UV-promoted radical formation, and near-IR-induced and spontaneous conformational isomerization in monomeric 9-methylguanine isolated in low-temperature Ar matrices

Maciej J. Nowak, Igor Reva, A. J. Lopes Jesus, Leszek Lapinski and Rui Fausto

Three low-energy isomers of 9-methylguanine, the amino-oxo (AO) form and two amino-hydroxy (AH1 and AH2) conformers, were trapped from the gas phase into low-temperature argon matrices. The AH1 and AH2 isomers, differing in the orientation of the OH group, were found to transform into each other upon excitation with near-IR light. The population of the AO form of the compound was not changed upon any near-IR irradiation of the matrix samples. Using monochromatic near-IR light, generated by a frequency-tunable laser source, it was possible to selectively induce the AH1 → AH2 or AH2 → AH1 conversion. Photoreversibility of this conformational transformation was then demonstrated. Exposure of matrix-isolated monomers of 9-methylguanine to broadband near-IR light also led to conformational conversions within the amino-hydroxy tautomeric form; the final stage of this process was always the same photostationary state independent of the initial ratio of AH1 and AH2 populations. Spontaneous conformational conversion, transforming the higher-energy AH2 form into the lower-energy AH1 isomer, was observed for matrix-isolated monomers of 9-methylguanine kept in the dark. The mechanism of this process must rely on quantum tunneling of the light hydrogen atom. Irradiation of matrix-isolated 9-methylguanine with UV laser light at \( \lambda = 288 \) or 285 nm led to a substantial consumption of the two AH forms, while the amount of AO isomer remained unchanged. On the other hand, a decrease in the population of the AO isomer occurred upon excitations at shorter wavelengths, \( \lambda = 280 \) or 275 nm. The spectral changes observed after UV-irradiation suggest the generation (and stabilization in the matrix) of a radical species, resulting from the photocleavage of the O–H or N1–H bonds, in the AH or AO isomer, respectively.

1. Introduction

Investigations of the structural and photochemical properties of guanine and its derivatives have attracted intense attention due to the role played by guanine as a “letter” of the genetic code. It was established that the relative populations of the tautomeric forms of guanine are strongly dependent on the molecular environment, similarly as it is in the case of other tautomerizing compounds, e.g., cytosine. For isolated molecules of unsubstituted monomeric guanine, the N9–H ↔ N7–H tautomerism dominates. In guanine derivatives, alkylated at the N9 position, the N9–H tautomerism is eliminated. An alkyl substituent attached at the N9 nitrogen atom mimics the sugar moiety in guanosine.

9-Methylguanine (9mG), the simplest 9-alkylated guanine derivative, exhibits prototropic tautomerism and may adopt amino-oxo (AO), amino-hydroxy (AH), imino-oxo (IO) or imino-hydroxy (IH) forms (see Scheme 1). Due to the possibility of OH group rotation, two conformational structures are possible for each of the hydroxy tautomers. Theoretical calculations predict the AO and AH forms as the most stable tautomers of isolated 9mG molecules. For the compound in the crystalline state, or dissolved in protic solvents, only the biologically relevant AO tautomer has been experimentally detected. In the gas phase, photoelectron and fluorescence investigations have suggested that the AH tautomer predominates, the AO form being also
One of the aims of the present work was the investigation of conformational transformations induced by excitation of matrix-isolated 9mG molecules with narrowband tunable near-infrared (near-IR) laser light. Stabilization of two rotamers of the AH tautomer of 9mG in a low-temperature inert environment allowed detailed investigation of the near-IR-induced conformational interconversion occurring by internal rotation of the OH group. Using frequency-tunable monochromatic light it was possible to selectively excite each one of the conformers and to induce its transformation into the other OH rotamer. Hence, it was possible to experimentally demonstrate the photoreversibility of the near-IR induced transformations converting the amino-hydroxy rotamers into each other. Matrices containing monomers of 9mG were also irradiated with broadband near-IR/mid-IR light. Upon such irradiations, the population ratio of the amino-hydroxy rotamers was changed towards a photostationary state.

Another objective of the current research was the investigation of the ultraviolet (UV) light-induced transformations of isolated 9mG molecules. For this purpose, monomers of 9mG isolated in Ar matrices were irradiated, at a chosen wavelength, with narrowband tunable UV laser light. According to the general perception, nucleic acid bases should be photochemically very stable, as the compounds chosen by prebiotic evolution. The assumed exceptional photostability had been, until recently, attributed to the short lifetime of the electronically excited states of these compounds. In the experimental studies on matrix-isolated cytosine21 and 1-methylcytosine,22 we have demonstrated that these compounds are not exceptionally photostable. The UV-induced transformations, observed for cytosine and 1-methylcytosine, are dominated by the oxo → hydroxy and the amino → imino phototautomerism. Also for 9-methylhypoxanthine,23 a molecule that is structurally very similar to 9mG, the oxo → hydroxy phototautomerism was found to be the dominant UV-induced photoreaction. Photochemical Norrish type I ring opening to the conjugated isocyanates and ketenes was observed for cytosines21,22 and isocytosine.24 Hence, it appeared interesting to check whether analogous photoreactions would also occur for 9mG.

