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Chemical Modification of Maltose. VII.[1] Synthesis of 4-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-D-glucopyranose (GlcNAcα1→4Glc)[2]

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1,6-Anhydro-2,3,3′-tri-*O*-benzyl-4′,6′-*O*-benzylidene-β-maltose (**5**), a maltose derivative having only one unprotected hydroxyl group at the C-2′ position, was synthesized from 1,6-anhydro-4′,6′-*O*-benzylidene-2′-*O*-tosyl-β-maltose (**2**) by benzylation followed by removal of the tosyl group with base. Compound **5** was converted into the corresponding ulose (**7**) by dimethylsulfoxide (DMSO)–Ac_2O oxidation. Treatment of **7** with hydroxylamine gave the 2′-oxime (**9**). Reduction of **9** with $LiAlH_4$ in ether and subsequent *N*-acetylation gave protected 1,6-anhydro-β-*N*-acetyl-glucosaminylglucose (**12**) and -mannosaminylglucose (**13**) in a yield ratio of *ca.* 6: 1. Debenzylidenation followed by debenzylation of **12** or **13** gave 1,6-anhydro-β-*N*-acetyl-glucosaminylglucose (**17**) or -mannosaminylglucose (**19**). The title sugar was obtained as white prisms by acetolysis of the 1,6-anhydro-β-linkage of peracetylated **17**, followed by de-*O*-acetylation.

Keywords——maltose; maltosan; 1,6-anhydroaminodisaccharide; GlcNAcα1→4Glc; ManNAcα1→4Glc; ^{1}H-NMR; ^{13}C-NMR; DMSO–Ac_2O oxidation

Wolfrom *et al.*[3] isolated two crystalline amino disaccharides from a hydrolyzate of carboxyl-reduced, partially desulfated heparin with hydrochloric acid and identified them as 2-amino-2-deoxy-4-*O*-α-D-glucopyranosyl-D-glucopyranose hydrochloride (maltosamine HCl, Glcα1→4GlcN HCl) and 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-glucopyranose hydrochloride (GlcNα1→4Glc HCl). However, the specific rotation and melting point reported for the *N*-acetyl derivative of the former *N*-acetylmaltosamine, Glcα1→4GlcNAc) were very different from those for *N*-acetylmaltosamine synthesized enzymically[4] or chemically.[5]

In Part V[6] of this series, the authors synthesized Glcα1→4GlcNAc from 1,6-anhydro-β-maltose and pointed out that the values of specific rotation and melting point of our sample were approximately consistent with those for the sample synthesized by Sinaÿ *et al.*,[5] but not with those of the material isolated from heparin hydrolyzate. In this paper, in order to ascertain the structure of the second amino disaccharide reported by Wolfrom *et al.*,[3] we describe an unequivocal synthesis of the *N*-acetyl derivative of GlcNα1→4Glc (GlcNAcα1→4Glc), a structural isomer of Glcα1→4GlcNAc, from 1,6-anhydro-β-maltose.

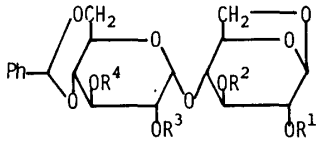
Synthesis of 1,6-Anhydro-2,3,3′-tri-*O*-benzyl-4′,6′-*O*-benzylidene-β-maltose (5), a Maltose Derivative having One Unprotected Hydroxyl Group at the C-2′ Position

In Part VI[1] of this series, we reported that tosylation of 1,6-anhydro-4′,6′-*O*-benzylidene-β-maltose (**1**)[7] using phase transfer catalysis gave the corresponding 2′-monotosylate (**2**) selectively in 64.2% yield. Subsequent benzylation of **2** with benzyl bromide and base in *N*,*N*-dimethylformamide (DMF) gave the tribenzylether (**3**) as the major product (80.6%) with a small amount (7.4%) of dianhydromaltose (**4**).

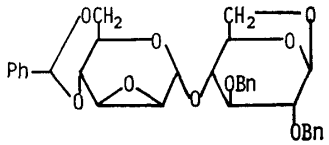
Compound **4** was crystallized from ether. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, the H-1 signal of the D-glucose (Glc) moiety bearing the 1,6-anhydro-β-linkage was observed at 5.46 ppm as a broad singlet, while a singlet at 5.00 ppm was assigned to H-1 of the benzylidene sugar moiety (H-1′). By reference to the reported chemical shifts of 2,3-anhydroglycopyranosides,[8] **4** was assigned as 1,6-anhydro-4-*O*-(2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranosyl)-2,3-di-*O*-benzyl-β-D-glucopyranose. Because this benzylation is carried

out under basic conditions, minor formation of the epoxide having D-*manno* configuration in the Glc moiety bearing the benzylidene group, in which the tosyl at C-2′ and the hydroxyl groups at C-3′ are located in a *trans* orientation, is in accord with expectation.

