Supplementary information for

The photoinduced transformation of fluorescent DNA base analogue tC triggers DNA melting

S. Preus, S. Jønck, M. Pittelkow, A. Dierckx, T. Karpkird, B. Albinsson, and L. M. Wilhelmsson

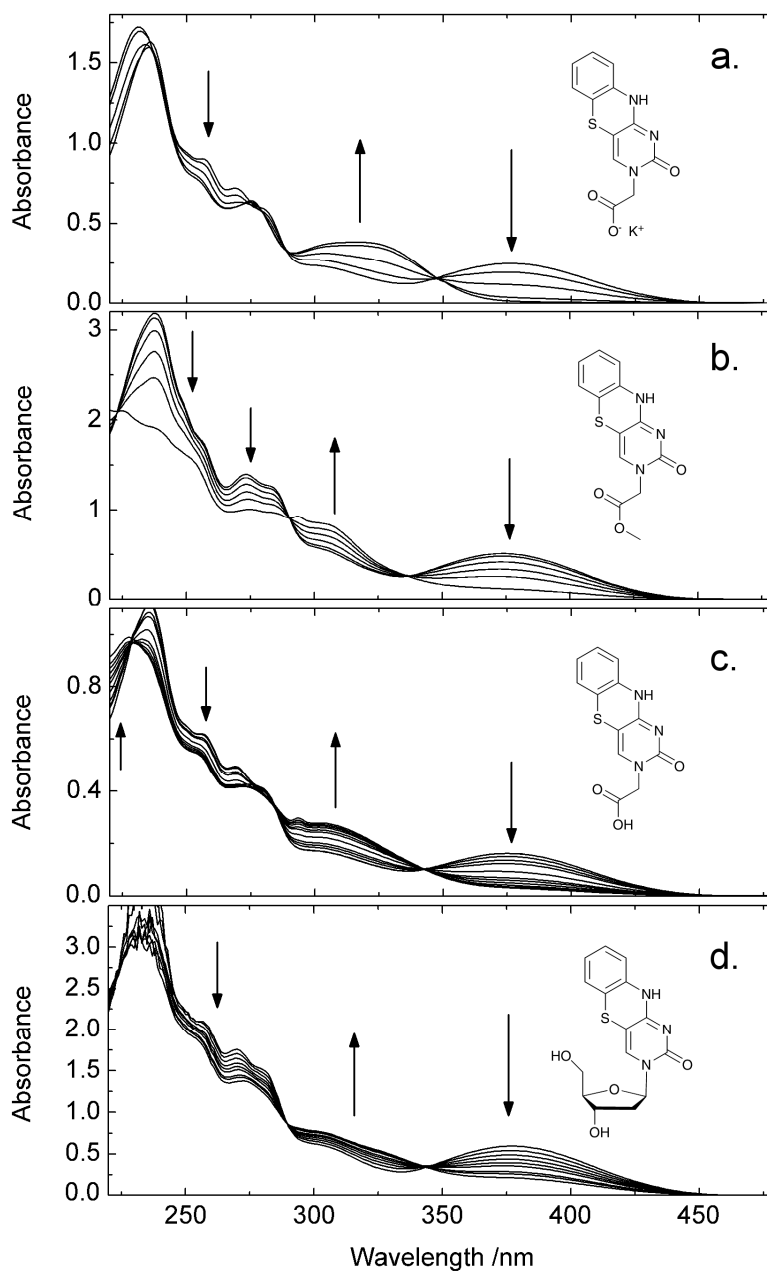**S1. UV-Vis absorption reaction curves of monomeric tC in H$_2$O**

Fig. S1 UV-Vis photodegradation curves of tC in H$_2$O. a) KtC, b) Me-tC, c) Ac-tC, d) Nuc-tC.

S2. Estimation of fluorescence quantum yield of tC[#]

The fluorescence quantum yield of tC[#] in aqueous solution was roughly estimated using monomeric tC[O] in H$_2$O as a reference (QY = 0.3, Table S1).[1]. The spectra used in the estimation of the quantum yield are shown in Figure S2 below. The experiment was performed using five different excitation wavelengths (300 nm, 305 nm, 340 nm, 370 nm and 375 nm). Since only tC and not the photoproduct absorbs at 375 nm exciting at several excitation wavelengths provided a means to test the contribution from any non-reacted tC left in the sample on the measured fluorescence intensity when exciting at 300-340 nm.

