

The Open University's repository of research publications and other research outputs

## Simultaneous Two-Photon Absorption of the Thioguanosine Analogue 2,3,5-TriOacetyl-6,8-dithioguanosine with Its Potential Application to Photodynamic Therapy

### Journal Item

#### How to cite:

Ando, Saki; Isozaki, Tasuku; Xu, Yao-Zhong and Suzuki, Tadashi (2020). Simultaneous Two-Photon Absorption of the Thioguanosine Analogue 2,3,5-TriOacetyl-6,8-dithioguanosine with Its Potential Application to Photodynamic Therapy. *The Journal of Physical Chemistry A* (Early Access).

For guidance on citations see [FAQs](#).

© 2020 American Chemical Society



<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Version: Accepted Manuscript

Link(s) to article on publisher's website:  
<http://dx.doi.org/doi:10.1021/acs.jpca.0c03747>

---

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

---

1  
2  
3 **Simultaneous Two-photon Absorption of Thioguanosine Analog**  
4  
5  
6 **2',3',5'-Tri-O-acetyl-6,8-dithioguanosine with Its Potential**  
7  
8  
9 **Application to Photodynamic Therapy**  
10  
11  
12  
13

14 Saki Ando,<sup>†</sup> Tasuku Isozaki,<sup>‡,\*</sup> Yao-Zhong Xu,<sup>§</sup> Tadashi Suzuki<sup>†,\*</sup>  
15  
16  
17

18  
19 <sup>†</sup> Department of Chemistry and Biological Science, Aoyama Gakuin University,  
20  
21 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5258, Japan  
22

23  
24 <sup>‡</sup> Division of Natural Sciences, College of Arts and Sciences, J. F. Oberlin University,  
25  
26 Tokiwa-machi, Machida, Tokyo 194-0294, Japan  
27

28  
29 <sup>§</sup> School of Life, Health and Chemical Sciences, The Open University,  
30  
31 Milton Keynes MK7 6AA, United Kingdom  
32  
33  
34

35 **ABSTRACT**  
36

37 2',3',5'-Tri-O-acetyl-6,8-dithioguanosine (taDTGuo) is an analog of nucleoside and  
38 currently under **investigation** as a potential agent for photodynamic therapy (PDT). Excitation  
39 by simultaneous two-photon absorption of visible near-infrared light would provide an  
40 efficient PDT for deep-seated tumors. The two-photon absorption spectrum of taDTGuo was  
41 obtained by optical-probing photoacoustic spectroscopy (OPPAS). A two-photon absorption  
42 band corresponding to the  $S_5 \leftarrow S_0$  transition was observed at 556 nm, and the two-photon  
43 absorption cross section  $\sigma^{(2)}$  was determined to be  $26 \pm 3$  GM, which was much larger than  
44 those of other nucleobases and nucleosides. Quantum chemical calculations suggested that the  
45 large  $\sigma^{(2)}$  value of taDTGuo was responsible for large transition dipole moments and small  
46 detuning energy resulting from the thiocarbonyl group at 6- and 8-positions. This is the first  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 report on two-photon absorption spectra and cross sections of thionucleoside analogs, which  
4  
5 could be used to develop a more specific PDT for cancers in deep region.  
6  
7  
8  
9

### 10 **KEY WORDS**

11  
12 Thio-substituted nucleoside, Thioguanosine, Two-photon absorption spectrum, Photoacoustic  
13  
14 spectroscopy, Two-photon absorption cross section, photodynamic therapy  
15  
16  
17  
18

19 \* Corresponding authors: [tisozaki@obirin.ac.jp](mailto:tisozaki@obirin.ac.jp) (T. Isozaki) and [suzuki@chem.aoyama.ac.jp](mailto:suzuki@chem.aoyama.ac.jp)  
20  
21 (T. Suzuki)  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## ■ INTRODUCTION

6-Thioguanine (6TG) is widely prescribed as an anticancer drug and immunosuppressive agent.<sup>1</sup> Through cellular metabolism,<sup>2,3</sup> 6TG can be converted into its nucleoside, 6-thioguanosine [2-amino-9-( $\beta$ -D-ribofuranosyl)purine-6-thiol] (6TGuo), which can be readily incorporated into cellular RNA and DNA.<sup>4-7</sup> 6TGuo localizing in tumor cell was reported to generate singlet molecular oxygen ( $^1\text{O}_2^*$ ) when exposed to UVA light, thus inducing cellular apoptosis.<sup>2,3</sup> Photo-induced therapeutic effect of 6TG and its nucleoside is based on its dominant relaxation pathway of intersystem crossing and the sequent energy transfer to molecular oxygen ( $^3\text{O}_2$ ).<sup>8</sup> Thus, 6TG would be an effective anti-cancer agent via its biochemical activity and photo-induced therapeutic effect.

