Practical Syntheses of Immunologically Relevant β-Glycosides of 2-Acetamido-2-deoxy-D-mannopyranose. Methyl *N*-Acetyl-β-D-mannosaminide, *N*-Acetyl-β-D-mannosaminyl-(1→6)-D-galactose, and Methyl *N*-Acetyl-β-D-mannosaminyl-(1→4)-α-D-glucopyranoside

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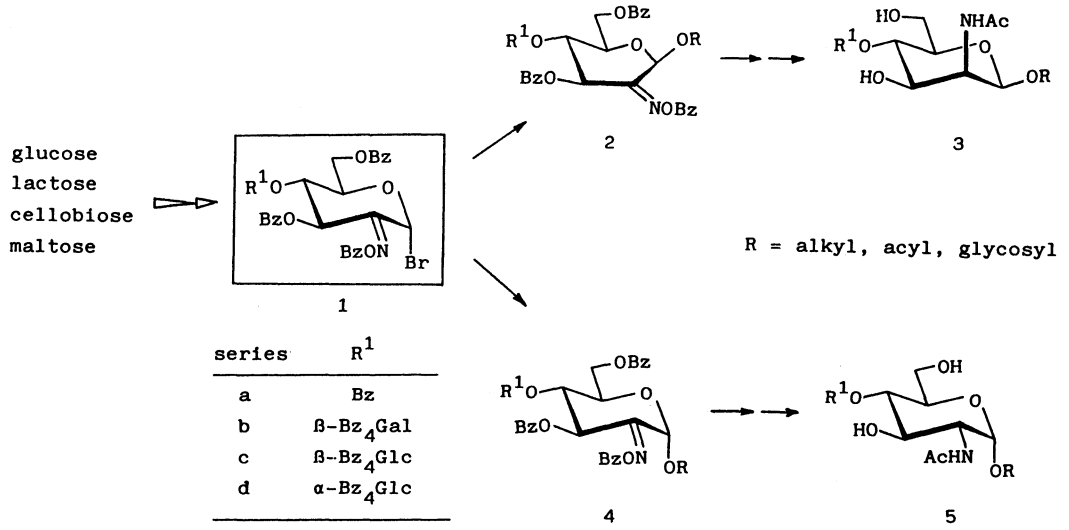
Highly stereoselective, expedient syntheses are described for three immunologically relevant β-glycosides of *N*-acetyl-D-mannosamine, i.e. β-D-Man*p*NAc-1-OMe (**8**), β-D-Man*p*NAc-(1→6)-D-Gal (**15**), and β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-1-OMe (**23**). Basic mannosamine progenitor in each case is 3,4,6-tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranosyl bromide (**1a**), which via the three step-sequence β-selective glycosidation → hydroboration → deblocking is converted to **8**, **15**, and **23** in overall yields of 61, 56, and 39% for the glycosyl acceptors methanol, 1,2:3,4-di-*O*-isopropylidene-α-D-galactose, and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside, respectively.

β-Glycosidically linked 2-acetamido-2-deoxy-D-mannopyranose (*N*-acetyl-β-D-mannosamine) carries a type-specific immunogeneity in various bacteria associated with invasive diseases.[1,2] Even simple alkyl glycosides such as methyl *N*-acetyl-β-D-mannosaminide acts as a specific hapten against the immunoglobulin of IgA class originated from a mouse myeloma MOPC 406;[3] more complex *N*-acetyl-β-D-mannosaminides constitute a number of bacterial K antigens of capsular polysaccharides[2] as well as O antigens of lipopolysaccharides.[4]

Investigations directed towards the molecular design of artificial antigens require as a principal prerequisite preparatively useful, chemical procedures for the practical construction of oligosaccharides containing *N*-acetyl-β-D-mannosamine. Such procedures are still lacking despite of the possibility of using 2-azido-2-deoxy-D-mannopyranosyl halides,[5,6] which are accessible in syrupy form via low yield, multistep reaction sequences, and furthermore, require careful optimization of glycosidation conditions to reach useful β-selectivities.[5] A similarly laborious approach[7] comprises the *a priori* construction of the respective disaccharide for example, and the *a posteriori* introduction of the 2-amino function via oxidation of a 2-hydroxyl group, oximation and subsequent reduction.[7]

Our approach to the problem of a practical synthesis for β-D-mannosaminides, that has been elaborated on lactose-derived derivatives,[8,9] comprises 2-(benzoyloxyimino)glycosyl bromides of type **1** as the key compounds readily accessible from glucose[10,11] or disaccharides[8] in a few high-yielding steps and in crystalline form. These key building blocks **1** are ideal β-D-mannosamine progenitors, since β-selective glycosidation, reduction of the benzoyloxyimino function to amino group, and deprotection yielding β-D-mannosamine-containing oligosaccharides, a protocol, which combines high stereoselectivity with efficiency of operation and large-scale adaptability



Scheme 1.

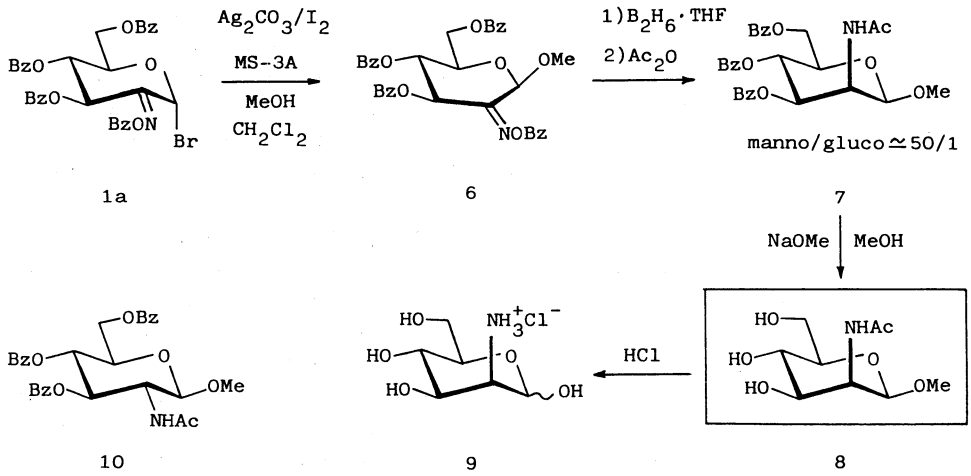
(Scheme 1).