Firstly, it is observed that the calculated quantum yield increases when going from low to high excitation wavelengths. Secondly, a fluorescence signal is observed not only when exciting tC[#] at 300-340 nm, but also when exciting the sample at 370 nm and 375 nm and in this case with a spectral appearance being identical to the emission spectrum of tC (Fig. S2b, blue). These observations demonstrate that there are small amounts of unreacted tC left in the tC[#] sample which hamper a proper quantitative evaluation of the fluorescence quantum yield of tC[#]. We can, however, conclude that tC[#] is only very slightly fluorescent with a blue-shifted emission spectrum compared to that of tC (which contributes to the broadening of the measured emission spectrum towards lower wavelengths in Fig. S2b).

Table S1 Fluorescence quantum yield measurements of tC[#] calculated at five different excitation wavelengths.

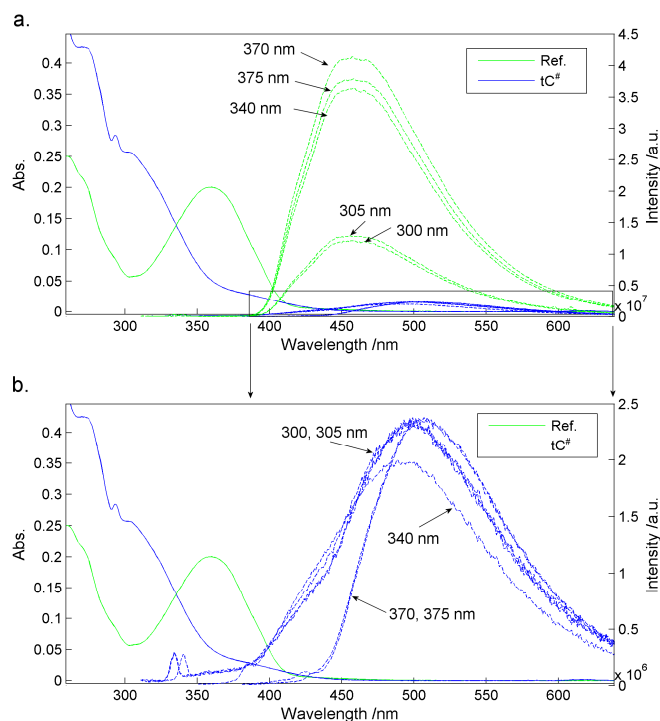| Exc. wavelength	Calculated QY
300 nm	0.0162
305 nm	0.0166
340 nm	0.0286
370 nm	0.0922
375 nm	0.0998

