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# Deuterium isotope effects in carbohydrates revisited. Cryoprobe studies of the anomerization and NH to ND deuterium isotope induced $^{13}C$ NMR chemical shifts of acetamidodeoxy and aminodeoxy sugars

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**Abstract**—Complete $^{1}H$ and $^{13}C$ NMR chemical shift assignments have been generated from a series of acetamidodeoxy and aminodeoxy sugar derivatives. For free sugars, the enhanced sensitivity of an NMR cryoprobe allowed simple 1D and 2D NMR spectra to be obtained from essentially single anomers, before significant mutarotation had occurred. The NMR assignments have been used to characterize deuterium isotope effects on $^{13}C$ chemical shifts measured under conditions of slow NH to ND exchange in single solutions. Within a range of 0 to −0.138 ppm, $\beta$, $\gamma$, $\delta$, and $\zeta$ deuterium isotope effects have been observed, thus providing additional reference data for assignment of the $^{13}C$ NMR spectra of nitrogenous saccharides.


*Keywords:* Acetamidodeoxy sugars; Anomerization; $^{13}C$ NMR chemical shifts; COSY; Cryoprobe; Deuterium isotope effects; $^{1}H$ NMR chemical shifts; HMBC; HSQC; TOCSY

## 1. Introduction

Secondary isotope effects on the $^{13}C$ chemical shifts of carbohydrates induced by OH to OD exchange have been extensively studied.[1–17] Such effects have been examined by two main methods: (a) detection of separate signals for OH and OD containing species in slow chemical exchange;[1–13] this is usually achieved by dissolution of the sugar in dimethyl sulfoxide, which is known to decrease the rate of OH proton exchange by hydrogen bonding of the solvent to the hydroxyl hydrogen atoms; (b) detection of the mixed $^{13}C$ signals of $H_2O$ and $D_2O$ solutions of sugars contained in concentric sample tubes, under conditions of rapid hydroxyl proton exchange.[14–17] Deuterium isotope effects on $^{13}C$ shifts due to *C*-deuteration are also well known.[18–21]

Acetamidodeoxy sugars are a common structural motif in bacterial polysaccharides,[22] but little data has been reported for isotope effects on $^{13}C$ chemical shifts induced by NH to ND chemical exchange in these sugars. As part of a broader study of nitrogenous polysaccharides as they relate to vaccine development, we have determined and confirmed the complete $^{1}H$ and $^{13}C$ NMR assignments of a series of *N*-acetylamino sugars and some selected secondary amino sugar derivatives by 2D NMR methods. The resulting spectral assignments have been used to characterize the isotope effects on $^{13}C$ chemical shifts produced by NH to ND exchange.

## 2. Experimental

### 2.1. Materials

2-Acetamido-2-deoxy-α- and β-D-glucopyranose (**1a**,**b**), 2-acetamido-2-deoxy-α- and β-D-mannopyranose (**2a**,**b**),

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2-acetamido-2-deoxy-α- and β-D-galactopyranose (**3a**,**b**), tetra-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (**4**), 4-nitrophenyl 2-acetamido-2-deoxy-α- and β-D-glucopyranoside (**5a**,**b**), 3-acetamido-3-deoxy-α- and β-D-glucopyranose (**6a**,**b**), methyl *N*-2,4-dinitrophenyl 3-amino-4,6-*O*-benzylidene-3-deoxy-2-*O*-methyl-α-D-altropyranoside[23] (**7**), and bis(methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranosid-3-yl)amine[24] (**8**). Compounds **1a**, **2b**, **3a**, and **6a**,**b** were obtained from Pfanstiehl Laboratories, Inc., Waukegan, IL and/or United Biochemical Corporation, Cleveland, OH. Their pyranose anomers were prepared by anomerization in solution. Derivatives **4**, **7**, and **8** were synthesized in house. Glycosides **5a** and **5b** were obtained from P-L Biochemicals, Inc., Milwaukee, WI (see Fig. 1).

## 2.2. NMR spectroscopy

All NMR spectra were acquired at 300 K by use of a Bruker DRX-500 NMR spectrometer equipped with a 5 mm TXI HCN cryoprobe with *z*-gradient coil, a Silicon Graphics O2 workstation, and Bruker XWINNMR software, version 3.5, patch level 6. During testing, this cryoprobe achieved a signal:noise of 3478:1 on 0.1% ethylbenzene solution, which was ~4 times the signal:noise specification for an equivalent, normal probe. $^{1}$H NMR spectra were acquired using 32,768 point data sets, a 30° pulse (2.7 μs), and a pulse recycle time of 6 s. NMR solvent volumes were 0.5 mL, and solute weights were 13–21 mg for compounds **1a**–**3b**, 21–23 mg for **4**, 18 mg for **5a** and **5b**, 15–17 mg for **6a** and **6b**, 22–27 mg for **7**, and 43 mg for **8**. The resolution of the spectra was enhanced

