Distinct photophysical behavior of nucleobase adenine and its constitutional isomer, 2-aminopurine, has been studied by using quantum chemical methods, in particular an accurate ab initio multiconfigurational second-order perturbation theory. After light irradiation, the efficient, ultrafast energy dissipation observed for nonfluorescent 9H-adenine is explained here by the nonradiative internal conversion process taking place along a barrierless reaction path from the initially populated $1(\pi\pi^* L_2)$ excited state toward a low-lying conical intersection (CI) connected with the ground state. In contrast, the strong fluorescence recorded for 2-aminopurine at 4.0 eV with large decay lifetime is interpreted by the presence of a minimum in the $1(\pi\pi^* L_2)$ hypersurface lying below the lowest CI and the subsequent potential energy barrier required to reach the funnel to the ground state. Secondary deactivation channels were found in the two systems related to additional CIs involving the $1(\pi\pi^* L_2)$ and $1(n\pi^* L_2)$ states. Although in 9H-adenine a population switch between both states is proposed, in 7H-adenine this may be perturbed by a relatively larger barrier to access the $1(n\pi^*)$ state, and, therefore, the $1(\pi\pi^* L_0)$ state becomes responsible for the weak fluorescence measured in aqueous adenine at ~4.5 eV. In contrast to previous models that explained fluorescence quenching in adenine, unlike in 2-aminopurine, on the basis of the vibronic coupling of the nearby $1(\pi\pi^*)$ and $1(n\pi^*)$ states, the present results indicate that the $1(n\pi^*)$ state does not contribute to the leading photophysical event and establish the prevalence of a model based on the CI concept in modern photochemistry.

**Conical intersections, DNA photophysics, fluorescence quenching, quantum chemistry, ultrafast decay**

Studies on the photostability of DNA and RNA bases after absorption of UV light have flourished since the early 1970s, when quenched DNA fluorescence at room temperature was first reported (1). Knowledge about the mechanisms that control nonradiative decay is fundamental, not only because of the extreme importance of photodamage in systems that form the genetic code but also because they have been used to establish the paradigm of the essentials of radiationless decay processes in nonadiabatic photochemistry (2). Adenine (6-aminopurine), the most studied nucleobase, maintains photostability by efficiently quenching its fluorescence, whereas a close constitutional isomer, 2-aminopurine, displays strong emission, and it is commonly used to substitute fluorescence, whereas a close constitutional isomer, 2-aminopurine, displays strong emission, and it is commonly used to substitute

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Conflict of interest statement: No conflicts declared.

Abbreviations: IC, internal conversion; CI, conical intersection; FC, Franck–Condon; PES, potential energy surface(s); MEP, minimum energy path; CASPT2, multiconfigurational second-order perturbation theory; CASSCF, complete active space self-consistent field.

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Fig. 1. Structure and atom labeling for 9H-adenine (Upper Left), 7H-adenine (Upper Right), and 9H-2-aminopurine (Lower).

chemical mechanisms and forms the basis for future theoretical studies on the dynamics of the system that may ultimately lead to the prediction of the time evolution of the process. In the present contribution, we illustrate the importance of a quantum chemical description of PES based on the computation of state minima, barrier heights, CIs, and, in particular, the proper calculation of minimum energy paths (MEPs) connecting the singular points in a way that allows us to carefully determine the most favorable paths for the energetic decay and understand the different behavior of systems as adenine and 2-aminopurine.

