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SYNTHESIS OF ANTIGENIC DETERMINANTS OF THE *Brucella* A ANTIGEN, UTILIZING METHYL 4-AZIDO-4,6-DIDEOXY-α-D-MANNOPYRANOSIDE EFFICIENTLY DERIVED FROM D-MANNOSE*,†

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ABSTRACT

A strategy for the synthesis of *Brucella* O-antigenic determinants containing 2-linked 4,6-dideoxy-4-formamido-α-D-mannopyranosyl residues is described. The approach adopted also permits the *N*-acyl moiety to be varied. A high-yield synthesis of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**7**) from D-mannose on the 10–20-g scale provided the key intermediate. Regioselective acetylation of **7** gave the 2-acetate **8**, which, on treatment with benzyl trichloroacetimidate, provided methyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**9**). This compound served as a common precursor to the glycosyl donor **12** and acceptor **10** molecules. Silver trifluoromethanesulphonate-promoted glycosylation of **10** by **12** gave a disaccharide derivative (**13**), hydrogenolysis of which gave methyl 4-amino-2-*O*-(4-amino-4,6-dideoxy-α-D-mannopyranosyl)-4,6-dideoxy-α-D-mannopyranoside (**16**) from which the *N*-formyl (**17**) and *N*-acetyl (**18**) derivatives were obtained. Deacetylation of **13** followed by glycosylation with **12** gave a trisaccharide derivative. The *N*-formylated disaccharide **17** inhibited the binding of *Brucella* O-polysaccharide to *Brucella*-specific monoclonal antibodies.

INTRODUCTION

The O-antigenic polysaccharides of *Brucella abortus*[1] and *Brucella melitensis*[2], although structurally distinct, both contain 2-*O*-linked 4,6-dideoxy-4-formamido-α-D-mannopyranosyl residues. Unambiguous identification of cattle infected by *Brucella* is based upon serological methods most often involving the cell-wall polysaccharide antigens[3]. The economic impact of Brucellosis is considerable in North

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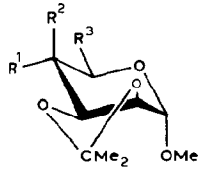
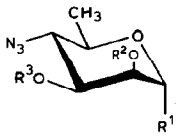
America and developing countries, and improved methods of detection and protection are highly desirable. As part of our studies of the polysaccharide antigens from the genus *Brucella*, we have begun synthesis work to investigate the potential of chemically defined oligosaccharides for diagnosis and as potential components of a vaccine[4].

Since 4-amino-4,6-dideoxy-D-mannose, the sole monosaccharide component of the *Brucella* O-antigens, is a rare sugar[5], its synthesis in acceptable yield was the first obstacle to the synthesis of oligosaccharides related to the *Brucella* polysaccharide antigens. A strategy was developed to provide this saccharide on the 10–20-g scale, suitably derivatized to permit its use directly in glycoside synthesis[4]. Furthermore, since the *Brucella abortus*[1] and *Vibrio cholera*[6] antigens share a common backbone structure, differing only in their *N*-acyl moiety, this approach would offer simultaneous access to determinants of the latter antigen. The use of a common precursor molecule for this purpose is reported together with the synthesis of disaccharide and trisaccharide glycosides.

RESULTS

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside (**3**) was synthesized by either of two routes. That described by Thiem *et al.*[7] involved the conversion of methyl 4,6-*O*-benzylidene-2,3-*O*-isopropylidene-α-D-mannopyranoside into the 6-bromo derivative followed by reduction and *O*-debenzoylation. Alternatively, methyl α-D-mannopyranoside was converted in a one-step procedure into the 2,3-*O*-isopropylidene acetal[8] **1**, from which methyl 6-deoxy-6-iodo-2,3-*O*-isopropylidene-α-D-mannopyranoside (**2**) could be readily produced on a 20-g scale using the procedure of Garegg and Samuelson[9]. The latter route in our hands was the method of choice, since **1** was converted into **2** within 5 min in a yield of 95%. Since **2** was also easily reduced in high yield, the overall procedure for preparing the methyl α-D-rhamnopyranoside derivative[10] **3** was well suited to large-scale synthesis. Initially, the oxidation of **3** to give the 4-keto derivative, which was not isolated, was performed by pyridinium chlorochromate according to a procedure reported[11] for the L isomer. Swern oxidation[12], however, was the method of choice for the transformation and, following borohydride reduction, the talopyranoside **4** was obtained in >80% yield from **3**. The crystalline trifluoromethanesulphonate **5** was readily displaced (→**6**) by potassium azide in *N,N*-dimethylformamide at room temperature, provided dicyclohexano-18-crown-6 was employed to enhance the solubility and nucleophilicity of potassium azide. The merits of crown ethers in the displacement reactions of less reactive leaving-groups from related compounds have been reported[13]. Fleet *et al.*[14] have reported a similar displacement of triflate from a substituted talopyranoside[14], and Paulsen and Lorentzen[15] have described the synthesis of a 4-azido-4,6-dideoxy-D-galactose derivative by azide displacement of a mesylate. An earlier synthesis of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**7**) exploited the reaction of methyl 3,4-anhydro-2-*O*-benzoyl-6-deoxy-α-D-talopyranoside[5] with sodium azide.