Not only the light-induced processes, but also a spontaneous conformational transformation, occurring by hydrogen-atom tunneling, were experimentally investigated in the current work. The structures of the two amino-hydroxy rotamers of 9mG differ only in the position of the hydrogen atom, which is a light particle able to cross energy barriers by quantum tunneling. In the present work, we observed a spontaneous conformational conversion involving those conformational isomers, occurring by H-atom tunneling for matrix-isolated 9mG monomers kept for several hours at low temperature and in the dark.

2. Experimental methods

A commercial sample of 9-methylguanine (Sigma-Aldrich, 98%) and argon (N60, supplied by Air Liquide) were used in the experiments. Crystals of 9-methylguanine (9mG) were placed in a miniature glass oven located inside the vacuum chamber of a closed-cycle helium refrigerator (APD Cryogenics) with a DE-202A expander. The oven was heated by a resistive wire to 500 K, and the vapor of 9mG was deposited onto a cold (12–14 K) CsI substrate, together with a large excess of argon. The temperature of the CsI window was measured directly at the sample holder with an accuracy of 0.1 K by using a silicon diode sensor connected to a digital controller (Scientific Instruments, Model 9650-1). The process of the matrix deposition took approximately 2 hours, and was controlled in real time by collecting mid-infrared (mid-IR) spectra using a Thermo Nicolet 6700 FTIR spectrometer equipped with a KBr beam splitter and a DTGS detector. For this purpose, the cold optical window of the cryostat was permanently exposed to the spectrometer beam. In one type of experiment, during deposition of the matrices, the spectra were recorded in the full mid-IR 4000–400 cm$^{-1}$ range. In other type of experiments, the spectra recorded during deposition of the matrices were collected only in the 1800–400 cm$^{-1}$ range. In these latter type of experiments, the matrices were protected from the spectrometer light source (Globar) by a longpass cutoff filter transmitting only IR light with wavenumbers lower than 1800 cm$^{-1}$. In all the experiments, the mid-IR spectra were recorded with a resolution of 0.5 cm$^{-1}$.

The near-infrared (near-IR) spectra were recorded using the same FTIR spectrometer, but equipped with a CaF$_2$ beam splitter and an InGaAs detector. The near-IR spectra were collected in the 12400–3800 cm$^{-1}$ range with a resolution of 1 cm$^{-1}$.

Matrix-isolated molecules of 9mG were irradiated with frequency-tunable, monochromatic (full width at half maximum FWHM $= 0.2$ cm$^{-1}$) near-IR light (pulse energy 10 mJ) generated as the idler beam of a Quanta-Ray MOPO-SL optical parametric...
oscillator (OPO). For the UV-irradiations, monochromatic, wavelength-tunable UV light [pulse energy 3 mJ] generated as the frequency-doubled signal beam of the same OPO was used. The OPO was pumped with a pulsed Nd:YAG laser [pulse duration 10 ns, repetition rate 10 Hz]. The near-IR irradiations were alternatively induced using a continuous wave tunable diode laser Toptica DL100 pro design (FWHM < 1 MHz, 40 mW). The wavelengths of the near-IR light were measured with a HighFinnesse WS-5 wavelength meter.

3. Computational methods

The quantum-mechanical calculations carried out in the present work were performed with the Gaussian 09 program package (Revision D.01). Geometries of $9\text{mg}$ isomers were fully optimized at the DFT level of approximation, with the B3LYP functional and the standard 6-31++G(d,p) basis set. Subsequently, the structures of the low-energy isomers were re-optimized using the MP2 method and the 6-31+G(d,p) basis set. Single-point energy calculations were further performed at the higher QCISD/6-31+G(d,p) level on the MP2/6-31+G(d,p) optimized geometries. To estimate the Boltzmann populations of the most stable isomers in the vapor phase, the theoretical Gibbs free energies were assessed (for the temperature of 500 K) using the B3LYP or MP2 calculated vibrational frequencies and rotational constants. The B3LYP harmonic wavenumbers (scaled by 0.950 and 0.980, above and below 3000 cm$^{-1}$, respectively) and respective IR intensities were used to simulate spectra in the mid-IR region. The spectral simulations were performed with the Chemcraft software, using Lorentzian functions with FWHM = 2 cm$^{-1}$, centered at the calculated (scaled) frequency and setting the intensity at the band maximum equal to the calculated IR intensity as taken from the Gaussian output. The description of the normal modes was based on the vibrational analysis provided by the VibAnalysis software, supported by animation of the vibrations using the ChemCraft software.

Near-IR spectra of the relevant isomers were also theoretically simulated using data obtained in DFT(B3LYP)/6-31+G(d,p) anharmonic vibrational computations, carried out for geometries optimized at the same level. A fully automated second-order vibrational perturbative approach (VPT2) of Barone and co-workers was applied in these calculations. This approach allows evaluation of anharmonic vibrational wavenumbers and anharmonic infrared intensities up to two quanta, including the first overtones and combination transitions. The computed anharmonic vibrational wavenumbers were not scaled.