Previous theoretical studies, pioneered by Ismail et al. (27), suggested the importance of CIs as responsible for ultrafast IC in the DNA bases, but the determination of its structure and nature has been controversial. Recently, the main role in the ultrafast relaxation of the nucleobases of low-energy CIs between the ground and a low-lying ($\pi^*\pi^*$) state has been established in cytosine, uracil, and adenine, whereas other states ($n^*\pi^*$, $\pi^*\pi^*$) have been proposed to contribute to the different decay pathways (28–35). Particularly relevant and valuable for the present work are recent multiconfigurational second-order perturbation theory (CASPT2) studies on adenine (34, 35) and the analyses of several structures of adenine and 2-aminopurine at the density functional theory/multireference configuration interaction level (33, 36). The most recent evidence highlights the relevance of out-of-plane distortions in all nucleobases (31, 33–35). To rationalize the different photophysical behavior of adenine and 2-aminopurine (see Fig. 1), the present work has been undertaken. For this purpose, the main IC channels in adenine and 2-aminopurine have been determined by computing specific MEPs on the PES of their low-lying singlet excited states, by describing the lowest relaxation pathways for the energy, and by locating the most accessible CIs and energy barriers. In a previous study (35), several aspects of the photophysics of adenine tautomers and derivatives were considered. Here we will focus on the careful comparison of the photophysics of adenine and 2-aminopurine, stressing similarities and discrepancies. The current scheme, based on a three-state model for these compounds, explains why adenine displays just a weak fluorescence, dissipating most of the absorbed energy by ultrafast, radiationless IC processes detected in the femtosecond and picosecond ranges (37, 38), whereas 2-aminopurine fluorescence can actually be recorded and is much more intense and is near 0.7 eV lower in energy (39, 40). The different properties of adenine and 2-aminopurine upon absorption of radiation make them good candidates to check the reliability of the models and mechanisms under study. CASPT2/complete active space self-consistent field (CASSCF) energy paths and profiles will be used to rationalize the photophysics of both compounds on the basis of their relaxation pathways from the initially excited singlet state and establish the prevalence of a model based on the CI concept.

Results and Discussion

Why 2-Aminopurine Is Highly Fluorescent, Whereas Adenine (6-Aminopurine) Is Not. 2-Aminopurine behaves as a highly emissive source. Measurements in water provided a fluorescence band origin at 3.70 eV (41) and a emission quantum yield ($\Phi_F$) of 0.66 (39, 40). A single-component fluorescence decay, with a large lifetime between 9.3 and 11.8 ns, was reported and assigned to the 9H-tautomer (6, 39, 42–44). Solvent polarity definitively favors emission, because, in the close derivative 9-ethyl-2-aminopurine, $\Phi_F$ varies from 0.010 in the nonpolar solvent cyclohexane to 0.68 in water (45). On the contrary, it was reported that emission is efficiently quenched in adenine, and this basic photophysical behavior is maintained in solution (1). An extremely weak fluorescence with low quantum yield, $\Phi_F = 2.9 \times 10^{-4}$ to $3.3 \times 10^{-4}$, is detected starting at 4.43 eV in aqueous environments (2, 46–51). Two decay lifetimes have been measured in solution, depending on the different techniques and wavelengths used: the ultrafast decay from 180 to 670 fs and the slower relaxation from 8.4 to 8.8 ps (2). At least two decay channels also have been measured in jet-cooled adenine after irradiation at 250 or 267 nm (4.95 and 4.64 eV, respectively), corresponding to a double exponential decay: $<100$ fs and 1.1 ps (37, 38). Therefore, the experimental evidence points out that adenine finds efficient ways to dissipate the absorbed energy different from emission.

According to Lim’s proximity effect model (8–10), the two close, lowest-lying $\pi^*\pi^*$ and $n^*\pi^*$ excited states were supposed to interact, coupled through certain low-energy, out-of-plane vibrational distortions, which ultimately led to an increasing overlap with the ground state vibration and large FC factors favoring radiationless IC to the ground state. Many of those conclusions are based on observed or computed vertical energy gaps between the states (11, 52), with emphasis on the fact that in adenine a small energy gap is found between the lowest $\pi^*\pi^*$ and $n^*\pi^*$ excited states, whereas in 2-aminopurine the lowest $\pi^*\pi^*$ state is much lower in energy and cannot interact with the $n^*\pi^*$ state properly.

