

## Supplementary Information

### **Spectroscopic studies of the color modulation mechanism of firefly (beetle) bioluminescence with amino-analogs of luciferin and oxyluciferin**

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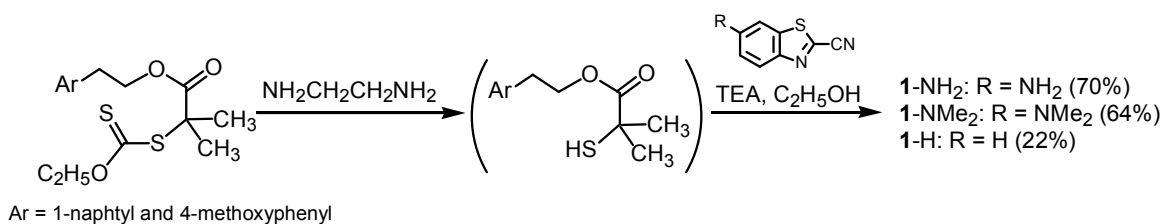
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## 1. General methods

Melting points were obtained with a Yamato MP-21 apparatus. IR spectra were measured with a Horiba FT-720 spectrometer. Electron ionization (EI) mass spectra and high-resolution electro-spray ionization (ESI) mass spectra were recorded with a JEOL JMS-600 and JEOL JMS-T100LC mass spectrometers, respectively.  $^1\text{H}$  NMR spectra were recorded on a JEOL ECA-500 (500 MHz) instrument. UV/visible absorption spectra were measured with a Varian Cary 50 spectrophotometer (scan speed, 600 nm/min; data interval, 1 nm). Fluorescence spectra were measured with a JASCO FP-6500 fluorescence spectrophotometer (excitation and emission bandpasses, 3 and 10 nm, respectively; scan speed, 500 nm/min) and were corrected according to manufacturer's instructions. Fluorescence quantum yields were determined relative to quinine sulfate in 0.10 M  $\text{H}_2\text{SO}_4$  ( $\Phi_{\text{F}} = 0.55$ ,  $\lambda_{\text{ex}} 366$  nm) as the standard.<sup>1</sup> Bioluminescence intensities were measured using an ATTO AB-2200 luminometer, and bioluminescence spectra were measured with an ATTO AB-1850 spectrophotometer (data interval, 1 nm). Spectroscopic measurements were made in a quartz cuvette (1 cm path length) at  $25 \pm 1$  °C. Spectral-grade solvents were used for measurements of UV/visible absorption and fluorescence. Quantum chemical calculations were performed on the Gaussian 09 program.<sup>2</sup> We used density functional theory (DFT): Beck's three-parameter functional combined with Lee, Yang and Parr's correlation functional (B3LYP) along with 6-31+G(d) basis set.<sup>3-5</sup> Molecular graphics were made using GaussView, Version 5.<sup>6</sup>

## 2. Syntheses of luciferin and oxyluciferin analogs

**2.1 Preparations of 5,5-dimethyloxyluciferin analogs.** Oxyluciferin analogs **1-NH<sub>2</sub>**, **1-NMe<sub>2</sub>**, and **1-H** were prepared by the procedure shown in Scheme S1.



**Scheme S1** Syntheses of **1-NH<sub>2</sub>**, **1-NMe<sub>2</sub>**, and **1-H**.

**Synthesis of 1-NH<sub>2</sub>.** *O*-Ethyl *S*-[2-[2-(1-naphtyl)ethoxycarbonyl]-2-propyl]dithiocarbonate<sup>7</sup> (145 mg, 0.40 mmol) was dissolved in ethylenediamine (2.2 mL) under Ar, and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated NH<sub>4</sub>Cl aqueous solution and 2-(1-naphtyl)ethyl 2-mercaptoisobutyrate was extracted with ether (20 mL × 3). The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue containing mercaptoisobutyrate (137 mg) was dissolved in ethanol (2 mL) under Ar and 6-amino-2-cyanobenzothiazol<sup>8,9</sup> (50 mg, 0.29 mmol) and triethylamine (60 μL, 0.43 mmol) was added to the solution. After heating at reflux for 6 h, the mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by TLC (SiO<sub>2</sub>, CHCl<sub>3</sub>/hexane/ethyl acetate) twice, to give **1-NH<sub>2</sub>** (55 mg, 70%) as red powder: mp. 214–216 °C. IR (KBr): 3438, 3452, 3230, 1707, 1637, 1608, 1543 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ (ppm): 1.77 (6 H, s), 4.19 (2 H, br s), 6.93 (1 H, dd, *J* = 2.3, 9.2 Hz), 7.13 (1 H, d, *J* = 2.2 Hz), 7.98 (1H, d, *J* = 9.2 Hz); MS EI *m/z* (%) 277 (M<sup>+</sup>, 100), 193 (54), 175 (49); HRMS ESI: calcd for C<sub>12</sub>H<sub>12</sub>N<sub>3</sub>OS<sub>2</sub> [M+H]<sup>+</sup>, 278.0422; found, 278.0433.

**1-NMe<sub>2</sub>.** Preparation with *O*-ethyl *S*-[2-[2-(1-naphtyl)ethoxycarbonyl]-2-propyl]dithiocarbonate<sup>7</sup> and 2-cyano-6-(dimethylamino)benzothiazol<sup>8,9</sup> with the similar procedure for **1-NH<sub>2</sub>** gave **1-NMe<sub>2</sub>** (64% yield) as red powder: mp. 195–198 °C. IR (KBr): 2970, 1925, 2860, 2808, 1716, 1608, 1545, 1508 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ (ppm): 1.73 (6 H, s), 3.13 (6 H, s), 7.05 (1 H, dd, *J* = 2.3, 8.1 Hz), 7.06 (1 H, brs), 8.02 (1H, dd, *J* = 2.3, 8.0 Hz); MS EI *m/z* (%) 305 (M<sup>+</sup>, 100), 221 (58), 203 (35); HRMS ESI: calcd for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>OS<sub>2</sub> [M+H]<sup>+</sup>, 306.0735; found, 306.0721.

