

**Supporting Information for
Ultrafast IR spectroscopy of short-lived transients formed by femtosecond UV
excitation of cytosine derivatives**

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Methods

Ps-TRIR measurements. Measurements were performed at the PIRATE ultrafast infrared absorption facility at the Rutherford Appleton Laboratory, Chilton which has been described in detail elsewhere.¹ Briefly, the sample was excited with 267-nm, 150-fs pulses with 2 μ J of energy at 0.5 kHz repetition rate, generated from the third harmonic of part of the output from a 1kHz, 800 nm, 150 fs, 1 mJ regenerative amplifier (Spectra Physics Tsunami/Spitfire) and probed with 150 cm^{-1} fwhm broadband infrared pulses generated by difference frequency mixing the signal and idler outputs of a BBO (β -BaB₂O₄) optical parametric amplifier, pumped with some residual 800 nm, in AgGaS₂ at 1 kHz. The spot size was 200 μ m and 150 μ m diameter for the pump and the probe beam, respectively. The difference signal pump-on minus pump-off was normalized on a shot-by-shot basis and typically accumulated for four successive rounds of 30 s data integration for a single time delay. The infrared beams were dispersed by 150 l/mm, 4000 nm blaze, gold grating monochromators and imaged onto 64 element MCT array. The data were collected in three 150 cm^{-1} spectral windows centred at 1646, 1552 and 1494 cm^{-1} using the delay line for optical delays between 2 ps and 1 ns, normally at 2, 3, 4, 5, 6.5, 8, 10, 12.5, 15, 20, 35, 50, 100, 150, 200, 500, and 1000 ps. The difference signal was calibrated using water lines present in the probe spectrum, and the spectral windows were interleaved after scaling using overlapping transients recorded at the same delay time. (This was done at 1554 cm^{-1} for dCyd, 1596 cm^{-1} for dCMP and at 1534 and 1585 cm^{-1} for Cyt.) The sample was raster scanned in *x*- and *y*- directions at an approximate rate of 100 mm/ms.

1. M. Towrie, D. C. Grills, J. Dyer, J. A. Weinstein, P. Matousek, R. Barton, P. D. Bailey, N. Subramaniam, W. M. Kwok, C. S. Ma, D. Phillips, A. W. Parker and M. W. George, *Applied Spectroscopy*, 2003, **57**, 367.

Computational Calculations. We have performed optimisations and vibrational frequency calculations using both B3LYP and MP2 with a 6-311+G(2d,p) basis set in the Gaussian 03 package. B3LYP and MP2 results are presented with all frequencies scaled by a factor of 0.9679 and 0.9427 respectively. The simulated spectra are obtained using the calculated intensity and a Gaussian distribution with a standard deviation of 15 cm^{-1} . To compare to experiment we have calculated the difference

spectra between the tautomers and 1-methylcytosine (Figs 4 and 5) and between the tautomers and dCMP (Fig 6).

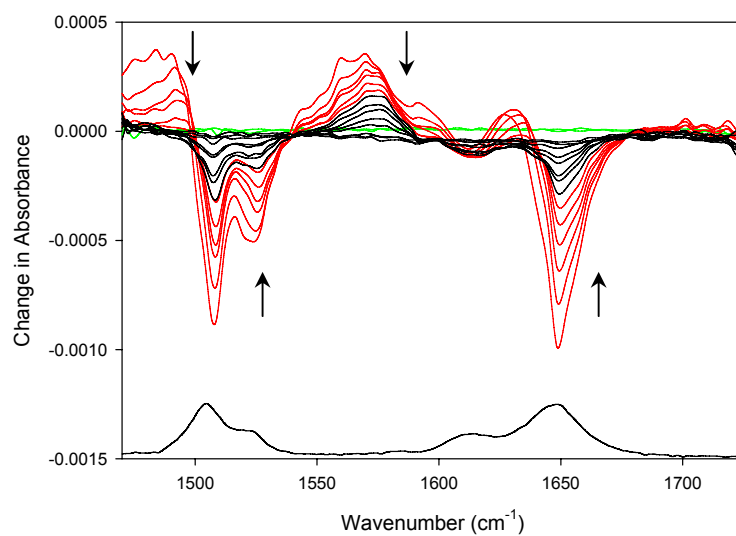


Fig 1. (a) ps-TRIR of 10 mM dCMP in 50mM phosphate buffer pH 7, FTIR below (baseline adjusted) Delays -50, -25 (green), 2, 3, 4, 5, 6.5, 8 (red), 10, 15, 20, 35, 50, 100, 500 and 1000 ps (black).

