, 123.75 (aromatic), 83.10 (C-1), 75.95, 71.67, 68.78 (C-3,4,5), 62.27 (C-6), 58.61 (C-2), 20.78, 20.64, and 20.43 (3 CO$\underline{C}H_3$).

**Methyl 2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (11).** Thioglycoside **8** (4.02 g, 7.61 mmol) and acceptor **9** (2.94 g, 6.33 mmol) were dissolved in dry dichloromethane (32 mL), pulverized activated molecular sieves (4Å, 3 g) and *N*-iodosuccinimide (3.56 g,

15.8 mmol) were added and the mixture, protected from light, was stirred for 30 min under an atmosphere of argon. The mixture was then cooled (0 °C; bath) and a solution of trifluoromethanesulphonic acid (71 μL), in dichloromethane (63 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, aqueous $NaHCO_3$, and aqueous $Na_2S_2O_3$ solution, and water, dried and concentrated to dryness. The foamy residue (containing **10**) so obtained was boiled for 3 h in a mixture of ethanol (100 mL) and hydrazine-hydrate (25 mL). The reaction mixture was then taken to dryness and the residue was dissolved in pyridine (100 mL) and acetic anhydride (50 mL) was added. After stirring overnight at room temperature excess acetic anhydride was decomposed by dropwise addition of methanol to the reaction mixture at 0 °C. Solvent was evaporated, and the solution of the residue in chloroform (200 mL) was successively washed with water, aqueous $NaHCO_3$, and water. Evaporation of the solvent and purification of the residue by chromatography (chloroform) gave **11** (4.5 g, 84.6%) which had physical data identical with those reported previously.[3] $^1H$ NMR (300 MHz in $CDCl_3$) δ 7.42-7.19 (m, 15H, arom), 5.46 (d, 1H, $J_{2,NH}$ = 7.5 Hz, NH), 5.17 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.64 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 2.03, 2.02, 2.0 (s, 3H each, 3 OAc), and 1.76 (s, 3H, NAc); $^{13}C$ NMR (75.5 MHz in $CDCl_3$) δ 171.38, 170.68, 170.21, 169.71 (4 $\underline{C}OCH_3$), 138.63, 138.49, 138.21 [3 quaternary aromatic (quat arom)], 98.39, 97.68 (C-1,1'), 78.27 (C-2), 75.15, 73.33, 71.42 (3 $Ph\underline{C}H_2$), 69.20 (C-6), 62.55 (C-6'), 56.48 ($OCH_3$), 54.96 (C-2'), 23.30, and 20.76 (3C) (4 $CO\underline{C}H_3$).

**2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl Bromide (12).** Bromide **12** was prepared from disaccharide **11** (in four steps) as described by Khan *et al.* and had physical and spectral data identical with those reported.[3]

**Phenyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-α,β-D-glucopyranoside (15).** To a cold (0 °C, bath), stirred solution of pentaacetate **13** (5 g, 12.81 mmol) in dry dichloroethane (25 mL) were added trimethylsilyl trifluoromethanesulfonate (5.9 mL, 30.74 mmol) and phenyl thiotrimethylsilane (7.3 mL, 38.43 mmol). After being stirred at O °C for 4 h, the mixture was allowed to warm to room temperature, and stirring was continued for an additional 8 h. The mixture was then diluted with dichloromethane (100 mL), successively washed with water, a saturated $NaHCO_3$ solution, and water, dried and concentrated to a syrup, which contained (TLC, solvent A) the faster-migrating product, as well as a slower-migrating contaminant. The crude product was taken up in methanol (50 mL) containing M sodium methoxide in methanol (20 mL), and stirred overnight. The base was neutralized with glacial acetic acid and the concentrated reaction mixture was

