^{15}N Chemical Shift Tensors in Nucleic Acid Bases

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Abstract: This paper presents measurements of the principal values of the ^{15}N chemical shift tensors in adenine, cytosine, guanine, and thymine. The assignment of the solid-state NMR resonances was done using the liquid values in the corresponding nuclesides and the results of quantum mechanical calculations of the ^{15}N tensors. The calculations were also used to assign the orientation of the principal axes of the tensors and to evaluate the effect of intermolecular interactions on the ^{15}N chemical shift tensors of the nucleic acid bases. The results indicate that calculations including intermolecular interactions produce results in better agreement with the experimental values than those in which these interactions are neglected.

Introduction

The study of nucleic acid bases by NMR has been reported in a number of monographs,[1] but very little information is available on the ^{15}N chemical shifts of the unsubstituted bases.[2] The low sensitivity of ^{15}N NMR spectroscopy, the long relaxation times exhibited by these compounds, and their poor solubility in common NMR solvents have posed the main impediments for these studies. Solid-state NMR eliminates solubility problems and recently introduced procedures of sample doping with free radical relaxation reagents reduce the relaxation times[3] and, hence, facilitate multiple-pulse experiments such us the 5-π pulse two-dimensional (2D) methods used in this paper. Moreover, solid-state NMR can be used to measure the principal values of the chemical shift tensors as well as the isotropic chemical shifts in the solid which, when compared with the liquid values, provide information on solid-state intermolecular interactions. The only information that seems to be available on the ^{15}N chemical shift tensors in nucleic acid bases has been obtained from IGLO calculations[4] and by recent work from this laboratory on uracil.[5]

Recent work on ^{15}N chemical shift tensors from this laboratory has proven that it is feasible to measure the principal values of these tensors using solid-state NMR techniques and that quantum mechanical calculations provide important information pertaining to the spatial assignment of these principal components. The calculations also provide valuable information for exploring the experimental NMR chemical shifts with the molecular geometry and environment.[6,7]

NMR chemical shifts are quite sensitive to intermolecular interactions.[8−10] Recent work from this laboratory demonstrated that the inclusion of intermolecular interactions is necessary to reproduce accurately the ^{15}N experimental chemical shift principal values.[11] These results suggest that it may be possible to obtain explicit relationships between ^{15}N chemical shifts and hydrogen bonding and helix parameters of nucleic acid oligomers.

This solid-state NMR study presents ^{15}N chemical shift tensor principal values in adenine, cytosine, guanine, and thymine along with new calculations of the shift parameters in all five nucleic acid bases. The quantum chemical calculations employed different geometrical models, some of which include intermolecular interactions in the calculations. These interactions have been approximated by explicitly adding the nearest neighbors into the calculations. The calculations including the intermolecular interactions give semiquantitative information on the effects of hydrogen bonding (HB) on the principal values of the nitrogen shift tensors. The computational work reported here provides the foundation for using the principal values of the nitrogen shift tensors as a diagnostics tool for nucleic acid characterization in complex oligomers.

Experimental Procedures

Adenine, cytosine, and guanine were purchased from Aldrich, and 98% ^{15}N labeled thymine was obtained from Cambridge Isotope Laboratory.

The NMR experiments on thymine and the 2D measurements on adenine and guanine were carried out on a Chemagnetics CMX400 NMR spectrometer with a ^{15}N Larmor frequency of 40.55 MHz. The shift tensor data on cytosine and the ^{1}H T_1 measurements on adenine and guanine were obtained on a Chemagnetics CMX200 NMR

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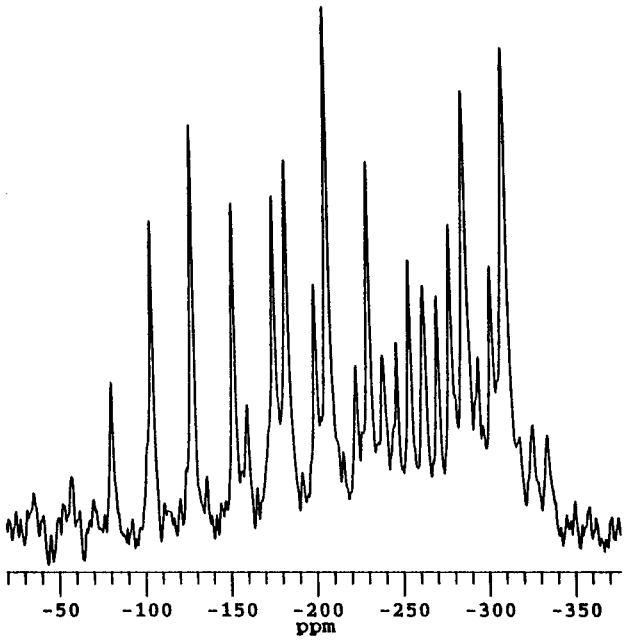

Figure 1. Slow spinning ^{15}N CP/MAS spectra of cytosine.

spectrometer with a nominal ^{15}N Larmor frequency of 20.27 MHz. The ^{1}H T_1's determined at 200 MHz are 137 s, 14 s, and 21 s for adenine, cytosine, and guanine, respectively. The ^{1}H T_1 in thymine, at 400 MHz, was estimated at approximately 20 s. All of the spectra were recorded using 38.5 kHz of power for cross polarization and 62.5 kHz for ^{1}H decoupling. Depending on the complexity of the ^{15}N spectrum, different types of experiments were chosen to extract the principal values of the ^{15}N chemical shift tensors. The details of the experimental procedures used in each compound are given below.

