Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information for:

Direct Observation of Intramolecular Charge Transfer State in Epigenetic Nucleobase N6-methyladenine

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1. Method to estimate the ground state equilibrium

The relative energies (ΔE in eV) can be used to estimate the ground state contribution of trans and cis conformation at room temperature via the following equation:

$$P = e^{-\Delta E/k_B T} \Big/ Z \quad (1)$$

$$Z = \sum_n e^{-\Delta E/k_B T} \quad (2)$$

where *P* represents the percentage of a particular species. *Z* is the sum population of all possible species in the ground state. k_B and T represent the Boltzmann's constant in $eV \cdot K^{-1}$ and temperature(298 K) respectively.[1] Song et al. have reported that the cis-from of 6MeAde is more stable than the trans-form by 12.44 kJ/mol based on their calculations. [2] Using equation (1) and (2), we estimated that the cis-6MeAde is exist in more than 99.4% in PBS buffer solution at room temperature.

2. Method to calculate the fluorescence quantum yield

The fluorescence quantum yields of 6MeAde in PBS buffer solution, D_2O, acetonitrile and 6MeAdo in PBS buffer solution were measured using a comparing method. The equation below was used to calculate the fluorescence quantum yield.[3, 4]

$$\Phi = \Phi_R \frac{I\, A_R n^2}{I_R\, A\, n_R^2}$$

where Φ is the quantum yield, *I* is the measured fluorescence intensity, *A* represents the absorbance of the sample, and *n* represents the refractive index. Here, adenine was adopted as reference and its quantum yield is reported to be $2.6 \Diamond 10^{-4}$.[5]

3. Method to evaluate the fitting results

The corrected Akaike Information Criterion (AICc) is adopted to evaluate the fitting results.[6] Similar method has also been used before.[7] AICc is calculated according to the following equation:

$$AICc = NIn\left(\frac{SS}{N}\right) + \frac{2KN}{N - K - 1}$$

In which *N* is the number of data points for fitting, *SS* represents the sum of square residuals, and *K* is the number of independently adjusted fitting parameters plus one. Usually, the better fitting results would give a smaller AICc value.

