energy will increase significantly due to much higher RR scattering intensity of the reduced form which implied a larger magnitude of $(\partial\alpha/\partial q)^2$ and thus larger dispersion-induced frequency shifts. The combined effect of these changes in repulsive and dispersion energies may result in a low-frequency shift of the Raman bands in the reduced form of the Cyt-c_3 compared to the Cyt-c. As the contribution of the induction term to the interaction energy depends on the dipole moment, it is expected to be much smaller than the dispersion term for nearly D_{4h} symmetry of individual hemes. Therefore, we conclude that the dispersion and repulsive terms contribute predominantly in the heme–heme vibrational coupling in Cyt-c_3.

The earlier attempts to detect vibrational coupling in the μ-oxo and μ-nitrido dimers of iron protoporphyrin[33] and μ-oxo dimer of iron tetraphenylporphyrin[34] were unsuccessful. Since most of these investigated dimers possess D_{4d} symmetry[35a,b] one could expect to observe only the in-phase component of vibrations of the two hemes in Raman scattering even if there were splittings of modes due to vibrational interactions. Although hemoglobin has four hemes in a molecule, each heme is surrounded by different polypeptide chains and direct heme–heme interaction cannot be expected. Cytochrome oxidase has two heme groups in a protein with different spin states and therefore vibrational coupling may not be detected. To our knowledge, other multi-heme systems have not been examined so carefully, specially with regard to the line width of Raman bands vis-ā-vis their corresponding monoheme systems.

Summarizing the above discussion, we have shown that the RR technique can be used, in suitale situations, to detect vibrational coupling in a multiheme system and to probe the electron-transfer process in redox proteins. The repulsive and dispersion interactions contribute predominantly for the heme–heme interaction in Cyt-c_3. Our conclusions about heme–heme interactions are also supported by an observation of exciton splitting of the Soret band in circular dichroism,[4] Mössbauer,[10] and other studies.[13]

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Registry No. Cyt-c_3, 9035-44-3; heme, 14875-96-8.

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Magnetic Properties in Terms of Localized Quantities. 9. The DNA Bases and the Protonation of Adenine

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Abstract: Chemical shift tensors and magnetic susceptibilities are calculated for some purine and pyrimidine bases by means of the IGLO method. The molecules studied are cytosine, uracil, thymine, adenine, and guanine. Protonation shifts of the ^{13}C and $^{14,15}N$ NMR spectra are calculated for adenine, protonated at N-1, N-3, and N-7. From a comparison with experimental data it is concluded that the second protonation site of adenine is N-3, N-7 being only marginally involved. It is demonstrated that double-ζ basis sets are sufficient only for the calculation of ^{13}C shifts, whereas triple-ζ plus polarization sets are necessary to achieve good agreement with experimental data for nitrogen (and oxygen) NMR shifts.

I. Introduction

Together with the two pentoses 2-desoxy-D-ribose and D-ribose, and with phosphoric acid, the pyrimidine bases thymine (T) (**1**), cytosine (C) (**2**), and uracil (U) (**3**) and the purine bases adenine (A) (**4**) and guanine (G) (**5**) are the essential constituents of the nucleic acids RNA and DNA. Hydrogen bonds between the pairs A-T and G-C are responsible for the helical structure of DNA.

The biochemical and pharmaceutical importance of the nucleobases is a challenge to theory to gain a more than qualitative insight into their spectroscopic properties. Nuclear magnetic resonance spectroscopy (^{13}C, $^{14,15}N$, ^{17}O) has become an extremely powerful tool for the elucidation of complex molecular structures and dynamical processes within the last few years. On the theoretical side gradient techniques facilitate the ab initio geometry optimization of medium sized molecules at the Hartree–Fock level.

However, ab initio calculations of magnetic properties like susceptibilities or NMR chemical shifts for molecules of this size have been very rare. This is due to the fact that conventional theories using one single gauge origin for the external magnetic field suffer from the lack of gauge invariance, i.e., magnetic properties, calculated with finite basis sets, are strongly dependent on the choice of the origin for the vector potential describing the external magnetic field. Even for small molecules large basis sets are necessary to arrive at results that are sufficiently invariant, but for the molecules of this study it is impossible to use methods with one common vector potential.

These computational difficulties have been overcome by the IGLO method.[1] (IGLO stands for *i*ndividual *g*auge for *l*ocalized molecular *o*rbitals.) We have applied it rather successfully to a number of different types of molecules and problems,[2,3] and in this paper we investigate the NMR chemical shift tensors for molecules **1–5**.

Up to now, the consecutive steps, i.e., investigations—on the same level of accuracy—of the pairs A-T and G-C as well as a study of the nucleosides corresponding to the bases **1–5** are beyond our possibilities. What we are intending to do in the near future is to calculate the NMR protonation shifts of all DNA bases. In the present paper we report on protonation effects in adenine **4**.

To give an example for the tremendously increasing demand in CPU time and peripheral storage, we estimate that one A-T or G-C IGLO calculation would require roughly 100 h of billing units at a CDC 205 vectorcomputer with 2MW core storage, and 10^9 two-electron integrals would have to be stored or recomputed in each iteration cycle. Vectorcomputers of the next generation,

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Table I. Geometries Used and Corresponding SCF Energies[a]

no.	molecule	geometry	DZ	II″	II′	II
1	cytosine	4	−392.041 24			
		5	−392.039 28	−392.661 24	−392.684 95	−392.695 51
2	thymine	8	−450.849 68		−451.589 97	
		6	−450.837 79			
3	uracil	6	−410.881 14		−412.545 38	
4	adenine	8	−463.845 05			
		7	−463.849 83		−464.602 34	
		6	−463.845 17			
5	guanine	8	−538.598 70	−539.461 73		

[a] Energies are in atomic units.

however, should be able to do this job in about 10–20 h.

This paper is organized as follows. In the next section we introduce our sign and naming conventions, geometries and basis sets used, and the IGLO terminology. After a short discussion of magnetic susceptibilities and characteristics of the magnetic shielding tensors common to all five molecules in section III, we will discuss ^{13}C and $^{14,15}N$ chemical shifts of the pyrimidine and purine bases and the effects arising from protonating adenine at the nitrogen atoms N-1, N-3, and N-7. Our Conclusions will be given in section IV.

II. Method, Basis Sets, and Geometries

The IGLO method for the ab initio calculation of 2nd-order magnetic properties is essentially of coupled Hartree–Fock type. We have described it in detail elsewhere,[1] so we can restrict ourselves to a short introduction of our nomenclature and our sign conventions.

In the IGLO framework we calculate the magnetic susceptibility χ as a sum of diamagnetic, paramagnetic, and nonlocal terms. The chemical shielding σ(A) of a nucleus A in a molecule consists of dia- and paramagnetic terms only. Our sign convention is such that diamagnetic (shielding) contributions are positive. Units are ppm cgs/mol for χ, whereas the dimensionless quantity σ (or δ) is given in ppm.

