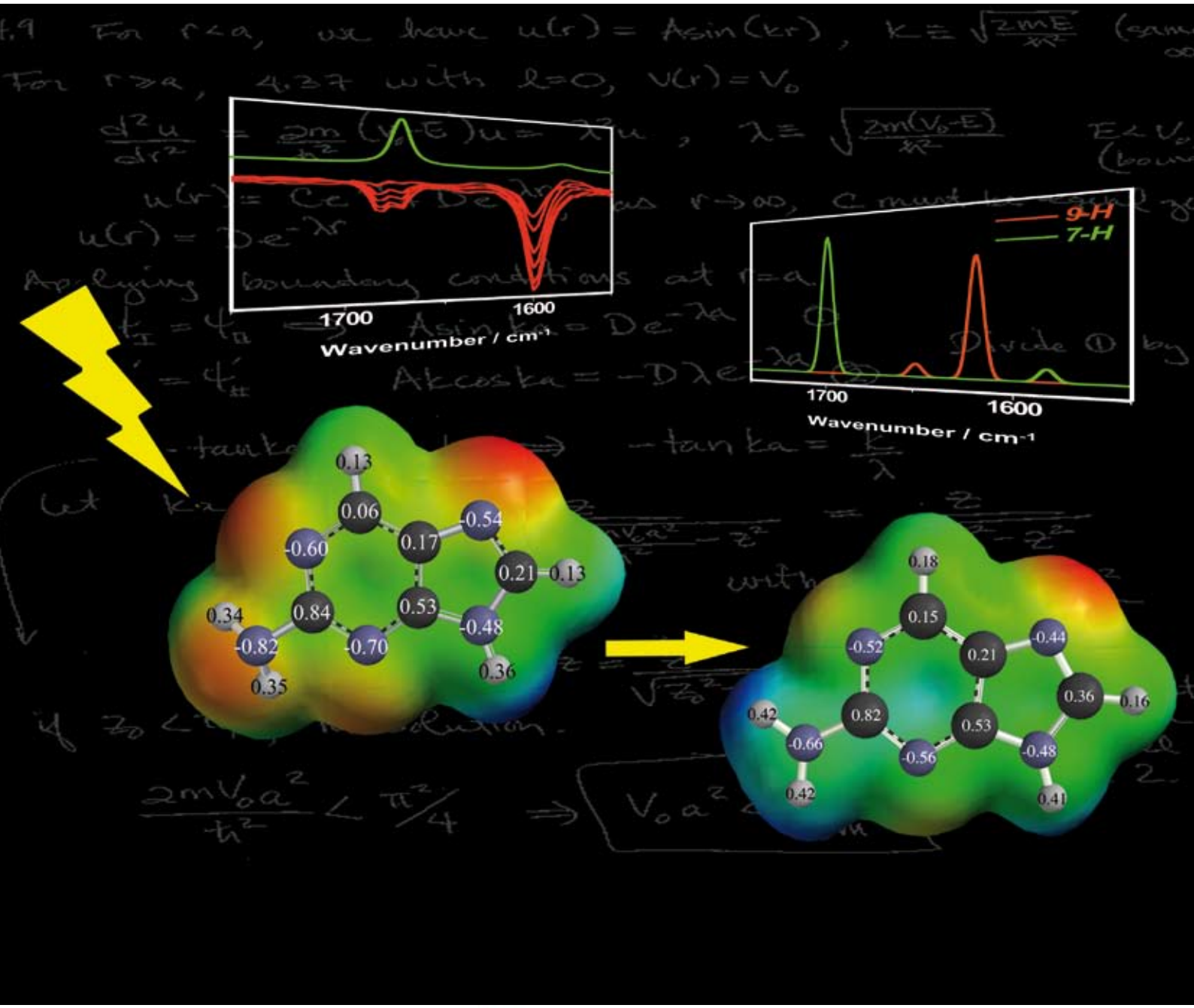
# Photochemical & Photobiological Sciences

An international journal

www.rsc.org/pps

Volume 6 | Number 9 | September 2007 | Pages 921–1036




ISSN 1474-905X

RSC Publishing

1474-905X(2007)6:9;1-R

# Picosecond time-resolved infrared study of 2-aminopurine ionisation in solution†‡

**Marina K. Kuimova,*§[a] Peter M. W. Gill,[a,c] Ching-Yeh Lin,[a,c] Pavel Matousek,[b] Michael Towrie,[b] Xue Zhong Sun,[a] Michael W. George[a] and Anthony W. Parker*[b]**

***Received 18th April 2007, Accepted 9th May 2007***
***First published as an Advance Article on the web 24th May 2007***
**DOI: 10.1039/b705801b**

Two photon ionisation of 2-aminopurine (2AP) has been monitored following 267 nm irradiation in neutral and acidic aqueous solutions using picosecond time-resolved infrared spectroscopy (ps-TRIR). The transient infrared spectra obtained in neutral and acidic conditions show significant differences that are consistent with the formation of different species, namely the 2AP radical cation, $2AP^{+\bullet}$, in acidic conditions and the uncharged radical, $2AP^{\bullet}(-H^{+})$, in neutral conditions. The ps-TRIR data indicate that deprotonation of $2AP^{+\bullet}$ in neutral solution takes place within <2 ps following photoionisation. DFT calculations (EDF1/6–31+G*) were used to support the assignment of the intermediates observed in these spectroscopic experiments.

