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Synthesis of C-3 nitrogen-containing derivatives of *N*-acetyl-α,β-D-mannosamine as substrates for *N*-acetylneuraminic acid aldolase

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Abstract

The synthesis of 3-azido-3-deoxy, 3-amino-3-deoxy and 3-*N*-*tert*-butyloxycarbonyl-3-deoxy derivatives of 2-acetamido-2-deoxy-α,β-D-mannose (*N*-acetyl-α,β-D-mannosamine, ManNAc), is presented. The 3-azido-3-deoxy- and 3-*N*-*tert*-butyloxycarbonyl compounds were further characterised as their peracetates. A preliminary study has found that these C-3 nitrogen-substituted derivatives of ManNAc not to be substrates for Neu5Ac aldolase.

Keywords: ManNAc derivatives; Neu5Ac aldolase; Sialic acid; *N*-Acetylneuraminic acid

1. Introduction

Aminodeoxy sugars have received considerable attention owing to their diverse structure and biological properties. Many differing structural types of such sugars frequently occur in antibiotics glycosidically linked to complex aglycon units or as simple derivatives in fermentation products.[1] As well as having therapeutic potential,[1] some aminodeoxy sugars are important synthetic intermediates to more elaborate systems. For example, aminodeoxy sugars have been employed as reaction intermediates in the synthesis of imino sugars (sometimes referred to as azasugars).[2] Imino sugars such as 1-deoxynojirimycin and 2,5-anhydro-2,5-imino-D-mannitol, a class of sugar mimics with a nitrogen in the ring, are inhibitors of a number of glycohydrolases.[3] It is not surprising, therefore, that efficient preparation of aminodeoxy sugars of natural and unnatural products has been of longstanding interest to organic and medicinal chemists.

Over the last decade, numerous papers dealing with the synthesis of derivatives of the aminodeoxy sugar, 2-acetamido-2-deoxy-α,β-D-mannose (*N*-acetyl-D-mannosamine, ManNAc) have appeared, particularly with respect to their use as substrates in the enzyme-catalysed construction of higher 3-deoxy-2-ulosonic acid derivatives.[4] Thus, as shown in Scheme 1, Neu5Ac aldolas_e catalyses the reversible aldol reaction of ManNAc and pyruvate, which results in the formation of

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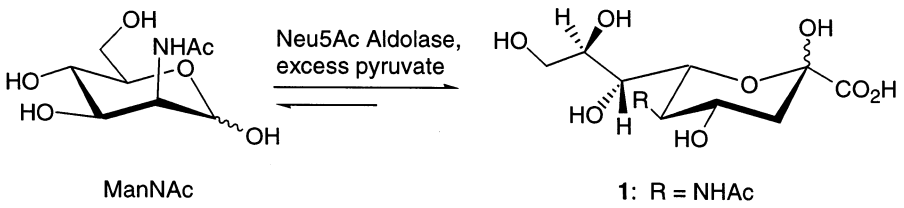
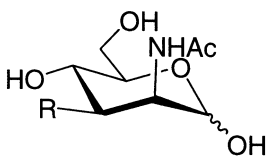
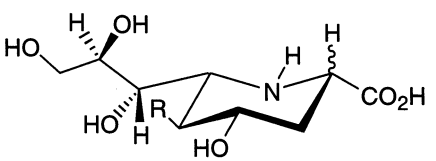


Scheme 1.

