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CONFORMATIONAL STUDIES OF PERTRIMETHYLSILYL DERIVATIVES OF 2-ACETAMIDO-2-DEOXYALDOHEXOPYRANOSES BY 220- AND 300-MHz P M.R SPECTROSCOPY

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ABSTRACT

The complete interpretation of high-resolution p.m r spectra and the accurate chemical shifts and coupling constants, obtained after computer simulation of the spectra, of the trimethylsilyl (TMS) derivatives of six 2-acetamido-2-deoxy-D-aldohexopyranoses are given. All pyranoid rings occur in the $^4C_1$(D) chair conformation. The preferred conformation of the C-5–CH_2OTMS group depends on the configuration at C-4 Only the chemical shifts and the line widths of the signals of NH and H-1 of the β-D-gluco- and the β-D-galacto-pyranose derivatives were found to be strongly dependent on the temperature This observation can be interpreted in terms of the preferred conformation of the acetamido group At room temperature, the cis and trans conformations of this group, with respect to the ring, are present in a dynamic equilibrium The intermediate conformations are populated to a very low extent Because of the similarity in coupling constants $J_{NH,2}$, it is proposed that this situation is also present in the α-D-gluco- and α-D-galacto-pyranose derivatives, whereas the trans conformation is populated in the D-mannopyranose derivatives.

INTRODUCTION

In the framework of p m r studies[1,2] of pertrimethylsilyl (TMS) derivatives of mono- and oligosaccharides, we now investigated 2-acetamido-2-deoxyaldohexopyranoses The determination of the structure of this type of sugar is relevant to the study of glycoproteins, which contain amino sugars A few p m r studies of the structures of amino sugars and their derivatives in solution have been reported Izumi[3] concluded that the $^4C_1$(D) chair conformation prevails for 2-acetamido-2-deoxy-α-D-galactopyranose in deuterium oxide This conformation was also found for derivatives of 2-amino-2-deoxy-D-glucopyranose[4–7], 2-amino-2-deoxy-D-galactopyranose[5], and 2-amino-2-deoxy-D-mannopyranose[5,6]. On the basis of the $J_{NH\ 2}$ coupling constant, Hirano[8] proposed that the acetamido group of a number of amino sugars occurs in the trans conformation with respect to the pyranoid ring

We now report on the structures of TMS derivatives of 2-acetamido-2-deoxyaldohexopyranoses deduced from accurate p m r data obtained by computer simulation of high-resolution p.m.r. spectra. The conformation(s) of the ring, the C-5 substituent, and the acetamido group were investigated. The influence of the latter group on the structure of the compounds was inferred from comparisons with TMS-aldohexopyranoses. To corroborate the conclusions about the ring structures, spectra were recorded at temperatures in the range of 25 to −80°.

RESULTS AND DISCUSSION

Spectrum interpretation. — The 220-MHz p.m.r. spectra of the 1,3,4,6-tetrakis-*O*-trimethylsilyl derivatives of 2-acetamido-2-deoxy-α-D-glucopyranose (**1**), -β-D-glucopyranose (**2**), -α-D-mannopyranose (**3**), -β-D-mannopyranose (**4**), -α-D-galactopyranose (**5**), and -β-D-galactopyranose (**6**) were recorded for solutions in acetone-d_6. The spectra of **1** and **3** showed much, high-order complexity, and therefore 300-MHz spectra were also measured.

The spectra can be subdivided into the following groups of signals: (*1*) four strong, sharp singlets for the TMS methyl groups at δ 0.1–0.2; (*2*) a singlet for the NAc-methyl group at δ 1.86–1.92, (*3*) the multiplets for the non-anomeric protons between δ 3.0–4.5, (*4*) a doublet for the anomeric proton at δ 4.7–5.2, and (*5*) a broad doublet for the NH proton at δ 6.2–7.2.

The initial parameters of the protons (groups *3* and *4*) attached to the carbon skeleton were obtained from a first-order, sub-spectral analysis. Refinement of these data was achieved by the calculation of theoretical spectra in an interactive, iterative procedure with the spin-simulation program SIMEQ[9] until a good agreement was obtained with the observed spectra. In these calculations, the spin system was treated as an eight-spin system XABCDEFG (NH, H-1, H-2, up to and including H-6′). All vicinal coupling constants were taken to be positive, and the geminal coupling constants to be negative. The observed and calculated 300-MHz spectra of the non-anomeric protons of the α-D-mannopyranose derivative **3** are shown in Fig. 1. The refined p.m.r. parameters and the first-order chemical shifts of the NH, TMS-methyl, and NAc-methyl protons of compounds **1–6** are given in Tables I and II.