Adenine. The long ^{1}H T_1, 137 s, of this compound combined with the very low natural abundance, i.e., 0.37%, and the low gyromagnetic ratio of ^{15}N precluded measuring the principal values at the natural abundance level using the pure compound. The ^{1}H T_1 was reduced from 137 s to approximately 0.6 s by doping the compound with $CuCl_2$ at a weight ratio of roughly 10%. The sample was doped by dissolving both adenine and $CuCl_2$ in DMSO and extracting the solvent, using a standard Rotavapor device. It has been demonstrated that this method of doping does not affect the principal values of the chemical shifts.[12] Since adenine contains five nonequivalent nitrogen sites, a 2D experiment which separates the anisotropy information according to the different isotropic chemical shift values must be used[13] to obtain the nitrogen principal values. Therefore, the recently developed 5-π 2D MAT experiment, explained in detail elsewhere[13] was also used in this study. This high resolution/high sensitivity experiment was carried out using a sample spinning at a rate of 480 Hz $\pm$ 0.5 Hz. Only eight increments were needed in the evolution dimension to secure all the required information for the TIGER method of tensor data extraction.[14] This procedure achieves comparable resolution to that obtained in the MAS spectrum. The total acquisition time was 31 h, corresponding to 14 000 scans for each evolution increment, using a recycle delay of 1 s. The spectral widths were 4 kHz in the evolution dimension and 25 kHz in the acquisition dimension. The signals over these spectral widths were digitized using 8 and 512 points, respectively. A cross polarization contact time of 4 ms was used for adenine.

Cytosine. A 5-π 2D MAT ^{15}N CP/MAS spectrum was acquired at a spinning rate of 480 Hz. This spinning rate was chosen because it provides adequate sideband families for each of the three ^{15}N sites, which are well separated from one another. The resultant spectrum is shown in Figure 1. This spectrum was obtained using the following experimental parameters: recycle delay time 10 s, 7600 scans, contact time 8 ms. The chemical shift principal values were obtained by analyzing the spinning sideband patterns using the Herzberg−Berger method.[15] The spectral width was 31.9 kHz; the signal over this spectral width was digitized using 2048 points.

Guanine. The short ^{1}H T_1 of guanine (21 s) facilitates the use of the new 5-π 2D experiment[14] at natural abundance without further treatment of the sample for relaxation enhancement. The experiment was carried out with a sample spinning rate of 960 $\pm$ 0.5 Hz. The total acquisition time was 44 h, corresponding to 1000 scans, using a recycle delay of 20 s and proton pulse flip back. The spectral widths were 8 kHz in the evolution dimension and 50 Khz in the acquisition dimension. The signals over these spectral widths were digitized using 8 and 512 points, respectively. A contact time of 4 ms was used.

Thymine. Using a 98% enriched compound, we measured the principal values of the ^{15}N shift tensor using the PHORMAT experiment[13] while spinning the sample at 30 Hz. Only 256 scans were required to obtain satisfactory S/N using a delay of 16 s. The spectral widths were 3 kHz in the evolution dimension and 80 kHz in the acquisition dimension. The signals over these spectral widths were digitized using 32 and 256 points, respectively. A contact time of 4 ms was used for the thymine experiments.

By using the PHORMAT or the 5-π 2D experiments, the principal values of the chemical shift tensors were obtained by fits of the one-dimensional slices of the 2D spectra at the isotropic values. This procedure gives principal values with errors of about 2−3 ppm for the individual components. The tensor principal components in cytosine, which were obtained by the Herzberg−Berger method, carry a 4−6 ppm error.

The experimental values for uracil were taken from the literature[5] and converted to the nitromethane scale using the value of −358.4 ppm for the relative shift of amonium nitrate (phase IV).

Computational Procedures

Chemical shift calculations and geometry optimizations were performed with the GAUSSIAN94[16] suite of programs using the DFT approach. The calculations were performed with an IBM SP and a SGI Origin 2000 using efficiently the parallel capabilities of these systems. The chemical shift calculations employed the method proposed by Cheeseman et al.[17] with the BLYP exchange-correlation functional[18] as implemented in the GAUSSIAN94 program.[16] This method makes use of an efficient implementation[19] of the gauge-including atomic orbitals (GIAO) methods.[20,21] The calculated ^{15}N chemical shielding values, σ_{calc}, were converted to the shift scale, $\upsilon_{nitromethane}$, by subtracting the shielding value of nitromethane, −135.8 ppm, obtained from the literature,[22] $\upsilon_{nitromethane} = -(\sigma_{calcd} + 135.8$ ppm).

