with oxygen throughout the entire irradiation period as was done in a similar experiment with acenaphthylene.[6] As a result, some triplet dimerization leading to the trans product may have occurred.

(17) For the purposes of discussion, Φ_{isc} is assumed to be 1.0. The linearity of the plot in Figure 4 indicates that Φ_{isc} remains relatively constant throughout the concentration range of ethyl iodide used. If Φ_{isc} is less than unity, a systematic error in our calculated rate constants will result, but the conclusions will not be affected.

(18) For a diffusion-controlled reaction, $k_q = 8RT/3000\eta$. The viscosity of cyclohexane was used in the calculation of this rate constant.

(19) This result is not unreasonable since Φ_{ST} for both the substituted and unsubstituted compounds should asymptotically approach unity at high concentrations of heavy-atom solvent. For acenaphthylene in 100 mol % ethyl iodide, $\Phi_{ST} = 0.81$.

(20) R. P. DeToma and D. O. Cowan, *J. Am. Chem. Soc.*, **97**, 3283, 3291 (1975).

(21) The triplet dimerization efficiency for 5,6-dichloroacenaphthylene in cyclohexane is 0.078 (see Table IV). A value of 0.21[2h] was obtained for acenaphthylene under the same conditions.

(22) The quantum yield of dimerization of 5,6-dichloroacenaphthylene in air-saturated cyclohexane is 0.003 as compared to a value of 0.013 in degassed cyclohexane.

(23) The quantum yield of intersystem crossing, Φ_{isc}, is equal to the quantum yield of dimerization from the triplet state divided by the triplet dimerization efficiency, as given in eq 18, where the terms in the denominator have the same meaning as previously given. The triplet dimerization efficiency is given in Table IV. The value of $\Phi_D{}^t$ was assumed to be 0.010, the difference between Φ_D in degassed cyclohexane and air saturated cyclohexane.

$$\Phi_{isc} = \frac{\Phi_D{}^t}{k_2[A]/(k_2[A] + k_{cq}[A] + k'_{isc}[HA] + k_d + k_q[Q])} \quad (18)$$

(24) (a) This same type of behavior was observed by Plummer for the cycloaddition of 5-bromoacenaphthylene to cyclopentadiene; see ref 5. (b) We have observed similar behavior in a preliminary study of 5,6-dibromoacenaphthylene. Quantum yields of 0.033, 0.011, and 0.0086 in cyclohexane, 10 mol % ethyl iodide–90 mol % cyclohexane, and ethyl iodide, respectively, were obtained indicating that $S_0 \leftarrow T_1$ is more sensitive than $T_1 \leftarrow S_1$ to internal heavy-atom perturbation when bromine is used.

(25) M. M. Dashevskii and G. P. Petrenko, *Ukr. Khim. Zh.* **21**, 370 (1955).

(26) (a) E. A. Braude, A. G. Brook, and R. P. Linstead, *J. Chem. Soc.*, 3569 (1954). (b) The reaction conditions for the dehydrogenation of acenaphthene as given by Braude were not optimized. The resulting reaction mixture contained both acenaphthylene and starting material which are very difficult to separate. We have found that by increasing the amount of quinone used to 1.5 equiv and by increasing the reaction time to 26 h, all of the starting material is consumed, thus eliminating this separation problem.

(27) G. P. Petrenko and E. N. Tellnyuk, *Zh. Org. Khim.*, **2**, 722 (1966).

(28) Cyclohexane was used as the cosolvent.

(29) A referee has commented that dimerization probably occurs through a stepwise reaction involving a dimeric triplet intermediate, $^3A_2$. Our data neither support nor disprove the existence of such an intermediate. If one replaces steps 5 and 6 of the mechanism in Chart II with the following,

$$^3A + A \xrightarrow{k_1} {}^3A_2,\ {}^3A_2 \xrightarrow{k_1'} A_2, \text{ and } {}^3A_2 \xrightarrow{k_A} 2A$$

one obtains a mechanism which is kinetically indistinguishable from the one presented here. In the triplet intermediate mechanism, $k_1 = k_2 + k_{cq}$ while k'_{isc} and k_d remain unchanged. Hence, this mechanistic choice does not alter the conclusions regarding the effects of heavy-atom perturbation.

Effect of ^{13}C–^{14}N Dipolar Interactions on Spin–Lattice Relaxation Times and Intensities of Nonprotonated Carbon Resonances

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Abstract: Spin-lattice relaxation times (T_1) and integrated intensities of the resonances in proton-decoupled natural-abundance ^{13}C Fourier transform NMR spectra of adenosine 5′-monophosphate and guanosine 5′-monophosphate (in H_2O and D_2O, at 40–44 °C, at 15.18 MHz, in 20-mm sample tubes) are compared with calculated values that take into account ^{13}C–^1H and ^{13}C–^{14}N dipolar relaxation. In each case, T_1 values of methine carbons of the base were used to obtain a rotational correlation time, which was then used, together with interatomic distances from crystallographic data, to compute T_1 values of nonprotonated carbons. Nonprotonated carbons which are directly bonded to nitrogens and which have no hydrogens two bonds removed yield theoretical T_1 values strongly affected by ^{13}C–^{14}N dipolar interactions. For carbons in this category, calculated T_1 values which include ^{13}C–^{14}N dipolar interactions are in much better agreement with experimental values than calculated values which consider only ^{13}C–^1H interactions. Integrated intensities were calculated by considering variations in the nuclear Overhauser enhancement that result from differences in relative contributions to $1/T_1$ from ^{13}C–^1H and ^{13}C–^{14}N dipolar relaxation. The calculated intensities are in excellent agreement with the experimental ones.

Measurements of ^{13}C spin-lattice relaxation times (T_1) of protonated carbons have yielded detailed information about overall rotation and internal motions of large molecules in solution.[1-4] The interpretation of T_1 values of protonated carbons of large molecules is relatively simple, because the relaxation of such carbons is overwhelmingly dominated by ^{13}C–^1H dipole-dipole interactions with the directly bonded hydrogens.[2] The contribution of dipole-dipole relaxation to $1/T_1$ is proportional to the inverse of the sixth power of the distance (r) between the nuclei involved. Values of r^{-6} are about 60 times greater for directly bonded C–H groups ($r \approx 1.09$ Å) than for the closest nonbonded C–H interactions.[5] Therefore, in general, the T_1 values of nonprotonated carbons are much longer than those of protonated ones.[2] One must investigate the possibility that contributions from relaxation mechanisms other than the ^{13}C–^1H dipolar one can be significant for nonprotonated carbons. There is evidence[2] that the lone nonprotonated carbon of sucrose (in H_2O) and the three nonprotonated carbons of cholesteryl chloride (in CCl_4) exhibit only ^{13}C–^1H dipolar relaxation at a resonance frequency of 15.1 MHz. On the other hand, there is also evidence[2,6] that mechanisms other than the ^{13}C–^1H dipolar one contribute significantly to the relaxation of two of the three nonprotonated carbons of adenosine 5′-monophosphate (AMP) in H_2O.