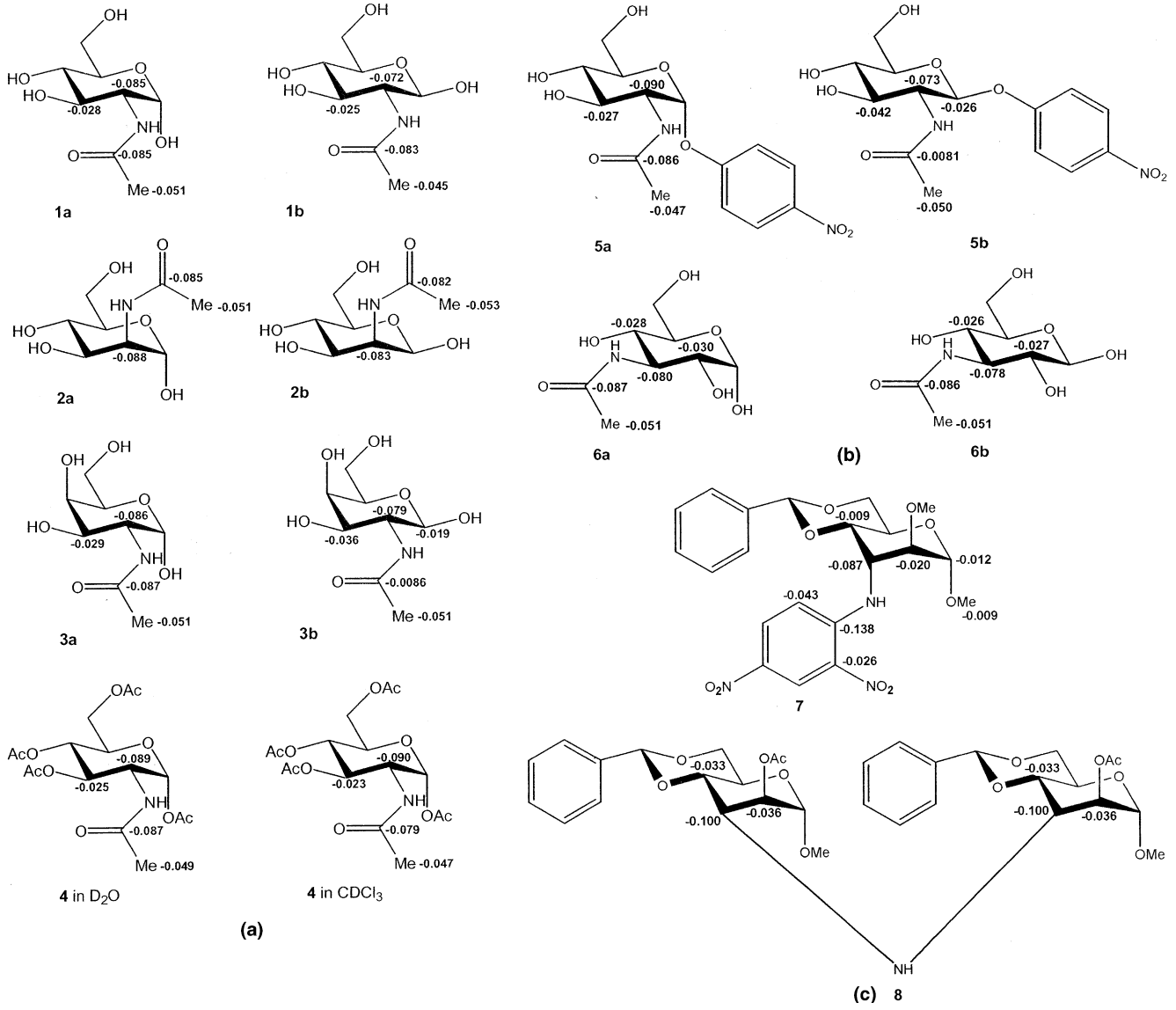


**Figure 1.** Structures of acetamidodeoxy and aminodeoxy carbohydrate derivatives **1a**–**8** and the incremental $^{13}$C NMR chemical shifts (ppm) produced by *N*-deuteration.

