On the Photochemistry of Purine Nucleobases

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We present R2PI spectra of a series of purine nucleobases, nucleosides, and related derivatives, including adenine, guanine, guanosine, 2-aminopurine, 2,6-diaminopurine, 9-ethyladenine, and 9-methylguanine. We compare the results with models involving two interacting excited states, which have been proposed to explain the short excited-state lifetime of most nucleobases. The amino group in the 2 position appears to have a strong effect on the excited-state potentials, as does any substitution in the 9 position. This explains the very different fluorescence characteristics of 2-aminopurine, but it raises new questions concerning the short lifetime of guanine in solution.

Introduction

All nucleobases involved in replication have very low fluorescence and phosphorescence quantum yields associated with excited-state lifetimes of the order of picoseconds.[1,2] Quenching occurs by internal conversion to the ground state.[3] It has been argued that evolution has arrived at this particular choice of building blocks for its genetic machinery, because the short lifetime protects them from photochemistry.[4] This is especially important in the wavelength range of 280 to 300 nm because solar irradiance at the earth surface is small but not zero in this range, while it is also the onset of absorption for many bases, notably, the purine-based ones.[3,5−8] Thus, this internal conversion is vital to the preservation of life on earth as we know it; nevertheless, its mechanism is not well understood.

Most nucleic acid components show fluorescence excitation spectra that are red-shifted relative to their absorption spectra. This suggests increased internal conversion of energy.[3] As compared to adenine (Ad), 2-aminopurine (2AP), has a much longer lifetime and larger quantum yield, each by 3 orders of magnitude, and absorbs further to the red by some 5000 cm^{-1}.[1,9] Some of the relevant data are summarized in Table 1, and the molecular structures are shown in Figure 1. The enhanced fluorescence of 2AP has been exploited extensively to incorporate this base as a fluorescent probe in DNA.[10] Lim has proposed that in adenine the first excited electronic state, of $\pi-\pi^*$ character, interacts with another excited state, of $n-\pi^*$ character, which in turn couples efficiently with the ground state.[11] Broo proposes that in 2AP the first excited state is lower in energy than in Ad, affecting the way in which the two excited states interact.[4] A similar model, involving two states, was also proposed by Andréasson et al. to explain the photophysical properties of alkyl substituted adenines.[12] To test these models, we need to gain insight into the excited state potentials. This requires spectroscopy at a resolution that allows observation of rovibronic transitions which is not possible in solution. Gas phase data have so far been scarce because of the difficulty of bringing these bases into the gas phase without thermal decomposition. Kim et al. have recently reported R2PI and LIF spectra of adenine seeded in a beam, but that technique does not work for all bases and all substituted bases.[13] We have circumvented this problem by combining laser desorption with jet cooling to record resonance enhanced multiphoton ionization (R2PI) and spectral hole burning (SHB) spectra of several purines, including the nucleobases guanine[14] (Gu) and adenine and their nucleosides.[15] The results, as will be discussed below, are consistent with a model involving two interacting excited

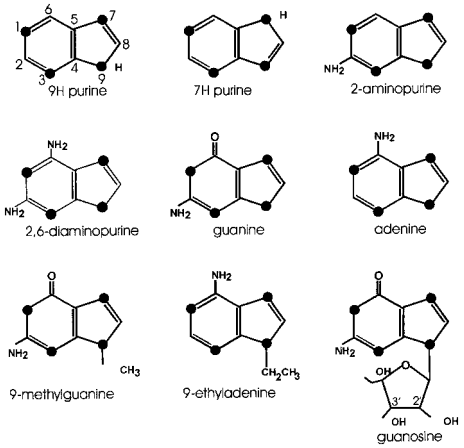

Figure 1. Diagrams of the purines studied in this work.

TABLE 1

	τ-singlet	QY	0-0 [cm^{-1}] (our work)
purine	1 ps[3]		
2-amino-purine	2.1 ns	0.66[33]	32368
	−24.6 ns[9]	(down 3−10× in DNA)	
guanine	5.7 ps[1]	3×10^{-4} [37]	32878
adenine	8.5 ps[1]	3×10^{-4} [21,37]	36101
adenosine	3.3 ps[1]	5×10^{-5} [12]	35550
guanosine			34492
2AP-riboside		0.68[32]	

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states for adenine, but this does not necessarily explain the short lifetime of guanine. Furthermore, we find significant differences in the spectroscopy for 9-substituted purines, including the purine-based nucleosides.

