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Synthesis of 2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-*β*-D-glucopyranose, a Useful Glycosyl Acceptor for 1,4-Linked, *N*-Acetylglucosamine-containing Oligosaccharides Synthesis

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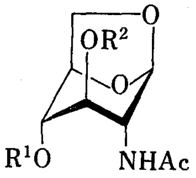
A facile synthesis of the title compound (**9**) was achieved. Tritylation of 1,6-anhydro-*β*-*N*-acetylglucosamine (**4**) with 3 molar equivalents of trityl chloride in pyridine at 85—90°C for 24 h afforded the corresponding 4-*O*-trityl ether (**5**) and 3-*O*-trityl ether (**6**) in 66.9 and 8.3% yields, respectively, after column chromatography. Benzylation of **5** followed by detritylation provided **9** *via* 3 steps from **4** in *ca.* 47% yield. Preparation of 2-acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-*β*-D-glucopyranose (**10**) from **6** was also carried out.

Keywords——synthesis; 1,6-anhydro-*β*-*N*-acetylglucosamine; 1,6-anhydro-3-*O*-benzyl-*β*-*N*-acetylglucosamine; 1,6-anhydro-4-*O*-benzyl-*β*-*N*-acetylglucosamine; tritylation; benzylation; detritylation; amino disaccharide; glycosyl acceptor; ^{13}C-NMR

Amino disaccharides having a monosaccharide attached to the C-4 position of *N*-acetylglucosamine are important in connection with studies of biologically active complex carbohydrates. For example, *N*,*N'*-diacetylchitobiose (**1**) exists in the internal region of many glycoproteins bearing *N*-acetylglucosaminyl-asparagine linkages, whereas *N*-acetyllactosamine (**2**) is often located at the non-reducing end of the sugar chain in glycoproteins or glycolipids[1]; **2** also exists in complex oligosaccharides isolated from human milk.[2] According to a paper by Wolfrom *et al.*,[3] *N*-acetylmaltosamine (**3**) was synthesized by acetylation of maltosamine hydrochloride prepared from a hydrolyzate of carbonyl-reduced, partially desulfated heparin with hydrochloric acid.

Recently, unequivocal syntheses of **2**,[4] **3**,[5] and the hexaacetate of **1**[6] *via* the corresponding 1,6-anhydro-*β*-derivatives were reported from this laboratory. However, the comparatively long synthetic routes are not very satisfactory for mass production of these compounds. In order to reduce the number of steps, we considered that a facile synthesis of the intermediate glycosyl acceptor, an *N*-acetylglucosaminide derivative having an unprotected hydroxyl group at the C-4 position, was required. In this paper, we report a useful synthesis of the title compound (**9**).



4: R¹=R²=H
5: R¹=Tr, R²=H
6: R¹=H, R²=Tr
7: R¹=Tr, R²=Bn
8: R¹=Bn, R²=Tr
9: R¹=H, R²=Bn
10: R¹=Bn, R²=H

Bn=benzyl
Tr=triphenylmethyl

Chart 1

1,6-Anhydro-*N*-acetyl-*β*-D-glucosamine (**4**), synthesized according to the method of Haga *et al.*,[7] was triphenylmethylated (tritylated) with 3 molar equivalents of triphenylchloromethane (trityl chloride) in pyridine at 85—90°C for 24 h. Thin-layer chromatography (TLC) showed the formation of two products with a trace of by-product. After column chromatography on silica gel, the major product (**5**) and the minor product (**6**) were isolated. The infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra of **5** and **6** suggested that they were monotrityl ethers of **4**. The by-product was assumed to be the corresponding ditrityl ether of **4**, but further studies were abandoned because of the ex-

Table I. ^{13}C-NMR Chemical Shifts of 1,6-Anhydro-β-*N*-acetylglucosamine Derivatives: δ (ppm) from TMS in Pyridine-d_5

Compounds	Sugar moiety						NHCOCH$_3$		$C_6H_5\underline{C}H_2$	$(C_6H_5)_3\underline{C}$
	C-1	C-2	C-3	C-4	C-5	C-6	C=O	CH$_3$	CH$_2$	C
4[a]	102.2	53.8	72.5	73.4	77.5	66.0	169.8	23.0		
5[b]	101.8	54.1	71.4	75.7	74.9	65.7	169.4	23.1		88.3
6[c]	101.5	52.0	75.3	68.9	76.8	65.3	168.5	22.8		89.3
9[d]	101.5	49.6	80.5	69.8	77.0	65.4	169.7	22.9	71.6	
10[e]	102.2	53.9	70.4	79.7	74.7	65.9	169.6	23.0	71.2	

a) 2-Acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose.
b) 2-Acetamido-1,6-anhydro-2-deoxy-4-*O*-trityl-β-D-glucopyranose.
c) 2-Acetamido-1,6-anhydro-2-deoxy-3-*O*-trityl-β-D-glucopyranose.
d) 2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose.
e) 2-Acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-β-D-glucopyranose.

tremely low yield.

