Nitrogen-15 Magnetic Resonance Spectroscopy. Natural-Abundance Nitrogen-15 Spectra of Some 2-Amino-2-deoxy-D-hexose Derivatives[1]

Robert E. Botto and John D. Roberts*

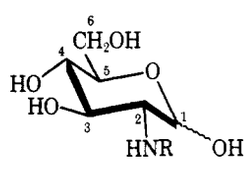
Contribution No. 5438 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California 91125

Received September 28, 1976

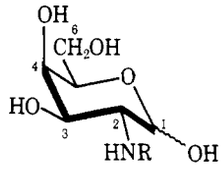
The nitrogen-15 nuclear magnetic resonance spectra of some 2-amino-2-deoxy-D-hexose hydrochlorides and 2-acetamido-2-deoxy-D-hexoses and *N*-benzoyl-D-glucosamine are reported. The differences in chemical shifts for the various hexopyranose structures are discussed in terms of steric, stereoelectronic, and hydrogen-bonding effects. These model compounds were used to study substituent effects related to amino sugar containing polysaccharides and antibiotics.

In recent years, the number of amino sugars which have been isolated from natural products has increased tremendously. In fact, the number of amino sugars characterized in living organisms is greater than that of all other sugars. This rapid development of knowledge in the field of amino sugar containing substances has inspired a rather comprehensive review[2] which surveys their chemistry and biological functions.

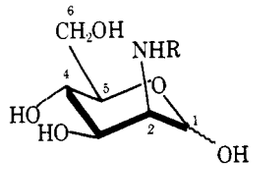
We are interested in the application of nitrogen-15 NMR spectroscopy for structural analysis of nitrogenous sugars, particularly polysaccharides and oligosaccharides such as heparin, and the neomycin-related antibiotics which contain deoxystreptamine and various other amino sugars. Recent improvements in the sensitivity of NMR instrumentation provide an opportunity to investigate these substances at their natural-abundance ^{15}N levels, and, with this end in mind, we have undertaken a study of a number of simple amino sugars. In particular, the chemical shifts and resonance-line intensities have been obtained for the pyranose anomers present at equilibrium for 25% aqueous solutions for the 2-amino-2-deoxy derivatives of D-glucose (GA·HCl), D-galactose (GalA·HCl), and D-mannose (MA·HCl), and for the respective 2-acetamido-2-deoxy derivatives (NAG, NAGal, NAM). The spectrum of *N*-benzoyl-α-D-glucosamine (NBG) in dimethyl sulfoxide (Me_2SO) has also been determined. Published data concerning the equilibrium composition of the aminohexopyranose anomers[3,4] have been correlated with the peak intensities in our spectra to corroborate the assignments.



R = H, GA·HCl
R = $COCH_3$, NAG
R = COPh, NBG



R = H, GalA·HCl
R = $COCH_3$, NAGal



R = H, MA·HCl
R = $COCH_3$, NAM

The nitrogen-15 chemical shifts of the α- and β-aminohexoses are shown in Table I. In general, the ^{15}N signal intensities allow estimates of the anomer concentrations at equilibrium, which are in qualitative agreement with ratios previously determined by proton nuclear magnetic resonance (^{1}H NMR)[3]. The ^{15}N spectra of the 2-acetamido-2-deoxy-D-hexoses have not indicated the presence of additional configurational isomers as a consequence of restricted rotation about the N–CO bond. Analogous studies by Bundle and coworkers[5] have shown that the ^{13}C chemical shifts of the methyl and carbonyl groups of the 2-acetamido function in 2-deoxy-D-hexoses are sensitive to the anomeric configuration and have established that the relative ^{13}C signal intensities allow estimation of the anomer ratios at equilibrium.

