Dependence of ^{13}C Chemical Shifts on Glycosidic Torsional Angles in Ribonucleic Acids

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Ribonucleic acids (RNAs) contain structurally diverse regions such as loops, bulges, and other less structured regions in addition to the canonical A-form duplex regions. These nonhelical structural regions play an important role in cellular processes, such as RNA catalysis.[1] However, these regions are also the most difficult to define structurally by standard NMR methods due to difficulties both in assignment of resonances and in detection of sufficient numbers of NOEs. Recently, the ability to uniformly $^{13}C/^{15}N$ label RNAs has helped to solve the assignment problem.[2] Double- and triple-resonance NMR experiments have facilitated an improvement in the resolution of the ribose region and have allowed unambiguous connection of base and ribose resonances. Connectivities can now be established both within the same residue and between residues using backbone directed assignment strategies that are relatively independent of secondary structure.[3] It now appears that the availability of labeled RNAs may provide additional structural constraints, not through additional NOEs but through systematic variation in heteronuclear chemical shifts. We present here an analysis that seems to correlate ^{13}C chemical shifts of purine bases with glycosidic torsion angles. These correlations could have applications in the qualitative assessment of RNA structural features in the future.

Interpretations of ^{13}C chemical shifts in RNAs have been based largely on the comparison of the effect of ring currents in stacked versus non-stacked regions of these molecules.[4] However, it is clear that these effects alone cannot completely explain the chemical shift variations in RNAs. Shifts for C(8) carbons of adenines and guanines found in loop or bulge regions of stem–loop structures can be quite anomalous. For example the C(8) carbon resonance of the guanine in the UNCG tetraloop, known to be in a syn conformation about its glycosidic bond, is found shifted 4 ppm downfield of other guanine C(8)'s.[5] We also note significant downfield shifts of resonances belonging to C(8) of guanines G_9 and G_{10} found in the loop region of the RNA I stem–loop (see supplementary material). The RNA I stem-loop is derived from RNA I, an antisense repressor molecule which functions together with RNA II and the ROM protein in the control of the replication of the Col E1 plasmid;[6,7] a structural characterization of RNA I has become a target of our own work. While a structure analysis of the RNA I stem–loop is not yet complete, it is significant that the shifts of C(8) carbons of G_9 and G_{10} are about 4 ppm downfield of guanines known to be in the stem region. Less than half these shifts can be explained on the basis of the loss of ring current effects seen in fully stacked and base-paired regions of nucleic acid structures.[4] We believe that these effects could be caused at least in part by local perturbations of the electronic structure that are correlated with glycosidic torsion angles. Similar effects have been shown to occur in several systems including aliphatic and alicyclic hydrocarbons,[8,9] amides,[10] and model peptides.[11]

To characterize the effect of changing the torsional angle about the glycosidic bond on the ^{13}C chemical shift of C(8) of a guanosyl (or adenyl) group (Figure 1a), chemical shift calculations were performed at the *ab initio* level on a model compound (Figure 1b). This simple model was adopted because the available computational resources were not sufficient to perform similar calculations on the entire guanosyl group. However, these calculations provide an indication that useful correlation of electronic effects with glycosidic torsional angle could be obtained.

The structure shown in Figure 1b was optimized at the HF/3-21G[12-14] level subject only to constraints on the torsional angle defined by atoms C(2), N(1), C(6), and O(7). This angle was constrained to have values between 0° and 360° in steps of 30°. No attempt was made to locate local energy minima or transition states accurately because of uncertainties in energies at this level of calculation. It has been shown, however, that due to cancelation of errors, a small split-valence basis set like the ones frequently used leads to geometries comparable to those obtained using much more sophisticated levels of theory like MP2/6-31G*.[15] The results are considerably better than those obtained using HF/6-31*G. These optimized geometries therefore provide a useful basis for chemical shift calculations. Each geometry optimization required 8–16 h of CPU time on a SGI Indigo R4000 workstation. The apparent minima found at torsion angles of 0° (energy = –452.1041 au) and 180° (energy = –452.1034 au) roughly correspond to the anti and syn conformations.

Chemical shift calculations were performed on each of the 13 optimized structures using the *ab initio* IGLO formalism of Kutzellnigg and Schindler[16,17] at the double-ζ level using a (7s3p/3s) Huzinaga Gaussian lobe basis set[18] in the contraction (4111;21/21). Previous calculations have shown that small basis sets such as the one used are usually sufficient for studying conformational effects on ^{13}C chemical shifts.[8-11] Each chemical shift calculation required 4–7 CPU h on a SUN SPARCstation 4.

The chemical shifts seem to show significant variation with the glycosidic torsional angle as depicted in Figure 2. The most pronounced changes occur in the range ±60° to either side of 0°. Near 0° the predicted resonance positions are markedly upfield, and a downfield shift of approximately 10 ppm occurs when the glycosidic torsional angle changes by ±60°. There is a smaller upfield shift as the torsional angle approaches 180°. Thus the apparent difference between minima near syn and anti confor-

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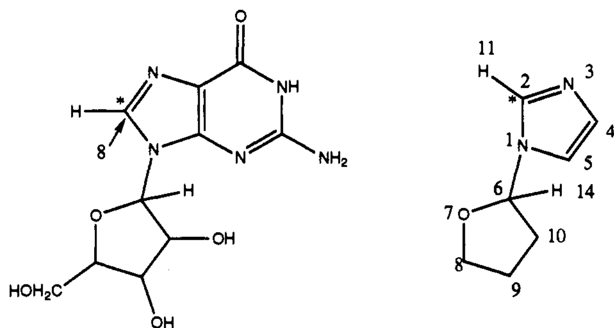

Figure 1. (a, left) Guanosine. (b, right) Model compound. The asterisk (*) indicates the atom under observation. It should be noted that the guanosyl C(8) corresponds to C(2) on the model compound. The H atoms on all the carbons except those connected to C(2) and C(6) of the model compound have been omitted for clarity, and only the analogous H atoms on the guanosyl group are shown.