In complementation of previous work concentrating on trisaccharides with central β-D-mannosamine units,[9] we here wish to describe[12] the utilization of the D-glucose-derived monosaccharide building block **1a** for the preparation of disaccharides with a nonreducing *N*-acetyl-β-D-mannosamine residue, of which one, i.e. **23**, constitutes a disaccharide unit of the capsular polysaccharide from *Streptococcus pneumoniae* type 9N[2] and 19F.[13]

Methyl *N*-Acetyl-β-D-mannosaminide (8) A preparatively reliable synthesis of methyl *N*-acetyl-β-D-mannosaminide (**8**), as of now, is not available, since direct glycosidation of 2-acetamido-2-deoxy-D-mannose with methanol in the presence of acid (Fischer method) results in a mixture of the four possible methyl *N*-acetyl-D-mannosaminides from which the β-D-pyranoside (**8**) is isolable in yields of 1—2% only.[14] The major isomers were α-D-pyranoside (9%), α-D-furanoside (8%), and β-D-furanoside (2—3%).

Our synthesis of **8** involves methanolysis of the building block **1a**, reduction of the resultant β-glycoside **6** to mannosaminide, *N*-acetylation, and *O*-debenzoylation, so that the synthetic efficiency depends on the β-selective methanolysis and manno-selective reduction (Scheme 2).

The glycosidation of **1a** with excess methanol (ten-fold molar amount) in the presence of several kinds of condensation promotors are summarized in Table 1. Of a variety of reaction conditions examined, only silver carbonate in dichloromethane resulted in good yield (93%) and high stereoselectivity ($\alpha:\beta \simeq 5:95$). Other conditions employed, such as silver triflate-tetramethylurea or 2,4,6-collidine-I_2 (cf. Runs 2—5,



Scheme 2.

Table 1. Glycosidation of Tri-*O*-benzoyl-2-benzoyloxyimino-2-deoxy-α-D-*arabino*-hexopyranosyl Bromide (**1a**) with Methanol and Partially Protected Sugars

Run	Alcohol	Molar Ratio (Alcohol/**1a**)	Promotor	Solvent	Time / h	Yield / %	α:β[a]
1	MeOH	10.0	Ag_2CO_3/I_2	CH_2Cl_2	72	93	5:95
2	MeOH	10.0	AgOTf/TMU[b]	CH_2Cl_2	24	87	20:80
3	MeOH	10.0	AgOTf/TMU	Dioxane	24	83	40:60
4	MeOH	10.0	2,4,6-Collidine/I_2	Dioxane	72	75	35:65
5	MeOH	10.0	$Hg(CN)_2$	Dioxane	72	17	35:65
6	6-OH Gal iP_2[c]	1.1	Ag_2CO_3/I_2	CH_2Cl_2	24	86	5:95
7	6-OH Gal iP_2	1.1	Ag_2CO_3/I_2	CH_2Cl_2	72	94	5:95
8	6-OH Gal iP_2	1.1	AgOTf/TMU	Dioxane	16	89	70:30
9	4-OH Glc Bz_3[d]	1.1	Ag_2CO_3/I_2	CH_2Cl_2	72	<24	e)
10	4-OH Glc Bz_3	1.1	$AgClO_4$/TMU	CH_2Cl_2	24	<50	e)
11	4-OH Glc Bz_3	1.1	AgOTf/TMU	Dioxane	16	<16	e)
12	4-OH Glc Bn_3[f]	1.1	Ag_2CO_3/I_2	CH_2Cl_2	48	52	5:95
13	4-OH Glc Bn_3	2.0	Ag_2CO_3/I_2	CH_2Cl_2	48	55	5:95
14	4-OH Glc Bn_3	1.1	$AgClO_4$/TMU	CH_2Cl_2	48	<24	e)
15	4-OH Glc Bn_3	1.1	Ag_2O/I_2	CH_2Cl_2	48	<44	e)
16	4-OH Glc Bn_3	1.5	Ag-silicate	CH_2Cl_2	48	<28	e)

a) Estimated on the basis of ^{1}H NMR signals for the anomeric protons. b) Silver trifluoromethanesulfonate/1,1,3,3-tetramethylurea. c) 1,2:3,4-Di-*O*-isopropylidene-α-D-galacto-pyranose (**11**). d) Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**17**). e) Contaminated with by-products that impeded the estimation of α:β ratio. f) Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**16**).