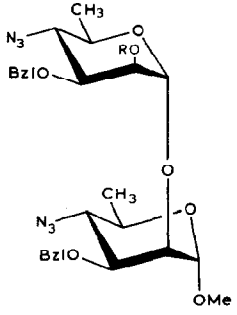


1 $R^1 = OH, R^2 = H, R^3 = CH_2OH$
2 $R^1 = OH, R^2 = H, R^3 = CH_2I$
3 $R^1 = OH, R^2 = H, R^3 = CH_3$
4 $R^1 = H, R^2 = OH, R^3 = CH_3$
5 $R^1 = H, R^2 = CF_3SO_3, R^3 = CH_3$
6 $R^1 = N_3, R^2 = H, R^3 = CH_3$

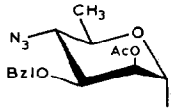
7 $R^1 = OMe, R^2 = H, R^3 = H$
8 $R^1 = OMe, R^2 = Ac, R^3 = H$
9 $R^1 = OMe, R^2 = Ac, R^3 = Bzl$
10 $R^1 = OMe, R^2 = H, R^3 = Bzl$
11 $R^1 = R^2 = Ac, R^3 = Bzl$
12 $R^1 = Cl, R^2 = Ac, R^3 = Bzl$

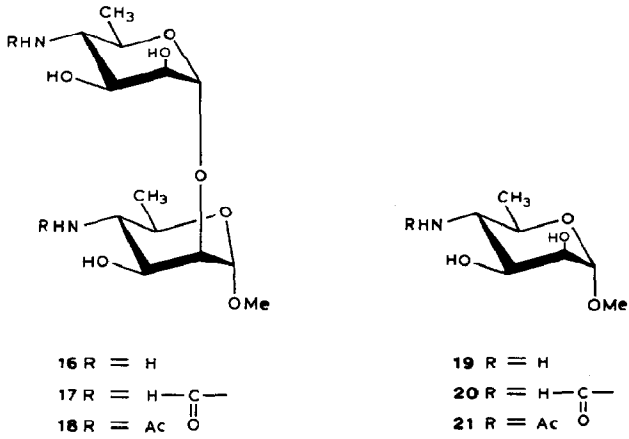
The selective protection of **7** was achieved by preparation of the 2,3-ortho-acetate followed by *in situ* regioselective opening by aqueous acetic acid[16,17]. Benzylation of the 2-acetate **8** to give **9** was accomplished under mildly acidic conditions using benzyl trichloroacetimidate[18,19]. Thus, the key intermediate **9** was obtained in >60% yield from **7**. Deacetylation of **9** afforded the alcohol **10**, which served as the glycosyl acceptor for disaccharide synthesis. Acetolysis of **9** gave the diacetate **11** quantitatively and reaction with α,α-dichloromethyl methyl ether gave 89% of the glycosyl chloride **12**. Alternatively, **11** was converted into **12** by the action of a saturated solution of HCl in ether in the presence of zinc bromide. The glycosyl donor **12** was characterized by n.m.r. spectroscopy and used immediately in glycosylation reactions.

The glycosyl chloride **12** was shown to be an effective glycosylating agent in a silver triflate-promoted reaction with **10**. The disaccharide glycoside **13** was ob-



13 R = Ac
14 R = OH

15 R = 



16 R = H
17 R = H—C—
18 R = Ac

19 R = H
20 R = H—C—
21 R = Ac

tained in ~85% yield after chromatography, and deacetylation afforded the disaccharide alcohol **14**. Glycosylation of **14** with **12** under the above conditions gave the protected trisaccharide **15** in 83% yield. The anomeric purity of the glycosides **13** and **15** was established from the $J_{C\text{-}1,H\text{-}1}$ values (171–174 Hz) which indicated that each possessed the α configuration[20].

Hydrogenolysis of **14** removed the benzyl group and also converted the azide group into an amino function to give **16**, *N*-formylation of which, to give **17**, was carried out using a mixed anhydride of formic and acetic acids[19]. *N*-Acetylation of **16** gave **18**. Compounds **16–18** were required as model compounds for ^{13}C-n.m.r. studies of the *Brucella* M antigen[2]. The corresponding derivatives **19–21** of the monosaccharide methyl glycoside were prepared directly from the azido derivative **7** by hydrogenation and acylation. The crystalline amino sugar **19** and its *N*-acetyl derivative **21** have been synthesized by Stevens *et al.*[5].

DISCUSSION

The synthesis of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**7**) from D-mannose in 30–40% overall yield forms the basis of the approach for the synthesis of α-(1→2)-linked di- and tri-saccharides of this rare monosaccharide. Iodination of **1** provided a convenient, rapid, and high-yield route to the D-rhamnopyranoside derivative **3**. Double inversion at C-4 by an oxidation–reduction and azide displacement sequence was similar to that employed by Fleet and co-workers[14] or Brimacombe *et al.* for the L isomer[21] and avoids the well-known ring contraction associated with direct displacement reactions at C-4 of *manno* compounds[10]. The azido derivative **9** was considered to be the most desirable intermediate for synthesis of 4-amino-4,6-dideoxyhexopyranosyl-containing oligosaccharides[22]. The azide function was selected over phthalimido or acetamido as the latent 4-amino functionality for several reasons, not least because of its availability directly from D-mannose. In addition, the conversion of the phthalimido or acetamido functions

into the *N*-formyl derivative would compromise the integrity of the bifunctional linking arm[23] intended for use in extension of this work to synthetic *Brucella* antigens.