The structure of the pyranoid rings. — The dihedral angles between the vicinal ring protons of compounds **1–6** (Table III*A*) were calculated from the observed, vicinal-coupling constants $J_{1,2}$ to $J_{4,5}$ (Table II) by using a modified Karplus relation*. For comparison, the data of the corresponding pentakis-TMS derivatives

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*For the calculation of coupling constants from dihedral angles and for the reversed procedure, the following, modified Karplus relation[1] was used.

$$J_{HH} = (6.6 - 1.0 \cos \phi + 5.6 \cos 2\phi)(1 - \sum_{i=1}^{4} f_i \Delta X_i),$$

in which ϕ is the dihedral angle between the protons in the fragment H-C–C′-H′, $\Delta X_i = X_R - X_H$ represents the difference in electronegativity X between a substituent R and hydrogen, f_i amounts to 0.15 when the dihedral angle θ between R and H in H-C–C′-R is larger than 90°, and 0.05 when θ is smaller than 90°. The following values for X, as determined by the method of Cavanaugh and Dailey[10], were used:

$X_H = 2.1$, $X_{OTMS} = 3.5$, $X_{NHAc} = 3.2$; $X_{-C-O-} = 2.5$, $X_{O_r} = 3.3$

TABLE I

PROTON CHEMICAL SHIFTS δ (P P M) OF THE TMS DERIVATIVES OF 2-ACETAMIDO-2-DEOXYALDOHEXOPYRANOSES IN ACETONE d_6

TMS derivative of		*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H 6*	*H-6'*	*NH*	*OTMS-methyl groups (number of protons)*				*NAc-methyl*
α D-GlcNAc*p*[a]	(1)	5 03	3 96	3 76	3 62	3 62	3 83	3 71	6 86	0 120(9)	0 129(9)	0 145(9)	0.182(9)	1 92
β D-GlcNAc*p*	(2)	4 80	3 50	3 77	3 58	3 20	3 79	3 79	6 98	0,116(9)	0,130(9)	0 136(9)	0 176(9)	1 88
α D-ManNAc*p*	(3)	5 12	4 10	4 05	3 72	3 65	3 69	3 78	6 77	0 109(9)	0 130(18)	0 185(9)		1 86
β D-ManNAc*p*	(4)	4 97	4 40	3 76	3 59	3 26	3 62	3 85	7 15	0 124(9)	0 130(18)	0 152(9)		1 87
α D-GalNAc*p*	(5)	5 05	4 38	3 90	3 98	3 84	3 55	3 64	6 28	0 110(9)	0 144(18)	0 150(9)		1.90
β D-GalNAc*p*	(6)	4 92	3 72	3.96	3 92	3 47	3 62	3 66	6 85	0 108(9)	0 111(9)	0 142(9)	0 149(9)	1 87