N-acetylneuraminic acid (Neu5Ac, **1**), a nine-carbon ulosonic acid. Ulosonic acids such as **1** and their derivatives are involved in a range of important biological processes such as molecular recognition[5] (Scheme 1). Besides ManNAc itself, Neu5Ac aldolase also accepts as substrates a range of ManNAc derivatives substituted at the 4- and 6-position.[4,6] This has provided an attractive enzymatic route to a number of natural and unnatural neuraminic acid derivatives. Studies employing various D-mannoses substituted at C-3 have found that the hydroxyl group at this position is essential for successful Neu5Ac aldolase-catalysed condensation.[7] To the best of our knowledge, no C-3 substituted derivatives of ManNAc have been investigated as potential substrates of Neu5Ac aldolase. Here, we attempt to delineate the reactivity of several C-3 substituted ManNAc derivatives as substrates of Neu5Ac aldolase. In particular, we have focussed on the C-3 nitrogen-substituted derivatives (**2**–**4**). Such ManNAc derivatives, if accepted by the enzyme, have the potential to generate the nitrogen isostere of Neu5Ac such as **5**. A 16-step total chemical synthesis of **5** starting from D-glucose has previously been reported[8] by Baumberger and Vasella, and this compound was found to be a modest inhibitor of sialidase from *Vibrio cholerae*. The synthesis of the ManNAc derivatives (**2**–**4**) themselves, per se, is also of interest, as this has not been previously reported. These compounds will allow us, for the first time, to investigate the substrate specificity of Neu5Ac aldolase for C-3 substituted ManNAc derivatives. Preliminary results of these experiments are presented.



