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Determination of the Anomeric Configurations of 2,3,4,6-Tetra-*O*-Acetyl-D-Mannopyranosyl Azide

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The structures of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide and 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide were determined using X-ray crystallographic and one-dimensional NOESY techniques.

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Glycosyl azides are frequently synthesized as precursors to glycosylamines and glycosylamides, many of which are biologically important.[1,2] Glycosyl azides may be reduced to the corresponding amine and then acylated using peptide-coupling methodologies. Alternatively, glycosyl azides can be coupled directly to an acid chloride by means of the Staudinger reaction.[3] The Staudinger reaction is the preferred method for the synthesis of β-glycosyl amides as it allows reaction of the acid chloride directly with the sugar azide, thereby eliminating the need for glycosyl amine intermediates, which are prone to anomerization and hydrolysis.[3] An excellent discussion on the synthesis and structure of glycosyl azides can be found in the review by Gyorgydeak et al.[4]

We have used glycosyl azides in the synthesis of a series of glycosylamides, for a program examining the inhibition of growth factors.[5] As part of our project we aimed to synthesise both α- and β-mannopyranosyl amides. This required preparation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide **3** and its C1 epimer 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **4**. Both **3** and **4** have been described previously;[6–12] however, uncertainty exists with regard to their anomeric configurations.[6,7] Despite this uncertainty, the anomeric configurations of **3** and **4** have nevertheless been assigned quite frequently in the literature.[6,7,10–12] In this paper we unambiguously assign the anomeric configuration of the acetylated mannopyranosyl azides **3** and **4**, using a combination of X-ray crystallography and NMR spectroscopy.

Typically, the anomeric configuration of a hexapyranose can be readily determined from ^{1}H NMR data if the proton at position 2 (H2) is axial. In that case the magnitude of the coupling constant (J) between H1 and H2 is relatively large ($J_{1,2} \sim 9$ Hz) if H1 is also axial. But the coupling constant is comparatively small ($J_{1,2} \sim 2$ Hz) if H1 is equatorial, as described by the Karplus equation.[13] Unfortunately, when H2 is oriented equatorially, as in the case of mannose derivatives, then the anomeric configuration cannot be predicted reliably from ^{1}H NMR data because $J_{1,2}$ axial–equatorial and $J_{1,2}$ equatorial–equatorial couplings are of similar magnitude. The use of ^{13}C chemical shifts and carbon–proton coupling constants ($^1J_{C1,H1}$) are similarly unreliable for establishing anomeric configuration in mannose derivatives.[7]

We synthesized both α- and β-mannopyranosyl azides (**3** and **4**) and were able to assign their anomeric configurations based on X-ray crystallography data and one-dimensional NOESY experiments. Scheme 1 illustrates the synthesis of **3** and **4**. The peracetylated sugar **1** was prepared following the literature.[14] The general method of Pfleiderer and Zondler[15] was used to synthesize the azides **3** and **4**.

The mixture of anomers **3** and **4** was partially separated by silica column chromatography to afford first a syrup which was pure in one anomer, and second a syrup that contained a mixture of anomers. The addition of hexane to this mixture caused crystallization of one anomer and allowed the anomers to be separated by trituration. Purification of the crystalline anomer was achieved by recrystallization from diethyl ether. A crystal suitable for X-ray diffraction was then grown from methanol. We report here the crystal structure of 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **4**.

Several crystals were screened before finally choosing the one used for this structure determination. Unfortunately the bulk sample lost crystallinity over a period of a week or so thus preventing further examination perhaps at low temperature. Despite compounded problems associated with crystal twinning, instability, and disorder, the stereochemistry of compound **4** was unequivocally established by X-ray crystallography (Fig. 1). All bond lengths and angles are as expected and the azide adopts an equatorial disposition. The carbonyl oxygen atoms and methyl carbon atom of the acetyl group attached to C4 exhibited abnormally large anisotropic thermal parameters during refinement and were subsequently