Experimental Section

The experimental setup has been described in detail elsewhere.[16] In brief, material is laser desorbed from a sample probe in front of a pulsed nozzle. All chemicals were obtained from Sigma-Aldrich Co. and used without further purification. The desorption laser is a Nd:YAG laser operated at its fundamental wavelength of 1064 nm. At this wavelength, one does not expect photochemical interaction with the compounds that we desorb while the graphite substrate absorbs effectively. Typical laser fluences are of the order of 1 mJ/cm^2 or less, which is significantly less than the fluences normally used for ablation. The laser is focused to a spot of the order of 0.5 mm diameter within 2 mm in front of the nozzle. The nozzle consists of a pulsed valve with a nozzle diameter of 1 mm. We usually operate with Ar as a drive gas at a backing pressure of about 5 atm. In earlier work, we optimized the geometry for effective entrainment by mapping entrained perylene with laser induced fluorescence.[17]

Downstream, ionization lasers intersect the beam inside the source region of a reflectron time-of-flight (TOF) mass spectrometer (R. M. Jordan Co.). A Nd:YAG-pumped dye laser, used for one color, two photon ionization, intersects the beam at right angles. By monitoring specific mass peaks while varying the two photon ionization wavelength, we obtain mass selected excitation spectra. We achieve spectral hole burning by using a second Nd:YAG-pumped dye laser, with a delay of about 200 ns between the two dye laser pulses. This results in two peaks in the time-of-flight spectrum, which we can monitor individually. The first laser pulse then serves as a "burn" laser, while the second serves as a "probe" laser. When both lasers are tuned to a resonance of the same conformer population, the burn laser causes a decrease in the signal of the probe laser.

Results

Adenine (6-aminopurine) and 2AP are isomers. They are both amino-substituted purines with the substitution in the 2 position for 2AP and in the 6 position for adenine. This difference in position appears to have a dramatic effect on the excited state potentials. To investigate the effect of amino substitution, we will first compare the R2PI spectra of these two compounds. Subsequently, we will compare their spectra with those of guanine (2-amino,6-hydroxypurine) and with 2,6-diaminopurine.

Figure 2a shows the R2PI spectrum of adenine. It is characterized by a very narrow wavelength range for which we observed resonant absorption. The main peaks in Figure 2a are spaced by 43, 105, and 185 cm^{-1}, respectively, relative to the redmost one at 36101 cm^{-1}. In Table 2 we compare these values with calculations by Mishra et al.[18] The lowest energy in-plane vibrations in the ground state are at about 330, 540, and 620 cm^{-1}, respectively.[19] Stepanek and Baumruk reported some of these frequencies in the excited state as well, but their data referred to diprotonated, rather than neutral, adenine and were not clearly resolved.[20] Kim et al. reported an LIF spectrum, which is essentially identical to our R2PI spectrum. They also report an R2PI spectrum that shows additional peaks with lower intensities, as will be discussed below.

We note that we cannot be certain which tautomer of adenine we ionize. The room temperature fluorescence that is observed for adenine in solution is mainly due to the 7H tautomer,