[a] Data from 300-MHz spectrum

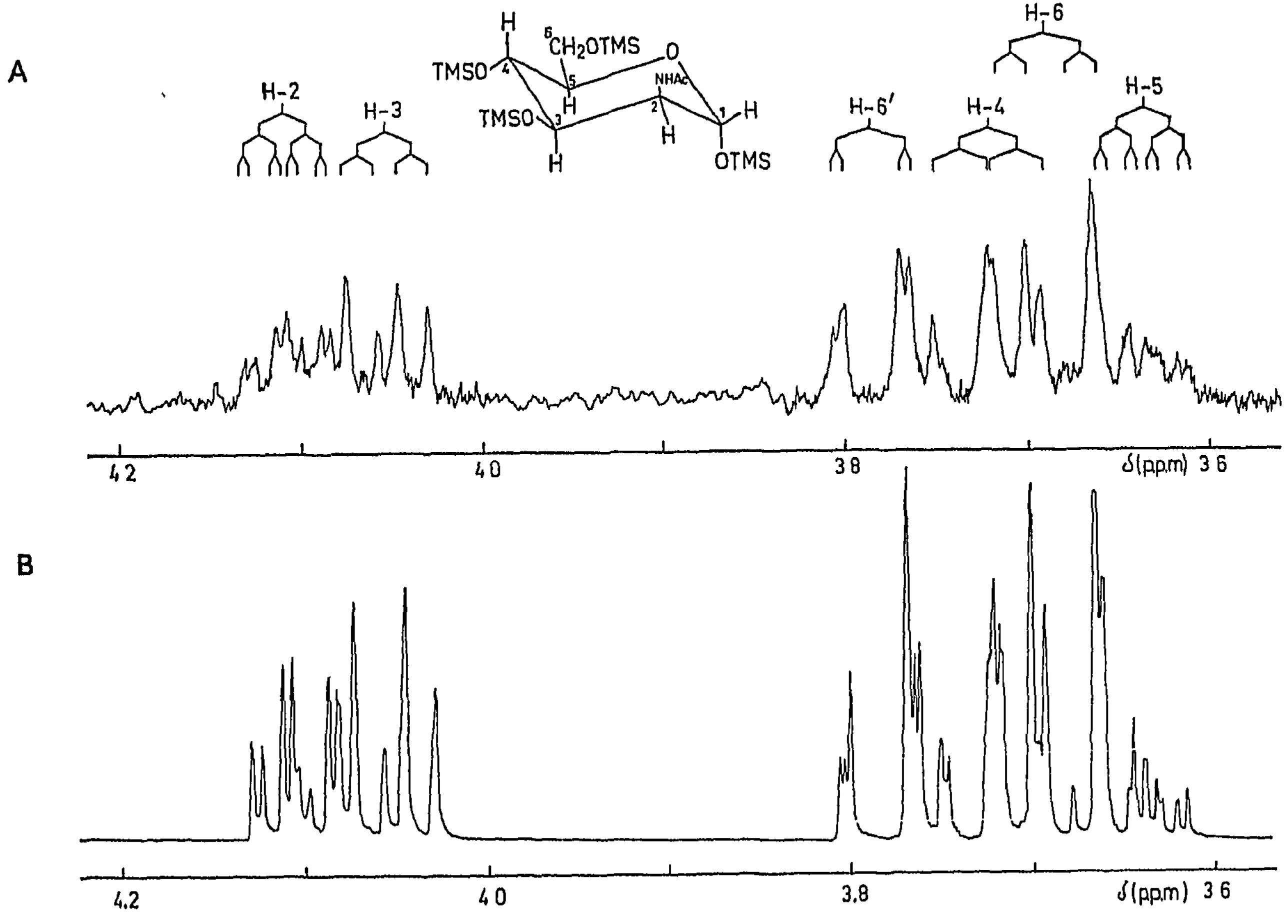

Fig 1 300-MHz p m r spectrum of the non-anomeric protons of the TMS derivative of 2 acetamido-2-deoxy-α-D-mannopyranose (3), A observed spectrum, B calculated spectrum

TABLE II

PROTON COUPLING CONSTANTS (HZ) OF THE TMS DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-ALDOHEXOPYRANOSES IN ACETONE-d_6

TMS derivative of		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6}$	$J_{6,6}$	$J_{NH,2}$
α-D-GlcNAc*p*[a]	(1)	3.5	10.0	8.6	—[b]	2.5	1.3	−11.8	10.0
β-D-GlcNAc*p*	(2)	8.0	8.7	9.4	8.7	2.7	2.7	—[b]	9.5
α-D-ManNAc*p*[a]	(3)	1.7	4.9	9.0	8.9	5.5	2.0	−11.4	7.8
β-D-ManNAc*p*	(4)	1.6	4.3	9.0	9.1	6.4	2.2	−11.0	10.0
α-D-GalNAc*p*	(5)	3.5	10.4	2.5	0.9	5.4	7.1	−9.6	10.0
β-D-GalNAc*p*	(6)	8.0	10.0	2.4	0.8	6.0	7.3	−9.3	9.5