**1-H.** Preparation with *O*-ethyl *S*-[2-[2-(4-methoxyphenyl)ethoxycarbonyl]-2-propyl] dithiocarbonate<sup>7</sup> and 2-cyanobenzothiazol<sup>10</sup> with the similar procedure for **1-NH<sub>2</sub>** gave **1-H** (22% yield) as yellow powder: mp. 148–149 °C. IR (KBr): 2970, 2927, 2866, 1722, 1521 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz) δ (ppm): 1.76 (6 H, s), 7.59 (1 H, dt, *J* = 1.2, 7.5 Hz), 7.63 (1 H, dt, *J* = 1.2, 7.5 Hz), 8.04 (1 H, dd, *J* = 1.2, 6.9 Hz), 8.24 (1H, dd, *J* = 1.2, 7.4 Hz); MS EI *m/z* (%) 262 (M<sup>+</sup>, 100), 247 (32), 178 (44); HRMS ESI: calcd for C<sub>12</sub>H<sub>11</sub>N<sub>2</sub>OS<sub>2</sub> [M+H]<sup>+</sup>, 263.0313; found, 263.0349.

## 2.2 Syntheses of aminoluciferin (ALH) and 5,5-dimethylaminoluciferin (Me<sub>2</sub>ALH).

Aminoluciferin (ALH) was prepared by the reported procedure.<sup>8,9</sup>

**Synthesis of Me<sub>2</sub>ALH.** To a solution of 6-amino-2-cyanobenzothiazol<sup>8,9</sup> (15 mg, 87 μmol) in methanol (3 mL) was added a solution of D-penicillamine (42 mg, 0.27 mmol) in 50 mM sodium phosphate buffer (pH 8, 3 mL) under Ar, and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with 50 mM sodium phosphate buffer (pH 8, 40 mL) and washed with ethyl acetate (30 mL × 3). The aqueous layer was acidified to pH 4 by adding 1 M HCl and the product was extracted with ethyl acetate (50 mL × 3). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was washed with hexane, to give Me<sub>2</sub>ALH (15 mg, 60%) as red powder: mp. 134–136 °C. IR (KBr): 3361, 3230, 2970, 2927, 2871, 1724, 1577 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz) δ (ppm): 1.43 (3 H, s), 1.71 (3 H, s), 4.93 (1 H, s), 5.83 (2 H, br s), 6.85 (1 H, dd, *J* = 2.3, 8.6 Hz), 7.07 (1 H, d, *J* = 2.3 Hz), 7.75 (1H, d, *J* = 8.5 Hz); MS EI *m/z* (%) 307 (M<sup>+</sup>, 10), 263 (100), 248 (85), 189 (85); HRMS ESI: calcd for C<sub>13</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub> [M+H]<sup>+</sup>, 308.0527; found, 308.0535.

## 3. <sup>1</sup>H-NMR Spectra

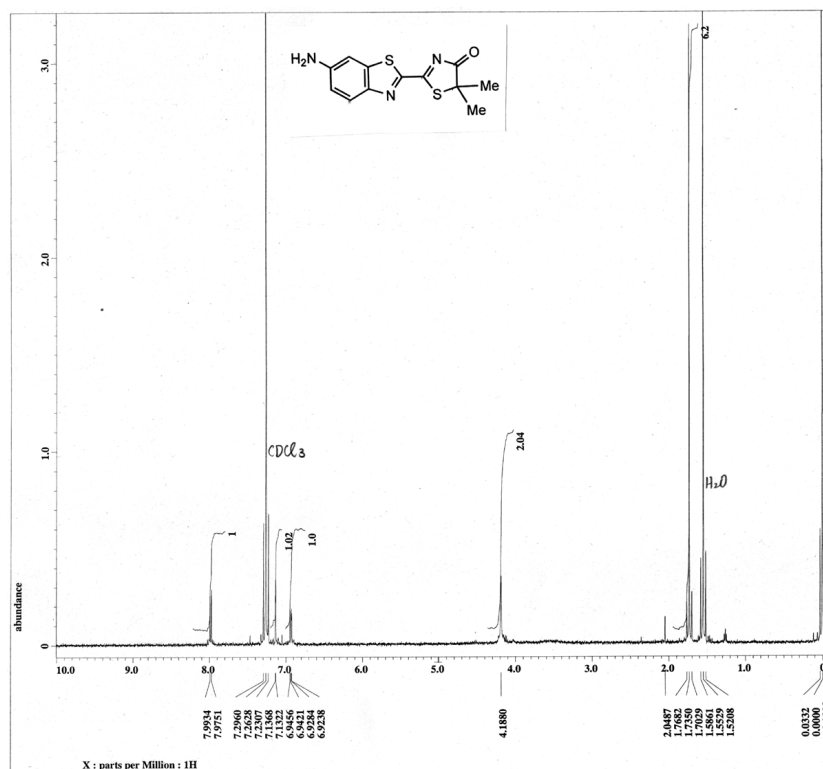


Fig. S1 <sup>1</sup>H NMR spectrum of 1-NH<sub>2</sub> in CDCl<sub>3</sub>.

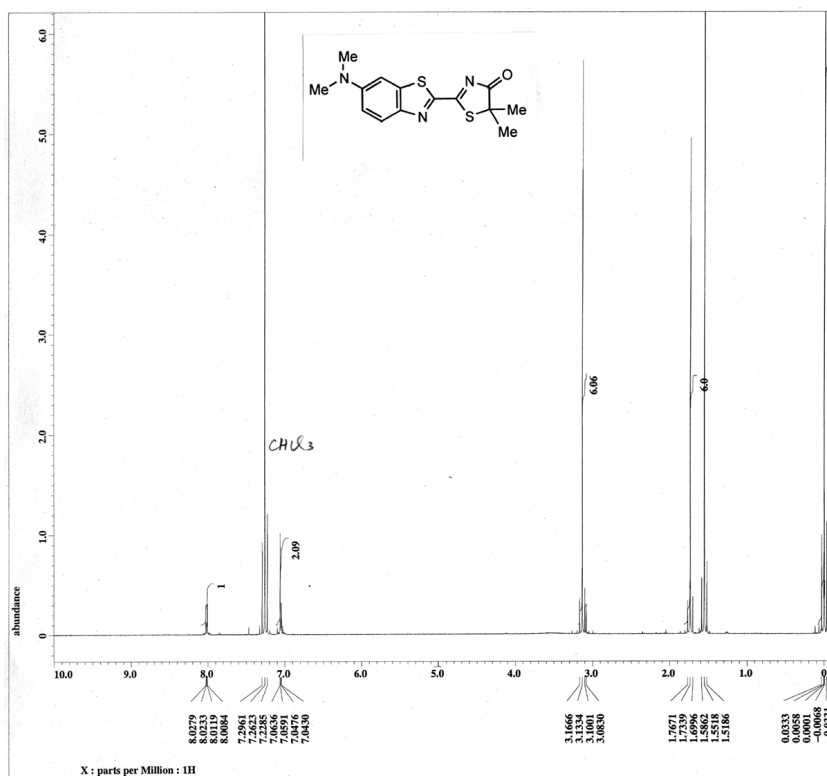


Fig. S2 <sup>1</sup>H NMR spectrum of 1-NMe<sub>2</sub> in CDCl<sub>3</sub>.