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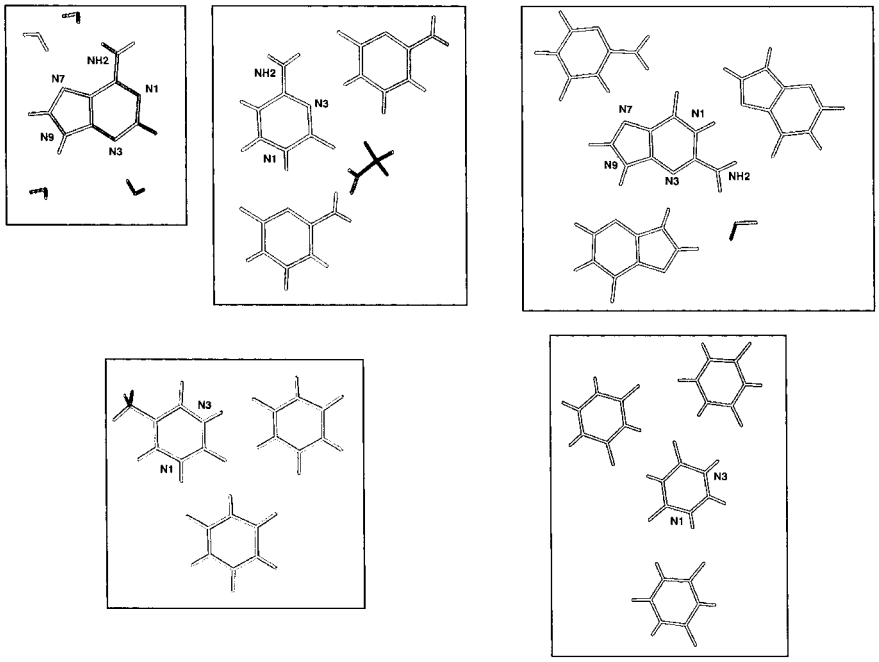

Figure 2. Numbering scheme for adenine (UL), cytosine (UC), guanine (UR), thymine (LL), and uracil (LR) used in this paper. The figure also shows the molecules added to create the models used to simulate the intermolecular interactions.