To establish relative NMR scales for our calculated absolute shieldings we use the following reference molecules: tetramethylsilane ($Si(CH_3)_4$) for ^{13}C and ^{29}Si, ammonia in its r_z geometry (NH_3) for ^{14}N and ^{15}N, and water (H_2O) for ^{17}O. Their NMR shieldings, calculated with the respective basis set, are taken as relative shifts of zero.

Although, to the best of our knowledge, for the DNA bases no experimental data on principal values of either carbon or nitrogen NMR shifts are available up to now, we have to mention that χ and σ are second-rank tensors. Anisotropies $\Delta\chi$ and $\Delta\sigma$ are defined as linear combinations of the respective principal values, i.e., $\Delta\sigma_1 = 2\sigma_{11} - \sigma_{22} - \sigma_{33}$, and $\Delta\sigma_2$ is defined analogously. In the experimental literature they are sometimes not given in their respective principal axis systems but in the molecular inertial or some other axis system. In our tables we list the principal values of the symmetric part of σ only, despite the fact that σ is, contrary to χ, an asymmetric tensor with nine independent components in general. Although $\mathrm{Tr}(\sigma)$ is invariant, the principal values of the unsymmetric tensor may differ from the symmetric ones.

Huzinaga Gaussian lobe basis sets[4] where used as follows:

Basis DZ:	C, N, or O	7s3p contracted to [4111,21]
	H	3s contracted to [21]
Basis II:	C, N, or O	9s5p1d contracted to [51111,2111,1], d-exponent: 1.0
	H	5s1p contracted to [311,1], p-exponent: 0.7
Basis II′:	C, N, or O	as basis II
	H	5s contracted to [311]
Basis II″:	C, N, or O	as basis II
	H	as basis DZ

In Table I we list the geometry references together with the corresponding SCF energies obtained with the basis sets DZ to II.

The experimental geometries[5-7] are mainly from crystal structure investigations of the corresponding dinucleosides and not of the bases themselves. This fact leads to an additional amount of uncertainty in explaining the observed differences between our calculated shieldings and the experimental data. Theoretical geometries,[8] optimized at the STO-3G level, have been used for N-1-, N-3-, and N-7-protonated adenine and, in order to get a feeling of the quality of the calculated geometries, for the neutral bases **1–5**, too.

Table II. Susceptibilities of the DNA Bases[a]

no.	molecule	χ_{DZ}	χ_{11}	χ_{11}	χ_{22}	χ_{33}
1	cytosine	76.0	66.6	84.2	58.3	57.2
2	thymine	85.0	76.0	90.4	74.5	63.1
3	uracil	69.8	62.5	77.1	56.6	53.9
4	adenine	102.0	91.0	141.6	66.6	64.7
5	guanine	105.7	*b*	149.9	87.1	79.9

[a] IGLO calculations with basis DZ (χ_{DZ}) and the respective best basis (χ_{II}). For units and sign conventions see text. The principal values (χ_{ii}, i = 1, 2, 3) are those obtained with the best basis sets. [b] Due to an error, the calculated basis II″ results for χ were overwritten by the program. In view of the considerable CPU time for this calculation we did not repeat it.

III. Discussion

1. Magnetic Susceptibilities. As there are no experimental data available on magnetic susceptibilities χ of DNA bases, we briefly discuss the basis set dependence of χ and its principal components χ_{ii} (i = 1, 3) as resulting from the calculations (Table II).

Using basis II (or II′) instead of basis DZ leads to a decrease in the susceptibility by about 10%. The pattern observed for the principal values χ_{ii}, a dominating, strongly diamagnetic component χ_{11} perpendicular to the molecular plane, and two smaller in-plane components of comparable magnitude, $\chi_{22} \approx \chi_{33}$, is similar to that of other heterocycles,[9] though more pronounced here. Thymine is somewhat exceptional because of its methyl group, which disturbes the planarity of the molecule.

2. Chemical Shifts. General Considerations. In addition to the "normal" experimental difficulties in obtaining ^{13}C and $^{14,15}N$ NMR spectra, i.e. complicated couplings due to the large number of nonequivalent nuclei for ^{13}C, quadrupolar broadening for ^{14}N, low abundance for ^{15}N, and low solubility for all DNA bases, several complications in the interpretation of the observed specra arise, which are due to the fact that different tautomeric equilibria are present under different experimental conditions for the pure DNA bases. By investigating nucleosides and nucleotides problems with tautomerism are reduced, but questions concerning the amount of protonation and deprotonation as well as the corresponding sites in the molecules remain, however.

In our calculations we determine the magnetic properties of an isolated molecule, frozen at a fixed geometry. Generally the latter is either a theoretically optimized structure or a geometry taken from microwave studies, or as for the neutral molecules of this study, it is obtained from crystal structure investigations. Unfortunately, for bases **1–5** the crystal structures available are those of the corresponding nucleosides or dinucleotides, and not of the bases themselves. This has to be kept in mind when we discuss our results and compare them with the experimental data.

Before treating each molecule in more detail, we want to comment on some general features shown by the shielding tensors of all bases (Figures 1–4).

The directions of the principal components of σ show a characteristic pattern. With the exception of the amine nitrogens and methyl carbons, the most shielded component σ_{11} is perpendicular

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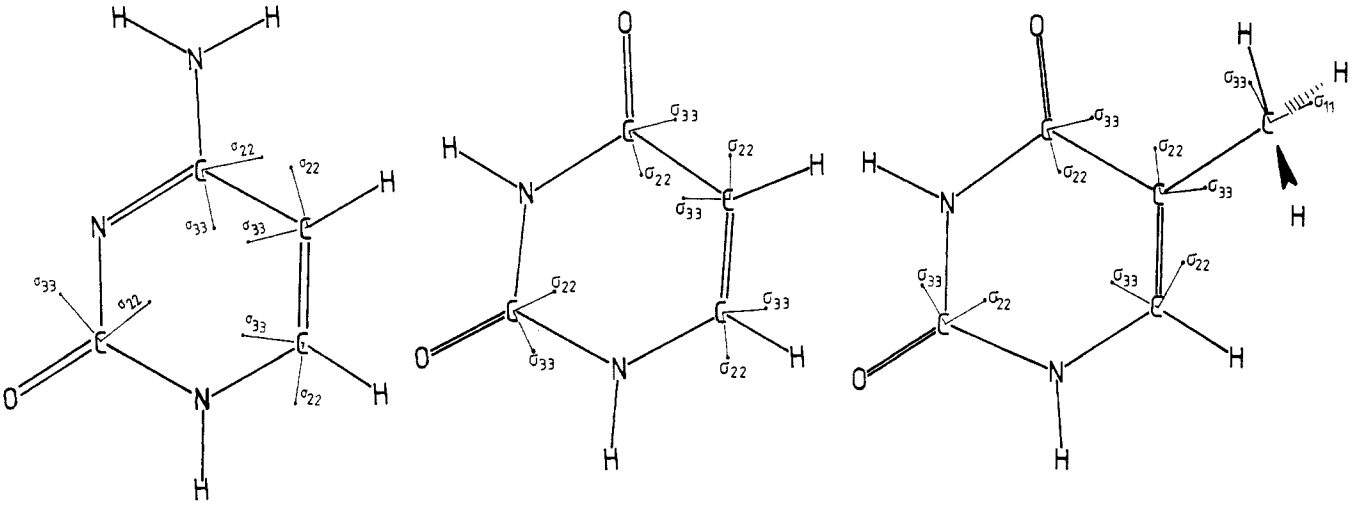

Figure 1. Principal carbon shielding axes in the pyrimidine bases as obtained from the symmetric part of the shielding tensors. The absolute shielding decreases in the series σ_{11}, σ_{22}, σ_{33}.

