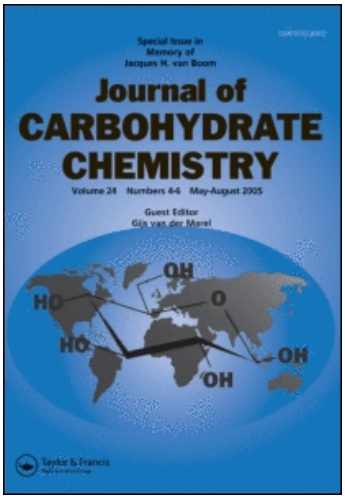

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Pawan K. Agrawal[a]; C. Allen Bush[a]
[a] Department of Chemistry and Biochemistry, The University of Maryland Baltimore County Campus, Baltimore, MD

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COMMUNICATION

^{1}H AND ^{13}C NMR ASSIGNMENTS OF 2,3-DIACETYLAMIDO-2,3-DIDEOXY-D-GLUCOPYRANOSE

Pawan K. Agrawal and C. Allen Bush*

Department of Chemistry and Biochemistry, The University of Maryland
Baltimore County Campus, Baltimore, MD 21228

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Lipid A exhibits the most important biological attributes of lipopolysaccharide (LPS) of gram-negative bacteria including endotoxicity, adjuvanticity and antitumor activity.[1] The lipid A backbone, in general, is found to consist of a pyranosidic β 1,6-linked D-glucosamine disaccharide [β-D-Glc$_p$N-(1$\rightarrow$6)-α-D-Glc$_p$N] phosphorylated at 1 and 4′ positions and bearing two amide bound and two ester linked hydroxy and/or acyloxy fatty acids.[2] However, the lipid A moiety of LPS from various strains of the two gram-negative, photosynthetic bacteria, *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*, possesses 2,3-diamino-2,3-dideoxy-D-glucose as a constituent sugar.[3] This diamino sugar has been also reported to occur in LPS from several other bacterial species.[4,5] Recently we found that the lipid A of *Brucella abortus* contains β(1$\rightarrow$6)-linked 2,3-diamino-2,3-dideoxy-D- glucopyranose disaccharide moiety with a phosphate group at the 4′ position and amide bound acyloxy and hydroxy fatty acids.[6]

The characterization of 2,3-diamino-2,3-dideoxy-D-glucose is usually carried out using mass spectral data[3,4] and CD techniques.[7] To the best of our knowledge, there has been no report determining the ^{1}H and ^{13}C NMR assignments for this biologically significant monosaccharide which occurs in the *N*-acylated form in lipid A. This prompted us to carry out detailed homo- and heteronuclear NMR studies[8] to achieve complete ^{1}H and ^{13}C NMR assignments for both anomers of this monosaccharide. Benzene-d_6+DMSO-d_6 was chosen as the solvent for the present