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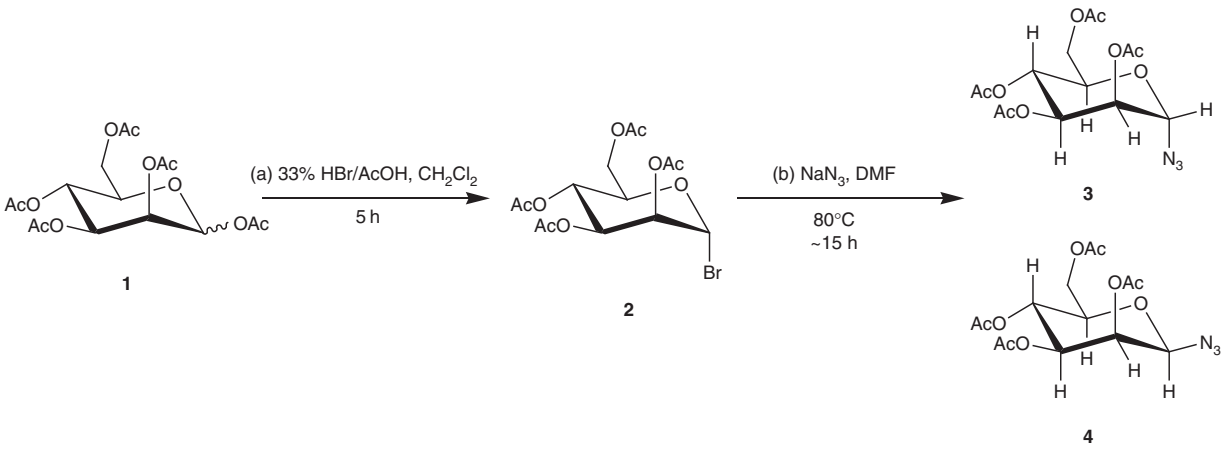

Scheme 1. The synthetic route for azides **3** and **4**.

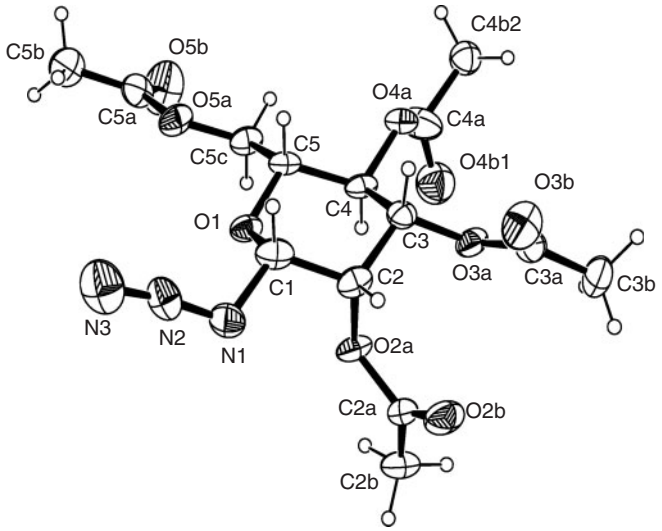

Fig. 1. *ORTEP* diagram of 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **4** (30% probability ellipsoids). Disorder of the acetyl group at C4 not shown for clarity.

resolved into two components that were refined with complementary occupancies. The major contributor is shown in Fig. 1. For comparison, the crystal structures of the isomeric 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide[16] and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide[17] have also been reported.

It could simply be assumed that the oily anomer was the α-azide **3**; however, to prove its anomeric configuration we conducted one-dimensional NOESY experiments. For comparison, one-dimensional NOESY experiments were also recorded on the β-anomer **4**. As illustrated in Fig. 2a, selective irradiation of the H1 resonance of the oily anomer **3** generated an nuclear overhauser enhancement (NOE) correlation with only the H2 resonance. In comparison, selective irradiation of the H1 resonance in the crystalline β-anomer **4** (Fig. 2b) generated NOE correlations with the H2, H3, and H5 protons. The presence of correlations with H3 and H5 in compound **4** indicates that H1 is situated on the α-face of the pyranose ring. Conversely the absence of H3 and H5 correlations in compound **3** indicates that H1 is on the β-face of the pyranose ring, and that this compound is therefore the α-anomer.