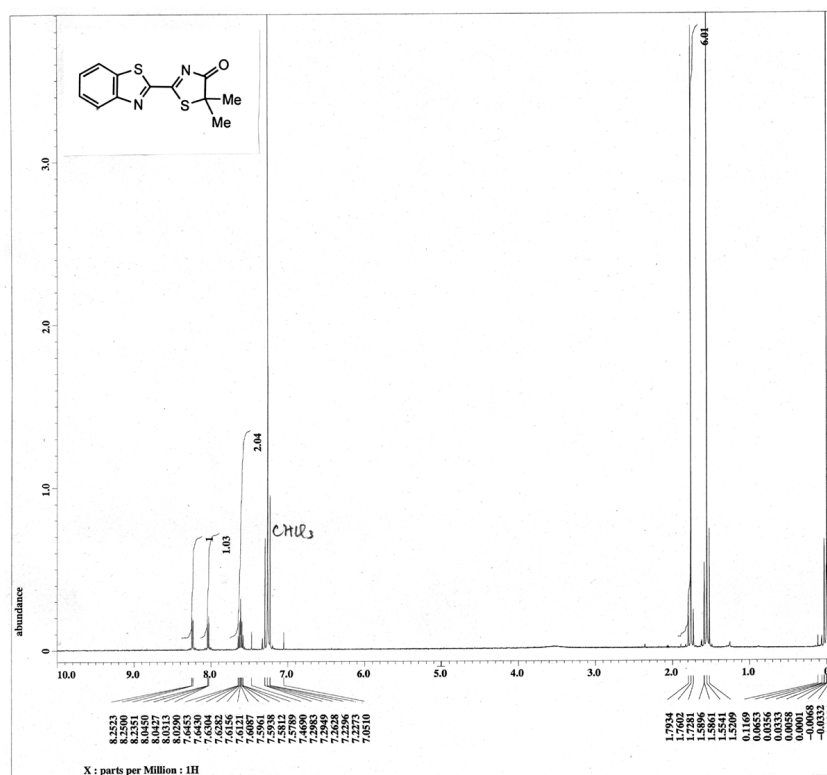
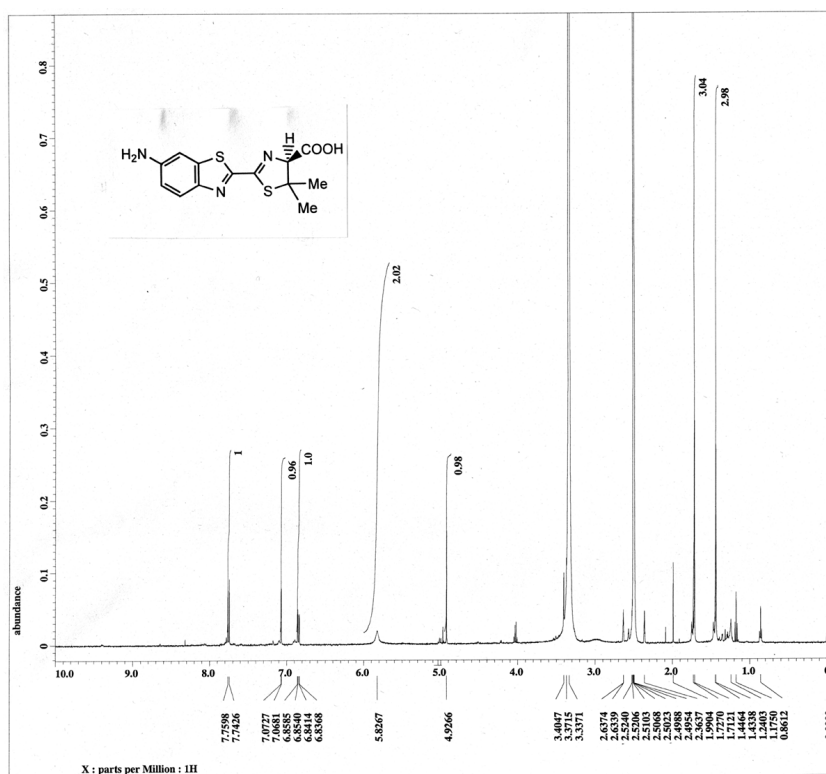


Fig. S3 <sup>1</sup>H NMR spectrum of 1-H in CDCl<sub>3</sub>.



**Fig. S4** <sup>1</sup>H NMR spectrum of Me<sub>2</sub>ALH in DMSO-*d*<sub>6</sub>.

#### 4. Measurements of bioluminescence emission intensities and spectra

Intensities of light emission by L-L reactions of LH<sub>2</sub>, ALH, and Me<sub>2</sub>ALH with a recombinant *Photinus pyralis* (Ppy) luciferase (QuantiLum recombinant luciferase, Promega, USA) and ATP magnesium salt (Mg·ATP) (Sigma, USA) were measured with the AB-2200 luminometer. The assays were performed by adding 40 μL of a Mg·ATP aqueous solution to a mixture (60 μL) of luciferin (and its analogs) and Ppy luciferase in potassium phosphate buffer (0.1 M, pH 5.0 and 8.0) at room temperature. Final concentrations were as followed: 80 μM Mg·ATP, 20 μM luciferin (and its analogs), and 30 nM Ppy luciferase. All assays were measured in triplicate. Relative intensities for bioluminescence of LH<sub>2</sub>, ALH, and Me<sub>2</sub>ALH were shown below.

	relative intensity of bioluminescence		
	LH <sub>2</sub>	ALH	Me <sub>2</sub> ALH
pH 8.0	1.0	0.13	0.0010
pH 5.0	1.0	0.90	0.0030

Bioluminescence spectra were measured with the AB-1850 spectrophotometer. Spectra for the L-L reactions of LH<sub>2</sub> and ALH with Ppy luciferase were measured in potassium phosphate buffer (0.1 M, pH 5.0 and 8.0) containing 80 μM Mg·ATP, 20 μM LH<sub>2</sub> and ALH, and 0.3 μM Ppy luciferase at room temperature. To measure bioluminescence spectra for Me<sub>2</sub>ALH, the concentrations of Me<sub>2</sub>ALH (100 μM) and Ppy (3 μM) were increased.