2 R = N_3
3 R = NH_2
4 R = NHBOC



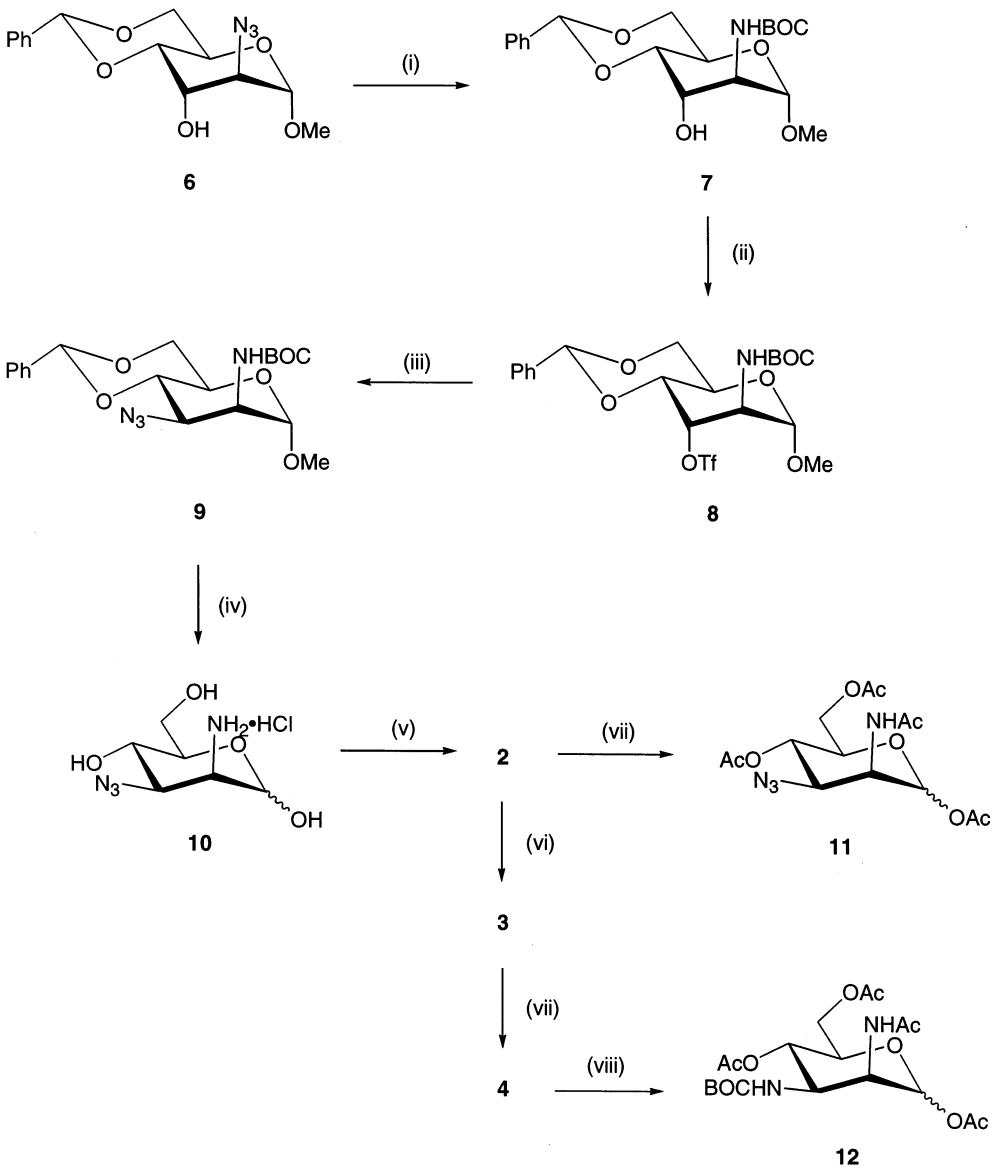
5: R = NHAc

2. Results and discussion

Two fundamental protocols for the synthesis of vicinal diamines are known: fission of epimino-derivatives by nucleophiles, e.g., azide ion,[9] and opening of an epoxide ring with a nitrogen-containing nucleophile.[10] In the latter case, the resulting alcohol is then converted into the diamino derivative via direct nucleophilic substitution by azide or an amino equivalent through intermediate sulfonate esters.

Retrosynthetic analysis of the ManNAc derivatives (**2**–**4**) suggested that the pyranose with an azido group at C-2, methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**6**),[11] was an obvious precursor (Scheme 2). Following previously published procedures,[11] this compound was conveniently prepared from the commercially available methyl α-D-glucopyranoside in four steps.

It was anticipated that direct S_N2 displacement with azide at C-3 via the methanesul-



Scheme 2. Reagents and conditions: (i) H_2, PtO_2, Boc_2O, rt, 40 h; (ii) Tf_2O, pyridine, $-30\,^{\circ}C \rightarrow 0\,^{\circ}C$; (iii) n-Bu_4NN_3, rt, 24 h; (iv) 4:1 HOAc–water, 100 °C, 24 h; 5 N HCl, 100 °C, 3 days; (v) Ac_2O, pyridine, MeOH, rt, 30 min; (vi) H_2, PtO_2, rt, 24 h; (vii) Boc-ON, rt, 48 h; (viii) Ac_2O, pyridine.

fonate ester of the 2,3-*trans*-diaxial 2-*N*-acetamido-2-deoxy altroside, methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (readily prepared via sequential reduction and acetylation of **6**) would lead to epimine formation.[12] Thus with the reacting groups being trans-diaxial, adjustment of the *N*-nucleophilicity at C-2 was necessary in order to favour intermolecular nucleophilic displacement over the competing intramolecular reaction. After some experimentation, it was found that a (*tert*-butoxycarboxy)amino group at this position was a suitable candidate. Following the one-pot catalytic hydrogenolysis of the azide **6** over platinum oxide and Boc-protection, the 2-*N*-(*tert*-butoxycarboxy)amine **7** was obtained in 90% yield.

The *altro*-configured alcohol **7** was then converted into the 3-*O*-trifluoromethanesulfonate **8** that underwent displacement with azide (tetrabutylammonium azide) in benzene to give, by inversion of configuration at C-3, the 3-azido-3-deoxy mannoside **9** in 57% yield (two steps). All of the protecting groups associated with mannoside **9** were then removed.

Thus, the benzylidene protecting group was removed by treating the mannoside **9** with aqueous acetic acid (4:1 HOAc–water) at 100 °C for 24 h. The volatiles were then removed under diminished pressure, and the residue was filtered through a short plug of silica gel. The product was isolated from the column using a combination of ethyl acetate and methanol as eluant. Conventional O-deglycosidation of the isolated methyl glycoside in refluxing 5 N HCl for 3 days resulted in the concomitant conversion of the 2-acetamido functionality to the corresponding amine hydrochloride **10**. Subsequent acetylation of **10** in pyridine with Ac_2O in the presence of methanol as a cosolvent gave, after chromatographic purification on silica gel, the corresponding 2-acetamide **2** as an amorphous solid in 63% yield (based on the mannoside **9**).