In conclusion we have resolved the long-standing ambiguity surrounding the anomeric configuration of mannopyranosyl azides (**3** and **4**). The literature previously assigned the anomeric configuration without definitive evidence to support the stereochemistry at C1. We have presented conclusive evidence for the anomeric configuration of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide **3** and its C1 epimer 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **4**.

Experimental

NMR experiments were recorded on a Bruker AVANCE 300 or 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) on a δ scale, relative to the solvent peak ($CDCl_3$ δ_H 7.24, δ_C 77.0; C_6D_6 δ_H 7.15). Coupling constants (*J*) are reported in Hertz. All assignments were confirmed with the aid of two-dimensional ^1H, ^1H (gCOSY), ^1H, ^{13}C (gHSQC), and selective ge-1D NOESY experiments. One-dimensional NOESY experiments were recorded using C_6D_6 as the solvent. For each one-dimensional NOESY experiment, 32 scans were acquired with a Gaussian-shaped pulse and a mixing time of 500 ms. Low- and high-resolution mass spectra were measured on a Finnigan API-3-sprayer mass spectrometer in positive electrospray ionization mode. Melting points were recorded on a Fischer Johns melting point apparatus. Optical rotations were determined on a Perkin–Elmer 141 Polarimeter.

2,3,4,6-Tetra-O-*acetyl-α-D-mannosyl Bromide 2*

A 33% solution of hydrogen bromide in acetic acid (30 mL) was added to 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose[14] **1** (9.0 g, 23 mmol) in CH_2Cl_2 (60 mL) which was stirred for 5 h at room temperature. The reaction was quenched with cold water, and extracted with CH_2Cl_2. The organic layer was washed with saturated solution of $NaHCO_3$ and brine before being dried ($MgSO_4$), filtered, and evaporated under vacuum to give the crude glycosyl bromide **2** as a yellow oil.

2,3,4,6-Tetra-O-*acetyl-α-D-mannopyranosyl Azide 3 and 2,3,4,6-Tetra*-O-*acetyl-β-D-mannopyranosyl Azide 4*

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranosyl bromide **2** (6.6 g, 16 mmol) was dissolved in DMF (30 mL) and stirred with NaN_3 (9.10 g, 140 mmole) at 80°C for 15 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were