A knowledge of the relative importance of various contributions to the relaxation of nonprotonated carbons will facilitate the use of ^{13}C T_1 values for making spectral assignments,[7] and the use of integrated intensities of nonprotonated carbon resonances for making carbon counts and for quantitative analysis. Carbon-13 NMR spectra of large molecules are nearly always recorded under conditions of proton decoupling, in order to remove complex splitting pat-

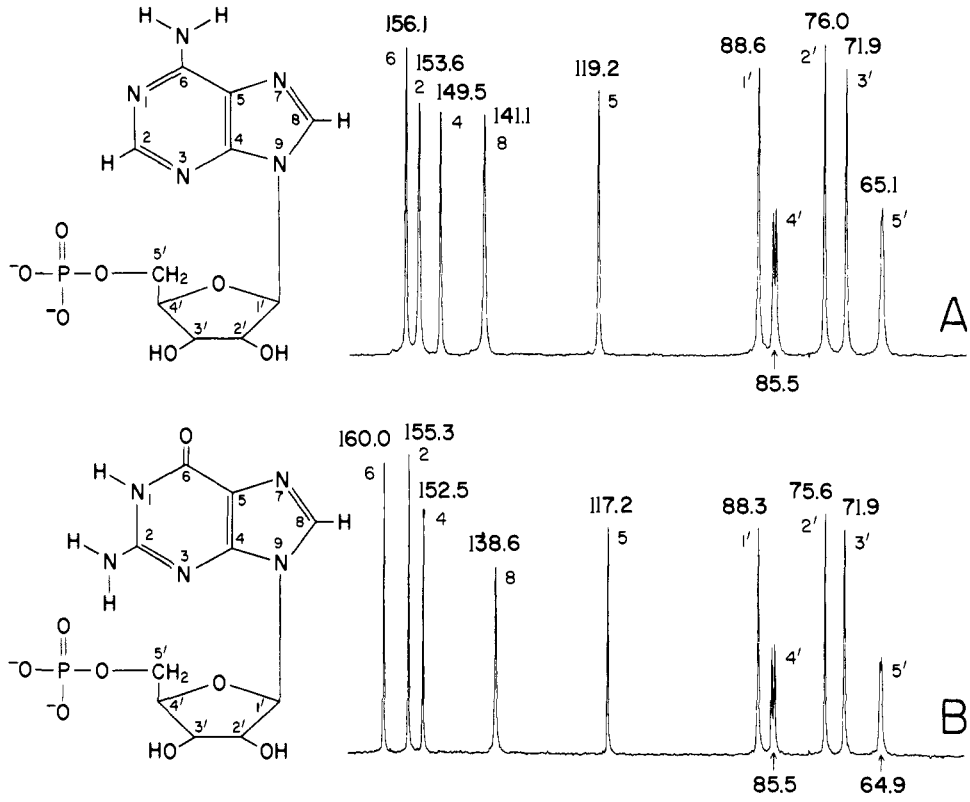

Figure 1. Proton-decoupled natural-abundance ^{13}C Fourier transform NMR spectra of AMP and GMP in H_2O. Spectra were recorded as described in the Experimental Section, with a 2994-Hz spectral width and 0.70-Hz digital broadening. Small numbers are assignments using the carbon designations shown in the structures. Large numbers are chemical shifts, in ppm downfield from Me_4Si, obtained from spectra (not shown) recorded after addition of dioxane as an internal standard. (A) 0.75 M AMP, pH 7.2, 41 °C, after 1600 accumulations, with a recycle time of 25 s. The resonance of C-4′ is split as a result of ^{13}C-^{31}P scalar coupling (J_{CP} = 8.4 Hz). The chemical shifts of AMP in D_2O are: C-2, 153.6; C-4, 149.5; C-5, 119.2; C-6, 155.9; C-8, 141.1; C-1′, 88.6; C-2′, 75.9; C-3′, 71.8; C-4′, 85.5; C-5′, 65.0. (B) 0.51 M GMP, pH 8.1, 40 °C, after 894 accumulations, with a recycle time of 42 s. The vertical gain in spectrum B is 1.75 times that of spectrum A. The resonances of C-4′ and C-5′ are split as a result of ^{13}C-^{31}P scalar coupling (J_{CP} is 8.2 Hz for C-4′ and 4.0 Hz for C-5′). Chemical shifts of GMP in D_2O are given in Figure 2.

terns caused by ^{13}C-^{1}H scalar coupling.[8] An important consequence of proton decoupling is the nuclear Overhauser enhancement (NOE).[1,9] Here we define the NOE as the *ratio* of the integrated intensity of a ^{13}C resonance under conditions of proton decoupling and the corresponding intensity in the absence of proton decoupling. The maximum value of the NOE is 2.988.[1,9] Different NOE values for various carbons within the same molecule will result in different integrated intensities for the resonances of these carbons. Two conditions must be satisfied in order to observe the maximum NOE of 2,988. First, ^{13}C relaxation must occur entirely by the ^{13}C-^{1}H dipolar mechanism.[1] Second, the correlation times for rotational reorientation of the vectors that connect the ^{13}C nucleus to the ^{1}H nuclei which cause relaxation must be small relative to the proton resonance frequency in radians/second (the extreme narrowing condition).[9,10] The extreme narrowing condition is normally satisfied for organic molecules in solution, because their correlation times for overall rotation (τ_R) are usually shorter than 10^{-10} s.[2,3] The extreme narrowing condition will not be satisfied for most carbons of native biopolymers.[10,11] A common misconception is that the resonances of nonprotonated carbons of organic molecules should always have one-third the intensity of the resonances of protonated carbons. However, this behavior is only expected (for large molecules[2]) if the ^{13}C-^{1}H dipolar mechanism contributes negligibly to the relaxation of nonprotonated carbons (NOE $\approx$ 1). It has been shown[2] that all carbons (including the nonprotonated ones) of cholesteryl chloride and sucrose have an NOE $\approx$ 3. As a result, the nonprotonated carbons have the same integrated intensities as the protonated ones in these two cases. On the other hand, two of the three nonprotonated carbons of AMP (in H_2O) have considerably lower intensities than the protonated carbons.[2]

In this report we show that ^{13}C-^{14}N dipolar interactions can contribute significantly to the relaxation of a nonprotonated carbon that is directly bonded to one or more nitrogen atoms. We show that if the T_1 values of the protonated carbons of a rigid large organic molecule are measured, then one can make fairly accurate predictions of the T_1 and NOE values of nonprotonated carbons within the same molecule, without invoking relaxation mechanisms other than the ^{13}C-^{1}H and ^{13}C-^{14}N dipolar ones (at 14.2 kG). Recently we have shown that ^{13}C-^{14}N dipolar interactions can contribute significantly to the relaxation of some nonprotonated side-chain carbons of proteins.[11] However, low signal-to-noise ratios prevented the accurate experimental determination of the importance of ^{13}C-^{14}N relaxation.[11] Here we present T_1 and NOE values of the nonprotonated carbons of concentrated solutions of adenosine 5′-monophosphate and guanosine 5′-monophosphate (GMP) in H_2O and in D_2O.