Table III. ^{13}C Chemical Shifts of DNA Bases[a]

no.	molecule		C-2	C-4	C-5	C-6	C-8	CH_3
1	cytosine	DZ	173.6	180.2	95.8	150.9		
		II″	166.5	172.0	85.0	150.5		
		II′	166.7	172.9	85.7	151.2		
		II	167.5	174.2	87.2	152.2		
		GIAO[f]	157	165	83	144		
		exptl[b]	166.9	171.0	93.9	158.1		
		exptl[g]	156.0	165.7	91.6	141.9		
		exptl[b,h]	156.9	166.7	95.7	142.8		
2	thymine	DZ	167.3	178.5	108.3	141.4		8.7
		II′	158.7	171.3	107.7	139.1		3.7
		exptl[b]	150.4	163.8	106.7	136.6		
		exptl[c]	154.04	168.71	111.93	140.66		12.52
3	uracil	DZ	167.8	173.3	106.2	145.7		
		II′	158.7	166.5	101.2	143.8		
		exptl[b]	152.7	165.2	100.9	143.0		
		exptl[c]	154.1	168.6	102.3	144.2		
4	adenine[g]	DZ	172.6	159.9	110.7	167.8	150.1	
		II′	165.5	156.3	107.9	164.1	141.4	
		exptl[k]	153.4	152.1	118.6	156.4	140.4	
		exptl[b]	152.4	151.3	117.6	155.3	139.3	
		exptl[e]	150.5	155.0	121.0	160.4	153.7	
		exptl[i]	143.8	150.5	113.5	151.8	145.5	
5	guanine[g]	DZ	173.8	170.4	124.4	159.7	150.1	
		II″	166.4	155.8	118.4	151.6	139.4	
		exptl[k,d]	154.6	152.3	117.5	157.8	136.9	
		exptl[e]	160.0	168.8	119.6	162.2	150.1	
		exptl[i]	155.8	149.6	108.1	153.8	137.8	

[a] In ppm with respect to TMS, the conversion constants $\delta(C_6H_6)$ = 128.5 ppm and $\eta(CS_2)$ = 192.5 ppm have been used when necessary. If not indicated otherwise, the solvent is DMSO. [b] Reference 26. [c] Reference 25. [d] The experimental data are for guanosine. [e] Reference 16, dissolved in D_2O, and NaOD (pD = 13). [f] Reference 11. [g] Reference 13. [h] The data are for cytidine. [g] Note that the numbering is different from the pyrimidine bases. [i] Reference 18, acidified solution. [k] Reference 23.

to the molecular plane. For the former σ_{11} corresponds approximately to the C–NH_2 vic. C–CH_3 bond.

While in the azoles and azines studied previously[9] the least shielding component σ_{33} of $\sigma(^{13}C)$ cum grano salis pointed to the center of the aromatic ring, this is true only for the non-carbonyl C atoms of the pyrimidine bases and those C atoms of the purine bases that are not involved in both rings. The carbonyl C atoms have σ_{22} directed along the C=O bond (Figures 1 and 2).

The directions of the nitrogen shielding tensor components show the same behavior as in the azoles and azines. Pyridine-like N atoms have σ_{22} directed into the ring, while this direction corresponds to σ_{33} for the pyrrole-like nitrogens (Figures 3 and 4).

On the whole, the agreement of our ^{13}C IGLO results and experiment is satisfactory to good for the larger basis sets and the pyrimidine bases, whereas we find deviations of up to 13 ppm for the ^{13}C shifts of adenine and guanine (Table III).

In the case of nitrogen a comparison with experiment is complicated for three reasons:

1. With the exception of adenine, no nitrogen NMR data are available for the DNA bases themselves. We thus have to compare our data with those of the corresponding nucleosides.

2. Nitrogen NMR shifts are much more susceptable to protonation or deprotonation than are carbon shifts. They are hence expected to be considerably more influenced by the solvent used, and by the acidity of the medium.

3. From our previous investigations we know that a correct theoretical description of the nitrogen shielding is more demanding than that of carbon shieldings, irrespective of effects of electron correlation which should play only a minor role for molecules of the type we are interested in here.

From these three reasons it is not surprising that we find deviations of up to 30 ppm between theory and experiment even for the larger basis sets (Table IV).

3. The Pyrimidine Bases Cytosine, Thymine, and Uracil. Despite the fact that we calculate the magnetic properties of a single molecule in space, frozen at a fixed geometry, whereas the

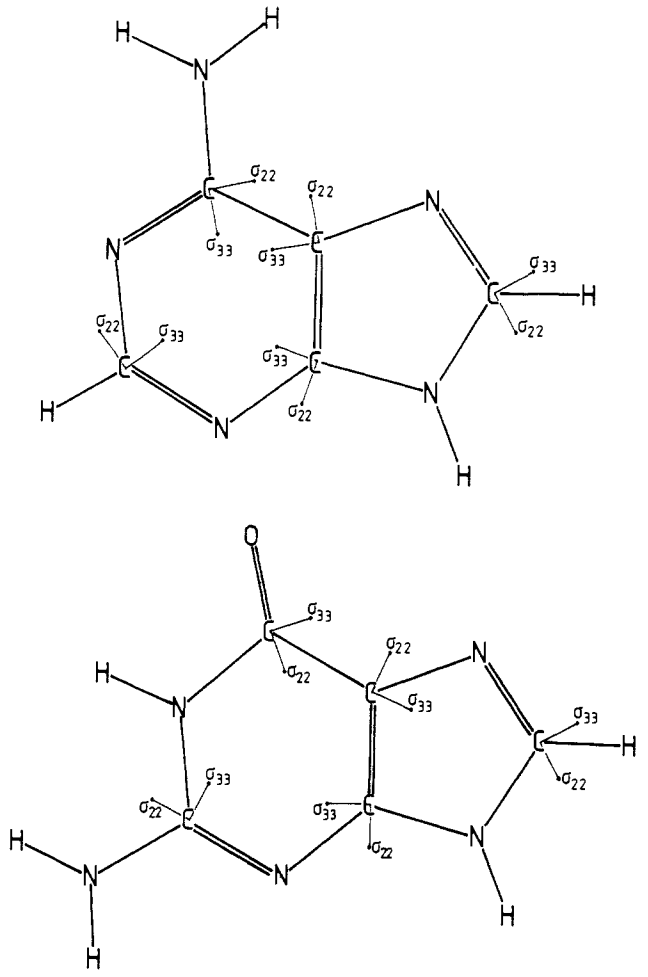

Figure 2. Principal carbon shielding axes in the purine bases as obtained from the symmetric part of the shielding tensors. The absolute shielding decreases in the series σ_{11}, σ_{22}, σ_{33}.

experimental data are recorded in DMSO solution, the agreement between theory and experiment is pretty good for the pyrimidine bases **1–3**, at least as far as ^{13}C is concerned. Obviously, for nitrogen NMR shifts a basis set of DZ quality is insufficent. Relative shifts in pyridine-like N atoms vary only slightly with variation of the basis set size, but for the pyrrole-like nitrogens we find variations of 35 to 45 ppm in the relative shifts, when we use the larger basis sets II″–II.