4. Additional experimental results

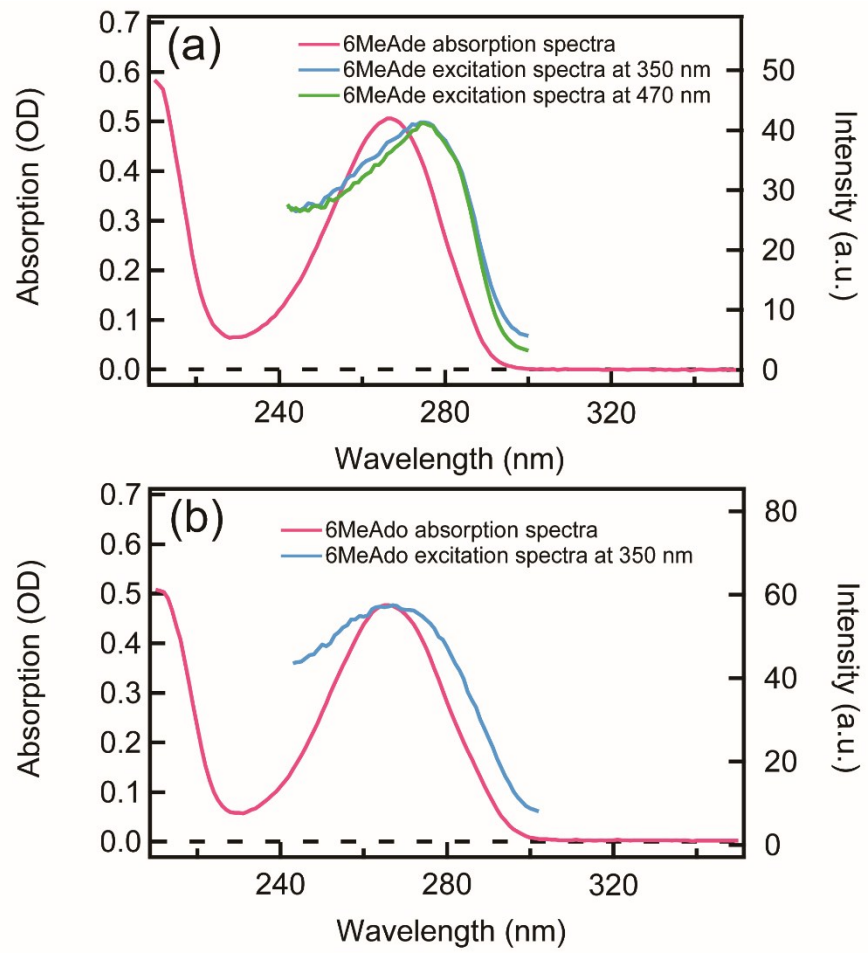

Figure S1. Steady state absorption and excitation spectra of 6MeAde (a) and 6MeAdo (b) in PBS buffer solution.

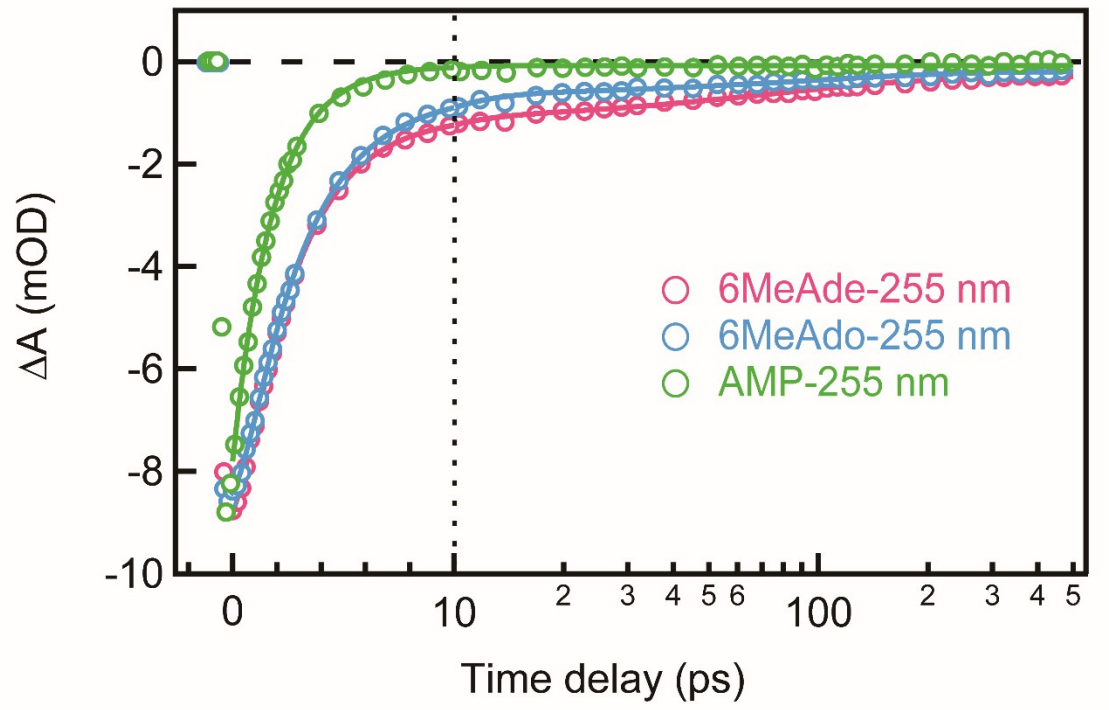

Figure S2. Transient absorption spectra at 255 nm for 6MeAde and 6MeAdo in PBS buffer solution.