Table 1 compiles relevant experimental data together with our computed CASPT2 values. Although the present study is focused on the major 9H-tautomer both for 2-aminopurine and adenine, some aspects of the photophysics of 7H-adenine also are analyzed, considering that it has distinct photophysics that influence measurements in polar media, unlike 2-aminopurine, in which the photophysics of both tautomers seems to be quite similar (43). 2-Aminopurine displays absorption maxima in stretched poly(vinyl alcohol) films (39) peaking at 4.1 (0.10), 4.5 (0.002), and 5.1 eV (0.07), where the oscillator strengths have been included in parentheses. Transition to three low-lying, singlet, excited states have been computed vertically at 4.33 (0.070), 4.46 (0.008), and 5.33 eV (0.148) at the CASPT2 level, in relative accordance with the experimental data. The features are assigned to the $^1(\pi^*\pi^*)_L_0$, $^1(n^*\pi^*)_L_0$ and $^1(\pi^*\pi^*)_L_0$ transitions, respectively. Platt’s nomenclature (53) allows us to distinguish between the two low-lying $\pi^*\pi^*$ states: $^1(\pi^*\pi^*)_L_0$ is described mainly (36%) by the highest occupied molecular orbital (HOMO)–lowest unoccupied molecular orbital (LUMO) one-electron promotion, whereas the $^1(\pi^*\pi^*)_L_0$ state wave function is basically formed by a linear combination (20%) of each of two configurations, $H\rightarrow L + 1$ and $H - 1 \rightarrow L$. The intensity of the bands in the low-energy range of the absorption spectra of 2-aminopurine seems to be distributed between the two $^1(\pi^*\pi^*)$ transitions. Regarding band origins, the resonant two-photon ionization spectrum of 2-aminopurine in the gas phase displayed an extended vibronic structure, with a band origin ($T_{10}$) for the recorded lowest-lying state at 4.01 eV (36, 52), red-shifted 0.5 eV below the corresponding feature in adenine. The band origin in water is red-shifted to ~3.70 eV (41). Our computed value of 3.89 eV for the adiabatic origin of the $^1(\pi^*\pi^*)_L_0$ state clearly agrees with the observed data in the
vapor, whereas our calculated radiative lifetime ($\tau_{\text{rad}}$), 25 ns, falls within the order of magnitude of the experimental value, 18 ns, estimated from quantum yields and intrinsic lifetimes in water (39).

We also computed the minima of the $|n\pi^*\rangle$ and $|\pi\pi^*\rangle_L_0\rangle$ states, leading to band origins at 4.04 and 4.84 eV, respectively. On the basis of these results, we can confirm that the observed fluorescence in 2-aminopurine starting at 4.01 eV in the gas phase and peaking near 3.4 eV in water (39, 41) takes place from the $|\pi\pi^*\rangle_L_0\rangle$ state but also that the origin of the $|n\pi^*\rangle$ state is above the $|\pi\pi^*\rangle_L_0\rangle$ origin by $\approx 0.1$ eV. A similar gap was estimated in jet-cooled adenine for the equivalent band origins (54–58), whereas for the vertical values the computed energy difference even increases (compare with Table 1) (35, 46–48, 59–61). It is therefore clear that both 9H-adenine and 9H-2-aminopurine have close-lying $|n\pi^*\rangle$ and $|\pi\pi^*\rangle_L_0\rangle$ states, although their photophysical behavior drastically differs. The energy gap criterion is certainly not solid enough to sustain the proximity effect model.