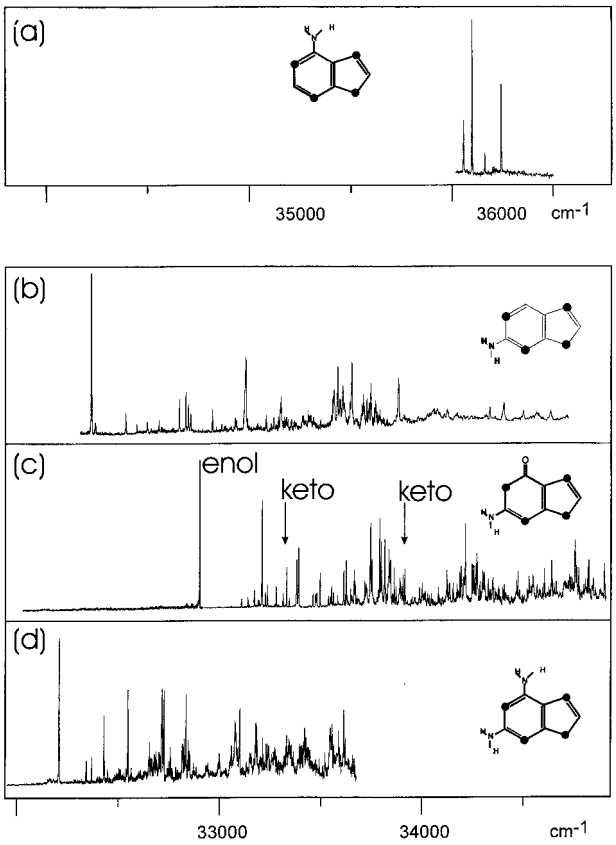

Figure 2. R2PI spectra of (a) adenine, (b) 2-aminopurine, (c) guanine, (d) 2-6-diaminopurine. Ion signals are shown in arbitrary units; spectra are not normalized with respect to each other.

TABLE 2: Excited State Vibrational Frequencies in Wavenumbers

adenine R2PI	Ad-9H[18]	2AP R2PI	2AP-9H[18]	
				scaled * 0.92
43			102	
105	110	169	164	
185	183	223	238	
	208	275	291	
	290	334	360	333
	338	434		
	502	465	497	462
	523	477	511	473
	540	490	533	493
	547	595	626	579
		758		

although the 9H tautomer is much more abundant.[18,21,22] According to calculations by Broo and Holmen,[23,24] the two tautomers are separated by 7−10 kJ/mol in solution and by about 35 kJ/mol in the absence of solvent effects. Given the abundance of the 9H tautomer in solution, we may thus expect an even greater abundance of the 9H tautomer under our gas phase and low-temperature conditions, similar to the situation in low-temperature matrixes.[25] This is also consistent with the observation of the 9H tautomer in the microwave spectra of jet-cooled purine[26] and jet-cooled adenine.[27] The same calculations predict a much smaller energy difference between the two tautomers in the excited state. The possibility has been suggested that the B state is actually due to the 7H tautomer, and both states can be coupled by phototautomerism.[18] On the other hand, we observe evidence of excited-state coupling in our gas-phase results, while phototautomerism could be expected to be particular to the solvent phase in which the proton transfer could be solvent assisted.

Figure 2b shows the R2PI spectrum of 2-aminopurine. Despite the similarity of the structure of this purine derivative to adenine, it has a much longer excited-state lifetime in solution and exhibits both a high fluorescence and a high phosphorescence quantum yield. The R2PI spectrum shows an extended vibronic structure and the origin appears at 32368 cm^{-1}, which is about 3700 cm^{-1} to the red of that of adenine. In Table 2, we compare the vibrational frequencies with values calculated by Mishra et al. for the 9H tautomer.[18]

The guanine R2PI spectrum, shown in Figure 2c, covers a much larger wavelength range than that of adenine. Spectral hole burning shows that the picture is somewhat more complex. The guanine spectrum consists of three overlapping spectra, due to three different tautomers. A detailed analysis of this spectrum will appear elsewhere.[28] The signal from the dominant tautomer in the spectrum covers a frequency range of about 2000 cm^{-1}, significantly more than the range in the spectrum of Ad and comparable with that of 2AP. The origin of Gu is red-shifted relative to the Ad spectrum by 3223 cm^{-1}, which is almost as much as in the case of 2AP. Therefore, both in terms of extent and in terms of red-shift, the Gu R2PI spectrum resembles that of 2AP, rather than that of Ad.

To further investigate the differences between 2AP and Ad, we have also studied 2,6-diaminopurine (2,6AP). This molecule can be considered as an intermediate structure between 2AP, which has the -NH_2 group in the 2 position, and Ad, which has the -NH_2 group in the 6 position. We find that its R2PI spectrum, shown in Figure 2d, resembles that of 2AP much more than that of Ad. It is red-shifted with respect to Ad even more than 2AP, by another 158 cm^{-1}, and its frequency range is similar to that of 2AP. We also recoded the R2PI spectrum of 6-*O*-methylguanine and found it to be very similar to that of guanine as well, with a slight blue-shift of 119 cm^{-1}. We did not obtain R2PI spectra for purine, but we note that in solution 2AP is red-shifted with respect to purine by about 5500 cm^{-1}, while 6AP is virtually not shifted with respect to purine.[29]

The R2PI signal intensities in our experiment are about an order of magnitude larger for guanine than for adenine. This could suggest that in the former case we have a $\pi-\pi^*$ transition while in the latter case we have an $n-\pi^*$ transition. However, caution is necessary when drawing conclusions from R2PI intensities, since the ionization probability from the excited state may differ as well. This is especially true in this case, where the ionization probabilities can be affected by excited-state lifetimes. Furthermore, laser desorption conditions may be different for the two compounds. According to calculations by Broo[4] the transition for Ad has $n-\pi^*$ character for the 9H tautomer, although it could be of $n-\pi^*$ character for the 7H tautomer.