To complete the synthesis, under catalytic hydrogenolysis conditions, the 3-azido-3-deoxy compound **2** was converted into the amine **3**, and **3** in turn was converted into the corresponding 3-*N*-(*tert*-butoxycarbonyl)-amine (**4**) with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON), in good overall yield (54%, two steps). Confirmation of the structures of both **2** and **4** was provided by the conversion of these compounds into their corresponding peracetylated derivatives (**11** and **12**, respectively) with Ac_2O and pyridine.

Finally, the substrate specificity of Neu5Ac aldolase for compounds **2**–**4** was investigated. In separate experiments, according to published procedures,[4,13] each of the compounds was incubated at pH 7.5 in the presence of excess pyruvate and Neu5Ac aldolase in a membrane-enclosed enzyme reactor (dialysis bag) at 37 °C, and the reaction was monitored by both TLC and ^{1}H NMR spectroscopy. Preliminary investigations have found that none of these compounds to be substrates of the enzyme.

In conclusion, through a simple series of functional group transformations of D-glucose, we have prepared three hitherto unknown C-3 nitrogen-containing derivatives of ManNAc, **2**–**4**. These compounds were not substrates for Neu5Ac aldolase, and this outcome suggests that a hydroxyl group at C-3 of the acceptor substrate is a necessary requirement for successful Neu5Ac aldolase-catalysed reactions. These conclusions are consistent with results from a previous study[7] that employed C-3-modified D-mannose derivatives as possible substrates for Neu5Ac aldolase.

3. Experimental

General.—^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra (in δ (ppm) downfield from Me_4Si) were recorded on a Bruker AMX 300 spectrometer at 303 K and were referenced using residual ^{1}H resonances in the solvent. *J*-values are in hertz (Hz). Low-resolution (LR) ESI mass spectra (cone voltage 30 V) were obtained using a Micromass Platform II electrospray spectrometer and high-resolution (HR) mass spectra were recorded on a Bruker BIO-APEX II FT ion cyclotron resonance mass spectrometer with an 'Analytica' ESI source. Specific optical rotations $[\alpha]_D$, quoted in 10^{-1} deg cm^2/g, were measured at rt using a JASCO DIP-370 polarimeter with a path length 50 mm. Concentrations are quoted in 10^{-2} g/mL. Elemental analyses were performed either by the Chemical and Microanalytical Service, Essendon, Victoria or by Microanalytical Service, Department of Chemistry, University of Queensland, Brisbane, Queensland. IR spectra were recorded using a Hitachi 270-30 spectrophotometer. Column chromatography was performed on E. Merck Silica Gel 60 (0.040–0.063 mm). Reactions were monitored by TLC on Kieselgel 60 F_{254} plates (E. Merck 5554) and visualised under ultraviolet (UV) irradiation and by spraying with 95% aq EtOH containing 5% H_2SO_4 and charring for several minutes at 180 °C. Hexane refers to the fraction of petroleum ether that boils in the range 60–80 °C and was distilled before use.

*Preparation of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-altropyranoside* (**6**).—The known title compound **6** was prepared according to the literature procedure.[11]

*Methyl 4,6-*O*-benzylidene-2-(*tert*-butoxycarbonyl)amino-2-deoxy-α-*D*-altropyranoside* (**7**).—A solution of the azide **6**, methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altro-