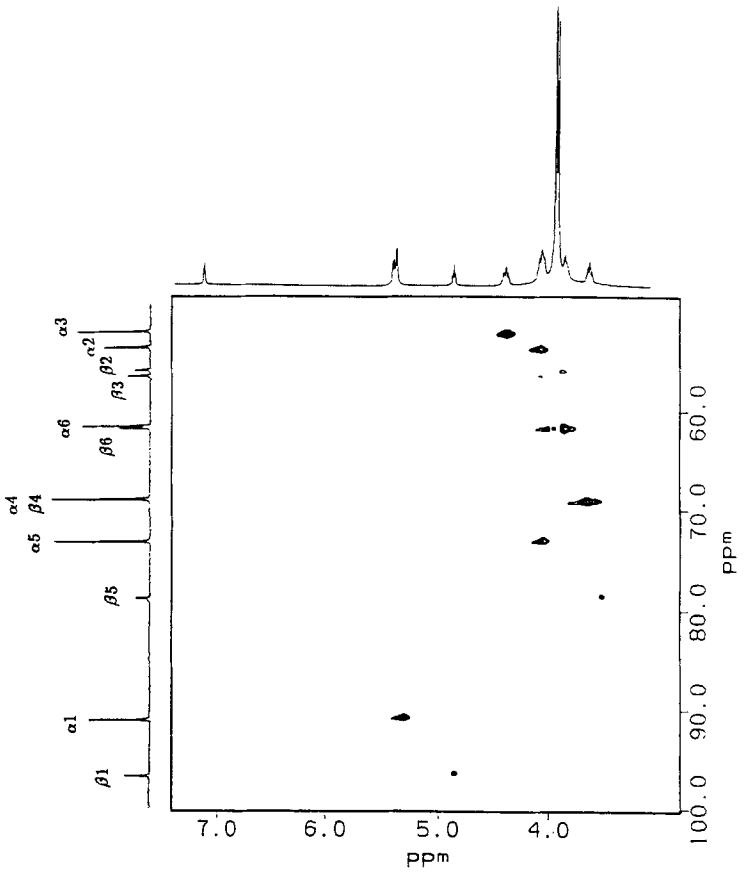

FIG. 1. Phase-sensitive ^{13}C-decoupled, ^{1}H-detected multiple-quantum correlation ($^{1}H[^{13}C]HMQC$) spectrum of 2,3-diacetylamido-2,3-dideoxy–D-glucopyranose.

investigation as this solvent was found to be suitable for the lipid A of *B. abortus*[6] and allowed us to assign exchangeable hydroxyl and amide protons.

The ^{13}C NMR (Fig.1) of 2,3-diacetylamido-2,3-dideoxy-D-glucose,[9] in addition to the two acetylamido methyl groups at 23.82 and 23.42 ppm, displayed two anomeric resonances at 90.63 and 96.78 ppm and four amido bearing carbon resonances at 51.94 and 53.52, and 55.78 and 56.38 ppm, which differed in their relative intensities, thus reflecting the presence of α, β anomers. The resonances at 90.63, 51.94 and 53.52 ppm were intense where as those at 96.78, 55.78 and 56.38 ppm were weak. Consideration of the chemical shifts and one-bond ^{1}H-^{13}C couplings

($^1J_{CH}$) 166.7 and 158.6 Hz for the anomeric carbon signals at 90.63 and 96.98 ppm, respectively, indicated that the former set of the signals correspond to the α-anomer (major anomer) and the latter set of the signals to the β-anomer (minor anomer). The rigorous ^1H and ^{13}C resonance assignments of the individual anomer were achieved by the analysis ^{13}C - ^1H correlation experiment (HMQC) as discussed below.

The ^1H NMR spectrum exhibited anomeric proton resonances at 5.28 (J = 3.22 Hz) and at 4.84 ppm, in addition to the two amide proton resonances at 7.98 and 7.51 ppm and two singlets for acetylamido methyl groups at 2.01 and 1.96 ppm. The identification of the ring protons of the individual anomer was achieved by the analysis of the DQF-COSY spectrum using amide ^1H signals (NHs) as the starting point. For instance, the amide resonance at 7.51 ppm exhibited a crosspeak at 4.05 ppm which was further correlated with the resonances at 5.28 and 4.36 ppm. The assignment of the former resonance to H-1 of the α–anomer was straight forward due to its characteristic chemical shift and its appearance as a doublet (J = 3.22 Hz) in 1D ^1H NMR spectrum. Moreover the H1/H2 crosspeak, in accordance with the above proposed α-anomeric configuration,[10] showed smaller active coupling (J = 3.2 Hz) and large passive coupling (J = 10 Hz). Thus, the resonance at 4.36 ppm can be assigned to H-3 as it exhibits correlation with the amide proton at 7.98 ppm and with H-4 at 3.62 ppm. The signal at 4.06 ppm could then be assigned to H-5 from the crosspeak between H-4 and H-5 at 3.62 ppm which showed crosspeaks with H-6 methylene protons at 3.84 and 3.93 ppm. The measurement of active and passive coupling constants in the H2/H3 and H3/H4 crosspeaks implied a chair conformation of the pyranose ring. The crosspeak connectivities also led to the identification of the hydroxylic protons at 6.94, 5.30 and 4.79 ppm and their assignments to OH-1, OH-4 and OH-6 respectively (Table 1). At a significantly lower contour level, long-range four-bond correlations were observed between the amide resonances at 7.98 (NH-3) and 7.51 (NH-2) with methyl groups at 2.01 and 1.96 ppm, leading to assignment of the acetylamido methyl groups at the 3- and 2- positions respectively.