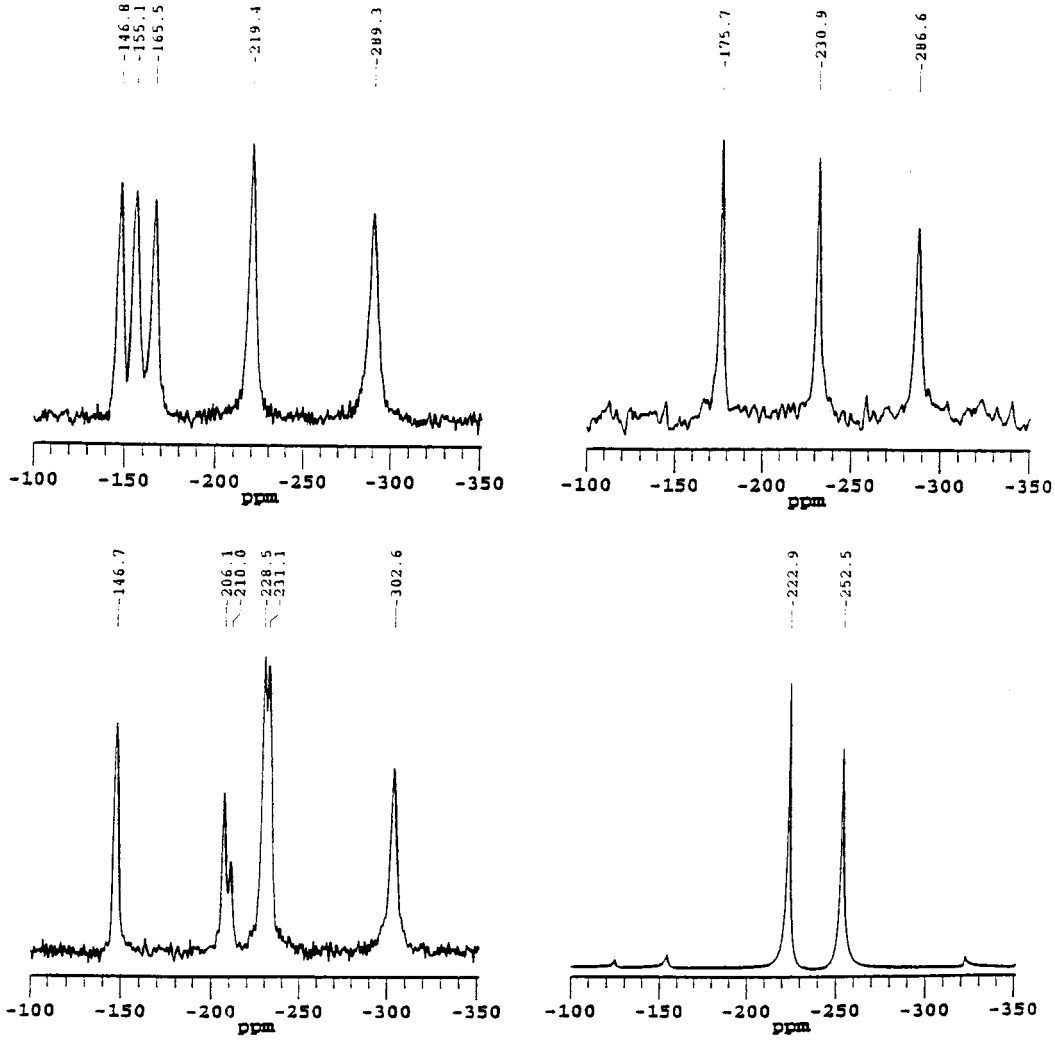

Figure 3. MAS spectra for adenine (UL), cytosine (UR), guanine (LL), and thymine (LR).

Calculations for the isolated molecules were done using molecular optimized geometries (OPT) obtained with the same computational method and basis set employed in the shielding calculations and using the X-ray structures (X-ray) taken from

the literature, i.e.; adenine,[23] cytosine,[24] guanine,[25] thymine,[26] and uracil.[27] Additional calculations were performed using model systems in which the intermolecular effects on the chemical shifts were taken into account by surrounding the molecule with molecular fragments representative of the nearest neighbors. Two molecular models were constructed, one using the corresponding X-ray structures (model A) as they are depicted in Figure 2 and a second one, (model B), in which the position of the protons in model A were optimized before performing the chemical shielding calculations.

Results and Discussion

Spectral Assignments. Figure 3 presents the CP/MAS spectrum of the four nucleic acid bases measured here. Table 1 contains the measured shift principal values, isotropic shifts, and MAS chemical shifts. These values are compared with the corresponding calculated values and with the isotropic values from the corresponding nucleosides in solution. The calculated values are those obtained using model B, which provides (see below) the best agreement between experimental and calculated values.

The MAS chemical shift values and the isotropic shifts, calculated as the trace, i.e., $^1/_3$ ($\delta_{11} + \delta_{22} + \delta_{33}$), of the principal values, agree within 2−3 ppm. These differences are comparable to the experimental errors observed in the determination of the principal values. Unfortunately, the difference between the values of the isotropic chemical shifts of the bases measured in the solid compared with the liquid values measured for the nucleosides is quite large, and therefore, the assignment of the solid resonances by comparison with chemical shifts in the nucleosides is not straightforward. These large ^{15}N chemical shift differences can be attributed to both intermolecular effects and the substituent effects in the free bases and the nucleosides. The assignment of the solid-state resonances proceeds as follows.

Adenine. The lines at −289 ppm and −219 ppm are clearly separated and can be assigned to NH_2 and to N9 by simple comparison with the liquid values. The other lines in the spectra are not sufficiently separated to use the chemical shifts of the nucleosides for their assignment. The line at −147 ppm, with a positive δ_{11} shift, can be assigned to N7 by comparison with the calculated principal values of this nitrogen and the value of δ_{11} found in imidazole, i.e., 19 ppm.[6] The lines at −155 ppm and −165 ppm can be assigned by comparing their δ_{22} and δ_{33} principal values with the respective calculated values, which suggest the tentative assignment of N1 (−155 ppm) and N3 (−165 ppm). These assignments are also consistent with the rank order observed in the liquid spectra of adenosine.

Cytosine. The separation of the two ^{15}N resonances in this compound and the excellent agreement between the solid shifts and those in solution for cytidine, makes the assignment of the two resonances obvious.

Guanine. The peaks at −146 ppm and −303 ppm are well separated, and they can be assigned to the N7 and NH_2 resonances, respectively, by comparison with the liquid values. The peak at −206 ppm is also well separated and can be assigned to N3 by comparison with the liquid value and its large δ_{33} principal component with the calculated principal values.

Table 1. Experimental and Calculated ^{15}N Principal Values of the Chemical Shifts in Nucleic Acid Bases[a]