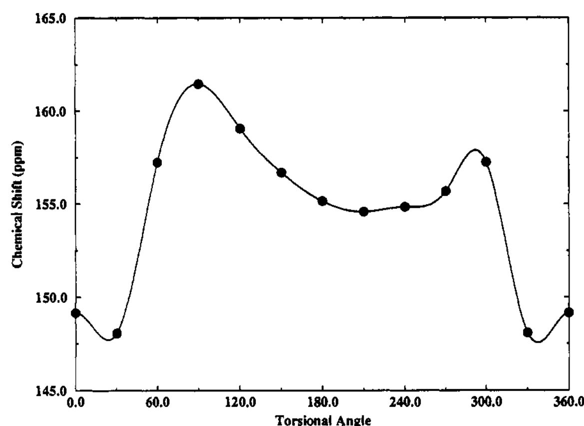

Figure 2. Variation of chemical shift of C(2) (referenced to methane) of the model compound with the glycosidic torsional angle. Splines through the data were constructed using Xmgr Version 2.07, P. J. Turner (1992).

mations seems to be about 6 ppm. Of course the absolute chemical shifts calculated for C(2) in the model compound are quite different from those actually observed in the guanine C(8)'s. Variations in the chemical shift should also be regarded as approximate. These differences may be due to inadequacies in the model, the small size of the basis set, or the fact that the calculations were performed on the isolated model compound without considering the effects of hydrogen bonding, ring currents, solvent, and remote groups. In principle these effects should be taken into account to refine the present approach. In some cases useful approaches for doing this have been presented.[4,19-20] These effects, though small, would play a significant role in the ranges where there is a sharp dependence of chemical shift on the glycosidic torsional angle. It is noteworthy, however, that, even with this preliminary calculation, the direction and magnitude of change are parallel to those observed in systems which have the guanosyl moiety in known syn and anti conformations about the glycosidic bond.[5]

In order to better understand the origin of the conformational effects, contributions of the molecular orbitals localized on particular bonds (LMOs) were also examined. The results are displayed in Table 1. The largest single contribution to the shielding seems to come from the inner shell 1s orbital of C(2) (200.8 ppm, not in Table 1). However, this is almost independent of the conformation about the glycosidic bond, in line with previously reported results.[11] The largest conformationally dependent contributions come from the N(3)–C(2) bond and the N(1)–C(2) bond. Large variations in these contributions are seen on going from a torsional angle of 0° to 90° (or from 270° to 360°), this is almost 7 ppm in the former and 2.5 ppm in the latter. This variation may be understood from the fact that when the torsional angle is 0°, there is an increased interaction between the p orbitals on C(2) which are perpendicular to the plane of the ring and those on O(7). This leads to an alteration in the utilization of the C(2) p orbitals for bonds with N(3) and N(1) resulting in a decrease in the paramagnetic contribution from the LMOs centered on these bonds to the chemical shielding. Significant variation is also seen in the contribution from the H(11)–C(2) (~1.6 ppm) bond. Since the last three contributions are paramagnetic (negative), an upfield shift near 0° and 360° is realized. Small but substantial paramagnetic contributions come from the N(3) lone pair, the N(1)–C(6) bond, and the N(3)–C(4) bond. The other LMOs contribute less than 4% toward the shielding of C(2).

Table 1. Major Contributions to the Chemical Shielding of C(2)[a]

dihedral angle	N(1)–C(2)	N(3)–C(4)	N(1)–C(6)	N(3)–C(2)	N(3)[b]	H(11)–C(2)
0	–41.60	–1.43	–2.29	–62.67	–3.77	–24.84
30	–41.35	–1.32	–2.29	–62.74	–3.88	–24.02
60	–43.43	–1.27	–2.33	–67.61	–4.13	–25.54
90	–44.25	–1.24	–2.27	–69.25	–4.27	–26.58
120	–43.66	–1.18	–2.38	–68.62	–4.23	–25.76
150	–43.87	–1.14	–2.53	–67.01	–4.19	–24.95
180	–42.51	–1.20	–2.15	–67.38	–4.17	–24.63
210	–41.89	–1.29	–2.31	–67.26	–3.98	–24.92
240	–42.26	–1.30	–2.54	–67.00	–3.88	–25.15
270	–42.23	–1.31	–2.36	–67.80	–3.96	–25.23
300	–43.42	–1.27	–2.33	–67.61	–4.13	–25.55
330	–41.36	–1.32	–2.29	–62.74	–3.88	–24.02
360	–41.60	–1.43	–2.29	–62.67	–3.77	–24.84

[a] Shielding referenced to bare nucleus. [b] MO localized on the N(3) lone pair.

In conclusion, it may be said that the changes in the electronic environment accompanying changes in the torsional angle about the glycosidic bond have a profound effect on the guanine C(8) chemical shift. This could be a possible contribution to the anomalous chemical shifts observed in C(8) of the guanine in the UNCG tetraloop[5] and in G_9 and G_{10} loop residues of RNA I under study in our laboratory. Similar calculations and analyses would be valid for DNA molecules as well.

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Supplementary Material Available: An expanded plot of a ^{13}C HSQC spectrum of the aromatic region of RNA I and optimized geometries and energies of the model compound and numerical values for the chemical shifts at various torsional angles (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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