Fig. S2 UV-Vis absorption and emission spectra used in the estimation of the fluorescence quantum yield of tC[#]. a) Overview of the comparison between the tC[O] reference (green) and the tC[#] spectra. b) Zoom of the fluorescence spectra of the tC[#] sample.
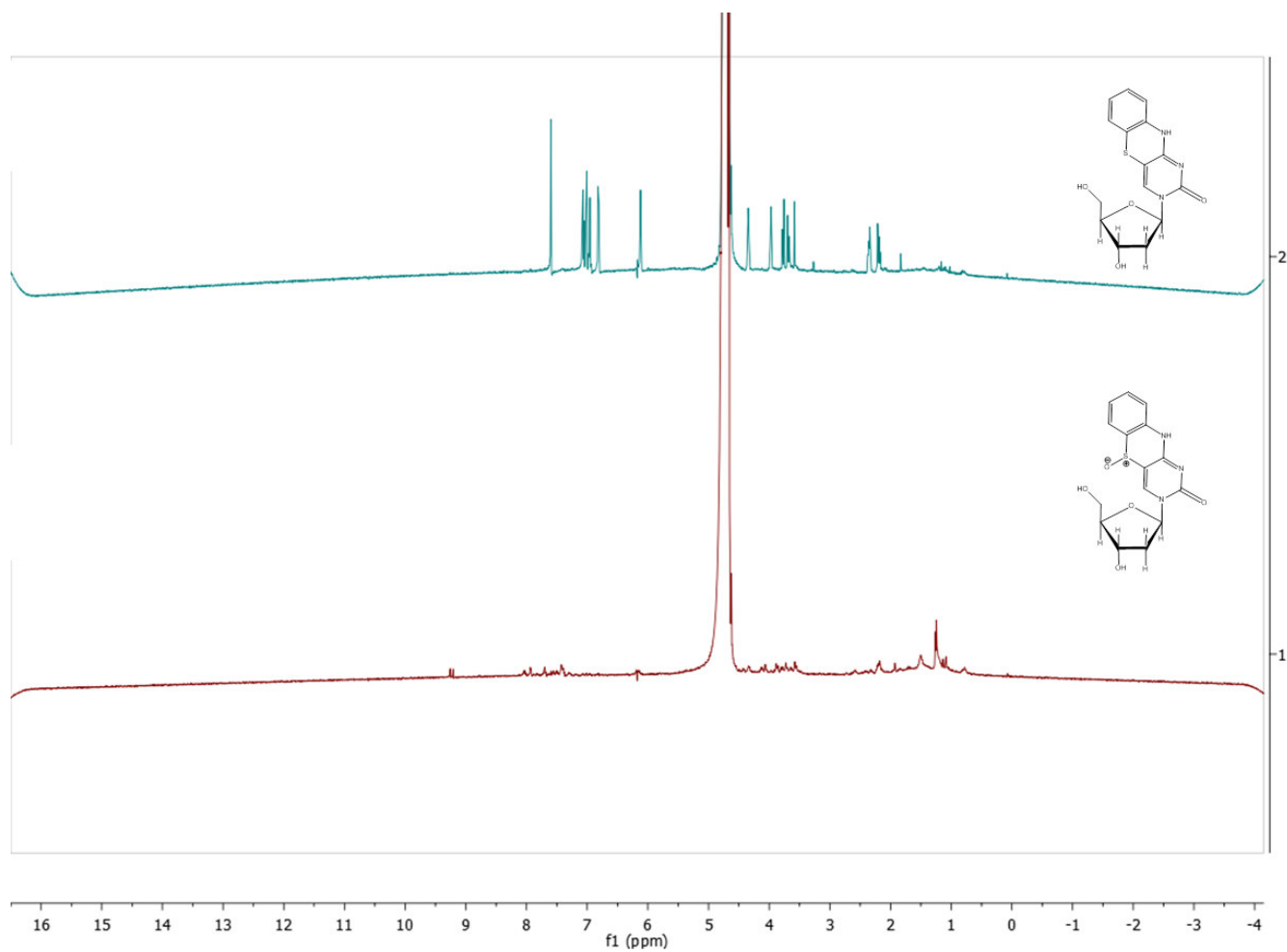
S3. NMR spectra