		calcd	exptl	iso. (calcd)	iso. (exptl)	MAS	liquid
adenine N1	δ_{11}	15	−16	−138	−158	−155	−144
	δ_{22}	−62	−76				
	δ_{33}	−367	−373				
N3	δ_{11}	−20	−26	−158	−167	−165	−157
	δ_{22}	−76	−79				
	δ_{33}	−379	−391				
N7	δ_{11}	72	31	−128	−149	−147	−140
	δ_{22}	−82	−98				
	δ_{33}	−373	−373				
N9	δ_{11}	−151	−142	−223	−221	−219	−209
	δ_{22}	−218	−200				
	δ_{33}	−299	−317				
NH_2	δ_{11}	−257	−221	−299	−291	−289	−297
	δ_{22}	−312	−324				
	δ_{33}	−328	−324				
cytosine N1	δ_{11}	−149	−147	−226	−231	−231	−230
	δ_{22}	−209	−211				
	δ_{33}	−320	−335				
N3	δ_{11}	−47	−61	−168	−176	−176	−172
	δ_{22}	−134	−139				
	δ_{33}	−324	−328				
NH_2	δ_{11}	−240	−216	−291	−287	−287	−289
	δ_{22}	−308	−306				
	δ_{33}	−324	−338				
guanine N1	δ_{11}	−154	−159	−227	−231	−231	−233
	δ_{22}	−244	−232				
	δ_{33}	−283	−302				
N3[b]	δ_{11}	−120	−86	−220	−206	−206	−215
	δ_{22}	−177	−160				
	δ_{33}	−362	−372				
N7	δ_{11}	48	6	−131	−146	−147	−133
	δ_{22}	−85	−102				
	δ_{33}	−357	−343				
N9	δ_{11}	−155	−135	−221	−229	−228	−211
	δ_{22}	−211	−227				
	δ_{33}	−298	−324				
NH_2	δ_{11}	−271	−250	−305	−303	−302	−307
	δ_{22}	−305	−320				
	δ_{33}	−339	−338				
thymine, N1	δ_{11}	−191	−168	−253	−251	−253	−236
	δ_{22}	−248	−258				
	δ_{33}	−321	−328				
N3	δ_{11}	−145	−153	−214	−222	−223	−224
	δ_{22}	−225	−231				
	δ_{33}	−273	−282				
uracil,[c] N1	δ_{11}	−155	−162	−236	−245	na	−236
	δ_{22}	−246	−244				
	δ_{33}	−307	−328				
N3	δ_{11}	−148	−158	−212	−221	na	−222
	δ_{22}	−218	−227				
	δ_{33}	−269	−279				

[a] Values in ppm. The liquid chemical shifts are from the corresponding nucleosides, taken from ref 2. The calculated values are those obtained using model B. [b] An additional line at −210 ppm has principal values −91, −167 and −373 ppm. [c] Experimental values from ref 5.

The less intense peak at −210 ppm is also attributed to N3, but its origin is unclear. Most likely, it is a consequence of magnetic inequivalences at the N3 site, but without a proper X-ray characterization of the NMR sample, it is not possible to determine its exact origin. The peaks at −228 ppm and −231 ppm are too close to make any conclusive assignment. Moreover, their principal components are quite similar, and therefore, the calculated values cannot be used to secure any valuable information on the assignment of these resonances. For purposes of comparison with the calculated values, the resonances at −231 ppm and −228 ppm have been assigned to N1 and N9, respectively. However, this arbitrary assignment, which follows the rank order observed in the liquid spectra of

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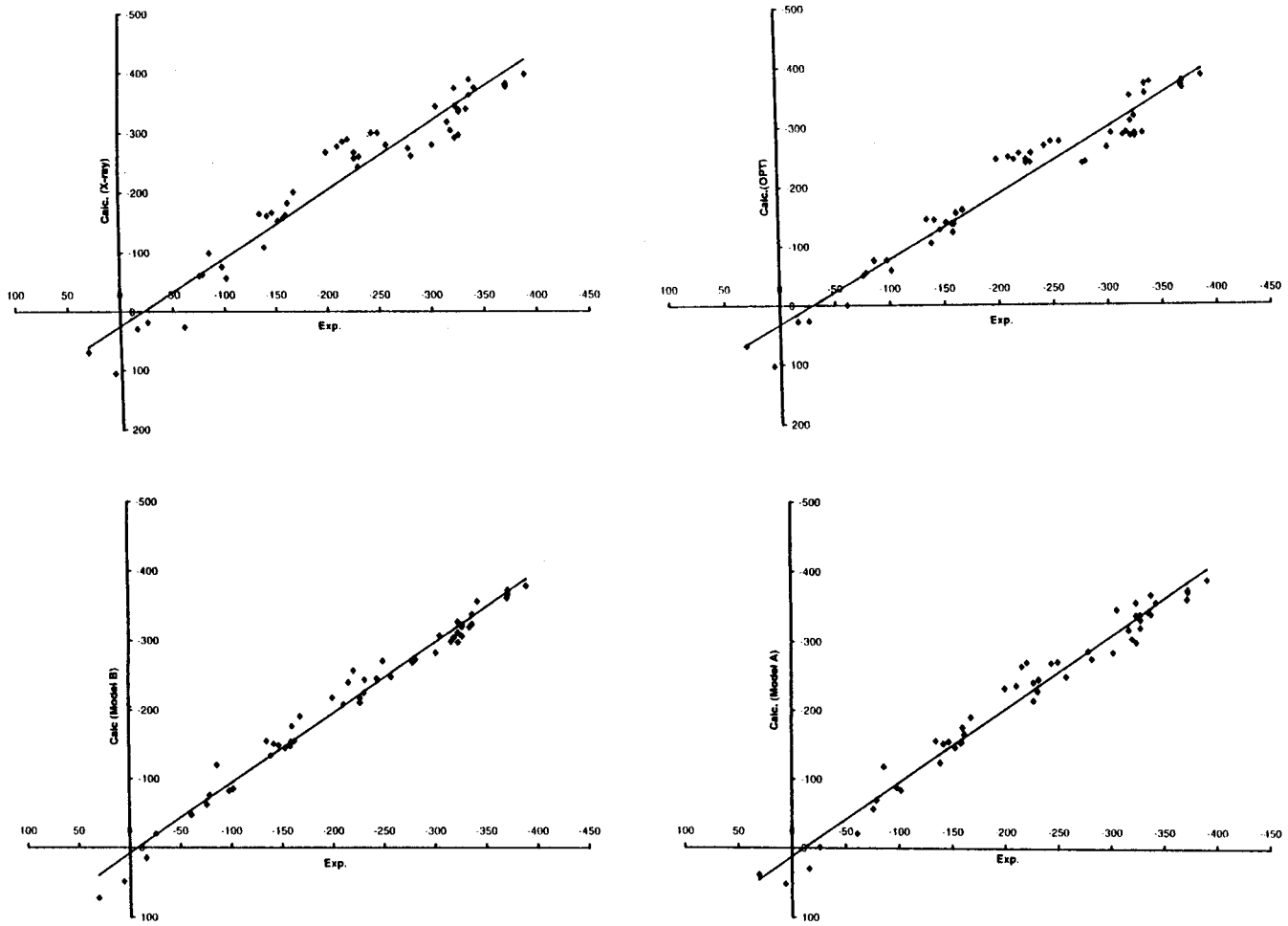