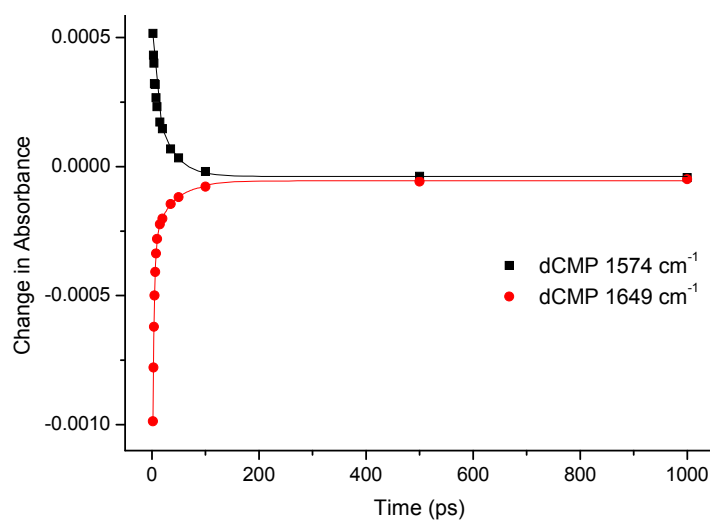


Fig 2. Kinetic analysis of 10 mM dCMP in 50 mM phosphate buffer pH 7 at 1574 and 1649 cm^{-1}

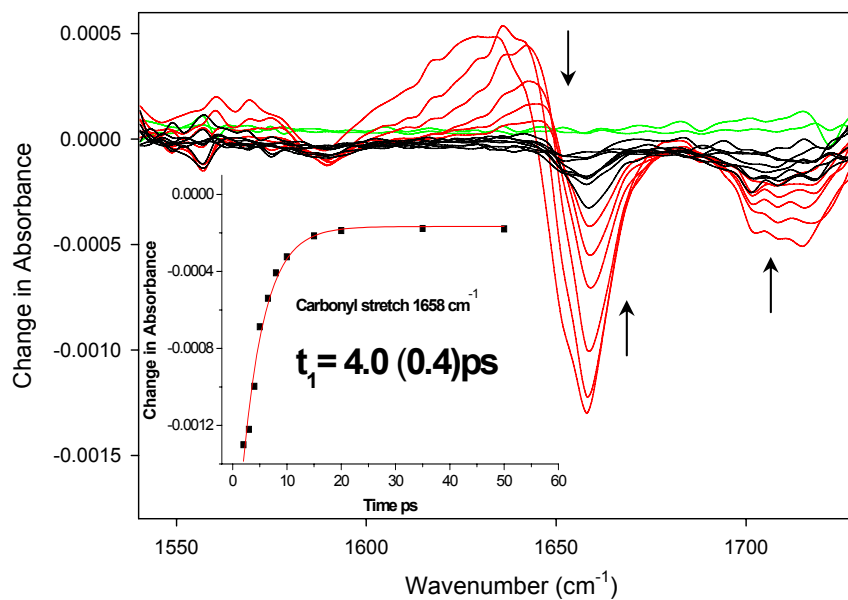


Fig 3. ps-TRIR of 10 mM dCMP under acidic conditions, 0.132 M H_3PO_4 in D_2O , and inset kinetics recorded at 1658 cm^{-1} . Delays at -50, -25 (green), 2, 3, 4, 5, 6.5, 8 (red), 10, 15, 20, 35, 50, 100, 500 and 1000 ps (black).