pyranoside[11] (11.2 g, 36.5 mmol) in EtOH (300 mL) was degassed (15 mm Hg/rt) before the addition of platinum oxide (50 mg) and di-*tert*-butyl dicarbonate (9.12 g, 41.8 mmol). The mixture was subjected to hydrogenolysis conditions (atmospheric H_2) for 40 h at rt. After filtration through a short plug of Celite, the clear solution was concentrated in vacuo, and the resulting amorphous solid was recrystallised (1:1 EtOAc–hexane) to give the title compound **7** as colourless needles (9.73 g, 70%): mp 170–171 °C; $[\alpha]_D$ +49° (*c* 3.49, $CHCl_3$); R_f 0.27 (1:2 EtOAc–hexane). ^1H NMR ($CDCl_3$): δ 1.46 (s, 9 H, $C(CH_3)_3$), 2.88 (br s, 1 H, OH), 3.44 (s, 3 H, OCH_3), 3.67 (m,1 H, H-4), 3.78 (dd, 1 H, $J_{6,5}$ 10.1, $J_{6,6'}$ 10.1, H-6), 4.08–4.18 (m, 2 H, H-2, H-5), 4.25 (m, 1 H, H-3), 4.34 (dd, 1 H, $J_{6',5}$ 5.1, H-6′), 4.64 (br s, 1 H, H-1), 4.78 (br, 1 H, NH), 5.63 (s, 1 H, H-7), 7.35–7.53 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 28.3 ($C(CH_3)_3$), 53.0 (C-2), 55.7 (OCH_3), 58.3 (C-3), 68.1 (C-5), 69.0 (C-6), 76.8 (C-4), 80.3 ($C(CH_3)_3$), 101.3 (C-1), 102.3 (C-7), 126.2, 128.2, 129.1, 137.1 (aromatic carbons), 154.5 (carbonyl). LRMS: 382 (MH^+, 13%), 326 (30), 294 (100); HRMS for $C_{19}H_{28}NO_7$ [$M^+ + H$] requires 382.1866, found 382.1855. Anal. Calcd for $C_{19}H_{27}NO_7$: C, 59.8; H, 7.1. Found: C, 59.9; H, 7.2.

*Methyl 3-azido-4,6-*O*-benzylidene-2-(*tert*-butoxycarbonyl)amino-2,3-dideoxy-α-*D*-mannopyranoside* (**9**).—A stirring solution of the alcohol **7** (7.00 g, 18.4 mmol) and pyridine (2.24 mL, 27.7 mmol) in dry CH_2Cl_2 (100 mL) was cooled to −24 °C (CCl_4–dry ice) prior to the addition of trifluoromethanesulfonic anhydride (3.24 mL, 19.2 mmol) dropwise over a period of 30 min. When the addition was complete, the cold bath was replaced with an ice bath, and stirring was continued for another 3 h. Et_2O (400 mL) was then added to the orange solution, and the resulting solids were filtered off. Evaporation of the orange solution under reduced pressure gave the corresponding 3-*O*-triflate **8** (9.13 g, 97%), which was used in the subsequent step without further purification: R_f 0.45 (1:2 EtOAc–hexane).

A mixture of the crude 3-*O*-triflate **8** (9.13 g, 17.8 mmol), sodium azide (2.34 g, 36.0 mmol), tetrabutylammonium hydrogensulfate (6.50 g, 19.1 mmol) and benzene (150 mL) was stirred vigorously at rt for 15 h. The reaction mixture was diluted with hexane (400 mL) and washed with water (50 mL). The organic phase was isolated and washed again with water (20 mL) before drying and concentrating to provide the crude azide **7** (6.01 g) as a straw-coloured foam. After column chromatography (1:8 → 1:6 EtOAc–hexane), pure azide **9** was obtained as an off-white solid (5.23 g, 70%); a small sample was recrystallised from hexane: mp 123–124 °C (colourless needles); $[\alpha]_D$ +25° (*c* 1.26, $CHCl_3$); R_f 0.60 (1:2 EtOAc–hexane). IR (ν_{max}, NaCl): 2103 cm^{-1} (N_3). ^1H NMR ($CDCl_3$): δ 3.38 (s, 1 H, OCH_3), 3.66 (dd, 1 H, $J_{4,3}$ 9.7, $J_{4,5}$ 9.7, H-4), 3.79 (dd, 1 H, $J_{6,5}$ 9.9, $J_{6,6'}$ 9.9, H-6), 3.89 (ddd, 1 H, $J_{5,6'}$ 4.4, H-5), 4.09 (dd, 1 H, $J_{3,2}$ 4.2, H-3), 4.19 (m, 1 H, H-2), 4.28 (dd, 1 H, H-6′), 4.69 (br s, 1 H, H-1), 4.73 (d, 1 H, $J_{NH,2}$ 7.5, NH), 5.61 (s, 1 H, H-7), 7.35–7.49 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 28.4 ($C(CH_3)_3$, 53.1 (C-2), 55.1 (OCH_3), 58.3 (C-3), 63.6 (C-5), 66.7 (C-6), 78.1 (C-4), 80.5 ($C(CH_3)_3$), 101.0, 102.0 (C-1, C-7), 126.1, 128.3, 129.1, 137.0 (aromatic carbons), 155.4 (carbonyl). LRMS: 407 (MH^+, 16%), 381 (82), 351 (50), 325 (100); HRMS for $C_{19}H_{27}N_4O_6$ [$M^+ + H$] requires 407.1931; found 407.1932. Anal. Calcd for $C_{19}H_{26}N_4O_6$: C, 56.15; H, 6.45. Found: C, 55.8; H, 6.3.

