Supporting information for:

Photophysical and structural properties of the fluorescent nucleobase analogues of the tricyclic cytosine (tC) family

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Temperature-dependent fluorescence quantum yield measurements

The temperature-dependent fluorescence quantum yields of tC, tC^O and tC_{nitro} were measured by acquiring absorption spectra of the compounds in an Oxford optistatDN cryostat using pure solvent in the cryostat at room temperature as baseline and acquiring emission spectra by exciting the fluorophores at 370 nm, 361 nm and 370 nm, respectively. The same setup was used for the reference fluorophore. The quantum yields at each temperature were then calculated by[1]

$$\Phi_{\mathrm{f}} = \frac{A_{\mathrm{ref}} \times I_{\mathrm{f}} \times n^2_{\mathrm{solvent}}}{A_{\mathrm{f}} \times I_{\mathrm{ref}} \times n^2_{\mathrm{ref\ solvent}}} \times \Phi_{\mathrm{ref}}$$

where A_f and A_{ref} are the absorbances at the excitation wavelength of the sample and the reference fluorophore, respectively, I is the integrated fluorescence intensity, n is the solvent refractive index and Φ_{ref} is the fluorescence quantum yield of the reference compound. A temperature-correction for the refractive index of MeTHF was made by systematically adding 0.00045 to the value of n at RT per degree decrease in temperature. Since no value for the volumetric temperature expansion coefficient of MeTHF was found in the literature a simple temperature-correction for the sample volume subtraction was done using a density-temperature-relationship obtained from a linear regression of the temperature-dependent densities of MeTHF measured previously.[2] The temperature-corrections resulted in a change in $\Phi_{f,0}$ from $\Phi_{f,0}$ = 0.77 to 0.71 and from $\Phi_{f,0}$ = 0.70 to 0.66 for tC and tC^O, respectively.

The absorption and emission spectra of each of the three fluorophores are shown in Figure S1-S3. In calculating the fluorescence quantum yields the value of the absorbance of the chromophores at the excitation wavelength was assumed to be the same at all temperatures (except for the temperature-correction for sample volume described above), and determined at T = 295 K for tC and tC^O and at T = 184 K for tC_{nitro}. For tC_{nitro} the absorption spectrum (Figure S1) does not change by varying the temperature supporting the approximation in using constant absorbance. For tC and tC^O the absorption spectra do change by lowering the temperature (Figure S2 and S3), however, as seen in the inserted enlargement of the spectra of tC (Figure S2, left insert) it is the light scattering of MeTHF (the solvent background) in the UV range that dominates the changes with temperature, and results in a concomitant error in the baseline used at lower temperature. The same baseline effect is seen in the absorption spectrum of the

tC^O sample upon lowering the temperature (Figure S3, left). The approximation that the oscillator strength is constant with temperature might introduce slight errors in the estimated quantum yields but, nevertheless, the resulting Arrhenius fits to the varying quantum yields show excellent correlation with the model functions (Figure 2 in the article).

1. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd edn., Springer, New York, 2006.
2. F. Comelli, R. Francesconi, A. Bigi and K. Rubini, Journal of Chemical and Engineering Data, 2007, 52, 639-644.

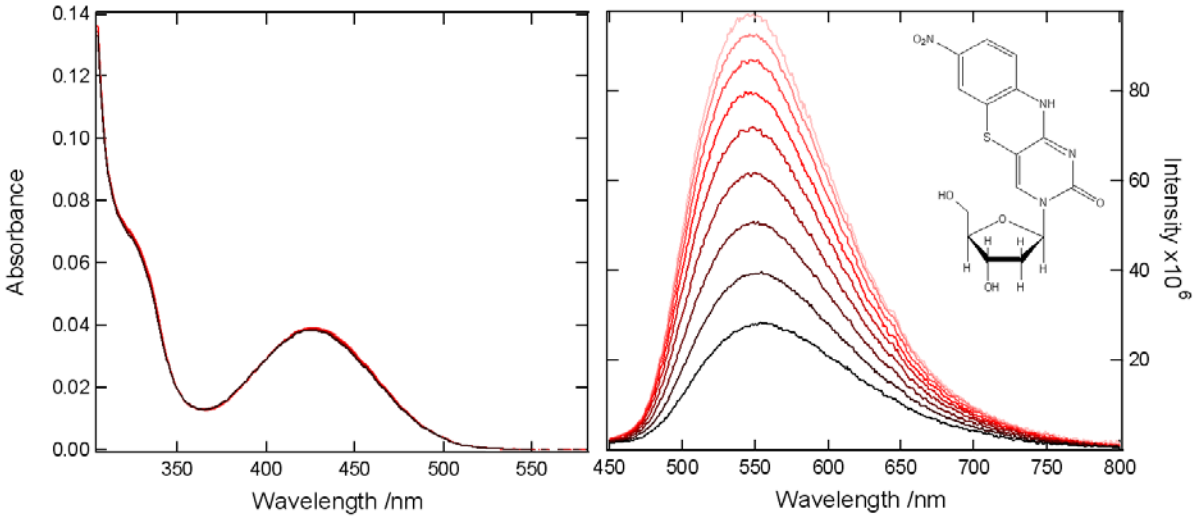

Figure 1. Absorbance (left) and emission (right) spectra of tC_{nitro} in PG glass at various temperatures. Absorbance spectra are shown in the interval from *T* = 184 K (black) going to *T* = 163 K (red). Emission spectra are shown in the interval from *T* = 212 K going to *T*= 150 K (black→red) using an excitation wavelength of 370 nm and same slit bandwidths at all *T*.