[a]Data from 300-MHz spectrum [b]Not observed

of D-aldohexopyranoses (TMS-aldohexopyranoses), which have the ${}^4C_1$(D) chair conformation[1], are given in Table III*B* Table III shows that corresponding angles in the two series of compounds have similar magnitudes This leads, in combination with the observation that $J_{1,2}$ is independent of temperature in the range of 25 to −80°, to the conclusion that the pyranoid rings of **1**–**6** occur in a single conformation, the ${}^4C_1$(D) conformation In the fragment C-1–C-2–C-3, the angles between vicinal axial–equatorial protons are smaller, and the angles between vicinal diaxial and vicinal diequatorial protons are larger in compounds **1**–**6** than in the corresponding TMS-aldohexopyranoses Consequently, the pyranoid rings in **1**–**6** are less flattened in the region C-1–C-2–C-3 This diminished flattening indicates that an acetamido group exhibits less steric hindrance with neighbouring OTMS groups than does an OTMS group

TABLE III

CALCULATED DIHEDRAL ANGLES ($\phi_{H,H}$) FOR THE TMS DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-ALDOHEXOPYRANOSES (*A*) AND ALDOHEXOPYRANOSES (*B*)

Configuration	A				B[a]			
	$\phi_{1,2}$	$\phi_{2,3}$	$\phi_{3,4}$	$\phi_{4,5}$	$\phi_{1,2}$	$\phi_{2,3}$	$\phi_{3,4}$	$\phi_{4,5}$
α-D-Glc*p*	46	161	151	—	50	154	151	156
β-D-Glc*p*	149	151	158	151	145	149	150	151
α-D-Man*p*	64	38	154	153	59	56	156	154
β-D-Man*p*	63	43	154	154	73	54	154	155
α-D-Gal*p*	46	166	56	74	49	158	55	67
β-D-Gal*p*	149	161	57	76	144	143	63	72

[a]Data taken from Ref 1

The preferred conformation of the C-5–CH$_2$OTMS group — The coupling constants $J_{5,6}$ and $J_{5,6'}$ are time-averaged parameters arising from the contributions of the various conformations of the C-5–CH_2OTMS group The three staggered rotamers 1, 2, and 3 (Fig 2) are the most important conformations[11,12] The mole fractions *n* (Table IV) of these rotamers can be inferred from the observed coupling constants $J_{5,6}$ and $J_{5,6}$ by means of the following, theoretical coupling constants[1] $(J_{5,6})_1 = (J_{5,6'})_2 = 2\ 0$ Hz, $(J_{5\ 6})_2 = (J_{5\ 6'})_1 = 11.1$ Hz, and $(J_{5,6})_3 = (J_{5\ 6'})_3 = 1\ 6$ Hz. The coupling constants $J_{5,6}$ and $J_{5,6'}$ and, by consequence, n_1 and n_2 can be interchanged (rotamer 3 contributes evenly to both coupling constants) In the *galacto* derivatives **5** and **6**, the assignment of $J_{5,6}$ and $J_{5,6'}$ can be made on the basis of the geminal coupling constants $J_{6,6'}$ Molecular orbital theory[13] predicts an algebraic increase of $J_{6,6}$ with the contribution of the rotamer in which the C-6–C-5–O-5 plane bisects the axis between H-6 and H-6′ (rotamer 1) The value for $J_{6,6'}$ in TMS-aldohexopyranoses varies[1 2] between −9 6 and −11 8 Hz The relatively large values (−9 6 and −9 3 Hz, respectively) found for **5** and **6** indicate that rotamer 1 is preponderant Rotamer 3 is strongly disfavoured in **5** and **6** because of the 1,3-parallel interaction between OTMS-4 and OTMS-6 It is reasonable to assume that this finding can be extrapolated to the D-gluco- and D-manno-pyranose derivatives (**1**–**4**) Because a 1,3-parallel interaction is present in rotamer 1 of these compounds, the lowest values for n_1 represent the most probable mole fractions (Table IV). Therefore, the configuration at C-4 is the crucial factor in the conformational preferences of the C-5–CH_2OTMS group, as was also found for TMS-aldohexopyranoses[1] Evidently, the replacement of an OTMS-group at C-2 by an acetamido group has no significant influence on the orientation of the substituent at C-5

TABLE IV

CALCULATED MOLE FRACTIONS (*n*) OF THE ROTAMERS 1, 2, AND 3 OF THE C-5–C-6 FRAGMENT OF THE TMS DERIVATIVES OF 2-ACETAMIDO-2-DEOXYALDOHEXOPYRANOSES