Recently, we synthesized tri-acetyl-protected thioguanosine derivatives: 2',3',5'-tri-*O*-acetyl-6-thioguanosine (ta6TGuo), 2',3',5'-tri-*O*-acetyl-8-thioguanosine (ta8TGuo), and 2',3',5'-tri-*O*-acetyl-6,8-dithioguanosine (taDTGuo) (Chart 1) and explored their photochemical and photophysical properties.<sup>9-12</sup> These three thioguanosine derivatives can absorb UVA light and generate  $^1\text{O}_2^*$  through energy transfer to  $^3\text{O}_2$ . taDTGuo is of particular interest because it has the longest wavelength for absorption maximum and the highest value in terms of molar absorption coefficient among all of the thiobases and thionucleosides so far reported in the literature.<sup>9,12,13</sup> It also generates  $^1\text{O}_2^*$  under low-pH<sup>10</sup> and low oxygen concentration<sup>11</sup> conditions like a tumor microenvironment. Therefore, these acetylated thio-guanosines, especially taDTGuo, are potential agents for light-induced therapies.

Since the penetration depth of UVA light into biological tissues is shallow, UVA light is not used to target deep-seated cancerous cells. The penetration depth is wavelength-dependent, thus, the disadvantage by UVA should be overcome by using the light with longer wavelengths such as visible or near-infrared light.<sup>14</sup> One potential solution to deep-seated tumors is to

1  
2  
3 exploit simultaneous two-photon excitation of thioguanosine derivatives in photodynamic  
4 therapy (PDT). For non-centrosymmetric molecules like thioguanosine derivatives,  
5 two-photon absorption is a way of accessing a given excited state by using photons with half  
6 the energy of the corresponding one-photon transition. Since two-photon absorption involve  
7 the simultaneous interaction of two photons, it increases with the square of the light intensity.  
8 Therefore, high spatial-selective treatments are also achieved by two-photon induced PDT.  
9

10  
11  
12 Two-photon absorption properties, such as the absorption wavelength and the cross section  
13 of thioguanosine derivatives are essential for the two-photon induced PDT. The study of  
14 two-photon absorption has been mostly based on the observation of direct absorption and  
15 radiative transitions, that is, open-aperture Z-scan and two-photon excited fluorescence.  
16 Generally, any molecules with the extended  $\pi$ -conjugated system would have large two-photon  
17 absorption cross section.<sup>15</sup> However, taDTGuo has a small  $\pi$ -conjugated system, thus, its  
18 two-photon absorption cross-section is likely to be small. Moreover, taDTGuo is less  
19 fluorescent, so conventional techniques for the two-photon absorption measurement are not  
20 applicable to this molecular system. From the above reasons, we applied photoacoustic  
21 spectroscopy (PAS), a highly sensitive photothermal calorimetric technique, for the  
22 measurement of two-photon absorption spectrum of taDTGuo.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

42 In this article, we investigated the two-photon excitation wavelength and the two-photon  
43 absorption cross section  $\sigma^{(2)}$  of taDTGuo by optical-probing photoacoustic spectroscopy  
44 (OPPAS),<sup>16-18</sup> a PAS technique detecting a transient angular deflection of the probe beam. We  
45 also simulated two-photon absorption spectra of thioguanine derivatives by using the quantum  
46 chemical calculation to better understand the nature of higher two-photon absorption cross  
47 sections for thioguanosines.  
48  
49  
50  
51  
52  
53  
54

## 55 ■ EXPERIMENTAL

56  
57  
58  
59  
60

1  
2  
3 2',3',5'-Tri-O-acetyl-6,8-dithioguanosine (taDTGuo) was prepared as described in the  
4 previous report.<sup>9</sup> One-photon absorption spectra were measured by a UV/Vis  
5 spectrophotometer (Shimadzu, UV-3100).  
6  
7