The introduction of a persistent blocking-group at O-3 was accomplished following the regio-selective opening of the 2,3-orthoacetate to the 2-acetate **8**. Benzyl trichloroacetimidate, a reagent well suited to the introduction of a benzyl ether group under slightly acidic conditions[18], converted **8** into **9** without affecting the ester group. Thus, **9** serves as a bifunctional precursor for generation of either the glycosyl acceptor **10** or, by two steps, the glycosyl donor **12**. The intermediates **6, 9, 10,** and **12** are thus well suited to the synthesis of the parent amino sugar and its α-(1→2)-linked oligosaccharides.

One purpose of the synthesis described here is to provide inhibitors with which to probe the antibody combining sites of monoclonal antibodies that bind the *Brucella* antigens[24]. To this end, derivatives specifically labelled by ^{2}H or ^{13}C are well suited for n.m.r. studies of the dynamics and kinetics of binding[25,26]. The double-inversion sequence noted above is well suited for the introduction of deuterium at C-4. Thus, borodeuteride reduction of the 4-keto intermediate derived from **3** leads to a labelled talopyranoside **4**. The *N*-formyl function can also serve as an n.m.r. reporter group by labelling with ^{2}H or ^{13}C.

The *N*-formylated di- and tri-saccharides were potent inhibitors of the binding of *Brucella* O-polysaccharide by *Brucella*-specific monoclonal antibodies[27]. The derivatives **16** and **19** provide a route to various *N*-acyl derivatives and, in particular, the synthesis of *Vibrio cholerae* related structures.

EXPERIMENTAL

General. — T.l.c. was performed on Silica Gel 60 F_{254} (Merck) with detection by u.v. light and/or charring with sulphuric acid. Silica Gel 60 (Merck, 70–230 mesh) and redistilled solvents were used for column chromatography. Hexane refers to a mixture of hexanes supplied by Home Oil Company, Wichita, Kansas.

Solvents were distilled before use and were dried where necessary by literature procedures[28]. Reactions performed with dried solvents were conducted under dry nitrogen.

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer spectropolarimeter.

^{1}H- (500 MHz) and ^{13}C-n.m.r. (125 MHz) spectra were recorded with a Bruker AM-500 n.m.r. spectrometer on solutions in $CDCl_3$ (internal Me_4Si) and D_2O (internal 10% acetone, δ_H 2.225). Chemical shifts and coupling constants are first-order values.

Methyl 6-deoxy-6-iodo-2,3-O-isopropylidene-α-D-mannopyranoside (**2**). — To a solution of **1** (20 g, 58.12 mmol) in toluene (600 mL) were added triphenylphosphine (28.8 g, 109.80 mmol), imidazole (20 g, 293.77 mmol), and iodine (25.6 g,

201.73 mmol). The mixture was boiled under reflux with vigorous stirring until the colour disappeared (12 min). A solution of sodium hydrogencarbonate (25 g) in water (300 mL) was then added and, after stirring for 5 min, iodine was added until the colour of the mixture remained purple. Aqueous 10% sodium thiosulfate was added dropwise with stirring until the purple colour was removed. The mixture was then diluted with ethyl acetate (600 mL), washed twice with water, and concentrated, and a solution of the residue in ether (400 mL) was cooled to −10°, filtered after 1 h, and concentrated. The syrupy residue was dissolved in ether (200 mL) and rapid column chromatography through silica gel (300 g) gave a product that crystallized from ether to give **2** (18.9 g, 95%), m.p. 111–112°, $[\alpha]_D^{20}$ +46° (*c* 1.3, chloroform). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 4.93 (d, 1 H, $J_{1,2}$ <1.0 Hz, H-1), 4.15–4.11 (m, 2 H, H-2,3), 3.59 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.56–3.45 (m, 2 H, H-4,5), 3.49 (s, 3 H, OMe), 3.32 (dd, 1 H, $J_{5,6b}$ 7.2 Hz, H-6b), 1.53 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3).

Anal. Calc. for $C_{10}H_{17}O_5$: C, 34.90; H, 4.98. Found: C, 35.02; H, 5.04.

*Methyl 6-deoxy-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**3**). — A solution of **2** (20 g, 58.11 mmol) in ethanol (200 mL) and triethylamine (10 mL) was hydrogenated in the presence of 5% Pd/C (2 g) at room temperature and normal pressure. T.l.c. (hexane–ethyl acetate, 2:1) showed that the reaction was finished after 12 h. The mixture was filtered and concentrated *in vacuo*, and column chromatography (hexane–ethyl acetate, 2:1) of the product gave **3** (11 g, 87%), isolated as a syrup, $[\alpha]_D^{20}$ +24° (*c* 1.7, chloroform); lit.[10] $[\alpha]_D^{27}$ +15.2°. ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 4.82 (s, 1 H, H-1), 4.09 (d, 1 H, $J_{2,3}$ 5.7 Hz, H-2), 4.03 (dd, 1 H, $J_{3,4}$ 7.1 Hz, H-3), 3.61 (dq, 1 H, $J_{4,5}$ 8.9, $J_{5,6}$ 6.4 Hz, H-5), 3.37 (ddd, 1 H, $J_{4,OH}$ 4.4 Hz, H-4), 3.36 (s, 3 H, OMe), 2.57 (d, 1 H, HO-4), 1.50 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.27 (d, 3 H, 3 H-6).