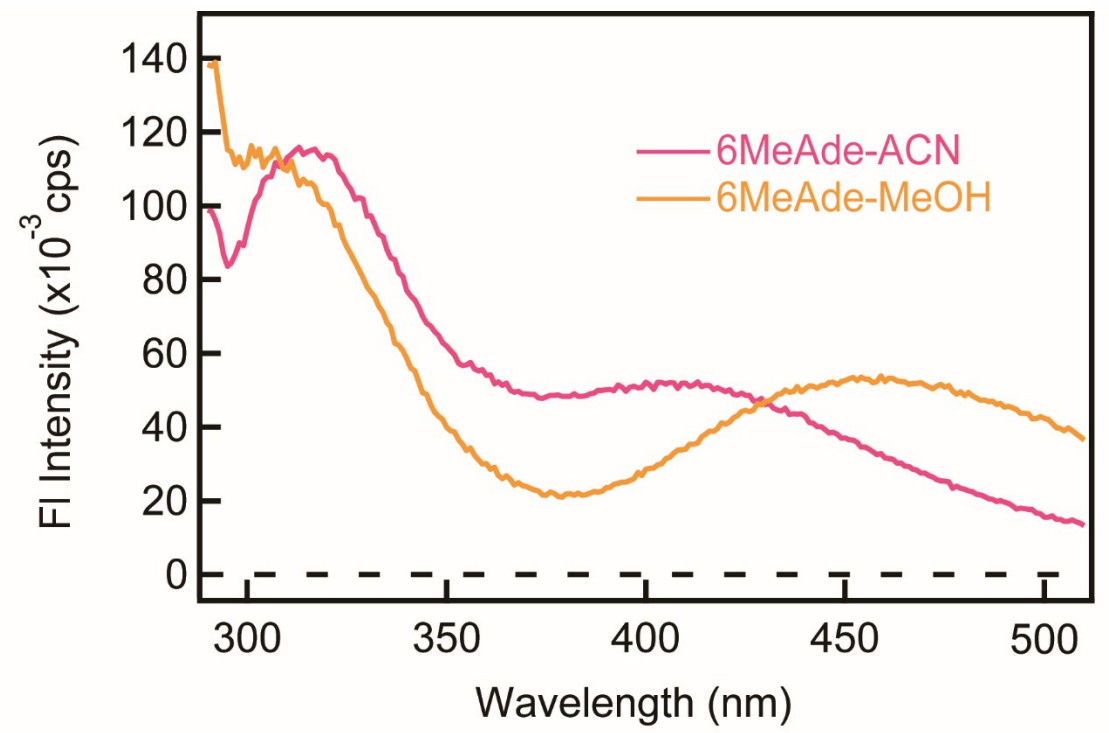

Figure S3. Steady state fluorescence spectra of 6MeAde in acetonitrile and methanol.