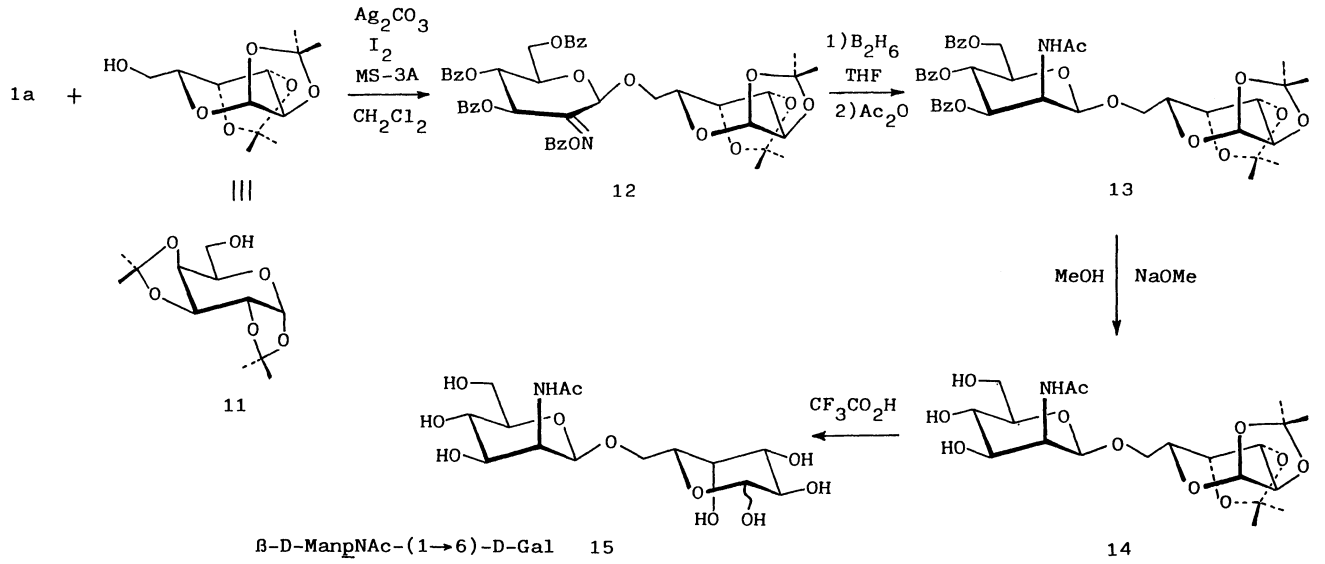
Table 1) gave anomeric mixtures with only partial predominance of β-anomers. β-Glycoside **6** was then subjected to hydroboration with twelve-fold molar excess diborane in tetrahydrofuran to afford on *N*-acetylation methyl *N*-acetyl-tri-*O*-benzoyl-β-D-mannosaminide (**7**) in 88% yield. The corresponding β-D-gluco isomer **10** was isolated in only 1.3% yield, so that the preference for the β-D-manno form is in the remarkable, practically very useful range of around 50:1. The subsequent *O*-debenzoylation of **7** was cleanly effected with 0.05 M sodium methoxide (1 M=1 mol dm^{-3}) in methanol (Zemplén method) to furnish methyl *N*-acetyl-β-D-mannosaminide (**8**) in 87% yield. Furthermore, **8** was hydrolyzed with 1 M hydrochloric acid giving D-mannosamine hydrochloride **9** in quantitative yield, which was unequivocally identified by comparison with an authentic sample.

The following stereochemical aspects are worthy of note: β-oriented methyl 2-(benzoyloxyimino)glycoside **6** seems to be distorted towards the twist-boat form as indicated in the formula considering from relatively small coupling constant of $J_{3,4}=J_{4,5}=5.7$ Hz. In contrast, **1a** and **7** prefer $^4C_1$ coformation, as evidenced by $J_{3,4}$ and $J_{4,5}$ values of 8.4—9.6 Hz. This may be attributed to substantial steric congestion caused by the 2-benzoyloxyimino and anomeric methoxyl groups in **6**. Similar conformational propensity for 2-(benzoyloxyimino)-β-glycosides were observed previously.[8,9]

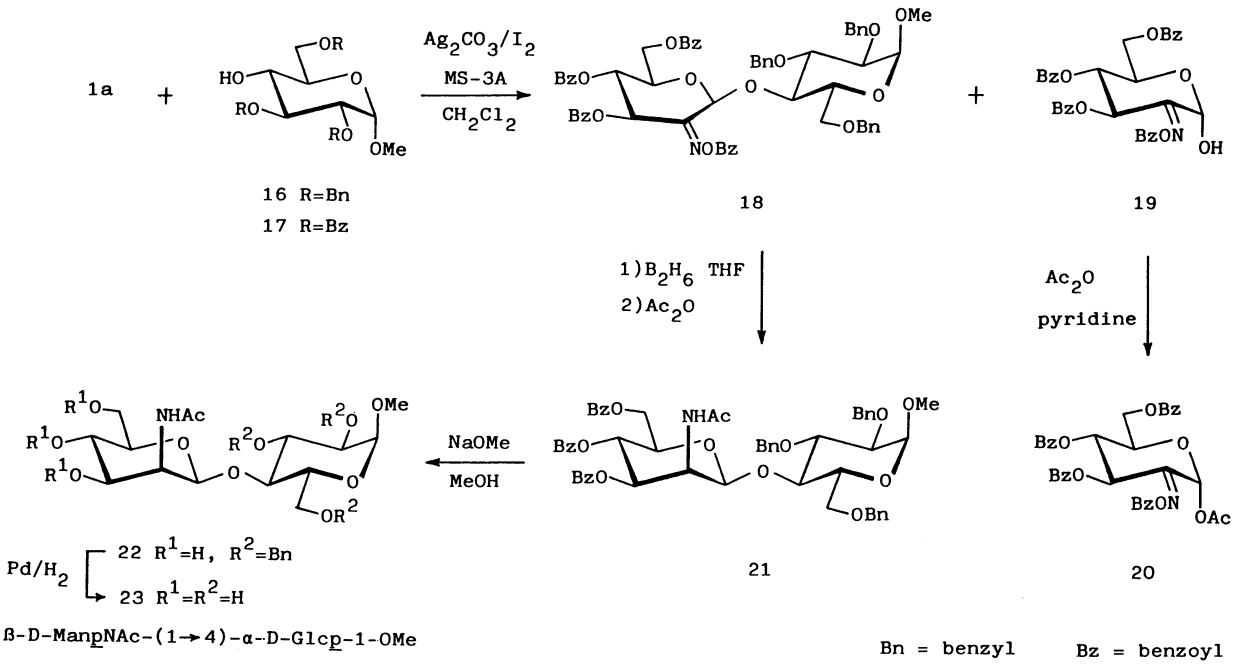
^1H and ^{13}C NMR spectra of methyl β-D-mannosaminide **8** were fully elucidated with the aid of ^1H-^1H and ^1H-^{13}C shift correlated two-dimentional (2D) NMR spectra. ^1H-^1H coupling of $J_{1,2}=1.5$ and $J_{2,3}=4.5$ Hz cogently reflect β-D-manno configuration, and a $J_{C1,H1}$ coupling of 163.6 Hz agrees well with that (ca. 160 Hz) of methyl β-glycopyranosides with axial H-1.[15] A calculation of differences of ^{13}C-chemical shifts ($\Delta\delta_c$) by $\delta_{gluco}-\delta_{manno}$ between methyl *N*-acetyl-β-D-mannosaminide and the corresponding glucosaminide[16] showed that major differences appeared at C-2 ($\Delta\delta_c=-4.1$ ppm) and C-4 (−3.2), whilst they are less pronounced at C-3 (−2.0), C-1 (−1.5), C-5 (+0.5), and C-6 (−0.4). These findings were found to be helpful in the structural elucidation of β-D-mannosamine-containing oligosaccharides.