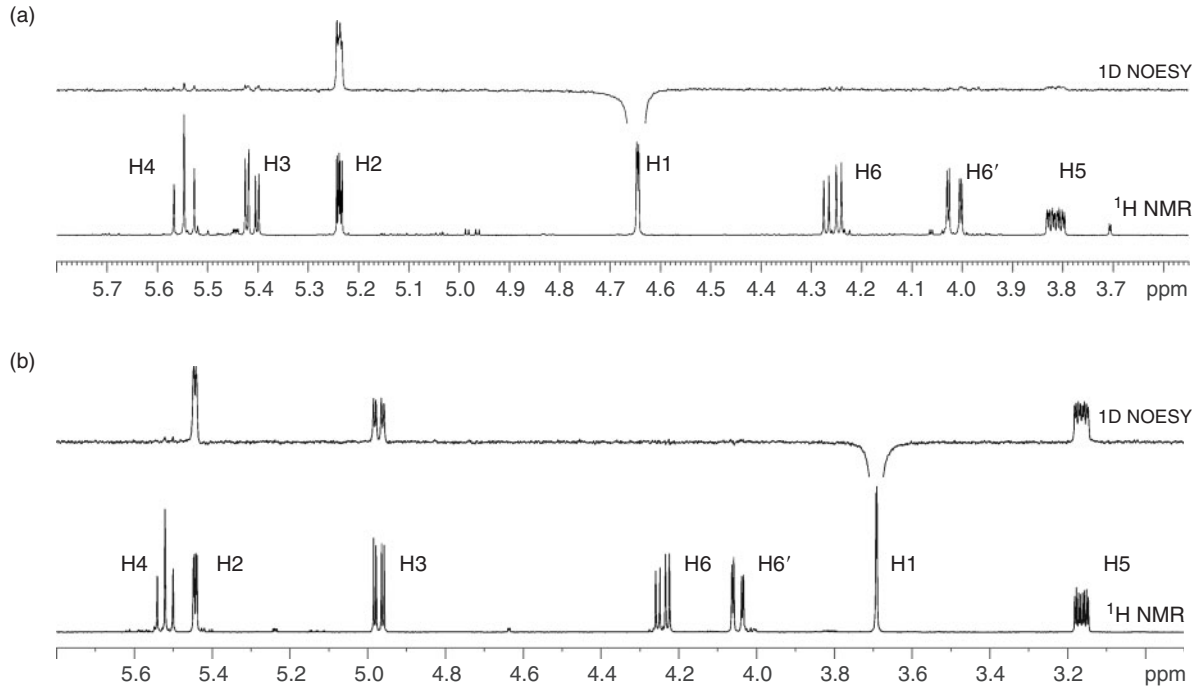

Fig. 2. ^{1}H NMR and one-dimensional NOESY spectra for (a) 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide **3** and (b) 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **4**.

washed with brine, dried ($MgSO_4$), filtered, and concentrated under vacuum to give an orange oil.* Partial separation of the two anomers was achieved by column chromatography (SiO_2; 2/3 EtOAc/light petroleum bp 40–60°C). Fractions containing a mixture of anomers were triturated with hexane. The crystalline material **4** that separated was further purified by recrystallization from diethyl ether.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl azide **3** was obtained as a yellow oil (0.5 g, 9%). R_F 0.57 (5/3/2 hexane/EtOAc/$CHCl_3$, phosphomolybdic acid and cerium sulfate dip). $[\alpha]_D$ +105.2° (*c* 0.1, $CHCl_3$; lit[9] +103°). *m/z* (ESI-MS) 396 ($[M+Na]^+$). HRMS calculated for $[M+Na]^+$ 396.1019, found 396.1024. δ_H (500 MHz, C_6D_6) 5.54 (1H, t, *J* 10.1, H4), 5.41 (1H, dd, $J_{3,4}$ 10.1, $J_{2,3}$ 3.4, H3), 5.24 (1H, dd, $J_{2,3}$ 3.4, $J_{1,2}$ 2.0, H2), 4.66 (1H, d, $J_{1,2}$ 1.9, H1), 4.26 (1H, dd, $J_{6,6'}$ 12.4, $J_{5,6}$ 5.2, H6), 4.03 (1H, dd, $J_{6,6'}$ 12.4, $J_{5,6'}$ 2.3, H6′), 3.83 (1H, m, H5), 1.70, 1.67, 1.63, 1.58 (12H, 4s, 4 × $COCH_3$). δ_H (500 MHz, $CDCl_3$) 5.36 (1H, d, $J_{1,2}$ 1.9, H1), 5.28–5.21 (2H, m, H3 + H4), 5.13 (1H, dd, $J_{2,3}$ 3.0, $J_{1,2}$ 2.0, H2), 4.28 (1H, dd, $J_{6,6'}$ 12.6, $J_{5,6}$ 5.7, H6), 4.15 (1H, dd, $J_{6,6'}$ 12.6 $J_{5,6'}$ 2.4, H6′) 4.14–4.10 (1H, m, H5), 2.14, 2.09, 2.03, 1.97 (12H, 4s, 4 × CH_3). δ_C (125 MHz, $CDCl_3$) 170.6, 169.8, 169.7, 169.6 (4 × C=O), 87.5 (C1), 70.7 (C5), 69.2 (C2), 68.3 (C3), 65.7 (C4), 62.2 (C6), 20.8, 20.7, 20.6, 20.6 (4 × CH_3).

