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Reference Data

Conformational analysis of 6-deoxy-6-iodo-D-glucose in aqueous solution

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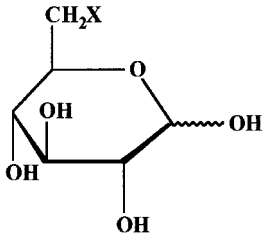
ABSTRACT: Complete ^{1}H and ^{13}C assignments for 6-deoxy-6-iodo-D-glucose, an analogue of glucose which is transported across the cell membrane, are given. These allowed rotameric populations around the C(5)—C(6) bond to be determined. Comparison with those observed for glucose suggests that the 6-OH group does not play a major role in glucose transport. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; conformational analysis; rotamer population; 6-deoxy-6-iodo-D-glucose

INTRODUCTION

Medical imaging of glucose-based biological events being of current interest, various iodinated glucose derivatives have been prepared for their possible use in SPECT (single photon emitted computed tomography) imaging. Among these analogues of glucose (**1**), 6-deoxy-6-iodo-D-glucose (**2**), has shown the required properties of a tracer of D-glucose transport[1] and we have undertaken an NMR study of **2**[2] to determine its conformation in aqueous solution, which could be of help in understanding on structural grounds why and how it mimics glucose. In order to compare the results with those for **1**, the spectrum of **1**[3–5] was analysed under identical conditions.



1 : X = OH
2 : X = I

RESULTS AND DISCUSSION

The ^{1}H resonances of **1** and **2** were assigned through the combined use of homonuclear COSY and 1D-TOCSY experiments, starting from the readily identified anomeric protons. The coupling constants *J* were determined after iterative analysis and spin simulation. The assignment of the prochiral H-6 protons (H-6*R* and H-6*S*) is based on the observed higher frequency of H-6*S* than H-6*R*.[3] These assignments are also consistent with the relative magnitudes of vicinal coupling constants, $^3J(5,6R)$ being larger than $^3J(5,6S)$.[4] Heteronuclear correlation experiments (HMQC, HMBC) were used to assign ^{13}C resonances, with the exception of C-2α and C-5α for **1** and C-2β and C-5β for **2**, which are not well enough resolved to allow their unambiguous assignment by these techniques. Therefore, selective irradiations were performed on each carbon and magnetization transfer to protons was observed. The complete proton and carbon assignments of **1** and **2** are presented in Tables 1 and 2.

Table 1. ^{13}C and ^{1}H chemical shifts for **1** and **2**

	^{13}C[a], δ (ppm)	^{1}H[b], δ (ppm)	Compound	^{13}C[a], δ (ppm)	^{1}H[b], δ (ppm)	Compound
1	91.99	5.2348	**1**α	92.11	5.2094	**2**α
	95.81	4.6390	**1**β	95.72	4.7095	**2**β
2	71.40	3.5375	**1**α	71.44	3.5456	**2**α
	74.06	3.2479	**1**β	74.11	3.2573	**2**β
3	72.68	3.7197	**1**α	72.15	3.7408	**2**α
	75.68	3.4929	**1**β	75.01	3.5196	**2**β
4	69.59	3.4145	**1**α	73.61	3.3196	**2**α
	69.54	3.4059	**1**β	73.27	3.3466	**2**β
5	71.35	3.8365	**1**α	69.49	3.5444	**2**α
	75.84	3.4685	**1**β	73.73	3.1997	**2**β
6*R*	60.55	3.7628	**1**α	7.93	3.4339	**2**α
	60.69	3.7248	**1**β	6.70	3.4086	**2**β
6*S*	60.55	3.8452	**1**α	7.93	3.5746	**2**α
	60.69	3.8989	**1**β	6.70	3.5896	**2**β

[a] Experimental data.
[b] Data obtained from simulated spectra.

Table 2. $J(^1H, ^1H)$ coupling constants (Hz) for **1** and **2**[a]

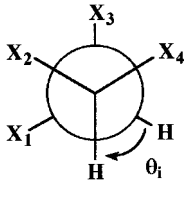
	1α	1β	2α	2β
J(1,2)	3.84	7.50	3.84	8.03
J(2,3)	9.23	9.00	9.65	9.32
J(3,4)	9.10	9.09	9.28	9.21
J(4,5)	9.90	9.80	9.50	9.21
J(5,6*R*)	5.40	5.95	5.80	5.77
J(5,6*S*)	2.20	1.95	2.90	2.72
J(6*R*,6*S*)	−11.80	−12.25	−11.00	−11.24

[a] Data obtained from simulated spectra.

As for glucose, **2** exists as pyranose ($\alpha : \beta = 41 : 59$) in the stable $^4C_1$ conformation. As the only structural difference between glucose and its iodinated analogue **2** pies in the C-6 substituent, rotameric populations around the C(5)—C(6) bond were determined using $^3J(5, 6R)$ and $^3J(5, 6S)$ vicinal coupling constants.[5] The experimental coupling constant $^3J_{exp}$ is linked to rotamer population by

$$^3J_{exp} = \sum_{i=1,2,3} p_i {}^3J_i \quad (1)$$

where p_i represents the population for each rotamer *gg*, *gt* and *tg* and J_i its theoretical coupling constant.



rotamer i

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As electronegative substituents modify the value of theoretical coupling constants J, the general Karplus equation as modified by Colucci *et al.*[6] was used to calculate the J_i parameter for each of the rotamer:[7]

$$^3J_i = A + B\cos\theta_i + C\cos 2\theta_i + \cos\theta_i[(\Delta S_1 + \Delta S_4)\cos(\theta_i - 120) + (\Delta S_2 + \Delta S_3)\cos(\theta_i + 120)] \quad (2)$$

where θ_i is the angle between the two C—H bonds in the rotamer i and ΔS_n is the contribution of each substituent X_n.