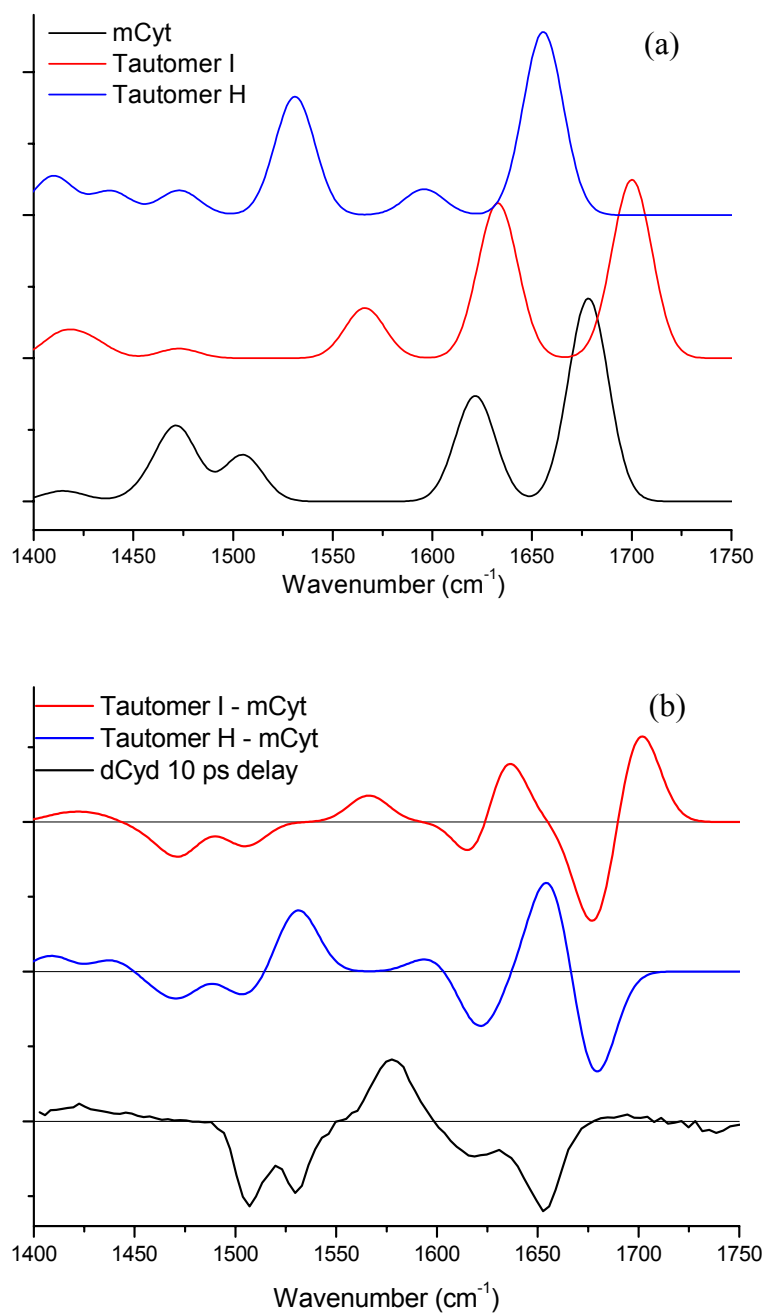


Fig 4. B3LYP Calculations (a) Calculated IR spectra of mCyt and tautomers I and H, and (b) difference spectra of tautomer I compared to mCyt, tautomer H compared to mCyt and experimental data for dCyd at 10 ps. (with both predicted spectra having a scaling factor of 0.96790 applied)