The tosyl group of **3** was removed by alkaline hydrolysis as used for an analogous tosylate[6] to afford the title compound of this section in 91% yield. Compound **5** was crystallized as white needles. In the infrared (IR) spectrum of **5**, an absorption due to the newly introduced hydroxyl group was seen, while that of the sulfonyl group, which was observed in the IR of **4**, was no longer present. Acetylation of **5** gave the monoacetate (**6**). The ^{1}H-NMR spectrum and elemental analyses of **6** were in good agreement with the assigned composition.



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	H	Ts	H
3	Bn	Bn	Ts	Bn
5	Bn	Bn	H	Bn
6	Bn	Bn	Ac	Bn



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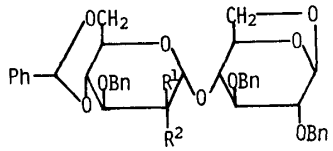
Ac=acetyl
Bn=benzyl
Ts=*p*-toluenesulfonyl

Chart 1

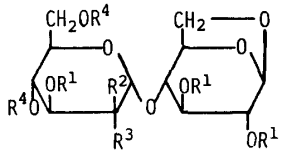
Synthesis of 1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-oxyimino-α-D-*arabino*-hexopyranosyl)-β-D-glucopyranose (9) *via* the Corresponding Ulose (7)

The unprotected hydroxyl group of **5** at the C-2′ position of maltose was selectively oxidized to ketone by dimethyl sulfoxide-acetic anhydride (DMSO–Ac_2O) reagent using a slightly modified procedure based on that used for the oxidation of the amino disaccharide derivative.[9] The protected ulose (**7**), 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-*arabino*-hexopyranosyl-2-ulose)-β-D-glucopyranose, was isolated in 87% yield as white needles. Thin-layer chromatography (TLC) of **7** showed two closely migrating spots: absorption of moisture probably occurred to form the hydrate (**8**) in the course of development on the plates.[10]

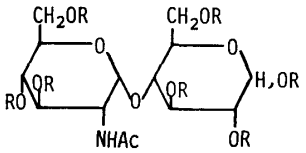
Treatment of the ulose (**7**) with hydroxylamine hydrochloride in pyridine–ethanol by reference to procedures for analogous compounds[11] gave the oxime (**9**) in 88.3% yield as an amorphous powder.



	R^1	R^2
7	O	
8	OH	OH
9	NOH	
10	H	NH_2
11	NH_2	H
12	H	NHAc
13	NHAc	H



	R^1	R^2	R^3	R^4
14	Bn	H	NHAc	H
15	Bn	H	NHAc	Ac
16	Ac	H	NHAc	Ac
17	H	H	NHAc	H
18	Ac	NHAc	H	Ac
19	H	NHAc	H	H



20 : R=Ac (α)
21 : R=Ac (β)
22 : R=H

Chart 2

Syntheses of the Isomeric Protected Acetamido Disaccharides (GlcNAcα1→4Glc and ManNAcα1→4Glc)

The oxyimino group of the oxime (**9**) was reduced with lithium aluminum hydride in ether to an amino group without affecting other protecting groups of **9**. The resultant reduction product was shown to be a mixture of two isomers, protected GlcNα1→4Glc (**10**) and 4-*O*-(2-amino-2-deoxy-α-D-mannopyranosyl)-D-glucopyranose (**11**, ManNα1→4Glc), on TLC. On column chromatography of the mixture, the former (52.8%), crystallizable as prisms, was isolated from the faster eluting fractions, and the latter (8.8%) was isolated from the subsequent fractions as an amorphous powder. They were respectively converted to the protected acetamido disaccharides, GlcNAcα1→4Glc (**12**) and ManNAcα1→4Glc (**13**). The configurations of amino groups in **10** and **11**, and those of acetamido groups in **12** and **13** were tentatively determined by ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopies of the deprotected acetamido disaccharides (**17** and **19**) derived from **12** and **13** by unequivocal synthetic routes as follows.