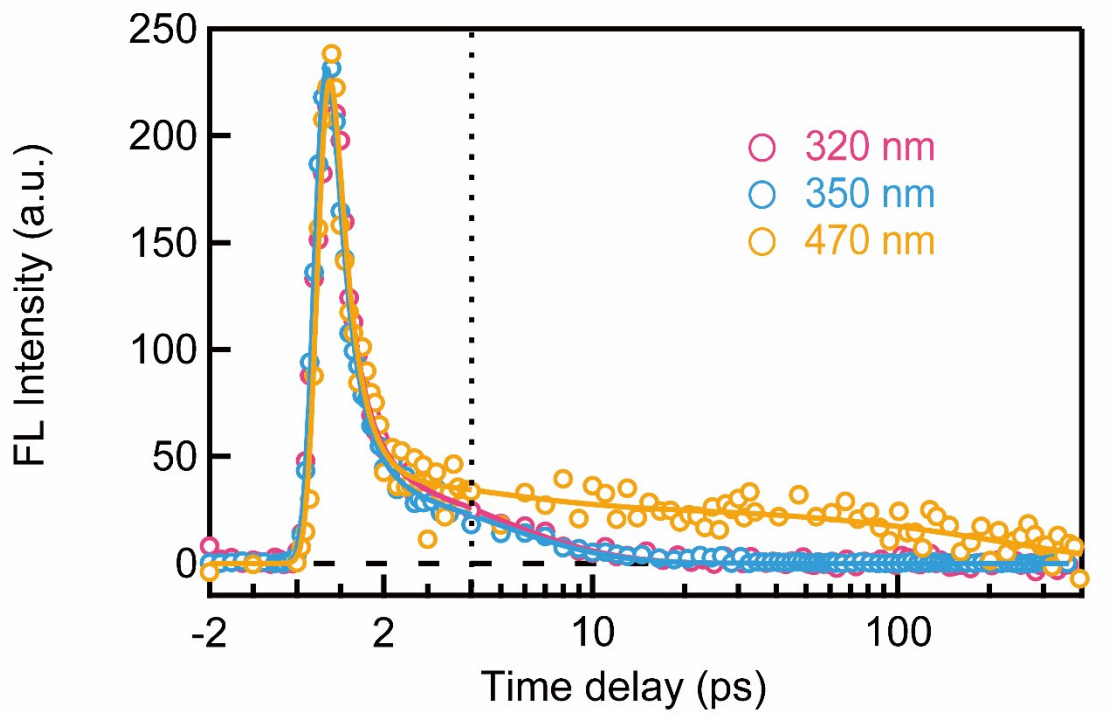

Figure S4. Fluorescence up-conversion spectra of 6MeAde in methanol.

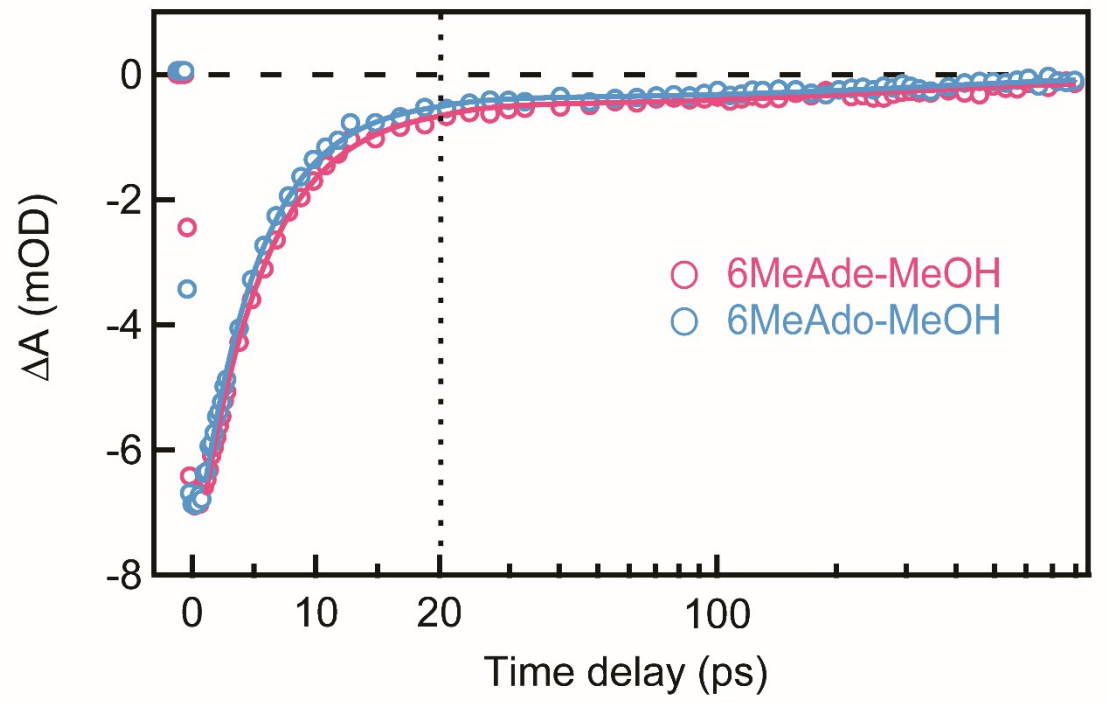

Figure S5. Transient absorption spectra at 255 nm for 6MeAde and 6MeAdo in methanol solution.