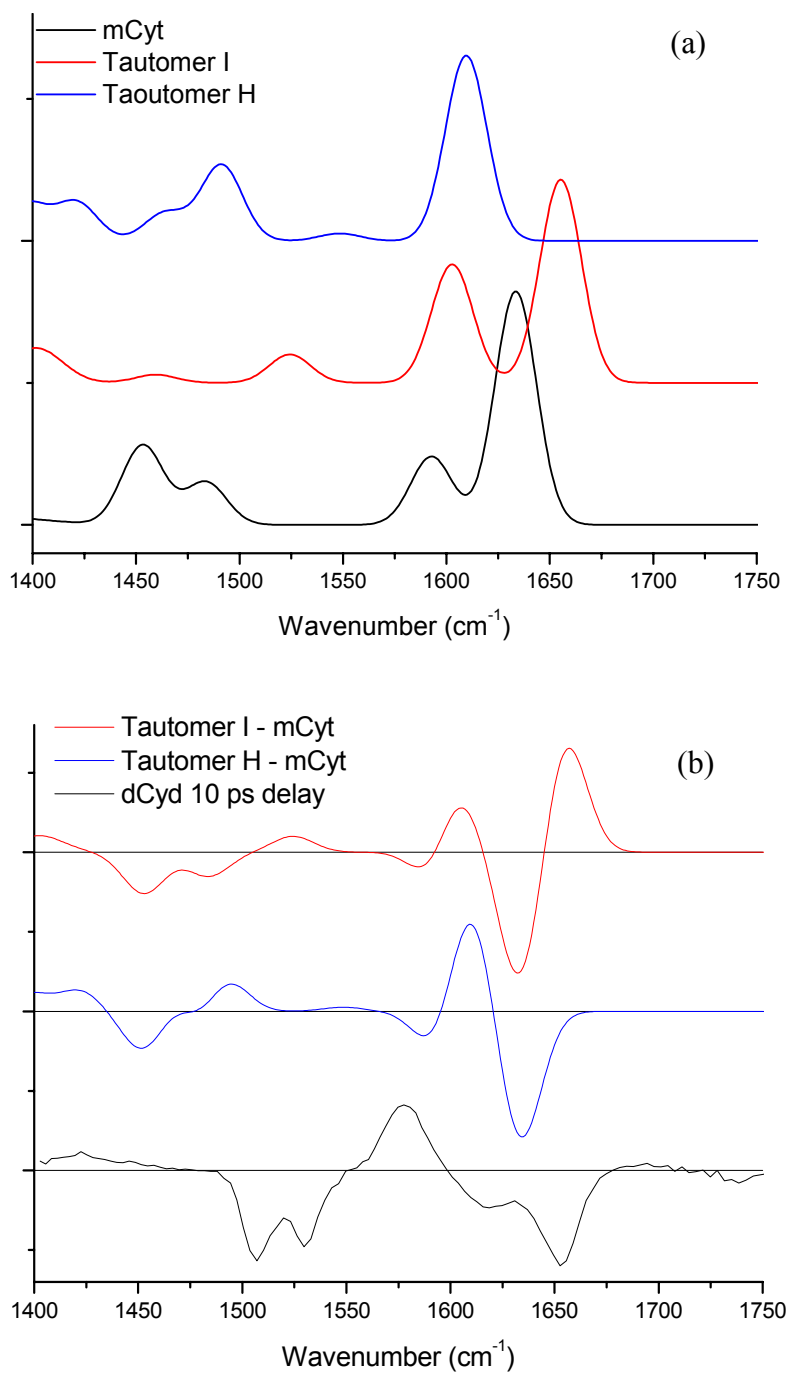


Fig 5. MP2 Calculations (a) Calculated IR spectra of mCyt and tautomers I and H, and (b) difference spectra of tautomer I compared to mCyt, tautomer H compared to mCyt and experimental data for dCyd at 10 ps. (with both predicted spectra having a scaling factor of 0.9427 applied)