Figure 4. Comparison between experimental and calculated ^{15}N chemical shift principal values for different molecular models. See text for the description of the molecular models used in the calculation of the ^{15}N chemical shifts.

Table 2. Correlation Parameters between Experimental and Calculated ^{15}N Chemical Shift Principal Values in Nucleic Acid Bases[a]

model	slope	intercept	rms
X-ray	1.15 ± 0.04	26 ± 10	33.6
OPT	1.10 ± 0.04	33 ± 9	28.7
A	1.07 ± 0.03	12 ± 6	20.4
B	1.02 ± 0.02	8 ± 5	16.6

[a] rms and intercept in ppm, models described in the text, models A and B include intermolecular interactions.

gaunosine and the calculated values in guanine, does not influence the quality of the agreement between experimental and calculated values (see below).

Thymine. The two resonance lines in the spectrum, at −253 ppm and −223 ppm, are well separated and can be assigned with confidence to N1 and N3, respectively, by comparison with the calculated and liquid values.

Agreement between Experimental and Calculated Values. In Figure 4, the correlation between the experimental and calculated ^{15}N chemical shift principal values is presented for the different molecular geometries and models used in this work. The corresponding parameters of the correlations between experimental and calculated values are presented in Table 2. From these results, it is apparent that the introduction of the intermolecular effects in the calculations plays a significant role in improving the correlation between the experimental and calculated values. Nonetheless, the calculations using model B still exhibit larger standard deviations than those observed in the calculation of ^{13}C chemical shifts.[7] Factors that may contribute to the larger discrepancies between calculated and experimental values in the nitrogen case are, (i) the higher sensitivity of ^{15}N chemical shifts to the intermolecular effects due to the higher polarizability of the nitrogen and (ii) the use of experimental X-ray information that may not be consistent with the crystalline state of the samples used in the NMR work. Note that the samples used in the NMR experiments have not been characterized by X-ray spectroscopy. The X-ray data used in the calculations corresponds in most cases to hydrates of the free bases. For adenine, the addition of free radicals to decrease the proton relaxation times may also cause unknown changes in the crystalline lattice. Even so, when taking into account the large range of ^{15}N chemical shifts values (∼450 ppm in these samples), the discrepancies between the experimental and theoretical data are only of the order of 4−5%. This value can be compared with the 2−3% deviations observed in the ^{13}C shift tensor values calculated using structures that have been carefully characterized. Hence, considering the level of inaccuracy in the structures used in this study, the quality of the correlation may be considered quite remarkable.

Orientation of the Principal Values. Experimental powder methods have no capacity to provide any information on the orientation of the chemical shift principal values; therefore, all of the information regarding the orientation of these principal axes is derived from the calculations. In the compounds of this study, the orientation of the principal axes follows the general trends found in the previous study of nitrogen heterocycles.[6]

Table 3. Comparison of the Calculated ^{15}N Chemical Shift Principal Values in Nucleic Acid Bases Using the X-ray Geometry with and without Including Intermolecular Interactions in the Calculations[a]