TABLE I. Physical Constants and ^{1}H-NMR Spectral Data of a Series of GlcNAc-Glc and ManNAc-Glc Derivatives

Compd. No.	mp (°C)	Specific rotation	Solvent	^{1}H-NMR δ (ppm) H-1	H-1′	OAc and/or NAc	NHAc[a]	C_6H_5CH
12	192—195	$[\alpha]_D^{23}$ +9.7° (c=0.60, $CHCl_3$)	$CDCl_3$	5.54 (1H, br s)	[b]	1.59 (3H, s)	5.83 (1H, d, J=9 Hz)	5.59 (1H, s)
13	Amorph.	$[\alpha]_D^{23}$ −31.3° (c=0.30, $CHCl_3$)	$CDCl_3$	5.46 (1H, br s)	[b]	2.04 (3H, s)	5.85 (1H, d, J=7 Hz)	5.59 (1H, s)
16	164—166	$[\alpha]_D^{24}$ +30.0° (c=0.50, $CHCl_3$)	$CHCl_3$	5.50 (1H, br s)	5.05 (1H, d, J=4 Hz)	2.00, 2.06, 2.10, 2.13 2.20 (18H, all s)	5.93 (1H, d, J=10 Hz)	—
18	101—103	$[\alpha]_D^{22}$ +6.0° (c=0.86, $CHCl_3$)	$CDCl_3$	5.50 (1H, br s)	5.41 (1H, d, J=4 Hz)	2.00, 2.07, 2.08, 2.12 2.20 (18H, all s)	6.07 (1H, d, J=8 Hz)	—
17	Amorph.	$[\alpha]_D^{23}$ +106.0° (c=0.87, MeOH)	py.-d_5	5.90 (1H, br s)	5.82 (1H, d, J=3.5 Hz)	2.12 (3H, s)	8.59 (1H, d, J=8 Hz)	—
19	Amorph.	$[\alpha]_D^{21}$ +34.3° (c=0.22, MeOH)	py.-d_5	5.79 (1H, br s)	5.74 (1H, d, J=2 Hz)	2.04 (3H, s)	8.57 (1H, d, J=8 Hz)	—

a) Exchangeable with D_2O.
b) Not observed : overlapped by other signals.

TABLE II. ^{13}C-NMR Chemical Shifts of 1,6-Anhydro-aminodisaccharides (**17** and **19**) and Related Compounds: δ (ppm) from TMS in Pyridine-d_5

Compounds	α-D-Glycosyl moiety C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	1,6-Anhydrosugar moiety C-1	C-2	C-3	C-4	C-5	C-6	NHCOCH$_3$ CO	CH$_3$	OCH$_3$
Me α-Glc[a]	101.3	73.7	75.3	72.0	74.0	62.8									55.0
α-GlcNAc[b]	92.1	55.3	72.0	71.4	72.8	61.9							175.7	23.3	
Me α-Man[c]	102.6	72.0	73.1	69.0	75.1	63.1									—
α-ManNAc[d]	94.3	54.4	70.1	68.0	73.2	61.7							175.9	23.2	
β-GlcAn[e]							104.0	73.3	75.2	73.6	78.1	66.3			
α-Glc-β-GlcAn[f]	99.9	73.3	75.3	72.1	74.9	62.9	104.0	73.7	72.1	79.1	76.7	66.4			
α-GlcNAc-β-GlcAn[g] (**17**)	97.7	55.3	72.7	71.3	74.9	62.7	103.5	73.0	72.7	77.7	76.4	66.0	171.2	23.1	
α-ManNAc-β-GlcAn[h] (**19**)	100.8	54.5	70.4	69.2	73.8	62.6	104.5	74.5	73.8	80.3	76.7	67.0	171.3	23.0	

a) Methyl α-D-glucopyranoside.
b) 2-Acetamido-2-deoxy-α-D-glucopyranose : data from ref. 16 (measured in D_2O).
c) Methyl α-D-mannopyranoside : data from ref. 17
d) 2-Acetamido-2-deoxy-α-D-mannopyranose : data from ref. 16 (measured in D_2O).
e) 1,6-Anhydro-β-D-glucopyranose.
f) 1,6-Anhydro-4-*O*-α-D-glucopyranosyl-β-D-glucopyranose (1,6-anhydro-β-maltose).
g) 1,6-Anhydro-4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose.
h) 1,6-Anhydro-4-*O*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)-β-D-glucopyranose.

The benzylidene group of **12** was smoothly removed by heating with aqueous acetic acid to give **14**, and subsequent acetylation of **14** yielded the crystalline diacetate (**15**). Hydrogenolytic debenzylation of **15**, followed by acetylation, gave the fully acetylated 1,6-anhydro-β-derivative of GlcNAcα1→4Glc (**16**) as needles. Compound **16** was also obtained directly from **12** by simultaneous hydrogenolytic debenzylidenation and debenzylation followed by acetylation. Deacetylation of **16** provided the 1,6-anhydro-β-derivative of GlcNAcα1→4Glc (**17**) as a hygroscopic amorphous powder.