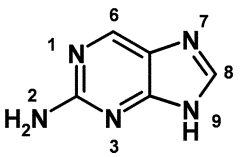
## Introduction

There is considerable research into DNA photochemistry[1,2] particularly since UV/visible irradiation of the DNA constituents has the potential to initiate events that may ultimately lead to mutations and the onset of cancer.[3] Its effects have been recently highlighted in a review by Møller and Mousseau reporting the biological consequences of Chernobyl over the last 20 years.[4]

Understanding the consequences of DNA damage brought about by radiation remains a serious intellectual challenge. It is known that one electron ionisation of the DNA bases produces the corresponding base radical cations[5] and these species can under suitable conditions easily lead to the fixation of genetic damage; for example, through the formation of mutagenic 8-oxo-7,8-dihydroguanine (8-oxo-G) produced by the reaction of guanine radical cation with water.[6]

Photoexcitation and ionisation of DNA induces "direct" damage to the genetic code. However, an alternative mechanism, "indirect" DNA damage is also possible when a chemical or photochemical reaction occurs between an activated agent and a DNA base. Indirect damage has been studied in a number of ways including using the DNA intercalator[7] $[Ru(phen)_2(1,4,5,8\text{-tetraazaphenanthrene})]^{2+}$ and modified DNA bases such as 4-pivaloylated thymidine[8] and 2-aminopurine.[9] The target of damage within the DNA double helix is *via* oxidation of guanine, the base with the lowest ionisation potential.[10]

2-Aminopurine (2AP) is a DNA base analogue with a structure very similar to that of adenine and guanine, see Scheme 1. The scheme depicts the most stable 9-H tautomer, however, for 2AP in solution phase a second tautomer is known to exist with a hydrogen atom located at the N7 position.[11]




**Scheme 1** Molecular structure of 2-aminopurine (2AP).

When incorporated into DNA, 2AP mimics A and G in so far as participating in the Watson–Crick base pairing with thymine or cytosine forming unperturbed double helical DNA structures.[12] However, unlike the natural DNA bases which do not sufficiently absorb light with $\lambda \gg 260$ nm, 2AP has an intense electronic absorption band at 305 nm ($\varepsilon = 6020\ M^{-1}\ cm^{-1}$).[13] This permits incorporation of 2AP in synthetic DNA sequences and the low energy absorption maximum of 2AP allows direct and selective excitation of this base mimic within DNA. Also, unlike the natural DNA bases, the lowest singlet excited state of 2AP is characterised by a long lifetime *ca.* 10 ns[14] and this has led to its extensive use in DNA studies. Thus 2AP has been used as an internal fluorescent probe for the study of DNA solvation[15] and structural dynamics studies including base-pairing interactions in DNA, structural perturbations resulting from the base bulges and protein induced DNA melting.[16,17] Furthermore, 2AP can be easily ionised with 308 nm laser light in a two-photon process that has been confirmed by detection of the solvated electron using time-resolved transient absorption.[9] The possibility to selectively ionise 2AP while it is incorporated into DNA strands provides a convenient way of

[a]*School of Chemistry, University of Nottingham, University Park, Nottingham, UK NG7 2RD*
[b]*Central Laser Facility, Science & Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK OX11 0QX. E-mail: a.w.parker@rl.ac.uk; Fax: +44 (0)1235-445808; Tel: +44 (0)1235-445109*
[c]*Research School of Chemistry, Australian National University, ACT, 0200, Australia*

† This paper was published as part of the special issue in honour of David Phillips.

‡ Electronic supplementary information (ESI) available: IR spectra calculated by DFT. See DOI: 10.1039/b705801b

§ Current address: Department of Chemistry, Imperial College London, Exhibition Road, South Kensington, UK SW7 2AZ. Email: m.kuimova@imperial.ac.uk; Tel: +44 (0)207-5895111; Fax: +44 (0)207-5945801.

injecting charge into the synthetic polynucleotide sequences and the redox potential of ionised 2AP ($2AP^{+\bullet}$ or the deprotonated form $2AP^{\bullet}(-H^{+})$) is such that it is capable of oxidising 8-oxo-G and guanine[9,18] but not the other DNA bases. This reactivity has been utilised to investigate the reversible electron transfer reaction from ionised 2AP to guanine with a series of synthetic oligonucleotides to demonstrate the distance dependence and the efficiency of this reaction.[9,17–19]

While several methods of injecting charge into DNA double helix exist monitoring the subsequent reactions and formation of damage products in real time is problematic due to the lack of sensitive versatile spectroscopic methods. Pump–probe fast reaction methods based upon monitoring the transients formed using UV/visible absorption (transient absorption TA) has been the most frequently used method to date employed to study DNA base intermediates produced either by ionisation, pulse radiolysis or indirect oxidative reactions.[5,20,21] However, spectroscopic bands observed in UV/Visible spectroscopy are often broad and featureless and provide little structural detail. While EPR spectroscopy provides a structural probe of the intermediates, its application to study ultrafast reactions of DNA is hampered by the limited time-resolution of this technique. Recently we have reported on the use of picosecond time resolved infrared spectroscopy (ps-TRIR) to monitor the effect of UV excitation to either produce singlet excited states[22] or ionised products[23] of DNA bases and polynucleotides. In addition we have also demonstrated that ps-TRIR can be used to monitor the indirect reaction of guanine with the important biological oxidant, carbonate radical.[23]

Although ionised 2AP has been used in many mechanistic investigations of electron transfer process in DNA, the nature of the intermediates involved in this reaction often remains ambiguous. Oxidising species might be either the positively charged radical cation $2AP^{+\bullet}$ or the neutral radical $2AP^{\bullet}(-H^{+})$. Indeed based on the observed isotope effect it was suggested that the electron transfer reaction between ionised 2AP and guanine is proton coupled[24] thus the nature of the 2AP species might play the crucial role in determining the rate of this process. Previously, ionisation of 2AP in solution was studied using UV/Visible TA techniques. Biphotonic ionisation of neutral aqueous solutions of 2AP (pH = 7) with 308 nm light results in the formation of the 2AP radical ($2AP^{\bullet}$), detected on the nanosecond timescale ($\lambda_{max}$ at 300–400 nm in TA), but not the radical cation $2AP^{+\bullet}$.[9] This was attributed to the fast deprotonation of $2AP^{+\bullet}$ and this rate is expected to be pH dependent. Consistent with this, $2AP^{+\bullet}$ was detected at low pH values and the p$K_a$ of 2AP radical cation ($\lambda_{max}$ at 355 nm in TA) was established to be 2.8.[13]

In this paper we apply ps-TRIR to study the photochemistry of 2AP in aqueous solution at different pD following a 267 nm laser irradiation in order to obtain the structural fingerprint and establish the nature of the different intermediates of the ionised mimic of DNA base.