In considering the implications of the photochemistry of nucleobases, we need to point out the fact that the actual chromophores in DNA are nucleosides, rather than nucleobases. Therefore, we have also investigated the jet cooled spectra of 9 substituted purines as well as of actual nucleosides. Figure 3 shows the spectra for (a) 9-methyl guanine, (b) 9-ethyl adenine, and (c) guanosine. Two observations follow from these data. (i) Substitution in the 9 position causes a large blue-shift for guanine and a small red-shift for adenine. We have found that these shifts increase with increasing size of the substituent group. Thus, unlike the purine bases themselves, the purine nucleosides do absorb in a similar wavelength region. (ii) Both 9-substituted guanine and adenine exhibit truncated spectra with peak broadening toward higher energies. The low energy peaks are due to the motion of the substituent with respect to the base and to different conformations.[15] A more detailed analysis of the 9-substituted purine spectra will be published elsewhere.

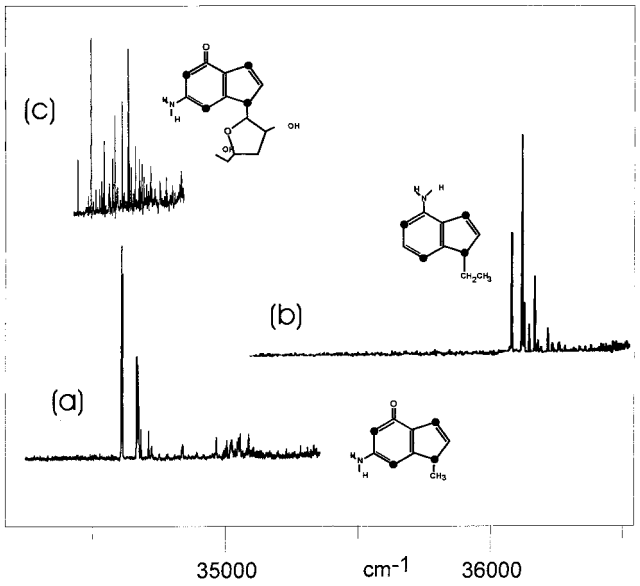

Figure 3. R2PI spectra of (a) 9-methyl guanine, (b) 9-ethyl adenine, (c) guanosine.

Discussion

Adenine. A detailed assignment of peaks in the adenine spectrum is fraught with two problems: First, these spectra may consist of peaks from more than one tautomer, Second, the possible interaction between nearby $n-\pi^*$ and $\pi-\pi^*$ states can result in vibronic coupling (proximity effect) which may dramatically affect the intensities of part of the vibronic transitions.[11] Our R2PI spectrum reveals only four peaks, identical to the LIF spectrum of Kim et al. The frequencies of these peaks are considerably smaller than what would be expected for even the lowest in-plain mode. This suggests that either the red-most peak may not be the origin of the $S_1 \leftarrow S_0$ transition or a strong interaction with a second excited state either perturbs the excited state potential or leads to vibronic mixing. In fact, according to the calculations by Broo, the adiabatic excited state (of mixed $n-\pi^*$ and $\pi-\pi^*$ character) is extremely flat, which would lead to drastically reduced vibrational spacings.[4] That assumption alone does not suffice to explain the anomalous intensity and frequency distribution so vibronic mixing is likely to play a role as well. Kim et al. report additional peaks at lower intensities in their R2PI spectrum, which they ascribe to an $n-\pi^*$ transition to a second excited state and part of which they interpret as a vibronic spectrum with frequencies corresponding to those in the ground state. However, Mishra et al. calculated excited-state vibrations for different tautomers of adenine and found them to be about half of the corresponding frequencies in the ground state.[18] The R2PI and LIF spectra of Kim et al. were recorded in two different machines, so it seems possible that they reflect two different distributions of tautomers. We are undertaking spectral hole burning experiments to further clarify this issue.