*Methyl 6-deoxy-2,3-*O*-isopropylidene-α-*D*-talopyranoside* (**4**). — (*a*) A solution of **3** (11 g, 50.4 mmol) in dry dichloromethane (150 mL) was stirred with molecular sieves (3 Å, 45 g), pyridinium chlorochromate (48 g, 222.7 mmol), and silica gel (5 g) for 18 h. After addition of ether (250 mL), the mixture was filtered over silica gel and concentrated. To a solution of the crude product in methanol (200 mL) was added sodium borohydride (15 g, 396.5 mmol). The mixture was stirred for 15 min, water (100 mL) was added, and, after stirring for another 30 min, the mixture was extracted with dichloromethane. The extract was dried (Na_2SO_4) and concentrated, and toluene was distilled from the residue to give **4** (7.9 g, 73%) as a syrup, $[\alpha]_D^{20}$ +40° (*c* 1.25, dichloromethane); lit.[29] for the L isomer, $[\alpha]_D$ −38.3° (chloroform). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 4.87 (s, 1 H, H-1), 4.15 (dd, 1 H, $J_{2,3}$ 6.4, $J_{3,4}$ 5.7 Hz, H-3), 3.97 (d, 1 H, H-2), 3.77 (q, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 3.50 (dd, 1 H, $J_{4,OH}$ 6.9 Hz, H-4), 3.35 (s, 3 H, OMe), 2.20 (d, 1 H, HO-4), 1.53 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.28 (s, 3 H, 3 H-6).

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.91; H, 8.22.

(*b*) A solution of oxalyl chloride (10.3 mL, 118.1 mmol) in dichloromethane (30 mL) was cooled to −78° under N_2. A mixture of methyl sulfoxide (16.1 mL,

226.9 mmol) and dichloromethane (30 mL) was added slowly and dropwise into the stirred solution. After stirring for another 30 min at −78°, a solution of **3** (11.9 g, 54.4 mmol) in dichloromethane (300 mL) was slowly added. *N,N*-Diisopropylethylamine (60 mL, 344.4 mmol) was then added dropwise and, after stirring for 3 h at −78°, the mixture was allowed to attain room temperature and stirred overnight. T.l.c. (hexane–ethyl acetate, 3:1) showed complete conversion of **3** into the corresponding ulose. The solution was washed with aqueous 15% $KHSO_4$, dried ($MgSO_4$), and concentrated *in vacuo* at room temperature. To a solution of the syrupy residue in ethanol (300 mL) was added sodium borohydride (2.5 g, 66 mmol), and the mixture was stirred for 2 h at room temperature. The reaction was monitored by t.l.c. (hexane–ethyl acetate, 3:1). The solution was filtered through Celite and concentrated. The crude material was extracted with hexane–ethyl acetate (3:1), the extract was filtered, and the insoluble material was washed several times with hexane–ethyl acetate (3:1). The combined filtrate and washings were concentrated. Column chromatography (hexane–ethyl acetate, 3:1) of the syrupy residue gave **4** (9.5 g, 80%).

Methyl 6-deoxy-2,3-O-isopropylidene-4-O-trifluoromethanesulfonyl-α-D-talopyranoside (**5**). — To a solution of **4** (5.9 g, 27 mmol) in dry dichloromethane (120 mL) containing pyridine (11 mL) at −70° was added trifluoromethanesulphonic anhydride (5.4 mL, 31.6 mmol) under nitrogen. The stirred mixture was warmed to room temperature within 1 h, then poured into ice–water (500 mL), and stirred for another 30 min. The dichloromethane solution was washed with M HCl and saturated aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and co-concentrated with toluene to give **5** (8.44 g, 89%), m.p. 77–80° (dec.), $[\alpha]_D^{20}$ +19° (*c* 1.4, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 4.85 (dd, 1 H, $J_{3,4}$ 5.5, $J_{4,5}$ 2.1 Hz, H-4), 4.85 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.38 (dd, 1 H, $J_{2,3}$ 6.9 Hz, H-3), 4.08 (dd, 1 H, H-2), 4.02 (dq, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 3.39 (s, 3 H, OMe), 1.56 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 1.32 (d, 3 H, 3 H-6).

Anal. Calc. for $C_{11}H_{17}F_3O_7S$: C, 37.72; H, 4.89; S, 9.15. Found: C, 37.67; H, 4.99; S, 9.01.

Methyl 4-azido-4,6-dideoxy-2,3-O-isopropylidene-α-D-mannopyranoside (**6**). — A solution of **5** (3.7 g, 10.6 mmol) in *N,N*-dimethylformamide (60 mL) containing potassium azide (2 g, 24.6 mmol) and dicyclohexano-18-crown-6 (0.1 g, 0.27 mmol) was stirred for 2 h at room temperature. The mixture was then poured into ice-cold 0.5M HCl and extracted with ether, the extract was washed with saturated aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated, and toluene was distilled from the residue to give **5** (2.5 g, 97%), $[\alpha]_D^{20}$ +23° (*c* 1.1, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 4.85 (s, 1 H, H-1), 4.10 (dd, 1 H, $J_{2,3}$ 5.4, $J_{3,4}$ 8.1 Hz, H-3), 4.05 (d, 1 H, H-2), 3.50 (dq, 1 H, $J_{4,5}$ 10.3, $J_{5,6}$ 6.2 Hz, H-5), 3.32 (s, 3 H, OMe), 3.13 (dd, 1 H, H-4), 1.51 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.26 (d, 3 H, 3 H-6).

Anal. Calc. for $C_{10}H_{17}N_3O_4$: C, 49.34; H, 7.04; N, 17.34. Found: C, 48.99; H, 6.90; N, 16.95.