The influence of axial substituents on ^{13}C shifts in various hexopyranose systems has received considerable attention in recent years.[5-7] Much less is known concerning the effects of substituents on the ^{15}N chemical shifts in nitrogen-containing hexopyranoses.[8] Lichter and Roberts[9] have established that shifts of simple, substituted amines are subject to the same kinds of steric and electronic influences as ^{13}C shifts. They demonstrated that γ_N substituent effects are upfield shifts which increase with increasing substituent electronegativity and can be attributed to stereoelectronic perturbations of the ^{15}N shielding. Similar arguments have been used previously to account for ^{13}C shieldings in substituted norbornanes.[10] However, there are other factors which can influence ^{15}N chemical shifts. For example, shift effects arising from inter- or intramolecular hydrogen bonding are well established.[11,12] Molecular association in neat liquids or in solvents where hydrogen bonding is expected to be extensive can result in either upfield or downfield shifts, depending on the type of nitrogen and the type of interactions involved.[13] Shift effects induced via stereoelectronic perturbations and/or those emanating from hydrogen bonding may contribute to the appreciable differences between the nitrogens of the α and β forms of NAM and MA·HCl, 6.7 and 7.4 ppm, respectively. Similar observations have been made in the ^{15}N spectra of *cis*- and *trans*-1,2-diaminocyclobutane;[9] however, both the electronegativity and the spatial arrangement of the adjacent amino group attached to the cyclobutane ring differ from those of the analogous hydroxyl group in the pyranose systems. In the β anomers, the anomeric hydroxyl and axial 2-amino function are gauche, and a considerable portion of the upfield shift observed for the ammonium (or acetamido) nitrogen relative to its shielding in the corresponding α isomer can be attributed to the γ effect associated with the gauche form. Thus, although the difference between the two aminomannose anomers will reflect a composite of steric, stereoelectronic, and hydrogen-bonding effects, the simplest interpretation is a predominant steric effect in the β anomer. A similar trend is found for aminopyranoses containing equatorial 2-amino functions, but $\Delta\delta$ between the anomers is much less pronounced. The β anomers of aminoglucose and aminogalactose derivatives are shielded by 0.7–1.6 ppm. The 1-OH and equatorial 2-ammonium (acetamido) groups are gauche in both the α and β forms. Consequently, it does not seem likely that an increase in the steric crowding of γ sub-

Table I. ^{15}N Chemical Shifts of Aminohexopyranoses in Water[a]

Sugar	δ_α (intensity)[b,e]	δ_β (intensity)[f]	$\Delta\delta$, ppm	Anomer ratio[c] α	β
GA·HCl	340.3 (61)	341.9 (39)	1.6	63	37
NAG	252.4 (69)	253.1 (31)	0.7	68	32
GalA·HCl	341.6 (55)	343.0 (45)	1.4	47	53
NAGal	252.6 (56)[d]	253.4 (44)	0.8	65	35
MA·HCl	344.2 (40)	351.6 (60)	7.4	43	57
NAM	255.6 (60)	262.3 (40)	6.7	57	43
NBG	260.8 (90)	261.2 (10)	0.4		

[a] In parts per million upfield from external nitric acid. [b] Relative peak height. [c] Determined by ^1H NMR. Data from ref 3. [d] If the integrated peak area is used, a ratio of 63:37 is obtained. [e] Registry no. are, respectively, 14131-62-5, 10036-64-3, 14131-59-0, 14215-68-0, 14131-65-8, 14131-64-7, 61949-16-4. [f] Registry no. are, respectively, 14131-63-6, 14131-68-1, 14257-79-5, 14131-60-3, 14131-67-0, 7772-94-3, 6847-14-9.

Table II. ^{15}N Shielding Differences (ppm) between Epimeric 2-Aminohexoses Derivatives[a]

α-GalA·HCl–α-GA·HCl	+1.3
β-GalA·HCl–β-GA·HCl	+1.1
α-NAGal–α-NAG	+0.2
β-NAGal–β-NAG	+0.3
α-MA·HCl–α-GA·HCl	+3.9
β-MA·HCl–β-GA·HCl	+9.7
α-NAM–α-NAG	+3.2
β-NAM–β-NAG	+9.2

[a] Obtained from data in Table I; positive values correspond to upfield shift differences in parts per million.

stituents in β relative to α anomers is responsible for these shielding effects. Moreover, a number of different modes of hydrogen bonding are possible and the stereoelectronic effects that influence ^{15}N shifts of aminohexopyranoses are complicated and not well understood.