by Gaussian multiplication of the free induction decay, using a line-broadening of −1.5 to −3.5 Hz, and a Gaussian truncation fraction of 0.3. $^{13}C$ NMR spectra were acquired by using 65,536 point data sets, a 90° pulse (14 μs), and a pulse recycle time of 1.5 s. Resolution was enhanced either by zero-filling to 65,536 data points, or by forward, complex linear prediction to 131,072 points, together with Gaussian multiplication up to a line-broadening of −4 Hz. The $^{1}H$ and $^{13}C$ chemical shifts of solutions in $D_2O$ were referenced to internal acetone, set to 2.225 and 31.0 ppm, respectively. Tetramethylsilane was used as an internal chemical shift reference for solutions in organic solvents, and was set to 0.0 ppm for both $^{1}H$ and $^{13}C$ NMR spectra. The digital resolution of most of the 1D $^{1}H$ and $^{13}C$ spectra was 0.00049 and 0.0015 ppm/point, respectively. $^{1}H$-coupled $^{13}C$ NMR spectra were acquired with the nuclear Overhauser effect (NOE) by use of gated, WALTZ-16 irradiation at the $^{1}H$ frequency. These spectra were used to measure $^{1}J_{C\text{-}1,H\text{-}1}$. All 2D NMR spectra were acquired by field gradient-selected methods. 2D Correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC) were used as needed to confirm $^{1}H$ and $^{13}C$ assignments. Forward linear prediction to larger data sizes was used to improve the resolution of 2D spectra, where necessary. 2D COSY, HSQC, and HMBC NMR spectra were obtained by using 2048 ($t_2$) × 512 ($t_1$) point data sets, zero-filled to 2048 ($F_2$) × 2048 ($F_1$) points. For 2D COSY, the spectral width was 4.25 kHz in each dimension and the read pulse was 30° (2.4 μs), whereas for 2D HSQC and HMBC, the $^{1}H$ and $^{13}C$ spectral widths were 4.25 kHz ($F_2$) and 25.1 kHz ($F_1$), respectively. 2D TOCSY NMR spectra were acquired by use of 16,384 ($t_2$) × 512 ($t_1$) point data sets, zero-filled to 32,768 ($F_2$) × 2048 ($F_1$) points, together with spectral widths of 3.21 kHz in each dimension. $F_2$ Slices were taken to generate separate 1D sub-spectra for anomers. 2D COSY and HMBC spectra were displayed in the magnitude mode, whereas the phase-sensitive, echo–anti-echo protocol was used for 2D TOCSY and HSQC spectra.

## 3. Results and discussion

This work was facilitated by the enhanced sensitivity of a cryoprobe, which allowed simple 1D and 2D $^{1}H$ and $^{13}C$ NMR spectra to be obtained rapidly from solutions of essentially single anomers of the acetamidodeoxy sugars before significant anomerization (10–15% content of

**Table 1.** $^{1}H$ chemical shifts[a] of amino sugar derivatives **1**–**8**

| | Solvent | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6′ | Me(NAc) | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | $D_2O$ | 5.197 | 3.872 | 3.757 | 3.483 | 3.853 | 3.817 | 3.779 | 2.046 | |
| **1b** | | 4.708 | 3.670 | 3.531 | 3.461 | 3.461 | 3.903 | ~3.739 | 2.044 | |
| **2a** | $D_2O$ | 5.121 | 4.319 | 4.047 | 3.620 | 3.863 | ~3.839 | ~3.839 | 2.048 | |
| **2b** | | 5.028 | 4.451 | 3.828 | 3.522 | 3.416 | 3.890 | 3.812 | 2.092 | |
| **3a** | $D_2O$ | 5.229 | 4.129 | 3.917 | 3.994 | 4.100 | ~3.743 | ~3.743 | 2.047 | |
| **3b** | | 4.642 | 3.871 | 3.714 | 3.933 | 3.686 | 3.792 | 3.753 | 2.045 | |
| **4** | $D_2O$ | 6.229 | 4.487 | 5.396 | 5.164 | 4.317 | 4.381 | 4.134 | 1.966 | Me-1,3,4,6[b] 2.230, 2.065, 2.100, 2.118 |
| | $CDCl_3$ | 6.172 | 4.491 | 5.251 | 5.211 | 4.005 | 4.253 | 4.071 | 1.945 | Me-1,3,4,6[b] 2.201, 2.060, 2.051, 2.095 |
| **5a** | $(CD_3)_2SO$ | 5.598 | 3.888 | 3.701 | 3.298 | 3.399 | 3.574 | 3.498 | 1.849 | NH 8.063, HO-3 5.014, HO-4 5.193, HO-6 4.564, Ar–H-2,6 7.274, H-3,5 8.223 |
| **5b** | $(CD_3)_2SO$–$D_2O$ (5:1 v/v) | 5.185 | 3.757 | 3.456 | 3.231 | 3.432 | 3.733 | 3.516 | 1.834 | NH 7.942[c], HO-3 5.254[c], HO-4 5.302[c], HO-6 4.803[c], Ar–H-2,6 7.178, H-3,5, 8.217 |
| **6a** | $D_2O$ | 5.240 | 3.576 | 4.093 | 3.432 | 3.897 | 3.828 | 3.762 | 2.056 | |
| **6b** | | 4.716 | 3.256 | 3.879 | 3.424 | 3.550 | 3.889 | 3.720 | 2.054 | |
| **7** | $CDCl_3$ | 4.879 | 3.636 | 4.472 | 4.266 | 4.106 | 4.314 | 3.872 | — | NH 9.862, Me-1 3.590, Me-2 3.552, Ph–H-2,6 7.190, H-3,5 7.240, H-4 7.285 Ph–C*H* 5.597, DNP[d] H-3 9.123, H-5 8.141, H-6 7.246 |
| **8** | $CDCl_3$ | 4.607 | 5.054 | 3.392 | 3.824 | 4.261 | 4.305 | 3.768 | — | NH 3.169, Me-1 3.378, Me-2 1.983, Ph–H-2,4,6 7.356–7.315, H-3,5 7.465–7.445 Ph–C*H* 5.547 |