## 5. Spectroscopic data

**Table S1** Electronic absorption of **1** in various solvents at 25 °C

solvent [ <i>E</i> <sub>T</sub> (30) <sup>a</sup> ]	$\lambda_{\text{ab}} / \text{nm} (\epsilon / 10^4)^{\text{b}}$					
	1-NMe <sub>2</sub>	1-NH <sub>2</sub>	1-H	1-OH <sup>c</sup>	1-O <sup>-c,d</sup>	1-OMe <sup>c</sup>
<i>p</i> -xylene [33.1]	464 (2.59)	415 (2.01)	326 (1.69)	366 (1.85)	ca. 500	368 (2.17)
benzene [34.3]	468 (2.64)	417 (2.03)	328 (1.84)	366 (1.95)	ca. 500	371 (2.24)
chloroform [39.1]	483 (2.63)	422 (2.14)	330 (1.90)	370 (2.04)	503	380 (2.12)
DMSO [45.1]	491 (2.42)	468 (2.39)	331 (1.74)	391 <sup>e</sup>	579	380 (2.11)
acetonitrile [45.6]	475 (2.50)	433 (2.19)	325 (1.88)	372 (2.01)	565	373 (2.14)
2-propanol [48.4]	479 (2.50)	464 (2.24)	327 (1.85)	391 <sup>e</sup>	527	380 (2.26)
methanol [55.4]	485 (2.46)	453 (2.23)	328 (1.86)	388 (1.93)	484	378 (2.11)
water [63.1]	513 <sup>d</sup>	436 <sup>d</sup>	326 <sup>d</sup>	--- <sup>f</sup>	484	380 <sup>d</sup>

<sup>a</sup> *E*<sub>T</sub>(30) in kcal mol<sup>-1</sup>.

<sup>b</sup> Extinction coefficient in dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>.

<sup>c</sup> Ref 7.

<sup>d</sup> Initial concentrations of 1-OH and 1,1,3,3-tetramethylguanidine were 5.0 × 10<sup>-6</sup> and 0.010 mol dm<sup>-3</sup>, respectively.

<sup>e</sup> The  $\epsilon$  value was not determined accurately because of decomposition of the substrate.

<sup>f</sup> Generation of 1-O<sup>-</sup> was observed at the same time.

<sup>g</sup> Only generation of 1-O<sup>-</sup> was observed.

**Table S2** Fluorescence of **1** in various solvents at 25 °C.

solvent [ <i>E</i> <sub>T</sub> (30) <sup>a</sup> ]	$\lambda_{\text{fl}} / \text{nm} (\Phi_{\text{F}})$				
	1-NMe <sub>2</sub>	1-NH <sub>2</sub>	1-OH <sup>b</sup>	1-O <sup>-b,c</sup>	1-OMe <sup>b</sup>
<i>p</i> -xylene [33.1]	535 (0.85)	494 (0.37)	ca. 420 <sup>d</sup>	563 (0.25)	weak <sup>d</sup>
benzene [34.3]	541 (0.90)	495 (0.48)	ca. 420 <sup>d</sup>	570 (0.25)	440 <sup>d</sup>
chloroform [39.1]	567 (0.77)	511 (0.78)	454 (0.001)	601 (0.53)	454 (0.002)
DMSO [45.1]	640 (0.31)	596 (0.76)	522 (0.07)	631 (0.51)	499 (0.008)
acetonitrile [45.6]	621 (0.43)	567 (0.78)	479 (0.03)	624 (0.22)	478 (0.02)
2-propanol [48.4]	634 (0.38)	596 (0.65)	504 (0.11)	624 (0.27)	487 (0.02)
methanol [55.4]	656 (0.11)	610 (0.36)	526 (0.19)	630 (0.10)	505 (0.12)
water [63.1]	690 <sup>e</sup>	634 <sup>e</sup>	--- <sup>f</sup>	637 <sup>e</sup>	535 <sup>e</sup>

<sup>a</sup> *E*<sub>T</sub>(30) in kcal mol<sup>-1</sup>.

<sup>b</sup> Ref 7.

<sup>c</sup> Initial concentrations of 1-OH and 1,1,3,3-tetramethylguanidine were 5.0 × 10<sup>-6</sup> and 0.010 mol dm<sup>-3</sup>, respectively.

<sup>d</sup> Quantum yield  $\Phi_{\text{F}}$  was lower than 0.001.

<sup>e</sup> The  $\Phi_{\text{F}}$  value was not determined accurately because of decomposition of the substrate.

<sup>f</sup> Only generation of 1-O<sup>-</sup> was observed.

## 6. DFT Calculation

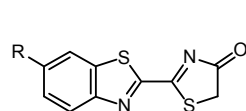
**Table S3** Dipole moment ( $\mu$ ), HOMO and LUMO levels, the allowed transitions to the excited singlet states with the lowest excitation energies, their vertical excitation energies ( $E_{\text{ex}}$ ), excitation wavelengths ( $\lambda_{\text{ex}}$ ), oscillator strengths ( $f$ ) and configurations predicted for oxyluciferin analogs **1** with DFT and TDDFT using B3LYP/6-31+G(d)

compd ( $\sigma_p$ )	$\mu$ /D	HOMO /eV	LUMO /eV	transition	$E_{\text{ex}}$ / eV	$\lambda_{\text{ex}}$ / nm	$f$	configuration <sup>a</sup>
1-NMe <sub>2</sub> (-0.83)	7.8	-5.75	-2.70	S <sub>0</sub> → S <sub>1</sub>	2.83	438	0.55	H → L (0.64)
1-NH <sub>2</sub> (-0.66)	6.5	-6.13	-2.80	S <sub>0</sub> → S <sub>1</sub>	3.09	401	0.49	H → L (0.63), H-1 → L (-0.13)
1-H (0.00)	4.1	-7.00	-3.05	S <sub>0</sub> → S <sub>3</sub>	3.65	340	0.14	H-1 → L (-0.26), H-2 → L (0.61)
1-OH <sup>b</sup> (-0.37)	3.6	-6.65	-2.96	S <sub>0</sub> → S <sub>2</sub>	3.37	367	0.39	H → L (0.66), H-1 → L (0.24)
1-OMe <sup>b</sup> (-0.27)	4.5	-6.52	-2.90	S <sub>0</sub> → S <sub>1</sub>	3.32	373	0.42	H → L (0.63), H-1 → L (0.16) H-2 → L (-0.25)

<sup>a</sup> Configuration of excitation. H, H-*n* and L denote the HOMO, HOMO-*n* and LUMO, respectively.