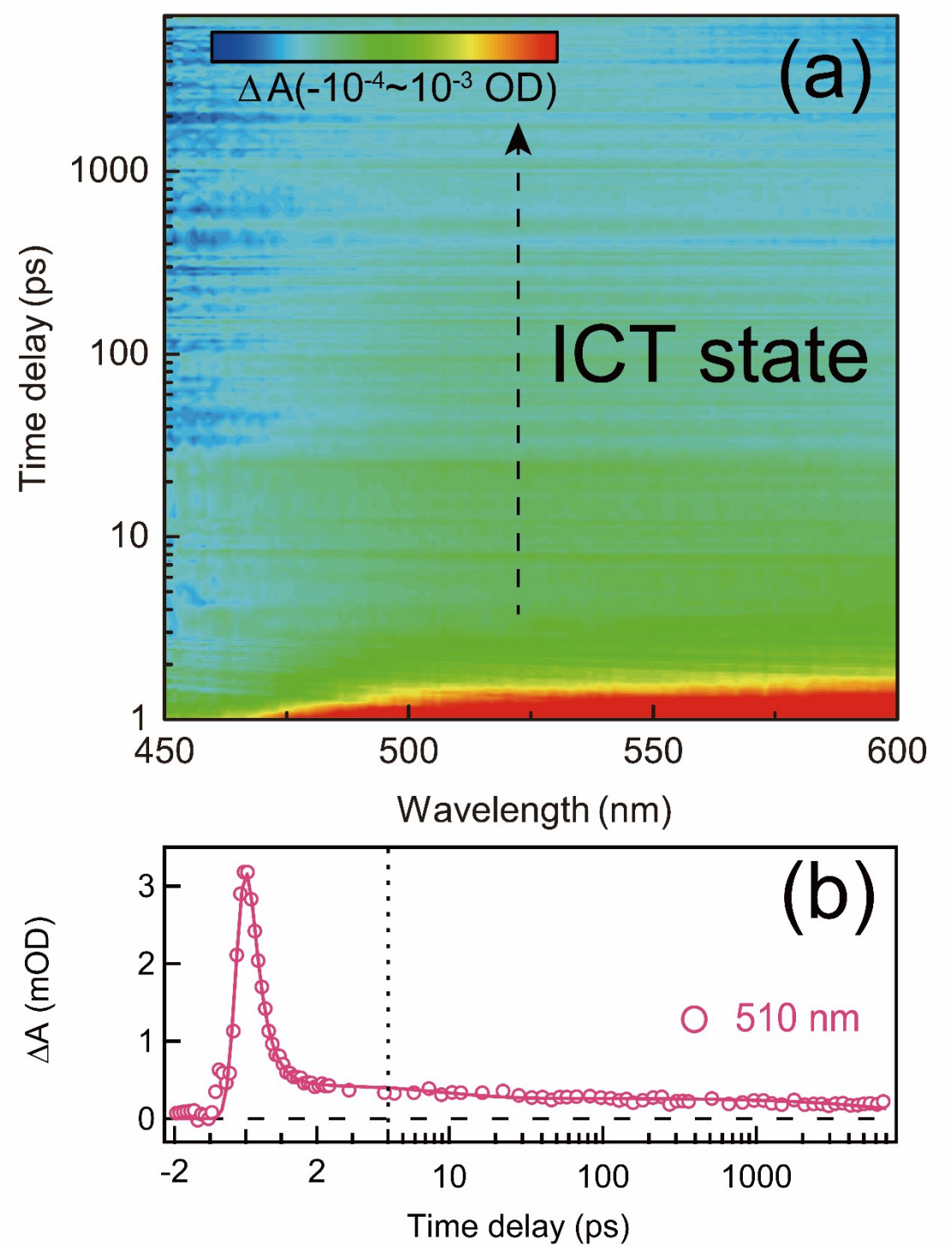

Figure S6. (a) Broadband transient absorption spectra of 6MeAde in acetonitrile. (b) Representative kinetic traces at 510 nm.

