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Note

^{1}H-N.m.r. study on (6*S*)-(6-$^2H_1$)-2-acetamido-2-deoxy-D-glucopyranose and conformational analysis of 2-acetamido-2-deoxy-D-glucopyranose

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In relation to biological interest in the mechanism of the catalytic action of lysozyme, the favored conformations of the substrate analog 2-acetamido-2-deoxy-D-glucopyranose (D-GlcNAc) have been extensively investigated[1–5]. These studies have led to the conclusion that D-GlcNAc adopts the $^4C_1$(D) conformation in both the solution and the crystalline state[1–5]. However, the favored orientation about the C-5–C-6 bond in solution has been a controversial problem[2,3].

Proton nuclear magnetic resonance (^{1}H-n.m.r.) spectroscopy provides a useful way by which to analyze the fractional rotamer population about the C-5–C-6 bond of hexopyranoses[3,6,7] in solution, using the vicinal coupling constants of H-6*proR* and H-6*proS* with H-5. In the two previous ^{1}H-n.m.r. studies[2,3] on D-GlcNAc in D_2O however, the difficulty in differentiating between the two prochiral protons at C-6 gave rise to different conclusions[2,3]. Perkins and co-workers[2] assigned the two protons as shown in Table I, based on the assumption that the *tg* conformation should be the least favored one among the three conformers possible, namely *gg*, *gt*, and *tg* (see Fig. 1), because of the *synperiplanar* repulsion between the OH-4 and OH-6 bonds. On the other hand, in more-recent work, Boyd and co-workers[3] reversed the assignments of the two protons and concluded that the populations of the *gg*, *tg*, and *gt* rotamer were ~75, 25, and 0%, respectively, on the basis that the other assignments gave a negative population for the *tg* rotamer, which would be stabilized by intramolecular hydrogen-bonding between OH-4 and OH-6. We had previously developed a general method of stereospecific deuteration at the hydroxymethyl groups of pentoses[8,9] and hexoses[10,11] which has enabled us to assign unequivocally the two prochiral protons in the ^{1}H-n.m.r. spectrum and to predict the rotamer populations about the C-5–C-6 bonds of hexoses[12] and 1,6-linked oligosaccharides[13,14].

We now describe the unambiguous assignments of the H-6*proR* and H-6*proS* atoms of D-GlcNAc in the ^{1}H-n.m.r. spectrum by use of our stereospecific deuteration method and of the rotamer populations based on our assignments.

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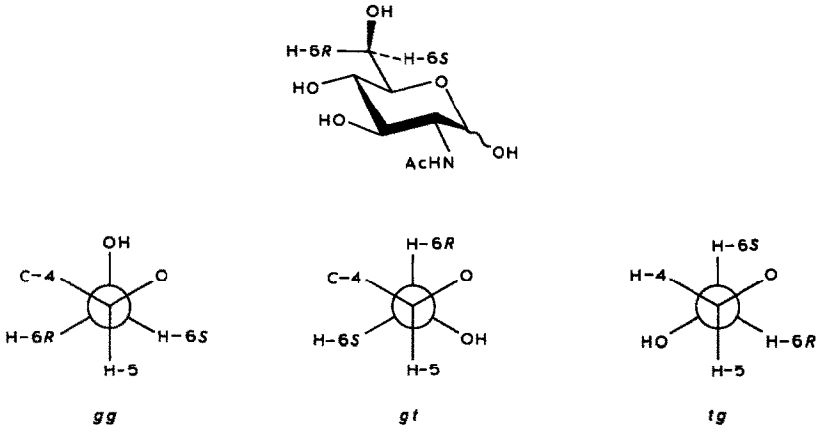


Fig. 1. Possible rotamers about the C-5–C-6 bond of 2-acetamido-2-deoxy-D-glucopyranose.