<sup>b</sup> They have the conformations of the OH and OMe groups more stable than those reported in Ref 7.

**Table S4** Dipole moment ( $\mu$ ), HOMO and LUMO levels, the allowed transitions to the excited singlet states with the lowest excitation energies, their vertical excitation energies ( $E_{\text{ex}}$ ), excitation wavelengths ( $\lambda_{\text{ex}}$ ), oscillator strengths ( $f$ ) and configurations predicted for OLH and its analogs with DFT and TDDFT using B3LYP/6-31+G(d)



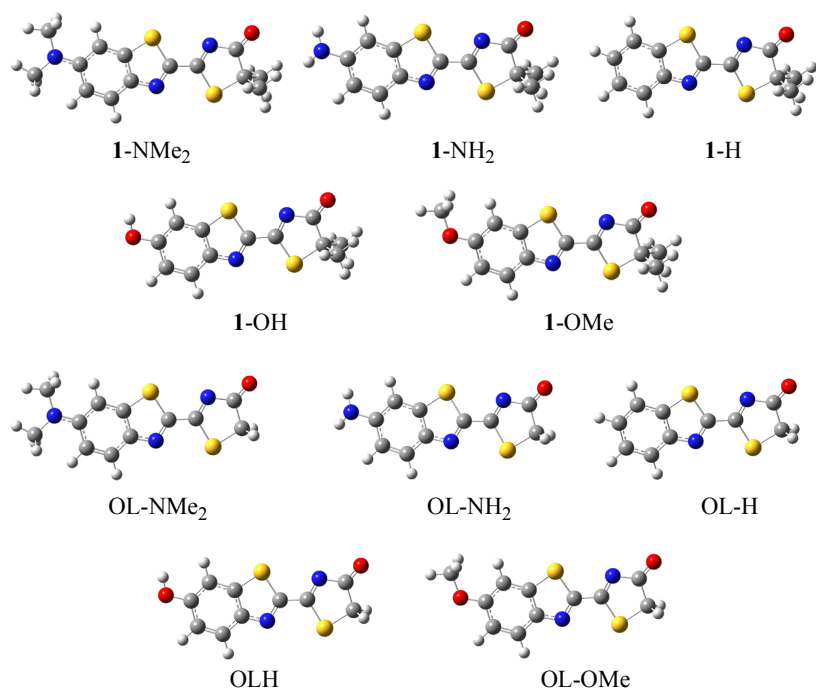
OLH: R = OH  
 OL-OMe: R = OMe  
 OL-NMe<sub>2</sub>: R = NMe<sub>2</sub>  
 OL-NH<sub>2</sub>: R = NH<sub>2</sub>  
 OL-H: R = H

compd	$\mu$ /D	HOMO /eV	LUMO /eV	transition	$E_{\text{ex}}$ / eV	$\lambda_{\text{ex}}$ / nm	$f$	configuration <sup>a</sup>
OL-NMe <sub>2</sub>	8.6	-5.80	-2.76	S <sub>0</sub> → S <sub>1</sub>	2.82	439	0.53	H → L (0.70)
OL-NH <sub>2</sub>	7.2	-6.19	-2.87	S <sub>0</sub> → S <sub>1</sub>	3.08	403	0.46	H → L (0.69), H-1 → L (-0.12)
OL-H	4.7	-7.07	-3.14	S <sub>0</sub> → S <sub>3</sub>	3.67	338	0.13	H-1 → L (0.30), H-2 → L (0.63)
OLH <sup>b</sup>	4.3	-6.71	-3.04	S <sub>0</sub> → S <sub>1</sub>	3.37	368	0.36	H → L (0.66), H-1 → L (0.23)
OL-OMe <sup>b</sup>	5.2	-6.58	-2.98	S <sub>0</sub> → S <sub>1</sub>	3.31	374	0.45	H → L (0.68), H-1 → L (0.17)

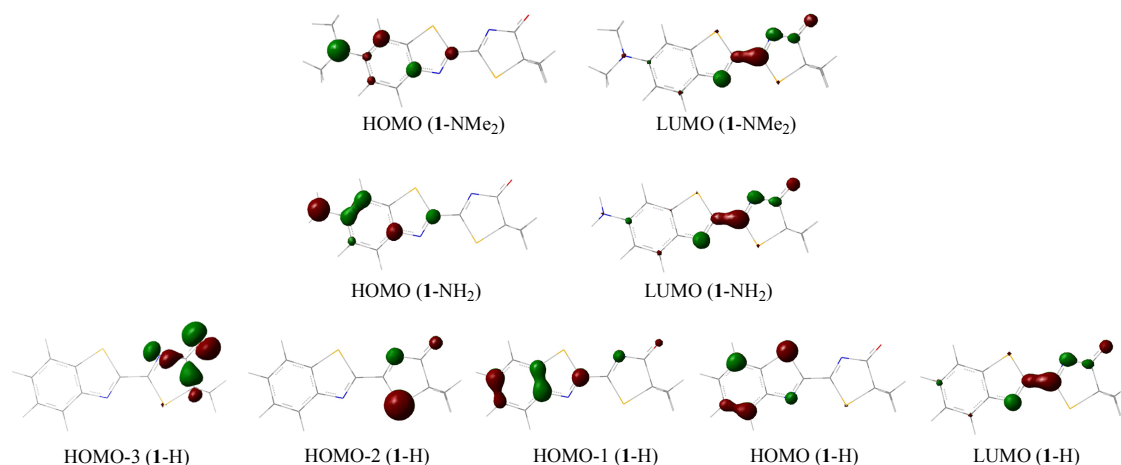
<sup>a</sup> Configuration of excitation. H, H-*n* and L denote the HOMO, HOMO-*n* and LUMO, respectively.

<sup>b</sup> They have the conformations of the OH and OMe groups more stable than those reported in Ref 11.