Fig. S3 ^{1}H-NMR spectra of the tC starting compound (top) and the tC$^{\#}$ photoproduct (bottom).
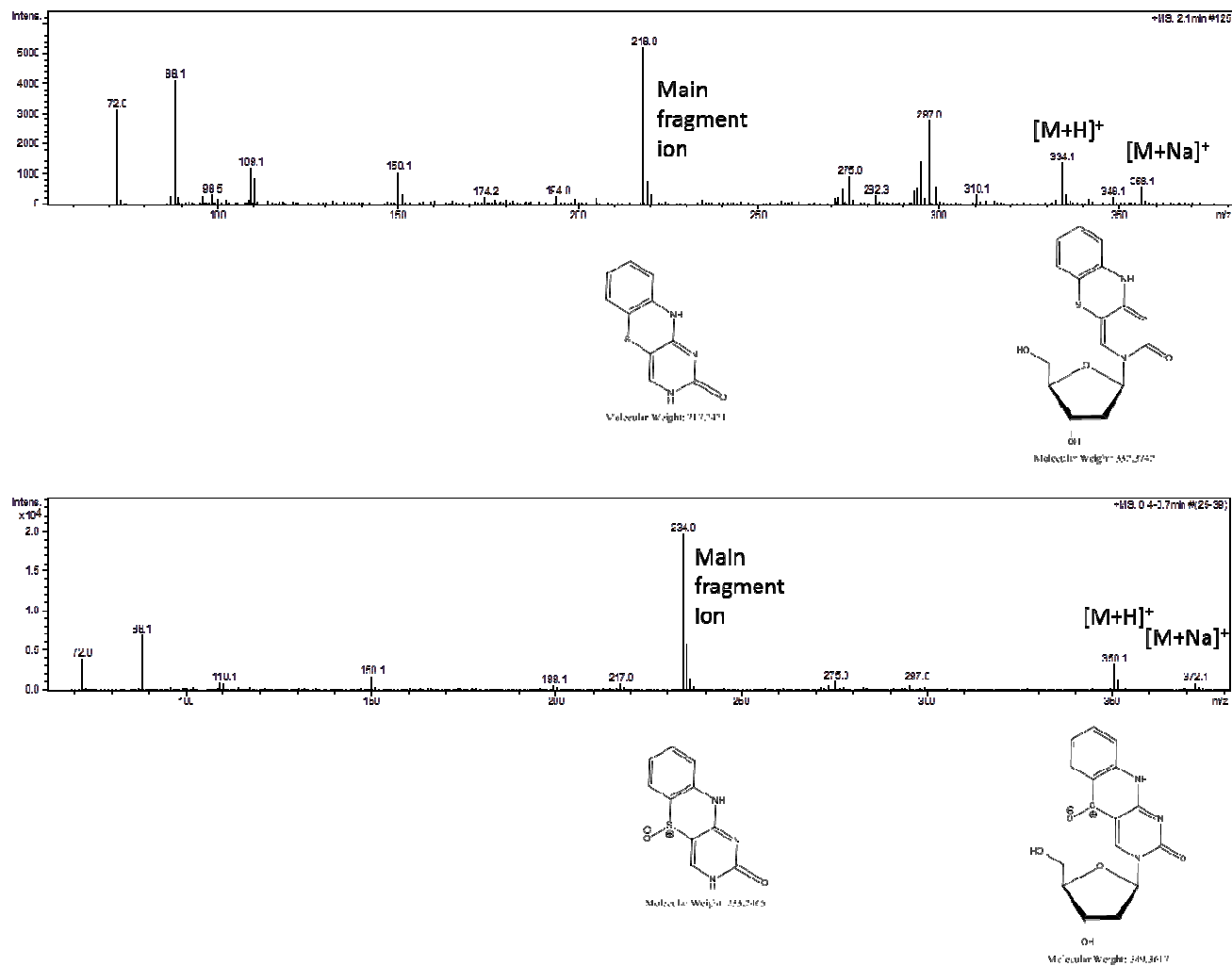
S4. Mass spectra of tC and tC#

Fig. S4 Mass spectra of the tC starting compound (top) and tC# photoproduct (bottom).

S5. Calculation of the oscillator strength of tC$^{\#}$ from the lowest energy absorption band

The oscillator strength of the lowest energy electronic transition of tC$^{\#}$ was estimated by representing the isolated absorption band using a Gaussian function. The Gaussian fit was performed in the spectral region between 25,000 cm^{-1} and 32,000 cm^{-1} (Fig. S5). The oscillator strength was then calculated using

$$f = 2303 \frac{m_e c^2}{N_A \pi e^2 n} \int \epsilon(v) dv \qquad \text{(Equation S1)}$$

where N_A is Avogadro's constant, n is the refractive index, v, is the frequency in cm^{-1} and ε is the extinction coefficient spectrum. The integration is performed over the full Gaussian band.
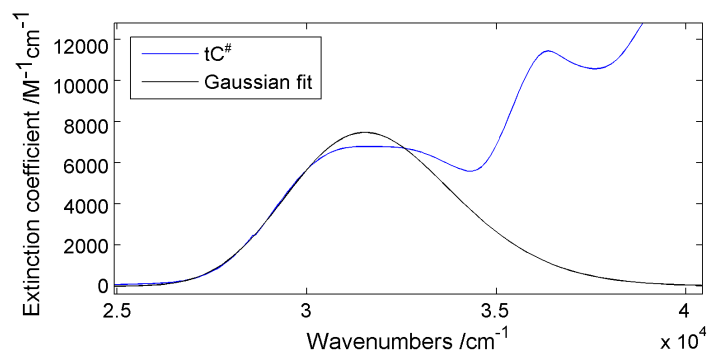

Fig. S5 The lowest energy absorption band of tC$^{\#}$ fitted to a Gaussian function in order to evaluate the oscillator strength using equation S1 above.

