# A Comparative Picosecond Transient Infra-red Study of 1-Methylcytosine and 5'-dCMP that Sheds Further Light on the Excited States of Cytosine Derivatives

Páraic M. Keane,[a] Michal Wojdyla,[a] Gerard W. Doorley,[a] Graeme W. Watson,[a] Ian P. Clark,[b] Gregory M. Greetham,[b] Anthony W. Parker,[b] Michael Towrie,[b] John M. Kelly,[a] and Susan J. Quinn.[c]*

[a]*School of Chemistry and Centre for Synthesis and Chemical Biology, Trinity College, Dublin 2, Ireland.;* [b]*Central Laser Facility, Science & Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK OX11 0QX.* [c]*School of Chemistry and Chemical Biology, Centre for Synthesis and Chemical Biology, University College Dublin, Dublin 4, Ireland.*

susan.quinn@ucd.ie

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ABSTRACT The role of N1-substitution in controlling the deactivation processes in photo-excited cytosine derivatives is explored by ps-TRIR. The simplest N1-substituted derivative, 1-methylcytosine, exhibits relaxation dynamics similar to the cytosine nucleobase and distinct from the biologically relevant nucleotide and nucleoside analogues that have longer excited-state intermediates. It is suggested that this is due to the sugar group either facilitating access to the long-lived $^1n_O\pi^*$ state or to retarding its crossover to the ground state.

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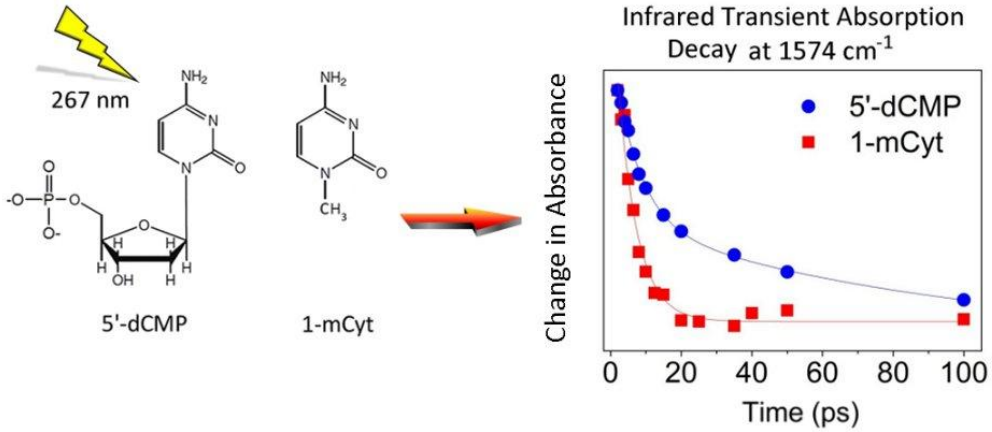
**Graphical Abstract**




Ultrafast internal conversion is the predominant decay mechanism of singlet ππ* nucleobase excited states of DNA and is believed to arise from a near-barrierless conical intersection with the ground state.[1-2] The parent nucleobases all have sub-picosecond lifetimes and this property is often invoked as the principal guardian against photo-damage. However, two recent observations challenge this assertion. First, the observation that thymine dimers can form on sub-picosecond timescales and second, that longer-lived non-emissive states are observed in pyrimidine nucleotides.[3-5] In the case of cytosine it is possible that such long-lived states may play a role in the production of mutagenic C<>C photodimers and subsequent conversion by deamination to uracil.[6]

Evidence of the non-emissive decay route for electronically excited cytosine derivatives has been obtained from both transient UV/vis (5'-CMP)[4] and time-resolved infrared (dCyd and 5'-dCMP) spectroscopies.[5] The relatively long-lived (34-37 ps) species has been assigned as a $^1n\pi^*$ state and is formed in moderate yields.[4] In our TRIR study a strong transient absorption band centred at 1574 $cm^{-1}$ was observed. However, this feature was found to be absent in the case of the nucleobase cytosine, leading us to conclude that N1 substitution has a major influence on the photophysics of cytosine derivatives, see Figure 1. In relation to this we now report experiments on the 1-methyl cytosine (1-mCyt) analogue, chosen to more fully investigate how the N1-substituent can influence the intramolecular dynamics through bond coupling of the pyrimidine ring system.