4. Results

4.1. The most stable amino-oxo and amino-hydroxy tautomers of 9-methylguanine and the AH1 $\leftrightarrow$ AH2 conformational conversions induced by selective excitation with monochromatic near-IR light

The relative energies of twenty-one different amino-oxo, amino-hydroxy, imino-oxo and imino-hydroxy isomers of 9-methylguanine ($9\text{mg}$) were calculated, within the current work, at the DFT(B3LYP)/6-31+G(d,p) level of theory. According to the results of these calculations (collected in Table S1 in the ESI†) there are only three low-energy isomers of $9\text{mg}$: AO, AH1 and AH2 (Table 1). The computed energy of any other isomer of $9\text{mg}$ is higher by more than 55 kJ mol$^{-1}$, in comparison with the energy of the most stable AO form. The energies of the AO, AH1 and AH2 isomers were also calculated at the higher QCISD/6-31+G(d,p)/MP2/6-31+G(d,p) level (see Table 1). From the theoretical QCISD results obtained in the current work, as well as from the computations performed elsewhere using the DFT, MP2 and G4(MP2) methods, one can conclude that the energies of the amino-oxo (N1H1, Fig. S1 in the ESI†) and amino-hydroxy tautomers of $9\text{mg}$ are very close to each other. The theoretically predicted energies of the AO and AH1 forms differ by less than 3 kJ mol$^{-1}$. As far as the theoretical computation of relative energies of different prototropic tautomers is concerned, the value of $\pm 3$ kJ mol$^{-1}$ represents the precision of the currently available theoretical methods. Usually more accurate are the theoretical predictions of the relative energies of structures (conformers) differing only by rotation of one atom. All the computations point at the AH1 conformer as being more stable than the other amino-hydroxy AH2 rotamer (see Table 1). Hence, one can expect that only the amino-oxo (AO) and the two amino-hydroxy (AH1 and AH2) isomers of $9\text{mg}$ should be non-negligibly populated in the gas phase and only these forms can be trapped in low-temperature matrices. Of the two amino-hydroxy conformers, the lower-energy AH1 form should be more populated than the AH2 rotamer. The gas-phase population of the higher-energy AH2 conformer can be preserved in low-temperature matrices only if the barrier separating

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relative electronic energies ($\Delta$Eel), zero-point corrected energies ($\Delta$Ezpc), and Gibbs energies at 500 K ($\Delta$G) calculated for the most significant forms of $9\text{mg}$ and the Boltzmann populations (Pop.) estimated at 500 K.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of theory</td>
<td>AO</td>
</tr>
<tr>
<td>B3LYP $\Delta$Eel</td>
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</tr>
<tr>
<td>$\Delta$Ezpc</td>
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<tr>
<td>Pop. (%)</td>
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<tr>
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<td>$\Delta$Ezpc</td>
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<tr>
<td>$\Delta$G (500 K)</td>
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<tr>
<td>Pop. (%)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>$\Delta$Ezpc</td>
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<tr>
<td>$\Delta$G (500 K)</td>
<td>0.00</td>
</tr>
<tr>
<td>Pop. (%)</td>
<td>57.2</td>
</tr>
</tbody>
</table>

* For the structures, see Scheme 1 [AO] and Fig. 1 [AH1 and AH2]. Electronic energies were calculated with the 6-31+G(d,p) basis set. All values are in kJ mol$^{-1}$, and are relative to the most stable AO isomeric form. Boltzmann populations were estimated at 500 K from the values of $\Delta$G computed at the same temperature. The Boltzmann populations of AO, AH1 and AH2 were assumed to totalize 100%. Relative populations of the AH1 and AH2 conformers are given in parentheses. Electronic energies calculated at the QCISD level on MP2 optimized geometries. Zero-point vibrational energies and thermal corrections to the Gibbs energies were calculated at the MP2 level.
the AH2 and AH1 forms is high enough to effectively hinder the AH2 \( \rightarrow \) AH1 transformation during formation of a low-temperature matrix. In the current work, the barrier between the AH2 and AH1 potential-energy minima has been estimated at the DFT(B3LYP)/6-31+G(d,p) level (see Fig. 1). The computed barrier (35 kJ mol\(^{-1}\)) is high enough, hence the AH2 \( \rightarrow \) AH1 conversion (conformational cooling)\(^{18}\) should not occur during temperature lowering by trapping 9mG molecules from the gas phase onto the cold matrix surface.

The mid-IR spectrum of 9mG monomers isolated in an Ar matrix is presented in Fig. 2a and 3b and its assignment is given in Table S2 (in the ESI\(^+\)). By taking into account the integrated intensities of some experimental absorption bands due to the AO, AH1, and AH2 forms, scaled by absolute intensities of the respective bands in the theoretically predicted spectra, it was possible to make an assessment of the population ratio of the AO and AH tautomers. This coarse assessment revealed that the populations of AO and AH are similar to each other, with a slight predominance of the AO form. The population ratio AO: (AH1 + AH2) can be roughly estimated as 1.3 ± 0.3. This result is in agreement with the theoretical prediction of the abundances of 9mG tautomers in the gas phase (see Table 1) and is similar to that reported previously for matrix-isolated 9mG.\(^{18}\)