*2-Acetamido-3-azido-2,3-dideoxy-α,β-*D*-mannopyranose* (**2**).—A mixture of methyl 3-azido-4,6-*O*-benzylidene-2-(*tert*-butoxycarbonyl)amino-2,3-dideoxy-α-*D*-mannopyranoside (**9**) (4.00 g, 9.85 mmol) and 4:1 HOAc–water (100 mL) was heated under reflux for 24 h and cooled. The volatiles were then evaporated under reduced pressure, and the residue was filtered through a short plug of silica gel. Elution with a combination of EtOAc and MeOH gave, after solvent removal, a straw-coloured foam (2.53 g).

A solution of the foam in 5 N HCl (100 mL) was heated for 3 days at 100 °C. The solution was then cooled, and the volatiles were evaporated in vacuo. The resulting residue was washed with Et_2O (3 × 20 mL) and dried under vacuum, leaving the corresponding amine hydrochloride **10** as a straw-coloured foam (2.34 g).

To a solution of the crude amine hydrochloride **10** (2.34 g) in MeOH (16 mL) and pyrid-

ine (8 mL) at rt, was added Ac_2O (8 mL) dropwise over 5 min. After 30 min, the volatiles were removed by co-distillation (× 2) with toluene. After drying under high vacuum, the residue was purified by column chromatography (85:12:3 EtOAc–MeOH–water), which gave the title compound as a straw-coloured foam (1.95 g, 82%): R_f 0.20 (85:12:3 EtOAc–MeOH–water). IR (ν_{max}, NaCl): 2112 cm^{-1} (N_3). ^1H NMR (D_2O): δ 2.05, 2.09 (s, 3 H, NHCOC$H_{3(\alpha,\beta)}$), 3.55–3.95 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 5.05, 5.11 (each d, 1 H, $J_{1,2}$ 1.4 and 1.7, H-$1_{(\alpha,\beta)}$). ^{13}C NMR (D_2O): δ 24.6 (NHCOCH_3), 54.3, 54.9 (C-$2_{(\alpha,\beta)}$), 63.0 (C-$6_{(\alpha,\beta)}$), 64.0, 66.9, 68.0, 68.2, 74.7, 79.7 (C-3, C-4, C-5), 95.2, 95.7 (C-$1_{(\alpha,\beta)}$), 177.1, 178.1 (carbonyls). LRMS: 247 (MH^+, 100%). 229 (89).

