Supporting information

Excited States of Thio-2'-deoxyuridine Bearing an Extended π -Conjugated System: 3',5'-Di-O-acetyl-5-phenylethynyl-4-thio-2'deoxyuridine

Daiki Nonoshita[†], Wataru Kashihara[†], Kazuhito Tanabe[†], Tasuku Isozaki[‡], Yao–Zhong Xu[§], and Tadashi Suzuki^{†, *}

[†] Department of Chemistry and Biological Science, Aoyama Gakuin University, Fuchinobe, Chuo–ku, Sagamihara, Kanagawa 252–5258, Japan

[‡] Division of Natural Sciences, College of Arts and Sciences, J. F. Oberlin University, Tokiwa-machi, Machida, Tokyo 194–0294, Japan

§ School of Life, Health and Chemical Sciences, The Open University, Milton Keynes MK7 6AA, United Kingdom

*Corresponding author e-mail: <u>suzuki@chem.aoyama.ac.jp</u> (Tadashi Suzuki)

List of Contents

Figure S1. (a) ¹H NMR and (b) ¹³C NMR spectra of $3^{,5}$ -di–*O*–acetyl–5–iodo–4–thio–2'–deoxyuridine (5).

Figure S2. (a) ¹H NMR and (b) ¹³C NMR spectra of 3',5'-di-O-acetyl-5- phenylethynyl-4-thio-2'-deoxyuridine (1c).

Figure S3. High–resolution mass spectrum for **1c** (The main peak of **1c** with Na⁺ was successfully determined to be 451.0949).

Figure S4. HPLC chart for synthesized products, respectively. Purple: 5-iodo-2'- deoxyuridine as a starting molecule, red: 3',5'-di-O-acetyl-5-iodo-2'-deoxyuridine (4), yellow: 3',5'-di-O-acetyl-5-iodo-4-thio-2'-deoxyuridine (5) and 3',5'-di-O-acetyl-5-phenylethynyl-4-thio-2'-deoxyuridine (1c). Retention time of 1c is 8.21 min.

Figure S5. Molecular orbitals of 1a, 2a, and 3a (H=HOMO, L=LUMO).

Figure S6. Absorption spectrum of 2a in acetonitrile, and computational vertical transition energies and oscillator strengths of 2a (scaling factor: 0.93).

Figure S7. Absorption spectrum of 3a in acetonitrile, and computational vertical transition energies and oscillator strengths of 3a (scaling factor: 1.08).

Figure S8. Energy diagrams based on the vertical excitation energies for **1a** and **3a** shown in Tables 1 and S5.

Figure S9. Plots of the TRTL signal intensities for the U_T and U_S components for (a) **1c** ($A_{355}=0.404$) and (b) benzophenone ($A_{355}=0.394$) against incident laser power (I_L) at 355 nm irradiation.

Figure S10. (a) Time evolution of TRTL signals of benzophenone in Ar–saturated acetonitrile emerged by 355 nm laser excitation. (b) Plots of the TRTL signal intensities for U_T/I_L and U_S/I_L components against absorptance of benzophenone at 355 nm irradiation.

Figure S11. Time evolution of TRTL signals of (a) **1c** and (b) benzophenone in Arsaturated acetonitrile emerged by 308 nm laser excitation.

Figure S12. Plots of the TRTL signal intensities for the U_T and U_S components for (a) **1c** (A_{308} =0.222) and (b) benzophenone (A_{308} =0.205) against incident laser power (I_L) at 308 nm irradiation.

Figure S13. Plots of the TRTL signal intensities for U_T/I_L and U_S/I_L components against absorptance of (a) **1c** and (b) benzophenone at 308 nm irradiation.

Figure S14. Plots of the emission intensity maxima (I_S^0) immediately after laser irradiation of (a) **1c** and (b) phenalenone (PN) in acetonitrile against incident laser power (I_L) at 355 nm.

Figure S15. Plots of the emission intensity maxima (I_S^0) immediately after laser irradiation of (a) **1c** and (b) PN in acetonitrile against incident laser power (I_L) at 308 nm.

Figure S16. (a) Phosphorescence decay profiles of ${}^{1}O_{2}^{*}$ measured at around 1275 nm of **1c** and PN in oxygen–saturated acetonitrile. (b) Plots of the $I_{S}{}^{0}/I_{L}$ value of **1c** and PN against the absorptance $(1-10^{-A})$ at excitation wavelength (308 nm).