**Fig. S5** Optimized structures of oxyluciferin (OLH) and its analogs.



**Fig. S6** Frontier orbitals of 1-NMe<sub>2</sub>, 1-NH<sub>2</sub>, and 1-H.

## 6.1 Geometries of oxyluciferin analogs in gas phase optimized with DFT using B3LYP/6-31+G(d)

### 6.1.1 Cartesian Coordinates (in Å) of 1-NMe<sub>2</sub> (R = NMe<sub>2</sub>)

	x	y	z
H	-4.903159	-1.413766	-1.698493
H	-5.455282	0.232345	-1.300806
H	-3.972666	-0.004722	-2.251320

H	-4.953502	-2.028085	0.797228
H	-5.507557	-0.380338	1.185576
H	-4.060073	-1.043215	1.975453
H	2.975768	-2.701940	-0.469216
H	5.319075	-2.023096	-0.214532
H	4.080872	2.024456	0.758526
O	-4.164129	1.900013	0.449887
C	-4.573040	-0.404689	-1.428052
C	-4.624098	-1.011290	1.037854
S	-2.194611	-1.357397	-0.303769
C	-3.782398	-0.424714	-0.108734
C	-3.343266	1.023637	0.258847
N	-1.959436	1.210960	0.347173
C	-1.303483	0.117397	0.092727
N	0.848170	-0.996266	-0.118702
C	0.146779	0.076219	0.127708
S	1.076203	1.536598	0.526083
C	2.535447	0.591699	0.337722
C	2.190082	-0.743444	-0.010085
C	3.224636	-1.678088	-0.206217
C	4.540928	-1.285949	-0.060525
C	4.895058	0.058504	0.285799
C	3.859585	0.999621	0.488646
N	6.220204	0.421478	0.415146
C	6.563342	1.775084	0.823259
H	6.168053	2.016511	1.821440
H	6.176254	2.517391	0.112160
C	7.275571	-0.573447	0.286336
H	7.186867	-1.369943	1.039711
H	8.241998	-0.085783	0.423099
H	7.273326	-1.038042	-0.708675
H	7.649208	1.876702	0.854069

### 6.1.2 Cartesian Coordinates (in Å) of 1-NH<sub>2</sub> (R = NH<sub>2</sub>)

	x	y	z
H	-4.983146	-1.734886	-1.283368
H	-5.510906	-0.033901	-1.280857
H	-4.051910	-0.512513	-2.175021
H	-4.982518	-1.735792	1.286558
H	-5.510157	-0.034775	1.285594
H	-4.050714	-0.514115	2.178617
H	2.905648	-2.806788	-0.000535
H	5.287599	-2.099638	-0.009741
H	4.115650	2.058960	-0.007421
O	-4.162658	1.984943	0.003579
C	-4.637551	-0.695380	-1.268497
C	-4.636854	-0.696299	1.272267
S	-2.243174	-1.387373	0.000835

C	-3.817384	-0.411582	0.001782
C	-3.356243	1.075702	0.002121
N	-1.967691	1.257912	0.000635
C	-1.330237	0.125517	-0.000061
N	0.808479	-1.034444	-0.001022
C	0.122078	0.074128	-0.001602
S	1.069367	1.575619	-0.004944
C	2.515622	0.596523	-0.004996
C	2.156059	-0.780581	-0.002966
C	3.174059	-1.754698	-0.001831
C	4.496371	-1.353807	-0.002096
C	4.852240	0.023977	-0.001190
C	3.849910	1.005081	-0.002018
N	6.193965	0.383527	-0.056602
H	6.427504	1.325158	0.231484
H	6.863442	-0.303629	0.265259

### 6.1.3 Cartesian Coordinates (in Å) of 1-H (R = H)

	x	y	z
H	4.570211	-1.802167	1.285305
H	5.134501	-0.113089	1.283602
H	3.665496	-0.560349	2.177494
H	4.570390	-1.802352	-1.284825
H	5.134705	-0.113281	-1.283263
H	3.665831	-0.560639	-2.177320
H	-3.315870	-2.721379	-0.000098
H	-5.699392	-1.965543	-0.000145
H	-4.458036	2.166367	-0.000095
O	3.826749	1.936354	0.000292
C	4.247158	-0.755559	1.271010
C	4.247351	-0.755736	-1.270721
S	1.839059	-1.396012	0.000006
C	3.434319	-0.453531	0.000061
C	3.005132	1.042544	-0.000087
N	1.617204	1.253232	0.000006
C	0.961487	0.134057	-0.000018
N	-1.200303	-0.983410	-0.000049
C	-0.498634	0.113452	-0.000032
S	-1.407733	1.630300	-0.000027
C	-2.873854	0.680674	-0.000070
C	-2.548913	-0.703688	-0.000070
C	-3.574805	-1.667022	-0.000097
C	-4.894237	-1.236288	-0.000123
C	-5.206662	0.140254	-0.000123
C	-4.207241	1.109700	-0.000097
H	-6.247955	0.451084	-0.000143

### 6.1.4 Cartesian Coordinates (in Å) of 1-OH (R = OH)

	x	y	z
H	-4.976610	-1.733509	-1.284889
H	-5.503058	-0.032260	-1.283401
H	-4.044322	-0.512320	-2.177118
H	-4.976504	-1.733509	1.285054
H	-5.502953	-0.032260	1.283608
H	-4.044144	-0.512317	2.177205
H	2.912999	-2.807240	-0.000047
H	5.315032	-2.081028	0.000032
H	4.126469	2.061199	0.000049
H	6.311424	1.272432	0.000104
O	6.175420	0.311569	0.000088
O	-4.151170	1.987022	0.000038
C	-4.630219	-0.694347	-1.270607
C	-4.630115	-0.694346	1.270743
S	-2.236853	-1.388323	-0.000032
C	-3.810581	-0.410483	0.000034
C	-3.347977	1.075900	0.000013
N	-1.957290	1.255970	-0.000024
C	-1.324145	0.122411	-0.000046
N	0.817407	-1.035803	-0.000069
C	0.131122	0.071058	-0.000055
S	1.073233	1.573943	-0.000066
C	2.519433	0.597522	-0.000012
C	2.167078	-0.779623	-0.000042
C	3.183657	-1.755940	-0.000027
C	4.507263	-1.356302	0.000019
C	4.841235	0.018487	0.000050
C	3.857377	1.007492	0.000033

### 6.1.5 Cartesian Coordinates (in Å) of 1-OMe (R = OMe)

	x	y	z
H	4.297985	-4.582412	1.646835
H	4.243697	-4.565269	3.426919
H	2.781933	-4.955597	2.494738
H	5.222627	-2.184330	1.651562
H	5.171121	-2.174132	3.431875
H	4.352628	-0.894626	2.509846
H	-1.891283	-0.857175	-1.930140
H	-4.235684	0.028130	-2.016152
H	-4.422166	0.112777	2.294701
O	-5.717163	0.594403	-0.069648
O	2.905604	-2.672321	4.900476
C	3.680900	-4.331441	2.516473
C	4.597428	-1.961442	2.523054
S	2.237953	-2.408589	1.087105
C	3.329983	-2.833344	2.521875
C	2.471101	-2.505596	3.778311