N-Acetyl-β-D-mannosaminyl-(1→6)-D-galactose (15) and Methyl N-Acetyl-β-D-mannosaminyl-(1→4)-α-D-glucopyranoside (23). Replacement of the glycosyl acceptor in the above glycosidation **1a**→**6** by suitably blocked sugar alcohols similarly gave the desired disaccharide derivatives in preparatively highly useful fashion. Thus, glycosylation of 1,2:3,4-di-*O*-isopropylidene-D-galactose (**11**)[17] with (benzoyloxyimino)glycosyl bromide **1a** in the presence of silver carbonate-iodine in dichloromethane (Run 7 in Table 1) cleanly gave the β-glycoside **12** in the satisfactory yield of 94% (Scheme 3). In contrast, silver triflate in dioxane (Run 8) gave rise to α-predominance (α:β≈70:30), a distinct promotor-dependent inversion, that was also observed in the glycosylation of **11** with the disaccharide donor **1b**.[9] The β-glycoside **12** was then subjected to hydroboration and subsequent *N*-acetylation as described for **7** to give **13** in 74% yield, whereby the presence of the β-D-gluco epimer could not be detected. *O*-Debenzoylation of **13** followed by removal of isopropylidene groups with 95% aqueous trifluoroacetic acid gave *N*-acetyl-β-D-mannosaminyl-(1→6)-D-galactose (**15**) in 81% yield. The structure of **15** was confirmed by ^{13}C NMR spectroscopy.

Scheme 4 depicts the synthetic pathway to methyl *N*-acetyl-β-D-mannosaminyl-(1→4)-α-D-glucopyranoside (**23**). Glycosidations of **1a** with partially protected methyl glucopyranosides with a free C-4 hydroxyl group were somewhat troublesome because of the low reactivity of the secondary hydroxyl function. After



Scheme 3.



Scheme 4.

much attempts shown in Table 1, the β(1→4)-glycoside (**18**) could be secured in 55% yield (Run 13 in Table 1) by the use of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**16**)[18] as an acceptor and silver carbonate-iodine as the promotor, whilst methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**17**)[19] afforded multi-component mixtures in all the cases tested (Runs 9—11). Other glycosylation catalysts, such as silver oxide, silver perchlorate, or the allegedly highly superior[20] silver silicate were noneffective (Runs 14—16).

Under the conditions of Run 13, i.e. glycosylation of **16** with two molar equivalents of **1a** and Ag_2CO_3 in dichloromethane, substantial amounts of 1-hydroxy derivative **19** was generated, its identity being established by its conversion into the 1-acetate **20α**, that in turn was unequivocally identified by comparison with an authentic sample prepared by reaction of **1a** with sodium acetate in dioxane. Hydroboration of **18** was carried out as for **6**→**7**, yet proceeded in an essentially stereospecific manner, to provide the protected *N*-acetyl-β-D-mannosaminide (**21**) in a yield of 81%. Subsequent debenzoylation readily gave **22** (96% yield), which was catalytically hydrogenated over palladium/carbon to quantitatively afford desired methyl *N*-acetyl-β-D-mannosaminyl-(1→4)-α-D-glucopyranoside (**23**).

The concise, practical route reported here for the chemical synthesis of methyl or glycosyl *N*-acetyl-β-D-mannosaminides, as of now, appears to be the most straightforward methodology for the construction of heterooligosaccharides with a terminal β-D-mannosamine unit; it can be expected to find application for the immunologically relevant repeating units of *Streptococcus pneumoniae*-derived capsular polysaccharide and a series of others, some of which we hope to report in due course.

Experimental

General. Melting points were determined on a Yamato MP-1 apparatus and are uncorrected. Spectral data were recorded on the following instruments; IR; Jasco IR-810 spectrophotometer; $[\alpha]_D$: Jasco DIP-180 digital polarimeter; MS: JMS D-100 Spectrometer; ^1H NMR: Varian EM-390 (90 MHz), VXR-300 (300 MHz), or XL-400 (400 MHz) spectrometers in chloroform-*d* solution unless otherwise noted. ^1H-^1H and ^1H-^{13}C shift correlated 2D NMR and ^{13}C NMR: Varian VXR-300 or XL-400 spectrometer. TLC was carried out on silica gel 60 F_{254} (Merck Art. 5735) developed with the same solvent systems as described for column chromatography in the individual experimental section. The spots were detected by UV light (254 nm) or charring with 10% aq. sulfuric acid. Column chromatography was achieved on a silica gel 60 (Merck Art. 7734). Paper chromatography (PC) was carried out on Toyo filter paper No. 525 and detected with 0.2% ninhydrin in pyridine solution at 100 °C for 10 min.