Figure S17. (a) Steady–state absorption spectrum of **1c** and fluorescence excitation spectrum of **1c**. (b) Fluorescence spectra excited at 355 nm and 308 nm.

Figure S18. Simulated two-photon absorption spectra of (a) 1a, (b) 2a, (c) 3a, (d) 2-thiouracil, (e) 2,4-dithiouracil, and (f) uracil.

 Table S1. Optimization of the reaction conditions for 1c.

Table S2. Transition orbitals of 1a.

Table S3. Spectroscopic properties for singlet and triplet excited states of 2a.

Table S4. Transition orbitals of 2a.

 Table S5. Spectroscopic properties for singlet and triplet excited states of 3a.

Table S6. Transition orbitals of **3a**.

A. Synthesis

NMR spectra were measured by a JEOL JNM–ECX 500 MHz spectrometer. The chemical shift (δ =7.26 ppm) in ¹H NMR and the chemical shift (δ =77.16 ppm) in ¹³C NMR were indicated as the corresponding values of the internal standard (CDCl₃).

Electrospray ionization mass spectrometry was performed by using Hitachi High– Technologies LIT–TOF mass spectrometer. Acetonitrile (HPLC–ACN) and pure water (HPLC–ACN/water=95:5) were used as a solvent to measure the accurate molecular weight for synthesized products.

Synthesis of 3',5'-di-O-acetyl-5-iodo-4-thio-2'-deoxyuridine (5). Lawesson's reagent (2.75 g, 6.81 mmol) was added to a solution of 3',5'-di-O-acetyl-5-iodo-2'deoxyuridine 4 (1.69 g, 3.86 mmol) in dry 1,4-dioxane (40 mL), and the mixture was kept for 4 h at 105 °C under N₂-saturated condition. The reaction was quenched by addition of water, and the whole mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate, and the solid was filtered off. After evaporation of the solvent, the crude product was purified by silica gel column 3',5'-di-O-acetyl-5-iodo-4-thio-2'chromatography $(CH_2Cl_2/MeOH=100:1),$ deoxyuridine 5 (1.37 g, 3.02 mmol, 78 %) was obtained as yellow solid. R_f=0.54 (CH₂Cl₂/MeOH=10:1): ¹H NMR (500 MHz, CDCl₃) : δ 10.65 (1H, s), 8.04 (1H, s), 6.21 (1H, dd, J=5.5, 8.0 Hz), 5.23–5.22 (1H, m), 4.41 (1H, q, J=14.5 Hz), 4.35 (2H, d, J=3.0 Hz), 2.58 (1H, ddd, J=2.5, 6.0, 14.5 Hz), 2.20 (1H, ddd, J=2.0, 8.5, 23.0 Hz), 2.11 (3H, s), 2.09 (3H, s). ¹³C NMR (125 MHz, CDCl₃): δ 188.3, 170.7, 170.5, 148.0, 139.9, 86.1, 83.2, 83.1, 74.2, 63.9, 38.6, 21.3, 21.0. HRMS (ESI): m/z [M+H]⁺ found 453.96 (calculated C₁₃H₁₅IN₂O₆S: 453.97).

Synthesis of 3',5'-di-O-acetyl-5-phenylethynyl-4-thio-2'-deoxyuridine (1c). A

mixture of 3',5'-di-O-acetyl-5-iodo-4-thio-2'-deoxyuridine 5 (403.8 mg, 0.89 mmol), Pd(pph₃)₄ (217.5 mg, 0.19 mmol), CuI (41.9 mg, 0.22 mmol), ethynylbenzene (0.40 mL, 3.64 mmol), and Et₃N (6 mL, 43.2 mmol) were added into 3 mL of dry DMF. The catalysts of Pd(pph₃)₄ and CuI were added portion-wise every hour and the total amount of the catalysts was 4 times as much as that for canonical nucleoside derivatives. After the reaction mixture was stirred at 40 °C for 4 h under N₂-saturated condition, the reaction was quenched by the addition of water, and the whole mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate, and the solid was filtered off. After evaporation of the solvent, the crude product was purified by silica gel column chromatography (CHCl₃/MeOH=100:1), 3',5'-di-O-acetyl-5-phenylethynyl-4-thio-2'-deoxyuridine 1c (85.8 mg, 0.20 mmol, 23 %) was obtained as brown solid. R_f=0.46 (CH₂Cl₂/MeOH=10:1): ¹H NMR (500 MHz, CDCl₃): δ 10.96 (1H, s), 8.47 (1H, s), 7.59 (2H, d, J=6.5 Hz), 7.54 (3H, ddd, J=2.0, 7.5, 7.5 Hz), 6.31 (1H, dd, J=5.5, 7.5 Hz), 5.26–5.24 (1H, m), 4.45 (1H, q, J=19.5 Hz), 4.45 (2H, d, J=3.0 Hz), 3.04 (1H, ddd, J=2.0, 5.5, 14.5 Hz), 2.13 (1H, ddd, J=5.0, 12.0, 27.0 Hz), 2.12 (3H, s), 2.06 (3H, s). ¹³C NMR (125 MHz, CDCl₃): δ 179.6, 170.6, 170.5, 152.4, 141.6, 132.3, 132.2, 132.1, 132.1, 128.7, 128.6, 126.3, 120.2, 113.6, 88.9, 83.7, 74.3, 63.8, 39.5, 21.0, 21.0. HRMS (ESI): $m/z [M+H]^+$ found 428.11 (calculated C₂₁H₂₀N₂O₆S: 428.10).