chromatographed (5→15% methanol in chloroform) to afford **14** (3.13 g) as a white amorphous solid. The solid was dried under vacuum then taken up in a mixture of acetic anhydride (25 mL) and pyridine (50 mL), and stirred overnight. Acetic anhydride and pyridine were evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene, and the residue was chromatographed (10→20% ethyl acetate in hexane) to afford **15** (4.8 g, 85%, based on **13**) as an amorphous solid that showed the presence of α,β mixture; TLC (solvent A): $R_F$ 0.31 and 0.25. The α,β ratio was estimated by $^1H$ NMR spectroscopy to be approximately 9:1; $^1H$ NMR (300 MHz in $CDCl_3$) δ 5.92 (d, $J_{1,2}$ = 6 Hz, H-1α) and 4.71 (d, $J_{1,2}$ = 10 Hz, H-1β), other spectral features were comparable to those reported in literature.[25] $^{13}C$ NMR (75.5 MHz in $CDCl_3$) δ 85.76 (C-1β) and 85.03 (C-1α).

**Benzyl *O*-β-D-Glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (18).** Reaction of alcohol **16** (0.492 g, 1 mmol) with thioglycoside **15** (0.53 g, 1.2 mmol), as described for the preparation of **10**, gave, after customary processing, a solid residue. This crude product (1.03 g, containing **17**) was taken up in 10 mM methanolic sodium methoxide (33 mL), and stirred overnight at room temperature. The base was neutralized by dropwise addition of glacial acetic acid and the solution was de-ionized with Amberlite IR-120 ($H^+$) cation-exchange resin. The resin was removed by filtration of (Celite bed) and thoroughly washed with methanol. The filtrate and washings were combined and concentrated and the concentrate was chromatographed (0→10% methanol in chloroform) to give first, unchanged **16** (0.1 g). Continued elution of the column gave a solid, which was dissolved in a little methanol. Addition of ether-hexane caused the precipitation of **18** (0.41 g, 62.9%), amorphous; $[\alpha]_D$ +90° (*c* 1.1, 1:1 chloroform-methanol); lit.[22] $[\alpha]_D$ +79° (*c* 1.2, chloroform); TLC (solvent B), $R_F$ 0.36; $^1H$ NMR (90 MHz in $CD_3OD$) δ 7.35 (br s, 15 H, arom) and 1.88 (s, 3H, NAc); $^{13}C$ NMR (50.3 MHz in $CD_3OD$) δ 171.5 ($NCOCH_3$), 129.58 (2C), 128.96 (3 quat arom), 103.62 (C-1'), 98.06 (C-1), 80.45 (C-4), 69.35 (C-6), 63.27 (C-6'), 54.22 (C-2), and 22.66 ($NCOCH_3$).

*Anal.* Calcd for $C_{35}H_{43}NO_{11}$. 0.5 $H_2O$: C, 63.43; H, 6.69; N, 2.11. Found: C, 63.75; H, 6.66; N, 2.11.

**Benzyl *O*-(4,6-*O*-Benzylidene-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (19).** To a stirred solution of **18** (2.41 g) in *N,N*-dimethylformamide (50 mL) was added 4-toluenesulfonic acid (0.08 g) and α,α-dimethoxytoluene (2.78 g), and the stirring was continued for 3.5 h. The acid was then neutralized with a little triethylamine, and the solution concentrated to a syrup which was dissolved in ethyl acetate. Addition of ether-hexane caused the crystallization of **19** (2.5 g, 91%); mp 146-148 ° C ; $[\alpha]_D$ +78° (*c* 1.3, chloroform); TLC

(solvent C), $R_F$ 0.3; $^1H$ NMR (90 MHz in $CDCl_3$) δ 7.39-7.22 (m, 20 H, arom), 5.32 (s, 1 H, PhCH), and 1.71 (s, 3 H, NAc).

*Anal.* Calcd for $C_{42}H_{47}NO_{11}$: C, 67.99; H, 6.39; N, 1.89. Found: C, 67.89; H, 6.45; N, 1.91.