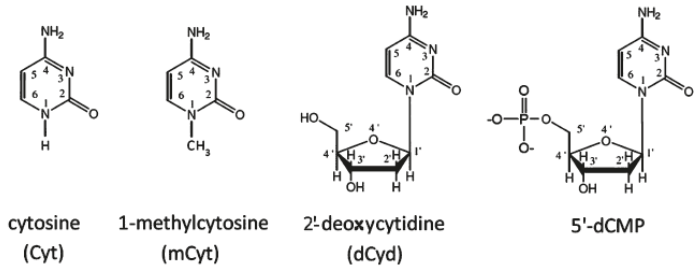



***Figure 1.*** Family of cytosine derivatives with different N1 substituents

We stress that whilst 1-mCyt is not a natural base this analogue is frequently used to represent and model the cytosine base in DNA work, for example in computational studies.[7] Thus there is a need to resolve the electronically excited state behaviour of 1-mCyt in relation to that of the nucleo-tide (-side) and the parent base cytosine. Solutions were prepared to a concentration of 10 mM in pH7 potassium phosphate buffered $D_2O$. The solutions are expected to be monomeric in this concentration range and Beer-Lambert law is obeyed, in contrast to what is found in chloroform solution.[8] The ps-TRIR spectrum of 1-mCyt was recorded following 267 nm excitation,[9] and is shown along with that of 5'-dCMP in Figure 2. Negative-going bands occur due to removal of ground-state population, mode assignments being carbonyl (1654 $cm^{-1}$) and ring stretches (1505, 1525 and 1612 $cm^{-1}$).

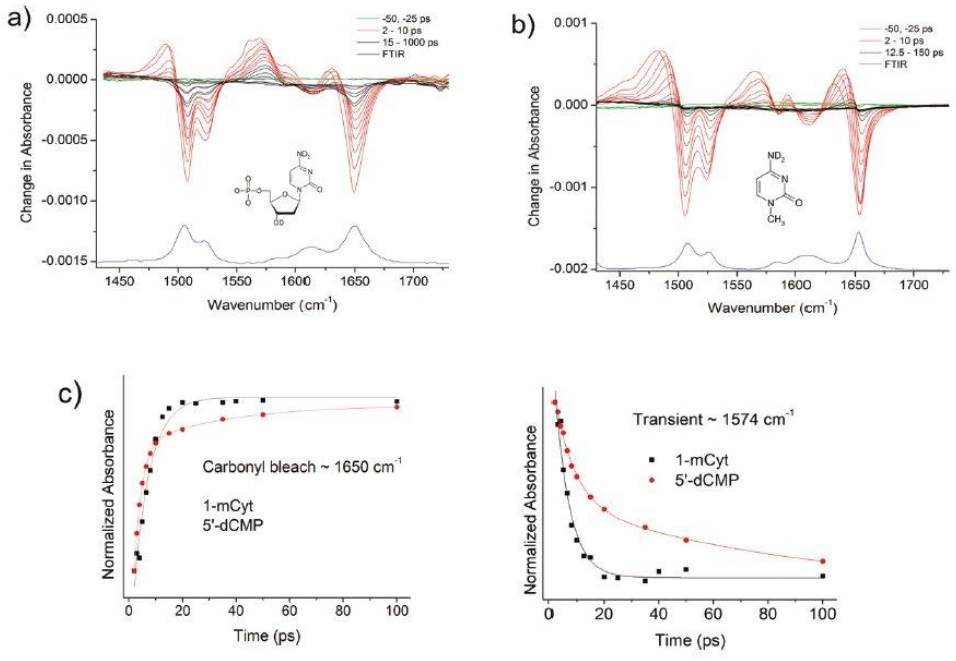



***Figure 2.*** (a) ps-TRIR of 10 mM 5'-dCMP in 50 mM potassium phosphate $D_2O$ buffer (pH 7). (b) ps-TRIR of 10 mM 1-mCyt, both with FTIR below. (c) comparative kinetics for the transient and bleach recoveries, fit at the band maxima.