As far as we know, earlier workers have not considered the possible importance of ^{13}C-^{14}N dipolar contributions to ^{13}C relaxation (of nonprotonated nitrogen-bearing carbons), probably because of the very low gyromagnetic ratio of ^{14}N. However, the short C-N bond length partly compensates for the low gyromagnetic ratio (see below).

Experimental Section

AMP (sodium salt, Type II) and GMP (sodium salt) were obtained from Sigma Chemical Co., St. Louis, Mo. D_2O (99.8% iso-

topic enrichment) was obtained from the U.S. Atomic Energy Commission, Savannah River Operations Office, Aiken, S.C. D_2O solutions were prepared with the use of nucleotides that were lyophilized twice from D_2O. The purpose of most of the steps described below was to minimize the presence of paramagnetic ions. H_2O was distilled, then passed through two columns of mixed bed ion-exchange resin (Research Model, Illinois Water Treatment Co., Rockford, Ill.). The D_2O was distilled, then extracted five times with an equal volume of 0.5% diphenylthiocarbazone (dithizone) in CCl_4 (spectroscopic grade). After each nucleotide was dissolved in H_2O or D_2O, the pH was adjusted with concentrated HCl (H_2O and D_2O solutions), or concentrated NaOH (H_2O solutions), or NaOD (D_2O solutions). Each solution was passed through a membrane filter (8-μm pore size, from Nuclepore Corp., Pleasanton, Calif.), and then extracted three times with an equal volume of 0.5% dithizone in CCl_4. EDTA (disodium salt) was added. The concentration of EDTA was 1 mM in each sample. Finally, each solution was degassed by bubbling argon through it for at least 6 min. The NMR tube, vortex preventing plug (Teflon), and glassware used in the final stages of sample preparation were soaked for at least 24 h in 50 mM EDTA (pH 8.5–9.0), rinsed with H_2O, and dried. pH measurements were made at 25 °C, as described previously.[12]

Natural-abundance ^{13}C Fourier transform NMR spectra were recorded at 15.18 MHz with the use of 20-mm sample tubes,[13] as described elsewhere.[11-13] All spectra were recorded under conditions of full proton decoupling.[11] Normal Fourier transform (NFT) NMR spectra were obtained with the use of 90° radiofrequency pulse excitation, and with the recycle time (interval between successive 90° pulses) sufficiently long, relative to the pertinent T_1 values, to ensure that the intensities were not measurably different from the equilibrium intensities.[14,15] Partially relaxed Fourier transform (PRFT) NMR spectra[16] were obtained in the same way as the NFT spectra, except that a 180° radiofrequency pulse was applied at a time τ before each 90° radiofrequency pulse. T_1 values of individual carbon resonances were obtained from PRFT spectra.[2] Separate sets of PRFT spectra were used for the protonated and nonprotonated carbons, with spectral widths of 2994 and 2000 Hz, respectively. Other details are given in the caption to Figure 2 and in Tables I and II.

Time-domain data were accumulated in 8192 addresses of a Nicolet 1085 computer. Fourier transformation was done on 16 384 time-domain addresses by placing 8192 addresses with a zero value at the end of each block of accumulated data points. In this way there was one point every 0.244 Hz (2000-Hz spectra) or 0.365 Hz (2994-Hz spectra). Integrated intensities were obtained digitally from NFT spectra recorded with spectral widths of 2994 Hz. Chemical shifts were obtained digitally and are reported in parts per million downfield from Me_4Si. Estimated accuracy is ±0.1 ppm. Dioxane (at 67.86 ppm downfield from external Me_4Si) was used as internal standard.

Results

We have measured the T_1 values and integrated intensities of the ^{13}C resonances of 0.75 M AMP in H_2O (pH 7.2, 41 °C), 0.77 M AMP in D_2O (pH meter reading 7.2, 44 °C), 0.51 M GMP in H_2O (pH 8.1, 40 °C), and 0.50 M GMP in D_2O (pH meter reading 8.0, 41 °C). T_1 values and integrated intensities of the ^{13}C resonances of 1 M AMP in H_2O (pH 5.2, 42 °C) have been reported by Allerhand, Doddrell, and Komoroski.[2] T_1 values of 0.4 and 0.2 M AMP (pH 7, 40 °C) have been reported by Hamill, Pugmire, and Grant.[17] In the next section we compare the previously published data[2,17] with our new results for AMP in H_2O.

Figure 1 shows typical NFT spectra used for obtaining integrated intensities. Assignments of the resonances are those of Dorman and Roberts,[18] as modified by Mantsch and Smith[19] and by Birdsall and Feeney.[20] Figure 2 shows typical NFT and PRFT spectra used for obtaining T_1 values of nonprotonated carbons. Note that the resonances of carbons attached to hydrogen-bearing oxygens and nitrogens undergo measurable upfield shifts when the labile hydrogens are replaced by deuterium (Figures 1 and 2). Similar effects have been observed in peptides[21,22] and proteins.[22]