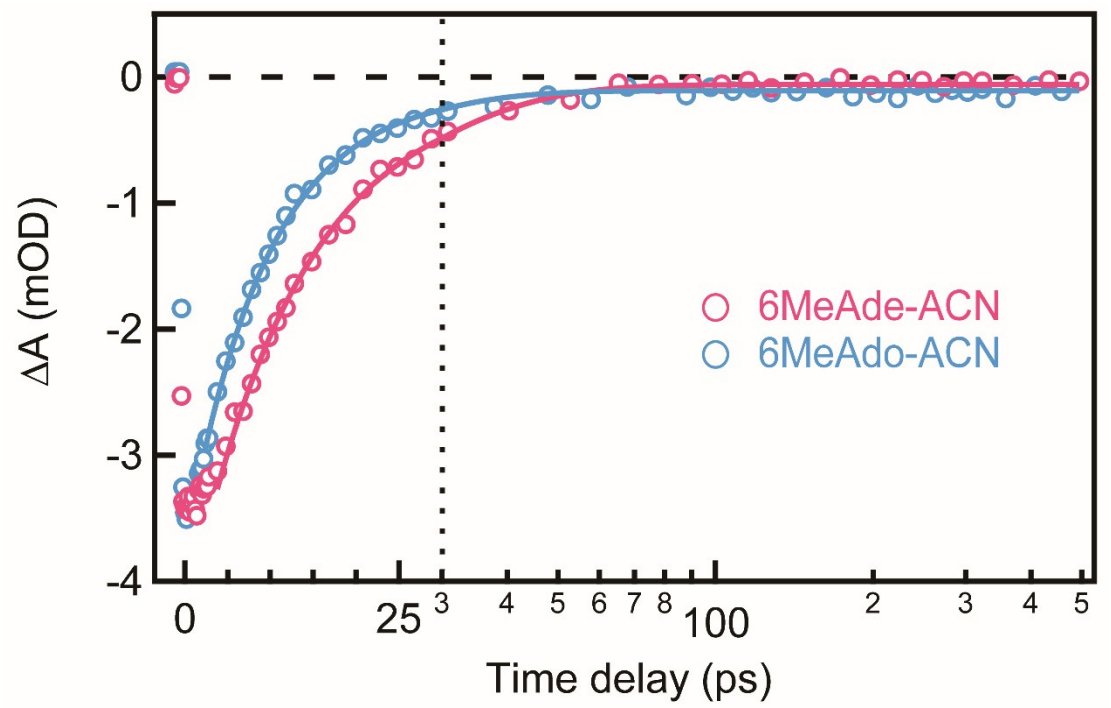

Figure S7. Transient absorption spectra at 255 nm for 6MeAde and 6MeAdo in acetonitrile solution.