N	1.187864	-2.015889	3.498406
C	0.971657	-1.930378	2.220681
N	-0.569894	-1.348350	0.428449
C	-0.298762	-1.447947	1.699319
S	-1.589219	-0.957332	2.811237
C	-2.571900	-0.589319	1.416666
C	-1.841314	-0.868605	0.232026
C	-2.447403	-0.643786	-1.022490
C	-3.737576	-0.156574	-1.069686
C	-4.457170	0.119121	0.122887
C	-3.882662	-0.093992	1.378314
C	-6.519193	0.899982	1.066526
H	-7.469587	1.258378	0.668642
H	-6.691687	0.006874	1.680521
H	-6.056989	1.686185	1.677033

### 6.1.6 Cartesian Coordinates (in Å) of OL-NMe<sub>2</sub> (R = NMe<sub>2</sub>)

	x	y	z
H	2.965100	-2.698904	-0.452545
H	5.310575	-2.026349	-0.202004
H	4.085344	2.025045	0.772915
O	-4.186147	1.889167	0.441888
S	-2.187084	-1.359868	-0.300706
C	-3.742083	-0.423498	-0.124808
C	-3.355140	1.024863	0.254858
N	-1.969538	1.216004	0.354128
C	-1.303215	0.130272	0.105822
N	0.843229	-0.986583	-0.098994
C	0.145359	0.088778	0.147046
S	1.079081	1.545357	0.545592
C	2.535300	0.595873	0.357174
C	2.185448	-0.738522	0.009748
C	3.217224	-1.676105	-0.188648
C	4.534549	-1.287540	-0.045326
C	4.893014	0.055838	0.301959
C	3.860502	1.000379	0.505420
N	6.218435	0.414112	0.432305
C	6.567505	1.773469	0.815790
H	6.163555	2.035529	1.804733
H	6.193267	2.505751	0.086955
C	7.273460	-0.575713	0.265971
H	7.190641	-1.391541	0.998370
H	8.240593	-0.091595	0.409669
H	7.263634	-1.013803	-0.741350
H	7.653605	1.867393	0.857889
H	-4.371880	-0.853459	0.658945
H	-4.304364	-0.419625	-1.062715

### 6.1.7 Cartesian Coordinates (in Å) of OL-NH<sub>2</sub> (R = NH<sub>2</sub>)

	x	y	z
H	2.892942	-2.802160	-0.000624
H	5.277531	-2.104881	-0.008646
H	4.123059	2.058928	-0.006991
O	-4.184217	1.974789	0.003420
S	-2.235307	-1.387448	0.000964
C	-3.777266	-0.413198	0.002037
C	-3.367665	1.077858	0.001980
N	-1.976796	1.266160	0.000322
C	-1.329011	0.142495	-0.000171
N	0.803503	-1.020556	-0.001609
C	0.121871	0.091410	-0.001989
S	1.074978	1.588138	-0.005904
C	2.517169	0.603083	-0.005408
C	2.151668	-0.772710	-0.003468
C	3.165712	-1.751225	-0.001871
C	4.489401	-1.355817	-0.001456
C	4.851106	0.020729	-0.000364
C	3.852889	1.006224	-0.001693
N	6.193688	0.374336	-0.054765
H	6.432436	1.315567	0.230131
H	6.861530	-0.315841	0.263843
H	-4.377368	-0.629867	-0.886043
H	-4.376405	-0.630080	0.890701

### 6.1.8 Cartesian Coordinates (in Å) of OL-H (R = H)

	x	y	z
H	-3.300880	-2.719026	-0.000081
H	-5.688195	-1.974762	-0.000159
H	-4.467683	2.163243	-0.000123
O	3.847812	1.926857	0.000164
S	1.830742	-1.394695	0.000086
C	3.393439	-0.453388	0.000049
C	3.016461	1.045446	0.000063
N	1.626100	1.262249	0.000081
C	0.960353	0.151924	-0.000001
N	-1.194265	-0.969891	-0.000021
C	-0.498489	0.131087	-0.000019
S	-1.414691	1.642354	-0.000004
C	-2.875964	0.685325	-0.000073
C	-2.544091	-0.697333	-0.000059
C	-3.565171	-1.666012	-0.000090
C	-4.886440	-1.241827	-0.000135
C	-5.205736	0.133367	-0.000149
C	-4.211452	1.107932	-0.000118
H	-6.248447	0.439128	-0.000181
H	3.988127	-0.684049	-0.888200

H	3.988127	-0.684057	0.888298
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### 6.1.9 Cartesian Coordinates (in Å) of OLH (R = OH)

	x	y	z
H	-2.464702	2.764331	0.000009
H	-4.826214	1.914672	0.000111
H	-3.425740	-2.160926	0.000031
H	-5.650522	-1.483994	0.000134
O	-5.562072	-0.517546	0.000135
O	4.863463	-1.613409	0.000327
S	2.734358	1.637755	-0.000074
C	4.327728	0.749579	0.000248
C	4.001198	-0.761501	-0.000263
N	2.620588	-1.025497	-0.000128
C	1.915288	0.061512	-0.000078
N	-0.281152	1.102223	-0.000084
C	0.460826	0.031552	-0.000082
S	-0.402420	-1.516890	-0.000134
C	-1.896793	-0.616033	-0.000026
C	-1.615477	0.777411	-0.000036
C	-2.680940	1.700553	0.000015
C	-3.981720	1.233089	0.000073
C	-4.244886	-0.157530	0.000085
C	-3.211797	-1.094767	0.000034
H	4.913930	0.999520	0.888898
H	4.914515	0.999947	-0.887893

### 6.1.10 Cartesian Coordinates (in Å) of OL-OMe (R = OMe)

	x	y	z
H	-1.883250	-0.856894	-1.927326
H	-4.225738	0.033233	-2.012857
H	-4.415905	0.107535	2.298183
O	-5.707418	0.596975	-0.066161
O	2.951745	-2.690014	4.870392
S	2.235994	-2.420810	1.060103
C	3.308360	-2.828952	2.478063
C	2.499326	-2.519393	3.758580
N	1.210394	-2.029445	3.489368
C	0.976113	-1.940955	2.217657
N	-0.565096	-1.355890	0.431537
C	-0.295079	-1.457788	1.702837
S	-1.584757	-0.967402	2.814218
C	-2.566221	-0.595759	1.419831
C	-1.835155	-0.873886	0.234989
C	-2.439818	-0.644831	-1.019662
C	-3.728771	-0.155031	-1.066516
C	-4.448988	0.119022	0.126329
C	-3.875984	-0.098164	1.381832