8  
9  
10 The experimental setup for OPPAS measurement has been described elsewhere.<sup>17,18</sup> An  
11 optical parametric oscillator (OPO) laser (Continuum, Panther EX OPO; linearly polarized,  
12 pulse width 3–5 ns, line width  $< 6 \text{ cm}^{-1}$ ) pumped by third harmonic of a Nd<sup>3+</sup>:YAG laser  
13 (Continuum, Surelite II ; pulse width 4–6 ns, repetition rate 10 Hz) and a He–Ne laser (LASOS,  
14 LGK 7634;  $\geq 2 \text{ mW}$ , cw 632.8 nm) were used as the excitation and the probe sources,  
15 respectively. The excitation laser power was attenuated with a variable neutral density filter to  
16 obtain the incident laser power dependence on the OPPAS signal. The intensity of the excitation  
17 laser, split by a beam sampler, was monitored with a thermopile sensor (Ophir, 3A-P). Both  
18 the excitation and the probe lights were focused into a quartz glass cell (optical path length 10  
19 mm) with 150 mm focal lenses and settled in parallel. An acoustic wave resulting from  
20 absorption was detected as a transient angular deflection of the probe beam. The temporal  
21 intensity profile of the probe light through a 100  $\mu\text{m}$  pinhole and a laser line filter was detected  
22 by a photomultiplier tube (Hamamatsu Photonics, R928). The signals from the photomultiplier  
23 tube and the thermopile sensor were averaged by a digital oscilloscope (Tektronix,  
24 DPO4104B-L), and the waveform was transferred to a personal computer. A differential  
25 amplifier (NF, 5307) was used to amplify the OPPAS signal. The sample solutions were  
26 deaerated with N<sub>2</sub> gas for 15 minutes prior to the measurements and flowed into the cell to  
27 avoid the contamination of photoproducts. The flow rate controlled by a syringe pump (YMC,  
28 YSP-101) was 2.0 mL/hour.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53

54 A mixed solvent of water and acetonitrile (Aldrich, >99%) [7:3 (v:v)] was used in order to  
55 prepare highly concentrated (10 mM) solution of taDTGuo. All of the measurements were  
56 carried out at ambient temperature.  
57  
58  
59  
60

To save calculation costs, calculations were performed without sugar moieties. Since the calculated vertical transition energies and oscillator strengths for singlet excited states of thioguanines were well reproduced the one-photon absorption spectra of corresponding tri-acetyl-protected thioguanosines,<sup>9</sup> this kind of simplification is considered to give little influence on the calculation results of optical properties. Ground- and excited-state calculations for purine bases [6,8-dithioguanine (DTG), 8-thioguanine (8TG), and 8-oxoguanine (8OG)] were performed using the Gaussian 09W program package.<sup>19</sup> Geometry optimization and vibrational frequency analysis in the ground state were performed at the B3LYP/6-311+G(d,p) level with the polarizable continuum model (PCM) in water. The vertical transition energies, the transition dipole moments to the excited singlet states, the transition dipole moments between excited states, and the permanent dipole moments of the ground and excited states necessary for the spectral simulation of two-photon absorption were estimated by the time-dependent DFT (TD-DFT) with Tamm-Dancoff approximation (TDA)<sup>20</sup> at the B3LYP/6-311+G(d,p) level. Simulation of the two-photon absorption spectra were performed using sum-over state (SOS) formalism<sup>21</sup> summing all intermediate and final states considered in the calculation (20 excited states). The dumping constant of the Lorentzian linewidth function was taken to be 0.25 eV.

## ■ RESULTS AND DISCUSSION

**1 Two-photon absorption spectrum of taDTGuo.** The one-photon absorption spectrum of taDTGuo in water/acetonitrile is illustrated in Figure 1. taDTGuo has an absorption maximum in the UVA region;  $\epsilon_{\max}=3.76\times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at  $\lambda_{\max}=377 \text{ nm}$ . Our previous article<sup>10</sup> reported that taDTGuo in the phosphate buffer solution was held in sequential acid dissociation equilibria among the neutral form, 1-imide anionic form, and 1,7-di-imide anionic form, and the equilibrium constants were determined to be  $7.02\pm 0.01$  and  $9.79\pm 0.01$ , respectively. Since

1  
2  
3 the one-photon absorption spectrum in water/acetonitrile well agreed to that of the neutral  
4 form,<sup>10</sup> the neutral form should mainly exist in this solution.

7  
8 Figure 2(a) shows the typical OPPAS signal of taDTGuo in water/acetonitrile following  
9 excitation at 556 nm. The OPPAS signal was observed at 1.7  $\mu$ s after the irradiation of visible  
10 light. The prompt photoacoustic signal amplitude,  $U$ , is proportional to the total quantity of heat  
11 released from excited molecules into the medium. Because taDTGuo has no significant  
12 absorption of a single photon at 556 nm (Figure S1(a) in Supporting Information), the observed  
13 signal should be responsible for multi-photon absorption of taDTGuo.

14  
15 The heat action spectrum of taDTGuo was measured by plotting the amplitude  $U$  against  
16 excitation wavelength (Figure 1 and Figure S2 in Supporting Information). The incident laser  
17 power,  $I$ , was kept constant in the range from 428 to 632 nm, though it decreased due to the  
18 limitation of the light source for the wavelength longer than 632 nm. An intense band at 556  
19 nm and a weak absorption to the red of the band were observed. Background signal resulting  
20 from the solvent was negligible under the experimental condition applied.