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S6. CD spectra of double-stranded DNA samples measured at 10 °C and 25 °C

CD spectra were averaged over 20 scans and corrected for background contributions. The scan rate was set to 0.5 s per point with a step size of 1 nm. The sequence of the tC-T mismatch sample is

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5'-CGCAAXATCG        (X = tC)
3'-GCGTTTTAGC
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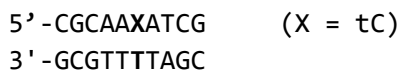
The melting temperature of the tC-T mismatch sample is T_m = 24 ^{o}C (melting curves in Figure S7). The CD spectra of the tC-T mismatch DNA duplex are very similar to the B-form spectra observed for tC and tC$^#$. Hence, the CD spectra do not give any indication of whether tC$^#$ is situated within or outside the DNA helix.
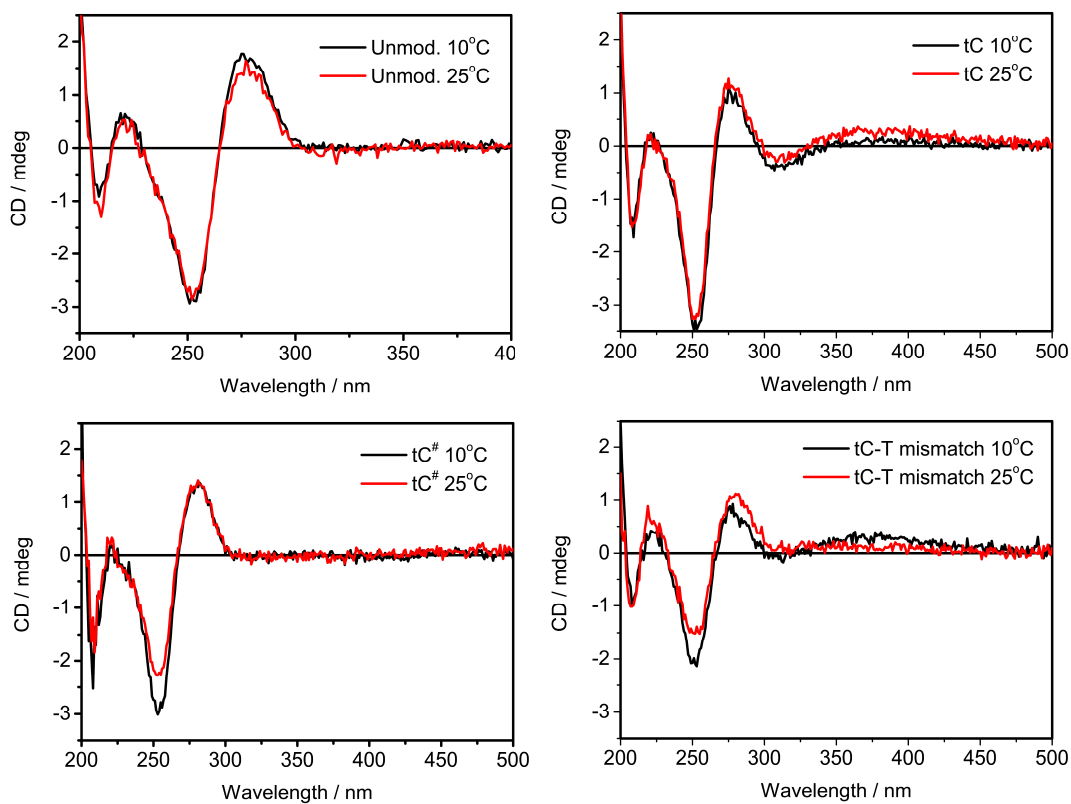

Fig. S6 CD spectra of the double-stranded DNA samples shown in Table 1 in the paper.
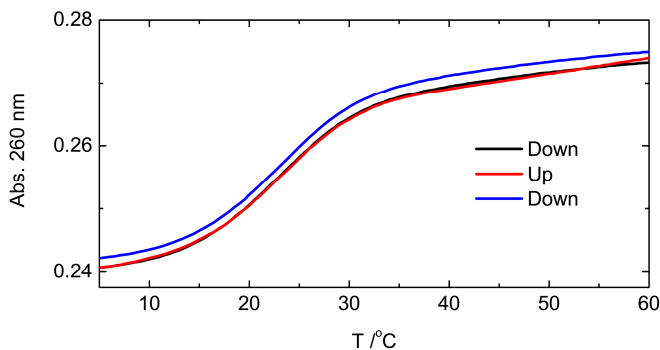

Fig. S7 Melting curve of the tC-T mismatch sample showing a T_m of 24 °C.