[a] The data were measured directly from the spectra by first-order analysis, and are for the ND forms, except that for derivative **5a** in $(CD_3)_2SO$, and **4**, **7**, and **8** in $CDCl_3$ solution, the NH forms were measured. Data reported for **1a**, **2b**, and **3a**, are for solutions of the predominant pyranoid anomer in the crystalline acetamidodeoxy sugar, measured before significant mutarotation had occurred.

[b] The assignments for the Me groups are specific and in respective order, and were made by 2D HMBC.

[c] Assignments for residual, non-exchanged NH and OH protons.

[d] DNP = 2,4-dinitrophenyl.

the second anomer) had occurred. For example, under the conditions of the NMR experiments, **1a** mutarotated to ~11% of **1b** in 13 min, **2b** to ~15% of **2a** in 11 min, and **3a** to ~13% of **3b** in 11 min. Typically, the use of the cryoprobe with one scan per free induction decay allowed data for each of the 2D experiments to be acquired from freshly dissolved samples, with good resolution, in about 9 min, following a 1–2 min sample dissolution, insertion, and lock equilibration time. This procedure afforded simple, readily interpretable 1D and 2D $^{1}$H and $^{13}$C spectra for compounds **1a**, **2b**, and **3a**, which were essentially single anomers in the crystal, but which underwent anomerization on dissolution in water. $^{1}$H and $^{13}$C chemical shift assignments are shown in Tables 1 and 2, and coupling constants in Table 3. Compounds **1a** and **1b** have been studied previously by NMR spectroscopy at 270 MHz, followed by iterative spectral analysis.[25] Our chemical shifts and assignments for **1a** and **1b** agree well with those of Perkins et al.[25] when allowance is made for the differing conditions of referencing, temperature, and concentration. For compounds **1a**–**3b**, our $^{13}$C assignments made by 2D methods agree with those of Bundle et al.,[26] which were partially obtained by use of synthetic, *C*-deuterated acetamidodeoxy sugars. More detailed $^{13}$C NMR assignments were obtained for **2a** and **2b** in the current work, because at a $^{13}$C Larmor frequency of 126 MHz, the C-1 resonances of these anomers are resolved (see Fig. 2), in contrast to the earlier work at 25 MHz. The anomeric configurations of the components of our solutions followed from the values of $^{1}J_{C\text{-}1,H\text{-}1}$, which fell in the range 169.7–179.3 Hz for α anomers, and 161.5–162.6 Hz for β anomers[27] (see Table 3). For compounds **1a**,**b**, **3a**,**b**, **4**, **5a**,**b**, **6a**, and **6b**, the anomeric configurations were also supported by the values of $^{3}J_{1,2}$ 3.4–3.7 Hz for α anomers and $^{3}J_{1,2}$ 7.9–8.5 Hz for β anomers.

During these studies, we observed that the resonances of $^{13}$C nuclei in and around the acetamido group or certain secondary amino groups undergo deuterium-induced isotope shifts (see Fig. 1 and Table 4) on NH to ND exchange. In contrast to previous measurements[14–17] of the OH to OD induced, differential deuterium isotope shifts of carbohydrates in aqueous solutions, which required the use of separate solutions in $H_2O$ and $D_2O$ contained in dual concentric cells, the NH and ND forms of the amino sugar derivatives could be detected directly as separate signals in the spectrum of one solution (see Figs. 2 and 3) because in these derivatives, NH to ND chemical exchange is slower[28,29] than OH to OD