In order to determine the structure, **5** was converted to authentic 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**9**)[8] through benzylation followed by detritylation. Therefore, **6** must be assigned as the 3-*O*-trityl ether of **4**, a positional isomer of **5**. The yield ratio of **5** to **6** was *ca.* 8: 1. Studies on partially substituted 1,6-anhydrohexopyranoses so far investigated have proved that the presence of the 1,6-anhydro bridge markedly decreases the reactivity of the axial hydroxyl group on C-3. Thus, when 1,6-anhydrohexopyranoses are treated with bulky chlorides, the C-3 position is substituted more slowly than the other axially oriented ones.[9] Consequently, in the partial tritylation of **4** mentioned above, preferential formation of **5** rather than **6** is in accord with expectation.

Benzylation of **6** followed by detritylation afforded 2-acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-β-D-glucopyranose (**10**). This product is also available as a glycosyl acceptor for 1,3-linked, *N*-acetylglucosamine-containing oligosaccharides synthesis.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **4**, **5**, **6**, **9**, and **10** were measured in pyridine-d_5 at room temperature. The chemical shifts are summarized in Table I. The resonances for C-4 of **5** and **10** were deshielded by 2.3 and 6.3 ppm, respectively, as compared with the chemical shift for C-4 of **4**. Similarly, those for C-3 of **6** and **9** were deshielded by 2.8 and 8.0 ppm, respectively, as compared with that for C-3 of **4**. The results provide an unequivocal proof of the positions of the newly introduced substituents.

Experimental

Melting points were determined with a Yanagimoto MP-S2 melting point apparatus and are uncorrected. Solutions were concentrated in a rotary evaporator below 45°C under a vacuum. Optical rotations were measured with a Union Giken PM-201 automatic digital polarimeter. IR spectra were recorded with a JASCO A-102 spectrometer. ^{1}H-NMR spectra were recorded at 100 MHz with a JEOL JNM-MH-100 spectrometer. ^{13}C-NMR spectra were recorded at 25 MHz with a JEOL JNM-FX-100 spectrometer. Tetramethylsilane was used as an internal standard. Chemical shifts are given on the δ scale. TLC was performed on pre-coated silica gel plates 0.25 mm thick (Kieselgel 60 F_{254}, Merck) using (A), benzene–ether (1: 4, v/v); (B), $CHCl_3$–acetone (3: 1); (C), $CHCl_3$–acetone (1: 1). Detection was effected with anisaldehyde–H_2SO_4–EtOH (1: 1: 18, v/v) spray reagent at 125°C,[10] or by UV irradiation at 254 nm. Column chromatography was performed on Kieselgel 60 (Merck, 70—230 mesh). Solvent combinations for elution are shown as v/v.

2-Acetamido-1,6-anhydro-2-deoxy-4-*O*-trityl-β-D-glucopyranose (**5**) **and 2-Acetamido-1,6-anhydro-2-deoxy-3-*O*-trityl-β-D-glucopyranose** (**6**)——A mixture of **4**[7] (1 g, 4.92 mmol) and trityl chloride (2.76 g, 9.90 mmol) in dry pyridine (70 ml) was stirred at 85—90°C with exclusion of moisture. After 16 and 22 h, further portions of trityl chloride (each 0.7 g, 2.51 mmol) were added, and the stirring was continued for 24 h. The mixture was then poured into ice-H_2O, and extracted with $CHCl_3$. The combined extracts were successively washed with ice-H_2O, ice-cold dil. HCl, satd. $NaHCO_3$, and H_2O. Desiccation ($MgSO_4$) and removal of the solvent provided a syrup, which was chromatographed on a column with benzene–ether (1: 4). The major fraction eluted provided **5** as a white powder (1.48 g, 66.9%), $[\alpha]_D^{21}$ −33.4° (c=0.7, $CHCl_3$). ^{1}H-NMR (pyridine-d_5): 2.13 (3H, s, –NHCOC$\underline{H}_3$), 4.88 (1H, br s, OH, exchangeable with D_2O), 5.66 (1H, s, H-1), 7.12—7.76 (15H, m, aromatic protons), 7.86 (1H, d, $J_{NH,2}$=9 Hz, NH, exchangeable with D_2O). ^{13}C-NMR: see Table I. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH, NH), 1666 (amide I), 1516 (amide II). TLC: *Rf* 0.14 (solvent A), 0.58 (B), 0.19 (C). *Anal.* Calcd for $C_{27}H_{27}NO_5 \cdot 1/4H_2O$: C, 72.06; H, 6.16; N, 3.11. Found: C, 71.95; H, 6.27; N, 2.87.