TMS derivative of		n_1	n_2	n_3
α-D-GlcNAc*p*	(**1**)	0 00	0 10	0 90
β-D-GlcNAc*p*	(**2**)	0 11	0 11	0 78
α-D-ManNAc*p*	(**3**)	0 02	0 41	0 57
β-D-ManNAc*p*	(**4**)	0 04	0 50	0 46
α-D-GalNAc*p*	(**5**)	0 56	0 38	0 06
β-D-GalNAc*p*	(**6**)	0 58	0 42	0 00

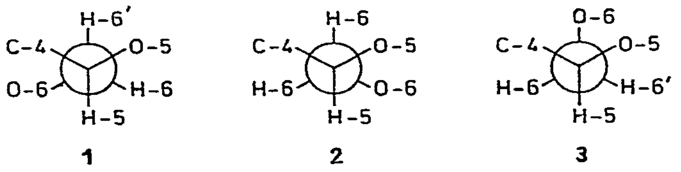

Fig 2 The three staggered rotamers (1, 2, and 3) of the C-5–CH_2OTMS group

The preferred conformation of the C-2-NHAc group — It is generally accepted that the acetamido group has a planar structure with NH and C=O in trans arrangement[8,14,15]. The proton–proton coupling in a fragment CH–NH is dependent on the dihedral angle, analogous to the coupling in a fragment CH–CH[15,16] The value of $J_{NH\,H}$ is ~0 Hz when the angle is 90°. Values of ~9–10 Hz or 9–11 Hz are observed

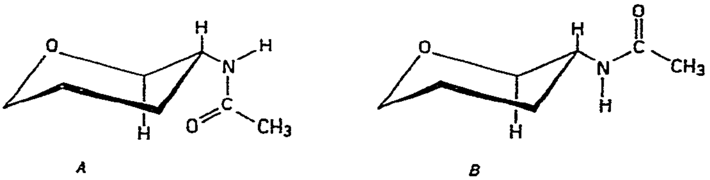

Fig 3 The cis (*A*) and trans conformation (*B*) of the acetamido group with respect to the ring in the TMS derivatives of 2-acetamido-2-deoxy-β-D-glucopyranose (2) and -β-D-galactopyranose (6)

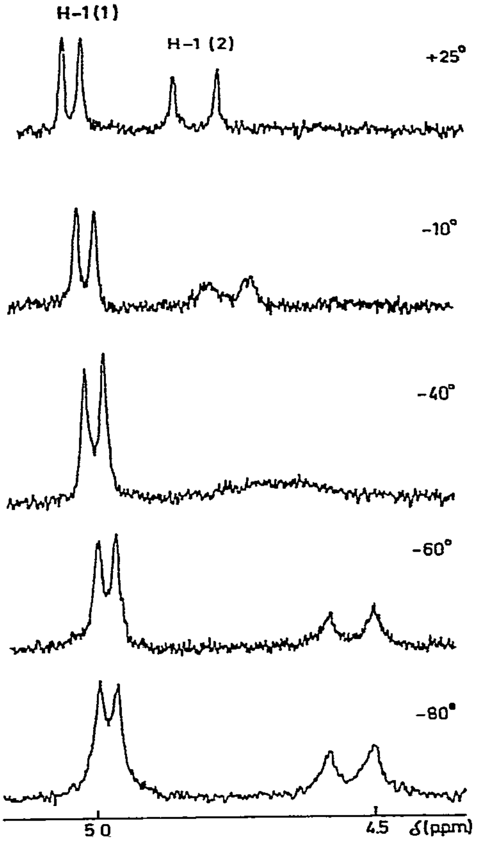

Fig 4 Temperature dependence of the signals of H-1 of the TMS derivatives of 2-acetamido-2-deoxy-α-D-glucopyranose (1) and -β-D-glucopyranose (2) in acetone-d_6

for angles of 0° or 180°, respectively The relatively large values found for $J_{\mathrm{NH},2}$ for compounds **1**–**6** (Table II) correspond to dihedral angles of ~0° or ~180°. For this observation, there are three alternative explanations the acetamido group adopts only the cis conformation with respect to the ring (Fig 3*A*); only the trans conformation is adopted (Fig 3*B*); both conformations are present and may exchange (intermediate orientations do not contribute significantly) Temperature experiments were carried out in order to obtain further information on these possibilities