Methyl 3,4,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-β-D-*arabino*-hexopyranoside (6). (Benzoyloxyimino)glycosyl bromide **1a**[10] (135 mg, 0.2 mmol) was added to a mixture of methanol (0.08 ml, 2 mmol), silver carbonate (279 mg, 1 mmol), and iodine (51 mg, 0.2 mmol) in dry dichloromethane (2 ml) containing Molecular Sieves 3A (100 mg, powder). The mixture was stirred in the dark at room temperature for 3 days, whereafter all the educt had been consumed (TLC). Dilution with dichloromethane (10 ml), filtration through Celite, consecutive washing of the filtrate with saturated aq. $NaHCO_3$ (10 ml) and water (3×10 ml), drying (Na_2SO_4) and evaporation to dryness in vacuo gave a residue which was eluted from a silica-gel column with toluene-ethyl acetate (8 : 1). The major fraction was concentrated and crystallized from diethyl ether-pentane: 116 mg (93%) of

6; mp 57—65 °C (decomp); $[\alpha]_D^{20}$ −42.8° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz) δ=3.70 (3H, s, CH_3), 4.51 (1H, m, H-5), 4.77 (1H, dd, H-6a), 4.84 (1H, dd, H-6b), 5.92 (1H, dd, H-4), 5.93 (1H, s, H-1), 6.25 (1H, d, H-3), 7.3—8.1 (aromatic H); $J_{3,4}=J_{4,5}=J_{5,6a}=5.5$, $J_{5,6b}=7.0$, $J_{6a,6b}=11.5$ Hz; ^{13}C NMR (100 MHz) δ=57.02 (OMe), 64.64 (C-6), 68.42 (C-3), 68.61 (C-4), 73.24 (C-5), 93.98 (C-1), 128.2—133.7 (phenyl C), 156.56 (C-2), 162.29, 164.52, 164.74, and 165.82 (benzoyl C=O).

Found: C, 67.09; H, 4.61; N, 2.36%. Calcd for $C_{35}H_{29}NO_{10}$: C, 67.41; H, 4.69; N, 2.25%.

Methyl 2-Acetamido-3,4,6-tri-*O*-benzoyl-β-D-mannopyranoside (7). A 1 M solution of diborane in tetrahydrofuran (15.5 ml) was added to a solution of **6** (807 mg, 1.3 mmol) in tetrahydrofuran (15 ml) at −10 °C under atmosphere of nitrogen. The mixture was stirred at −10 °C for 0.5 h and at ambient temperature for 2 h. Excess reductant was quenched with methanol (15 ml) followed by addition of acetic anhydride (8 ml) for *N*-acetylation. After stirring for 1 h at ambient temperature, the mixture was passed through a basic resin (Amberlite IR-45), and washed with methanol. The eluate was concentrated in vacuo and the residue was purified by elution from a silica-gel column with chloroform-ethyl acetate (1 : 1). The major fraction was concentrated and the residue crystallized from ethyl acetate-diethyl ether (1:2)-pentane to give 622 mg (88%) of **7**: mp 95—105 °C; $[\alpha]_D^{22}$ −55.8 ° (*c* 0.5, $CHCl_3$); IR (KBr) 3450, 3380 cm^{-1} (NH); ^1H NMR (400 MHz) δ=2.03 (3H, s, $COCH_3$), 3.55 (3H, s, OCH_3), 4.11 (1H, td, H-5), 4.57 (1H, dd, H-6a), 4.64 (1H, dd, H-6b), 4.79 (1H, d, H-1), 4.98 (1H, td, H-2), 5.47 (1H, dd, H-4), 5.87 (1H, d, NH), 7.3—8.1 (aromatic H); $J_{1,2}=2.0$, $J_{2,3}=4.0$, $J_{2,NH}=8.5$, $J_{3,4}=9.5$, $J_{4,5}=9.0$, $J_{5,6a}=6.0$, $J_{5,6b}=3.5$, $J_{6a,6b}=12.0$ Hz; ^{13}C NMR (100 MHz) δ=23.38 ($CO\underline{C}H_3$), 50.19 (C-2), 57.15 (OCH_3), 63.46 (C-6), 67.45 (C-4), 71.92 (C-3), 72.60 (C-5), 100.12 (C-1), 128.2—133.5 (phenyl C), 165.32, 165.53, and 165.86 (3×C=O), 170.28 (NHCO).

Found: C, 65.70; H, 5.35; N, 2.84%. Calcd for $C_{30}H_{29}NO_9$: C, 65.80; H, 5.34; N, 2.56%.

On concentration of the minor fraction, dissolution of the residue in diethyl ether, and addition of pentane, β-D-gluco isomer **10** was obtained in a yield of 9.4 mg (1.3%): mp 80—88 °C; $[\alpha]_D^{22}$ +8.8° (*c* 0.25, $CHCl_3$); ^1H NMR (90 MHz) δ=1.85 (3H, s, $COCH_3$).

Methyl 2-Acetamido-2-deoxy-β-D-mannopyranoside (8). A solution of **7** (212 mg, 0.39 mmol) in 0.05 M methanolic sodium methoxide (20 ml) was stirred at ambient temperature for 20 h and was subsequently neutralized with a dry acidic resin (Dowex 50W×8) and filtered. The filtrate was evaporated to dryness and the residue was partitioned between dichloromethane (30 ml) and water (30 ml). The aqueous layer was separated, washed with dichloromethane (2×40 ml), and concentrated to dryness. The residue was purified by elution from a silica-gel column with chloroform-methanol (1 : 1), followed by concentration and crystallization from methanol-diethyl ether to give 71 mg (74%) of **8** as 1/2 hydrate: mp 123—127 °C; $[\alpha]_D^{23}$ −70° (*c* 0.25, H_2O; lit,[14] $[\alpha]_D^{24}$ −68 °(*c* 1.5, H_2O)); ^1H NMR (D_2O, 400 MHz) δ=1.95 (3H, s, $COCH_3$), 3.29 (1H, td, H-5), 3.29 (3H, s, OCH_3), 3.40 (1H, dd, H-4), 3.69 (1H, dd, H-3), 3.70 (1H, dd, H-6a), 3.80 (1H, dd, H-6b), 4.37 (1H, dd, H-2), 4.58 (1H, d, H-1); $J_{1,2}=1.5$, $J_{2,3}=4.5$, $J_{3,4}=J_{4,5}=9.5$, $J_{5,6a}=5.0$, $J_{5,6b}=2.5$, $J_{6a,6b}=12.0$ Hz; ^{13}C NMR (D_2O, 100 MHz), δ=22.82 ($CO\underline{C}H_3$), 53.83 (C-2), 57.81 (OCH_3), 61.24 (C-6), 67.60 (C-4), 72.83 (C-3), 77.22 (C-5), 101.24 (C-1), 176.24 (NHCO); $J_{Cl,H1}=163.6$ Hz.

