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Note

Two-dimensional *J*-resolved ^{1}H-nuclear magnetic resonance spectroscopy of α,β-D-glucose at 500 MHz

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The use of ^{1}H-n.m.r. spectroscopy as a primary method for structural studies of carbohydrates has been hampered by the poor resolution of the nonanomeric resonances, which fall in a narrow-chemical shift range of ~1.0 p.p.m. Despite this limitation, much useful information about ring conformation and anomeric composition has been obtained from analysis of the anomeric resonances[1]. Furthermore, empirical correlation of the anomeric resonances of complex oligosaccharides with proposed or known structures has aided in structural confirmation[2,3]. In the case of simple sugars, some spectral information for the nonanomeric protons can be obtained by a combination of approaches, *e.g.*, selective deuteration[4], or INDOR (indirect detection of resonance), and spectral simulation[5].

Recently, two-dimensional n.m.r. techniques[6] have been successfully applied to carbohydrates[7,8]. These two-dimensional techniques provide large improvements in spectral resolution, and hold great promise for the advancement of n.m.r. spectroscopy as a nondestructive technique for structural studies of underivatized carbohydrates. In this Note, we present conventional and two-dimensional *J*-resolved spectra at 500 MHz of the simple anomeric mixture, α,β-D-glucose, in deuterium oxide. The spectral resolution obtained at 500 MHz is impressive, and provides encouragement that previously intractable carbohydrate structural problems will now be approachable.

In Fig. 1 is presented the nonanomeric region of the 500-MHz ^{1}H-n.m.r. spectrum of α,β-D-glucose. This spectrum has been resolution-enhanced by applying Lorentzian and Gaussian lineshape-transformations. The spectrum is almost completely resolved, and has been assigned by selective decoupling. The increased resolution obtained as a result of the high field-strength (500 MHz) is significant. For example, the 4α–4β resonances at δ 3.43–3.38 are totally resolved at 500 MHz,

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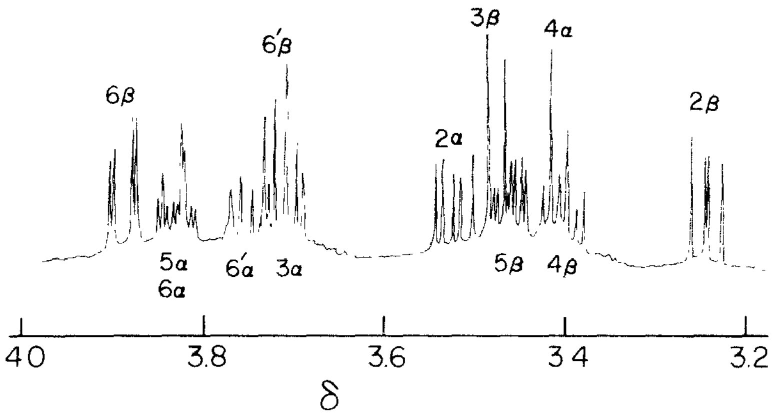

Fig. 1. Nonanomeric portion of the 500 MHz ^{1}H-n.m.r. spectrum of α,β-D-glucose for a deuterium oxide solution at 30°.