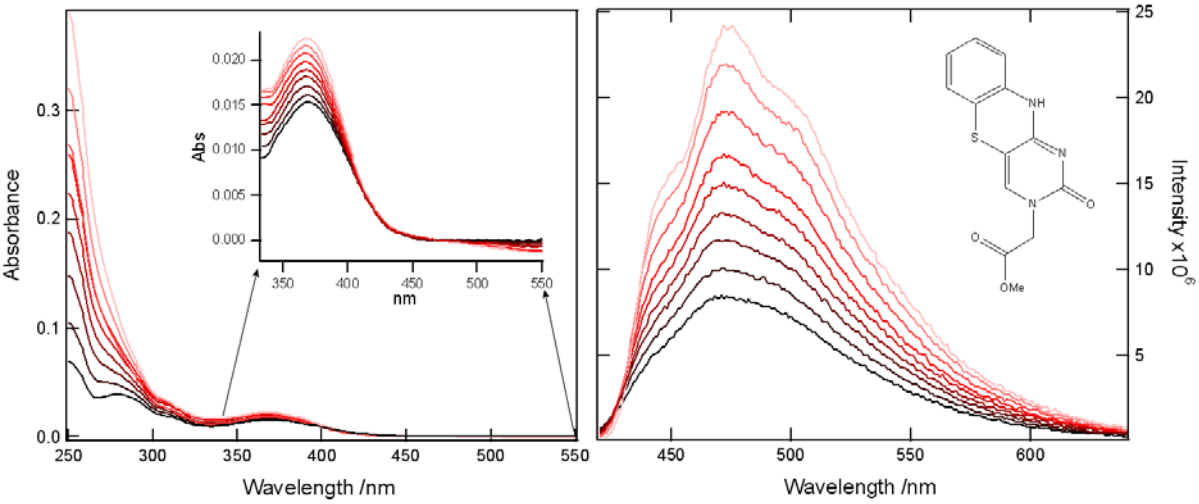

Figure 2. Spectral evolution of the tC in MeTHF as the temperature is lowered from *T* = 295 K to *T* = 153 K (black→red). Left: Absorption spectra. Insert shows a magnified area of the spectrum. Right: Fluorescence spectra acquired using an excitation wavelength of 370 nm and same slit bandwidths at all temperatures.

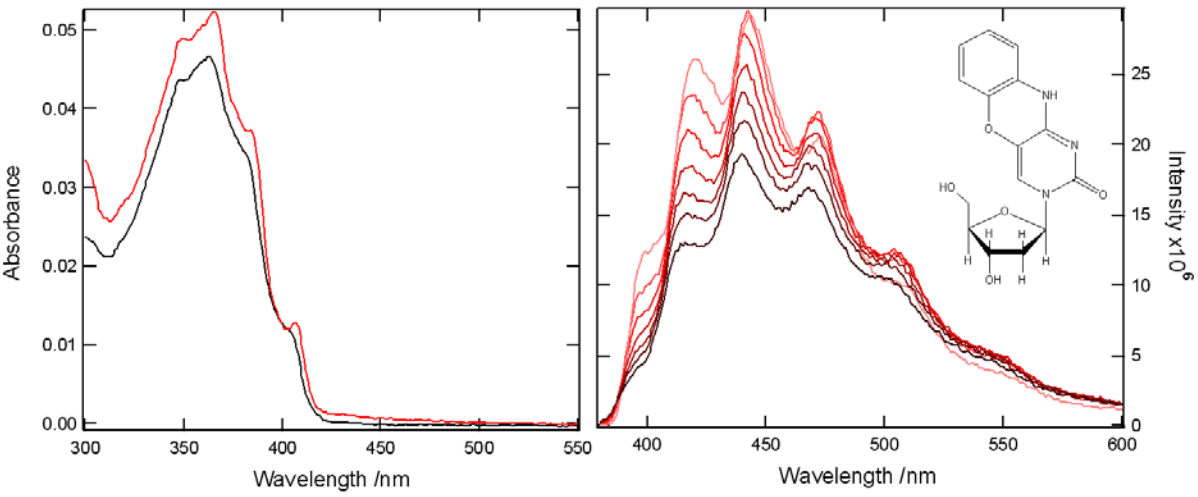

Figure 3. Left: Absorption spectra acquired of the nucleoside of tC^O at 295 K (black) and at 218 K (red). Right: Spectral evolution of tC^O emission in MeTHF as the temperature is lowered from 295 K to 175 K (black→red). Spectra were acquired using an excitation wavelength of 361 nm and same slit bandwidths at all temperatures.