Entry	Phenylacetylene	Pd(pph3)4	CuI	Time	Temperature	Yield
	/ mol%	/ mol%	/ mol%	/ h	/ °C	/ %
1	4	0.1	0.2	4.5	40	0
2	7	0.3	0.3	4.5	40	4
3	7	0.3	0.3	4.5	70	0
4	7	0.3	0.3	15	40	0
5	10	0.4	0.4	4.0	40	23
6	10	0.5	0.5	3.0	40	15

 Table S1. Optimization of the reaction conditions for 1c.

B. Characterization



Figure S1. (a) ¹H NMR and (b) ¹³C NMR spectra of 3',5'-di-*O*-acetyl-5-iodo-4-thio-2'-deoxyuridine (**5**).



Figure S2. (a) ¹H NMR and (b) ¹³C NMR spectra of 3',5'-di-O-acetyl-5- phenylethynyl-4-thio-2'-deoxyuridine (1c).



Figure S3. High–resolution mass spectrum for **1c** (The main peak of **1c** with Na⁺ was successfully determined to be 451.0949).

Newly synthesized product **1c** was analyzed using a HPLC system with a UV–vis detector (Shimadzu, SPD–10A). Samples with the mobile phase consisting of a mixed solvent of acetonitrile/water (2/1v/v) passed through a column (Kanto Chemical; Mightysil, RP–18 GP 150–4.6 5µm) at 40°C and were quantified by the UV detection at 308 nm. The mobile phase was delivered at a flow–rate of 0.1 mL min⁻¹.



Figure S4. HPLC chart for synthesized products, respectively. Purple: 5–iodo–2'– deoxyuridine as a starting molecule, red: 3',5'–di–*O*–acetyl–5–iodo–2'–deoxyuridine (**4**), yellow: 3',5'–di–*O*–acetyl–5–iodo–4–thio–2'–deoxyuridine (**5**) and 3',5'–di–*O*–acetyl–5–phenylethynyl–4–thio–2'–deoxyuridine (**1c**). Retention time of **1c** is 8.21 min.

C. Quantum chemical calculations



Figure S5. Molecular orbitals of 1a, 2a, and 3a (H=HOMO, L=LUMO).

Transition from S ₀		Transition orbitals ^a	Composition / %		
$\overline{S_1}$	$n\pi^*$	H−1→L	82.0		
		$H-1 \rightarrow L+1$	18.0		
S_2	$\pi\pi^*$	H→L	85.6		
		$H-2\rightarrow L$	14.4		
S_3	$\pi\pi^*$	$H \rightarrow L+1$	100		
S_4	$n\pi^*$	$H-1 \rightarrow L+1$	81.5		
		$H-1 \rightarrow L$	18.5		
S 5	$\pi\pi^*$	$H-2\rightarrow L$	79.6		
		$H-2 \rightarrow L+1$	20.4		
S ₆	$n\pi^*$	H−3→L	70.5		
		$H \rightarrow L+2$	17.9		
		$H-3 \rightarrow L+1$	11.6		
\mathbf{S}_7	$n\pi^*$	$H-5 \rightarrow L+1$	85.0		
		$H-5\rightarrow L$	15.0		
S_8	$\pi\pi^*$	$H \rightarrow L+2$	41.5		
		$H-3\rightarrow L+1$	33.1		
		$H-3 \rightarrow L$	17.1		
		$H-3 \rightarrow L+3$	8.3		
S 9	$n\pi^*$	$H-2\rightarrow L+1$	80.5		
		H–2→L	19.5		
T_1	$\pi\pi^*$	H→L	65.5		
		$H-2\rightarrow L$	21.9		
		$H \rightarrow L+1$	12.6		
T_2	$\pi\pi^*$	$H-1 \rightarrow L$	78.0		
		$H-1 \rightarrow L+1$	22.0		
T_3	$n\pi^*$	$H \rightarrow L+1$	55.9		
		$H-2\rightarrow L$	32.3		
		$H-3\rightarrow L+2$	11.8		
T_4	$\pi\pi^*$	$H-2\rightarrow L$	32.1		
		$H-2\rightarrow L+1$	16.5		
		$H \rightarrow L+1$	16.0		
		H→L	15.0		
		H–4→L	13.5		
		$H-2 \rightarrow L+3$	6.9		