The 7150–6700 cm\(^{-1}\) region of the near-IR spectrum of the compound is shown in Fig. 4a. According to the anharmonic calculation of the vibrational overtones (Fig. 4c and Table S3 in the ESI\(^+\)), the prominent experimental absorption at 6965/6957 cm\(^{-1}\) should be assigned to the 2-\(\nu_{\text{OH}}\) vibration of the AH1 isomer. Irradiation of an Ar matrix, containing monomers of 9mG, with monochromatic 6965 cm\(^{-1}\) laser light led to substantial changes in the intensities of the bands observed in the mid-IR spectrum (see Fig. 2 and 3). The intensities of one group of bands (assigned to AH1) decreased, the intensities of the other group of bands (assigned to AH2) increased, whereas the intensities of the third group of bands (assigned to AO) stayed unchanged upon near-IR irradiation at 6965 cm\(^{-1}\). These experimentally observed changes in IR-band intensities are the spectral indications of the AH1 \( \rightarrow \) AH2 conformational transformation that occurs upon excitation of the 2-\(\nu_{\text{OH}}\) overtone of AH1. The spectral width (ca. 10 cm\(^{-1}\)) of the experimental absorption due to 2-\(\nu_{\text{OH}}\) is larger than the spectral width (0.2 cm\(^{-1}\)) of the laser light used for irradiation. Hence, irradiations at other wavelengths from the range 6966–6956 cm\(^{-1}\) also led to the same AH1 \( \rightarrow \) AH2 conversion. This conformational transformation was also induced by near-IR excitation of other overtones or combination modes, such as those corresponding to the bands at 7071 cm\(^{-1}\) (2\(\nu_{\text{sNH2}}\)) or 6881 cm\(^{-1}\) (2\(\nu_{\text{NH2}}\)).

Moreover, the photoreversibility of the near-IR-induced AH1 \( \leftrightarrow \) AH2 conformational transformation (see Scheme 2) has also been experimentally demonstrated. In the near-IR spectra of Ar matrices containing monomers of 9mG, an absorption band grows at 6982–6976 cm\(^{-1}\) (Fig. 4a and b), following the increase of AH2 population caused by irradiations at 6965 and 6957 cm\(^{-1}\). According to the theoretical anharmonic calculations (Fig. 4c), this growing band should be assigned to the 2-\(\nu_{\text{OH}}\) mode of the AH2 isomer. Irradiation of the matrix with monochromatic near-IR
light from the 6982–6976 cm\(^{-1}\) range induced the AH\(_2\) → AH\(_1\) conversion (Fig. 5c, and Fig. S2 in the ESI†). The latter one is a transformation in the reverse direction with respect to the AH\(_1\) → AH\(_2\) conversion caused by excitation at 6966–6956 cm\(^{-1}\).

Not only excitation of the 2\(\nu\)OH overtone of AH\(_2\), but also excitation of the 2\(\nu\)aNH\(_2\) or 2\(\nu\)sNH\(_2\) overtones of AH\(_2\) (at 7080 and 6884 cm\(^{-1}\)) led to the AH\(_2\) → AH\(_1\) phototransformation (Fig. 4b).

Spectral changes, reflecting the AH\(_1\) → AH\(_2\) and AH\(_2\) → AH\(_1\) phototransformations (Fig. 2, 3 and Fig. S2 in the ESI†) allowed reliable assignment of many IR bands to the AH\(_1\), AH\(_2\) and AO isomers of 9mG. Comparison of the experimental and theoretical spectra leaves no doubt about the correctness of the assignment of the spectrum growing upon 6965 cm\(^{-1}\) irradiation to AH\(_2\), the spectrum decreasing upon this irradiation to AH\(_1\) and the spectrum not affected by this irradiation to AO.

Bands of the AO form were found at 3543/3535 cm\(^{-1}\) (\(\nu\)aNH\(_2\)), 3440/3435 (\(\nu\)N\(_1\)H), 3430/3427 (\(\nu\)sNH\(_2\)), 1752/1741 (\(\nu\)C==O), 1622, 1582, 1561, 1540, 1376/1373, 1310, 1239, 1137, 777, 662, and 643/642 cm\(^{-1}\) (see Fig. 2, 3, 5 and Table S2 for more details, ESI†). The mid-IR spectra of each of the AH\(_1\), AH\(_2\) and AO forms are quite densely spaced. Hence, alongside the IR absorptions due to a single isomer, there are numerous IR absorption features that originate from overlapping bands due to different 9mG isomers. The assignment of the different band components to a specific form could only be possible thanks to the spectral changes induced by the selective near-IR irradiations.

4.2 AH\(_1\) ↔ AH\(_2\) conformational conversions induced by exposure of matrix-isolated monomers of 9-methylguanine to broadband near-IR/mid-IR light

An Ar matrix containing isolated monomers of 9mG was subjected to a series of irradiations with monochromatic near-IR light with wavenumbers in the range of 6982–6976 cm\(^{-1}\). Upon these irradiations the AH\(_2\) isomer was nearly totally converted into AH\(_1\) (Fig. 6a). The matrix prepared this way was subsequently kept for 4 hours exposed to the broadband near-IR/mid-IR light of the FTIR spectrometer beam. During this period, the population of AH\(_2\) increased and the population of AH\(_1\) correspondingly decreased (Fig. 6e). However, the scale of these changes was not very large. With the time of exposure...
to the broadband near-IR/mid-IR light, the changes in the 
AH1 : AH2 population ratio were becoming smaller and smaller.
After 4 hours of monitoring, the system was already very close to
the photostationary ratio of AH1 and AH2 populations (Fig. 6b).