Table II illustrates the effect which results from the introduction of an axial substituent to the 2-amino-2-deoxy-D-hexose derivatives, using GA·HCl and NAG epimers as reference compounds. Shielding effects of +1.3 and +1.1 ppm are observed in the comparison with the analogous anomers of GalA·HCl. We assume that the orientation of the hydroxyl group is responsible for these upfield shifts, because the C-2 ammonium group remains unchanged with respect to the remaining substituents on the oxane ring, at least as a first approximation. However, there may be slight differences in bond angles in the galactosamine derivatives as a consequence of ring flattening to relieve 1,3-nonbonded interactions with the axial C-4 hydroxyl group. The shielding effects associated with change in stereochemistry of a C-4 hydroxyl group (δ substituent), although opposite in direction, are comparable in magnitude to those involving γ substituents, as can be seen from the shifts of the anomeric aminoglucose and aminogalactose derivatives in Table I. This is a large effect for a substituent far removed from the nitrogen in question. The orientation in space of the 4-OH group should not affect the steric environment of the equatorial C-2 ammonium function directly, and thus should have very little influence on its chemical shift. Furthermore, hydrogen bonding from the ammonium group in these 2-deoxyhexopyranoses to O-4 is sterically impossible.[14] These shielding differences may reflect some distant stereoelectronic perturbation of the ^{15}N chemical

Table III. Nitrogen-15 Shieldings of the 4-*tert*-Butylcyclohexylamines and Their Derivatives[a]

Derivative (solvent)	Trans[d]	Cis[e]	$\Delta\delta$[b]
$-NH_2$[c] (cyclohexane)	334.6	343.4	8.8
-NHAc (chloroform)	240.5	248.8	8.3
$-NH_3^+Cl^-$ [c] (CH_3OH)	329.5	335.4	5.9

[a] In parts per million from external nitric acid. [b] $\Delta\delta = \delta_N^{cis} - \delta_N^{trans}$. [c] Unpublished research by Dr. R. Duthaler. [d] Registry no. are, respectively, 2163-34-0, 2163-33-9, 31023-36-6. [e] Registry no. are, respectively, 31023-35-5, 54572-02-0, 61886-14-4.

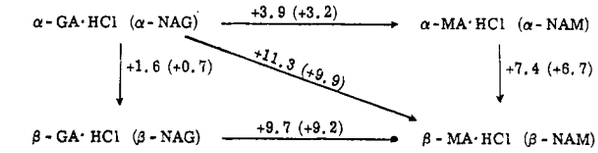


Figure 1. Nitrogen-15 chemical shift (ppm) relationships between aminoglucose and aminomannose derivatives.

shift, and it is conceivable that the spatial configuration between the hydroxyl substituent, C-4 and C-2, has an important bearing on the mechanism of these shift changes. The ^{19}F chemical-shift data for 4-substituted 1,1-difluorocyclohexanes[15] and 4-substituted 1-fluorobicyclo[2.2.2]octanes[16] have similar downfield shifts of the ^{19}F shieldings when polar substituents are oriented in a W arrangement. When the ^{15}N shifts of α- and β-NAGal are compared with those of analogous anomers of NAG, considerably smaller $\Delta\delta$ values are derived. If such shift effects are inductive effects of the electronegative C-4 hydroxyl group, then these smaller differences may, in part, reflect a change in polarization of the C-2–N bond for the change in substituent $-NH_3^+Cl^- \rightarrow -NHCOCH_3$.

Considerably larger shielding differences are observed between derivatives of mannosamine and glucosamine. To evaluate ^{15}N shielding differences for axial and equatorial C-2 amino groups for amino sugars in the absence of any additional perturbations caused by the changing relationships of γ-OH groups, experimental shifts of the β-mannosamine derivatives can be compared with expected shifts derived from the anomeric aminoglucose derivatives. Axial–equatorial differences of +12.9 and +10.6 ppm were obtained for the amino sugar hydrochlorides and their acetyl derivatives.[17] Analogous shift comparisons are reported in Table III for the amine nitrogen in *cis*- and *trans*-4-*tert*-butylcyclohexylamines as well as the hydrochloride salts and *N*-acetates, for which values are +8.8, +5.9, and +8.3, respectively. In every case, the axial nitrogen absorbs at higher field. For the cyclohexyl series, nonbonded interactions of the axial hydrogens at C-3 and C-5 are sufficient to perturb the electron distribution about the axial nitrogen nucleus such that its shielding is increased. The larger shielding differences between epimeric aminohexopyranose structures are not likely to arise solely from an interaction of a single axial hydrogen, and consequently, it seems likely that a substantial part of this effect can be ascribed to the gauche interaction of the ring oxygen in the axial compounds.