Analysis of the TRIR spectrum of 1-mCyt shows it is almost fully recovered within c.20 ps. By fitting each time-delay to asum of overlapping Lorentzian functions (see Figure 3) we see that the transient bands can all be assigned as hot ground states. The temporal changes of the spectral profile are consistent with a combination of a fast intramolecular vibrational relaxation (IVR) process and also subsequent cooling of the molecule as excess energy is dissipated into the solvent. Both processes cause a shift to higher frequency due to the anharmonicity within the initially excited vibrational modes and the cooling of excited low frequency modes (unobserved) that couple to the higher modes we observe. Hence the infrared transient absorption maxima are found to shift to higher wavenumber with time as the molecules thermally equilibrate. (Similar behaviour has been observed previously with 5'-GMP and other nucleotides.)[10] The lifetimes of the decay of the fitted bands were calculated and each demonstrated monoexponential kinetics of 4.0 ± 0.4 ps. This is in good agreement with that measured for the parent nucleobase cytosine, which undergoes rapid single exponential decay with a lifetime of 4.4 ± 0.4 ps.[5] The similarity in kinetics between cytosine and 1-mCyt leads us to assign the observed TRIR activity to rapid crossing from the electronic excited potential energy surface (PES) to the ground-state PES (*via* internal conversion from the $^1\pi\pi^*$ state) creating vibrationally hot ground state molecules, formed with up to 34,000 $cm^{-1}$ excess energy.[10b]

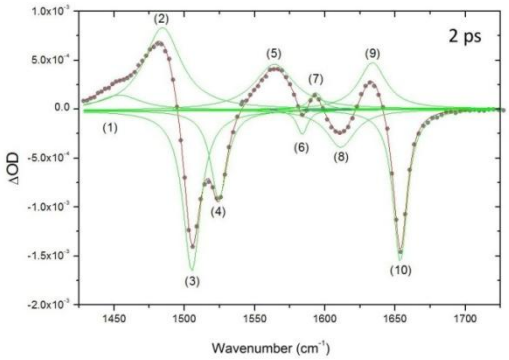



***Figure 3.*** Band fitting using Lorentzian function at 2 ps of the ps-TRIR data of 10 mM 1-mCyt in 50 mM potassium phosphate $D_2O$ buffer (pH 7).

Turning our attention to 5'-dCMP, we see that the TRIR spectra (Figure 2a) are clearly different from that of 1-mCyt. A long-lived IR transient absorption is present in the case of 5'-dCMP (black spectra at longer time delays) at 1574 $cm^{-1}$ that has a double exponential decay of 5.0 ± 0.4 and 39 ± 5 ps for the deuterated solvent in close agreement with our previous work.[5] However, this longer-lived transient feature is absent in the 1-mCyt spectrum.

We now discuss these results. As described above, 1-mCyt is frequently used as an analogue to the biologically relevant derivatives of cytosine. The $CH_3$ group blocks oxo-hydroxy tautomerism between the N1 and O7 sites and hence 1-mCyt has access to the same tautomeric forms available to dCyd and 5'-dCMP.[7] However, the current work shows that 1-mCyt does not form the longer-lived state with significant yield highlighting the fact that simple N1-methyl substitution does not have the same effect as sugar substitution. In line with other reports we suggest that this longer-lived species is an $n\pi^*$ state. We previously made a tentative assignment to the $n_N\pi^*$ state.[5] This was argued on the basis that the transient band at 1574 $cm^{-1}$ was due to a lowering of the ground state carbonyl stretch from 1650 $cm^{-1}$. While in the case of the $n_O\pi^*$state, this would be expected to have a vibrational signature at a much lower frequency. Configuration Interaction Singles (CIS) calculations with the 6-311++G(d,p) basis set have now been performed to estimate the expected band positions for the $n_O\pi^*$ and the $n_N\pi^*$states. These calculations (Figure 4) highlight two key points (i) The vibration of the carbonyl bond for the $n_O\pi^*$state is expected to be displaced to a very low wavenumber < 1400 $cm^{-1}$ and (ii) In the $n_N\pi^*$state, at least in the gas phase, the carbonyl stretch vibration is at higher wavenumber than the ground state.[11] Taking account of these findings we conclude the transient species we observe is the singlet $n_O\pi^*$state, rather than the $n_N\pi^*$species, though higher level calculations taking into account solvent influences are required for complete validation.