Before another period of exposure to the broadband near-IR/mid-IR light of the FTIR spectrometer beam, the matrix was
subjected to a series of monochromatic irradiations at 6966–
6956 cm⁻¹. Upon these irradiations, the population of AH2
substantially grew at the cost of decreasing population of AH1
(Fig. 6c). Subsequently, such an Ar matrix with a large initial
population of AH2 was kept for 4 hours exposed to the broad-
band light of the spectrometer beam. The evolution of AH1 and
AH2 populations was systematically monitored by recording
mid-IR spectra. During the period of 4 hours, the population of
AH1 increased and the population of AH2 correspondingly
decreased (Fig. 6f). Hence, in this run, the direction of the
net population changes was opposite with respect to the evolution
observed in the previous run (compare Fig. 6e and f). The final ratio
of the AH1 and AH2 populations was very similar to that achieved in
the previous run (compare Fig. 6b and d).

The results of the experiments carried out with Globar as a
source of broadband near-IR/mid-IR light used for irradiation
of the matrix clearly demonstrate that exposure to such broad-
band radiation induces both AH1 → AH2 and AH2 → AH1
conformational transformations. Independent of the initial
ratio of the AH1 and AH2 populations, the final ratio of the
populations of these conformers (achieved after prolonged
exposure to the broadband near-IR/mid-IR light) is always the
same. This final population ratio corresponds to the photo-
stationary state.³⁹ In the case of 9mG and the Globar-type
source of near-IR/mid-IR light, the population of AH1 at the
photostationary state is approximately 4 times greater than the
population of AH2 (an AH1 : AH2 ratio of 80 : 20, see horizontal
dashed lines in Fig. 6e and f).

4.3 Spontaneous AH2 → AH1 conformational conversions
occurring in the dark by hydrogen-atom tunneling

To conveniently observe the spontaneous transformation of the
higher-energy conformer AH2 into the lower-energy AH1 form, it
is good to start from the matrix with a high relative population
of the AH2 conformer. Such a matrix (see spectrum “a” in Fig. 7)
was prepared by a series of monochromatic irradiations with
near-IR light in the range of 6966–6956 cm⁻¹. Then, the matrix
sample at 12 K was kept in the dark for several hours. The
changes in the AH1 and AH2 populations were periodically
monitored by recording the mid-IR spectra in the 1800–400 cm⁻¹
range. During collection of the spectra, the matrix was protected
from exposure to near-IR/mid-IR light with wavenumbers higher
than 1800 cm⁻¹ by a cut-off filter placed between the outer window
of the cryostat shroud and the Globar spectrometer source. The
cutoff energy of this filter (21 kJ mol⁻¹) is much lower than the
energy barrier separating the AH1 and AH2 conformers (see dashed
lines in Fig. 1). This means that one can safely exclude the
possibility of an over-the-barrier conformational isomerization
occurring due to IR-excitation of 9mG molecules, which are
invariably exposed to the filtered radiation of the Globar source
during collection of the spectra. When spectra were not recorded,
the spectrometer beam was blocked by a metal shutter. The
spontaneous AH2 → AH1 transformation was monitored for
12 hours. During this whole period the population of AH1
was monotonously increasing at the cost of the decreasing population of AH2 (see the evolution of this population pre-
sented in the lower panel of Fig. S3 in the ESI†). After 720 min
of keeping the matrix in the dark, the initially small population
Fig. 5 The photoreversibility of the conformational $\text{AH}_1 \leftrightarrow \text{AH}_2$ transformations induced in molecules of $9\text{mG}$ isolated in an Ar matrix by selective excitation with monochromatic near-IR light. Fragments of the IR spectra recorded: (a) after deposition of the matrix; (b) after a series of irradiations with near-IR light from the 6966–6956 cm$^{-1}$ range; (c) after a series of irradiations with near-IR light from the 6982–6976 cm$^{-1}$ range. The bands that did not change upon any near-IR irradiation are due to the AO tautomer. $\text{AH}_1^*$ and $\text{AH}_2^*$ indicate bands due to $\text{AH}_1$ and $\text{AH}_2$ forms partially overlapping with bands of other forms.

![Image of IR spectra](image_url)

Fig. 6 Conformational transformations induced in monomers of $9\text{mG}$ isolated in an Ar matrix by exposure to the broadband near-IR/mid-IR light of the FTIR spectrometer beam. A fragment of the mid-IR spectrum: (a) recorded after a series of near-IR irradiations at 6982–6976 cm$^{-1}$ and (b) after subsequent 240 min of exposure of the sample to the broadband near-IR/mid-IR radiation; (c) recorded after a series of near-IR irradiations at 6966–6956 cm$^{-1}$ and (d) after subsequent 240 min of exposure of the sample to the broadband near-IR/mid-IR radiation. (e and f) Evolution of the populations of two amino-hydroxy conformers: $\text{AH}_1$ (closed blue circles) and $\text{AH}_2$ (open red circles); the relative populations of $\text{AH}_2$ and $\text{AH}_1$ were estimated from the ratio of integrated intensities of the experimental bands at 1448/1445 cm$^{-1}$ ($\text{AH}_2$) and 1441/1440 cm$^{-1}$ ($\text{AH}_1$), scaled by the intensities of the corresponding theoretical bands. Horizontal dashed lines represent the relative abundances estimated for the two conformers at the photostationary state (≈80% $\text{AH}_1$, 20% $\text{AH}_2$). Spectra (a) and (b) correspond to 0 min and 240 min of the population evolution shown in panel (e). Spectra (c) and (d) correspond to 0 min and 240 min of the population evolution shown in panel (f).