The 1,6-anhydro-β-derivative of ManNAcα1→4Glc (**19**) was similarly obtained as an amorphous powder by removal of the protecting groups from **13**. In **17** and **19**, the conformations of H-1′ and H-2′ are in equatorial–axial and *trans*-diequatorial orientations, respectively. Therefore, the observed small coupling constants (**17**: $J_{1',2'}$=3.5 Hz, **19**: $J_{1',2'}$=2 Hz) are in accord with expectation. In addition, these individual values are in good agreement with the reported values for alkyl α-D-glucopyranosides[14,15] and -D-mannopyranosides,[15] respectively. The data are listed in Table I.

The ^{13}C-NMR spectra of **17** and **19** were measured in pyridine-d_5 at room temperature. The chemical shifts of the individual carbons were assigned by comparison with the observed

TABLE III. Comparison of the Acid Hydrolyzates of **17** or **19** with Reference Component Monosaccharides on TLC[a]

Samples	*Rf* values of spots [solvent : phenol–1% NH_4OH (2:1v/v)]		
D-Glucose	0.37[b]		
D-Glucosamine·HCl		0.56[b,c]	
D-Mannosamine·HCl			0.60[b,c]
Acid hydrolyzate of **17**	0.36[b]	0.57[b,c]	
Acid hydrolyzate of **19**	0.37[b]		0.61[b,c]

a) TLC on precoated microcrystalline cellulose plates 0.25 mm thick (Avicel SF, Funakoshi Yakuhin Ltd., Tokyo).
b) Detectable with alkaline silver nitrate.
c) Detectable with 0.2% ninhydrin in aq. BuOH.

TABLE IV. Physical Constants and Elemental Analyses of the Products

Compd. No.	Cryst. form	mp (°C) (recrystn. solvent)	Specific rotation (in $CHCl_3$)	Formula	Analysis (%) Calcd (Found) C	H	N
3	White needles	118—120 (ether-hexane)	$[\alpha]_D^{19}$ −36.8° (c=1.1)	$C_{47}H_{44}O_{12}S$	67.44 (67.61	5.78 5.56	— —)
4	Cryst. powder	138—140 (ether)	$[\alpha]_D^{18}$ +26.1° (c=0.68)	$C_{33}H_{34}O_9$	68.98 (68.86	5.96 5.81	— —)
5	White needles	120—122 (ether-hexane)	$[\alpha]_D^{22}$ +3.5° (c=1.01)	$C_{40}H_{42}O_{10}$	70.37 (70.23	6.20 5.99	— —)
6	Glass	—	$[\alpha]_D^{21}$ +28.9° (c=0.38)	$C_{42}H_{44}O_{11}$	69.60 (69.32	6.12 6.12	— —)
7	White needles	109—110 (EtOH)	$[\alpha]_D^{23}$ −37.3° (c=0.66)	$C_{40}H_{46}O_{10}$	70.57 (70.56	5.92 5.87	— —)
9	Amorph.	—	$[\alpha]_D^{23}$ −45.6° (c=0.52)	$C_{40}H_{41}NO_{10}$	69.05 (69.32	5.94 5.95	2.01 2.25)
10	White prisms	135—136 (ether-hexane)	$[\alpha]_D^{19}$ +2.4° (c=0.52)	$C_{40}H_{43}NO_9$	70.46 (70.27	6.36 6.57	2.05 2.13)
11	Amorph.	—	$[\alpha]_D^{21}$ −13.8° (c=0.66)	$C_{40}H_{43}NO_9$	70.46 (70.96	6.36 6.75	2.05 1.79)
14	White needles	176—178 (AcOEt)	$[\alpha]_D^{20}$ +17.1° (c=0.91)	$C_{35}H_{41}NO_{10}$	66.13 (65.53	6.51 6.73	2.20 2.25)
15	White needles	165—167 (EtOH)	$[\alpha]_D^{20}$ +21.1° (c=0.84)	$C_{39}H_{45}NO_{12}$	65.08 (64.88	6.30 6.54	1.95 1.83)

values for 1,6-anhydro-β-maltose,[6] GlcNAc,[16] and ManNAc.[16] The data are listed in Table II.

The configuration of the amino group was finally ascertained by identification of the component amino monosaccharides. Thus, in the hydrochloric acid hydrolyzates of **17** and **19**, GlcN·HCl and ManN·HCl were respectively identified on TLC as component amino monosaccharides. The *Rf* values of reference compounds are listed in Table III.