## Experimental

2-Aminopurine, $D_2O$ (99.99%), DCl (25%), NaCl (all Aldrich) were used as received without further purification. UV/Visible measurements were carried out on a Lambda Perkin Elmer Spectrometer. FTIR measurements were carried out on Nicolet Avatar 360 spectrometer; prior to data acquisition the spectrometer sample compartment was purged with nitrogen gas for at least 15 min until a negligible change in the background spectrum in the water vapour absorption region (1500–1900 $cm^{-1}$) was achieved. pD was adjusted by adding small aliquots of DCl to the solution of the sample. pH was measured on Hanna Instruments pH210 microprocessor pH meter (±0.02 accuracy) and pD was calculated from these values according to eqn (1).[25]

$$pD = pH + 0.4 \quad (1)$$

### ps-TRIR measurements

The picosecond TRIR experiments were carried out on the PIRATE apparatus at the Central Laser Facility of the CCLRC Rutherford Appleton Laboratory. This apparatus has been described in detail previously.[26] Part of the output from a 1 kHz, 800 nm, 150 fs, 2 mJ Ti-Sapphire oscillator/regenerative amplifier (Spectra Physics Tsunami/Spitfire) was used to pump a white light continuum seeded β-$BaB_2O_4$ (BBO) optical parametric amplifier (OPA). The signal and idler produced by this OPA were difference frequency mixed in a type I $AgGaS_2$ crystal to generate tuneable mid-infrared pulses (*ca.* 150 $cm^{-1}$ FWHM, 1 μJ), which were split to give probe and reference pulses. Third harmonic generation of the residual 800 nm light provided 267 nm pump pulses. The broad absorption spectrum of $e_{aq}$ allows monitoring its formation at 800 nm probe wavelength, achievable within the PIRATE system and away from absorption of 2AP intermediates.[9,13] Both the pump and probe pulses were focused to a diameter of 200–300 μm in the sample. Changes in infrared absorption at various pump–probe time delays were recorded by normalising the outputs from a pair of 64-element MCT infrared linear array detectors on a shot-by-shot basis. In all TRIR experiments static samples were used *i.e.* a small volume of solutions saturated in 2AP (*ca.* 1 mM) were placed between two $CaF_2$ windows of an IR cell (Harrick Scientific Corp.), *ca.* 56 μm pathlength. The samples were monitored by FTIR spectroscopy and changed frequently to prevent decomposition of 2AP.

### DFT calculations

The EDF1/6-31+G* functional,[27] which has been shown to be accurate for frequency calculations,[28] was used for calculating the harmonic vibrational frequencies of 2-aminopurine and related molecules. The calculations were performed using Q-Chem 2.1 software package.[29] The exchange–correlation quadrature was performed using an Euler–Maclaurin–Lebedev grid[30] with 100 radial and 194 angular points on each atom. Each frequency calculation was preceded by a geometry optimization. The Onsager[31] and Langevin[32] solvation models were used to predict solvatochromic shifts in order to facilitate comparisons with the experimental spectra. In the Onsager model the solute is placed in a spherical cavity surrounded by a continuous solvent medium characterised by a dielectric constant. Three parameters are required to define the system: the cavity radius, the polarizability, and the dielectric constant.[31] The Langevin dipole solvation model is based on the evaluation of interactions between the electrostatic field of the solute and point dipoles placed on a cubic grid surrounding the solute. This grid of dipoles is surrounded by a dielectric continuum.

The solute electrostatic field is generated from the point charges placed at the atomic nuclei. To obtain the solvation free energy, all the effects must be summed up, namely the electrostatic part of the solvation energy, the outer continuum dielectric, van der Waals, hydrophobic, and solute-polarization terms.[32]

## Results and discussion

Fig. 1 shows the series of FTIR spectra recorded for 2AP in $D_2O$ following pD change from 7 to 2. The decrease in pD leads to the reduction in intensity of the bands centred at 1582 and 1626 $cm^{-1}$, while two new bands centred at 1597 and 1670 $cm^{-1}$ grow in. The positive shift of the IR bands of 2AP with decreasing pD is consistent with its protonation. Protonation can result in either a positive or a negative shift of the IR bands depending on whether the bonds weaken or strengthen. In purines it is generally observed that skeletal stretching motions move to higher wavenumber upon protonation.[33,34]

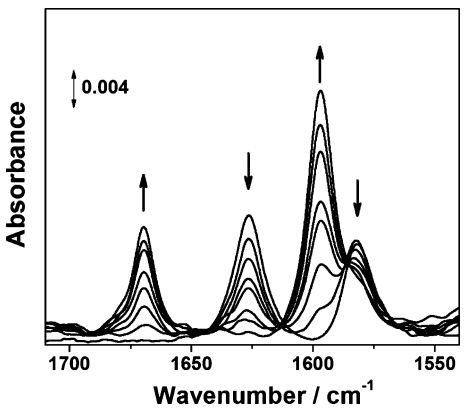



**Fig. 1** A series of FTIR spectra recorded for 1 mM 2-aminopurine solution in $D_2O$ following decrease in pD from pD = 7 to pD = 2 (pD = 7.3; 5.9; 4.7; 4.4; 4.2; 4.0; 3.9; 3.7; 2.0).

This could be explained by an increase in the electron density of the carbon atom adjacent to the nitrogen atom that is protonated.[35] Our result is consistent with a $pK_a$ of 3.8 for protonation of 2AP as previously reported.[13] Lowering the pD of the solution is accompanied by only minor changes in the UV/Visible absorption spectrum and thus in both neutral and acidic solutions we expect to populate the π–π* singlet excited state with 267 nm excitation.