In contrast with 5'-dCMP or 2'-dCyd both 1-mCyt and Cyt show rapid routes to the vibrationally excited ground state with no evidence of a long-lived $^1n\pi^*$ state. This could be due to the $n\pi^*$ state either not forming or alternatively decaying on a sub-picosecond timescale.

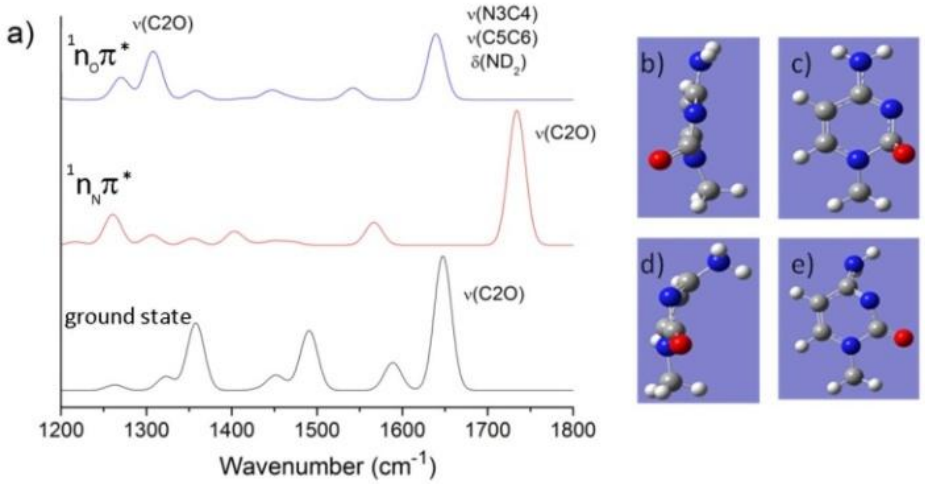



***Figure 4.*** (a) Calculated gas-phase IR spectra of the ground state, and $^1n_N\pi^*$ and $^1n_O\pi^*$ excited states of 1-mCyt. Carbonyl stretches and the strong ring vibration of the $^1n_O\pi^*$ state are labeled. (b) edge-on view of $^1n_O\pi^*$. (c) face-on view of $^1n_O\pi^*$. (d) edge-on view of $^1n_N\pi^*$ (e) face-on view on $^1n_N\pi^*$.

The results presented herein, combined with previously reported results for Cyt and dCyd, categorically show that N1 substitution alone is not sufficient to cause the generation of longer-lived (>10 ps) species. Previously, Kohler and co-workers reported that 1-cyclohexyluracil and 1,3 dimethyluracil show similar kinetics to uracil, while much longer lived decay occurs with UMP.[4] Our observations with cytosine mirror those of uracil, and now confirm that the ribose group has major significance in the photostability of nucleic acids.