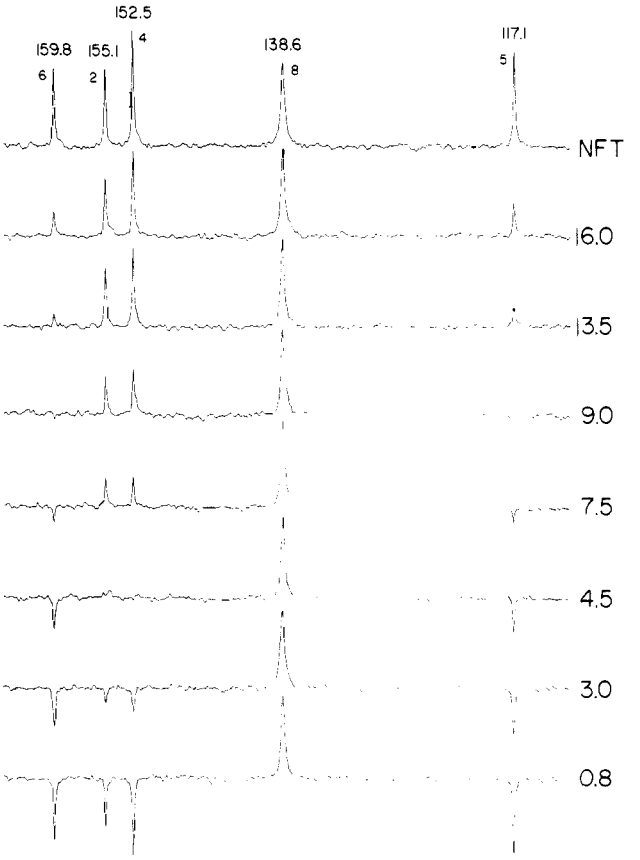

Figure 2. Regions of base carbon resonances in the proton-decoupled natural-abundance ^{13}C NFT and PRFT NMR spectra of 0.50 M GMP in D_2O at a pH meter reading of 8.0, and 41 °C. Each spectrum was recorded as described in the Experimental Section, with a 2000-Hz spectral width, 64 accumulations, and a digital broadening of 2.33 Hz. The recycle time was 60 s for the NFT (top) spectrum and (τ + 60 s for each PRFT spectrum. The large number to the right of each PRFT spectrum is τ, in seconds. The small numbers next to the peaks in the NFT spectrum are the assignments, using the carbon designations of Figure 1. The large numbers in the NFT spectrum are chemical shifts, in ppm downfield from Me_4Si, obtained after addition of dioxane as an internal standard. The chemical shifts of the ribose carbons are: C-1′, 88.4; C-2′, 75.5; C-3′, 71.8; C-4′, 85.4; C-5′, 64.9.

Integrated intensities of the nonprotonated and protonated carbon resonances are given in Tables I and II, respectively. Except in the case of C-8 of nucleotides in D_2O, each value is the arithmetic average from two or three spectra. When D_2O is the solvent, the hydrogen attached to C-8 is slowly replaced by deuterium.[23] As a result, the sharp resonance of C-8 in our spectra of nucleotides in D_2O gradually decreased, and a broad signal became visible under the sharp resonance. The broad component is the resonance of C-8 when this carbon is bonded to deuterium.[24,25] From the decrease in the intensity of the sharp component, we estimated that about 20% of the hydrogens attached to C-8 of the nucleotides in D_2O had been replaced by deuterium at the completion of measurements (which took about 3 days for each sample in D_2O). The integrated intensities given in Table II for C-8 of AMP and GMP in D_2O were obtained from the first spectrum in each case. All integrated intensities of Tables I and II were normalized to give a value of 3.0 for the arithmetic average of the intensities of the protonated carbons (C-8 of D_2O solutions was not included in the arithmetic average).

Table I. Observed and Calculated Spin–Lattice Relaxation Times and Integrated Intensities of the Nonprotonated Carbon Resonances of AMP and GMP

Compd	Carbon[a]	T_1 (obsd)[b]	T_1 (calcd)/T_1 (obsd)[c]		Intensity	
			$^{13}C-^{1}H$ only[d]	With $^{13}C-^{14}N$[e]	Obsd[f]	Calcd/obsd[g]
AMP, H_2O[h]	4	5.7	1.91–2.05	1.25–1.32	2.1	1.11–1.13
	5	7.4	1.78–1.93	1.36–1.47	2.4	1.06
	6	2.2	1.12–1.21	0.99–1.06	2.7	1.00–1.01
AMP, D_2O[i]	4	5.1	1.80–1.99	1.15–1.23	2.0	1.14–1.16
	5	9.7	3.07–3.12	1.63–1.71	1.7	1.21–1.24
	6	5.9	9.35–9.51	1.47[j]	1.5	0.89[k]
GMP, H_2O[l]	2	2.2	0.85–0.98	0.76–0.86	2.8	0.99–1.00
	4	8.6	1.68–2.49	1.07–1.34	2.1	0.98–1.07
	5	14.1	2.36–2.59	1.46–1.55	2.1	1.05–1.07
	6	6.0	0.86–1.03	0.79–0.92	2,7	1.04–1.05
GMP, D_2O[o]	2	6.7	22.9–44.5	1.08–1.16[m]	1.3	0.80–0.83[n]
	4	7.3	1.89–2.96	1.09–1.38	1.9	1.01–1.13
	5	15.3	2.79–3.24	1.38–1.48	1.7	1.15–1.20
	6	15.5	17.0–22.6	1.53–1.62[p]	1.3	0.86–0.90[q]

[a] Carbon designations are given in Figure 1. [b] Experimental T_1 value, in seconds. Estimated accuracy is ±10%. One of the five NFT spectra and seven of the 11 PRFT spectra used for getting T_1 values of GMP in D_2O are shown in Figure 2. Unless otherwise stated below, instrumental conditions of all other spectra used for obtaining T_1 values were the same as in Figure 2. The four PRFT spectra of GMP in D_2O that are not shown in Figure 2 had τ values (in s) of 2, 6, 12, and 20. For GMP in H_2O, five NFT spectra with a recycle time of 42 s, and 12 PRFT spectra with a recycle time of τ + 42 s were used. τ values (in s) were: 0.2, 0.6, 1.0, 1.8, 2.4, 3.2, 4.0, 5.2, 7.0, 8.5, 10.5, and 13.5. For AMP in H_2O, four NFT spectra with a recycle time of 25 s, and 12 PRFT spectra with a recycle time of τ + 25 s, were used. τ values (in s) were: 0.25, 0.60, 1.25, 1.80, 2.25, 2.75, 3.0, 4.2, 5.4, 6.6, 7.8, and 9.0. For AMP in D_2O, five NFT spectra with a recycle time of 40 s and 12 PRFT spectra with a recycle time of τ + 40 s were used. τ values (in s) were: 0.7, 2.0, 3.0, 3.5, 4.5, 5.4, 6.4, 7.8, 9.1, 10.5, 11.7, and 13.5. [c] Ratio of calculated T_1 value and the corresponding observed value. The ranges of calculated values arise from the use of more than one set of atomic coordinates (see Discussion). For D_2O solutions, we assumed complete replacement of OH and NH hydrogens by deuterium. [d] Calculated T_1 values consider only $^{13}C-^{1}H$ dipolar interactions. [e] Calculated T_1 values consider $^{13}C-^{1}H$ and $^{13}C-^{14}N$ dipolar interactions. For D_2O solutions, $^{13}C-^{2}H$ dipolar interactions with deuterium two bonds removed were also included in the calculations for C-6 of AMP and C-2 and C-6 of GMP (carbon–deuterium distances were assumed to be the same as carbon–hydrogen distances). The corresponding T_1 values which do not consider the effect of $^{13}C-^{2}H$ interactions are given in footnotes *j*, *m*, and *p*. [f] Observed integrated intensity, normalized as described in the text. Estimated accuracy is ±10%. Each value is the arithmetic average from two or three spectra recorded as follows: 1408 and 1600 accumulations (with a recycle time of 25 s) for AMP in H_2O; 960, 890, and 900 accumulations (with a recycle time of 40 s) for AMP in D_2O; 894 and 1024 accumulations (with a recycle time of 42 s) for GMP in H_2O; 610, 600, and 674 accumulations (with a recycle time of 60 s) for GMP in D_2O. Other spectral conditions are given in the caption of Figure 1. [g] Ratio of calculated and experimental intensity. Calculated values were obtained from eq 7, with T_{1H} taken as the spin–lattice relaxation time which considers only $^{13}C-^{1}H$ dipolar interactions, and T_1 taken as the spin–lattice relaxation time which considers $^{13}C-^{1}H$ and $^{13}C-^{14}N$ dipolar interactions. For D_2O solutions, $^{13}C-^{2}H$ dipolar interactions with deuterium two bonds removed were included in the calculation of T_1 values for C-6 of AMP and C-2 and C-6 of GMP. The corresponding calculated intensities which do not consider the effect of $^{13}C-^{2}H$ interactions are given in footnotes *k*, *n*, and *q*. [h] 0.75 M AMP in H_2O, pH 7.2, 41 °C. [i] 0.77 M AMP in D_2O, pH meter reading 7.2, 44 °C. [j] 1.96–2.0 if $^{13}C-^{2}H$ dipolar interactions are not included. [k] 0.97 if $^{13}C-^{2}H$ dipolar interactions are not included. [l] 0.51 M GMP in H_2O, pH 8.1, 40 °C. [m] 1.57–1.64 if $^{13}C-^{2}H$ dipolar interactions are not included. [n] 0.81–0.86 if $^{13}C-^{2}H$ dipolar interactions are not included. [o] 0.50 M GMP in D_2O, pH meter reading 8.0, 41 °C. [p] 2.33–2.42 if $^{13}C-^{2}H$ dipolar interactions are not included. [q] 0.92–0.96 if $^{13}C-^{2}H$ dipolar interactions are not included.