Fig. 2 shows a series of ps-TRIR spectra obtained following 267 nm excitation of 2AP in $D_2O$ at pD = 7 (a) and pD = 2 (c). The inserts show the kinetic TRIR recovery traces obtained from the IR band maxima. It is clear that in both neutral and acidic environments the ground state bands are bleached immediately following excitation and that partial recovery occurs on the timescale of the ps-TRIR experiment (2 ns).

Ionisation of 2AP following 267 nm irradiation was confirmed by monitoring the absorption of solvated electron at 800 nm which was considerably more intense than that for the control experiment obtained following 267 nm irradiation of the pure solvent (Fig. 3). We monitored the absorbance of the solvated electron while varying the laser energy and found that ionisation was consistent with a biphotonic process at both pD = 7 and pD = 2.

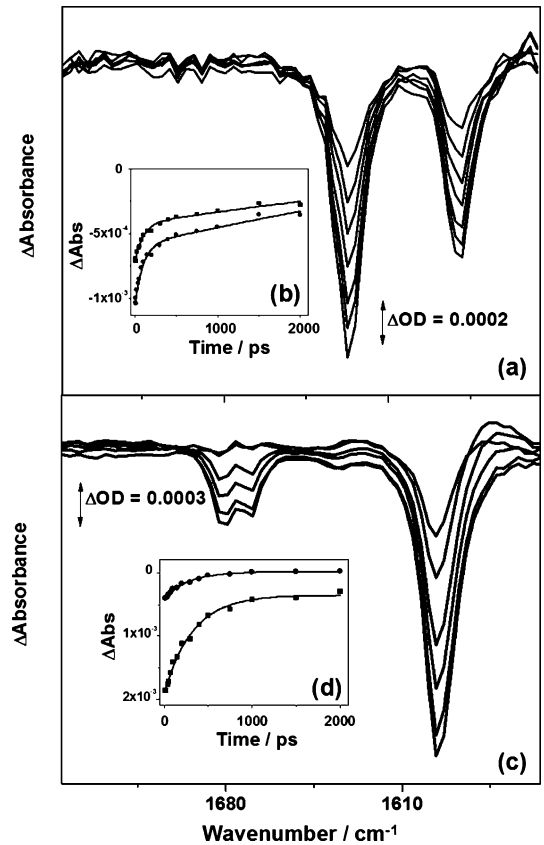



**Fig. 2** Series of TRIR spectra obtained following 267 nm excitation of 1 mM solution of 2-aminopurine in $D_2O$ at (a) pD = 7 at time delays of 5, 20, 75, 200, 400, 1000 and 2000 ps and (c) pD = 2 at 5, 20, 75, 200, 750 and 1500 ps. Inserts show the single point kinetic recovery traces in the IR band maxima at (b) pD = 7 and (d) pD = 2.

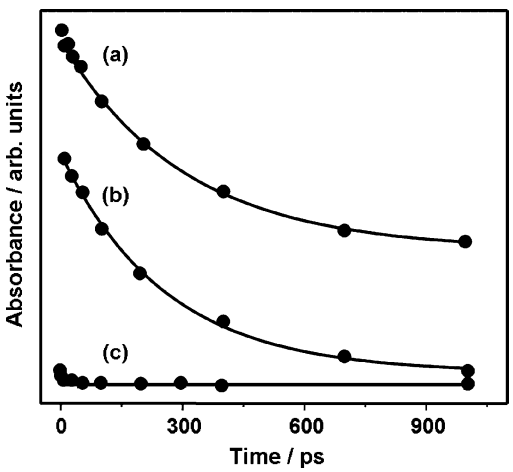



**Fig. 3** Kinetic decay traces obtained at 800 nm (monitoring $e_{aq}$) following 267 nm irradiation of (a) 1 mM 2-aminopurine in $D_2O$ at pD = 7, (b) 1 mM 2-aminopurine in $D_2O$ at pD = 2 and (c) pure $D_2O$.

At neutral pD no strong positive transient bands in the region studied (1450–1700 $cm^{-1}$) were observed in the ps-TRIR spectra. Multi-curve analysis of these ps-TRIR spectra did not reveal any transient features hidden beneath the bleached bands, Fig. 4.

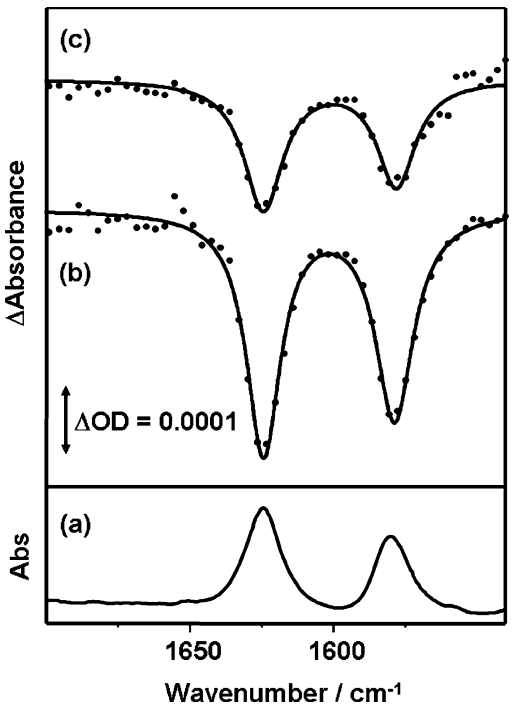
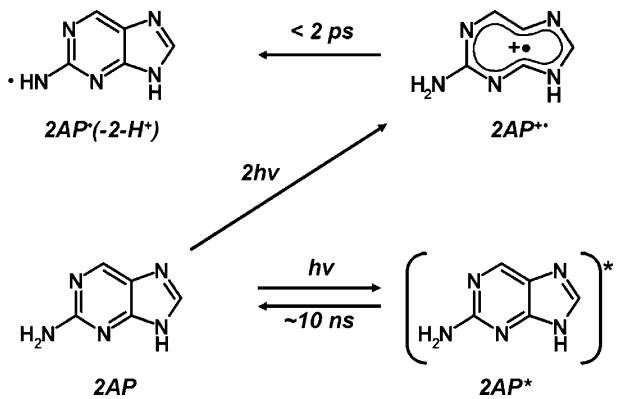



**Fig. 4** (a) FTIR and TRIR spectra obtained (b) 2 ps and (c) 2000 ps following 267 nm irradiation of 1 mM solution of 2-aminopurine in $D_2O$ at pD = 7 (●). Solid lines in (b) and (c) represent multi-curve fit of these data.