**Table 2.** $^{13}$C chemical shifts[a] of amino sugar derivatives **1**–**8**

| | Solvent | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | Me | C=O | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | $D_2O$ | 91.608 | 55.850 | 71.462 | 70.857 | 72.319 | 61.371 | 22.656 | 175.230 | |
| **1b** | | 95.683 | 57.453 | 74.653 | 70.594 | 76.703 | 61.498 | 22.927 | 175.508 | |
| **2a** | $D_2O$ | 93.873 | 53.967 | 69.610 | 67.521 | 72.742 | 61.140 | 22.663 | 175.533 | |
| **2b** | | 93.378 | 54.838 | 72.807 | 67.250 | 77.087 | 61.125 | 22.794 | 176.453 | |
| **3a** | $D_2O$ | 91.767 | 51.028 | 68.167 | 69.356 | 71.294 | 62.015 | 22.725 | 175.429 | |
| **3b** | | 96.192 | 54.446 | 71.924 | 68.648 | 75.941 | 61.796 | 22.979 | 175.690 | |
| **4** | $D_2O$ | 91.336 | 50.927 | 71.449 | 69.016 | 70.004 | 62.560 | 22.377 | 175.201 | Me-1,3,4,6[b] 20.916, 20.745, 20.832, 20.845<br>C=O-1,3,4,6[b] 172.852, 173.901, 173.464, 174.404 |
| | $CDCl_3$ | 90.680 | 51.040 | 70.656 | 67.450 | 69.703 | 61.516 | 23.056 | 169.993 | Me-1,3,4,6[b] 20.956, 20.733, 20.580, 20.709<br>C=O-1,3,4,6[b] 168.660, 171.726, 169.110, 168.660 |
| **5a** | $(CD_3)_2SO$ | 96.289 | 53.272 | 70.203 | 70.097 | 74.316 | 60.351 | 22.474 | 169.687 | Ar–C-1 161.855, C-2,6 116.958,<br>C-3,5 125.666, C-4 141.714 |
| **5b** | | 98.288 | 55.109 | 73.737 | 69.893 | 77.286 | 60.382 | 22.956 | 169.276 | Ar–C-1 162.168, C-2,6 116.467,<br>C-3,5 125.687, C-4 141.689 |
| **6a** | $D_2O$ | 92.296 | 70.621 | 54.558 | 68.675 | 72.390 | 61.268 | 23.006 | 175.887 | |
| **6b** | | 97.200 | 73.162 | 57.936 | 68.803 | 77.798 | 61.467 | 22.953 | 175.734 | |
| **7** | $CDCl_3$ | 98.196 | 78.578 | 51.133 | 76.535 | 59.227 | 68.964 | — | — | Me-1 55.233, Me-2 58.583, Ph–C-1 136.757,<br>C-2,6 125.738, C-3,5 128.191, C-4 129.166,<br>PhCH 101.944<br>DNP[c] C-1 148.841, C-2 130.796, C-3 123.964,<br>C-4 136.141, C-5 129.137, C-6, 115.782 |
| **8** | $CDCl_3$ | 98.932 | 72.524 | 55.513 | 77.698 | 58.315 | 69.156 | 21.000 | 169.331 | Me-1 55.253, Ph–C-1 137.861, C-2,6 136.347,<br>C-3,5 128.159, C-4 128.863, Ph–*C*H 102.030 |

[a] Data are for the ND forms, except that for derivatives **5a** and **5b** in $(CD_3)_2SO$, and **4**, **7**, and **8** in $CDCl_3$ solution, the NH forms were measured. The data for **1a**, **2b**, and **3a**, are for solutions of the predominant pyranoid anomer in the crystalline acetamidodeoxy sugar, measured before significant mutarotation had occurred.

[b] The assignments for the Me and C=O groups are specific and in respective order, and were made by the 2D HMBC and HSQC methods.

[c] DNP = 2,4-dinitrophenyl.

**Table 3.** Coupling constants[a] of amino sugar derivatives **1**–**8**

| | Solvent | $J_{1,2}$ | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $J_{5,6}$ | $J_{5,6'}$ | $J_{6,6'}$ | $^{1}J_{C\text{-}1,H\text{-}1}$ | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | $D_2O$ | 3.5 | 10.7 | 9.2 | 10.1 | NR[b] | 5.2 | 12.1 | 171.0 | |
| **1b** | | 8.4 | 10.3 | 8.9 | 9.7 | 1.8 | 5.3 | 12.3 | 162.2 | |
| **2a** | $D_2O$ | 1.7 | 4.7 | 9.8 | 9.5 | NR | NR | NR | 172.2 | |
| **2b** | | 1.7 | 4.4 | 9.7 | 9.9 | 2.3 | 5.0 | 12.3 | 162.6 | |
| **3a** | $D_2O$ | 3.7 | 11.1 | 3.2 | 1.3 | ~5.8[c] | ~6.4[c] | NR | 171.4 | $J_{1,3}$ 0.6 |
| **3b** | | 8.4 | 11.0 | 3.4 | 1.1 | 7.8 | 4.6 | 11.6 | 161.5 | $J_{2,4}$ 0.5 |
| **4** | $D_2O$ | 3.7 | 10.9 | 9.4 | 10.2 | 3.9 | 2.2 | 12.7 | 179.3 | |
| | $CDCl_3$ | 3.7 | 10.7 | 9.6 | 9.8 | 4.0 | 2.4 | 12.5 | 178.9 | $J_{2,NH}$ 9.1 |
| **5a** | $(CD_3)_2SO$ | 3.4 | 10.8 | 8.6 | 9.9 | 2.1 | 5.2 | 11.9 | 173.3[d] | $J_{2,NH}$ 7.9 $J_{3,OH}$ 5.9, $J_{4,OH}$ 5.8, $J_{6,OH}$ 5.7, $J_{6',OH}$ 5.9 |
| **5b** | $(CD_3)_2SO$–$D_2O$ (5:1 v/v) | 8.5 | 10.2 | 8.9 | 9.8 | 1.9 | 5.8 | 11.9 | 162.1[e] | $J_{2,NH}$ 9.1[f], $J_{3,OH}$ 5.6[g], $J_{4,OH}$ 5.4[g], $J_{6,OH}$ 5.7[g], $J_{6',OH}$ 5.7[g] |
| **6a** | $D_2O$ | 3.6 | 10.5 | 10.4 | 9.9 | 2.4 | 5.0 | 12.3 | 169.7 | |
| **6b** | | 7.9 | 10.4 | 10.1 | 9.8 | 2.3 | 5.7 | 12.3 | 161.6 | |
| **7** | $CDCl_3$ | 1.2 | 2.9 | 3.6 | 9.9 | 5.1 | 10.0 | 10.4 | 170.2 | $J_{3,NH}$ 8.5 |
| **8** | $CDCl_3$ | 1.1 | 2.8 | 3.7 | 9.5 | 5.2 | 10.1 | 9.7 | 170.2[h] | |