In Figure 1, data from Tables I and II are combined to illustrate the various chemical-shift relationships among anomeric aminomannose and aminoglucose derivatives. Much larger $\Delta\delta$ values are observed between β-mannosamine and β-glucosamine derivatives—+9.7 (+9.2) ppm compared with +3.9 (+3.2) ppm for the α derivatives. As noted previously, $\Delta\delta$ between anomers of either 2-deoxymannose derivative illustrates the pronounced γ-shielding effect associated with

Table IV. ^{13}C Chemical Shifts of NBG in Me_2SO[a]

Anomer (rel signal inten)	C_1	C_2	C_3	C_4	C_5	C_6	C=O
α (90)	91.3	46.2	71.9	71.0	72.9	62.1	167.4
β (10)	96.3	58.6	75.0	[b]	77.7	62.1	167.4

[a] In parts per million from external Me_4Si. [b] This signal must coincide with another reported.

the gauche form. The relief of this gauche interaction accounts for the smaller $\Delta\delta$ values between the α-aminomannoses and their epimeric gluco brethren. That is, the shielding effect associated with the change in the bonding relationship of the C-2 amino group with respect to the ring is partially cancelled by a concomitant (but opposing) effect resulting from the relief of the gauche interaction with the 1-OH substituent.

To observe both anomers of NBG in dimethyl sulfoxide solution, the original sample was maintained at room temperature for a period of 2 months, at which time the α-D anomer was preponderant. The ^{13}C NMR spectrum confirmed the ^{15}N NMR results which indicated that both anomers were present in an α:β ratio of 90:10. The ^{15}N chemical-shift difference between anomers of NBG is 0.4 ppm, compared with values of 1.6 and 0.7 ppm, respectively, for anomers of GA·HCl and NAG; thus $\Delta\delta_{-NH_3^+} > \Delta\delta_{-NHAc} > \Delta\delta_{-NHCOPh}$.

The shielding differences in these 2-amino-2-deoxy-D-hexoses could well be a function of the orientation of the nitrogen about the N–C-2 bond and, because the shielding differences are often larger than 1 ppm, they could be a source of stereochemical information.

Experimental Section

All the 2-amino-2-deoxy-D-hexose derivatives were purchased from Sigma Chemical Co., Inc., and were used without further purification. The ^{15}N NMR spectra were recorded in 25-mm sample tubes at temperatures of ca. 30–40 °C on a Bruker WH-180 spectrometer equipped with a Nicolet B-NC 12 computer with 24K memory (16K for spectrum accumulation), operating at 18.25 MHz in a pulsed Fourier transform mode with complete broad-band, proton noise decoupling. The computer allowed acquisition of 8192 data points for a spectrum having a sweep width of 10 000 Hz. A typical experiment required 1–2 h of data acquisition, using a pulse width of 20 μs (20° flip angle) at pulse intervals of 2.0 s. Chemical shifts are reported in parts per million (ppm) upfield from external nitric acid (1 M 98% ^{15}N-enriched nitric acid in deuterium oxide) capillary in which deuterium oxide was used to produce the field lock signal. Spectra of 25% solutions of the 2-acetamido-2-deoxy-D-hexoses and the 2-amino-2-deoxy-D-hexose hydrochlorides in water were recorded after reaching their mutarotational equilibria. A 20% solution of *N*-benzoyl-2-amino-2-deoxy-α-D-glucose in dimethyl sulfoxide mutarotated to ca. 10% of the β anomer after standing at room temperature for 2 months.

The ^{13}C NMR spectrum of NBG was recorded for the same sample used for the ^{15}N spectrum with the Bruker WH-180 spectrometer operating at 45.28 MHz. The chemical shifts of the skeletal carbons of the pyranose ring and the carbonyl carbon in both anomers are reported in Table IV.