		X-ray	model A	Δ
adenine N1	δ_{11}	30	29	−1
	δ_{22}	−60	−57	3
	δ_{33}	−378	−376	2
N3	δ_{11}	19	−1	−20
	δ_{22}	−62	−70	−8
	δ_{33}	−399	−391	8
N7	δ_{11}	70	38	−32
	δ_{22}	−75	−88	−13
	δ_{33}	−383	−374	9
N9	δ_{11}	−162	−152	10
	δ_{22}	−268	−233	35
	δ_{33}	−319	−319	0
NH_2	δ_{11}	−290	−271	19
	δ_{22}	−345	−340	5
	δ_{33}	−375	−358	17
cytosine N1	δ_{11}	−167	−155	12
	δ_{22}	−278	−237	41
	δ_{33}	−340	−345	−5
N3	δ_{11}	27	−21	−48
	δ_{22}	−109	−124	−15
	δ_{33}	−339	−334	5
NH_2	δ_{11}	−286	−265	21
	δ_{22}	−344	−348	−4
	δ_{33}	−390	−369	21
guanine N1	δ_{11}	−161	−154	7
	δ_{22}	−261	−246	15
	δ_{33}	−281	−286	−5
N3	δ_{11}	−99	−118	−19
	δ_{22}	−163	−176	−13
	δ_{33}	−381	−363	18
N7	δ_{11}	106	51	−54
	δ_{22}	−56	−84	−28
	δ_{33}	−376	−358	18
N9	δ_{11}	−165	−156	−9
	δ_{22}	−258	−215	43
	δ_{33}	−293	−301	−8
NH_2	δ_{11}	−301	−272	29
	δ_{22}	−305	−306	−1
	δ_{33}	−364	−342	−22
thymine N1	δ_{11}	−201	−191	10
	δ_{22}	−281	−250	31
	δ_{33}	−298	−322	−24
N3	δ_{11}	−154	−146	8
	δ_{22}	−243	−229	14
	δ_{33}	−262	−276	−14
uracil N1	δ_{11}	−183	−166	17
	δ_{22}	−301	−270	31
	δ_{33}	−336	−341	−5
N3	δ_{11}	−159	−153	6
	δ_{22}	−268	−242	26
	δ_{33}	−275	−288	−13

[a] All values in ppm referenced to nitromethane as explained in the text. X-ray and model A geometries/models used for the calculations are described in the text. $\Delta = \delta$ (model A) $- \delta$ (X-ray).

For protonated nitrogens, δ_{11} lies approximately along the N−H bond, δ_{22} is in the plane of the molecule and approximately perpendicular to the N−H bond, and δ_{33} is nearly perpendicular to the plane of the ring. In the nonprotonated nitrogens, the orientation of δ_{11} and δ_{22} is reversed, while δ_{33} remains about perpendicular to the molecular plane. The calculated orientations of the principal values presented here are also in agreement with the orientations determined in previous calculations on these compounds using the IGLO method.[4]

Calculated Intermolecular Effects on the Principal Values. Table 3 displays a comparison of the calculated ^{15}N chemical shift principal values in nucleic acid bases using the X-ray geometry with and without including the intermolecular interactions in the calculations. The X-ray structure has been used for both calculations to isolate the direct intermolecular effects from those that may arise indirectly through changes in the molecular geometries induced in the calculations when optimized geometries are used. The results presented here were not corrected by BSSE (basis set superposition error)[28] because previous experience[29] shows that these corrections (a few ppm) are much smaller than the large (10−20 ppm) intermolecular effects observed in the ^{15}N chemical shifts.

For the compounds studied, the most important intermolecular interaction arises from hydrogen bonding (HB) between adjacent molecules or with water molecules occluded in the crystalline structure. According to the results in Table 3, the calculations predict significant intermolecular effects for all of the nitrogen shift tensors considered here, except for N1 in adenine. This is not unduly surprising because A:N1 is the only nitrogen without a HB interaction (see Figure 2). The intermolecular effects for the other nitrogens show significant similarities within each type of nitrogen. For the protonated (N−H) nitrogens, which behave as proton donors, the δ_{22} principal shift component shows the largest intermolecular effects. This component lays approximately perpendicular to the N−H direction which is always along the direction of the HB forces, and it shows positive Δ values, i.e., the HB interaction produces a deshielding in this direction. The deshielding effect ranges from 43 to 14 ppm. The δ_{11} shift component also exhibits positive but smaller Δ values, ranging from 17 to 6 ppm, while the component perpendicular to the plane of the ring, δ_{33}, show negative Δ values ranging from 0 to −24 ppm.

For the nonprotonated nitrogens, which behave as proton acceptors, the HB effects on the in-plane shift components, δ_{11} and δ_{22}, are negative with the exception of δ_{22} in adenine N1. They range from −55 to −1 ppm for δ_{11} and from −28 to +3 ppm for δ_{22}. The effects on the perpendicular component are positive, ranging from 7 to 18 ppm. For the nonprotonated nitrogens, the largest effects are observed along a direction perpendicular to the HB in the plane of the molecule, a similar effect to that noted for the protonated nitrogens. For the NH_2 nitrogens, δ_{11} and δ_{33}, which lie in the amine plane, show positive Δ values ranging from 17 to 29 ppm and δ_{22}, which lies perpendicular to the amine plane shows very small Δ values ranging from −4 to 5 ppm.