To understand photoinduced processes, the evolution pathways with respect to the relevant reaction coordinates have to be known. We have therefore computed MEPs along the most relevant singlet excited states of the molecules. Fig. 2 displays the energies of the low-lying singlet excited states of 9H-2-aminopurine computed at the CASPT2 level from the FC ground-state geometry along the CASSCF MEP on the $|\pi\pi^*\rangle_L_0\rangle$ hypersurface, followed by a reaction path through a transition state and a CI with the ground state. When directly populated, the H--L $|\pi\pi^*\rangle_L_0\rangle$ state is the lowest one at all geometries (see also Table 2), whereas the $|\pi\pi^*\rangle_L_0\rangle$ state is placed energetically too high to be of primary photophysical importance. The $|\pi\pi^*\rangle_L_0\rangle$ state evolves from the FC structure to an almost planar minimum, $|\pi\pi^*\rangle_L_{\text{min}}\rangle$. From that region, the system has to release the energy either by emitting radiation or by reaching a higher-lying CI, $|gs/\pi\pi^*\rangle_L_{\text{CI}}\rangle$, by surmounting a barrier of 5.0 kcal mol$^{-1}$ through an intermediate transition state, $|\pi\pi^*\rangle_L_{\text{TS}}\rangle$ (see Fig. 2). As it was discussed previously, the computed band origin for the $|\pi\pi^*\rangle_L_0\rangle$ state, 3.89 eV, matches quite well the experimental measurement, 4.01 eV. Initially, the deformation leading from the FC region to the minimum involved an enlargement of the N$_1$C$_2$ and C$_2$C$_3$ bonds, which can be understood by analyzing the change for these bonds from a bonding to an antibonding character in the H and L orbitals, respectively. From the minimum, the out-of-plane vibrational modes leading to the basic deformation at the CI involve the twisting of the N$_1$C$_2$ bond, moving the amino group to a perpendicular disposition (dihedral angle C$_6$C$_1$C$_2$N$_2$) with respect to the rings, a structure that represents a large distortion when evolving from the almost planar $|\pi\pi^*\rangle_L_0\rangle$ minimum.

The same strategy to compute $|\pi\pi^*\rangle_L_{\text{CI}}\rangle$ MEPs was followed in 9H- and 9H-adenine (35). In the major 9H-tautomer, the obtained path after crossing the $|n\pi^*\rangle$ and $|\pi\pi^*\rangle_L_0\rangle$ states’ PES at high energies leads directly to a CI with the ground state, $|gs/\pi\pi^*\rangle_L_{\text{CI}}\rangle$. The path has a steepest-descendent nature and, consequently, the $|\pi\pi^*\rangle_L_0\rangle$ state has no minimum (see Fig. 3). This feature is the corner stone of adenine photochemistry. Taking into consideration

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Table 1. Computed properties for the low-lying singlet excited states of 9H-adenine, 7H-adenine, and 9H-2-aminopurine at the CASPT2/CASSCF(16/13)/6-31G(d,p) level

<table>
<thead>
<tr>
<th>State</th>
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<th>Experiment</th>
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<td>9H-adenine</td>
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<td>(gs)</td>
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<tr>
<td>$</td>
<td>n\pi^*\rangle$</td>
<td>4.33</td>
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<tr>
<td>$</td>
<td>\pi\pi^*\rangle_L_0\rangle$</td>
<td>4.46</td>
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<tr>
<td>7H-adenine</td>
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<td>(gs)</td>
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<td>$</td>
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<tr>
<td>$</td>
<td>\pi\pi^*\rangle_L_0\rangle$</td>
<td>5.41</td>
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<tr>
<td>9H-2-aminopurine</td>
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<td></td>
<td>(gs)</td>
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1 Absorption band origins for jet-cooled adenine, assigned to 9H-adenine (54–58).
2 Absorption band maxima in adenine (47, 59–61).
3 Geometry optimization leads to $(gs/\pi\pi^*\rangle_L_{\text{CI}}\rangle$ in adenine (see Results and Discussion and ref. 35).
4 Absorption band origin for jet-cooled adenine and fluorescence in water (both assigned to 7H-adenine): $\phi_F = 2.6\times 10^{-4}$, $\tau_{\text{rad}} = 8.8$ ps, $\tau_{\text{rad}} = \phi_F$ (47, 48).
5 Absorption band maxima for 2-aminopurine in poly(vinyl alcohol) films (39) and in water (40).
6 Jet-cooled 9H-2-aminopurine. Fluorescence in water: $\tau_0 = 3.70$ eV (41), $\phi_F = 0.66$, $\tau = 11.8$ ns, $\tau_{\text{rad}} = \tau_F/\phi_F$ (39).