Table II. Observed Spin–Lattice Relaxation Times and Integrated Intensities of the Protonated Carbon Resonances of AMP and GMP

Compd[a]	NT_1 (intensity)[b]							τ_R[c]
	1'	2'	3'	4'	5'	2	8	
AMP, H_2O	0.18 (3.0)	0.22 (3.0)	0.21 (3.0)	0.18 (3.0)	0.20 (3.0)	0.16 (3.0)	0.14 (3.0)	0.30
AMP, D_2O	0.14 (3.1)	0.18 (3.1)	0.18 (3.0)	0.14 (3.0)	0.17 (2.9)	0.13 (3.0)	0.12 (2.8)	0.38
GMP, H_2O	0.24 (3.1)	0.29 (3.0)	0.30 (2.9)	0.24 (2.9)	0.32 (2.9)	*d*	0.17 (3.1)	0.26
GMP, D_2O	0.17 (3.1)	0.22 (3.0)	0.22 (2.9)	0.17 (3.0)	0.24 (2.9)	*d*	0.13 (2.8)	0.34

[a] Sample conditions are given in the text and footnotes *h*, *i*, *l*, and *o* of Table I. [b] NT_1 values, in seconds, are given outside the parentheses. *N* is the number of directly attached hydrogens (2 for C-5' and 1 for all other protonated carbons). Values in parentheses are observed integrated intensities, normalized as described in the text. Estimated accuracy of T_1 values and intensities is ±10%. Number above each column of T_1 values and intensities is the carbon designation of Figure 1. In each case, T_1 values were obtained from 9 or more PRFT spectra and two NFT spectra, recorded with 128 accumulations (AMP in H_2O and D_2O, GMP in H_2O) or 256 accumulations (GMP in D_2O) per spectrum, a recycle time of 2.0 s (AMP) or 2.2 s (GMP), and a digital broadening of 1.4 Hz. Integrated intensities were obtained from the same sets of NFT spectra as used for the nonprotonated carbons (see footnote *f* of Table I), except that the intensity of C-8 of each nucleotide in D_2O was obtained from the initial spectrum only. Digital accumulation of these initial spectra began 37 and 15 h after preparing the D_2O solutions of AMP and GMP, respectively. [c] Rotational correlation time (in nanoseconds) of the base, calculated with the use of eq 3, r = 1.084 Å, the T_1 values of C-8 of GMP, and the arithmetic averages of the T_1 values of C-2 and C-8 of AMP. [d] Nonprotonated carbon. T_1 value is given in Table I.

The T_1 values of the nonprotonated carbons are given in Table I. These values were obtained from sets of NFT and PRFT spectra described in footnote *b* of Table I. The T_1 values of the protonated carbons are given in Table II. These values were obtained from sets of NFT and PRFT spectra described in footnote *b* of Table II. Measurements of T_1 values of protonated carbons of AMP and GMP in D_2O were started 50 and 5 h, respectively, after sample preparation. Each set of PRFT spectra required less than 2 h of signal accumulation. The *change* in the extent of deuteration during the PRFT experiments was negligible for both nucleotides, although about 10% deuterium incorporation at C-8 had occurred by the time the PRFT spectra of AMP were recorded. The resonance of deuterated C-8 was

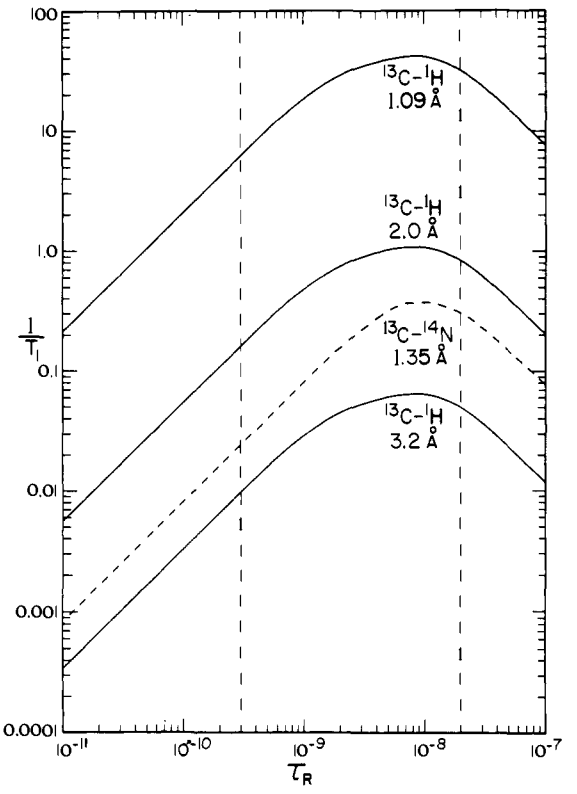

Figure 3. Contributions to $1/T_1$ (in s^{-1}) of a ^{13}C nucleus from dipolar interaction with a directly bonded hydrogen (r = 1.09 Å), a typical hydrogen two bonds removed (r = 2.0 Å), a typical hydrogen three bonds removed (r = 3.2 Å), and a typical directly bonded nitrogen (r = 1.35 Å). τ_R is in seconds. The magnetic field strength is 14.2 kG. Equation 1 was used. Broken vertical lines indicate typical τ_R values of aqueous mononucleotides (3×10^{-10} s) and small biopolymers (2×10^{-8} s).

broad (see above) and did not interfere with intensity measurements of the sharp resonance of the protonated C-8. Within our experimental error of ±10%, the T_1 values of C-8 of the nucleotides in D_2O were not affected by deuterium exchange.