[a] The data were measured directly from the spectra by first-order analysis, and are for the ND forms, except that for derivative **5a** in $(CD_3)_2SO$, and **4**, **7**, and **8** in $CDCl_3$ solution, the NH forms were measured. The data for **1a**, **2b**, and **3a**, are for solutions of the predominant pyranoid anomer in the crystalline acetamidodeoxy sugar, measured before significant mutarotation had occurred.
[b] Not resolved.
[c] Values interchangeable.
[d] Measured on the ND form in 5:1 $(CD_3)_2SO$–$D_2O$.
[e] Measured on the NH form in $(CD_3)_2SO$.
[f] Measured from the residual, non-exchanged NH doublet.
[g] Measured from the residual, non-exchanged OH multiplets.
[h] Measured on a 1:2 mixture of NH and ND forms.

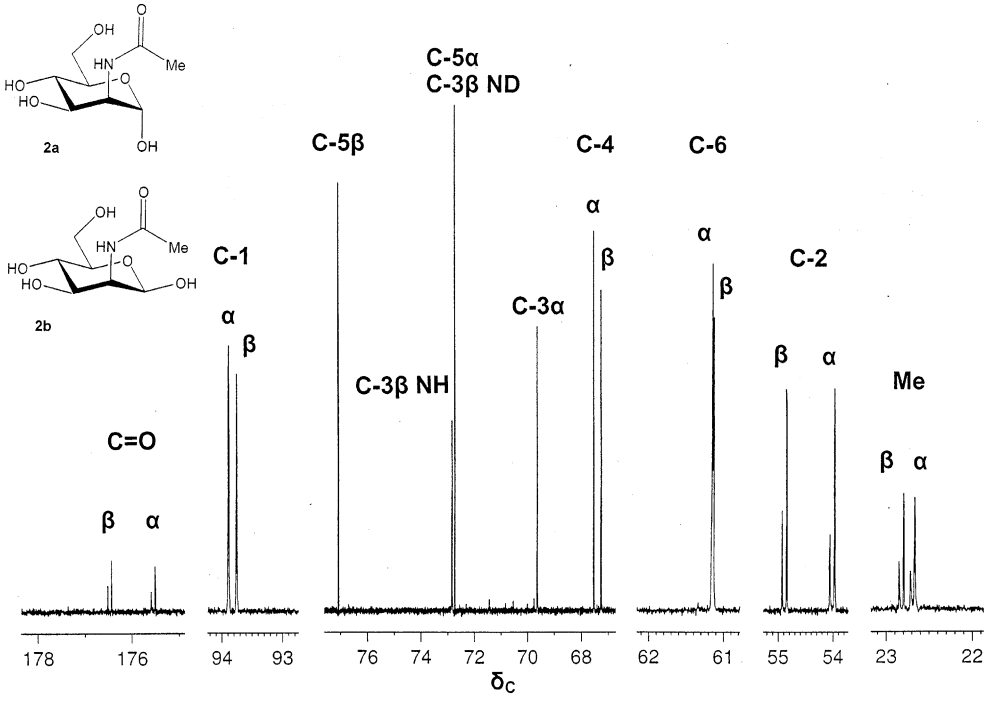


**Figure 2.** $^{13}C$ NMR spectrum of 2-acetamido-2-deoxy-D-mannopyranose equilibrated in 2:1 (v/v) $D_2O$–$H_2O$ for 13 days.

exchange. This is an experimental advantage compared with the use of concentric cells. The selectivity of detection of NH- to ND-induced $^{13}C$ shifts for **2a** and **2b** equilibrated in $D_2O$ is illustrated in Figure 2, in which