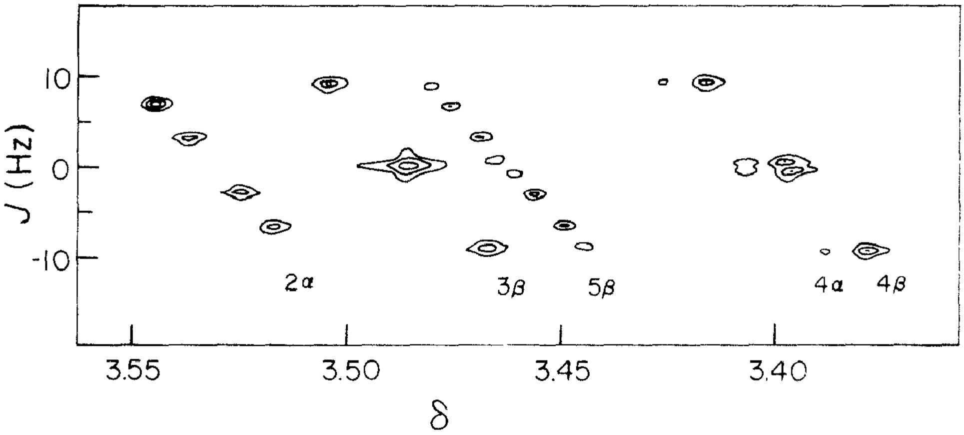

Fig. 2. Portion of the 500-MHz, two-dimensional *J*-resolved ^{1}H-n.m.r. spectrum of α,β-D-glucose at 30°. This spectrum was obtained with a 90°–N*T*–180°–N*T*-acquisition-pulse sequence, where *T* was 10 ms and N was incremented from 0 to 127: Sweep width, 3600; recycle delay, 1 s; number of acquisitions, 24; and size, 8192 data points.

whereas at 270 MHz (not shown) they are buried under a complex 3β–5β–4α–4β envelope. Note that the 5α and 6α region (δ 3.85–3.81) is not first-order, even at 500 MHz.

Two-dimensional *J*-resolved spectroscopy is a technique used to increase the resolution available in an n.m.r. experiment by expanding the spectrum along a second dimension. The *J*-resolved spectrum of the nonanomeric region of α,β-D-glucose was obtained at 500 MHz. Fig. 2 presents a contour plot of the 2α–3β–5β–4α–4β region. Chemical shift and *J*-coupling values are on the abscissa, and *J*-coupling values alone on the ordinate. The individual multiplets are inclined at 45° with respect to each axis. In contrast to the one-dimensional spectrum, the H-5β octet is completely resolved from the H-3β multiplet, and all eight resonances appear.

TABLE I

^{1}H-N.M.R. SPECTRAL PARAMETERS FOR α,β-D-GLUCOSE

Chemical shifts (δ)[a]	*Conformer*	
	α	β
H-1	5.228	4.640
H-2	3.530	3.241
H-3	3.709	3.485
H-4	3.406	3.396
H-5	[b]	3.463
H-6	[b]	3.893
H-6′	3.756	3.717
Coupling constants (Hz)		
$J_{1,2}$	3.8	8.0
$J_{2,3}$	9.9	9.2
$J_{3,4}$	9.6	9.1
$J_{4,5}$	9.6	9.8
$J_{5,6}$	2.2	2.3
$J_{5,6'}$	5.5	5.8
$J_{6,6'}$	12.3	12.3

[a]Relative to the signal of 4,4-dimethyl-4-silapentane-1-sulfonic acid. [b]Not first order.

The H-4α and -4β resonances are completely resolved. Furthermore, increased resolution in the *J*-dimension clearly demonstrates that both H-4α and -4β are doublets of doublets, as expected, but not observed in the one-dimensional spectrum. The chemical shifts and *J*-couplings for α,β-D-glucose are summarized in Table I. These values are similar to those reported previously, but were obtained without selective deuteration[4] or spectral simulation[9]. Our results with the simple sugar α,β-D-glucose demonstrate that the increased resolution afforded at 500 MHz will greatly increase the complexity of carbohydrate-structural problems that can be approached by two-dimensional *J*-spectroscopy.

EXPERIMENTAL

A solution of α-D-glucose (Sigma Chemical Co., St. Louis, MO 63178) in 99.8% deuterium oxide was lyophilized two times, and the residue finally dissolved in 100% deuterium oxide to give a 0.1M concentration. ^{1}H-N.m.r. experiments were performed with a home-built spectrometer operating at a proton-observe frequency of 500 MHz, as previously described[10].

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