Synthesis of GlcNAcα1→4Glc (22)

The 1,6-anhydro-β-linkage of **16** was acetolyzed with a cold acetolysis mixture to give the anomeric mixture of hepta-*O*-acetyl-acetamido disaccharides (**20**, α-acetate; **21**; β-acetate) in 56% yield. On column re-chromatography of the mixture, **20** was isolated in 34.1% yield from the faster eluting fractions. From the subsequent fractions (after **20** had emerged), **21** was isolated in 64.4% yield. The values of specific rotations and coupling constants ($J_{1,2}$) of **20** and **21** were respectively consistent with the assigned acetoxyl configurations at C-1.

De-*O*-acetylation of the anomeric mixture (**20** and **21**) provided the title acetamido disaccharide (**22**) in 64.3% yield. The product was crystallized as white prisms having mp 215—217°C and $[\alpha]_D^{20}$ +141° (H_2O). These values were not in agreement with those [mp 124—125°C, $[\alpha]_D^{20}$ +129°→+75° (H_2O)] reported by Wolfrom *et al.* for GlcNAcα1→4Glc.

Experimental[18]

1,6-Anhydro-2,3,3′-tri-*O*-benzyl-4′,6′-*O*-benzylidene-2′-*O*-(*p*-toluenesulfonyl)-β-maltose (3)——Benzyl bromide (10 ml, 84 mmol) was added under stirring to a suspension of **2**[21] (2 g, 3.53 mmol), BaO (8.8 g, 57 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (3.5 g, 11 mmol) in DMF (100 ml). The mixture was stirred vigorously at 0°C for 2 h, then stirring was continued at 10°C for a further 40 h. The mixture was diluted with $CHCl_3$ (200 ml), poured into ice–H_2O (300 ml), stirred for 2 h, and then filtered. The residue was washed with $CHCl_3$ (30 ml×2), and the combined filtrate and washings were successively washed with 10% HCl (50 ml×4) and H_2O (50 ml×2), then dried ($MgSO_4$), and concentrated to a syrup. The residue was chromatographed on a column of silica gel with hexane–ether (2: 1). From the faster eluting fractions, **4** (0.15 g, 7.4%) was isolated. The product was crystallized from ether as a crystalline powder. ^{1}H-NMR ($CDCl_3$): 5.00 (1H, s, H-1 of α-Man), 5.46 (1H, s, H-1 of β-Glc), 5.55 (1H , s, $C_6H_5C\underline{H}$), 7.26—7.42 (15H, aromatic protons).

From the subsequent major fractions containing a product with *Rf* 0.27 [solvent: hexane–ether (1: 3)], **3** (2.37 g, 80.6%) was isolated. The product was crystallized from ether–hexane as white needles. ^{1}H-NMR ($CDCl_3$): 2.32 (3H, s, $C\underline{H}_3C_6H_4SO_2$), 5.37 (1H, d, $J_{1,2}$=3.5 Hz, H-1 of α-Glc), 5.49 (2H, s, H-1 of β-Glc and $C_6H_5C\underline{H}$), 7.02—7.74 (24H, aromatic protons). IR ν_{max}^{Nujol} cm^{-1}: 1594 (C=C), 1176 (SO_2).

1,6-Anhydro-2,3,3′-tri-*O*-benzyl-4′,6′-*O*-benzylidene-β-maltose (5)——A mixture of **3** (730 mg, 0.87 mmol) and 8 N KOH (15 ml) in dioxane (40 ml) and MeOH (20 ml) was gently heated to reflux for 5 h. The mixture was concentrated to half the initial volume, then extracted with CH_2Cl_2 (20 ml×3). The combined extracts were washed with H_2O (30 ml×3), dried ($MgSO_4$), and concentrated to dryness. The residue was chromatographed on a column of silica gel with hexane–ether (1: 1). From the major fractions, **5** (540 mg, 90.8%) was isolated. The product was crystallized from ether–hexane as white needles. IR ν_{max}^{Nujol} cm^{-1}: 3400—3480 (OH), no absorption due to sulfonate.

1,6-Anhydro-2′-*O*-acetyl-2,3,3′-tri-*O*-benzyl-4′,6′-*O*-benzylidene-β-maltose (6)——Acetylation of **5** (50 mg, 0.073 mmol) with Ac_2O and pyridine (each 1 ml) under stirring was carried out first at 0°C and then at room temperature overnight. The mixture was concentrated by repeated co-distillation with toluene to give a syrup, which was chromatographed on a column of silica gel with CH_2Cl_2–acetone (60: 1). From the major fractions, **6** (45.6 mg, 86%) was isolated as a glass. ^{1}H-NMR ($CDCl_3$): 1.84 (3H, s, OAc), 5.23 (1H, d, $J_{1',2'}$=4 Hz, H-1 of α-Glc), 5.47 (1H, s, H-1 of β-Glc), 5.56 (1H, s, $C_6H_5C\underline{H}$), 7.18—7.46 (20H, aromatic protons).