Discussion

In Figure 3 we compare the contributions to $1/T_1$ (of a ^{13}C nucleus) from ^{13}C-^{1}H dipolar interactions with a directly bonded hydrogen (r = 1.09 Å), with a typical hydrogen two bonds removed (r = 2.0 Å), and with a typical hydrogen three bonds removed (r = 3.2 Å). Also shown in Figure 3 is the contribution to $1/T_1$ from a ^{13}C-^{14}N dipolar interaction with a typical directly bonded nitrogen (r = 1.35 Å). The theoretical results of Figure 3 were obtained with the use of eq 1.[1,24]

$$1/T_{ij} = (2/15)\hbar^2 S_j(S_j + 1)\gamma_C{}^2\gamma_j{}^2 r^{-6}\chi_j \qquad (1)$$

Here $1/T_{ij}$ is the contribution to $1/T_1$ of the carbon from a dipolar interaction between the ^{13}C nucleus and nucleus j. S_j is the spin quantum number of nucleus j, γ_C and γ_j are the gyromagnetic ratios of ^{13}C and nucleus j, respectively, r is the distance between the ^{13}C nucleus and nucleus j, and χ_j is given by eq 2.

$$\chi_j = \frac{\tau_R}{1 + (\omega_j - \omega_C)^2\tau_R{}^2} + \frac{3\tau_R}{1 + \omega_C{}^2\tau_R{}^2} + \frac{6\tau_R}{1 + (\omega_j + \omega_C)^2\tau_R{}^2} \qquad (2)$$

Here ω_C and ω_j are the resonance frequencies (in radians/second) of ^{13}C and nucleus j, respectively, and τ_R is the correlation time for overall rotational reorientation. Equation 1 was derived for the case of isotropic rotational reorientation, and without allowing for internal motions.[1,24]

It follows from Figure 3 that in the case of a proton-bearing carbon it is a good approximation to neglect dipolar contributions from nonbonded nuclei. It also follows from Figure 3 that when dealing with a nonprotonated carbon which has one or more directly bonded nitrogens, the contribution to $1/T_1$ from ^{13}C-^{14}N dipolar interactions may be comparable to or even greater than the contribution from ^{13}C-^{1}H dipolar interactions. Figure 3 indicates that ^{13}C-^{14}N dipolar relaxation may be particularly important for a nonprotonated carbon that lacks hydrogens two bonds removed but has one or more directly bonded nitrogens.

The total T_1 of a protonated carbon is given by[2]

$$1/T_1 = (1/10)N\hbar^2\gamma_C{}^2\gamma_H{}^2 r^{-6}\chi_H \qquad (3)$$

Here N is the number of directly bonded hydrogens, r is the CH bond length, and χ_H is given by eq 2. We have assumed that in the case of a CH_2 or CH_3 group, the value of r is the same for all CH bonds. Equation 3 can be used to compute τ_R from a measured T_1 value of a protonated carbon whose CH vectors are not undergoing internal rotation at a rate comparable to or faster than the rate of overall rotation.[10]

For each of the four samples, the NT_1 values of the ribose carbons are slightly greater than the corresponding values of the methine carbons of the purine moiety (Table II). This result indicates that the nucleotide molecules in our samples do not behave strictly as isotropic rigid rotors. The longer NT_1 values of the ribose carbons may reflect pseudorotation in the ribofuranose rings,[26] or anisotropic rotation caused by base stacking.[17] Nevertheless, we have used the isotropic rigid rotor model in our calculations of the T_1 and NOE values of the nonprotonated carbons of the purine moieties.

We used eq 3 and the T_1 values of the methine carbons of the purine moiety (Table II) to calculate τ_R (in the case of AMP, we used the arithmetic average of the T_1 values of C-2 and C-8). Introduction of these T_1 values into eq 3 yielded two solutions for τ_R in each case: about 3×10^{-10} s and about 10^{-7} s. The second solution was rejected not only on the basis of known correlation times for molecules of comparable molecular weight,[2,3] but also because the computed line widths corresponding to the two solutions are about 2 and 150 Hz, respectively, while the experimental methine carbon line widths were about 3 ± 1 Hz. The accepted solutions for τ_R are given in Table II. Note that τ_R of each nucleotide is slightly longer in D_2O than in H_2O. Part or all of the difference may arise from the higher viscosity of D_2O.[27] If there is a deuterium isotope effect on base stacking[28] equilibria, then this factor may also influence the rotational correlation time.

We introduced the appropriate τ_R value from Table II into eq 1 to calculate each dipolar contribution to $1/T_1$ of a nonprotonated carbon. Internuclear distances for AMP were obtained from the crystal structures of adenosine[29] and AMP.[30] The relative orientation of the adenine and ribose groups in both cases is anti.[31] The torsion angle about the glycosidic bond between C-1′ of the ribose and N-9 of the purine (ϕ_{CN}) is 9.9° for adenosine and −18° for AMP. A number of studies[28,32-34] indicate that the conformation of AMP in solution is also anti. At the pH used in our experiments, the adenine ring is in its neutral form.[35] In the case of GMP, internuclear distances were obtained from the crystal structure of guanosine.[36] There are two different conformations in the guanosine crystal, with ϕ_{CN} values of −123.3 and −43.9°.[36] In the crystal of deoxyguanosine 5′-monophosphate, the orientation of the guanine and deoxyri-

bose rings is anti.[37] Studies of the conformation of GMP in solution indicate that the anti conformation predominates,[28,32] although the syn conformation may contribute more than in AMP.[38] We calculated T_1 values for GMP with the use of the coordinates of both crystalline forms of guanosine,[36] and also for a structure with $\phi_{CN} = 9.9°$, in order to test the effect of large variations in ϕ_{CN}. The guanine ring in GMP deprotonates at N-1 with a pK_a of about 9.4.[35] In H_2O at pH 8.1, more than 95% of the ring should be in the neutral form. A similar situation is expected for GMP in D_2O at a pH meter reading of 8.0. We have ignored the small quantities of ionized guanine ring in calculating the T_1 values of Table I.