**Benzyl *O*-(2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (20).** Compound **19** (1.38 g) was acetylated, as described for **14** (to yield **15**), to afford a solid residue which was dissolved in small amount of dichloromethane. Addition of ether-hexane caused the precipitation of **20** (1.4 g, 91%); amorphous: $[\alpha]_D$ +37° (*c* 1.4, chloroform); TLC (solvent C), $R_F$ 0.51; $^1H$ NMR (90 MHz in $CDCl_3$) δ 7.33-7.20 (m, 20 H, arom), 5.26 (s, 1 H, PhCH), 1.96, 1.93 (s, 3 H each, 2 OAc), and 1.73 (s, 3 H, NAc).

*Anal.* Calcd for $C_{46}H_{51}NO_{13}$: C, 66.89; H, 6.22; N, 1.69. Found: C, 66.67; H, 6.20; N, 1.56.

**Benzyl *O*-(2,3-Di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (21).** Compound **20** (1.3 g) was taken up in 60% aqueous acetic acid (80 mL) and heated for 2 h at 70 °C. Acetic acid was evaporated under reduced pressure, the last traces being removed by coevaporation with several added portions of toluene to leave a residue which crystallized from ethyl acetate-hexane to give **21** (0.86 g, 78%): mp 159-160 °C; $[\alpha]_D$ +72° (*c* 1.2, chloroform); TLC (solvent B), $R_F$ 0.49; $^1H$ NMR (300 MHz in $CDCl_3$) δ 7.44-7.22 (m, 15H, arom), 5.27 (d, 1H, $J_{2,NH}$ = 9.0 Hz, NH), 4.89 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 2.06, 1.96 (s, 3H each, 2 OAc), and 1.78 (s, 3H, NAc); $^{13}C$ NMR (75.5 MHz in $CDCl_3$) δ 171.32, 169.90, 169.51 (3 $COCH_3$), 138.75, 137.68, 137.16 (3 quat arom), 99.81 (C-1'), 97.08 (C-1), 77.72 (C-4), 74.39, 73.31, 69.86 (3 $PhCH_2$), 67.44 (C-6), 61.81 (C-6'), 52.20 (C-2), 23.29, 20.87, and 20.78 (3 $COCH_3$).

*Anal.* Calcd for $C_{39}H_{47}NO_{13}$: C, 63.49; H, 6.42; N, 1.90. Found: C, 63.64; H, 6.24; N, 1.79.

**Benzyl *O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3-di-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (22).** A stirred mixture of diol **21** (0.61 g, 0.82 mmol), powdered $Hg(CN)_2$ (0.21 g, 0.82 mmol) and 4Å molecular sieves (0.8 g) in 1:1 benzene-nitromethane (90 mL) was boiled until 25 mL of the solvent had distilled off. After cooling to room temperature bromide **12** (0.86 g, 1.23 mmol) in 1:1 benzene-nitromethane (18 mL) was added and stirring was continued for 14 h at 40-45 °C. The mixture was filtered through a bed of Celite, the solids throughly washed with benzene, and the filtrate and washings were combined and diluted with benzene to a total volume of 300 mL. The

organic solution was successively washed with water, M KI solution, an aqueous $NaHCO_3$ solution, and water, dried, and concentrated to give a solid residue. TLC (solvent B) of the crude mixture showed the presence of a major product, slightly faster migrating than **21**; small proportions of faster and slower migrating contaminants, as well as of **21**, were also revealed by TLC. The crude product was purified by chromatography (0→2% methanol in chloroform) to give a solid, which was dissolved in chloroform and precipitated by the addition of ether to furnish **22** (0.72 g, 64%): amorphous; $[\alpha]_D$ +30.5° (*c* 0.4, chloroform); TLC (solvent B), $R_F$ 0.56; $^1H$ NMR (300 MHz in $CDCl_3$) δ 7.47-7.22 (m, 15H, arom), 5.91 (d, 1H, $J_{2,NH}$ = 8.75 Hz, NH), 5.74 (d, 1H, $J_{2,NH}$ = 9.25 Hz, NH), 4.91 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.86 ( br s, 1H, H-1"), 2.12, 2.07, 2.04, 2.03, 2.02, 1.99, 1.95, 1.93 (s, 3H each, 8 OAc), 1.80, and 1.68 (s, 3H, 2 NAc).