Guanine. In contrast to adenine all 2-amino substituted purines exhibit extensive vibronic spectra as well as significant red-shifts. The spectrum of guanine is discussed in detail elsewhere so here we only summarize the main findings.[14,28] The spectrum consists of contributions from three tautomers, as determined by spectral hole burning and tautomeric blocking by methyl substitution. The dominant tautomer is the 9H enol,

while the other two are the 7H and 9H keto tautomers, with their origins at +405 and 1044 cm^{-1}, respectively, with respect to the enol origin at 32878 cm^{-1}. Their vibrational spectra can be partially assigned by comparison with ab initio calculated vibrational frequencies.[28]

2-Aminopurine. We compare our R2PI spectrum of 2AP with the computation of excited state vibrational frequencies by Mishra et al. as summarized in Table 2 for the 9H tautomer. The calculated 7H tautomer frequencies do not differ significantly from these.[18] The authors state that their results should probably be scaled by a factor of the order of 0.9. In fact, when scaling with a factor of 0.92, all in-plane vibrational frequencies come to within a few wavenumbers of peaks in our spectrum, as tabulated in Table 2. The peaks below 300 cm^{-1} are most likely due to overtones and combinations of the lowest out-of-plane vibrations, the fundamentals of which are too weak to be observed. The peak at 434 cm^{-1} falls outside the computed sequence and may thus well be the origin of another tautomer. We plan hole burning experiments to investigate this question.

Two observations stand out from these results: on one hand the spectrum of adenine lacks clear vibrational progressions while on the other hand 2-amino substitution of the purine moiety results in spectra that are red-shifted and show extensive vibrational transitions. Most of these general spectral features can be interpreted with a model involving excitation to an excited state, A, which interacts with a second excited state B (this differs from the notation by Andréasson et al.). The latter can couple with the electronic ground state to quench fluorescence and phosphorescence. The A state is likely to be of $\pi-\pi^*$ character and the B state of $n-\pi^*$ character. The absorption characteristics of the purines and their excited state lifetimes then depend strongly on two parameters: (i) the relative energies of these two states and (ii) the amount of electronic coupling between them. Possible arrangements are shown schematically in Figure 4. A curve crossing, as sketched schematically in Figure 4b, will typically lead to spectra that are diffuse or show a cutoff at absorption energies larger than that of the crossing, indicated by E_c. If, on the other hand, as sketched in Figure 4a, the interaction between the two excited states is weak, or if the A state is much lower in energy than the B state, then we may expect a sharp and extended vibronic spectrum. Finally, the other extreme is sketched in Figure 4c: If the interaction is strong and the A state is higher in energy, then it is possible for the vibronic spectrum to be completely structureless.

Within this picture the striking photochemical difference of purine and adenine as compared to 2-aminopurines is the result of the relative energies of the $\pi-\pi^*$ and the $n-\pi^*$ states. The fact that the origin for the 2AP spectrum is significantly to the red of the adenine spectrum, implies that the A state is lower in this case. Furthermore, the extent of the spectrum suggest that 2AP does *not* exhibit a curve crossing as sketched in Figure 2b, as opposed to adenine. Therefore our gas-phase results suggests that the amino substitution in the 2 position of purine lowers the excited state potential of the $\pi-\pi^*$ state, while substitution in the 6 position has little effect. Possibly the more drastic change in electronic structure is the result of the formation of a structure in which the C2 carbon has three nitrogen neighbors. This is fully consistent with previous solution results, as well as with calculations by Jean and Hall[30] and with circular dichroism measurements by Holmen et al.[31] which both identify a $\pi-\pi^*$ state about 4000 cm^{-1} below the $n-\pi^*$ state for 2AP.