For the nonprotonated carbons of AMP and GMP (in H_2O and D_2O) we calculated T_1 values which take into account $^{13}C-^{1}H$ dipolar interactions with all hydrogens, and $^{13}C-^{14}N$ dipolar interactions with directly bonded nitrogens (Table I). For C-6 of AMP in D_2O, and for C-2 and C-6 of GMP in D_2O, $^{13}C-^{2}H$ dipolar relaxation was also included. Theoretical values which consider only $^{13}C-^{1}H$ interactions were also tabulated. Each theoretical T_1 value of Table I has been divided by the corresponding experimental value.

When H_2O is the solvent, C-6 of AMP has two hydrogens two bonds removed (Figure 1A), C-2 of GMP has three such hydrogens, and C-6 of GMP has one (Figure 1B). As a result, when H_2O is the solvent the theoretical T_1 values of these nonprotonated carbons are not significantly affected by $^{13}C-^{14}N$ relaxation, and there is excellent agreement between the experimental and theoretical values even when $^{13}C-^{14}N$ dipolar relaxation is not included in the computed values (Table I). The presence of more than one hydrogen, two bonds removed from C-6 of AMP and C-2 of GMP (H_2O solutions), results in particularly short T_1 values for these carbons.

As expected on the basis of Figure 3, nonprotonated carbons without hydrogens two bonds removed yield theoretical T_1 values strongly affected by $^{13}C-^{14}N$ dipolar relaxation (Table I). For all carbons in this category, the theoretical T_1 values which include the effect of $^{13}C-^{14}N$ dipolar interactions are in much better agreement with the experimental values than calculated values which consider only $^{13}C-^{1}H$ dipolar interactions (Table I). When H_2O is the solvent, C-4 and C-5 are the only nonprotonated carbons of AMP and GMP that do not have hydrogens two bonds removed, and therefore they are the only carbons with important $^{13}C-^{14}N$ dipolar contributions to $1/T_1$. When D_2O is the solvent, none of the nonprotonated carbons of AMP and GMP has hydrogens two bonds removed. The effect of $^{13}C-^{14}N$ dipolar relaxation is particularly striking for C-6 of AMP in D_2O and for C-2 and C-6 of GMP in D_2O. In each of these three cases, the theoretical T_1 values that take into account only $^{13}C-^{1}H$ dipolar interactions differ by about an order of magnitude or more from the corresponding experimental values (Table I). In contrast, the theoretical T_1 values which include the effect of $^{13}C-^{14}N$ dipolar interactions are in reasonable agreement with the experimental values (Table I). $^{13}C-^{14}N$ dipolar interactions dominate the relaxation of C-6 of AMP in D_2O and of C-2 and C-6 of GMP in D_2O. When D_2O is the solvent, $^{13}C-^{14}N$ dipolar interactions contribute significantly to $1/T_1$ of all nonprotonated carbons of AMP and GMP (Table I).

It follows from Table I that when the T_1 value of at least one protonated carbon has been measured, it is possible to make reasonable predictions of the T_1 values of the nonprotonated carbons. Some calculated T_1 values differ by 25% or less from the experimental ones (Table I). We discuss below some possible sources of the relatively large discrepancies between the experimental and theoretical values for C-5 (in H_2O and D_2O) and C-6 (in D_2O) of both nucleotides. First, however, we will show that accurate predictions of the variations of the intensities of the resonances in proton-decoupled ^{13}C NMR spectra of nucleotides can be made on the basis of relative contributions of $^{13}C-^{1}H$ and $^{13}C-^{14}N$ dipolar relaxation.

Note that the τ_R values of Table II are short enough to satisfy the extreme narrowing condition. As a result, eq 2 yields $\chi_j = 10\tau_R$, and eq 1 becomes

$$1/T_{1j} = \tfrac{4}{3}\hbar^2 S_j(S_j + 1)\gamma_C^2\gamma_j^2 r^{-6}\tau_R \quad (4)$$

If nucleus j is a proton, eq 4 becomes

$$1/T_{1j} = \hbar^2\gamma_C^2\gamma_H^2 r^{-6}\tau_R \quad (5)$$

The integrated intensity of a resonance in a proton-decoupled ^{13}C NMR spectrum depends on the value of the NOE. The NOE is a function of the rotational correlation time and the relative contributions of $^{13}C-^{1}H$ dipolar and other relaxation mechanisms.[1] When rotational motion is sufficiently fast to satisfy the extreme narrowing condition, the NOE becomes independent of the rotational correlation time, and is given by[1]

$$\text{NOE} = 1 + \tfrac{1}{2}(\gamma_H/\gamma_C)(T_1/T_{1H}) = 1 + 1.988(T_1/T_{1H}) \quad (6)$$

Here T_1 is the total spin-lattice relaxation time and $1/T_{1H}$ is the contribution to $1/T_1$ from $^{13}C-^{1}H$ dipolar interactions. We will use an approximate form of eq 6:

$$\text{NOE} = 1 + 2T_1/T_{1H} \quad (7)$$

Note that even though the calculation of T_1 and T_{1H} from eq 4 and 5 requires a knowledge of τ_R, the ratio T_1/T_{1H} is a function of gyromagnetic ratios and interatomic distances only (in the extreme narrowing limit). Consequently, a knowledge of molecular geometry is sufficient for predicting relative intensities of resonances in proton-decoupled ^{13}C NMR spectra (if only nuclear dipole-dipole contributions to $1/T_1$ are important, the extreme narrowing condition applies, overall rotation is fairly isotropic, and internal motions can be neglected).

The resonances of all protonated carbons of nucleotides should have the same integrated intensity ($T_1 = T_{1H}$, NOE = 3).[2] Within our experimental error of ±10%, the resonances of all protonated carbons of AMP and GMP (in H_2O and D_2O) do indeed have the same intensity (Table II). The experimental integrated intensities of the nonprotonated carbons are given in Table I. These intensities have been normalized so as to give a value of 3.0 to the average of the intensities of the protonated carbons (see Results). Under the safe assumption[2] that all protonated carbons of the nucleotides have an NOE of 3, this normalization procedure yields integrated intensities of nonprotonated carbon resonances which can be taken as experimental NOE values, and which can be compared directly with theoretical NOE values computed from eq 7 (Table I). The experimental intensities of most nonprotonated carbon resonances of AMP and GMP are considerably lower than those of protonated carbons (compare Tables I and II), and there are differences in the intensities of the various nonprotonated carbons. In addition, some of these carbons undergo intensity changes when going from H_2O to D_2O solution (Table I). These results are qualitatively consistent with $T_1 < T_{1H}$ for the nonprotonated carbons, as a consequence of significant contributions to $1/T_1$ from $^{13}C-^{14}N$ dipolar relaxation. Furthermore, the intensities of nonprotonated carbon resonances calculated with the use of eq 7 are in excellent agreement with the experimental values (Table I). For most nonprotonated carbons, the difference between the theoreti-

cal and experimental values is comparable to the experimental uncertainty of ±10%. The largest discrepancy is about 20% (C-5 of AMP in D_2O). We conclude that the low integrated intensities of nonprotonated carbon resonances of nucleotides, relative to the intensities of protonated carbon resonances, can be explained by invoking ^{13}C-^{14}N dipolar relaxation. These results invalidate the earlier suggestion[2] that chemical shift anisotropy may be an important relaxation mechanism for the nonprotonated carbon resonances of aqueous AMP. Note that our results were obtained at 14.2 kG. Chemical shift anisotropy may be an important relaxation mechanism for these carbons at higher magnetic field strengths.