The different behavior of the two types of nitrogen atoms with respect to the quality of the basis used requires special care when we try to calculate protonation shifts, i.e., the influence on ^{13}C, ^{14}N, or ^{15}N NMR shifts of protonating the parent base. We will comment on this in more detail in the next section.

Cytosine. Cytosine has been the subject of previous theoretical investigations.[10,11] Its NMR shielding constants have been calculated by the GIAO (Gauge Invariant Atomic Orbital) method.[12] The split valence AO basis set used, however, was rather small. This means that the paramagnetic contributions to σ are described rather poorly. Consequently, the GIAO results exhibit a systematic shift to high field as compared to our data (Table V, basis II).

This becomes especially clear from a comparison of ^1H shieldings. The computed IGLO values for σ(H) are up to 10 ppm smaller than the GIAO data, only a few ppm of which can be accounted for by the shorter X–H distances used in the GIAO calculations. In the ammonia molecule, for example, the variation of σ(H) with the length of one N–H bond is about −30 ppm/Å. Changing all N–H bonds simultaneously leads to a variation of σ(H) of −40 ppm/Å (IGLO, basis II).

In cytosine, the hydrogen shielding values are (GIAO results in parentheses) $\sigma(N_1H)$ = 23.82 (33.0) ppm, $\sigma(NH_{2a})$ = 25.61 (35.8) ppm, $\sigma(NH_{2b})$ = 26.79 (36.4) ppm, $\sigma(C_5H)$ = 26.41 (33.7) ppm, $\sigma(C_6H)$ = 24.52 (27.1) ppm. Intermolecular effects should reduce the proton shieldings as compared to an isolated molecule. Probably these interactions are stronger for the N–H than for the C–H protons. Accordingly, we find large deviations of 3.1 ppm (N_1H) and 2.0 ppm (NH_2) from experiment[13] for the former and smaller ones of 0.9 ppm (C_5H) and 0.8 ppm (C_6H) for the latter.

Table IV. $^{14,15}N$ Chemical Shifts of DNA Bases[a]

no.	molecule		N-1	N-3	N-7	N-9	NH_2
1	cytosine	DZ	86.2	216.4			41.6
		II″	123.2	207.6			65.9
		II′	126.0	211.0			68.7
		II	127.3	215.4			69.8
		GIAO[f]	91	209			30
		exptl[b,h]	151.8	207.9			93.0
		exptl[c]	152.1	206.7			93.0
		exptl[k]	123 ± 4	189 ± 4			74 ± 4
2	thymine	DZ	105.9	74.2			
		II′	150.0	112.5			
		exptl[b,h]	154.5	142.7			
3	uracil	DZ	100.0	64.6			
		II′	145.1	105.4			
		exptl[b,h]	160.0	132.4			
4	adenine[g]	DZ	223.9	220.9	246.9	95.9	31.3
		II′	243.4	230.6	266.2	139.6	61.6
		exptl[e,h]	235.8	230.8	226.8	170.8	79.8
		exptl[e,i]	224.7	223.8	215.2	172.1	78.0
		exptl[b,h]	237.4	224.5	242.1	170.5	82.0
		exptl[d]	234.4	221.3	239.3	168.4	80.2
5	guanine[g]	DZ	101.8	155.1	281.8	103.3	32.2
		II″	133.7	167.0	297.0	143.4	57.7
		exptl[b,h]	146.0	164.5	245.4	168.7	72.0
		exptl[c]	146.2	164.8	246.0	169.0	72.3

[a] In ppm, experimental data are referenced to liquid NH_3 (using $\delta(NH_3)$ = 380.2 ppm w.r.t. nitromethane), theoretical ones to NH_3 in its r_z geometry. [b] Reference 27, the data are for the corresponding nucleosides. [c] Reference 29, the data are for cytosine and guanosine. [d] Reference 28, the data are for the nucleosides. [e] Reference 15, converted to the NH_3-scale using $\delta(DNO_3)$ = 375.8 ppm. [f] Reference 11, split-valence basis. [g] See footnote *g* of Table III. [h] Solvent DMSO. [i] Solvent neutral water. [k] Reference 31, solvent H_2O at pH 8.

Table V. Principal Values of Shielding Tensors in Cytosine[a,b]

atom	σ	σ_{11}	σ_{22}	σ_{33}	δ
O	−7.3	273.8	−119.2	−176.6	313.1
	12.9	368.2	−144.0	−183.9	370.9
N-1	121.8	192.9	142.1	30.4	127.3
	183.2	255.0	194.3	100.2	91
N-3	33.7	224.1	−10.0	−113.1	215.4
	65.0	273.0	38.0	−116.0	209
NH_2	179.3	244.2	181.9	111.6	69.8
	243.7	303.4	243.8	183.9	30
C-2	25.2	105.7	50.7	−80.8	167.5
	61.7	141.9	92.7	−49.3	157
C-4	18.6	148.7	−7.8	−85.2	174.2
	53.8	175.3	34.4	−48.3	165
C-5	105.5	178.0	124.7	13.7	87.2
	135.6	219.1	144.4	43.4	83
C-6	40.5	162.8	42.6	−84.0	152.2
	75.5	193.5	76.8	−44.0	144

[a] In ppm; for the absolute shielding, $\sigma_{ii} > 0$ corresponds to shielding with respect to the naked nucleus. Reference molecules for relative shifts δ are discussed in the text. [b] IGLO (basis II) and GIAO[11] (split-valence) results are shown in consecutive lines.