TABLE I

^{1}H-N.M.R. DATA FOR 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE

Compound	*δ*		*J (Hz)*		*Populations (%)*		
	*H-6*R	*H-6*S	$J_{H\text{-}5,H\text{-}6R}$	$J_{H\text{-}5,H\text{-}6S}$	gg	gt	tg
α-D-GlcNAc	3.776	3.835	4.8	2.4	62	32	6[a]
					64	33	−7[b]
					67	40	−7[c]
β-D-GlcNAc	3.735	3.895	5.5	1.4	62	43	−5[a]
					65	55	−20[b]
					68	51	−19[c]

[a]Calculated with the following equations[17]:

$$1.3\,gg + 2.7\,gt + 11.7\,tg = J_{H\text{-}5,H\text{-}6S} \quad (1)$$
$$1.3\,gg + 11.5\,gt + 5.8\,tg = J_{H\text{-}5,H\text{-}6R} \quad (2)$$
$$gg + gt + tg = 1 \quad (3)$$

[b]Calculated with the following equations[6]:

$$2.8\,gg + 3.1\,gt + 10.7\,tg = J_{H\text{-}5,H\text{-}6S} \quad (1)$$
$$0.9\,gg + 10.7\,gt + 5.0\,tg = J_{H\text{-}5,H\text{-}6R} \quad (2)$$
$$gg + gt + tg = 1 \quad (3)$$

[c]Calculated with the following equations[7]:

$$^3J_{H,H} = 13.22\cos^2\phi - 0.99\cos\phi + \Sigma\Delta xi\{0.87 - 2.4\cos^2(\xi\phi + 19.91\,\Delta xi)\}$$

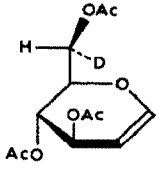
where $\phi = 180°$ *(gg)*; $\phi = -60°$ *(gt)*; $\phi = +60°$ *(tg)*; $\Delta xi = 0.08$ (C-4); $\Delta xi = 1.22$ (O-5); $\Delta xi = 1.22$ (O-6).

$$2.94\,gg + 2.95\,gt + 11.22\,tg = J_{H\text{-}5,H\text{-}6S} \quad (1)$$
$$1.0\,gg + 11.22\,gt + 4.89\,tg = J_{H\text{-}5,H\text{-}6R} \quad (2)$$
$$gg + gt + tg = 1 \quad (3)$$

RESULTS AND DISCUSSION

Our previous ^{1}H-n.m.r. studies[12,14] on D-glucose, D-mannose, and their derivatives, which have an equatorial OR-4 group and exist in the $^4C_1$(D) conformation, gave the general features of δH-6*proS* > δH-6*proR* and $^3J_{H\text{-}5,H\text{-}6proR}$ ~5–6 Hz > $^3J_{H\text{-}5,H\text{-}6proS}$ ~2 Hz, indicating that the *tg* rotamer was very disfavored, due to the 1,3-*syn* repulsion between OR-4 and OR-6. Although it was expected that D-GlcNAc, which, in the $^4C_1$(D) conformation, has an equatorial OH-4 group, would give the same results as do other D-*gluco* and D-*manno* derivatives, it was considered important to make sure whether replacement of OH-2 by NHAc-2 would affect the chemical shifts of the protons on C-6 and the values of $^3J_{H,5\text{-}H\text{-}6proR}$ and $^3J_{H\text{-}5,H\text{-}6proS}$, resulting in change of rotamer populations about the C-5–C-6 bond.

In order to assign unequivocally the signals of H-6*proR* and H-6*proS* in the ^{1}H-n.m.r. spectra, (6*S*)-(6-$^2H_1$)-D-GlcNAc (**5**) was prepared from (6*S*)-(6-$^2H_1$)-D-glucose[11] *via* azidonitration[15] of (6*S*)-(6-$^2H_1$)-tri-*O*-acetyl-D-glucal (**1**). The azido compounds **2** and **3** were obtained as a mixture of D-*gluco*- and D-*manno* derivatives, but treatment of **4** with $Ba(OMe)_2$ in MeOH gave the desired (6*S*)-(6-$^2H_1$)-D-GlcNAc (**5**) in 70% yield by epimerization[16] of the C-NHAC group.



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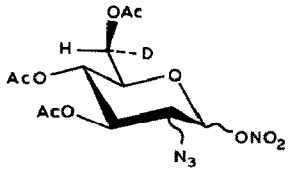

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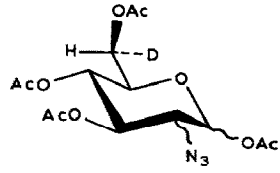

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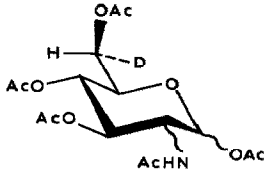

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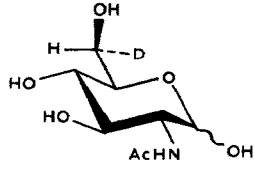