On decreasing the temperature, $J_{\mathrm{NH}\ 2}$ remained constant, whereas the NH signals in **2** and **6** broadened more than in the other compounds below −10°, $J_{\mathrm{NH}\ 2}$ could not be measured in **2** and **6** Surprisingly, the broadening of the NH signals in **2** and **6** is accompanied by a strong, upfield shift of the signal for H-1 (Fig 4 and 5*A*),

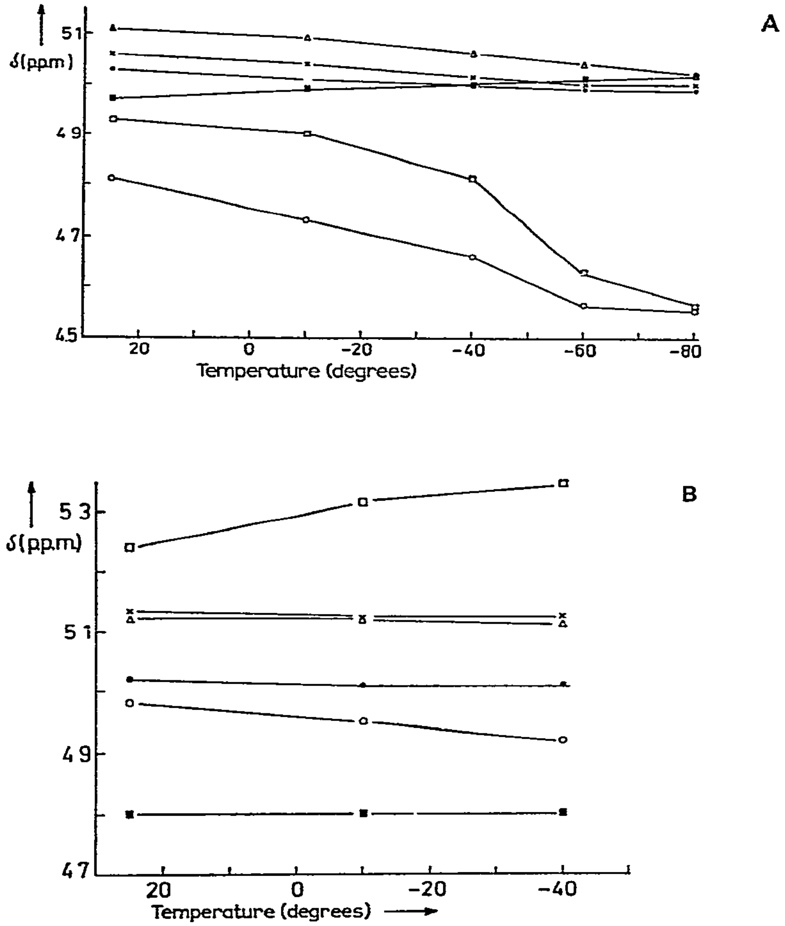

Fig 5 Temperature dependence of the chemical shifts of H-1 of the TMS derivatives of 2-acetamido-2-deoxy-α-D-gluco- (**1**, —·—), -β-D-gluco- (**2**, —○—), -α-D-manno- (**3**, —△—), -β-D-manno- (**4**, —■—), -α-D-galacto- (**5**, —×—), and -β-D-galactopyranose (**6**, —□—) in acetone-d_6 (A) and in chloroform-*d* (B)

while the lines of this doublet broaden and coalesce at −40°. Below this temperature, a gradual sharpening of the coalesced lines is observed (Fig 4) For the other compounds, no such temperature dependence is found (*cf.* H-1 of **1**, and H-1 of **2**, in Fig 4) It has to be noted that, for all compounds, $J_{1,2}$ is temperature independent