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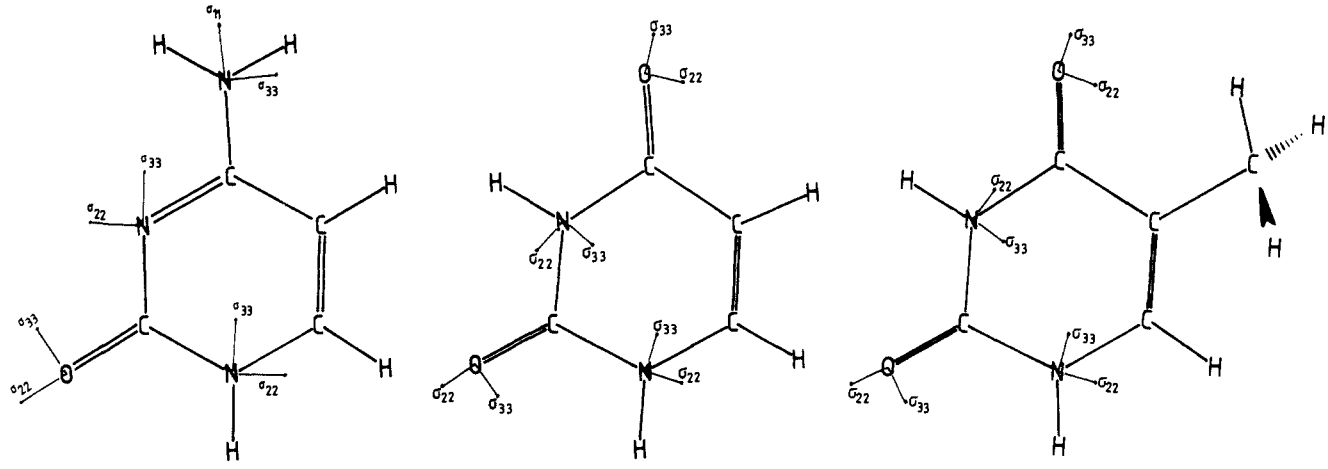

Figure 3. Principal nitrogen and oxygen shielding axes in the pyrimidine bases as obtained from the symmetric part of the shielding tensors. The absolute shielding decreases in the series σ_{11}, σ_{22}, σ_{33}.

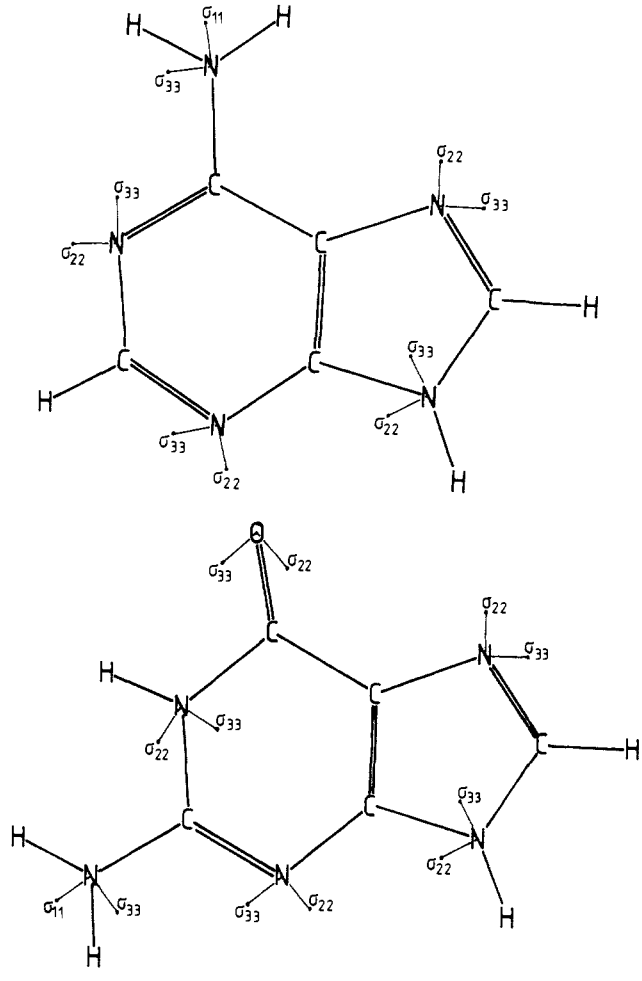

Figure 4. Principal nitrogen and oxygen shielding axes in the purine bases as obtained from the symmetric part of the shielding tensors. The absolute shielding decreases in the series of σ_{11}, σ_{22}, σ_{33}.

It is interesting to notice that basis II gives the correct (experimental) relative order of the proton shieldings in cytosine, i.e., $\sigma(H_5) > \sigma(NH_2) > \sigma(H_6) > \sigma(H_1)$, whereas with the smaller DZ basis we obtain the same wrong one which was found in the split valence GIAO calculation.

The deviations between the IGLO and GIAO calculations (Table V) are smaller by a factor of 2 for σ(C) (30–35 ppm) than they are for nitrogen (10–60 ppm). By converting the absolute shieldings σ to the corresponding relative scales, these deviations are reduced to 6–40 ppm for nitrogen and to 4–9 ppm for carbon. The relative order of the carbon shifts in cytosine as obtained from the IGLO calculations, i.e., δ(C-4) > δ(C-2) > δ(C-6) > δ(C-5), is in good agreement with the GIAO calculations and the experimental data. However, the remaining discrepancies between IGLO and GIAO for nitrogen shifts must be attributed to the insufficient quality of the split valence basis set used in the latter calculations. We have similar difficulties in describing N-1 andNH_2 with the same accuracy as N-3 when we use our DZ basis.

The deviations between IGLO and GIAO are larger for the principal components σ_{ii} than for the isotropic σ itself. We can see no systematics in the varying magnitudes of the differences. With the noteable exception of σ_{22} and σ_{33} at the oxygen atom and σ_{33} in σ(N-3) which are deshielding, the GIAO values for the σ_{ii} are always more shielding than the corresponding IGLO components. In view of the preceding discussion and our previous experiences with nitrogen-containing heterocycles we consider anisotropies $\Delta\sigma$ from calculations with small basis sets as completely unreliable.

Being one of the smaller DNA bases, cytosine was used to test the influence of polarization functions, located at the hydrogen atoms, and the importance of the size of the hydrogen s-basis set, on the calculated ^{17}O, $^{14,15}N$, and ^{13}C shifts. The corresponding basis sets are denoted II′ and II″ respectively. As can be seen from Table III, the resulting variations of the chemical shifts are pretty small. The changes in the directions of the shielding principal axis systems are even smaller. For that reason, and for computational economy, we did not perform basis II calculations on the larger DNA bases.

Thymine and Uracil. The effect of methyl substitution at C-5 on the ^{13}C resonance in C-2 is negligible in thymine as compared to uracil. At C-5, C-4, and C-6 we calculate effects of 6.5, 4.8, and −4.7 ppm, while the experimental data are 9.6, 0.1, and −3.5 ppm (Table III). For nitrogen (Table IV) we find a deshielding effect of 4.9 ppm, whereas the observed shift is shielding by 5.5 ppm. At N-3 our calculation gives 7.1 ppm, to be compared with 10.3 ppm experimentally. However, this agreement is fortituous, since the experimental data correspond to the respective nucleosides.