 Table S2. Transition orbitals of 1a

^aH=HOMO, L=LUMO.



Figure S6. Absorption spectrum of **2a** in acetonitrile, and computational vertical transition energies and oscillator strengths of **2a** (scaling factor: 0.93).

Transition from S ₀	$\lambda_{\max}{}^a$	ε ^b	$E_{\rm calc}$ ^{c, d}	f_{calc}^{e}
	/ nm (eV)	$/ 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	/ eV	
$S_1 = \pi \pi^*$	305 (4.07)	1.54	4.07	0.7435
S ₂ $\pi\pi^*$	263 (4.71)	1.12	4.77	0.3744
S ₃ $n\pi^*$			4.95	0.0003
S ₄ $n\pi^*$			5.16	0.0000
S ₅ $n\pi^*$			5.33	0.0000
$\mathrm{S}_6 \pi\pi^*$			5.43	0.0392
S ₇ $\pi\pi^*$			5.60	0.0369
$S_8 n\pi^*$			5.80	0.0003

Table S3. Spectroscopic properties for singlet and triplet excited states of 2a.

^a Wavelengths of maximum absorption band. ^b Molar absorption coefficient at the absorption peak. ^c The values were calculated by the TD–DFT method for the molecular configuration optimized in the S₀ state at the PCM/B3LYP/6–311+G (d,p) level for **2a**. ^d Values are scaled by 0.93. ^e Oscillator strength was obtained by the TD–DFT calculation for **2a**.

Transition from S ₀		Transition orbitals ^a	Composition / %
S 1	$\pi\pi^*$	H→L	100
S_2	$\pi\pi^*$	$H \rightarrow L+1$	100
S ₃	$n\pi^*$	H–1→L	43.3
		$H-1 \rightarrow L+1$	13.8
		$H \rightarrow L+2$	42.9
S4	$n\pi^*$	$H-4\rightarrow L+1$	17.5
		H−3→L	82.5
S 5	$n\pi^*$	H–4→L	64.3
		$H-4\rightarrow L+1$	13.2
		$H - 3 \rightarrow L + 1$	22.5
S ₆	$\pi\pi^*$	H–1→L	44.4
		$H-1 \rightarrow L+1$	16.0
		$H \rightarrow L+2$	39.6
S_7	$\pi\pi^*$	H−2→L	64.5
		$H \rightarrow L+3$	35.5
S_8	$n\pi^*$	$H \rightarrow L + 4$	100

Table S4. Transition orbitals of 2a.

^aH=HOMO, L=LUMO.



Figure S7. Absorption spectrum of **3a** in acetonitrile, and computational vertical transition energies and oscillator strengths of **3a** (scaling factor: 1.08).

Transition from S ₀		$\lambda_{\max}{}^a$	ε^{b}	$E_{\rm calc}$ ^{c, d}	f_{calc}^{e}
		/ nm (eV)	$/ 10^4 \text{ M}^{-1} \text{ cm}^{-1}$	/ eV	
$S_1 n$	π^*			2.82	0.0000
$S_2 = \pi$	π^*	330 (3.76)	1.67	3.79	0.4228
S ₃ π	π^*			4.49	0.0152
S ₄ n	π^*			4.50	0.0004
S5 π	π^*			4.74	0.0775
S ₆ n	π^*			4.99	0.0010
S ₇ <i>n</i>	π^*			5.16	0.0715
$S_8 \pi$	π^*			5.26	0.0070
S_9 n	π^*			5.68	0.0443
$T_1 = \pi$	π^*			2.76	
$T_2 \pi$	π^*			3.01	
T_3 n	$2\pi^*$			4.12	
$T_4 \pi$	$\pi\pi^*$			4.85	

Table S5. Spectroscopic properties for singlet and triplet excited states of 3a.