5

The 400-MHz ^{1}H-n.m.r. spectra of **5** and D-GlcNAc (see Fig. 2) showed clearly the disappearance of a one-proton signal centered at ~3.90 p.p.m. for D-GlcNAc, and at ~3.84 p.p.m. for **5**. Because the signals that disappeared are assigned to those of H-6*proS*, the more-deshielded H-6 atom, having the smaller coupling constant ($^3J_{H\text{-}5,H\text{-}6}$), was unequivocally assigned to H-6*proS*. The assignment agrees with the general features already described and, furthermore, with the results of Perkins and co-workers[2]. Although accurate values for $^3J_{H\text{-}5,H\text{-}6proR}$ and

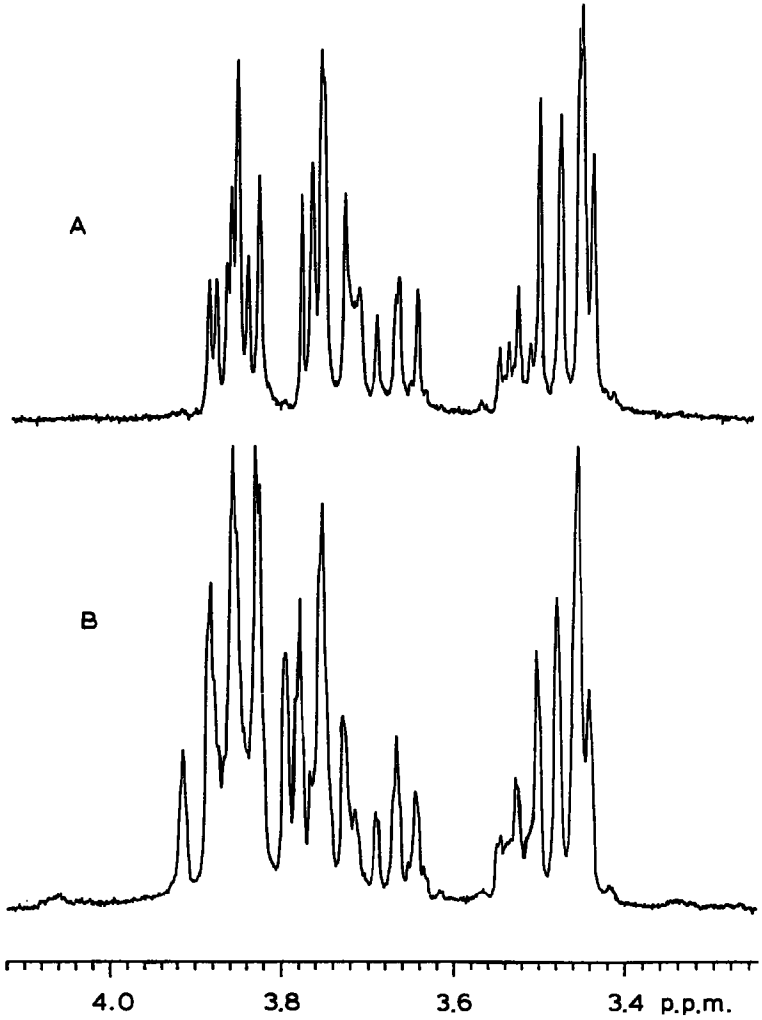

Fig. 2. Partial 400-MHz ^{1}H-n.m.r. spectra of 2-acetamido-2-deoxy-D-glucopyranoses in D_2O: A, (6*S*)-(6-$^2H_1$)-2-acetamido-2-deoxy-D-glucopyranose (**5**); B, 2-acetamido-2-deoxy-D-glucopyranose.

$^3J_{H\text{-}5,H\text{-}6proS}$ could not be obtained by first-order analysis of our spectra, the data reported by Perkins and co-workers[2] on the basis of spin-simulation enabled us to calculate the rotamer populations[6,7,17,18] of α- and β-D-GlcNAc as shown in Table I.

EXPERIMENTAL

Melting points were determined with a Yanako Model P hotplate apparatus and are uncorrected. ^{1}H-N.m.r. spectra were recorded with a JEOL JNM-FX 100 or a JEOL GX 400 instrument, with Me_4Si as the internal standard in $CDCl_3$, or acetone (at 2.220 p.p.m.) as the internal standard in D_2O. Merck silica gel 60 (Art.

