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

L. SZILÁGYI
Department of Organic Chemistry, L. Kossuth University, Debrecen, Hungary

P. HERCZEK
Antibiotics Research Group of the Hungarian Academy of Sciences, L. Kossuth University, Debrecen, Hungary

and

GY. BUJTÁS
Central Chemical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary

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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 studies 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. Alt and Richardson proposed a rather complicated route for the synthesis of 2. The well-known epimerisation 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 glucos and mannos epimers, respectively. Coxon and Hough suggested, without experimental proof, the following plausible scheme for the mechanism of epimerisation.

\[
\text{H} \quad \xrightarrow{\text{COXON and HOUGH}} \quad \text{H} \\
\text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H}
\]

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 (\(\delta \approx 5.2, J_{12} = 2.5 \text{ Hz}\)) began to collapse immediately when a D2O 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 (a- and \(\beta\)-anomer) and H-3 (a-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 D2O 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-\(\beta\)-D-glucopyranose (6) prepared from 2. Comparison of the residual H-1 doublet to the main H-1 ”singlet” showed that 6...
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 CH2DCOO. The deuterium content of 3 was >99%1. 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 ±2%.

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 Hough4 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 described8,6 for the preparation of the unlabelled compounds. 4 was prepared from 31 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 Śpiyak and Rośman9. 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, [α]D + 47.8° (H2O, equilibrium). Horton5 gives for 1 m.p. 203-205 °C, [α]D + 41°. Further 1.85 g was obtained in two crops from the mother liquors (overall yield 70%).

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, [α]D + 8.9° (H2O, equilibrium). Lit.5 gives for 7 m.p. 128-129 °C, [α]D + 9.7 °C. 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).

* The agreement between the NMR and mass spectral data can be regarded as satisfactory considering the integration accuracy of both methods.
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2. Y. Ali and A. C. Richardson, Carbohydr. Res. 5, 441 [1967].