The magnetic environment of an axial H-1 proton next to an equatorial acetamido group (in **2** and **6**) is strongly temperature dependent, in contrast to the magnetic environments of an equatorial H-1 proton next to an equatorial acetamido group (in **1** and **5**) and of an axial H-1 or an equatorial H-1 proton next to an axial acetamido group (in **3** and **4**, respectively) It is reasonable to assume that the orientation of the carbonyl function of the acetamido group with respect to H-1 is responsible for this effect In **2** and **6**, this function is located quite close to H-1 when NH and H-2 are cis (Fig 3*A*), whereas in trans orientation this function is far removed (Fig 3*B*) In the other compounds (**1**, **3**, **4**, and **5**), the carbonyl group is far removed from H-1 in the cis as well in the trans conformation The observed temperature effect on the chemical shift can be interpreted as a change of the relative proportions of the cis and trans conformations At room temperature, the chemical shift of H-1 of **2** and **6** arises from both conformational extremes Below −80°, the chemical shift results from the presence of only one conformer Presumably, the energetically favourable trans-form prevails at low temperatures, because of less steric hindrance with neighbouring groups The coalescence of the signal for H-1 indicates that, at intermediate temperatures, the rate of rotation around the C-2–N bond becomes of the order of magnitude of the chemical-shift difference of H-1 in the two environments Such an exchange phenomenon would also explain the observations for the NH signals (see above)

The effect of temperature on line broadening and chemical shifts of H-1 are unaltered when NHAc is replaced by NDAc Hence, a strong dipole–dipole relaxation between NH and H-1, caused by a relatively slow rotation around the C-2–N bond, can be excluded as an explanation of the line-width phenomenon

To determine whether acetone is an essential factor in the observed effects on the signals for H-1, spectra for solutions in chloroform were recorded In the latter solvent, the same variation of line width with temperature is observed However, the chemical-shift effects are less pronounced (Fig 5*B*)

In conclusion, it is proposed that, at room temperature, the cis and trans conformations of the acetamido group in **2** and **6** are present in a dynamic equilibrium The similarity of the $J_{NH,2}$ values for **1**, **2**, **5**, and **6** suggest that the conformational preferences of the acetamido groups in all these compounds are identical In the mannopyranose derivatives **3** and **4**, the situation is different, as the steric interactions between the acetamido group and other ring-substituents make the cis arrangement very unlikely The large value (10 Hz) for $J_{NH,2}$ in **4** indicates that the trans arrangement is almost exclusively populated The somewhat smaller value (7 8 Hz) observed in **3** shows that, besides the trans form, neighbouring conformations also contribute considerably

EXPERIMENTAL

Preparation of the pertrimethylsilyl derivatives — An aqueous solution of the 2-acetamido-2-deoxyaldohexopyranose was anomerized during 24 h and then lyophilized, and the residue was treated with hexamethyldisilazane and chlorotrimethylsilane in dry pyridine[17] For the temperature experiments, 12% (w/v) solutions of the anomeric mixture in acetone-d_6 or in chloroform-*d* were used For the exchange of the amide hydrogen in the acetamido group by deuterium, 60 mg of the anomeric mixture was dissolved in chloroform-*d* and shaken for 8 h with deuterium oxide and a trace of triethylamine This procedure was repeated three times with renewal of the layer of deuterium oxide. The degree of deuteration was checked by p m r spectroscopy For the 220- and 300-MHz p m r spectra, the pure anomers were separated from the anomeric mixtures by preparative g l c A Pye Model 105 Gas Chromatograph was used, provided with a flame-ionization detector and a glass column (2 m×9.6 mm outer diameter) containing 10% of OV-17 on Chromosorb W/NAW (30–60 mesh) at 230° Nitrogen was used as carrier gas Solutions (3–6%) of the anomers were prepared in acetone-d_6

P m r. spectroscopy — The 220- and 300-MHz p m r spectra were run on a Varian HR-220 spectrometer (TNO Central Laboratories, Delft, The Netherlands) and a Varian HA-300 spectrometer (Laboratory of NMR spectroscopy, Ghent, Belgium), at a probe temperature of ~25° Spectrum simulations were run on a 16 k Varian 620i computer coupled with an XL-100 spectrometer, using a modified SIMEQ spin-simulation program[9] Chemical shifts are given relative to that of tetramethylsilane (indirect to acetone-d_6: δ 2 05) with an accuracy of ~0 005 p p m The accuracy of the coupling constants is ~0 1 Hz The temperature and deuteration experiments were performed with a Varian HA-100 spectrometer.

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