*Methyl 4-azido-4,6-dideoxy-α-*D*-mannopyranoside* (**7**). — Compound **6** (6.5 g, 26.75 mmol) was stirred for 10 min with trifluoroacetic acid and water (65 mL, 9:1). The solution was then concentrated and toluene was distilled from the residue. Filtration through silica gel and crystallisation from ether gave **7** (4.6 g, 85%), m.p. 79–80°, $[\alpha]_D^{20}$ +127° (*c* 1, dichloromethane); lit.[30] m.p. 81.5–82.5°, $[\alpha]_D^{20}$ +127° (methanol). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$, exchangeable protons were deuterated): δ 4.68 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.90 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 3.82 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.56 (dq, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 6.2 Hz, H-5), 3.42 (s, 3 H, OMe), 3.29 (dd, 1 H, H-4), 1.34 (d, 3 H, 3 H-6).

Anal. Calc. for $C_7H_{13}N_3O_4$: C, 41.34; H, 6.44; N, 20.75; Found: C, 41.34; H, 6.43; N, 20.57.

*Methyl 2-*O*-acetyl-4-azido-4,6-dideoxy-α-*D*-mannopyranoside* (**8**). — A solution of **7** (2.62 g, 12.9 mmol) in dry *N,N*-dimethylformamide (50 mL) was stirred with triethyl orthoacetate (3 mL, 16.4 mmol) and toluene-*p*-sulphonic acid (50 mg) for 2 h at 50°. Triethylamine (2 mL) was added and the mixture was concentrated to a syrup that was dissolved in aqueous acetic acid (20 mL, 80%). After 10 min, the mixture was concentrated and toluene was distilled several times from the residue to give **8** as a syrup (3.1 g, 98%), $[\alpha]_D^{20}$ +71° (*c* 0.9, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$; exchangeable protons were deuterated): δ 5.03 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,1}$ 3.5 Hz, H-2), 4.62 (d, 1 H, H-1), 3.98 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.54 (dq, 1 H, $J_{4,5}$ 10.1, $J_{5,6}$ 6.2 Hz, H-5), 3.32 (s, 3 H, OMe), 3.28 (dd, 1 H, H-4), 2.13 (s, 3 H, Ac), 1.33 (d, 3 H, 3 H-6).

Anal. Calc. for $C_9H_{15}N_3O_5$: C, 44.05; H, 6.16; N, 17.20. Found: C, 43.97; H, 6.04; N, 16.96.

*Methyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**9**). — A mixture of **8** (7 g, 28.6 mmol), carbon tetrachloride (20 mL), and cyclohexane (85 mL) was stirred with benzyl trichloroacetimidate (14.5 g, 7.87 mmol) for 12 h at room temperature during which time trifluoromethanesulphonic acid (2 mL) was added dropwise. The mixture was filtered through glass wool, triethylamine (1 mL) was added, the solution was concentrated, and a solution of the residue in dichloromethane was washed with aqueous sodium hydrogencarbonate and dried (Na_2SO_4). Non-carbohydrate components were removed by crystallisation with ether–hexane, and column chromatography (ethyl acetate–hexane, 1:4) of the filtrate gave **9** (6.1 g, 64%), $[\alpha]_D^{20}$ +99° (*c* 0.9, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 7.42–7.20 (m, 5 H, Ph), 5.35 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.3 Hz, H-2), 4.69 (d, 1 H, *J* 11.1 Hz, PhCH_aH$_b$), 4.65 (d, 1 H, H-1), 4.53 (d, 1 H, PhCH$_a$$H_b$), 3.81 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.54 (dq, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 6.2 Hz, H-5), 3.42 (dd, 1 H, H-4), 3.34 (s, 3 H, OMe), 2.12 (s, 3 H, Ac), 1.35 (d, 3 H, 3 H-6).

Anal. Calc. for $C_{16}H_{21}N_3O_5$: C, 57.27; H, 6.31; N, 12.58. Found: C, 57.14; H, 6.19; N, 12.29.

*Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**10**). — Compound **9** (0.5 g, 1.49 mmol) was treated with methanolic 0.01M sodium methoxide (10 mL) for 2 h at room temperature. After neutralizing the solution with Rexyn

101 (H^+) resin and filtration, solvent removal gave **10** as a syrup (0.450 mg, ~100%), $[\alpha]_D^{20}$ +141° (*c* 0.5, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$, exchangeable protons were deuterated): δ 7.44–7.20 (m, 5 H, Ph), 4.71 (d, 1 H, *J* 11.3 Hz, PhCH_aH$_b$), 4.70 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.66 (d, 1 H, PhCH$_a$$H_b$), 3.97 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 3.71 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 3.53 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.41 (dd, 1 H, H-4), 3.34 (s, 3 H, OMe), 1.34 (d, 3 H, 3 H-6).

Anal. Calc. for $C_{14}H_{19}N_3O_4$: C, 57.29; H, 6.53; N, 14.38. Found: C, 57.40; H, 6.49; N, 14.15.