![Image of population evolution](image_url)

This demonstrates that the spontaneous process occurring in the dark and at low-temperature (12 K) is strictly unidirectional.

The only particle significantly changing its position during the $\text{AH}_2 \rightarrow \text{AH}_1$ transformation is the light hydrogen atom. Taking into account the height (35 kJ mol$^{-1}$) of the barrier for the $\text{AH}_2 \rightarrow \text{AH}_1$ conversion and the low temperature (12 K) at which the process occurs, one can come to the conclusion that the quantum tunneling effect must play a crucial role in this transformation.

Molecules of $9\text{mG}$ are trapped in Ar matrices in two spectroscopically distinct sites “A” and “B” (see the fragments of the mid-IR spectra shown in Fig. 7). The spontaneous $\text{AH}_2 \rightarrow \text{AH}_1$ tunneling conversion occurring in the dark was found to be faster for monomers of $9\text{mG}$ isolated in site “B” than for the molecules trapped in site “A”. Moreover, the microenvironments surrounding the isolated $9\text{mG}$ monomers are not exactly the same, even within the same spectroscopically distinguishable site (“A” or “B”). That is why, the Ar matrix environment cannot be treated as homogeneous, even if the consideration is restricted to a single spectroscopically distinguishable site. Consequently, the experimental curves representing the decay of the $\text{AH}_2$ population [expressed as $n(\text{AH}_2)$], due to the spontaneous $\text{AH}_2 \rightarrow \text{AH}_1$ tunneling process, are not very accurately reproduced (Fig. 7) by eqn (1) of single-exponential-decay:

$$
\frac{n(\text{AH}_2)}{n(\text{AH}_2)_{t=0}} = e^{-t/\tau}
$$

(1)

Using eqn (1), the time constant ($\tau$) was estimated to be 275 min for $9\text{mG}$ molecules trapped in site “A” and 188 min for $9\text{mG}$ molecules trapped in site “B”. The tunneling decay of $\text{AH}_2$ is much better reproduced (Fig. 7) by eqn (2) of dispersive kinetics,$^{39-41}$ as follows:

$$
\frac{n(\text{AH}_2)}{n(\text{AH}_2)_{t=0}} = e^{-B\tau^n}
$$

(2)

For the present experiments, $B = 0.008$, $\alpha = 0.86$ for $9\text{mG}$ molecules trapped in site “A”, and $B = 0.018$, $\alpha = 0.78$ for $9\text{mG}$ molecules trapped in site “B”. The dispersive time constant of $\text{AH}_2$ tunneling decay $\tau_{\text{disp}} \equiv \alpha B$ is equal to 107 min (for $9\text{mG}$ molecules trapped in the site “A”) and 43 min (for $9\text{mG}$...
molecules trapped in the site “B”). The empirical parameter $\alpha$, introduced by Siebrand and Wildman,\textsuperscript{40} accounts for the environmental effects. In matrix-isolation experiments, $\alpha$ usually has a value between 0.5 and 1, depending on the matrix, the temperature, and the time when measuring the kinetics is started, as pointed out by Sander \textit{et al.}\textsuperscript{42} Indeed, the $\alpha$ values of 0.86 and 0.78, obtained in the present work for 9mG molecules, fall in the same range as the values of 0.89 and 0.80 obtained earlier for pyruvic acid\textsuperscript{43} and cytosine,\textsuperscript{39} respectively.

4.4 Phototransformations induced by UV irradiation of matrix-isolated 9mG

Matrix-isolated monomers of 9mG were also irradiated with monochromatic UV light of a chosen wavelength. Irradiations at 316, 312, 308 and 304 nm induced no changes in the sample. Excitations at 300, 296 and 292 nm, corresponding to the long-wavelength edge of the UV absorption spectrum of the compound,\textsuperscript{18} resulted in the AH1 $\rightarrow$ AH2 net shift of populations of the amino-hydroxy conformers (Fig. 8b). Irradiations at shorter UV wavelengths (288 or 285 nm) led to fast decomposition of the amino-hydroxy tautomer of the compound. The IR bands due to both AH1 and AH2 amino-hydroxy isomers decreased rapidly upon such irradiations (10 min of irradiations consumed more than 75% of the initial amount of both AH forms in the Ar matrix). At the same time, the changes in the intensities of the IR bands at 3535 and 3435 cm$^{-1}$, reflecting the amount of AO population, were very small (Fig. 8c). These effects are similar to those induced in an earlier work\textsuperscript{18} by broadband UV irradiations of 9mG monomers trapped in Ar matrices.

