A Convenient Route to N-Acetyl-D-glucosamine-2-C-d and N-Acetyl-D-mannosamine-2-C-d

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Title compounds were prepared through epimerisation of 2-acetamido-2-deoxy-D-glucose in NaOD. The isotopic purity of the products was determined by NMR and mass spectroscopy. The mechanism of epimerisation is briefly discussed.

In connection with our studies¹ on the interaction of lysozyme with deuterium-labelled monosaccharide inhibitors, we needed gram quantities of 2-acetamido-2-deoxy-D-glucose-2-C-d (**2**) in fairly high isotopic purity. ALI and RICHARDSON² proposed a rather complicated route for the synthesis of **2**. The well-known epimerisation^{3,4} of 2-acetamido-2-deoxy-D-glucose (**1**) offers a much simpler alternative. This reaction takes place under basic conditions and results in a 4:1 mixture of the *gluco* and *manno* epimers, respectively. COXON and HOUGH suggested⁵, without experimental proof, the following plausible scheme for the mechanism of epimerisation.

If this mechanism is correct, complete exchange between the sugar C-2 proton and water protons should occur during the epimerisation process. This is exactly what we observed. The anomeric proton

doublet in the ^1H NMR spectrum of **1** ($\delta \sim 5.2$, $J_{12} = 2.5$ Hz) began to collapse immediately when a D_2O solution of **1** was made basic with NaOD. This phenomenon indicated that fairly rapid proton exchange occurred at C-2 of **1**. More detailed information could, however, be obtained by observing the ^1H NMR spectrum of 2-acetamido-2-deoxy-D-mannose (**7**) under similar conditions. This spectrum (Fig. 1A) displays, in addition to the anomeric proton signals, well-resolved resonances for H-2 (α - and β -anomer) and H-3 (α -anomer). Since the spectrum of **1** is empty in this region the course of the epimerisation can be conveniently monitored through changes in the ^1H NMR spectrum of **7**. The intensity of the H-2 signals decreased rapidly when NaOD was added to a solution of **7** in D_2O with concomitant collapse of the H-1 (to singlets) and the H-3 (to a doublet) resonances. Fig. 1B shows the ^1H NMR spectrum of the D-manno epimer (**8**), isolated pure from the reaction mixture.

The exact percentage of the deuterium incorporated could not be determined from this spectrum alone, however. A quantitative estimate was obtained from the ^1H NMR spectrum of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy- β -D-glucopyranose(**6**) prepared⁶ from **2**. Comparison of the residual H-1 doublet to the main H-1 "singlet" showed that 6

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contained ~70–75% deuterium at C-2. The exact percentage was determined through ^2H NMR. For this purpose, 2-(acetyl-d-amino)-2-deoxy-D-glucopyranose-2-C-d (**4**) (and the D-manno analogue (**9**)) was prepared by treating 2-(acetyl-d-amino)-2-deoxy-D-glucopyranose (**3**) with NaOD. Compound **3** was obtained¹ by N-acetylation from 2-amino-2-deoxy-D-glucose using $(\text{CH}_2\text{DCO})_2\text{O}$. The deuterium content of **3** was $\geq 99\%$. A reliable ^2H intensity reference label was thus introduced into compound **2**. Integration of the proton noise-decoupled FT ^2H NMR spectrum of **4** showed its deuterium content at C-2 to be $71 \pm 2\%$.

Fig. 1. Partial 100 MHz ^1H NMR spectra of 2-acetamido-2-deoxy-D-mannose (**7**) (A) and 2-acetamido-2-deoxy-D-mannose-2-C-d₁ (**8**) (B) in D₂O solution.

Obviously, the dilution of D₂O due to the five exchangeable protons in **1** (or **3**) may have contributed considerably to the incomplete deuterium incorporation at C-2. When the exchangeable protons in **3** were replaced by deuteriums prior to epimerisation, integration of the D-2 signals against the CO-CH₂D signal in the ^2H NMR spectrum of **4** indicated $94 \pm 2\%$ D at C-2. This figure was substantiated by mass spectral data of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy- α -D-glucopyranose-2-C-d (**5**) obtained by epimerisation and subsequent acetylation from per-O,N-deuterated **1**. Both EI and CI (NH₃) mass spectra of **5** and its unlabelled counterpart were recorded and the abundance of

their ions M-43, M-148, M-233 (EI), M+1 and M+18 (CI) compared (correction for the naturally occurring isotopes was carried out). The mean value of the D content was found to be $89 \pm 2\%$ for each of these ions in **5**.