7734) was used for column chromatography and Merck silica gel 60 F_{254} (Art. 5548) was used for both preparative and analytical thin-layer chromatography (t.l.c.).

(6S)-(6-$^2H_1$)-1,3,4,6-Tetra-O-acetyl-2-azido-2-deoxy-D-glucopyranose (**3a**) *and -mannopyranose* (**3b**). — (6*S*)-(6-$^2H_1$)-3,4,6-Tri-*O*-acetyl-D-glucal (**1**; 2.1 g, 7.7 mmol) in acetonitrile (5 mL) was added to a mixture of sodium azide (0.75 g, 11.5 mmol) and ceric ammonium nitrate (12.7 g, 28.1 mmol) at −10° and the mixture was vigorously stirred for 10 h at 0°. Cold ether (50 mL) and water (10 mL) were added, and the ether layer was washed three times with cold water (10 mL), dried (anhydrous $MgSO_4$), and evaporated, to afford a yellow syrup (**2**; 2.1 g); R_F 0.7 in 2:1 benzene–ether (R_F of **1**, 0.67). A mixture of this syrup (2.1 g) and anhydrous sodium acetate (800 mg) in glacial acetic acid (12 mL) was stirred for 1.5 h at 100° and cooled to room temperature. Sat. aq. sodium acetate (30 mL) was added and the mixture was extracted with chloroform (50 mL). The extract was washed successively with water and sat. sodium hydrogencarbonate, dried ($MgSO_4$), and evaporated. Column chromatography of the residual yellow syrup with 15:1 benzene–ethyl acetate gave **3a** (540 mg, 21%) m.p. 113° and **3b** (440 mg, 25%) m.p. 128–130°.

The ^{1}H-n.m.r. spectrum of **3a** showed doublet signals at 6.28 (*J* 3.7 Hz) and 5.54 (*J* 8.6 Hz) which were respectively, assigned to the anomeric protons of α and β anomer, in the ratio of ~3:4. The ^{1}H-n.m.r. spectrum of **3b** showed doublet signals at 6.11 (*J* 1.7 Hz) and 6.24 (*J* 1.3 Hz) which were respectively, assigned to the anomeric protons of the α and β anomer, in the ratio of ~10:1.

(6S)-(6-$^2H_1$)-2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranose (**4a**) *and* D-*mannose* (**4b**). — A mixture of **3a** (500 mg, 1.8 mmol) and palladium-black (10 mg) in dry methanol (50 mL) was hydrogenated at 101.3 kPa and room temperature. After the starting material had disappeared, (~20 min), acetic anhydride (0.5 mL) was added, and the mixture was stirred for 3 h, filtered, the filtrate evaporated, and the residue co-evaporated five times with absolute ethanol (5 mL). The residual syrup crystallized from ether–petroleum ether to give **4a** (470 mg, 90%), m.p. 118–128°. The ^{1}H-n.m.r. spectrum of **4a** showed doublet signals at 6.18 (*J* 3.4 Hz) and 5.69 (*J* 8.8 Hz) which were respectively, assigned to the anomeric protons of the α and β anomer, in the ratio of ~2:3. Compound **4b** (α:β = ~7:1), a syrup, was obtained by the same treatment of **3b**. The ^{1}H-n.m.r. spectrum of **3b** showed doublet signals at 6.03 (*J* 1.7 Hz) and 6.15 (*J* 1.6 Hz) which were assigned to the anomeric protons.

(6S)-(6-$^2H_1$)-Acetamido-2-deoxy-D-glucose (**5**). — To a solution of **4a** (100 mg, 0.26 mmol) in abs. methanol (10 mL) was added barium metal (1 mg), and the mixture was stirred for 1 h at room temperature, made neutral with Amberlite IR-120 (H^+) resin, filtered, and the filtrate evaporated. The residual syrup crystallized from ethanol–ether to give **5** (40 mg, 70%); m.p. 205–208°, $[\alpha]_D^{22}$ +40.0° (*c* 0.1, water); lit.[16] for 2-acetamido-2-deoxy-D-glucose, m.p. 208°, $[\alpha]_D^{22}$ +40.5° (water).

Compound **5** was also obtained in 70% yield by the same treatment of **4b**.

ACKNOWLEDGMENTS

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