*1,2-Di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranose* (**11**). — A solution of **9** (500 mg, 1.49 mmol) in acetic anhydride–acetic acid–sulfuric acid (50:20:0.5, 10 mL) was stirred for 3 h at room temperature, and then poured into ice-cold aqueous potassium carbonate. The product was extracted with dichloromethane, the extract was dried (Na_2SO_4) and concentrated, and toluene was distilled from the residue to give **11** (560 mg, ~100%), $[\alpha]_D^{20}$ +89° (*c* 1, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 7.36–7.10 (m, 5 H, Ph), 6.00 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 5.30 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.69 (d, 1 H, *J* 11.1 Hz, PhCH_aH$_b$), 4.49 (d, 1 H, PhCH$_a$$H_b$), 3.78 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 3.59 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.45 (dd, 1 H, H-4), 2.11 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 1.32 (d, 3 H, 3 H-6).

Anal. Calc. for $C_{17}H_{21}N_3O_6$: C, 56.16; H, 5.82; N, 11.61. Found: C, 56.01; H, 5.64; N, 11.40.

*2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl chloride* (**12**). — (*a*) A solution of **11** (0.54 g, 1.49 mmol) in dry dichloromethane (5 mL) was stirred with zinc bromide (20 mg) and dichloromethyl methyl ether (0.5 mL, 5.23 mmol) for 1 h at room temperature. Filtration over glasswool and freeze-drying gave crude **12** (0.45 g, 89%), which was used directly for the glycosylation reactions.

(*b*) A solution of **11** (0.16 g, 0.44 mmol) in saturated ethereal HCl (10 mL) was stirred with zinc bromide (10 mg) at room temperature for 0.5 h. Filtration through glasswool and concentration *in vacuo* gave the crude **12** (0.14 g, 93%), which was used directly for glycosylation reactions.

*Methyl 2-*O*-(2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**13**). — A solution of **10** (300 mg, 1.0 mmol) in anhydrous dichloromethane (5 mL) was stirred with molecular sieves (4 Å) for 1 h. After adding silver trifluoromethanesulfonate (340 mg, 1.32 mmol), the mixture was cooled to −30° and a solution of **12** (400 mg, 1.18 mmol) in dichloromethane (2 mL) was added. After 18 h, the mixture was filtered through Celite. Column chromatography (hexane–ethyl acetate, 4:1) of the product gave **13** (320 mg, 54%). Another fraction, which also contained **12** but which required re-chromatography, gave more (200 mg, 33%) **13**, $[\alpha]_D^{20}$ +89° (*c* 1.2, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$): δ 7.37–7.26 (m, 10 H, 2 Ph), 5.40 (dd, 1 H, $J_{1',2'}$ 1.7, $J_{2',3'}$ 3.2 Hz, H-2'), 4.84 (d, 1 H, H-1'), 4.70 (d, 1 H, *J* 11.1 Hz, PhCH_2), 4.67 (d, 1 H, *J* 11.5 Hz, C*H*Ph), 4.60 (d, 1 H, *J* 11.5 Hz, PhCH_2), 4.56

(d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.53 (d, 1 H, *J* 11.1 Hz, PhCH_2), 3.84 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.78 (dd, 1 H, $J_{3',4'}$ 9.9 Hz, H-3'), 3.71 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.58 (dq, 1 H, $J_{4',5'}$ 10.0, $J_{5',6'}$ 6.2 Hz, H-5'), 3.45 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.38 (dd, 1 H, H-4'), 3.32 (dd, 1 H, H-4), 3.29 (s, 3 H, OMe), 2.07 (s, 3 H, Ac), 1.29 (d, 6 H, 3 H-6,6').

Anal. Calc. for $C_{29}H_{36}N_6O_8$: C, 58.38; H, 6.08; N, 14.09. Found: C, 58.11; H, 5.95; N, 13.84.

*Methyl 4-azido-2-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl)-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**14**). — A solution of **13** (325 mg, 0.545 mmol) in methanol (20 mL) was treated with methanolic 0.1M sodium methoxide (2 mL) for 8 h at room temperature. Neutralisation with Rexyn (H^+) resin, concentration, and distillation of toluene from the residue gave **14** (300 mg, ~100%), $[\alpha]_D^{20}$ +79° (*c* 0.9, dichloromethane). ^{1}H-N.m.r. data (500 MHz, $CDCl_3$, OH was converted into OD): δ 7.40–7.29 (m, 10 H, 2 Ph), 4.93 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.71 (d, 1 H, *J* 11.3 Hz, PhCH_2), 4.66 (d, 1 H, *J* 11.3 Hz, C*H*Ph), 4.64 (d, 1 H, *J* 11.5 Hz, PhCH_2), 4.60 (d, 1 H, *J* 11.5 Hz, PhCH_2), 4.58 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.97 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.89 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.71 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.71 (dd, 1 H, $J_{3',4'}$ 9.9 Hz, H-3'), 3.59 (dq, 1 H, $J_{4',5'}$ 10.0, $J_{5',6'}$ 6.3 Hz, H-5'), 3.44 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.3 Hz, H-5), 3.42 (dd, 1 H, H-4'), 3.30 (s, 3 H, OMe), 3.29 (dd, 1 H, H-4), 1.30 (d, 3 H, 3 H-6), 1.29 (d, 3 H, 3 H-6').

Anal. Calc. for $C_{27}H_{34}N_6O_7$: C, 58.47; H, 6.18; N, 15.15. Found: C, 58.13; H, 6.13; N, 14.99.