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Fig. 2. Low-lying singlet excited states of 9H-2-aminopurine computed at the CASPT2/CASSCF level from the FC ground-state geometry along the MEP on the $|\pi\pi^*\rangle_L_0\rangle$ state [up to $|\pi\pi^*\rangle_L_{\text{min}}\rangle$ and along the reaction coordinate including $|\pi\pi^*\rangle_L_{\text{TS}}\rangle$ and $(gs/\pi\pi^*\rangle_L_{\text{CI}}\rangle$. 

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that upon light irradiation the $^1(\pi\pi^* L_a)$ state has the major population, the energy will be deactivated to the ground state through the CI ($gs/\pi\pi^* L_a$)$_{CI}$, in an efficient, ultrafast, nonradiative IC process that quenches most of the fluorescence. The initial downhill profile of the $L_a$ PES in 9H-adenine is equivalent to the one found for 9H-2-aminopurine. As in 9H-2-aminopurine, it is the twisting and wagging of the N6C4 bond that ultimately leads to the CI ($gs/\pi\pi^* L_a$)$_{CI}$, although in adenine it is just an hydrogen atom bonded to C2 that becomes perpendicular to the rings (see dihedral angle C5C2C4H2 in Fig. 3). The outer-of-plane deformation generating the CI structures is characterized by coupled torsional and wagging motions of the center’s substituent (either H in adenine or NH$_2$ in 2-aminopurine). Unlike in 2-aminopurine, where the wagging of the amino group was forced, in adenine the distortion implies outer-of-plane modes involving the H$_2$ atom, and there is no barrier hindering the access to the CI and therefore preventing the ultrafast nonradiative decay. Differently, in 7H-adenine, the computed path leads to a local minimum on the $^1(\pi\pi^* L_a)$ hypersurface, $^1(\pi\pi^* L_a NH_2)_{min}$, having a planar conformation for the rings while the NH$_2$ hydrogen atoms are twisted near 60° and the amino nitrogen lone-pair electrons face hydrogen H7 (see Supporting Methods, which is published as supporting information on the PNAS web site). The character of the state remains the same, that is, the $\pi\pi^*$ excitation is restricted to the rings without charge transfer from the NH$_2$ moiety. The PES around the region of the $^1(\pi\pi^* L_a NH_2)_{min}$ minimum is quite flat for the three computed singlet excited states, which become almost degenerate and cross at energies similar to those computed in 9H-adenine. From those crossings, one can expect that part of the energy can reach the minima of the $^1(\pi\pi^*)$ and $^1(\pi\pi^* L_a)$ states along their respective paths.

One basic difference, therefore, can be expected in the photophysics of both adenine tautomers. Although in 9H-adenine most of the energy will be quickly transferred to the ground state via a barrierless path along the $^1(\pi\pi^* L_a)$ PES and through the CI ($gs/\pi\pi^* L_a$)$_{CI}$, explaining the observed ultrafast decays (<100 fs) in 9H-adenine and 9-methyladenine (38), in 7H-adenine the presence of the $^1(\pi\pi^* L_a NH_2)_{min}$ minimum represents a barrier to surmount, hindering the fast relaxation. No equivalent ultrafast decay has been measured in 7-methyladenine (62, 63), and we may attribute this absence to the profile of the reaction path itself, together with the fact that part of the population may be transferred to the $^1(n\pi^*)$ and $^1(\pi\pi^* L_a)$ states in the region of the plateau. Because of this feature, the 7H-tautomer can contribute to the emitting properties of adenine in solution, as it is explained in Secondary Pathways to Dissipate the Energy.