*2-Acetamido-4,5,6-tri-*O*-acetyl-3-azido-2,3-dideoxy-α,β-*D*-mannopyranose* (**11**).—To a stirring solution of 2-acetamido-3-azido-2,3-dideoxy-α,β-D-mannopyranose (**2**) (100 mg, 0.41 mmol) in pyridine (1 mL) at rt was added Ac_2O (0.5 mL). After 3 h, the reaction mixture was concentrated to dryness, and the residue was purified by column chromatography (1:2 → 1:1 EtOAc–hexane) to give **11** as an α/β anomeric mixture (110 mg, 73%), α:β = 4:1 (by ^1H NMR spectroscopy): R_f 0.58 (EtOAc). A small sample was recrystallised from 1:1 EtOAc–hexane, which gave the α anomer, 2-acetamido-4,5,6-tri-*O*-acetyl-3-azido-2,3-dideoxy-α-D-mannopyranose (**11**) as fine colourless needles: mp 162–163 °C; $[\alpha]_D$ + 64° (*c* 1.31, $CHCl_3$). IR (ν_{max}, NaCl): 2108 cm^{-1} (N_3). ^1H NMR for the α anomer ($CDCl_3$): δ 1.54 (s, 9 H, $C(CH_3)_3$), 2.09, 2.10, 2.15, 2.17 (s, each 3 H, $OCOCH_3 \times 3$), 3.99 (ddd, 1 H, $J_{5,4}$ 9.8, $J_{5,6}$ 2.6, $J_{5,6'}$ 5.1, H-5), 4.10–4.35 (m, 2 H, H-3, H-6), 4.26 (dd, 1 H, $J_{6',6}$ 12.4, H-6′), 4.62 (ddd, 1 H, $J_{2,1}$ 1.7, $J_{2,3}$ 4.4, $J_{2,NH}$ 9.1, H-2), 5.07 (dd, 1 H, $J_{4,3}$ 10.0, H-4), 5.68 (d, 1 H, NH), 6.04 (d, 1 H, H-1). Selected ^1H NMR data for the β anomer ($CDCl_3$): δ 5.02 (dd, 1 H, $J_{4,3}$ 9.6, $J_{4,5}$ 9.6, H-4), 5.78 (d, 1 H, $J_{1,2}$ 2.1, H-1). ^{13}C NMR ($CDCl_3$): δ 20.6, 20.7, 23.1 ($OCOCH_3 \times 3$), 49.8 (C-5), 59.2 (C-2), 62.3 (C-6), 66.9 (C-4), 70.0 (C-3), 91.5 (C-1), 167.9, 169.7, 170.4 (carbonyls). LRMS: 373 (MH^+, 29%), 347 (61), 313 (100); HRMS for $C_{14}H_{21}N_4O_8$ [$M^+ + H$] requires 373.1359; found 373.1372. Anal. Calcd for $C_{14}H_{20}N_4O_8$: C, 45.16; H, 5.41. Found: C, 44.9; H, 5.3.

*2-Acetamido-3-(*tert*-butoxycarbonyl)amino-2,3-dideoxy-α,β-*D*-mannopyranose* (**4**).—A solution of the azide, 2-acetamido-3-azido-2,3-dideoxy-α,β-D-mannopyranoside (**2**) (2.00 g, 8.1 mmol) in MeOH (50 mL) was degassed (15 mm Hg/rt) before the addition of Pt_2O (30 mg), and the mixture was subjected to hydrogenolysis conditions (atmospheric H_2) until TLC analysis showed the disappearance of starting material. After filtration, the solution was concentrated in vacuo, which gave the corresponding amine **3** as a straw-coloured foam (1.76 g, 98%).

To a stirring solution of the amine 3 (1.76 g, 8.0 mmol) in 1:1 CH_3NO_2–water (10 mL) was added NEt_3 (2.23 mL, 16.0 mmol) and Boc-ON (2.96 g, 12.0 mmol). After 2 days at rt, the white precipitate was filtered off, and the solution was concentrated under reduced pressure. The remaining residue was washed with Et_2O (2 × 20 mL) and then applied onto a silica gel column. Elution with 10:2:1 EtOAc–2-PrOH–water gave the title compound **4** as a colourless amorphous solid (1.40 g, 54%): R_f 0.20 (85:12:3 EtOAc–MeOH–water). ^1H NMR (D_2O): δ 1.37 (s, 9 H, $C(CH_3)_3$), 1.97, 2.01 (s, 3 H, NHCOC$H_{3(\alpha,\beta)}$), 3.40–3.95 (m, 5 H), 4.25–4.38 (m, 1 H), 5.04 (br s, 1 H, H-1). Selected ^{13}C NMR (D_2O): δ 24.9 (NHCOCH_3), 30.8 ($C(CH_3)_3$), 54.7, 55.5 (C-$2_{(\alpha,\beta)}$), 63.6 (C-6), 67.3, 67.4 (C-$3_{(\alpha,\beta)}$), 75.5, 80.6 (C-4, C-5), 95.8, 96.2 (C-$1_{(\alpha,\beta)}$), 81.0 ($C(CH_3)_3$).