Methyl O*-(2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl)-(1→2)-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl)-(1→2)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**15**). — A solution of **14** (140 mg, 0.252 mmol) in dichloromethane (5 mL) was glycosylated, as in the preparation of **13**, using silver trifluoromethanesulphonate (150 mg, 0.52 mmol) and **12** (150 mg, 0.44 mmol), but the reaction period was limited to 4 h. Work-up then gave **15** as a syrup (180 mg, 83% yield), $[\alpha]_D^{20}$ +83° (*c* 1, dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): ^{1}H (500 MHz), δ 7.37–7.28 (m, 15 H, 3 Ph), 5.37 (dd, 1 H, $J_{1'',2''}$ 2.1, $J_{2'',3''}$ 3.2 Hz, H-2''), 4.94 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.80 (d, 1 H, H-1''), 4.69 (d, 1 H, *J* 11.8 Hz, PhCH_2), 4.69 (d, 1 H, *J* 11.1 Hz, PhCH_2), 4.61 (d, 1 H, *J* 11.8 Hz, PhCH_2), 4.58–4.54 (m, 2 H, PhCH_2), 4.52 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.51 (d, 1 H, *J* 11.1 Hz, PhCH_2), 3.84 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.82 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.74 (dd, 1 H, $J_{3'',4''}$ 9.9 Hz, H-3''), 3.71 (dd, 1 H, $J_{3',4'}$ 9.9 Hz, H-3'), 3.66 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.51 (dq, 1 H, $J_{4',5'}$ 10.0, $J_{5',6'}$ 6.1 Hz, H-5'), 3.48 (dq, 1 H, $J_{4'',5''}$ 10.0, $J_{5'',6''}$ 6.2 Hz, H-5''), 3.41 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.34 (dd, 1 H, H-4''), 3.33 (dd, 1 H, H-4'), 3.28 (s, 3 H, OMe), 3.20 (dd, 1 H, H-4), 2.08 (s, 3 H, Ac), 1.28 (d, 3 H, 3 H-6), 1.26 (d, 3 H, 3 H-6'), 1.17 (d, 3 H, 3 H-6''); ^{13}C (125 MHz), δ 100.24 (d, 1 C, $J_{C\text{-}1'',H\text{-}1''}$ 174 Hz, C-1'), 99.79 (d, 1 C, $J_{C\text{-}1',H\text{-}1'}$ 171 Hz, C-1'), 99.08 (d, 1 C, $J_{C\text{-}1,H\text{-}1}$ 173 Hz, C-1).

Anal. Calc. for $C_{42}H_{57}N_9O_{77}$: C, 58.80; H, 5.99; N, 14.69. Found: C, 59.02; H, 6.01; N, 15.59.

Methyl 4-amino-2-O-(4-amino-4,6-dideoxy-α-D-mannopyranosyl)-4,6-dideoxy-α-D-mannopyranoside (**16**). — A solution of **14** (120 mg, 0.216 mmol) in methanol (20 mL) was hydrogenated over 5% Pd/C (20 mg) at atmospheric pressure and room temperature for 2 days. The solution was filtered through Celite and concentrated to give **16** (50 mg, 72%), which was characterized as the formamido (**17**) and acetamido (**18**) derivatives although the ^{1}H- and ^{13}C-n.m.r. data indicated that the material was pure. ^{1}H-N.m.r. data (500 MHz, D_2O): δ 5.01 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.79 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.02 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.90 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.90 (dq, 1 H, $J_{4',5'}$ 10.0, $J_{5',6'}$ 6.3 Hz, H-5'), 3.85 (dd, 1 H, $J_{3,4}$ 10.2 Hz, H-3), 3.85 (dd, 1 H, $J_{3',4'}$ 10.1 Hz, H-3'), 3.77 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.40 (s, 3 H, OMe), 2.99 (dd, 1 H, H-4), 2.97 (dd, 1 H, H-4'), 1.30 (d, 3 H, 3 H-6), 1.28 (d, 3 H, 3 H-6').

Methyl 4,6-dideoxy-2-O-(4,6-dideoxy-4-formamido-α-D-mannopyranosyl)-4-formamido-α-D-mannopyranoside (**17**). — A solution of **16** (30 mg, 0.093 mmol) in methanol (5 mL) was treated with the mixed anhydride[18] (0.5 mL) prepared from acetic anhydride (20 mL) and formic acid (10 mL). After 3 h, concentration and distillation of toluene from the residue gave **17** (30 mg, 85%). Column chromatography (ethyl acetate–methanol–water, 6:3:1) gave pure **17**, $[\alpha]_D^{20}$ +39° (*c* 0.5, methanol). The *ZE*-ratio was ~3.4:1.0. ^{1}H-N.m.r. data (500 MHz, D_2O): *Z*-isomer, δ 8.21 (s, 1 H, NHC*H*O), 8.20 (s, 1 H, NHC*H*O), 5.04 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.84 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.13 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.11 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.98 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 3.97–3.93 (m, 3 H, H-2,3,4), 3.95 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4'), 3.90 (dq, 1 H, $J_{5',6'}$ 6.0 Hz, H-5'), 3.79 (dq, 1 H, $J_{5,6}$ 6.3 Hz, H-5), 3.40 (s, 3 H, OMe), 1.23 (d, 3 H, 3 H-6), 1.22 (d, 3 H, 3 H-6'); *E*-isomer, δ 8.04 (s, 1 H, NHC*H*O), 8.03 (s, 1 H, NHC*H*O), 5.05 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.82 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.09 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.98 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 3.97–3.93 (m, 2 H, H-2,3), 3.93 (m, 1 H, H-5'), 3.83 (m, 1 H, H-5), 3.40 (s, 3 H, OMe), 3.39 (m, 2 H, H-4,4'), 1.29 (d, 3 H, 3 H-6), 1.27 (d, 3 H, 3 H-6').