Fig. 4 summarizes the basics of the photophysics of 9H-adenine and 9H-2-aminopurine designed on the basis of the present calculations. Regarding the main relaxation paths of the molecules, both mechanisms relate with the spectroscopic $^1(\pi\pi^* L_a)$ states, and their profiles account for the different emissive properties. In 9H-2-aminopurine, the path from the minimum of the $^1(\pi\pi^* L_a)$ state to reach the lowest CI has a computed barrier of 5.0 kcal mol$^{-1}$, and the CI lies above the minimum. The theoretical results are consistent with the measured fluorescence and the corresponding slow decay, reported in water with a lifetime 11.8 ns. On the contrary, in 9H-adenine, the analogous $^1(\pi\pi^* L_a)$ state has no minimum, and the lowest-lying, singlet excited state structure corresponds to a CI that allows relaxation of the system toward the ground state in a barrierless, ultrafast (subpicosecond) manner. Fluorescence is surely enhanced in polar media (45) for 2-aminopurine because of the larger stabilization of the $^1(\pi\pi^* L_a)_{min}$ structure in polar solvents, as compared with the barrier or the CI. Elucidation of the effects of the solvent on the PES profiles will require a more detailed theoretical study.

Secondary Pathways to Dissipate the Energy. We have shown that reaction paths related to the $^1(\pi\pi^* L_a)$ state form the primary mechanism for energy relaxation as a fluorescent source in 9H-2-aminopurine and in a radiationless way in 9H-adenine. Secondary relaxation mechanisms can be found along the other low-lying singlet states PES. Table 2 and Fig. 4 summarize the computed structures and the energy barriers required to reach the PES funnels.

In 9H-2-aminopurine, the $^1(n\pi^*)$ state lies vertically slightly above the $^1(\pi\pi^* L_a)$ state, and it will not be largely populated by direct absorption considering the almost forbidden character of the corresponding transition. A CI between the two states, $^1(n\pi^* L_a/\pi\pi^*)_{CI}$, at a nearly planar structure, intermediate between the FC geometry and the $^1(n\pi^*)$ minimum conformation, has been found, however, and it may lead to a partial population switch toward the low-lying $^1(\pi\pi^* L_a)$ state. From the slightly distorted $^1(n\pi^*)$ state minima, $^1(n\pi^*)_{min}$ (see Fig. 4), it is also possible to reach a CI with the ground state, ($gs/\pi\pi^* L_a$)$_{CI}$, having a structure in which the N6C4 bond has been twisted, leaving H$_2$ perpendicular to the rings. The barrier to access the CI from $^1(n\pi^*)_{min}$ through the transition state structure, $^1(\pi\pi^*)_{TS}$, has been computed at 1.9 kcal mol$^{-1}$ above the minimum. In the gas phase, there is enough excess vibrational energy to surmount the barrier, and the expected lifetimes should not be too large. In polar environments, however, the $^1(n\pi^*)$ state clearly will be destabilized, and the profile will change accordingly, probably increasing the relaxation times.