*Anal.* Calcd for $C_{65}H_{82}N_2O_{29}$: C, 57.60; H, 6.10; N, 2.07. Found: C, 57.44; H, 6.09; N, 2.23.

The last fraction that emerged from the column was unchanged **21** (0.08 g).

**Benzyl *O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (23).** Compound **22** (0.63 g) in 20 mM methanolic sodium methoxide (104 mL) was stirred overnight at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid. The resulting solution was deionized with Amberlite IR-120 ($H^+$) cation exchange resin. The resin was removed by filtration through a bed of Celite. It was throughly washed with methanol, and the filtrate and washings were combined and concentrated. The concentrate was chromatographed (solvent D) to afford **23** (0.35 g, 68.5%): amorphous; $[\alpha]_D$ +39° (*c* 0.8, chloroform); TLC (solvent E), $R_F$ 0.32; $^1H$ NMR (300 MHz in $D_2O$) δ 7.52-7.22 (m, 15H, arom), 4.83 (br s, 1H, H-1"), 4.18 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 2.03, and 1.77 (s, 3H each, 2 NAc); $^{13}C$ NMR (75.5 MHz in $D_2O$) δ 175.61, 174.41 (2 $N\underline{C}OCH_3$), 139.01, 138.21, 137.79 (3 quat arom), 103.12 (C-1'), 100.50 (C-1'"), 97.48, 97.04 (C-1,1"), 73.76 (2C), 70.73 (3 $Ph\underline{C}H_2$), 68.46 (C-6), 66.22 (C-6'), 62.29 (C-6"), 61.04 (C-6'"), 56.19 (C-2'"), 53.06 (C-2), 23.19, and 22.74 (2 $NCO\underline{C}H_3$).

*Anal.* Calcd for $C_{49}H_{66}N_2O_{21}$: C, 57.75; H, 6.53; N, 2.75. Found: C, 58.00; H, 6.56; N, 2.58.

***O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (6).** A mixture of **23** (0.2 g) and 10% Pd-C (0.2 g) in glacial acetic acid (15 mL) was shaken under $H_2$ at 345 kPa for 2 days at room temperature. The suspension was filtered through a bed of Celite, the solid thoroughly washed with glacial acetic acid and methanol, and the filtrate and washings were combined and concentrated.

The residue was chromatographed (solvent E→F), to give a solid which was dissolved in a small volume of methanol. Addition of ether caused the precipitation of **6** (0.1 g, 68%): amorphous; $[\alpha]_D$ +6° (initial)→ +7° (40 h, *c* 0.5, water); TLC (solvent G), $R_F$ 0.12; FAB-MS m/z 771 $[M+Na]^+$; $^1H$ NMR (500 MHz in $D_2O$) δ 5.21 (d, $J_{1,2}$ = 2.9 Hz, H-1α), 4.92 (s, H-1"), 4.71 (d, H-1β), 4.55 (d, $J_{1''',2'''}$ = 8.5 Hz, H-1'''), 4.54 (d, $J_{1',2'}$ = 8 Hz, H-1'α), 4.53 (d, $J_{1',2'}$ = 9.4 Hz, H-1'β), 4.09 (dd, $J_{1'',2''}$ = 1.6 Hz, $J_{2'',3''}$ = 3.4 Hz, H-2"), 2.06 (NAc, C-2α), 2.057 (NAc, C-2β), and 2.046 (NAc, C-2"), $^{13}C$ NMR data are presented in Table I.

*Anal.* Calcd for $C_{28}H_{48}N_2O_{21}$: C, 44.92; H, 6.46; N, 3.74. Found: C, 45.21; H, 6.48; N, 3.49.

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