The crosspeaks corresponding to the less abundant β-anomer were visible at the lower contour level and in cross sections. Starting with amide proton resonances at 7.976 and 7.974 ppm, the ^1H signal assignments up to H-4 for the β-anomer were traced out in a similar manner as discussed for α-anomer. The H-4 at 3.62 ppm,

Table 1. ^{1}H and ^{13}C NMR spectral data for α-and β-2,3- diacetylamido- 2,3-dideoxy-D-glucopyranose

	α			β		
Atom no	^{1}H	^{13}C	$^{1}J_{CH}$ [Hz]	^{1}H	^{13}C	$^{1}J_{CH}$ [Hz]
1	5.28	90.63	166.7	4.84	96.78	158.6
2	4.05	53.52	140.5	3.86	55.78	140.8
3	4.36	51.94	137.4	4.05	56.38	138.7
4	3.62	68.76	142.8	3.62	68.76	142.8
5	4.06	73.01	141.6	3.49	78.50	140.4
6	3.84,3.93	61.53	140.3	3.84,3.93	61.71	140.3
2NHCO	-	171.44	-	-	171.23	-
3NHCO	-	172.44	-	-	171.71	-
$3CH_3$	2.01	23.42	128.8	2.01	23.42	128.8
$2CH_3$	1.96	23.82	126.1	1.96	23.82	126.1
NH2	7.51	-	-	7.976	-	-
NH3	7.98	-	-	7.974	-	-
OH1	6.94	-	-	6.95	-	-
OH4	5.30	-	-	5.30	-	-
OH6	4.79	-	-	4.79	-	-

in addition to the intense cross peaks for H-3 (4.36 ppm) and H-5 (4.06 ppm) of α-anomer, showed cross peaks at 4.05 and 3.49 ppm. Since the resonance at 3.49 ppm exhibited cross peaks with methylene protons at 3.84 and 3.93 ppm, assignment of the resonance at 3.49 ppm to H-5 of the β-anomer is straightforward.

The ^{13}C-^{1}H one-bond correlation through a ^{13}C-decoupled ^{1}H detected heteronuclear multiple-quantum coherence ($^{1}H[^{13}C]$ HMQC) spectrum (Fig. 1) led to assignment of all the ^{13}C resonances. Of particular importance were the correlations of α–H1 at 5.28 ppm with ^{13}C resonances at 90.63 ppm, and β–H1 at 4.84 ppm with

^{13}C resonance 96.78 ppm in keeping with the generalization that the β-anomeric carbon resonance appears at lower field than its α-anomer.[11] These correlations also confirmed the coincidence of the C-4 and H-4 resonances for the two anomers. A comparison of one-bond ^{1}H-^{13}C coupling constants ($^{1}J_{CH}$) for the acylamino carbons, C-2 and C-3, suggests that they can be distinguished by the value of $^{1}J_{CH}$ which is lower by 2-3 Hz for C-3. The lower field carbonyl resonance at 172.44 ppm is tentatively assigned to the acetylamido carbonyl at the 3-position. The assignment of both the acetylamido carbonyl and acetylamido methyl of position-3 to resonances at a deshielded position with respect to analogous resonances of position-2 is in accord with similar observations for acetylated α- and β-glycopyranosides.[12] It can also be mentioned here that the H-1, H-2, H-3 and H-5 absorb at lower field by 0.44, 0.19, 0.31 and 0.57 ppm in the α-anomer relative to the β-anomer whereas C-2, C-3, C-5 and C-6 resonances resonate at 2.26, 4.44, 5.49 and 0.18 ppm lowerfield in the β-anomer relative to the α-anomer. These chemical shift differences may be of significance in establishing anomeric configuration of 2,3-diacetylamido-2,3-dideoxy-D-glucopyranosides.

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