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-*arabino*-2-hexulopyranosyl)-β-D-glucopyranose (7)——A solution of **5** (1 g, 1.46 mmol) in DMSO–Ac_2O (2: 1, v/v, 45 ml) was kept for 4 d at room temperature. The mixture was concentrated to a syrup by repeated co-distillation with toluene. After trituration of the residue with H_2O (30 ml) and $CHCl_3$ (50 ml), the separated organic layer was washed with H_2O (30 ml×3), dried ($MgSO_4$), and concentrated to a syrup. On silica gel column chromatography with hexane–ether (1: 1), **7** (0.87 g, 87%) was isolated. The product was crystallized from EtOH as white needles. TLC showed two closely migrating spots, probably corresponding to ulose (**7**) and its hydrate (**8**). IR ν_{max}^{Nujol} cm^{-1}: 1743 (C=O). ^{1}H-NMR ($CDCl_3$): 5.43 (1H, s, H-1 of β-Glc), 5.51 (1H, s, $C_6H_5C\underline{H}$), 7.18—7.46 (20H, aromatic protons).

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-oximino-α-D-*arabino*-hexopyranosyl)-β-D-glucopyranose (9)——Compound **7** (1.25 g, 1.84 mmol) was added at 25°C to a solution of

$NH_2OH \cdot HCl$ (1.2 g, 17.3 mmol) in a mixture of pyridine and EtOH (each 36 ml). After standing at 25°C for 30 min, the mixture was heated to reflux on a steam bath for 3 h, and then concentrated to a syrup. The residue was triturated with H_2O, and the whole was extracted with ether (30 ml × 3). The combined extracts were successively washed with ice-cold 10% H_2SO_4 (20 ml × 2), H_2O (20 ml), aq. $NaHCO_3$ (20 ml), and H_2O (20 ml × 2), and then dried ($MgSO_4$). Removal of the solvent gave a syrup, which was chromatographed on a column of silica gel with hexane–ether (1: 1) to give pure **9** (1.13 g, 88.3%) as an amorphous powder. IR ν_{max}^{Nujol} cm^{-1}: 3290 (OH), 1705 (C=N). ^{1}H-NMR ($CDCl_3$): 5.46 (1H, s, H-1 of β-Glc), 5.56 (1H, s, C_6H_5CH), 6.18 (1H, s, H-1 of ulose), 7.18—7.42 (20H, aromatic protons).

Reduction of the Oxime (9) with Lithium Aluminum Hydride——$LiAlH_4$ (340 mg, 8.96 mmol) was added in small portions to a solution of **9** (580 mg, 0.83 mmol) in dry ether (60 ml). The mixture was heated to reflux gently over a steam bath for 80 min. Excess $LiAlH_4$ was then completely decomposed by successive careful additions of AcOEt (20 ml) and ice–H_2O (5ml) at 0°C. The mixture was stirred for 30 min, and the precipitated salts were removed by filtration. The residue was washed with ether (20 ml × 2). The combined filtrate and washings were concentrated to a thin syrup, which was dissolved in CH_2Cl_2 (40 ml). The solution was washed with H_2O (20 ml × 2), dried ($MgSO_4$), and concentrated to a syrup. On silica gel column chromatography, 1,6-anhydro-2,3-di-*O*-benzyl 4-*O*-(2-amino-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (**10**, 300 mg, 52.8%) was isolated from the major fractions containing a product with *Rf* 0.15 [solvent: benzene–ether (1: 3)]. The product was crystallized from ether–hexane as white prisms. IR ν_{max}^{Nujol} cm^{-1}: 3350, 3280 (NH_2).

From the subsequent fractions containing a product with *Rf* 0.04, 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2-amino-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranosyl)-β-D-glucopyranose (**11**, 49 mg, 8.8%) was isolated as an amorphous powder.