^a Wavelengths of maximum absorption band. ^b Molar absorption coefficient at the absorption peak. ^c The values were calculated by the TD–DFT method for the molecular configuration optimized in the S₀ state at the PCM/B3LYP/6–311+G(d,p) level for **3a**. ^d Values are scaled by 1.08. ^e Oscillator strength was obtained by the TD–DFT calculation for **3a**.

Transition from S ₀		Transition orbitals ^a	Composition / %	
S 1	$n\pi^*$	H→L	100	
S_2	$\pi\pi^*$	H–1→L	100	
S ₃	$\pi\pi^*$	H–2→L	100	
S 4	$n\pi^*$	$H \rightarrow L+1$	100	
S5	$\pi\pi^*$	$H-1 \rightarrow L+1$	100	
S ₆	$n\pi^*$	$H - 3 \rightarrow L + 1$	100	
S 7	$\pi\pi^*$	$H \rightarrow L+2$	78.2	
		$H \rightarrow L+3$	11.8	
S_8	$n\pi^*$	$H-1\rightarrow L+2$	100	
S 9	$\pi\pi^*$	$H \rightarrow L+2$	13.9	
		$H \rightarrow L+3$	49.5	
		$H \rightarrow L+4$	13.0	
		$H\rightarrow L+5$	23.6	
T_1	$\pi\pi^*$	H–1→L	100	
T_2	$n\pi^*$	H→L	100	
T_3	$\pi\pi^*$	H–2→L	73.1	
		$H-1\rightarrow L+1$	26.9	
T_4	$\pi\pi^*$	H–2→L	23.7	
		$H-2\rightarrow L+1$	23.9	
		$H \rightarrow L+1$	52.4	

Table S6. Transition orbitals of 3a.

a H = HOMO, L = LUMO.



Figure S8. Energy diagrams based on the vertical excitation energies for **1a** and **3a** shown in Tables 1 and S5.



Figure S9. Plots of the TRTL signal intensities for the $U_{\rm T}$ and $U_{\rm S}$ components for (a) 1c ($A_{355}=0.404$) and (b) benzophenone ($A_{355}=0.394$) against incident laser power ($I_{\rm L}$) at 355 nm irradiation.



Figure S10. (a) Time evolution of TRTL signals of benzophenone in Ar–saturated acetonitrile emerged by 355 nm laser excitation. (b) Plots of the TRTL signal intensities for $U_{\rm T}/I_{\rm L}$ and $U_{\rm S}/I_{\rm L}$ components against absorptance of benzophenone at 355 nm irradiation.



Figure S11. Time evolution of TRTL signals of (a) **1c** and (b) benzophenone in Ar-saturated acetonitrile emerged by 308 nm laser excitation.



Figure S12. Plots of the TRTL signal intensities for the $U_{\rm T}$ and $U_{\rm S}$ components for (a) **1c** (A_{308} =0.222) and (b) benzophenone (A_{308} =0.205) against incident laser power ($I_{\rm L}$) at 308 nm irradiation.



Figure S13. Plots of the TRTL signal intensities for U_T/I_L and U_S/I_L components against absorptance of (a) **1c** and (b) benzophenone at 308 nm irradiation.



E. Time-resolved near-infrared emission measurements

Figure S14. Plots of the emission intensity maxima (I_S^0) immediately after laser irradiation of (a) **1c** and (b) phenalenone (PN) in acetonitrile against incident laser power (I_L) at 355 nm.



Figure S15. Plots of the emission intensity maxima (I_S^0) immediately after laser irradiation of (a) **1c** and (b) PN in acetonitrile against incident laser power (I_L) at 308 nm.



Figure S16. (a) Phosphorescence decay profiles of ${}^{1}O_{2}^{*}$ measured at around 1275 nm of **1c** and PN in oxygen–saturated acetonitrile. (b) Plots of the $I_{S}{}^{0}/I_{L}$ value of **1c** and PN against the absorptance (1–10^{-A}) at excitation wavelength (308 nm).

F. Comparison of fluorescence spectra excited at 355 nm and 308 nm, and absorption and fluorescence excitation spectra



Figure S17. (a) Steady–state absorption spectrum of **1c** and fluorescence excitation spectrum of **1c**. (b) Fluorescence spectra excited at 355 nm and 308 nm.

G. Two-photon absorption property



Figure S18. Simulated two–photon absorption spectra of (a) **1a**, (b) **2a**, (c) **3a**, (d) 2– thiouracil, (e) 2,4–dithiouracil, and (f) uracil.