Thus, for glucose (**1**), ΔS is zero for H, 4.60 for OR, 3.28 for C(OR) and C(O)R groups and 5.20 for OH with coefficients A, B and C being 8.17, −1.96 and 6.54 respectively;[6] for example, application of these data to **2** (ΔS being 2.80 for iodine)[6] gives the following equation system from which rotameric populations can be extracted:

$$^3J(5, 6R) = 4.13p_{tg} + 1.04p_{gg} + 11.33p_{gt} \quad (3)$$

$$^3J(5, 6S) = 11.33p_{tg} + 2.03p_{gg} + 3.14p_{gt} \quad (4)$$

$$p_{tg} + p_{gg} + p_{gt} = 1 \quad (5)$$

The calculated relative populations p_{tg}, p_{gg} and p_{gt} of **1** and **2** are displayed in Table 3. It can be seen that the *tg* population, which reflects the *syn* diaxial interaction between the C-6 substituent and the 4-OH, is negative but such negative values have frequently been observed for glucose derivatives[5] and, although not being of physical significance, they can be used to assay *relative* populations. Variations in rotameric populations upon comparison of **1** and **2** (Table 3) are found to be minimal, which suggests that, as the transport of carbohydrates across the cell membrane is controlled by hydrogen bonding, the 6-substituent (hydroxyl for glucose and iodine for **2**) does not play a significant role in their entry into the cell. Whether bulkier and/or more lipophilic groups (such as affinity markers) can be introduced at position 6 remains to be investigated.

EXPERIMENTAL

Materials

D-Glucose was from a commercial source and 6-deoxy-6-iodo-D-glucose was prepared according to the literature procedure.[2a] In aqueous solution, **2** equilibrates to a mixture of anomers which, as for glucose, can be separated by HPLC; however, each isolated anomer mutarotated to produce the initial mixture;[2a] therefore, all NMR experiments on **1** and **2** were performed on pre-equilibrated aqueous solutions of mixture of anomers.

Spectra

NMR spectra were recorded in 5 mm tubes ($c = 0.17$ mol l^{-1} in D_2O) at 37 °C using a Varian UNITY$^+$ spectrometer operating at 499.89 MHz (^{1}H) and 125.6 MHz (^{13}C). The residual signal of *H*OD was used as internal reference (δ = 4.663 ppm at 37 °C), after temperature calibration using sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropanoate in D_2O.

^{1}H NMR spectra were acquired using a 1230 Hz spectral width, 32 K data points, 13 s acquisition time, 1 s relaxation delay and 10 μs for a 90° pulse. Zero-filling was performed without apodization to give a resolution of 0.037 Hz per point. ^{13}C spectra were obtained using a 15 kHz spectral width, 20 K data points, 0.7 s acquisition time, 1 s relaxation time and 20 μs for a 60° pulse. Zero-filling and 0.4 Hz exponential line broadening apodization provided a resolution of 0.9 Hz per point. T_1 relaxation times were obtained by the inversion–recovery method.

^{1}H–^{1}H COSY spectra were recorded on 2K data points for 1K increments in the F_1 dimension and processed using a 4096 × 4096 transformed matrix with zero-filling in each dimension. Sinusoidal multiplication was performed before Fourier transformation.

HMQC and HMBC spectra were recorded without proton decoupling, on the spectral windows used for 1D spectra acquisition, using a 2048 × 256 matrix. Delay was optimized for ^nJ(H,C) = 5 Hz and ^1J(C,H) = 150 Hz. Zero-filling in both dimensions (4096 × 2048 matrix) and sine multiplication were performed prior to Fourier transformation. A forward linear prediction on 1024K in the ^{13}C dimension enhanced resolution.

^{1}D TOCSY experiments were recorded using a selective 'e-burp'-shaped pulse (pulse width = 240 ms) on each anomeric proton; the mixing time was incremented from 10 to 100 ms.

Selective irradiations of individual ^{13}C sites followed by polarization transfer to proton were performed using the reverse detection mode pulse sequence proposed by Blechta *et al.*[8a] and Nishida *et al.*,[8b] in which some modifications were introduced: broadband WALTZ homodecoupling was applied during the preparation delay (3 s), then a half-Gaussian selective excitation (94 ms) was applied to a selected ^{13}C site under decoupled conditions. The polarization transfer used a 3.4 ms = 0.5/J(C,H) delay. A 1230 Hz spectral width was retained and a slight exponential broadening (0.6 Hz) was applied prior to Fourier transformation.

Table 3. Rotamer populations about C5—C6 calculated for **1** and **2** in solution

	p_{tg}(%)	p_{gg}(%)	p_{gt}(%)	Compound
α	−1	47	54	**1**
	4	51	45	**2**
β	−7	45	62	**1**
	2	53	45	**2**

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