Regarding the higher-lying $^1(\pi\pi^* L_a)$ state, a CI has been
computed connecting the two low-lying $1(n\pi^*)$ states, $(\pi\pi^+ L_0/\pi\pi^+ L_b)_{CI}$, placed 4.3 kcal mol$^{-1}$ above the $(\pi\pi^+ L_0)$ state minimum, $(\pi\pi^+ L_0)_{\text{min}}$. Taking into account the large oscillator strength calculated for the $(\pi\pi^+ L_0)$ virtual transition, such a funnel may become important when the system is irradiated with a short wavelength to additionally populate the $(\pi\pi^+ L_0)$ state and so contribute to the final fluorescence. Recent transient-absorption experiments in aqueous environments (64) identified three low-lying excited states in 2-aminopurine. A nonfluorescent long-lived state that we assign as $(n\pi^*)$, with excited-state absorption polarized perpendicular to the ground-state absorption, was detected in minor proportion and decayed with a lifetime of $>15$ ns (64), a channel that also has been attributed to other species (43). In contrast, another state displaying stimulated emission was measured, and it evolved in 1.3 ps into a new fluorescent state that finally decays in $6.5$ ns (64). According to the present results, the two pathways can be related to the $(\pi\pi^+ L_0)$ and $(\pi\pi^+ L_b)$ states of 9H-2-aminopurine, respectively, whereas the computed CIs connecting both states and finally the ground state represent the funnels for efficient IC.

With respect to 9H-adenine and along the MEPs of the $(\pi\pi^+ L_0)$ state, a fraction of the population can be transferred to the $(n\pi^*)$ and $(\pi\pi^+ L_b)$ routes mediated by the CIs $(n\pi^*/\pi\pi^+ L_0)_C$ and $(\pi\pi^+ L_0/\pi\pi^+ L_b)_C$ toward their respective minima (compare with Fig. 4) (35). The $(\pi\pi^+ L_0)$ state minima also can be populated by direct absorption (54–58), in particular in 7H-adenine, in which transition to $(\pi\pi^+ L_0)$ is computed with a relatively larger oscillator strength. From $(\pi\pi^+ L_b)_{\text{min}}$, the MEP shows a barrier to reach the CI with the $(n\pi^*)$ state, 3.2 kcal mol$^{-1}$ above $(\pi\pi^+ L_0)_{\text{min}}$ in 9H-adenine, and 7.6 kcal mol$^{-1}$ in 7H-adenine (35). Therefore, because of the presence of the barrier and, consequently, the corresponding decrease in the excess of vibrational energy available to reach the CI, one could expect a longer lifetime associated to this decay when the $(\pi\pi^+ L_0)$ state band origin would become directly populated. Indeed, this assumption has been corroborated experimentally. At long excitation wavelengths (277 nm (4.48 eV)), a single decay has been measured with a lifetime of $>2$ ps (it was 1.1 ps at shorter wavelengths) (37). The fact that the barrier is larger in 7H-adenine, together with the profile of the $(\pi\pi^+ L_b)$ MEP in the 7H tautomer, including a plateau where an efficient population transfer from the $(\pi\pi^+ L_0)$ to the $(\pi\pi^+ L_b)$ degenerate states can take place (see Fig. 5, which is published as supporting information on the PNAS web site), leads us to suggest that the weak fluorescence ($\phi_F \approx 3 \times 10^{-4}$) observed in aqueous adenine starting near 4.43 eV (46, 52) comes essentially from the $(\pi\pi^+ L_b)$ state of the minor 7H-tautomer. This assignment is also confirmed by comparing the computed and measured radiative lifetimes (see Table 1) and by our calculated band origin at 4.60 eV, lower than for 9H-adenine, as discussed elsewhere (35). Experimental evidence also supports that 7-substituted adenines fluoresce more intensely than 9-substituted adenines, whereas adenine nucleoside and nucleotide (9-substituted species) are known to fluoresce less than the monomer (2, 46, 47).