In order to help the assignment of the transient spectra we performed DFT calculations, see electronic supplementary information (ESI).‡ The calculations of the IR spectra of 2AP in the ground state show good agreement with experiment (ESI,‡ Fig. 1). Above we noted that 2AP in aqueous solution exists in two tautomeric forms, the 7-H and the 9-H forms. Time resolved fluorescence studies have demonstrated that the ratio of these two species in aqueous solution is *ca.* 4 : 6, respectively.[11] The IR spectra calculated for 7-H and 9-H tautomers in the ground state (ESI,‡ Fig. 1) do not possess any characteristic features to enable an unequivocal assignment of the experimental spectrum to either of the two forms.[36]

Photoionisation of 2AP in neutral solution has been previously shown to produce the deprotonated form, $2AP^{\bullet}(-H^+)$, on the nanosecond timescale.[13] The calculated IR spectra of the four different tautomers of $2AP^{\bullet}(-H^+)$ with proton excluded from different possible sites are given in the ESI.‡ Of these four species the lowest energy tautomer, 2AP radical deprotonated from amino group, $2AP^{\bullet}(-2\text{-}H^+)$, was previously reported to form following deprotonation.[13] Our calculations for this species both in the gas phase and in the solution phase do not predict intense IR bands in the spectral region of interest, 1500–1700 $cm^{-1}$, Table 1, while the spectra calculated for the three other tautomers ($2AP^{\bullet}(-6\text{-}H^+)$, $2AP^{\bullet}(-8\text{-}H^+)$ and $2AP^{\bullet}(-9\text{-}H^+)$, see ESI‡) predict intense vibrational bands in this region. The formation of $2AP^{\bullet}(-2\text{-}H^+)$ is therefore in agreement with our experimental TRIR spectrum in neutral solution showing no intense IR transient bands. The spectrum of $2AP^{\bullet}(-H^+)$ should be contrasted with the initial product of ionisation $2AP^{+\bullet}$ for which IR bands are observed in this region (see below).

We interpret our results to indicate that the main species formed following 267 nm excitation in neutral $D_2O$ solution (pD = 7) is $2AP^{\bullet}(-H^+)$, the deprotonated radical cation, and that deprotonation occurs within 2 ps following excitation. In addition to the ionisation product we also expect to generate the excited state of 2AP by a monophotonic process. The processes which follow an excitation of 2AP in neutral aqueous solution are outlined in Scheme 2.




**Scheme 2** Processes following absorption of 267 nm light by 2-aminopurine in aqueous solution at pH = 7. Monophotonic excitation (*hv*) results in the formation and subsequent decay of the excited state, while two photon excitation (2*hv*) leads to the ionised product which deprotonates within 2 ps. The proton loss can be suppressed in acidic solution at pH = 2.

**Table 1** Ground state and transient FTIR band positions obtained following UV irradiation of 2-aminopurine in fluid $D_2O$ solution at room temperature

| FTIR 2AP (pD = 7) | Calculated IR band positions 2AP[a,b] | Calculated IR band positions 2AP[a,c] | TRIR 2AP (pD = 2)[a,d] | Calculated IR band positions $2AP^{+\bullet}$[a,b] | Calculated IR band positions $2AP^{+\bullet}$[a,c] |
|---|---|---|---|---|---|
| 1626 | 1643 (354) | 1642 (453) | 1670 | 1648 (17) | 1650 (19) |
| | 1632 (195) | 1638 (14) | 1580 | 1617 (156) | 1617 (171) |
| 1582 | 1578 (107) | 1575 (92) | | | |
| 1522 | 1512 (139) | 1515 (83) | | | |
| 1437 | 1478 (104) | 1476 (66) | | | |
| 1426 | 1431 (207) | 1432 (137) | | | |
| 1377 | 1359 (41) | 1392 (17) | | | |

[a] Calculated IR band position ($cm^{-1}$) and relative intensity in parentheses. [b] The calculated values shown use the Onsager solvent model (all other calculations can be found in the ESI‡). [c] The calculated values shown use the Langevin solvent model (all other calculations can be found in the ESI‡). [d] Measured with ps-TRIR in the spectral region 1500–1800 $cm^{-1}$.

At neutral pD the reformation of the bleached ground state IR bands does not conform to single exponential decay, Fig. 2. An initial fast recovery of the bleach, *ca.* 100 ps, is accompanied by a much slower component (*ca.* 5–10 ns). The fast component is likely to be due to the geminate recombination of solvated electron with the 2AP ionised product. However, the nature of the slower component of the decay is less clear. This could be either due to the decay of the excited singlet state of 2AP which is known to have a lifetime of 10 ns,[14] or may correspond to the back reaction with the solvated electron. Our experimental set-up was unable to monitor delays >2.5 ns and as such we were unable to resolve this issue.