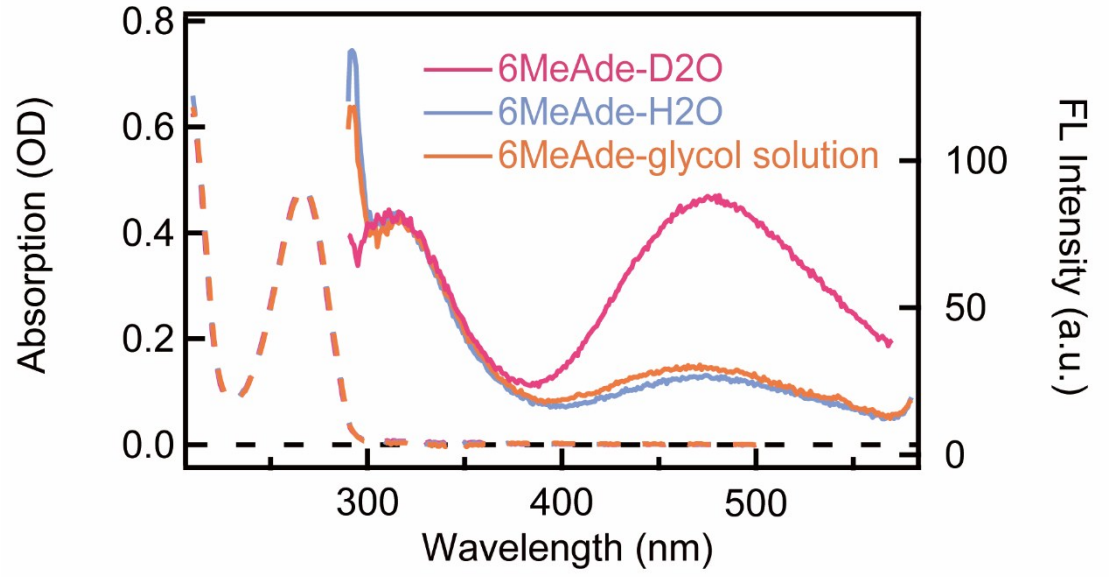

Figure S8. Steady state absorption (dash line) and emission spectra (solid line) of 6MeAde in D2O, H2O and glycol aqueous mixture solution.

Table S1. Fluorescence quantum yields of 6MeAde and 6MeAdo in PBS buffer, D_2O, acetonitrile and methanol solutions.

	Φ_{Total} (10^{-4})	Φ_{LE} (10^{-4})	Φ_{ICT} (10^{-4})
Adenine-PBS[a]	2.6		
6MeAde-PBS	1.5	0.6	0.9
6MeAde-D2O	2.8	0.8	2.0
6MeAde-ACN	3.1	0.9	2.2
6MeAde-MeOH	1.9	0.7	1.2
6MeAdo-PBS	1.3	0.8	0.5

[a] The fluorescence quantum yield of adenine was taken from reference 5.

Table S2. Best-fit parameters for transient absorption traces of 6MeAde and 6MeAdo.[a]

	λ (nm)	a_1 (%)	τ_1 (ps)	$\mathbf{a_2}$ (%)	$\boldsymbol{\tau_2}$ (ps)	$\mathbf{a_3}$ (%)	$\boldsymbol{\tau_3}$ (ps)	$\mathbf{a_4}$ (%)
6MeAde in PBS buffer	255	-	-	-94%	2.5 ± 0.1	-5%	75 ± 5	-1%
	510	77%	0.5 ± 0.1	1%	1.3 ± 0.9	13%	104 ± 22	9%
	680	85%		10%		-	-	5%
6MeAdo in PBS buffer	255	-	-	-96%	2.8 ± 0.1	-3%	93 ± 10	-1%
	510	65%	0.6 ± 0.2	31%	1.5 ± 0.6	-	-	4%
	680	57%		38%		-	-	5%
6MeAde in acetonitrile	255	-	-	-99%	13.0 ± 0.3	-	-	-1%
	510	93%	0.34 ± 0.04	3%	8.9 ± 6.8	-	-	4%
6MeAdo in acetonitrile	255	-	-	-96%	9.4 ± 0.2	-	-	-4%
6MeAde in methanol	255	-93%	5.3 ± 0.1	-	-	-5%	300 ± 100	-2%
6MeAdo in methanol	255	-95%	4.9 ± 0.1	-	-	-4%	387 ± 190	-1%
AMP in PBS	255	-99%	1.8 ± 0.1	-	-	-	-	-1%

[a] Amplitude percentage was calculated by $A_i = {A_i}/{\sum |A_n|}$

Table S3. Fit parameters for up-conversion traces of 6MeAde in methanol.

	λ (nm)	a_1 (%)	τ_1 (ps)	a_2 (%)	τ_2 (ps)	a_3 (%)	τ_3 (ps)
6MeAde in methanol	320	86%	0.41 ± 0.04	14%	4.0 ± 0.7	-	-
	350	88%		12%		-	-
	470	90%		4%		6%	222 ± 36

Reference

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