2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl azide **4** was isolated as colourless crystals (0.82 g, 14%), mp 115°C (lit.[11] 124°C). R_F 0.43 (5/3/2 hexane/EtOAc/$CHCl_3$, phosphomolybdic acid and cerium sulfate dip). $[\alpha]_D$ −69° (*c* 0.1, $CHCl_3$) (lit.[11] −77.0°). *m/z* (ESI-MS) 396 ($[M+Na]^+$). HRMS calculated for $[M+Na]^+$ 396.1019, found 396.1023. δ_H (500 MHz, C_6D_6) 5.52 (1H, t, *J*10.2, H4), 5.44 (1H, dd, $J_{2,3}$ 3.3, $J_{1,2}$ 1.3, H2), 4.97 (1H, dd, $J_{3,4}$ 10.2, $J_{2,3}$ 3.3, H3), 4.24 (1H, dd, $J_{6,6'}$ 12.5, $J_{5,6}$ 5.2, H6), 4.05 (1H, dd, $J_{6,6'}$ 12.5, $J_{5,6'}$ 2.3, H6′), 3.68 (1H, d, $J_{1,2}$ 1.3, H1), 3.16 (1H, m, H5), 1.69, 1.67, 1.66, 1.62 (12H, 4s, 4CH_3). δ_H (500 MHz, $CDCl_3$) 5.43 (1H, dd, $J_{2,3}$ 3.3, $J_{1,2}$ 1.3, H2), 5.24 (1H, t, *J*10.1, H4), 5.02 (1H, dd, $J_{3,4}$ 10.1, $J_{2,3}$ 3.3, H3), 4.71 (1H, d, $J_{1,2}$ 1.3, H1), 4.26 (1H, dd, $J_{6,6'}$ 12.3, $J_{5,6}$ 5.6, H6), 4.19 (1H, dd, $J_{6,6'}$ 12.3, $J_{5,6'}$ 2.6, H6′), 3.74 (1H, ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 5.7, $J_{5,6'}$ 2.6, H5), 2.19, 2.09, 2.03, 1.97 (12H, 4s, 4CH_3). δ_C (125 MHz, $CDCl_3$) 170.6 (1 × C=O), 169.9 (2 × C=O), 169.5 (1 × C=O), 85.1 (C1), 74.7 (C5), 71.0 (C3), 69.2 (C2), 65.4 (C4), 62.3 (C6), 20.7 (2 × CH_3), 20.6 (CH_3), 20.5 (CH_3).

Crystallography

Compound **4**: $C_{14}H_{19}N_3O_9$, *M* 373.32, *T* 293 K, orthorhombic, space group $P2_12_12_1$ (no. 19), *a* 8.562(3), *b* 9.400(4), *c* 22.67(1) Å, *V* 1825(1) Å^3, D_c (Z 4) 1.359 g cm^{-3}, *F*(000) 784, $\mu(Mo_{K\alpha})$ 0.115 mm^{-1}, 2532 unique data ($2\theta_{max}$ 46°), 1434 with $I > 2\sigma(I)$; *R* 0.0784 (obs. data), wR_2 0.2182 (all data), goodness of fit 1.03.

Intensity data at 293 K were collected on an Enraf–Nonius CAD4 four-circle diffractometer using graphite monochromated $Mo_{K\alpha}$ radiation (λ 0.71073 Å) in the $\omega - 2\theta$ scan mode within the range $3 < 2\theta < 46°$. A twinned specimen was chosen (from several attempts) and lattice dimensions were determined by a least-squares fit of the setting parameters of 20 independent reflections. Indexing was made possible with the program *DIRAX*.[18] Data reduction and empirical absorption corrections (ψ-scans) were performed with the *WINGX* package.[19] The structure was solved by direct methods with *SHELXS* and refined by full matrix least-squares analysis with *SHELXL97*.[20] All non-H atoms were refined with anisotropic thermal parameters except those involved in disorder (specifically the acetyl group at position 4 on the ring). H-atoms were constrained at estimated positions. The absolute configuration was assigned on the basis of the starting material. The atomic nomenclature is defined in Fig. 1 drawn with *ORTEP3*.[21] Crystallographic data in CIF format are available from the Cambridge Crystallographic Data Base (CCDC deposition number 604525).

Acknowledgments

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* CAUTION: While concentrating the crude product under high vacuum, a small amount of colorless liquid collected in the cold trap of the vacuum pump. This liquid detonated the following day during disassembly of the cold trap. We suspect that the explosive liquid was diazidomethane, and we strongly recommend that azide ion not be allowed to contact halogenated solvents, even during workup of reactions involving its use. For a discussion of the hazards of diazidomethane, see http://pubs.acs.org/cen/safety/index.html

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