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S7. Melting temperature determination of double-stranded DNA with tC[#]

The T_m of tC[#] in double-stranded DNA was determined for two different samples (Fig. S8). Each sample was cycled four times going from low to high and high to low temperatures. The melting temperature was determined from the first derivative of each of the UV-Vis melting curves (Fig. S9 and Table S2). As seen in Fig. S9 two distinct melting temperatures are observed in all the tC[#] modified DNA duplexes ('1' and '2'). The dominant component at 26 °C is not observed in the sample with unmodified DNA or the sample with tC-modified DNA. The small component '2' is located at the same melting temperature as the tC-DNA. Component '2' is thus interpreted as being either unreacted tC or an isomer in which the oxygen atom of tC[#] is directed towards the 3' end in double-stranded DNA, in this case not causing a sterical clash with the neighbouring base (see Supplementary Information S8).
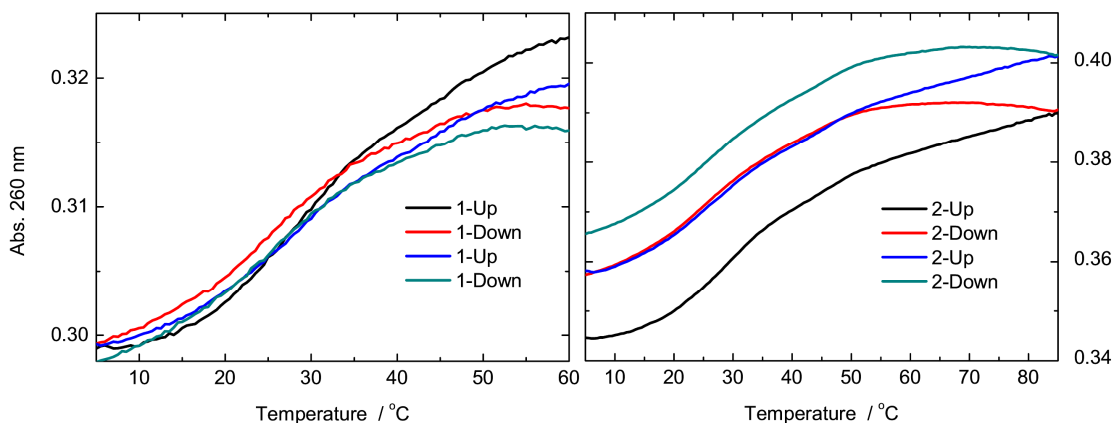

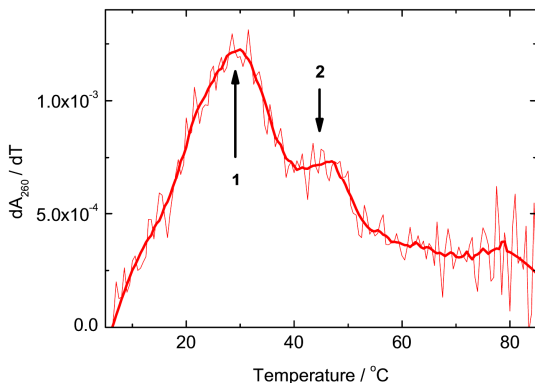**Fig. S8** Melting curves of tC[#]-DNA performed on two different samples (left and right).

Fig. S9 Representative example of the first derivative of a melting curve of tC[#]-DNA.

Table S2 Melting temperatures of DNA with the sequence 5'-CGCAAYATCG (Y = tC[#]) measured in 25 mM phosphate buffer (pH 7.5, 0.1 M Na^{+}). All temperatures are given in degrees Celsius.

Step:	1-Up	1-Down	1-Up	1-Down	2-Up	2-Down	2-Up	2-Down	Avg.[§]
	28	25	27.5	26	29.5	25	26.5	25.5	26.0

[§]T_m determined for 1-Up and 2-Up were not included in the calculation of the average since samples were not fully annealed at the start of the first ramp. If these values are taken into account the average T_m is 26.6 °C.