C	-6.510810	0.900911	1.069814
H	-7.459839	1.262096	0.671310
H	-6.685757	0.006288	1.680816
H	-6.048031	1.684583	1.683042
H	4.220701	-2.226308	2.464197
H	3.586898	-3.886346	2.465332

## 6.2 TDDFT calculations for excitations to the first three singlet-excited states of oxyluciferin and its analogs using B3LYP/6-31+G(d)

The TDDFT calculations of **1-H**, **1-OH**, and **OL-H** showed that the  $S_0 \rightarrow S_1$  transition of **1-OH** and the  $S_0 \rightarrow S_1$  and  $S_0 \rightarrow S_2$  transitions of **1-H** and **OL-H** are forbidden, whose oscillator strengths ( $f$ ) are below 0.006. The  $S_0 \rightarrow S_1$  transitions of **1-H**, **1-OH**, and **OL-H** are of a  $\sigma-\pi^*$  type. While the  $S_0 \rightarrow S_2$  transitions of **1-H** and **OL-H** are of a  $\pi-\pi^*$  type, the nodes of the occupied and unoccupied orbitals related to the transition are perpendicular to each other. Therefore, we predict that these singlet states of **1-H**, **1-OH**, and **OL-H** don't participate in their photophysics.

### 6.2.1 Excitation of **1-NMe<sub>2</sub>** (**R = NMe<sub>2</sub>**)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	2.8321 eV	437.79 nm	f=0.5530
80 -> 81		0.64087			
Excited State	2:	Singlet-A	3.3498 eV	370.12 nm	f=0.0004
77 -> 81		0.68606			
77 -> 84		0.10284			
Excited State	3:	Singlet-A	3.5715 eV	347.14 nm	f=0.1196
78 -> 81		-0.13491			
79 -> 81		0.64960			

### 6.2.2 Excitation of **1-NH<sub>2</sub>** (**R = NH<sub>2</sub>**)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.0894 eV	401.33 nm	f=0.4874
71 -> 73		-0.12564			
72 -> 73		0.63611			
Excited State	2:	Singlet-A	3.3375 eV	371.49 nm	f=0.0004
69 -> 73		0.68643			
Excited State	3:	Singlet-A	3.6005 eV	344.36 nm	f=0.1392
70 -> 73		-0.15291			
71 -> 73		0.63867			



### 6.2.3 Excitation of 1-H (R = H)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.2865 eV	377.26 nm	f=0.0004
65 -> 69		0.68589			
65 -> 71		-0.10015			
Excited State	2:	Singlet-A	3.4263 eV	361.87 nm	f=0.0047
68 -> 69		0.66707			
Excited State	3:	Singlet-A	3.6467 eV	339.99 nm	f=0.1407
66 -> 69		0.61268			
67 -> 69		-0.25563			

### 6.2.4 Excitation of 1-OH (R = OH)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.3140 eV	374.12 nm	f=0.0004
69 -> 73		0.69454			
69 -> 76		-0.10721			
Excited State	2:	Singlet-A	3.3745 eV	367.41 nm	f=0.3865
71 -> 73		0.23538			
72 -> 73		0.66038			
Excited State	3:	Singlet-A	3.6160 eV	342.87 nm	f=0.2028
70 -> 73		-0.18401			
71 -> 73		0.63760			
72 -> 73		-0.21959			

### 6.2.5 Excitation of 1-OMe (R = OMe)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.3224 eV	373.17 nm	f=0.4208
73 -> 77		-0.25127			
75 -> 77		0.16278			
76 -> 77		0.63203			
Excited State	2:	Singlet-A	3.3250 eV	372.89 nm	f=0.0646
73 -> 77		0.64735			
73 -> 79		0.10139			
76 -> 77		0.24686			
Excited State	3:	Singlet-A	3.5827 eV	346.07 nm	f=0.1275
74 -> 77		-0.14614			
75 -> 77		0.66478			
76 -> 77		-0.16109			

### 6.2.6 Excitation of OL-NMe<sub>2</sub> (R = NMe<sub>2</sub>)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	2.8230 eV	439.19 nm	f=0.5254
72 -> 73		0.69933			
Excited State	2:	Singlet-A	3.4547 eV	358.88 nm	f=0.0002
69 -> 73		0.69304			
69 -> 76		0.11539			
Excited State	3:	Singlet-A	3.5662 eV	347.66 nm	f=0.1227
70 -> 73		-0.12082			
71 -> 73		0.68198			

### 6.2.7 Excitation of OL-NH<sub>2</sub> (R = NH<sub>2</sub>)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.0800 eV	402.55 nm	f=0.4590
63 -> 65		-0.12237			
64 -> 65		0.69214			
Excited State	2:	Singlet-A	3.4433 eV	360.07 nm	f=0.0002
61 -> 65		0.69299			
Excited State	3:	Singlet-A	3.5955 eV	344.83 nm	f=0.1429
62 -> 65		-0.14364			
63 -> 65		0.67263			
64 -> 65		0.11678			

### 6.2.8 Excitation of OL-H (R = H)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.3972 eV	364.97 nm	f=0.0002
57 -> 61		0.69261			
57 -> 63		0.11070			
Excited State	2:	Singlet-A	3.4131 eV	363.26 nm	f=0.0060
60 -> 61		0.69523			
Excited State	3:	Singlet-A	3.6696 eV	337.87 nm	f=0.1349
58 -> 61		0.63197			
59 -> 61		0.29756			

### 6.2.9 Excitation of OLH (R = OH)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.3674 eV	368.18 nm	f=0.3594
63 -> 65		0.22896			
64 -> 65		0.66260			
Excited State	2:	Singlet-A	3.4231 eV	362.20 nm	f=0.0002
61 -> 65		0.69304			
61 -> 68		0.11379			
Excited State	3:	Singlet-A	3.6096 eV	343.49 nm	f=0.1982
62 -> 65		-0.17552			
63 -> 65		0.64159			
64 -> 65		-0.21447			

### 6.2.10 Excitation of OL-OMe (R = OMe)

Excitation energies and oscillator strengths:

Excited State	1:	Singlet-A	3.3148 eV	374.03 nm	f=0.4522
67 -> 69		0.17004			
68 -> 69		0.67977			
Excited State	2:	Singlet-A	3.4325 eV	361.21 nm	f=0.0002
65 -> 69		0.69310			
65 -> 71		0.11552			
Excited State	3:	Singlet-A	3.5748 eV	346.83 nm	f=0.1281
66 -> 69		-0.13349			
67 -> 69		0.66787			
68 -> 69		-0.15901			

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