The series of ps-TRIR spectra obtained following 267 nm excitation of 2AP in $D_2O$ solution at pD = 2 are also shown in Fig. 2. As in the case of 2AP in neutral solution, both parent bands at 1597 and 1670 $cm^{-1}$ are bleached immediately following excitation. However, the TRIR spectrum observed at pD = 2 is different to that of pD = 7. The transient band of low intensity centred at *ca.* 1580 $cm^{-1}$ appears following 267 nm excitation. In addition, the comparison of TRIR spectra with ground state FTIR spectrum of 2AP recorded at pD = 2 reveals that the ratio of the intensities of the two parent bands in the bleached state is not maintained. The high energy band is bleached to approximately half of its intensity of the band centred at 1597 $cm^{-1}$. Both parent bleaches partially recover on the timescale of the experiment and while the band at 1597 $cm^{-1}$ remains bleached, a positive feature becomes apparent near 1670 $cm^{-1}$. These data were analysed using multi-curve Lorentzian fit of the TRIR spectra and this analysis clearly shows the formation of a transient band at 1670 $cm^{-1}$, Fig. 5. This analysis also indicates the presence of a second band at 1580 $cm^{-1}$ which appears to grow in at the same time as the 1670 $cm^{-1}$ band.

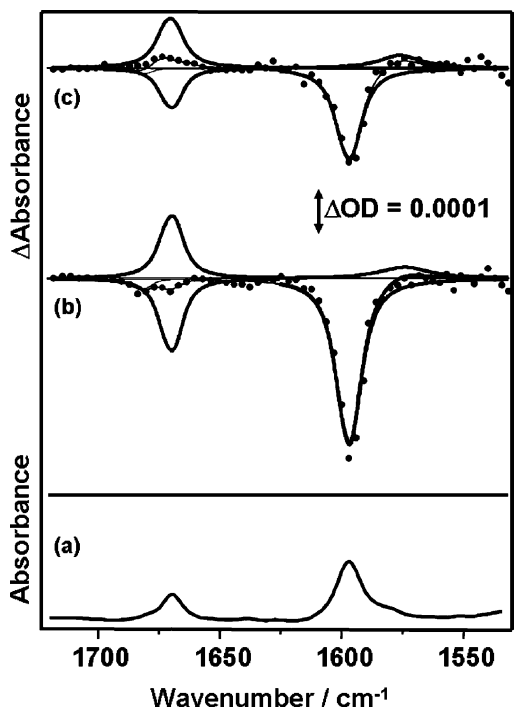



**Fig. 5** (a) FTIR and TRIR spectra obtained (b) 50 ps and (c) 1000 ps following 267 nm irradiation of 1 mM solution of 2-aminopurine in $D_2O$ at pD = 2 (●). Solid lines represent multi-curve fit of these data with individual Lorentzian components shown as positive and negative bands.

The present study clearly shows that following 267 nm excitation of 2AP in acidic and neutral solution the TRIR spectra are strikingly different, with two transient bands observed at pD = 2 and no transient bands observed at pD = 7. This indicates the formation of different species in these different environments. These results are consistent with the TA study where it was shown that $2AP^{+\bullet}$ rapidly deprotonates to give a neutral radical, $2AP^{\bullet}(-H^{+})$ in neutral solution and that proton loss in $2AP^{+\bullet}$ is suppressed in acidic solution at pD = 2 (below $pK_a = 2.8$).[13] This allows us to assign the transient bands observed in ps-TRIR at pD = 2 to $2AP^{+\bullet}$.

We have again used DFT calculations in an attempt to assign our spectral observations. The predicted IR spectra of initially formed $2AP^{+\bullet}$ both in the gas phase and in the solution phase using both Onsager and Langevin solvation models are in reasonable agreement with the transient IR spectrum that was observed for 2AP following 267 nm excitation in solution at pD = 2 and we tentatively assign these bands to be due to this species. We note here that the intensity pattern in the IR spectra calculated for 7-H tautomer of $2AP^{+\bullet}$ appears to agree better with experimental spectrum than that calculated for 9-H tautomer. However at this stage further experiments are necessary to confirm this observation.

## Conclusions

This work shows that ps-TRIR spectroscopy can be used to monitor the ionisation of DNA base mimic 2-aminopurine following 267 nm laser irradiation. Moreover, the neutral radical and radical cation of 2AP were identified with TRIR in different environments and the rate of deprotonation was altered by changing the acidity of these solutions. The use of TRIR for identification of different species is combined with DFT calculations of vibrational spectra of ionised intermediates, which provides the support for the assignment of the species observed with TRIR. This study paves the way to exploit the full potential of 2AP as an internal trigger of DNA damage and to monitor the electron transfer between 2AP and guanine through molecularly specific vibrational spectroscopy.

## Acknowledgements

We thank Professor P. O'Neill for helpful discussions and Mr A. J. Cowan and D. Jacopo for assistance with some of the ps-TRIR experiments. We are grateful to STFC, EPSRC (GR/M40486) and ORS (MKK) for financial support.

## References

1 (*a*) C. E. Crespo-Hernandez, B. Cohen, P. M. Hare and A. K. Kohler, Ultrafast excited-state dynamics in nucleic acids, *Chem. Rev.*, 2004, **104**, 1977–2019; (*b*) C. J. Burrows and J. G. Muller, Oxidative nucleobase modifications leading to strand scission, *Chem. Rev.*, 1998, **98**, 1109–1151.

2 H. Görner, Photochemistry of DNA and related biomolecules-quantum yields and consequences of photoionisation, *J. Photochem. Photobiol., B*, 1994, **26**, 117–139.