On the whole, the discrepancies between experimental and calculated T_1 values (with ^{13}C-^{14}N contributions) of nonprotonated carbon resonances (Table I) are greatest for the longest calculated T_1 values, i.e., for carbons which have no hydrogens closer than about 3 Å and which have only one directly bonded nitrogen (such as C-5 of both nucleotides and C-6 of GMP in D_2O). In such cases, relaxation mechanisms other than ^{13}C-^{1}H and ^{13}C-^{14}N dipolar interactions have the greatest opportunity for contributing significantly to $1/T_1$. Chemical shift anisotropy[1] can be ruled out as an important relaxation mechanism at our low magnetic field strength, because measurements on AMP (1 M, pH 7, 32-35 °C, in H_2O and D_2O) did not yield detectable differences between the T_1 values at 15.18 and 25.2 MHz.[6] However, intermolecular ^{13}C-^{1}H dipolar interactions may contribute significantly to $1/T_1$, as a result of base stacking.[28] Relaxation by paramagnetic metal ion impurities[39] may contribute to $1/T_1$ of some of the nonprotonated carbons, even though steps were taken to remove metal ions from the solutions (see Experimental Section), and 1 mM EDTA was present. The adenine and guanine rings have a metal ion binding site in the vicinity of N-7.[40] A comparison of our T_1 values for AMP in H_2O with previously published results for unpurified AMP[2,17] suggests that paramagnetic metal ions may contribute very significantly to $1/T_1$ of C-5 of unpurified nucleotide solutions. In Table III we compare our data for 0.75 M AMP (pH 7.2, 41 °C, 15.2 MHz) with the T_1 values of Allerhand, Doddrell, and Komoroski[2] for 1.0 M AMP (pH 5.2, 42 °C, 15.1 MHz) and the values of Hamill, Pugmire, and Grant[17] for 0.4 M AMP (pH 7, 40°, 25.2 MHz). Both previous studies[2,17] were done on AMP not treated for removal of paramagnetic metal ions, although molecular oxygen was removed by Hamill et al.[17] Because of the concentration dependence of the rotational correlation time of AMP,[17] the three sets of T_1 values cannot be compared directly. However, if we neglect intermolecular contributions to $1/T_1$, then the *ratios* of the T_1 values of nonprotonated and methine carbons of the adenine ring should be independent of concentration, in the absence of paramagnetic ion effects. Indeed, C-4 and C-6 do exhibit this concentration-independent behavior (Table III). However, the T_1 of C-5 divided by the average T_1 of C-2 and C-8 is much shorter for the 1.0 and 0.4 M solutions of unpurified AMP than for our 0.75 M purified AMP (Table III). Our suggestion that these differences are caused by contributions from paramagnetic metal ions to $1/T_1$ of C-5 of the unpurified samples is consistent with the proximity of C-5 to the metal ion binding site at N-7.[40]

We have shown that ^{13}C-^{14}N dipolar interactions can contribute significantly to the relaxation of nonprotonated carbons of molecules that are sufficiently small to satisfy the extreme narrowing condition. It follows from Figure 3 that for biopolymers ($\tau_R \gtrsim 10^{-8}$ s) the contribution to $1/T_1$ from ^{13}C-^{14}N dipolar interactions, relative to the contribution from ^{13}C-^{1}H dipolar interactions, is even greater than for small molecules. We have already suggested[11] that ^{13}C-^{14}N dipolar interactions contribute significantly to $1/T_1$ of some nonprotonated nitrogen-bearing carbons of proteins. ^{13}C-^{14}N dipolar interactions should greatly shorten the T_1 values of some nonprotonated carbons of nucleic acids (relative to T_1 values based on ^{13}C-^{1}H dipolar interactions alone). We have calculated the ^{13}C-^{1}H and ^{13}C-^{14}N dipolar contributions to $1/T_1$ of some nonprotonated carbons of a guanine group of aqueous tRNA. A value of 3×10^{-8} was used for τ_R.[41] For tRNA in H_2O, the calculated T_1 of C-4 is 1.4-1.6 s if ^{13}C-^{14}N interactions are included, and 3.4-5.0 s if only ^{13}C-^{1}H interactions are considered. For tRNA in D_2O, the calculated T_1 of C-2 is about 1.4 s if ^{13}C-^{14}N interactions are included, but 70-90 s if only ^{13}C-^{1}H interactions are considered. With typical NMR instruments, a given signal-to-noise ratio can be obtained in less accumulation time if the T_1 values are about 1 s than if they are considerably longer. Because of the long τ_R values of native biopolymers, the NOE should be about 1.2 or less (in the absence of effects from internal rotations) even if relaxation is purely ^{13}C-^{1}H dipolar.[10,11] Therefore, a decrease in the NOE as a result of ^{13}C-^{14}N dipolar relaxation can be no more than a 20% effect.[11] On the whole, the presence of ^{13}C-^{14}N dipolar interactions should have a favorable effect on spectral sensitivity when using nonprotonated carbon resonances for studying nucleic acids in solution.[41] The limitations in the use of protonated carbon resonances of native biopolymers have been discussed elsewhere.[11]

Table III. Effect of Sample Conditions on the T_1 Values of Nonprotonated Carbons of AMP in H_2O

Nonprotonated carbon	Nonprot T_1/methine T_1[a]		
	This work[b]	Ref 2[c]	Ref 17[d]
4	38.8	34.2	35.7
5	50.6	30.3	29.3
6	15.2	15.5	16.8

[a] T_1 value of nonprotonated carbon divided by the arithmetic average of the T_1 values of the two methine carbons of the base (C-2 and C-8). [b] T_1 values of nonprotonated and protonated carbons were taken from Tables I and II, respectively. [c] 1.0 M AMP, pH 5.2, 42 °C, 15.08 MHz. Nucleotide was not treated for removal of paramagnetic metal ions. Reported accuracy of T_1 values is ±10–25%.[2] [d] 0.4 M AMP, pH 7, 40 °C, 25.2 MHz. Sample was degassed, but nucleotide was not treated for removal of paramagnetic metal ions. Reported accuracy of T_1 values is better than ±20%.[17]

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References and Notes

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