While these observations satisfactorily explain the enhanced fluorescence of 2-aminopurine, they introduce the need for an alternative explanation as to why guanine exhibits a very efficient quenching of the S_1 state. It is, however, possible that the proposed quenching mechanism does apply to all purine nucleosides, including guanosine, even when it does not apply to guanine. We note that 2-aminopurine is not affected in the same way by 9-substitution, allowing it to remain fluorescent even when part of DNA, albeit with a quantum yield that is reduced by an order of magnitude.[32,33] It is also possible for other mechanisms to be involved with the spectral changes upon N9 substitutions. For example, if phototautomerism plays a role in the excited state mixing, the N9 substitution would block the 7H tautomer. However, this is unlikely to be a major effect because we found 7-methyl guanine to exhibit a R2PI spectrum that is similar to that of guanine. Furthermore, the drastic change in the vibronic spectrum upon N9 substitution of the purine chromophore suggests that the lone pair electrons on the N9 nitrogen play an important role in the excited state. Since those electrons would mainly contribute to the $n-\pi^*$ transition it would not be unreasonable that the excited state mixing would be affected. One possible hypothesis might be that the A state is lowered by electron donating groups at C2 and that this effect is balanced by a second electron donating group at N9. In the case of 2AP, the effect of the C2 substitution would still be dominant.

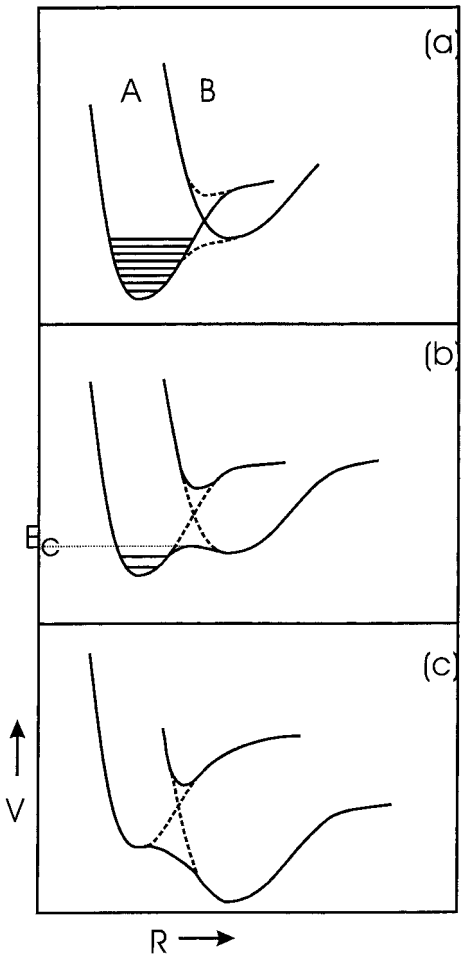

Figure 4. Schematic potential curves, showing different possible mechanisms, as discussed in the text.

The pyrimidines absorb further to the blue, and thus they are less sensitive to photochemistry due to absorption of solar irradiance. Nevertheless, they have the same short lifetimes as do the purine bases. The R2PI spectra of uracil and thymine were measured by Levy,[34] who found them to be very broad.

This suggests a strong coupling between excited states, as indicated in Figure 2c. The fact that the pyrimidines also may have a similar excited state quenching mechanism raises questions about irradiance conditions in early evolution.

The transition from the A state to the B state may occur as readily in the isolated molecules as in solution, but the internal conversion from the B state to the ground state may be another matter. We have measured excited state lifetimes by exciting with one laser and ionizing with another laser at 193 nm, after a variable delay. The maximum observable delay in our apparatus is of the order of 5 μs because the excited molecules in the beam leave the ionization region after 5 μs. The lifetime of all nucleobases appears to be of this order of magnitude or larger. This suggests that after coupling with the B state both guanine and adenine can also couple with the triplet state; however, in solution the coupling between the ground state and the B state is significantly more efficient. In other words, the solvent is required to carry off the excess energy. This is also consistent with the observation that in solution at low temperatures the lifetime increases.[35,36]

Summary

The short excited state lifetime of most nucleobases, with the notable exception of 2-aminopurine, has been explained by models involving interactions between two excited states. To test these models, we have here reported R2PI spectra of a series of laser desorbed, jet cooled nucleobases and nucleosides. All nucleosides as well as adenine exhibit truncated spectra, consistent with a curve crossing between two excited states. 2-Aminopurine has a spectrum with a more extensive vibronic structure and a significantly red-shifted origin. This is consistent with a lower energy first excited state and could explain its extended lifetime. The R2PI spectra of all 2-amino-substituted purines are qualitatively similar to that of 2-aminopurine, including the spectrum of guanine. The latter thus requires an alternative explanation for the short excited-state lifetime of this nucleobase.

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