Photochemical consumption of the amino-hydroxy form of 9mG, occurring upon excitation at 288 or 285 nm, was accompanied by generation of photoproduct(s). Most of the new IR bands, growing in the experimental spectra of the matrices irradiated at 288 or 285 nm (Fig. 9a), are well reproduced by the theoretical IR spectra (Fig. 9b) computed for the 9mG$^*$ radical (Scheme 3) with the hydrogen atom of the OH group detached from the molecule. The most characteristic bands of 9mG$^*$ are found at 3444, 1520, 1366 and 1320 cm$^{-1}$ (see Fig. 8c, d and 9a). These values are in good correspondence with the positions of

![Figure 7](image_url)  
**Fig. 7** Upper panel: the Fragment of the mid-IR spectrum of 9mG isolated in an Ar matrix at 12 K recorded: (a – red) after a series of monochromatic irradiations with near-IR laser light at wavenumbers from the 6966–6956 cm$^{-1}$ range; (b – blue) after subsequently keeping the sample in the dark for 720 min. Letters A and B identify band components ascribed to molecules isolated in spectroscopically distinct trapping sites (A or B). Lower panel: Evolution of the population of the AH2 conformer trapped in site A (triangles) and site B (squares). The amount of AH2 trapped in site A at the beginning of the process (time = 0) occurring in the dark was set to 1. Similarly, the amount of AH2 trapped in site B at the beginning of the process (time = 0) occurring in the dark was also set to 1. During the collection of the mid-IR spectra, the matrix sample was protected by a longpass cutoff filter transmitting only IR light with wavenumbers lower than 1800 cm$^{-1}$. When the spectra were not recorded the matrix was kept in the dark. Experimental data were fitted using equations of dispersive kinetics (solid curves, coefficients of determination: $R^2 = 0.997$ and 0.999) and single exponential kinetics (dashed curves, coefficients of determination: $R^2 = 0.985$ and 0.992).

![Figure 8](image_url)  
**Fig. 8** Effects of UV irradiation of 9mG monomers isolated in an Ar matrix: (a) the fragment of the infrared spectrum recorded after deposition of the matrix; (b) the spectrum recorded after irradiation at 292 nm minus the spectrum recorded before this irradiation; (c) the spectrum recorded after irradiations at 288 and 285 nm minus the spectrum recorded before these irradiations; (d) the spectrum recorded after irradiations at 280 and 275 nm minus the spectrum recorded before these irradiations. The emerging band marked with an asterisk is assigned to the 9mG$^*$ radical. The theoretically predicted counterpart of this band appears at 3440 cm$^{-1}$, with IR intensity of 155 km mol$^{-1}$.
intense IR-bands of the radical, predicted at 3440, 1517, 1367/1356, and 1319 cm$^{-1}$ (see Fig. 9b).

This suggests that such a radical is photogenerated from UV-excited 9mG. UV-induced generation of the analogous radicals, for matrix-isolated compounds bearing an O–H group connected to an aromatic ring, has been previously reported for phenol$^{44}$ and hydroxyquinolines.$^{45,46}$

Exposure of the matrices to UV light of shorter wavelengths ($\lambda = 280$ and 275 nm) led to photodecomposition of all AH1, AH2 and AO forms of 9mG. This was manifested by the intensity decrease of the IR bands due to these isomers (Fig. 8d). Interestingly, the decrease of the intensity of the bands assigned to the AO form was much slower than the intensity decrease of the bands due to the AH forms. In fact, 10 min of irradiation at $\lambda = 280$ and 275 nm consumed only $\sim 15\%$ of the population of the AO form initially present in the matrix. No spectral indications of UV-induced transformation of AO into other tautomeric forms of 9mG were detected. It is also worth noticing that consumption of the AO form was accompanied by a slight growth of the bands due to 9mG*, which had already appeared during UV irradiations at longer wavelengths. This demonstrates that the 9mG* radical is photogenerated not only upon UV-excitation of the AH tautomer (by photodetachment of the H-atom from the O–H group), but also upon UV-excitation of the AO form (by photodetachment of the H-atom from the N1–H group, see Scheme 3).

Besides the emerging absorptions of the radical, during the 288–275 nm irradiations, two new broad bands were also identified in the region between 2300 and 2100 cm$^{-1}$: one centered at $\sim 2263$ cm$^{-1}$, and the other appeared in the 2180–2120 cm$^{-1}$ region (see Fig. S4 in the ESI$^\dagger$). Bands at these positions have been identified upon UV-excitation of various heterocyclic carbonyl compounds analogous to 9mG$^{23,47,48}$ and are generally ascribed to the formation of open-ring conjugated isocyanates (higher frequency band) and ketenes (lower frequency band). The possible formation of carbon monoxide from photodecomposition processes can also contribute to the broad absorption appearing in the 2180–2120 cm$^{-1}$ region.

An oxo $\rightarrow$ hydroxy phototautomeric transformation has been observed in the previous studies on isocytosine,$^{24}$ cytosine$^{21}$ hypoxanthine,$^{48}$ 9-methylhypoxanthine$^{23}$ and 4[(3H)-pyrimidin-1-yl]-pyrimidine$^{49,50}$ isolated in low-temperature Ar matrices and excited with UV light. For each of these compounds, excitation of the oxo tautomeric form, which usually absorbs at longer UV wavelengths, led to a decrease of population of this tautomer accompanied by an increase of the population of the hydroxy form. Despite the fact that no intermediates in the above reactions were reported, it is likely that the oxo-hydroxy phototautomerism in those molecules goes through formation of the radical (and an H-atom) followed by a fast recombination at different positions in the molecule.

In spite of the structural similarity of 9mG and the compounds listed above, no signs of oxo $\rightarrow$ hydroxy (or hydroxy $\rightarrow$ oxo) phototautomerism were detected in the experiments carried out in the present work. A plausible explanation for the observed behavior is that photooxidation of the AH and AO tautomeric forms of 9mG results in the formation of a radical, which is considerably more stable in the matrix than those produced from the other analogous molecules. To the best of our knowledge, this photochemical behavior is unique.