Anal. Calc. for $C_{15}H_{26}N_2O_9$: C, 47.61; H, 6.93; N, 7.40. Found: C, 47.55; H, 6.67; N, 7.03.

Methyl 4-acetamido-2-O-(4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-4,6-dideoxy-α-D-mannopyranoside (**18**). — A solution of **16** (20 mg, 0.062 mmol) in methanol (2 mL) was treated with acetic anhydride (0.2 mL). After 2 h at room temperature, the solution was concentrated, and co-distillation with toluene gave **18** (18 mg, 72%), $[\alpha]_D^{20}$ +37° (*c* 0.65, methanol). ^{1}H-N.m.r. data (500 MHz, D_2O): δ 4.90 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.71 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 3.98 (dd, 1 H, $J_{2,3}$ 3.1 Hz), 3.94 (dd, 1 H, $J_{3,4}$ 10.6, $J_{4,5}$ 10.0 Hz, H-4), 3.92–3.80 (m, 4 H, H-2',3',4',5'), 3.83 (dd, 1 H, H-3), 3.81 (dq, 1 H, $J_{5,6}$ 6.1 Hz, H-5), 3.40 (s, 3 H, OMe), 2.04 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 1.22 (d, 3 H, 3 H-6), 1.18 (d, 3 H, $J_{5',6'}$ 5.8 Hz, 3 H-6').

Anal. Calc. for $C_{17}H_{30}N_2O_9$: C, 50.24; H, 7.44; N, 6.89. Found: C, 50.15; H, 7.29; N, 6.71.

Methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**19**). — A solution of **7** (500 mg, 2.46 mmol) in methanol (20 mL) was hydrogenated over 5% Pd/C (70 mg) at atmospheric pressure for 18 h. The mixture was filtered through Celite and concentrated to give **19** (385 mg, 88%), m.p. 151–153°, $[\alpha]_D^{20}$ +86° (*c* 1, methanol); lit.[5] m.p. 152–153°, $[\alpha]_D^{27}$ +82.5° (methanol). ^{1}H-N.m.r. data (500 MHz, D_2O): δ 4.69 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.83 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.59 (dq, 1 H, $J_{4,5}$ 10.0, $J_{5,6}$ 6.2 Hz, H-5), 3.58 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 3.38 (s, 3 H, OMe), 2.74 (dd, 1 H, H-4), 1.28 (d, 3 H, 3 H-6).

Anal. Calc. for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 47.33; H, 8.54; N, 7.73.

Methyl 4,6-dideoxy-4-formamido-α-D-mannopyranoside (**20**). — A solution of **15** (200 mg, 1.13 mmol) in methanol (20 mL) was treated for 1 h at 0° with the mixed anhydride (2 mL) prepared from acetic anhydride (20 mL) and formic acid (10 mL) mixed at 0°, heated for 15 min at 50°, and then cooled to 0°. Concentration and distillation of toluene from the residue gave a product which crystallized from ethanol–ether to give **20** (200 mg, 86%), m.p. 171–172°, $[\alpha]_D^{20}$ +85.5° (*c* 0.9, methanol). The ^{1}H-n.m.r. data showed a *ZE*-ratio of ~2.5:1.0. ^{1}H-N.m.r. data (500 MHz, D_2O): *Z*-isomer, δ 8.20 (s, 1 H, NHC*H*O), 4.72 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.91 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.89 (dd, 1 H, $J_{3,4}$ 10.2, $J_{4,5}$ 10.0 Hz, H-4), 3.81 (dd, 1 H, H-3), 3.77 (dq, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 3.38 (s, 3 H, OMe), 1.11 (d, 3 H, 3 H-6); *E*-isomer, δ 8.03 (s, 1 H, NHC*H*O), 4.72 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.93 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.79 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 3.77 (dq, 1 H, $J_{4,5}$ 10.5, $J_{5,6}$ 6.5 Hz, H-5), 3.38 (s, 3 H, OMe), 3.34 (dd, 1 H, H-4), 1.17 (d, 3 H, 3 H-6).

Anal. Calc. for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83. Found: C, 46.69; H, 7.33; N, 6.72.

Methyl 4-acetamido-4,6-dideoxy-α-D-mannopyranoside (**21**). — A solution of **19** (150 mg, 0.85 mmol) in methanol (5 mL) was treated with acetic anhydride (0.5 mL) for 2 h at 0°. Concentration, distillation of toluene from the residue, and crystallization from ether–hexane gave **21** (150 mg, 81%), m.p. 185–186°, $[\alpha]_D^{20}$ +89° (*c* 0.9, methanol); lit.[5] m.p. 184–186°, and $[\alpha]_D^{26}$ +85.2° (methanol). ^{1}H-N.m.r. data (500 MHz, D_2O): δ 4.72 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.91 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.84 (dd, 1 H, $J_{3,4}$ 10.2, $J_{4,5}$ 10.1 Hz, H-4), 3.78 (dd, 1 H, H-3), 3.71 (dq, 1 H, $J_{5,6}$ 6.2 Hz, H-5), 3.39 (s, 3 H, OMe), 2.03 (s, 3 H, AcN), 1.20 (d, 3 H, 3 H-6).

Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; N, 7.82; N, 6.39. Found: C, 49.10; H, 7.78; N, 6.24.

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