S8. Stereoisomers of tC#

The DFT calculations predict that tC# exists as four stereoisomers: The tC parent compound exists in two folded conformations (enantiomers) as predicted previously[2] and each of these enantiomers exists as two stereoisomers in which the oxygen atom is directed in either direction relative to the aromatic plane (tC#1 and tC#2, Figure S10). Here, tC#1 is the isomer proposed to cause a significant destabilization of the DNA helix while tC#2 should not cause any major destabilization according to the proposed mechanism of destabilization.

B3LYP 6-31G(d,p) calculations of the two isomers in Figure S8 predicts that tC#1 has a ground-state energy of 2 kJ/mol lower than tC#2. In addition to this, when positioned in B-DNA the folded isomer in which the tC framework is directed into the major groove was previously suggested to be the dominating species.[2, 3] In combination, these two predictions point towards that the tC#1 isomer is the dominating conformation of tC# in double-stranded DNA. Based on the calculated ground-state energies of tC#1 and tC#2, and the Boltzmann distribution at room temperature, the tC#1 and tC#2 stereoisomers exist in an approximate 70:30 ratio when in their monomeric forms.

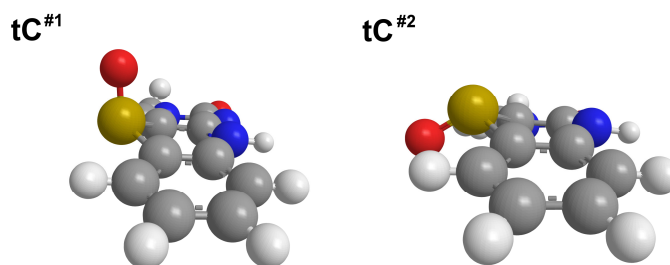**Fig. S10** Two predicted stereoisomers of tC#.

S9. Fast photoconversion of tC positioned in double-stranded DNA

Using a regular 150 W Xe lamp the photoconversion of tC positioned in double-stranded DNA required an irradiation time of up to 24 hours. To demonstrate the photoconversion can be achieved on more practical time-scales, an excitation volume of 60 µl was irradiated with 7 ns laser pulses of 420 nm at a repetition frequency of 10 Hz and an intensity of 2.1 mJ per pulse originating from a Q-switched Nd:YAG laser (Continuum Surelite II-10) and consecutive wavelength tuning with an OPO (Surelite). A high O_2 concentration was ensured in the DNA sample by bubbling O_2 (g) through the solution every 2-4 minutes. The resulting absorption spectra of tC at timescales from 0-12 minutes are shown in Figure S11.
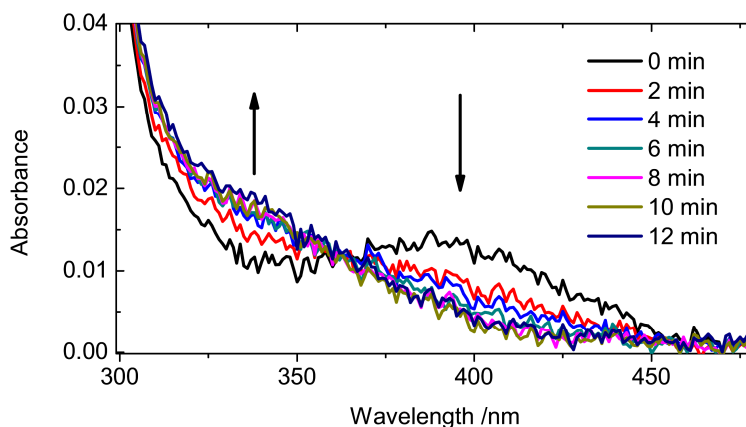

Fig. S11 Time-evolution of the absorption spectrum of tC positioned in double-stranded DNA irradiated with 7 ns laser pulses of 420 nm at a repetition frequency of 10 Hz and an intensity of 2.1 mJ per pulse.

References:

1.   P. Sandin, K. Börjesson, H. Li, J. Mårtensson, T. Brown, L. M. Wilhelmsson and B. Albinsson, *Nucleic Acids Res*, 2008, **36**, 157-167.
2.   S. Preus, K. Kilså, L. M. Wilhelmsson and B. Albinsson, *Physical chemistry chemical physics : PCCP*, 2010, **12**, 8881-8892.
3.   S. Preus, K. Kilså, F. A. Miannay, B. Albinsson and L. M. Wilhelmsson, *Nucleic Acids Res*, 2013, **41**, e18.