References and Notes

(1) Supported by the Public Health Service, Research Grant GM-11072, from the Division of General Medical Sciences, and by the National Science Foundation.
(2) R. W. Jeanloz and E. A. Balazs, "The Amino Sugars", Vol. IA, IB, IIA, and IIB, Academic Press, New York, N.Y., 1969.
(3) D. Horton, J. S. Jewell, and K. D. Philips, *J. Org. Chem.*, **31**, 4022 (1966).
(4) D. Horton, J. S. Jewell, and K. D. Philips, *J. Org. Chem.*, **31**, 3843 (1966).
(5) D. R. Bundle, H. J. Jennings, and I. C. Smith, *Can. J. Chem.*, **51**, 3812 (1973).
(6) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
(7) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).
(8) B. Coxon, *Carbohydr. Res.*, 35 (1974).
(9) R. L. Lichter and J. D. Roberts, *J. Am. Chem. Soc.*, **94**, 2495 (1972).
(10) M. Christl, H. J. Reich, and J. D. Roberts, *J. Am. Chem. Soc.*, **93**, 3463 (1971).
(11) W. M. Litchmann, M. Alei, Jr., and A. E. Florin, *J. Am. Chem. Soc.*, **91**, 6574 (1969).
(12) M. Alei, Jr., A. E. Florin, and W. M. Litchmann, *J. Am. Chem. Soc.*, **92**, 4828 (1970).
(13) Two fundamental types of hydrogen bonding are possible, >N:···H–X and >N–H···:X. Such interactions are known to influence nitrogen resonance-line positions in opposite directions: R. O. Duthaler and J. D. Roberts, unpublished results.
(14) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis", Interscience, New York, N.Y., 1965, p 421.
(15) K. Grohmann and J. D. Roberts, unpublished results.
(16) G. L. Anderson and L. M. Stock, *J. Am. Chem. Soc.*, **90**, 212 (1968).
(17) In derivatives of β-mannosamine, the axial amino function has a cis-bonding relationship to both the C-1 and C-3 hydroxyl groups; therefore, a shift value must be estimated for a system in which an equatorial amino function retains the same bonding relationships to these hydroxyl groups. In the latter case, the C-1 and C-3 hydroxyl groups have an axial-bonding relationship with respect to the pyranose ring. A value of −1.6 (−0.7) ppm can be derived from the structural change 1-OH_e → 1-OH_a from the chemical shifts of the α and β forms of 2-amino-2-deoxyglucose. If an analogous change in the bonding of the 3-OH results in a similar shift difference, an additional factor of −1.6 (−0.7) ppm should be introduced into the shielding values of the α-aminoglucose derivatives. Thus, $\Delta\delta = \delta_{\beta\text{-MA·HCl}} - (\delta_{\alpha\text{-GA·HCl}} - 1.6) = +12.9$ ppm; $\Delta\delta = \delta_{\beta\text{-NAM}} - (\delta_{\alpha\text{-NAG}} - 0.7) = +10.6$ ppm.

^{15}N Nuclear Magnetic Resonance Spectroscopy. Natural-Abundance ^{15}N Nuclear Magnetic Resonance Spectra of Enamines[1]

Philip W. Westerman and John D. Roberts*

Contribution No. 5471 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California 91125

Received November 30, 1976

The ^{15}N chemical shifts of 18 cyclic enamines have been determined at the natural-abundance level of ^{15}N using the Fourier transform technique. The shifts depend on the size of both the cycloalkene and nitrogen-containing rings. Methyl substituents on the cycloalkene ring also influence the chemical shifts of enamines. Tertiary amines formed on hydrogenation of cyclic enamines are found to have ^{15}N chemical shifts 3.9–19.7 ppm upfield of the shift for the corresponding unsaturated compound. A carbonyl group in conjugation with an enamine group results in a large downfield shift of approximately 30–40 ppm for the nitrogen resonance of the enamine.

The nitrogen lone-pair electrons of an enamine can interact with the π electrons of the enamine double bond, enhancing the electron density of the β-alkenic carbon and making this position available for introduction of a substituent by a wide variety of electrophilic reagents.[2] The reactivities of enamines formed from cyclic ketones depend on the ring size and substitution pattern of both the ketone and amine parts.[3-7]