3 (*a*) J. Thacker, Sources and effects of ionizing radiation, in *United Nations Scientific Committee on the Effects of Atomic Radiation*,

New York, 2000, vol. II, pp. 1–72; (*b*) K. B. Beckman and B. N. Ames, Oxidative decay of DNA, *J. Biol. Chem.*, 1997, **272**, 19633–19636.
4 A. P. Møller and T. A. Mousseau, Biological consequences of Chernobyl: 20 years on, *Trend Eco. Evol.*, 2006, **21**, 200–207.
5 L. P. Candeias and S. Steenken, Ionization of purine nucleosides and their components by 193 nm laser photolysis in aqueous solution-model studies for oxidative damage of DNA, *J. Am. Chem. Soc.*, 1992, **114**, 699–704; T. Melvin, S. Botchway, A. W. Parker and P. O'Neill, Migration of photoinduced oxidative damage in models for DNA, *J. Chem. Soc., Chem. Commun.*, 1995, 653–654.
6 (*a*) R. P. Hickerson, F. Prat, J. G. Muller, C. S. Foote and C. J. Burrows, Sequence and stacking dependence of 8-oxoguanine oxidation: Comparison of one-electron *vs.* singlet oxygen mechanisms, *J. Am. Chem. Soc.*, 1999, **121**, 9423–9428; (*b*) J. Cadet, F. Odin, J. F. Mouret, M. Polverelli, A. Audic, P. Giacomoni, A. Favier and M. J. Richard, Chemical and biochemical postlabeling methods for singling out specific oxidative DNA lesions, *Mutat. Res.*, 1992, **275**, 343–354.
7 I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron and A. Kirsch-DeMesmaker, $[Ru(TAP)_2(dppz)]^{2+}$: a DNA intercalating complex, which luminesces strongly in water and undergoes photo-induced proton-coupled electron transfer with guanosine-5′-monophosphate, *J. Chem. Soc., Dalton Trans.*, 2004, 668–676.
8 B. Giese and A. Biland, Recent developments of charge injection and charge transfer in DNA, *Chem. Commun.*, 2002, 667–672.
9 V. Shafirovich, A. Dourandin, W. D. Huang, N. P. Luneva and N. E. Geacintov, Oxidation of guanine at a distance in oligonucleotides induced by two-photon photoionization of 2-aminopurine, *J. Phys. Chem. B*, 1999, **103**, 10924–10933.
10 V. M. Orlov, A. N. Smirnov and Y. M. Varshavsky, Ionization potentials and electron-donor ability of nucleic bases and their analogues, *Tetrahedron Lett.*, 1976, 4377–4378.
11 (*a*) R. K. Neely, S. W. Magennis, D. T. F. Dryden and A. C. Jones, Evidence of tautomerism in 2-aminopurine from fluorescence lifetime measurements, *J. Phys. Chem. B*, 2004, **108**, 17606–17610; (*b*) A. Broo, A theoretical investigation of the physical reason for the very different luminescence properties of the two isomers adenine and 2-aminopurine, *J. Phys. Chem. A*, 1998, **102**, 526–531.
12 (*a*) S. M. Law, R. Eritja, M. F. Goodman and K. J. Breslauer, Spectroscopic and calorimetric characterizations of DNA duplexes containing 2-aminopurine, *Biochemistry*, 1996, **35**, 12329–12337; L. C. Sowers, Y. Boulard and G. V. Fazakerley, Multiple structures for the 2-aminopurine-cytosine mispair, *Biochemistry*, 2000, **39**, 7613–7620; (*b*) D. G. Xu, K. O. Evans and T. M. Nordlund, Melting and premelting transitions of an oligomer measured by DNA-base fluorescence and absorption, *Biochemistry*, 1994, **33**, 9592–9599; (*c*) R. Eritja, B. E. Kaplan, D. Mhaskar, L. C. Sowers, J. Petruska and M. F. Goodman, Synthesis and properties of defined DNA oligomers containing base mispairsinvolving 2-aminopurine, *Nucleic Acids Res.*, 1986, **14**, 5869–5884.
13 V. Shafirovich, A. Dourandin, N. P. Luneva and N. E. Geacintov, Acid–base equilibria in aqueous solutions of 2-aminopurine radical cations generated by two-photon photoionization, *J. Chem. Soc., Perkin Trans. 2*, 2000, 271–275.
14 E. Nir, K. Kleinermanns, L. Grace and M. S. de Vries, On the photochemistry of purine nucleobases, *J. Phys. Chem. A*, 2001, **105**, 5106–5110.
15 S. K. Pal, L. Zhao, T. B. Xia and A. H. Zewail, Site- and sequence-selective ultrafast hydration of DNA, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 13746–13751.
16 (*a*) R. A. Hochstrasser, T. E. Carver, L. C. Sowers and D. P. Millar, Melting of a DNA helix terminus withing the active-site of a DNA polymerase, *Biochemistry*, 1994, **33**, 11971–11979; (*b*) M. W. Frey, L. C. Sowers, D. P. Millar and S. J. Benkovic, The nucleotide analogue 2-aminopurine as a spectroscopic probe of nucleotide incorporation by the Kenlow fragment of Eschericia coli polymerase I and bacteriophage T4 DNA polymerase, *Biochemistry*, 1995, **34**, 9185–9192; D. P. Millar, Fluorescence studies of DNA and RNA structure and dynamics, *Curr. Opin. Struct. Biol.*, 1996, **6**, 322–326; (*c*) E. L. Rachofsky, R. Osman and J. B. A. Ross, Conformation and dynamics of abasic sites in DNA investigated by time-resolved fluorescence of 2-aminopurine, *Biochemistry*, 2001, **40**, 946–967; (*d*) Y. G. Jiao, S. Stringfellow and H. T. Yu, Distinguishing "looped-out" and "stacked-in" DNA bulge conformation using fluorescent 2-aminopurine replacing a purine base, *J. Biomol. Struct. Dyn.*, 2002, **19**, 929–934; (*e*) N. P. Johnson, W. A. Baase and P. H. von Hippel, Low-energy circular dichroism of 2-aminopurine dinucleotide as a probe of local conformation of DNA and RNA, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 3426–3431.