A rather large change (22 ppm) is found for σ(O) attached to C-2. Contrary to carbon, where the principal values of C-6 are affected isotropically by similar amounts, σ_{22} of O-2 is shifted by about 45 ppm, while σ_{11} is virtually unchanged, and σ_{33} is found downffield by 20 ppm in thymine as compared to uracil (Tables VI and VII).

4. The Purine Bases Adenine and Gaunine. The purines[14] and the purine bases adenine and guanine[15,16–23] have been investigated

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Table VI. Principal Values of Shielding Tensors in Thymine[a]

atom	σ	σ_{11}	σ_{22}	σ_{33}	δ
O=C_2	2.4	299.0	−134.5	−157.3	
O=C_4	−94.7	346.7	−242.8	−388.0	
N-1	97.4	138.7	129.6	23.8	150.0
N-3	134.9	178.8	172.9	52.9	112.5
C-2	33.5	93.2	71.9	−64.7	158.7
C-4	20.9	102.4	49.3	−89.1	171.3
C-5	84.5	167.1	93.4	−7.0	107.7
C-6	53.1	148.0	80.7	−69.5	139.1
CH_3	188.5	204.0	182.2	179.3	3.7

[a] See footnote *a* of Table V.

Table VII. Principal Values of Shielding Tensors or Uracil[a]

atom	σ	σ_{11}	σ_{22}	σ_{33}	δ
O=C-2	24.6	298.8	−88.5	−136.6	
O=C-4	−89.4	336.1	−239.6	−364.6	
N-1	102.3	139.8	139.0	27.9	145.1
N-3	141.9	193.0	160.3	72.5	105.4
C-2	33.5	105.8	61.2	−66.5	158.7
C-4	48.4	163.7	73.2	−91.7	143.8
C-5	91.0	170.7	117.1	−14.7	101.2
C-6	25.7	111.0	52.2	−86.1	166.5

[a] See footnote *a* of Table V.

Table VIII. Principal Values of Shielding Tensors in Adenine and in Protonated Adenine[a]

atom	σ	σ_{11}	σ_{22}	σ_{33}	δ
N-1	3.9	253.9	−94.5	−147.6	243.4
N_1H^+	105.7	200.0	127.2	−10.1	
N-3	16.8	280.7	−81.9	−148.3	230.6
N_3H^+	123.4	216.9	133.9	19.4	
N-7	−18.8	264.0	−73.0	−247.4	266.2
N_7H^+	101.7	202.6	128.8	−26.3	
N-9	107.7	176.4	116.6	30.2	139.6
NH_2	185.8	242.8	191.9	122.8	61.6
C-2	26.7	136.6	24.7	−81.3	165.5
C-4	35.9	145.9	17.6	−55.6	156.3
C-5	84.3	161.6	54.5	36.8	107.9
C-6	28.1	148.1	−12.1	−51.6	164.1
C-8	50.8	132.0	69.0	−48.5	141.4

[a] See footnote *a* of Table V.

both by ^{13}C and ^{15}N NMR spectroscopy. From the theoretical side, geometry optimizations on the HF STO//4-31G level have been performed for neutral and protonated bases.[8] Very recently, even the potential energy surface for the C-G pair, including the effect of partial geometry relaxation, was computed with ab initio methods on loosely coupled arrays of processors (LCAP).[24] Magnetic property calculations of molecules containing nitrogen and oxygen in addition to hydrogen and carbon atoms, however, require larger basis sets than do geometry optimizations. So we can report only on NMR chemical shifts of isolated DNA bases and on calculations of protonation effects on the NMR shifts of adenine (Tables VIII–X).

The experimental ^{13}C shifts of adenine, (**4**) and guanine (**5**) have been obtained in the solvents DMSO and water of varying acidity. Those arising from saturated solutions in DMSO or in neutral water,[23,25,26] which, by the way, differ only marginally from each other (cf. Table III), are probably closest to a hypothetical NMR spectrum of gaseous **4** or **5**.

Table IX. Principal Values of Shielding Tensors in Guanine[a]

atom	σ	σ_{11}	σ_{22}	σ_{33}	δ
N-1	111.1	164.4	79.1	89.8	133.7
N-3	77.8	260.6	10.8	−38.1	167.0
N-7	−52.2	242.5	−113.8	−285.4	297.0
N-9	101.4	166.7	28.8	108.8	143.4
NH_2	187.1	257.6	174.0	129.5	57.7
C-2	19.4	119.3	36.0	−77.7	166.4
C-6	36.4	131.5	28.4	−50.8	155.8
C-5	73.8	135.7	56.1	29.7	118.4
C-4	40.6	135.5	74.9	−88.7	151.6
C-8	52.8	129.0	78.9	−49.4	139.4

[a] See footnote *a* of Table V.

While in adenine the relative order of the ^{13}C shifts remains unchanged upon changing the pH (or pD) value of the solution, in guanine C-2, C-4, and C-8 exhibit large shifts to high field upon basification,[16] and C-6 moves downfield upon acidification.[18] Our calculations give δ(C-4 > δ(C-6) which is observed experimentally only under strongly basic conditions. Because of the size of the molecule, at the moment we cannot carry out calculations of protonation shifts in **5** with sufficient accuracy.

The influence of the solvent used is much more pronounced for nitrogen than for carbon. In neutral water the pyridine-like nitrogens N-1, N-3, and N-7 of adenine exhibit upfield shifts of up to 11.5 ppm as compared to DMSO as solvent[15] (Table IV). This reflects the ability of building hydrogen bonds and is in parallel with the much larger protonation shifts observed for nitrogen than for carbon resonances.

While the ^{13}C shifts of **4** and **5** from our calculations agree reasonably well with experiment, there are remarkable deviations for the nitrogen shifts.[15,27-29] The N atoms of the six-membered rings are described fairly well by theory, whereas the nitrogens N-7 and N-9, both in adenine and guanine, show deviations of about 40 ppm to low (N-7) and 30 ppm to high field (N-9). Fortunately, this does not stem from basis set deficiencies or from shortcomings of the theory but from the fact that in DMSO solution approximately 15% of adenine is present as the N_7-H tautomer.[15] Although we did not perform a calculation on N_7-H adenine, it is most likely that averaging of the corresponding nitrogen NMR shifts would reduce the differences between theory and experiment considerably.

5. The Protonation of Adenine. Using geometries optimized[8] at the STO-3G level, we have calculated the NMR chemical shifts of adenine, protonated at N-1, N-3, and N-7. The theoretical protonation shifts are compared with experimentally observed changes in the nitrogen and ^{13}C NMR spectra obtained upon acidification of the neutral solution of adenine in DMSO or water (Table X).