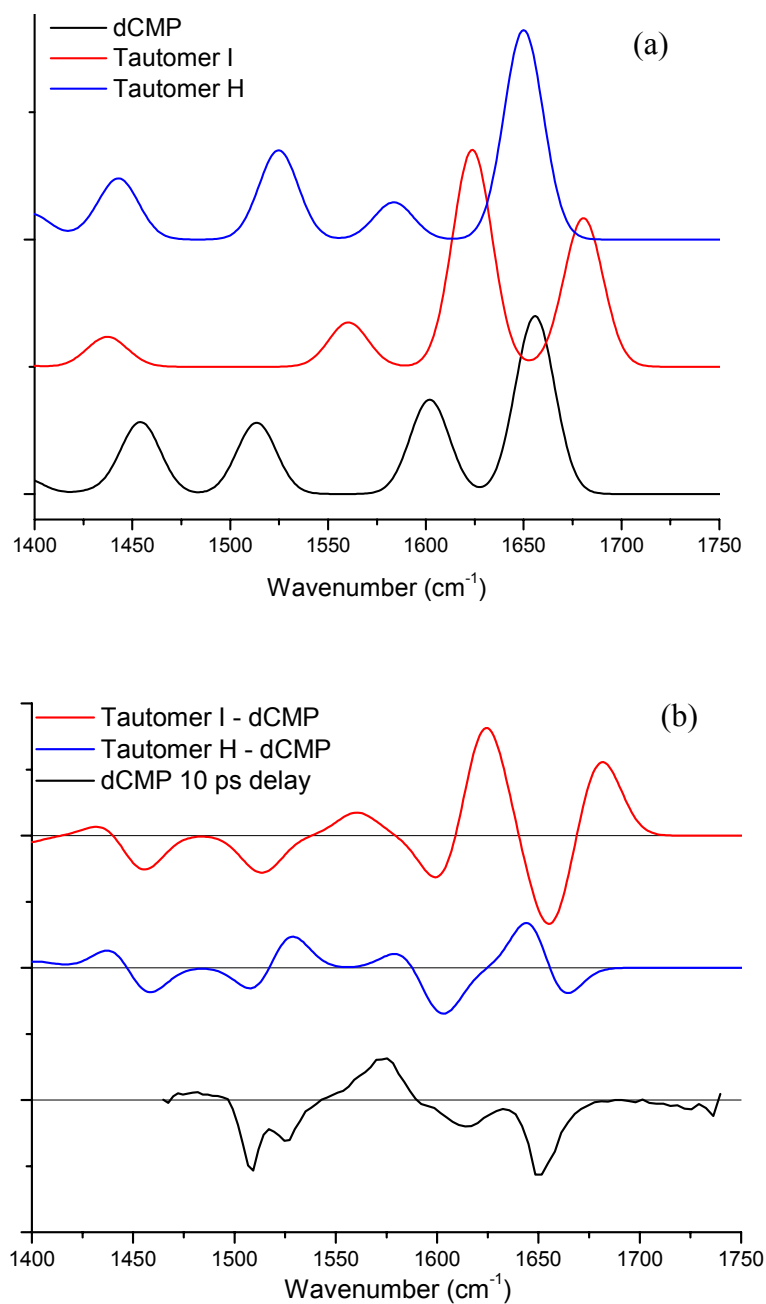
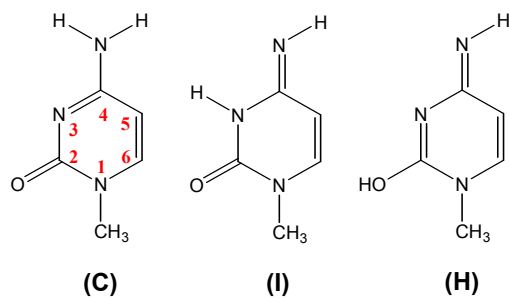


Fig 6. B3LYP Calculations (a) Calculated IR spectra of dCMP and tautomers I and H, and (b) difference spectra of tautomer I compared to dCMP, tautomer H compared to dCMP and experimental data for dCMP at 10 ps. (with both predicted spectra having a scaling factor of 0.96790 applied)



mCyt (cm ⁻¹)	Principal Components	Tautomer I (cm ⁻¹)	Principal Components	Tautomer H (cm ⁻¹)	Principal Components
1678	C2-O	1700	N3-C2,	1656	C5-C6, C2-N3
1621	C5-C6, C4-NH ₂	1633	C5-C6, C4-NH	1596	C4-NH, C2-N3
1505	C4-C5 C5-C1 C4-NH ₂	1566	C4-NH, C5-C6	1531	C4-NH, C2-N3 C5-C6

Table 1. B3LYP calculated IR vibrational frequencies with principal mode assignment. A Scaling factor of 0.96790 has been applied to all frequencies.