**Table 4.** Incremental $^{13}C$ chemical shifts[a] of amino sugar derivatives **1**–**8** induced by NH to ND exchange

| | Solvent | C-1 | C-2 | C-3 | C-4 | Me | C=O | Others |
|---|---|---|---|---|---|---|---|---|
| **1a** | $D_2O$ | | 0.085 | 0.028 | | 0.051 | 0.085 | |
| **1b** | | | 0.072 | 0.025 | | 0.045 | 0.083 | |
| **2a** | $D_2O$ | | 0.088 | | | 0.051 | 0.085 | |
| **2b** | | | 0.083 | | | 0.053 | 0.082 | |
| **3a** | $D_2O$ | | 0.086 | 0.029 | | 0.051 | 0.087 | |
| **3b** | | 0.019 | 0.079 | 0.036 | | 0.051 | 0.086 | |
| **4** | $D_2O$ | | 0.089 | 0.025 | | 0.049 | 0.087 | |
| | $CDCl_3$ | | 0.090 | 0.023 | | 0.047 | 0.079 | |
| **5a** | $(CD_3)_2SO$ | | 0.090 | 0.027 | | 0.047 | 0.086 | |
| **5b** | | 0.026 | 0.073 | 0.042 | | 0.050 | 0.081 | |
| **6a** | $D_2O$ | | 0.030 | 0.080 | 0.028 | 0.051 | 0.087 | |
| **6b** | | | 0.027 | 0.078 | 0.026 | 0.051 | 0.086 | |
| **7** | $CDCl_3$ | 0.012 | 0.020 | 0.087 | 0.009 | 0.009 (Me-1) | | DNP[b] C-1 0.138, C-2 0.026, C-6 0.043 |
| **8** | $CDCl_3$ | | 0.036 | 0.100 | 0.033 | | | |

[a] All data shown are negative, that is, the upfield shifts in ppm produced by NH to ND conversion.
[b] DNP = 2,4-dinitrophenyl.

splitting of the Me, C=O, C-2, and C-3 resonances is observed (except for C-3 of **2a**).

We investigated several solvent systems for NH to ND (and concurrent OH to OD) exchange, including $D_2O$–$H_2O$, $(CD_3)_2SO$–$D_2O$–$H_2O$, and $CDCl_3$–$D_2O$–$H_2O$. The use of the latter two-phase mixture for detection of the isotope induced $^{13}C$ shifts of a fully blocked carbohydrate derivative is illustrated for the secondary amine **7** in Figure 3. For this 3-amino-3-deoxy derivative, deuterium-induced splittings of the C-1, C-2, C-3, C-4, and $H_3CO$-1 resonances of the altropyranosyl ring, and C-1, C-2, and C-6 signals of the 2,4-dinitrophenyl ring were observed (see Table 4).

An exchange solvent containing 2:1 (v/v) $D_2O$–$H_2O$ was useful for identifying which $^{13}C$ resonances belonged to the NH and ND forms, but for the ready detection of small splittings between these forms, the use of 1:1 (v/v) $D_2O$–$H_2O$ was optimum. The rates of NH to ND exchange varied widely under different conditions of solvent and $D_2O$ availability, and these rates were monitored by $^1H$ and $^{13}C$ NMR spectroscopy.

The magnitudes of the NH- to ND-induced $^{13}C$ shifts found (Table 4) were $\beta$ (CH and C=O) −0.072 to −0.100 ppm, $\gamma$ (CH) 0 to −0.043 ppm, $\gamma$ ($CH_3$) −0.045 to −0.053 ppm, $\delta$ 0 to −0.012 ppm, and $\zeta$ 0 to −0.009 ppm. The $\beta$ $^{13}C$ shift for C-1 of an *N*-(2,4-dinitrophenyl) group was unusually large at −0.138 ppm. For the C-2 resonances, the absolute magnitudes of the deuterium isotope-induced $^{13}C$ shifts were observed to be 0.003–0.017 ppm larger for the α anomers than for the β anomers (Table 4). Similarly, the incremental shifts for the $^{13}C$=O signals are 0.001–0.005 ppm larger for the α anomers than for the β. These differences are small and may not be structurally useful. Sites (e.g., C-3 and Me) that are more remote from the anomeric carbon atom did not show consistent α/β differences (Table 4). In general, the magnitudes of the NH- to ND-induced $^{13}C$ shifts are comparable with those induced by OH to OD exchange.[1–17]

## 4. Summary

The enhanced sensitivity of an NMR cryoprobe has been used to obtain simple 1D and 2D $^1H$ and $^{13}C$ NMR spectra of $D_2O$ solutions of three acetamidodeoxy sugars that occur commonly in bacterial polysaccharides, before significant anomerization of the anomers initially present in the crystalline sugars had taken place. Mixtures of equilibrated anomers and other related aminodeoxy sugar derivatives have been studied, complete $^1H$ and $^{13}C$ chemical shift assignments generated, and $^1H$–$^1H$ and $^{13}C$–$^1H$ coupling constants measured. The $^{13}C$ assignments have been used to define the deuterium induced, incremental $^{13}C$ chemical shifts produced by NH to ND exchange. Detection of the NH to ND induced isotope effects on the $^{13}C$ shifts does not require the use of concentric cells containing $H_2O$ and $D_2O$ solutions, because the rate of NH to ND exchange is slower than that of the OH to OD exchange studied earlier.[14–17] Observation of the NH- to ND-induced isotope effects on $^{13}C$ shifts has been extended to two secondary amino sugar derivatives. The data reported should be useful for the NMR analysis of saccharides in general, and specifically for selective confirmation of the $^{13}C$ NMR assignments for nitrogenous saccharides in aqueous solution, for situations in which 2D NMR correlation methods yield ambiguous results.