In conclusion, it can be stated that the high percentage of deuterium incorporation found by NMR and mass spectroscopy* provides strong support in favour of the mechanism advanced by COXON and HOUGH⁵ for the base-catalysed epimerisation of amino sugars. On the other hand, this reaction is potentially useful for the preparation of amino sugars specifically labeled with tritium and possessing high specific activity.

This manuscript had been under preparation when the paper of SALO *et al.*⁷, reporting similar use of the epimerisation reaction, came to our attention.

Experimental

Compounds **5** and **6** were obtained by procedures described^{8,6} for the preparation of the unlabelled compounds. **4** was prepared from **3**¹ by the procedure described below for **2**.

2-Acetamido-2-deoxy-D-glucopyranose-2-C-d (**2**)

This compound was prepared essentially by the method of SPIVAK and ROSEMAN⁹.

10 g of **1** was lyophilised 3 times from D₂O then dissolved with gentle warming (30–35 °C) in 25 ml of 0.1 mol/dm³ NaOD in D₂O. The pH of the solution was 11–11.5. After standing at room temperature for 48 hrs it was treated with BIO-RAD AG 50 W (H⁺-form) cation exchange resin (to pH 5–5.5) and evaporated under reduced pressure until incipient crystallisation. 5.1 g of a white crystalline product was obtained; m.p. 203–205 °C, $[\alpha]_D + 47.8^\circ$ (H₂O, equilibrium). HORTON⁶ gives for **1** m.p. 203–205 °C, $[\alpha]_D + 41^\circ$. Further 1.85 g was obtained in two crops from the mother liquors (overall yield 70%). ^1H NMR δ 5.2 s, H-1(α); δ 4.75 s, H-1(β).

2-Acetamido-2-deoxy-D-mannopyranose-2-C-d (**8**)

The mother liquors from **2** were evaporated to dryness and worked up as described by KUHN and BASCHANG¹⁰. Crystallisation from water-aceton yielded 1.0 g (yield 10%) of a product, m.p. 125–127 °C, $[\alpha]_D + 8.9^\circ$ (H₂O, equilibrium). Lit.⁹ gives for **7** m.p. 128–129 °C, $[\alpha]_D + 9.7^\circ$. The ^1H NMR spectrum of **8** is shown in Fig. 1 B.

EI mass spectrum of **5** (the mass spectrum of the unlabeled compound was published¹¹).

m/e(I%): 348(0.6), 347(3.3), 346(1.2), 330(1.5), 329(0.4), 319(1.1), 318(0.2), 289(0.5), 288(2.3),

* The agreement between the NMR and mass spectral data can be regarded as satisfactory considering the integration accuracy of both methods.

- 1 $R^1 = R^2 = R^3 = H$; $R^4, R^5 = H, OH$
- 2 $R^1 = R^2 = H$; $R^3 = D$; $R^4, R^5 = H, OH$
- 3 $R^1 = R^2 = H$; $R^3 = D$; $R^4, R^5 = H, OH$
- 4 $R^1 = H$; $R^2 = R^3 = D$; $R^4, R^5 = H, OH$
- 5 $R^1 = COCH_3$; $R^2 = D$; $R^3 = R^4 = H$; $R^5 = OCOCH_3$
- 6 $R^1 = COCH_3$; $R^2 = D$; $R^3 = R^5 = H$; $R^4 = OCOCH_3$

- 7 $R^2 = R^3 = H$
- 8 $R^2 = D$; $R^3 = H$
- 9 $R^2 = R^3 = D$

287(1.8), 286(0.3), 245(1.9), 244(0.8), 243(4), 242(36), 241(5), 230(1), 229(1), 228(1), 211(1), 210(0.3), 200(12), 199(11), 198(2), 183(2), 182(12), 181(3.5), 169(1.6), 168(2.5), 167(2.8), 166(1), 158(4), 157(37), 156(5), 150(2), 140(26), 139(4), 127(8), 126(3), 115(60), 114(6), 109(2.5), 108(2), 103(7), 102(3), 101(1.5), 100(5), 99(10), 98(30), 97(15), 96(2), 85(20), 84(7), 73(13), 72(2), 60(20), 43(100).

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