Found: C, 44.23; H, 7.22; N, 5.62%. Calcd for $C_9H_{17}NO_6$ $1/2H_2O$: C, 44.26; H, 7.43; N, 5.73%.

The monohydrate of **8** was also isolated in a yield of 13 mg (14%) along with the 1/2 hydrate; Found: C, 42.84; H, 7.61; N, 5.40%. Calcd for $C_9H_{17}NO_6$ H_2O: C, 42.68; H, 7.56; N, 5.53%.

2-Amino-2-deoxy-D-mannopyranose Hydrochloride (9). Methyl *N*-acetyl-β-D-mannosaminide (**8**) 1/2 hydrate (30 mg, 0.124 mmol) was dissolved in 1 M HCl (10 ml) and heated at 95 °C for 2 h. After concentration of the solution, the residue crystallized from methanol-diethyl ether to afford 27 mg (quantitative yield) of **9** as colorless crystals: mp 173—175 °C (decomp); $[\alpha]_D^{24}$ −4.2° (*c* 0.5, H_2O), (lit,[21] mp 178—180 °C, $[\alpha]_D^{23}$ −3.0° (*c* 2.0, H_2O)); PC (*n*-BuOH-pyridine-H_2O, 6 : 4 : 3 v/v): R_{Glc}=0.73 (cf., authentic D-Man*p*N HCl: R_{Glc}=0.73, D-Glc*p*N HCl: R_{Glc}=0.68).

6-*O*-[3,4,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-β-D-*arabino*-hexopyranosyl]-1,2 : 3,4-di-*O*-isopropyridene-α-D-galactopyranose (12). A mixture of diacetonegalactose (**11**)[17] (573 mg, 2.2 mmol), silver carbonate (2.76 g, 10 mmol), iodine (508 mg, 2.0 mmol), and (benzoyloxyimino)glycosyl bromide **1a** (1.35 g, 2.0 mmol) in dry dichloromethane (20 ml) with Molecular Sieves 3A (2.0 g, powder) was stirred in the dark at ambient temperature for 3 days. Subsequent dilution with dichloromethane (50 ml), filtration through celite, washing of the filtrate with 1 M aqueous $Na_2S_2O_3$ (50 ml), water (50 ml), saturated aqueous $NaHCO_3$ (50 ml), and water (3×100 ml) was followed by drying (Na_2SO_4) and evaporation to dryness. The residue was eluted through a silica-gel column with toluene-ethyl acetate (6 : 1). Concentration of the major fraction, followed by crystallization from diethyl ether-pentane gave 1.61 g (94%) of **12**: mp 83—86 °C; $[\alpha]_D^{22}$ −95.6° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz) δ=1.27, 1.28, 1.35, and 1.40 (each 3H, s, 2×Me_2C), 3.90 (1H, dd, H-6a), 4.04 (1H, td, H-5), 4.12 (1H, dd, H-4), 4.24 (1H, dd, H-6b), 4.28 (1H, dd, H-2), 4.47 (1H, m, H-5′), 4.54 (1H, dd, H-3), 4.84 (2H, m, H-6′a,b), 5.46 (1H, d, H-1), 5.92 (1H, t, H-4′), 6.09 (1H, s, H-1′), 6.25 (1H, d, H-3′), 7.3—8.2 (aromatic H); $J_{1,2}=5.0$, $J_{2,3}=2.5$, $J_{3,4}=8.0$, $J_{4,5}=2.0$, $J_{5,6a}=6.8$, $J_{5,6b}=5.5$, $J_{6a,b}=9.7$, $J_{3',4'}=J_{4',5'}=5.3$ Hz; ^{13}C NMR (100 MHz) δ=24.32, 24.89, 25.88, and 25.96 (2×Me_2C), 64.55 (C-6′), 66.74 (C-5), 68.65 (C-3′), 68.86 (C-4′ and 6), 70.45 (C-2), 70.60 (C-3), 70.89 (C-4), 73.03 (C-5′), 93.19 (C-1′), 96.22 (C-1), 108.65 and 109.45 (2×$Me_2\underline{C}$), 128.0—134.0)phenyl C), 156.21 (C-2′), 162.68, 164.70, 164.92, and 165.91 (4×C=O).

Found: C, 64.60; H, 5.29; N, 1.59%. Calcd for $C_{46}H_{45}NO_{15}$: C, 64.86; H, 5.32; N, 1.64%.

6-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-1,2 : 3,4-di-*O*-isopropylidene-α-D-galactopyranose (13). A solution of the disaccharide **12** (426 mg, 0.5 mmol) in dry tetrahydrofuran (6 ml) was treated with a 1 M solution of diborane in tetrahydrofuran (6 ml) as described for **6**→**7**. Termination with methanol (6 ml) and subsequent *N*-acetylation with acetic anhydride (3 ml) followed by processing of the mixture as described for **7** gave a syrup, which crystallized from ethyl acetate-diethyl ether (1 : 2)-pentane providing 288 mg (74%) of **13**: mp 171—173 °C; $[\alpha]_D^{22}$ −66.1° (*c* 0.5, $CHCl_3$); ^1H NMR (90 MHz) δ=1.29 and 1.40 (each 6H, s, 2×Me_2C), 1.98 (3H, s, $COCH_3$), 4.27 (1H, dd, H-2), 4.53 (1H, dd, H-3), 4.55—4.70 (2H, m, H-6a,b), 4.90 (1H, td, collapsed to dd on deuteration), 7.2—8.1 (aromatic H), 3.7—4.2 (other protons); $J_{1,2}=5.1$, $J_{2,3}=2.4$, $J_{3,4}=7.8$, $J_{1',2'}=3.0$, $J_{2',3'}=3.6$,

$J_{2',NH}$=8.4, $J_{3',4'}$=7.2, $J_{4',5'}$=7.8 Hz.

Found: C, 63.23; H, 5.81; N, 1.64%. Calcd for $C_{41}H_{45}NO_{14}$: C, 63.48; H, 5.85; N, 1.81%.