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (12)——Acetylation of **10** (167 mg, 0.24 mmol) with Ac_2O and pyridine (each 1.5 ml) was carried out as described for the preparation of **6**. The crude acetate was purified by silica gel column chromatography with CH_2Cl_2–acetone (40: 1) to yield pure **12** (167 mg, 94.4%), which was crystallized from EtOH. IR ν_{max}^{Nujol} cm^{-1}: 3290 (NH), 1643 (amide I), 1540 (amide II). *Anal.* Calcd for $C_{42}H_{45}NO_{10}$: C, 69.69; H, 6.27; N, 1.94. Found: C, 69.67; H, 6.57; N, 1.82.

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranosyl)-β-D-glucopyranose (13)——Acetylation of **11** (48 mg, 0.07 mmol) as described for the preparation of **12** gave **13** (44.1 mg, 87%) as an amorphous powder. IR ν_{max}^{Nujol} cm^{-1}: 3430 (NH), 1670 (amide I), 1496 (amide II). *Anal.* Calcd for $C_{42}H_{45}NO_{10}$: C, 69.69; H, 6.27; N, 1.94. Found: C, 69.98; H, 6.40; N, 1.93.

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (14)——A suspension of **12** (167 mg, 0.23 mmol) in 80% (v/v) AcOH (10 ml) was heated on a steam bath for 2 h to carry out debenzylidenation. The mixture was concentrated to dryness, and the residue was chromatographed on a column of silica gel with CH_2Cl_2–acetone mixtures (10: 1, 150 ml; 5: 1, 100 ml; 3: 1, 100 ml) to isolate pure **14** (124 mg, 86.5%), which was crystallized from AcOEt as white needles.

1,6-Anhydro-2,3-di-*O*-benzyl-4-*O*-(2-acetamido-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (15)——Acetylation of **14** (115 mg, 0.18 mmol) with Ac_2O and pyridine (each 1 ml) as described for the preparation of **6**, followed by column chromatographic purification with CH_2Cl_2–acetone (30: 1), yielded **15** (122 mg, 93.8%). The product was crystallized from EtOH as white needles. ^{1}H-NMR ($CDCl_3$): 1.55, 2.00, 2.08 (9H, each s, 2 × OAc, NAc), 5.58 (1H, s, H-1 of β-Glc), 5.76 (1H, d, J=8 Hz, NHAc, exchangeable with D_2O).

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (16)——A solution of **15** (105 mg, 0.15 mmol) in AcOH (10 ml) was hydrogenated with a Pd catalyst at room temperature under atmospheric pressure for 40 h; the catalyst was freshly prepared from $PdCl_2$ (100 mg) by the method of Schmidt and Staab.[19] The catalyst was removed by filtration and washed with AcOH (5 ml), and the combined filtrate and washings were concentrated to a syrup. Subsequent acetylation of the syrup with Ac_2O and pyridine (each 5 ml) gave **16** (68 mg, 81.8%), which was crystallized from benzene as white needles.

Compound **16** (190 mg, 95.6%) was also obtained from **12** (250 mg, 0.35 mmol) in AcOH (25 ml) by hydrogenolytic debenzylidenation and debenzylation with a Pd catalyst followed by acetylation. *Anal.* Calcd for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.78; N, 2.43. Found: C, 49.93; H, 5.66; N, 2.46.

1,6-Anhydro-4-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (17)——A 0.5 N methanolic solution of MeONa (0.3 ml) was added to a suspension of **16** (110 mg, 0.19 mmol) in MeOH (10 ml), and the mixture was stirred at room temperature for 5 h with exclusion of moisture. After neutralization with Amberlite IR-120 (H^+) resin, the filtrate (free from the resin) was concentrated to dryness to yield **17** (65.1 mg, 93.5%) as a hygroscopic amorphous powder.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranosyl)-β-D-glucopyranose (18)——A solution of **13** (79 mg, 0.11 mmol) in AcOH (8 ml) was hydrogenated with a Pd catalyst. Subsequent acetylation as described for the preparation of **16** yielded **18** (50 mg, 79.6%). The product was crystallized from EtOH as colorless prisms. *Anal.* Calcd for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.78; N, 2.43. Found: C, 49.66; H, 5.72; N, 2.50.

1,6-Anhydro-4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-β-D-glucopyranose (19)——De-*O*-acetylation of **18** (35 mg, 0.06 mmol) in MeOH (3 ml) with 0.5 N methanolic solution of MeONa (0.1 ml) was carried out as described for the preparation of **17** to yield **25** (20 mg, 89%) as an amorphous powder.