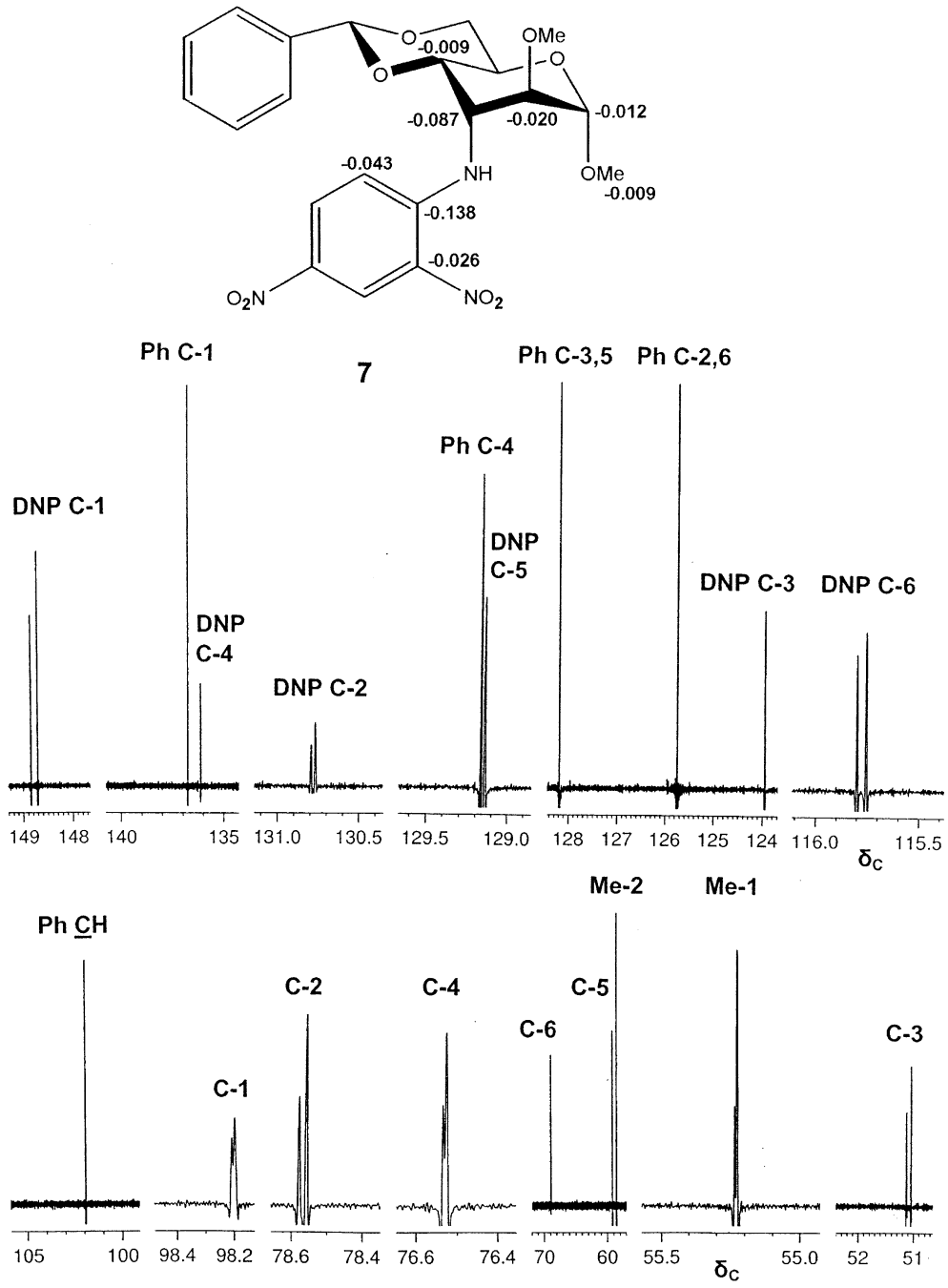


**Figure 3.** $^{13}C$ NMR spectrum of a solution of methyl *N*-2,4-dinitrophenyl 3-amino-4,6-*O*-benzylidene-3-deoxy-2-*O*-methyl-α-D-altropyranoside (**7**) in chloroform-*d* with a $D_2O$ overlayer. The spectrum represents the data obtained by averaging of partial NH to ND exchange over a weekend, using 5708 scans, a 45° pulse, and a pulse recycle time of 40 s to ensure relaxation of $^{13}C$ nuclei having long $T_1$ values. Deuterium-induced splittings (ppm) of the resonances of $^{13}C$ nuclei around the nitrogen atom are shown.

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