The IGLO calculations have to be considered as being of preliminary nature, and our protonation shifts can hardly be expected to resemble the experimental ones. The main reason for this restriction results from the fact that in solution simultaneously both tautomeric equilibria (N_7-H and N_9-H adenine) and pH-dependent protonation equilibira $A + H^+ \rightleftharpoons AH^+$ and probably $AH^+ + H^+ \rightleftharpoons AH_2^{2+}$, with at least two protonation sites (N-1 and N-3[15] or N-1 and N-7[17] or N-1, N-3, and N-7), are present. This explains the largely varying pattern of nitrogen protonation shifts found in several N-7-, N-9-, and N-6-substituted adenines, and in adenine[15] itself. On the other hand, a comparison

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Table X. Calculated and Observed Protonation Shifts in Adenine and Adenosine[a]

		AH^+			$\Delta\delta_{calcd}$			experiment[b]			
atom	A	N-1	N-3	N-7	N-1	N-3	N-7	$\Delta\delta^c$	$\Delta\delta^d$	$\Delta\delta^e$	$\Delta\delta^f$
N-1	243.4	141.7	275.3	273.6	−101.7	31.9	30.2	−47.8	−57.6	−77.2	−71.3
N-3	230.6	270.8	124.0	248.0	40.2	−106.6	17.4	−19.3	−28.9	0.0	1.0
N-7	266.2	277.3	281.1	145.6	11.1	14.9	−120.6	2.0	8.6	2.5	4.2
N-9	139.6	155.7	137.6	158.1	16.1	−2.0	18.5	11.7	−11.2	7.6	7.0
NH_2	61.6	74.1	95.6	72.3	12.5	34.0	10.7	20.0	20.1	9.4	7.8
								$\Delta\delta^g$	$\Delta\delta^h$		$\Delta\delta^i$
C-2	165.5	157.0	159.3	176.0	−8.5	−6.2	10.5	−10.4	−7.3		−6.4
C-4	156.3	162.1	149.8	160.3	5.8	−6.5	4.0	−3.7	−0.2		−0.4
C-5	107.9	116.0	115.7	104.4	8.1	7.8	−3.5	−4.9	0.9		0.1
C-6	164.1	162.2	181.5	165.9	−1.9	17.4	1.8	−5.9	−4.7		−4.6
C-8	141.4	153.2	146.3	153.0	11.8	4.9	11.6	4.0	3.4		2.5

[a] Relative shifts in ppm. Calculated shifts (basis II') according to protonation at the position indicated; experimentally, no definite statement concerning the amount and location of protonation is possible in general. $\Delta\delta_{N-x} = \delta(AH^+_{N-x}) - \delta(A)$. [b] Reference 15. [c] Adenine in DMSO, the concentration of trifluoroacetic acid (TFA) ranges from 0 to 1.0 mol/L. [d] 7-Ethyladenine; TFA concentration from 0 to 3.1 mol/L. [e] 9-Ethyladenine; TFA concentration from 0 to 1.33 mol/L. [f] Adenosine. [g] Reference 21. [h] Adenosine in aqueous solution with 3% DCl,[22] compared to a neutral aqueous solution of ATP.[19] [i] Reference 23.

between theory and experiment becomes almost impossible.

Even if only one cationic species were present in an acidic solution, such a comparison would be difficult enough, since solvent effects that are supposed to be more important for charged molecules than for neutral ones are not accounted for in our calculations. From our experience with carbocations,[2] however, we know that the influence of counterions is less important the more the positive charge is delocalized. Hence we expect no extremely strong effect for adenine, protonated at N-1, N-3, or N-7.

Nevertheless, the geometry optimized for a single AH^+ molecule may be different from the structure of AH^+ in a solvent, not only because of the rather modest basis set used in the optimization but also due to solute–solvent interactions.

There is another possible source of error that must be taken care of when performing the calculations. Upon protonating a pyridine-like nitrogen atom this becomes pyrrole-like. We known from previous investigations[9,30] that the basis set dependencies of calculated NMR shifts are different for both types of nitrogens. Whereas relative shifts of pyridine-like atoms change by about 10 ppm to low field, when we use basis II instead of a DZ basis, the corresponding changes for pyrrole-like nitrogen atoms are of the order of 40 ppm. Thus, calculations of protonation effects using basis sets without polarization functions overestimate the shifts to high field considerably and are hence completely unreliable.

Keeping all the above mentioned precautions in mind, we will start with a discussion of the calculated protonation shifts in adenine and try to compare them with experimental data.

The calculated isotropic upfield shifts of the protonated nitrogen atoms N-1, N-3, and N-7 are in a range of −100 to −120 ppm. The most dramatic changes upon protonation, however, occur in their principal components σ_{ii} (Table VIII). While the high-field component, σ_{11}, which remains perpendicular to the molecular plane, is deshielded by about 60 ppm, σ_{22} is shielded by 213 ppm, and σ_{33} by 175 ppm, as compared to the σ_{ii} of N-1, N-3, or N-7 in the neutral adenine molecule. In addition, σ_{33} is now directed along the new N–H bond in all protonated adenines. We thus find the pattern that is characteristic for pyrrole-like nitrogens.[9] All other nitrogen atoms, with the exception of N-9 in $AH^+_{N\text{-}3}$ with a slight upfield shift, show resonances shifted downfield. Here σ_{11} is nearly unaffected by the protonation, the changes being mainly in σ_{33} and, to a smaller extent, in σ_{22}, without altering the principal shielding axis orientation.

While $\Delta\delta(N\text{-}1) = \delta(AH^+_{N\text{-}1}) - \delta(A) = 31$ ppm both in $AH^+_{N\text{-}3}$ and in $AH^+_{N\text{-}7}$, and $\Delta\delta(N\text{-}7) = 13$ ppm in $AH^+_{N\text{-}1}$ and $AH^+_{N\text{-}3}$, at N-9 $\Delta\delta = 17$ ppm in $AH^+_{N\text{-}1}$ and in $AH^+_{N\text{-}7}$, but −2 ppm in $AH^+_{N\text{-}3}$. Most of the latter effect can be explained by looking at just one MO contribution to σ(N-9). The lone pair at N-3 of adenine, which is deshielding for σ(N-9) by −1 ppm, is replaced by a slightly (0.1 ppm) shielding N–H bond contribution in $AH^+_{N\text{-}3}$.

We do not have a satisfactory explanation for the large downfield shift of the NH_2 resonance in $AH^+_{N\text{-}3}$ (and correspondingly the unusual magnitude of the ^{13}C-6 resonance) as compared to $AH^+_{N\text{-}1}$. The important MO contributions to σ(C-6), i.e., the bond contributions C_6–NH_2, C_3–N_1, and C_6–C_5, are −54, −45, and −62 ppm in $AH^+_{N\text{-}1}$, and −60, −54, and −64 ppm in $AH^+_{N\text{-}3}$, accounting for a difference of 17 ppm of a total of 20 ppm.

A first attempt to correlate the calculated shift with changes in the partial charges failed, since upon protonation, according to a Mulliken population analysis, the partial charges at C-6 change by 0.2 unit in $AH^+_{N\text{-}1}$ and by −0.1 unit in $AH^+_{N\text{-}3}$. If a correlation would exist, it should have led to a prediction just opposite to the calculated effects.