6-*O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-D-galactopyranose (15). A solution of **13** (343 mg, 0.44 mmol) in 0.05 M methanolic sodium methoxide (20 ml) was stirred at ambient temperature for 20 h. Subsequent neutralization (Dowex 50 W×8), filtration through Celite, and evaporation to dryness gave a residue, which was eluted through a silica-gel column with chloroform–methanol (4:1). Concentration of the major fraction gave debenzoylated product **14** as a colorless syrup (200 mg, quantitative), which was subjected to further deblocking of the isopropylidene groups without further purification.

The syrup was dissolved in 95% aqueous trifluoroacetic acid (3 ml) and stirred at ambient temperature for 20 min. Evaporation of the mixture was followed by co-evaporation with methanol to give a residue, which crystallized from ethanol–diethyl ether affording 140 mg (81%) of **15** as a monohydrate: mp 170 °C (decomp, with sintering at 115 °C): $[\alpha]_D^{21}$−14° (*c* 0.25, H_2O): ^{1}H NMR (D_2O, 90 MHz) δ=1.90 (3H, s, $COCH_3$), 7.73 (1H, d, NH); ^{13}C NMR (D_2O, 25.4 MHz) δ=22.8 ($CO\underline{C}H_3$), 53.9 (C-2′), 61.3 (C-6′), 67.6 (C-4′), 69.1 and 69.6 (C-6 for respective anomers), 77.3 (C-5′), 93.2 (C-1 for α-anomer), 97.3 (C-1 for β-anomer), 101.6 (C-1′), 176.5 (NHCO). The anomeric carbon signals of the reducing end suggested that the ratio of the anomers was estimated as α:β=ca. 2:3.

Found: C, 41.68; H, 6.50; N, 3.38%. Calcd for $C_{14}H_{25}NO_{11}$ H_2O: C, 41.89; H, 6.78; N, 3.49%.

Methyl 4-*O*-[3,4,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-β-D-*arabino*-hexopyranosyl]-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (18). Silver carbonate (138 mg, 0.5 mmol) and iodine (25 mg, 0.1 mmol) were added to a stirred solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**16**)[18] (93 mg, 0.2 mmol) in dry dichloromethane (1 ml) containing Molecular Sieves 3A (100 mg, powder) and then stirred in the dark for 1 h under atmosphere of nitrogen. A solution of (benzoyloxyimino)glycosyl bromide **1a** (67 mg, 0.1 mmol) in dry dichloromethane (1 ml) was added and the mixture was further stirred at ambient temperature for 2 days. The mixture was diluted with dichloromethane (10 ml) and filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ (10 ml) and water (3×15 ml). Drying (Na_2SO_4) and evaporation in vacuo gave a syrup, which was eluted through a silica-gel column with toluene–ethyl acetate (6:1). The major fraction eluted first was concentrated and the residue crystallized from diethyl ether–pentane to give 58 mg (55%) of **18**: mp 57—59 °C; $[\alpha]_D^{20}$+21.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR (90 MHz), δ=3.29 (3H, s, OCH_3), 4.98 (1H, s, H-1), 5.92 (1H, t, H-4′), 6.22 (1H, d, H-3′), 6.74 (1H, s, H-1′), 3.4—4.9 (15H, other protons), 6.9—8.2 (aromatic H); $J_{3',4'}$=$J_{4',5'}$=5.4 Hz.

Found: C, 70.51; H, 5.44; N, 1.33%. Calcd for $C_{62}H_{57}NO_{15}$: C, 70.69; H, 5.20; N, 1.30%.

The minor fraction eluted next was treated as above to give 10.5 mg (18%) of **19** as 1/2 hydrate: mp 177—179 °C (decomp); $[\alpha]_D^{20}$ +32.4° (*c* 0.25, $CHCl_3$); IR (KBr) 3400 cm^{-1} (OH); ^{1}H NMR (DMSO-d_6, 90 MHz), δ=4.5—4.7 (2H, m, H-6a,b), 4.75 (1H, m, H-5), 5.87 (1H, t, H-4), 6.36 (1H, d, H-3), 6.50 (1H, t, H-1), 8.27 (1H, d, OH, disappeared on deuteration), 7.3—8.2 (aromatic H); $J_{1,OH}$=6.0, $J_{3,4}$=$J_{4,5}$=10.5 Hz.

Found: C, 66.29; H, 4.42; N, 2.19%. Calcd for $C_{39}H_{27}NO_{10}$ 1/2H_2O: C, 66.02; H, 4.56; N, 2.26%.

Acetylation of **19** with acetic anhydride–pyridine in a usual manner gave the corresponding 1-acetate **20α** which proved to be identical with the product obtained by acetylation of **1a** as described below.

1-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-2-(benzoyloxyimino)-2-deoxy-α-D-*arabino*-hexopyranose (20α) and Its β-Anomer (20β). A mixture of (benzoyloxyimino)glycosyl bromide **1a** (1.0 g, 1.5 mmol), sodium acetate (369 mg, 4.5 mmol), and Molecular Sieves 3A (1 g, powder) in dry dioxane (20 ml) was stirred at ambient temperature for 3 days. The resulting mixture was diluted with dichloromethane (50 ml) and filtered through Celite. The filtrate was washed with water (3×50 ml), dried (Na_2SO_4), and evaporated to dryness. Elution of the residue through a silica-gel column with toluene–ethyl acetate (6:1) provided both anomeric isomers, which crystallized from diethyl ether–pentane to afford 365 mg (37%) of **20α** and 200 mg (20%) of the corresponding β-anomer **20β**.

20α: Mp 75—77 °C; $[\alpha]_D^{22}$ +53.4° (*c* 0.3, $CHCl_3$); ^{1}H NMR (90 MHz) δ=2.25 (3H, s, $COCH_3$), 4.4—4.7 (2H, m, H-6a,b), 4.68 (1H, m, H-5), 5.98 (1H, dd, H-4), 6.44 (1H, d, H-3), 7.2—8.2 (H-1 and aromatic H); $J_{3,4}$=9.6, $J_{4,5}$=9.0 Hz.