5. Concluding discussion

The experiments carried out within the current work unequivocally demonstrated that there are three low-energy isomers of 9mG trapped from the gas phase into low-temperature Ar matrices. The infrared spectra of these isomers, the amino-oxo tautomer and two rotamers of the amino-hydroxy form, could be separated from one another thanks to the effects of near-IR or UV irradiation of
the matrix-isolated monomers. Monochromatic near-IR selective excitations of a chosen amino-hydroxy conformer (AH1 or AH2) led to the transformation of this species into the other rotamer (AH2 or AH1, respectively). In addition, the broadband near-IR/ mid-IR irradiation of the matrix resulted in AH1 → AH2 and AH2 → AH1 conformational transformations, which were observed to occur simultaneously. Prolonged exposure of an Ar matrix containing monomers of 9mG to the broadband near-IR/mid-IR irradiation was always leading to a photostationary state. The ratio of AH1 and AH2 populations at this photostationary state was always the same, independent of the ratio of populations of these rotamers present in the matrix before irradiation with broadband near-IR/mid-IR light. The molecules adopting the amino-oxo (AO) tautomeric form were not affected by any irradiation of the matrix with monochromatic or broadband near-IR light. Hence, it was possible to reliably assign a set of IR bands, which do not change upon any near-IR irradiation, to the AO form of 9mG.

It was easy to notice that there are two amino-hydroxy conformers of 9mG populated in low-temperature matrices. But in order to say which of the experimentally observed conformers has the AH1 and which has the AH2 structure, one needed to rely on the theoretical calculations. The assignment of the AH1 structure to the form more populated after deposition was based on computations (Table 1), which predict the energy of AH1 to be lower than that of AH2. This assignment was supported by comparison of the theoretical spectra of AH1 and AH2 with two sets of experimental bands decreasing and increasing upon near-IR irradiation. In the high-wavenumber range of the mid-IR spectrum, the bands due to the amino-hydroxy conformer, which is more abundant after deposition of the matrix, are placed at lower wavenumbers (3568 and 3563 cm⁻¹) than the bands assigned to the less populated form, which are found at 3576 and 3571 cm⁻¹ (Fig. 2a and b). The same is the sequence of the wavenumbers theoretically predicted for the ωOH bands due to AH1 and AH2 (Fig. 2c). Comparison of the relatively strong bands at 1174 and 1275 cm⁻¹, which belong to different amino-hydroxy forms (Fig. 3), with the corresponding theoretical bands calculated for AH1 and AH2, leads to the same conclusion: the AH1 structure should be assigned to the form more populated directly after deposition of the matrix (the form that has the bands at 3568, 3565 and 1174 cm⁻¹ in its spectrum), whereas the AH2 structure should be assigned to the form less populated directly after deposition of the matrix (the form that has the bands at 3576, 3571 and 1275 cm⁻¹ in its spectrum).

The assignment of the AH1 and AH2 structures to the two amino-hydroxy conformers, manifesting themselves by different mid-IR spectra, was possible only by comparison with the results of the theoretical calculations of the infrared spectra and of the relative energies of the two forms. The presently available theoretical methods are accurate enough to allow a reliable identification of the AH1 and AH2 conformers. In an earlier attempt to identify the AH1 and AH2 conformers, the spectra of which were separated by UV irradiation, the authors had no possibility to refer to reliable theoretical calculations. Hence, no trustworthy identification of AH1 and AH2 has been given in ref. 18.

In the current work, a spontaneous conformational transformation was observed for monomers of 9mG isolated in a low-temperature Ar matrix (at 12 K) and kept in the dark. This spontaneous transformation concerned the conversion of the higher-energy AH2 conformer into the lower-energy AH1 form. Because the only part of the molecule significantly changing its position during the AH2 → AH1 transformation is the light hydrogen atom and the barrier separating the AH2 and AH1 forms is quite high (35 kJ mol⁻¹), the conversion is possible only via quantum tunneling of the H-atom. So far, conformational conversions occurring via hydrogen-atom tunneling were observed for a number of carboxylic acids51 (such as formic,52,53 acetic,54 glycolic,55 or furoic56 acids) as well as small amino-acids glycine57 and alanine.58,59 In all these tunneling processes, the conversion of the higher-energy conformer to the lower-energy form occurs via 180° rotation of the OH group, being a part of the O=C–O–H carboxylic fragment. A significantly smaller number of reports concern conformational transformations, occurring by tunneling of a hydrogen atom, which does not make part of any carboxylic group. Such spontaneous conformational transformations were observed for matrix-isolated cytosine,39 5-methylcytosine,60 5-fluorocytosine,60 pyrimidine,61 2-cyano-phenol,61 hydroquinones,62,63 and 9-methylguanine investigated in the current work.

In contrast to the typical photochemical behavior of the molecules analogous to 9mG, no UV-induced o xo → hydroxy or hydroxy → oxo phototautomerization has been observed for matrix-isolated 9mG. Instead, the spectroscopic results strongly suggest the generation and stabilization of the 9mG* radical, photoproduced by H-atom detachment from the O–H or N–H groups. Generation of such radical species upon UV-excitation of guanine derivatives is unprecedented.

Conflicts of interest

There are no conflicts to declare.

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