Compound **6** was eluted with the same solvent after **5** had emerged. Removal of the solvent and washing of the residue with ether provided **6** as prisms (180 mg, 8.3%), mp 279—281°C (dec.), $[\alpha]_D^{20}$ −72.8° (c=0.77, $CHCl_3$). ^{1}H-NMR (pyridine-d_5): 1.74 (3H, s, –NHCOC$\underline{H}_3$), 4.95 (1H, br s, OH, exchangeable with D_2O), 5.75 (1H, s, H-1), 7.08—7.80 (15H, m, aromatic protons). ^{13}C-NMR: see Table I. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH, NH), 1653 (amide I), 1525 (amide II). TLC: *Rf* 0.05 (solvent A), 0.56 (B), 0.13 (C). *Anal.* Calcd for $C_{27}H_{27}NO_5$: C, 72.79; H, 6.11; N, 3.14. Found: C, 72.50; H, 6.22; N, 3.18.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-4-*O*-trityl-*β*-D-glucopyranose (7)——Benzyl bromide (0.6 ml, 5 mmol) was added dropwise with stirring at 0°C to a suspension of **5** (1 g, 2.24 mmol), BaO (3 g, 19.8 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (1.2 g, 3.8 mmol) in dry DMF (8.5 ml). After being stirred for 24 h at room temperature, the mixture was diluted with $CHCl_3$ and filtered. The filtrate was successively washed with ice-cold dil. HCl, satd. $NaHCO_3$, and H_2O. Desiccation ($MgSO_4$) and removal of the solvent gave a syrupy residue which was crystallized by trituration with MeOH as white needles (0.87 g, 72.3%), mp 199—201.5°C, $[\alpha]_D^{18}$ −36.1° (c=1.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.97 (3H, s, –NHCOC$\underline{H}_3$), 5.34 (1H, s, H-1), 6.26 (1H, d, $J_{NH,2}$=10 Hz, NH, exchangeable with D_2O), 7.00—7.60 (20H, m, aromatic protons). TLC: *Rf* 0.49 (solvent A), 0.84 (B), 0.71 (C). *Anal.* Calcd for $C_{34}H_{33}NO_5$: C, 76.24; H, 6.21; N, 2.61. Found: C, 76.00; H, 6.21; N, 2.72.

2-Acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-3-*O*-trityl-*β*-D-glucopyranose (8)——Compound **6** (300 mg, 0.67 mmol) in DMF (2 ml) was benzylated with benzyl bromide (0.2 ml, 1.67 mmol), BaO (750 mg, 4.95 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (300 mg, 0.95 mmol) as described for the preparation of **7** to afford prisms (273 mg, 75.8%), mp 197.5—199.5°C, $[\alpha]_D^{17}$ −99.3° (c=0.61, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.75 (3H, s, –NHCOC$\underline{H}_3$), 5.30 (1H, s, H-1), 5.86 (1H, d, $J_{NH,2}$=9.6 Hz, NH, exchangeable with D_2O), 7.00—7.56 (20H, m, aromatic protons). TLC: *Rf* 0.42 (solvent A), 0.82 (B), 0.62 (C). *Anal.* Calcd for $C_{34}H_{33}NO_5$: C, 76.24; H, 6.21; N, 2.61. Found: C, 76.39; H, 6.05; N, 2.65.

2-Acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-*β*-D-glucopyranose (9)——A mixture of **7** (500 mg, 0.93 mmol) in 80% (v/v) AcOH (10 ml) was warmed at 80—85°C for 2 h under stirring. After being concentrated to a syrup by repeated co-distillation with toluene, the residue was chromatographed on a column with $CHCl_3$–MeOH (40: 1). The major fraction eluted gave **9** as a hygroscopic amorphous powder (276 mg, 98%), $[\alpha]_D^{20}$ −91.1° (c=1.71, $CHCl_3$) [lit. 8, syrup, $[\alpha]_D^{21}$ −88° (c=0.86, $CHCl_3$)]. ^{1}H-NMR (pyridine-d_5): 1.99 (3H, s, –NHCOC$\underline{H}_3$), 5.72 (1H, s, H-1), 7.04—7.64 (5H, m, aromatic protons), 7.93 (1H, d, $J_{NH,2}$=10 Hz, NH, exchangeable with D_2O). ^{13}C-NMR: see Table I. IR ν_{max}^{KBr} cm^{-1}: 3340 (OH, NH), 1645 (amide I), 1532 (amide II). TLC: *Rf* 0.04 (solvent A), 0.42 (B), 0.12 (C). The product was indistinguishable from authentic 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-*β*-D-glucopyranose[8] by IR and TLC.

2-Acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-*β*-D-glucopyranose (10)——Compound **8** (273 mg, 5.1 mmol) was treated with 80% AcOH (5 ml) as described for the preparation of **9**. The detritylated product was chromatographed on a column with $CHCl_3$–MeOH (30: 1). The major fraction eluted gave **10** as a syrup, which was crystallized by treatment with AcOEt–hexane as white needles (143 mg, 96%), mp 120—121°C, $[\alpha]_D^{17}$ −31.4° (c=0.61, $CHCl_3$). ^{1}H-NMR (pyridine-d_5): 1.99 (3H, s, –NHCOC$\underline{H}_3$), 5.76 (1H, s, H-1), 7.24—7.52 (5H, m, aromatic protons), 7.89 (1H, d, $J_{NH,2}$=10 Hz, NH, exchangeable with D_2O). ^{13}C-NMR: see Table I. IR ν_{max}^{KBr} cm^{-1}: 3310 (NH), 3180 (OH), 1650 (amide I), 1550 (amide II). TLC: *Rf* 0.06 (solvent A), 0.40 (B), 0.10 (C). *Anal.* Calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.42; H, 6.61; N, 4.80.

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