The ultrafast photophysics of pyrimidines has been the subject of a number of detailed computational studies.[12] In the pyrimidine bases the major non-radiative decay pathway is internal conversion from the ππ* state to the ground state through a conical intersection arising from pyramidalization of the C5 and/or torsion of the C5-C6 bond. The out-of-plane motion of the 5-substituent, which accompanies this torsion (ethylenic path) is deemed to be the critical mechanistic step toward the conical intersection. Indeed Gustavsson et. al. have correlated the access to the conical intersection from the $S_1$ minimum to the nature of the 5- and 1-substituents.[13] Additionally, Nieber and Doltsinis found the mechanism of ultrafast decay of uracil and uridine to be dominated by large out-of-plane ring distortionswhich appear to be closely coupled to changes in the excited and ground state energy gap.[14] Interestingly, no qualitative difference between the mechanisms governing nonradiative decay in the gas phase and in aqueous solution were observed. This suggests that the excited-state lifetimes of bases, and more complex forms, are less dictated by hydrogen bonding influences but rather are linked with their capacity to adopt conformational changes so as to reach the conical intersection.

It is notable that the ultrafast decay of the ππ* state (< 1 ps) is reported to occur in both cytosine and 5'-dCMP,[1,15] The 39 ps decay observed in dCMP may thus be due to an additional competing pathway.[16,17] Indeed recent ab initio multiple spawning dynamics calculations by Hudock and Martinez have shown cytosine, in contrast to uracil and thymine, to possess multiple simultaneous competing relaxation pathways with most trajectories acquiring $n_O\pi^*$ or ππ* character before quenching through the $n_N\pi^*/S_0$ conical intersection.[18]

One pathway is predicted to proceed through the $n_O\pi^*$ minimum and therefore should be detectable by picosecond spectroscopy. This is similar to that observed for dCyd and 5'-dCMP, but different for Cyt and 1-mCyt. It is noteworthy that in this model the wavefunction acquires $n_O\pi^*$ character by movement along the N-H out-of-plane vibration. It is intriguing to suggest that the presence of the sugar promotes passage to the $n_O\pi^*$ minimum, whereas that of the H atom or methyl group are insufficient to direct the molecule to the $n_O\pi^*$ metastable structure. (Alternatively it may be that the $n_O\pi^*$ state is indeed formed for 1-mCyt and Cyt, but that its decay to the ground state is very rapid (< 1ps) for 1-mCyt, but much slower for

5'-dCMP and dCyd. A distinction between these alternatives is currently not possible given the time resolution of our measurements.)

The role of the ribose group in the decay of the excited state remains unclear. It may be a simple consequence of the mass of the sugar, or some steric property that restricts certain ring motions resulting in different nuclear configurations. Hare et al. suggested that hydrogen bonding between sugar and solvent may aid vibrational relaxation of the hot nπ* state in pyrimidines, resulting in trapping on the nπ* surface.[4a] TD-DFT calculations on pyrimidine systems have also found that mixing may occur between non-bonding electrons on the base and electrons on the ribose[19] It is clear that none of these factors are sufficiently influenced by the presence of a methyl substituent at the 1- position.

The participation of different excited states in the photophysics of natural DNA is only beginning to be more rigorously explored with the advancement of spectroscopic techniques, and the role of nπ* states in DNA systems was again recently highlighted.[20] The postulate of a localized cytosine excited state in double-stranded $[poly(dG\text{-}dC)]_2$ suggests that it may also be present in genomic DNA.[10a] However there is still much to be understood of the behaviour in the monomer species.

Importantly, the family of 1-methyl-nucleobase derivatives are commonly employed in computational studies.[21] This is primarily to avoid the inherent difficulties associated with performing calculations of very large molecules. Our work highlights that some caution is needed in modeling electronic excited dynamics on the photophysics of such systems. The full potential of transient infrared to characterize excited states requires support from computational studies that appropriately account for solvent to allow prediction of structural bands. We recommend revisiting the role of the 1-substitution further using high-level calculations to specifically address the issues highlighted here and thus shed further light on the factors that influence conical intersections.

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**Supporting Information Available:** Experimental details and computational methods, kinetics analysis of 1- mCyt, and Calculated gas-phase IR spectra of the ground state, and $^1n_N\pi^*$ and $^1n_O\pi^*$ excited states of 1-mCyt. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Note: the $n_O\pi^*$state has a vibration in approximately the same region as the observed transient, but this is due to a pyrimidine ring vibration.

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