From the results presented above, the following qualitative observations follow: (i) the largest HB effects are observed in the chemical shift components that are in the plane of the molecule and perpendicular to the direction of the HB and (ii) for NH and NH_2, which act as H donors, the HB interaction decreases the chemical shielding along the directions in the molecular plane and slightly increases the shielding in the perpendicular direction. For nonprotonated nitrogens that act as H acceptors, the shielding effects of the HB interactions behave in the opposite way.

There is strong evidence that intermolecular effects are important in determining the ^{15}N chemical shifts of free nucleic acid bases and that some systematic trends appear from the analysis of the calculated chemical shift principal values. The results also indicate that the HB effects on the ^{15}N chemical shift are highly anisotropic. Unfortunately, the absence of experimental values for the chemical shifts in an inert environment and the lack of characterization of the crystalline lattice of the NMR samples precludes an extensive investigation of

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Table 4. Comparison of the Calculated ^{15}N Chemical Shift Principal Values in Nucleic Acid Bases by Nitrogen Type[a]

	δ_{11}	δ_{22}	δ_{33}	δ_{iso}
6-n				
A: N1	15	−62	−367	−158
A: N3	−20	−76	−379	−167
C: N3	−47	−134	−324	−176
G: N3	−120	−177	−362	−206
pyridine[b]	200	33	−422	−63
average	−43	−112	−358	−177
SD	57	53	24	21
6-p				
C: N1	−149	−209	−320	−231
G: N1	−154	−244	−283	−231
T: N1	−191	−248	−321	−251
T: N3	−145	−225	−273	−222
U: N1	−155	−246	−307	−245
U: N3	−148	−218	−269	−221
pyridinium[b]	−30	−97	−381	−169
average	−157	−232	−296	−234
SD	17	17	23	12
5-n				
A: N7	72	−82	−373	−149
G: N7	48	−85	−357	−146
imidazole[b]	19	−55	−359	−132
average	60	−84	−365	−148
SD	17	2	11	2
5-p				
A: N9	−151	−218	−299	−221
G: N9	−155	−211	−298	−229
imidazole[b]	−118	−186	−320	−208
average	−153	−215	−299	−225
SD	3	5	1	6
NH_2				
A: NH_2	−257	−312	−328	−291
C: NH_2	−240	−308	−324	−287
G: NH_2	−271	−305	−339	−303
benzamide[c]	−176	−321	−326	−274
average	−256	−308	−330	−294
SD	16	4	8	8

[a] All values in ppm. The type of nitrogen is indicated as in ref 6; 6-n, nonprotonated nitrogens in a six-membered ring; 6-p, protonated nitrogens in a six-membered ring; 5-n, nonprotonated nitrogens in a five-membered ring; and 5-p, protonated nitrogens in a five-membered ring. [b] Values from ref 6. [c] Values from ref 11.

the quantitative relationships between the crystalline structure of these compounds and the experimental values of their ^{15}N chemical shifts.

Comparison of Principal Values of Related Compounds. In Table 4 the experimental values of the ^{15}N chemical shift tensor principal values are presented by nitrogen type using the notation from ref 6. The values can be compared with their average values, their standard deviations, and the chemical shift values in similar nitrogens. Unfortunately, the comparison with values from the literature is quite sparse because there have been very few reported studies of the ^{15}N chemical shift tensors. From the table, the following observations follow: for nitrogens in six-membered rings, the standard deviations of the principal values are quite large, in some cases a factor of 2 larger than those of the isotropic values. But by comparing the average shift tensor values in nucleic acid bases with those in pyridine and pyridinium, it is apparent that the in-plane values in the bases are quite different from those in the pyridinic compounds. The differences between the principal values in pyridine and pyridinium and the corresponding average values in the nucleic acid bases are always larger than the corresponding standard deviations. For the nitrogens in five-membered rings, the standard deviations in the principal components are smaller and closer to those observed for the isotropic values. In this case, significant differences are also observed (albeit, these differences are smaller than those for the nitrogens in the six-membered rings) between the principal values in imidazole and the average values in the nucleic acid bases. From these observations, it is apparent that the ^{15}N chemical shift principal values are quite sensitive to small variations in the molecular structure and environment and that, even within the same nitrogen type, variations of 50 to 100 ppm can be expected. A better understanding of the factors determining these large changes in the chemical shifts of similar nitrogens must wait for the development of a comprehensive collection of ^{15}N chemical shift data in a larger set of molecules.

Conclusions

The results presented in this paper show that it is possible to measure the principal values of ^{15}N chemical shifts in nucleic acid bases using natural abundance and/or labeled materials, depending on the relaxation times and complexity of the compound. The experimental results show that the ^{15}N chemical shifts in nucleic acid bases are very sensitive to small changes in molecular geometry and environment. Even for nitrogen with similar hybridization, differences of up to 50 ppm can be expected in the corresponding principal shift components. The calculations presented here using different molecular models also support this claim. The agreement between the experimental and calculated values is highly dependent on the molecular model used in the calculations, and molecular models that include the intermolecular interactions show superior correlation with the experimental values.

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