17 M. A. O'Neill and J. K. Barton, 2-aminopurine: A probe of structural dynamics and charge transfer in DNA and DNA: RNA hybrids, *J. Am. Chem. Soc.*, 2002, **124**, 13053–13066.
18 V. Shafirovich, J. Cadet, D. Gasparutto, A. Dourandin, W. D. Huang and N. E. Geacintov, Direct spectroscopic observation of 8-oxo-7,8-dihydro-2′-deoxyguanosine radicals in double-stranded DNA generated by one-electron oxidation at a distance by 2-aminopurine radicals, *J. Phys. Chem. B*, 2001, **105**, 586–592.
19 T. Fiebig, C. Z. Wan and A. H. Zewail, Femtosecond charge transfer dynamics of a modified DNA base: 2-aminopurine in complexes with nucleotides, *ChemPhysChem*, 2002, **3**, 781–788.
20 S. Steenken, Purine-bases, nucleosides and nucleotides-aqueous solution redox chemistry and transformation reactions of their radical cations and $e^-$ and OH adducts, *Chem. Rev.*, 1989, **89**, 503–520.
21 L. P. Candeias and S. Steenken, Electron transfer in di(deoxy)nucleoside phosphates in aqueous solution-rapid migration of oxidative damage (*via* adenine) to guanine, *J. Am. Chem. Soc.*, 1993, **115**, 2437–2440.
22 M. K. Kuimova, J. Dyer, M. W. George, D. C. Grills, J. M. Kelly, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie and A. M. Whelan, Monitoring the effect of ultrafast deactivation of the electronic excited states of DNA bases and polynucleotides following 267 nm laser excitation using picosecond time-resolved infrared spectroscopy, *Chem. Commun.*, 2005, 1182–1184.
23 M. K. Kuimova, A. J. Cowan, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie and M. W. George, Monitoring the direct and indirect damage of DNA bases and polynucleotides by using time-resolved infrared spectroscopy, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 2150–2153.
24 (*a*) V. Shafirovich and N. E. Geacintov, Long-Range Charge Transfer in DNA II, *Top. Curr. Chem.*, 2004, **237**, 129–158; (*b*) V. Shafirovich, A. Dourandin, W. D. Huang, N. P. Luneva and N. E. Geacintov, *Phys. Chem. Chem. Phys.*, 2000, **2**, 4399; (*c*) V. Shafirovich, A. Dourandin, N. P. Luneva and N. E. Geacintov, *J. Phys. Chem. B*, 2000, **104**, 137.
25 P. K. Glasoe and F. A. Long, Use of glass electrodes to measure acidities in deuterium oxide, *J. Phys. Chem.*, 1960, **64**, 188–190.
26 M. Towrie, D. C. Grills, J. Dyer, J. A. Weinstein, P. Matousek, R. Barton, P. D. Bailey, N. Subramaniam, W. M. Kwok, C. Ma, D. Phillips, A. W. Parker and M. W. George, Development of a broadband picosecond infrared spectrometer and its incorporation into an existing ultrafast time-resolved resonance Raman, UV/visible, and fluorescence spectroscopic apparatus, *Appl. Spectrosc.*, 2003, **57**, 367–380.
27 R. D. Adamson, P. M. W. Gill and J. A. Pople, Empirical density functionals, *Chem. Phys. Lett.*, 1998, **284**, 6–11.
28 (*a*) T. M. Watson and J. D. Hirst, Density functional theory vibrational frequencies of amides and amide dimers, *J. Phys. Chem. A*, 2002, **106**, 7858–7867; (*b*) M. A. Phillips, N. A. Besley, P. M. W. Gill and P. Moriarty, Empirical density functional and the adsorption of organic molecules on Si(100), *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2003, **67**, 035309.
29 J. Kong, C. A. White, A. I. Krylov, D. Sherrill, R. D. Adamson, T. R. Furlani, M. S. Lee, A. M. Lee, S. R. Gwaltney, T. R. Adams, C. Ochsenfeld, A. T. B. Gilbert, G. S. Kedziora, V. A. Rassolov, D. R. Maurice, N. Nair, Y. H. Shao, N. A. Besley, P. E. Maslen, J. P. Dombroski, H. Daschel, W. M. Zhang, P. P. Korambath, J. Baker, E. F. C. Byrd, T. Van Voorhis, M. Oumi, S. Hirata, C. P. Hsu, N. Ishikawa, J. Florian, A. Warshel, B. G. Johnson, P. M. W. Gill, M. Head-Gordon and J. A. Pople, Q-Chem 2.0: A high-performance *ab initio* electronic structure program package, *J. Comput. Chem.*, 2000, **21**, 1532–1548.
30 V. I. Lebedev, Spherical quadrature formulas exact to order 25–29, *Sib. Math. J.*, 1977, **18**, 99–107.
31 L. Onsager, Electric moments of molecules in liquids, *J. Am. Chem. Soc.*, 1936, **58**, 1486–1493.
32 J. Florian and A. Warshel, Langevin dipoles model for ab initio calculations of chemical processes in solution: Parametrization and application to hydration free energies of neutral and ionic solutes and conformational analysis in aqueous solution, *J. Phys. Chem. B*, 1997, **101**, 5583–5595.
33 A. Lautie and A. Novak, Infrared and Raman spectroscopic studies on protonation and complex formation of purine in some crystallized compounds, *J. Chim. Phys. Phys.-Chim. Biol.*, 1971, **68**, 1492–1499.

34 N. Kottmair and W. Beck, Tungsten carbonyl complexes of 2′,3′-*o*-isopropylideneguanosine and 6-mercaptopurine, *Inorg. Chim. Acta*, 1979, **34**, 137–144.
35 W. Adam, A. Grimison and G. Rodriguez, Effects of protonation on electron densities in nitrogen heterocyclic molecules, *Tetrahedron*, 1967, **23**, 2513–2521.
36 The DFT calculations for 7-H tautomer using Onsager model produce IR spectra significantly different from the gas phase and Langevin models. This is due to the change in the optimised geometry of 2AP. For 7-H tautomer within Onsager model the solvent forces 2AP to adopt a puckered conformation, while in all the other cases 2AP molecule remains flat.