Since intermolecular effects which stabilize the positive charge are not accounted for in the calculations, we expect that the theoretical protonation shifts are larger than the observed ones. This is certainly true for $\Delta\delta$(N) (Table X). The missing counterions are, however, perhaps not the only reason for this finding, especially as far as N-1 and N-3 are concerned. Obviously a combination of $\Delta\delta$(N-1) and $\Delta\delta$(N-3) leads to a better agreement with experimental for adenine, and $\Delta\delta$(N-1) alone is sufficient for a description of adenosine and 9-ethyladenine. Here both theory and exeriment show a large upfield shift of the N-1 resonance and downfield shifts for the other nitrogen atoms.

The calculated effects due to protonation exclusively at N-3 or N-7 do not permit a direct comparison with an experimental counterpart. In adenine, where N-1 and N-3 or N-7 protonated species are postulated to be present simultaneously, shielding and deshielding effects compensate to some extent, thus explaining the experimental finding of smaller protonation shifts in adenine than in adenosine and leading to a rather satisfactory agreement between theory and experiment.

The ^{13}C resonances show much smaller variations upon protonation than do the nitrogen shifts. We find upfield shifts for the next neighbors of the nitrogen atoms actually being protonated, and downfield shifts elsewhere. An exception is C-8 in $AH^+_{N\text{-}7}$ which shows a downfield shift of almost 12 ppm, and which cannot be explained in a simple way.

On the whole, the correlation of calculated and observed protonation shifts of the ^{13}C resonances is not overwhelmingly good whereas it is quite satisfactory for ^{14,15}N.

Apparently there are some discrepancies in explaining the experimental data concerning the nature of the second and third protonation sites of adenine. According to crystallographic studies, and ^{1}H and ^{13}C NMR investigations,[17] besides the protonation at N-1, N-7 should be the second and N-3 the third protonation site. This seems to be in contrast to ^{15}N NMR studies of Roberts

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Table XI. CPU Times[a]

no.	N^b	int[c]/[10^6]	ZGM[d]	SCF	2e[e]	1e[f]	iter[g]	Σ(CPU)[h]	Σ(STU)[i]
C	186	51.8	2964	1657	1367	680	2044	8795	12760
C	206	74.2	3970	1935	1865	830	2300	10995	15635
U	188	54.1	3005	1150	1400	650	1875	8150	11370
T	216	81.4	4375	1780	2215	1135	2410	12030	16970
A	235	118.0	6505	2450	3420	1560	4325	18410	25795
G	252	131.0	7570	3360	4095	2170	4520	21890	31190

[a] The CPU time for the Cyber 205 is given in seconds. [b] Number of basis functions. [c] Number of electron-repulsion integrals stored. [d] Vectorized two-electron integral program, ref 32. [e] Construction of the first-order Fock operators. [f] Construction of the individual one-electron perturbations. [g] Iterative generation of the (x, y, z, components of the) first-order wave function. [h] Sum of CPU times. [i] STUs are the billing units that account for CPU and (implicit or explicit) I/O time.

et al.,[15] who find that adenine both in DMSO and aqueous solutions protonates at N-1 and N-3, the N-7 resonance moving only slightly downfield in DMSO and slightly upfield in aqueous solution.

Our calculations give support to the latter interpretation. A protonation at nitrogen N-7 should result in rather large, i.e., 120 ppm, upfield shifts of the ^{14,15}N resonance, to an upfield shift of −3.5 ppm at C-5, and to a downfield shift of 11.6 ppm at C-8 (cf. Table X), as compared to the adenine molecule.

The calculated ^{14,15}N NMR pattern of protonation shifts is in satisfactory agreement with experimentally observed data if one assumes that mostly $AH^+_{N\text{-}1}$ and some $AH^+_{N\text{-}3}$ species are present simultaneously. The calculated ^{14,15}N shifts for $AH^+_{N\text{-}7}$ do not fit into this pattern.

As we do not find a good correlation between calculated and observed protonation shifts of the ^{13}C resonances, it were rather keen to draw a conclusion from only these data. However, the agreement of the $AH^+_{N\text{-}7}$ ^{13}C protonation shifts is even worse than that for $AH^+_{N\text{-}1}$ and $AH^+_{N\text{-}3}$.

From a comparison of our calculations with the experimental data we hence believe that we can exclude a sizable protonation at N-7.

It is well-known that in adenosine only N-1 is involved in the protonation, and we may thus compare our calculated shifts due to protonation at N-1 more conveniently with adenosine data from experiment. The trends are reproduced correctly by IGLO, though the magnitudes are largely overestimated. However, this ought to be so, since protonation of a single molecule in space will cause much larger effects than the same process taking place in the liquid state, where the charge can be stabilized by counterions or solvent molecules that are absent in our calculation.

IV. Conclusions

We have shown that it is possible to calculate the magnetic properties of biochemically interesting molecules like the DNA bases by means of an ab initio method achieving a rather high degree of accuracy. The complete shielding tensors of all atoms in a molecule and the molecular magnetic susceptibility are obtained from a single calculation, the computational effort of which is comparable to that needed for a normal SCF calculation with the same basis set.

Protonation shifts of adenine have been calculated for all species that are postulated to be present in acidic solvents.

For the protonated and neutral DNA bases effects of electron correlation seem to be of only minor importance, whereas medium effects are rather pronounced. The latter fact prevents an easy comparison of calculated and experimental NMR data.

In order to describe pyridine- and pyrrole-like nitrogen atoms with comparable accuracy, it is mandatory to use basis sets having polarization functions at the non-hydrogen atoms. As for any SCF-type calculation the demand of CPU time and/or mass-storage increases with the fourth power of the number of basis functions, and this requirement (cf. Table XI) hinders a straightforward application to the calculation of base pairs or nucleotides. We are confident, however, that the sp basis set used in this study can be reduced without significant loss of accuracy.

It is somewhat problematic to compare calculated and observed chemical shifts for molecules whose structures depend strongly on solvents and pH. While in different solvents the tautomeric equilibria, and correspondingly the various protonation equilbria, are different for **1–5**, just one single species in a definite conformation is present in a calculation. We thus get a very detailed view of the magnetic properties of isolated, neutral, or charged molecules whose theoretically optimized structures might be different from those present in solution.

Of the two ways out of this dilemma, i.e., either to model and calculate whole an ensemble of molecules or to modify the experimental conditions in order to observe only very few distinct molecules, only the last one seems to be viable, at least in the near future. One can imagine low-temperature investigations that would provide the theoreticians with both accurate structures and principal components of the shielding tensors for neutral DNA bases and their protonated forms.

On the other hand, it is not unlikely that new hardware architectures will enhance computer performance by the two or three orders of magnitude that are necessary to permit a simulation of small clusters of molecules by ab initio methods. These calculations should permit a sound explanation and, of course, prediction of experimental observations.

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Registry No. 1, 65-71-4; **2**, 71-30-7; **3**, 66-22-8; **4**, 73-24-5; **5**, 73-40-5.