*2-Acetamido-4,5,6-tri-*O*-acetyl-3-(*tert*-butoxycarbonyl)amino-2,3-dideoxy-α,β-*D*-mannopyranose* (**12**).—A solution of 2-acetamido-3-(*tert*-butoxycarbonyl)amino-2,3-dideoxy-α,β-D-mannopyranose (**4**) (100 mg, 0.313 mmol) in pyridine (1.0 mL) was treated with Ac_2O (0.5 mL) at rt for 16 h and then concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2, and the solution was washed with water (2 × 10 mL) and then dried. After evaporation of the solvent, the crude product (130 mg) was purified by column chromatography (2:1 EtOAc–hexane) to give first, the α anomer of the title com-

pound **12** as a colourless crystalline solid (95 mg), followed by the β anomer (24 mg), giving a combined total yield of 85%. Physicochemical data for the α anomer: mp 183–184 °C; $[\alpha]_D$ +46° (*c* 1.00, $CHCl_3$); R_f 0.27 (3:1 EtOAc–hexane). ^1H NMR ($CDCl_3$): δ 1.42 (s, 9 H, $C(CH_3)_3$), 2.07, 2.08, 2.10, 2.16 (s, each 3 H, $NHCOCH_3$, $OCOCH_3 \times 3$), 4.04 (dd, 1 H, $J_{6,5}$ 2.0, $J_{6,6'}$ 12.0, H-6), 4.08 (m, 1 H, H-5), 4.26–4.36 (m, 2 H, $J_{6',5}$ 4.3, H-3, H-6′), 4.54 (m, 1 H, H-2), 4.81 (d, 1 H, $J_{NH,3}$ 9.0, NH_a), 4.90 (dd, 1 H, $J_{4,3}$ 10.0, $J_{4,5}$ 10.0, H-4), 5.75 (d, 1 H, $J_{NH,2}$ 9.1, NH_b), 5.95 (br s, 1 H, H-1). ^{13}C NMR δ 20.5, 20.6, 20.8 ($OCOCH_3 \times 3$), 23.2 ($NHCOCH_3$), 28.2 ($C(CH_3)_3$), 49.5 (C-3), 50.6 (C-2), 62.5 (C-6), 67.1 (C-4), 70.4 (C-5), 80.0 ($C(CH_3)_3$), 91.7 (C-1), 155.4, 168.3, 170.5, 170.6, 170.9 (carbonyls). LRMS: 447 (MH^+, 4%), 402 (12), 380 (35), 347 (10), 331 (100), 280 (65), 239 (12), 60 (25). Anal. Calcd for $C_{19}H_{30}N_2O_{10}$: C, 51.12; H, 6.77; N, 6.27. Found: C, 51.0; H, 6.9; N, 6.2.

Physicochemical data for the β anomer: R_f 0.18 (3:1 EtOAc–hexane). ^1H NMR ($CDCl_3$): δ 1.39 (s, 9 H, $C(CH_3)_3$), 2.03, 2,04, 2.06, 2.13 (s, each 3 H, $NHCOCH_3$, $OCOCH_3 \times 3$), 3.82 (m, 1 H, H-5), 3.98–4.12 (m, 2 H, H-3, H-6), 4.32 (m, 1 H, $J_{6',5}$ 5.0, $J_{6',6}$ 12.3, H-6′), 4.64 (m, 1 H, H-2), 4.82 (dd, 1 H, $J_{4,3}$ 10.4, $J_{4,5}$ 10.4, H-4), 4.91 (br d, 1 H, $J_{NH,3}$ 7.0, NH_a), 5.81 (br s, 1 H, H-1), 6.32 (d, 1 H, $J_{NH,2}$ 9.2, NH_b). ^{13}C NMR: δ 20.7 ($OCOCH_3 \times 3$), 23.5 ($NHCOCH_3$), 28.2 ($C(CH_3)_3$), 51.2 (C-3), 53.0 (C-2), 62.1 (C-6), 66.9 (C-4), 74.3 (C-5), 80.3 ($C(CH_3)_3$), 91.9 (C-1), 155.3, 168.3, 170.5, 170.6, 171.5 (carbonyls). LRMS: 447 (MH^+, 3%), 380 (22), 347 (32), 331 (35), 280 (65), 239 (34), 141 (27), 60 (100).

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