Identification of the Component Monosaccharides in 17 and 19——Amino disaccharides (**17** and **19**, each 1 mg) in 1 N HCl (0.5 ml) were heated at 95°C for 7 h. The mixture was concentrated to dryness by repeated co-distillation with MeOH. The hydrolyzates were chromatographed on cellulose plates. The results are summarized in Table III.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-α- and β-D-glucopyranoses (20 and 21)——Compound **16** (200 mg, 0.35 mmol) was added in small portions to an ice-cold acetolysis mixture [6 ml, H_2SO_4–Ac_2O–AcOH (1: 70: 30, v/v)] at 0°C under stirring. After being stirred at 5°C for 2 h, the mixture was poured into ice–H_2O (50 ml) under stirring, stirred for a further 2 h, and then neutralized with $NaHCO_3$. The whole was extracted with CH_2Cl_2 (15 ml×2). The combined extracts were washed with H_2O, dried ($MgSO_4$), and concentrated to a syrup, which was chromatographed on a column of silica gel with CH_2Cl_2–acetone (10: 1) to yield a mixture of **20** and **21** (132 mg, 56%) as an amorphous powder. *Anal.* Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.48; H, 5.72; N, 2.05.

The anomeric mixture in CH_2Cl_2 (3 ml) was re-chromatographed with CH_2Cl_2–acetone (20: 1). From the faster eluting fractions, the α-anomer (**20**, 45 mg, 34.1%) was isolated. Crystals, mp 89—91°C and $[\alpha]_D^{21}$ +114.2° (c=0.56, $CHCl_3$), were obtained from ether–hexane. ^{1}H-NMR ($CDCl_3$): 1.96, 2.00, 2.01, 2.02, 2.03, 2.10, 2.14, 2.25 (24H, each s, 7×OAc, NAc), 5.59 (1H, d, J=9 Hz, NHAc), 6.31 (1H, d, $J_{1,2}$=4 Hz, H-1 of α-Glc).

From the subsequent fractions (after **20** had emerged), the β-anomer (**21**, 85 mg, 64.4%) was isolated as an amorphous powder, $[\alpha]_D^{21}$ +53° (c=1.04, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.96, 2.04, 2.05, 2.12, 2.13, 2.16 (24H, each s, 7×OAc, NAc), 5.72 (1H, d, J=9 Hz, NHAc), 5.81 (1H, d, $J_{1,2}$=8 Hz, H-1 of β-Glc).

4-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-D-glucopyranose (22)——De-*O*-acetylation of the anomeric mixture (**20** and **21**, 55 mg, 0.08 mmol) in MeOH (3 ml) with a 0.5 N methanolic solution of MeONa (0.1 ml) was carried out as described for the preparation of **17** to yield **22**. The product was crystallized from EtOH as white prisms (20 mg, 64.3%), mp 215—217°C, $[\alpha]_D^{20}$ +141° (c=0.2, H_2O). *Anal.* Calcd for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.65. Found: C, 43.36; H, 6.51; N, 3.86. Paper partition chromatography: *Rf* 0.33 (BuOH–pyridine–H_2O, 6: 4: 3, v/v). *cf.* *Rf* 0.40 (Glc); 0.51 (GlcNAc); 0.30 (Mal); 0.40 (Glcα1→4GlcNAc).

TABLE V. *Rf* Values of the Products on Precoated Silica Gel Plates 0.25 mm Thick (Kieselgel 60F_{254}, Merck)[a]

Compd. No.	Solvent system[b]					
	A	B	C	D	E	F
3	0.72	—	0.27	0.62	—	—
4	0.66	—	0.34	0.60	—	—
5	0.62	—	0.17	0.52	—	—
6	0.67	—	0.29	0.60	—	—
7	0.61	—	0.12	0.42	—	—
9	0.57	—	0.29	0.60	—	—
10	0.28	—	—	0.15	—	—
11	0.15	—	—	0.04	—	—
12	0.42	—	—	0.24	—	—
13	0.25	—	—	0.13	—	—
14	0.01	0.04	—	0.01	0.57	—
15	0.23	0.38	—	0.11	0.75	—
16	0.05	0.10	—	0.01	0.69	—
18	0.06	0.11	—	0.01	0.61	—
20	0.08	0.22	—	0.03	0.72	—
21	0.10	0.16	—	0.05	0.69	—
17	—	—	—	—	—	0.41
19	—	—	—	—	—	0.38
22	—	—	—	—	—	0.27
Maltose	—	—	—	—	—	0.27

a) Detection was effected with H_2SO_4 or by UV irradiation (short wavelength).
b) Solvent systems (v/v): A, CH_2Cl_2–acetone (9:1); B, CH_2Cl_2–acetone (5:1); C, hexane–ether (1:3); D, benzene–ether (1:3); E, $CHCl_3$–MeOH (5:1); F, AcOEt-2-PrOH-H_2O (5:7:3).

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