Also in adenine, after the switch to the $(n\pi^*)$ state through the $(\pi\pi^+ L_b/\pi\pi^+ L_b)_{\text{CI}}$, the $(n\pi^*)$ state minima can be reached through a reaction path similar to that computed for the equivalent state in 9H-2-aminopurine. A CI $(gs/n\pi^*)_{C}$ can be accessed from $(n\pi^*)_{\text{min}}$ after surmounting a small barrier of 1.9 kcal mol$^{-1}$ through the transition state, $(n\pi^*)_{TS}$. The deformation leading to the CI is, like in 2-aminopurine, the rotation of the N1C6 bond. Here, however, the amino group, NH$_2$, substitutes C6, and the associated normal modes leading to a NH$_2$ structure perpendicular to the ring structure have larger energy (see Figs. 6 and 7, which are published as supporting information on the PNAS web site). We tentatively propose that the path leading from $(\pi\pi^+ L_b)$ to the $(n\pi^*)$ state, finally reaching the CI with the ground state, $(gs/n\pi^*)_{C}$, is responsible for the second, slower, relaxation decay measured in jet-cooled adenine with a lifetime of 1.1 ps (38). A piece of strong evidence in favor of such an assignment is the measured decay lifetimes in jet-cooled N,N-dimethyldenine: 200 fs and 3.1 ps (38). Although in this system the ultrafast decay remains similar to that in adenine because the deformation in the N$_2$C$_2$H$_2$ structure is equivalent, the second relaxation triples its lifetime in N,N-dimethyldenine, a logical consequence of the role of a relatively hindered $(gs/n\pi^*)_{C}$ CI because of the presence of two methyl groups substituting N1.

**Final Remarks.** Although adenine efficiently quenches fluorescence, 2-aminopurine (a similar molecule) is a highly emissive source used to probe protein-induced conformational changes in DNA. We have rationalized their different photophysical behavior by mapping the PES at the highest available theoretical level, ab initio.
CASPT2, based on carefully computed MEPs, state minima, CIs, transition states, and barriers. A three-state model comes out for both systems, explaining (i) the strong fluorescence measured in adenine and 2-aminopurine because of the presence of a (ππ*) state minimum below the lowest accessible CI; (ii) how in adenine a barrierless pathway from the initially populated excited state leads to the analogous CI and consequently to an efficient IC toward the ground state dissipating most of the energy; (iii) that the two observed decay mechanisms are mediated by two distinct CIs with the ground state; (iv) how the 7H-tautomer has a major contribution to the weak fluorescence recorded in solvated adenosine.

Up to quite recently, a limited scheme like Lim's proximity effect model involving the coupling of nearby ππ* and ππ* states still has been claimed to explain quenching of fluorescence in these important systems. It has been shown here both in adenine and 2-aminopurine that the ππ* states do not play an important role in the leading photophysical event, whereas the character, relative location, and accessibility of the lowest CI do. Until more elaborated theoretical studies including time scale can be performed in systems of this size using quantum chemical parameters, at least as accurate as the present ones, knowledge of the photochemical reaction path (i.e., MEP after the initial irradiation, minimum energy barriers required to access CIs, and crossing seams) represents the most powerful theoretical approach presently available to interpret photochemical processes.

**Methods**

Optimizations of minima, PES crossings, and MEPs have been performed at the CASCF level of theory. MEPS have been built as steepest descendent paths in which each step requires the minimization of the PES on a hyperspherical cross section of the PES centered at the initial geometry and characterized by a predefined radius (see Supporting Methods). At the obtained points, CASPT2 calculations on several singlet states were carried out to include the necessary dynamical correlation effects. The protocol is usually named CASPT2/CASSCF and has proved its accuracy repeatedly (65–71). The CI searches were carried out first at the CASCF level by obtaining minimum energy crossing points and later by performing CASPT2 calculations around the CASSCF-optimized structures. The one-electron atomic basis set 6–31G(d,p) was used throughout. Unless otherwise specified, the final results used an active space of 11 orbitals and 12 electrons. No symmetry restrictions were imposed during the calculations, which in all cases used the MOLCAS-6 set of programs (72). From the calculated CASCF transition dipole moments and the CASPT2 excitation energies, the radiative lifetimes have been estimated by using the Strickler–Berg relationship (73), which is basically valid under the hypothesis that no excited-state deactivated occurs. A detailed description of additional technical details can be found in Supporting Methods and Tables 3–5. These were published as supporting information on the PNAS web site, together with the selected geometries computed for the different systems.

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