Found: C, 66.46; H, 4.49; N, 2.18%. Calcd for $C_{36}H_{29}NO_{11}$: C, 66.36; H, 4.49; N, 2.15%.

20β: Mp 64—66 °C (with sintering at 60 °C); $[\alpha]_D^{23}$ −43.2° (*c* 0.5, $CHCl_3$); ^{1}H NMR (90 MHz) δ=4.5—5.0 (3H, m, H-5 and 6a,b), 5.88 (1H, dd, H-4), 6.28 (1H, d, H-3), 7.2—8.2 (H-1 and aromatic H); $J_{3,4}$=5.1, $J_{4,5}$=4.8 Hz.

Found: C, 66.74; H, 4.46; N, 2.21%. Calcd for $C_{36}H_{29}NO_{11}$: C, 66.36; H, 4.49; N, 2.15%.

Methyl 4-*O*-(2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (21). A solution of **18** (618 mg, 0.59 mmol) in dry tetrahydrofuran (8 ml) was treated with a 1 M solution of diborane in tetrahydrofuran (7.1 ml) as described for **6**→**7**. Subsequent acetylation with acetic anhydride (4 ml) followed by processing of the mixture as described for **7** gave 461 mg (81%) of syrupy **21**. Crystallization from diethyl ether afforded 411 mg of **21** as colorless prisms: mp 163—164 °C (decomp); $[\alpha]_D^{22}$ −21.2° (*c* 0.25, $CHCl_3$); IR (KBr) 3430 (NH), 1720 (ester C=O), 1685 cm^{-1} (amide C=O); ^{1}H NMR (300 MHz) δ=1.83 (3H, s, $COCH_3$), 3.37 (3H, s, OCH_3), 3.48 (1H, m, H-5′), 3.49 (1H, dd, H-2), 3.65—3.75 (3H, m, H-5 and H-6a,b), 3.88 (1H, t, H-3), 4.01 (1H, t, H-4), 4.24 (1H, dd, H-6′a), 4.39 (1H, dd, H-6′b), 4.55, 4.72, and 4.91 (each 2H, dd, 3×$C\underline{H}_2$Ph), 4.61 (1H, d, H-1), 4.81 (1H, td, H-2′), 4.86 (1H, d, H-1′), 5.12 (1H, dd, H-3′), 5.49 (1H, t, H-4′), 5.70 (1H, d, NH), 7.2—8.0 (aromatic H); $J_{1,2}$=3.5, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.3, $J_{1',2'}$=1.5, $J_{2',NH}$=8.7, $J_{2',3'}$=4.0, $J_{3',4'}$=$J_{4',5'}$=10.0, $J_{5',6'a}$=5.0, $J_{5',6'b}$=3.5, $J_{6'a,b}$=12.0 Hz; ^{13}C NMR (75 MHz) δ=23.05 ($CO\underline{C}H_3$), 51.09 (C-2′), 55.29 (OCH_3), 63.25 (C-6′), 67.26 (C-4′), 68.45 (C-6), 69.35 (C-5), 72.34 (C-5′) 72.43 (C-3′), 73.44, 73.56, and 75.00 (3×$C\underline{H}_2$Ph), 76.51 (C-4), 79.38 (C-2), 80.41 (C-3), 98.27 (C-1), 98.87 (C-1′), 126.6—139.2 (aromatic C), 165.64, 165.64, and 165.90 (3×C=O), 170.34 (NHCO).

Found: C, 70.00; H, 5.87; N, 1.42%. Calcd for $C_{57}H_{57}NO_{14}$: C, 69.85; H, 5.86; N, 1.43%.

Methyl 4-*O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (22). A 0.05 M solution of sodium methoxide in dry methanol (18 ml) was added to **21** (352 mg, 0.36 mmol). The solution was stirred at

ambient temperature for 20 h, neutralized with acidic resin (Dowex 50W×8), and filtered through Celite. The filtrate was concentrated in vacuo to give a residue, which was eluted through a silica-gel column with chloroform-methanol (8:1). Concentration of the major fraction, followed by recrystallization from methanol-pentane gave 210 mg (87%) of **22** as colorless needles: mp 170—172 °C; $[\alpha]_D^{22}$ +14.2° (*c* 0.5, CH_3OH); IR (KBr) 3400—3300 (NH and OH), 1640 cm^{-1} (amide C=O); ^1H NMR (DMSO-d_6, 90 MHz) δ=1.83 (3H, s, $COCH_3$), 3.29 (3H, s, OCH_3), 7.04 (1H, s, NH), 7.1—7.5 (aromatic H), 2.8—5.1 (other protons).

Found: C, 64.98; H, 6.89; N, 2.04%. Calcd for $C_{36}H_{45}NO_{12}$: C, 64.75; H, 6.79; N, 2.10%.

Methyl 4-*O*-(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-α-D-glucopyranoside (23). A solution of **22** (172 mg, 0.26 mmol) in acetic acid (10 ml) was hydrogenated in the presence of 10% palladium on carbon (85 mg) under atmosphere of hydrogen (3.45×10^5 Pa) for 24 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give a syrup, which was purified by elution from a silica-gel column with chloroform-methanol (1:2). The major fraction was concentrated and the residual solid was washed with diethyl ether to give 110 mg (quantitative) of **23** as 1/2 hydrate: mp 190 °C (decomp, with sintering at 110 °C); $[\alpha]_D^{23}$ +45.2° (*c* 0.25, H_2O); ^{13}C NMR (D_2O, 25.4 MHz) δ=22.8 ($CO\underline{C}H_3$), 54.1 (C-2′), 55.8 (OCH_3), 60.9 and 61.3 (C-6 and 6′), 67.5 (C-4′), 70.8 (C-2), 71.9, 72.5, and 72.8 (C-3, C-3′, and C-5), 77.4 (C-5′), 79.6 (C-4), 99.2 (C-1), 100.2 (C-1′), 176.4 (CONH).

Found: C, 44.62; H, 7.13; N, 3.16%. Calcd for $C_{15}H_{27}NO_{11}$ $1/2H_2O$: C, 44.33; H, 6.94; N, 3.45%.

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