21  
22 Figure 2(b) shows the log-log plots of the OPPAS signal intensity against the incident laser  
23 power. The signal intensity increased nonlinearly with the incident laser power, indicating that  
24 multi-photon absorption occurs. For a simplified  $n$ -photon absorption process, the OPPAS  
25 signal intensity is proportional to the  $n$ -th power of the incident laser power. The slopes of the  
26 solid lines in Figure 2(b), which were the best-fitting curves obtained by the least-square's  
27 method, were estimated to be 1.98 (460 nm), 2.16 (556 nm), and 2.35 (584 nm). This reveals  
28 that the nonlinear absorption is attributed to the simultaneous two-photon absorption process.  
29 The slope for 584 nm exceeds 2, implying that additional one-photon absorption following the  
30 two-photon absorption, called as excited-state absorption, would slightly contribute to the  
31 OPPAS signal.



1  
2  
3 **2 Comparison of one- and two-photon absorption spectra.** The two-photon absorption  
4 spectrum of taDTGuo is compared with the one-photon absorption spectrum (Figure 1). The  
5 observed spectral profile was quite similar with that of the one-photon absorption, revealing  
6 that the excited states are the same for the one- and two-photon absorption. This is consistent  
7 with the fact that the exclusive selection rule on one- and two-photon absorption should be  
8 inappropriate to non-centrosymmetric molecules like taDTGuo. Our previous work  
9 documented that taDTGuo generates singlet molecular oxygen by one-photon excitation of  
10 UVA light. Since the excited state following the one-photon absorption was the same as that  
11 following the two-photon absorption, taDTGuo excited by the two-photon absorption of  
12 visible light is also expected to generate singlet molecular oxygen.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

28 **3 Estimation of two-photon absorption cross section  $\sigma^{(2)}$ .** The two-photon absorption cross  
29 section of taDTGuo was estimated. The OPPAS signal intensity resulting from the two-photon  
30 absorption process  $U^{(2)}$  is described as  
31  
32  
33  
34  
35  
36

$$U^{(2)} = K^{(2)} \sigma^{(2)} C \alpha^{(2)} b I^2 \quad (1)$$

37  
38  
39  
40  
41  
42 where  $K^{(2)}$  is the instrumental function,  $\sigma^{(2)}$  is the two-photon absorption cross section,  $C$   
43 is the concentration of the solution,  $b$  is the coefficient that reflects thermoelastic properties  
44 of the solvent, and  $I$  is the incident laser power.  $\alpha^{(2)}$  is the heat conversion efficiency for  
45 two-photon absorption, namely the ratio of the energy released as heat into the medium against  
46 the absorbed energy. The  $\sigma^{(2)}$  of taDTGuo can be determined by the following equation with  
47 a reference of 1,4-bis(2-methylstyryl)benzene (Bis-MSB;  $\sigma^{(2)}=68 \text{ GM at } 556 \text{ nm}^{22}$ ),  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

$$\sigma_{\text{sam}}^{(2)} = \frac{U_{\text{sam}}^{(2)} / I_{\text{sam}}^2}{U_{\text{ref}}^{(2)} / I_{\text{ref}}^2} \times \frac{c_{\text{ref}}}{c_{\text{sam}}} \times \frac{\alpha_{\text{ref}}^{(2)} b_{\text{ref}}}{\alpha_{\text{sam}}^{(2)} b_{\text{sam}}} \times \sigma_{\text{ref}}^{(2)} \quad (2)$$

where the subscripts of sam and ref represent the parameters of sample (taDTGuo) and reference (Bis-MSB), respectively. Since Bis-MSB is insoluble in water/acetonitrile, the measurements for Bis-MSB were performed in 1,4-dioxane. The ratio of  $b_{\text{ref}}/b_{\text{sam}}$  for the mixed solvent water/acetonitrile and 1,4-dioxane was estimated by comparing the signal intensity of a calorimetric standard 2-hydroxybenzophenone (2HBP) with the 355 nm light ( $\alpha_{355}^{(1)} = 1.0$ <sup>23</sup>). Assuming that the heat conversion efficiency for two-photon absorption,  $\alpha^{(2)}$ , was identical to that for one-photon absorption at 355 nm,  $\alpha_{355}^{(1)}$ , the heat conversion efficiencies for the two-photon absorption of taDTGuo and Bis-MSB were determined to be  $0.60 \pm 0.02$  and  $0.18 \pm 0.02$ , respectively. The detailed procedure for the measurements is described in Supporting Information (Figures S3 and S4), and the key results are summarized in Table 1.

The OPPAS signal intensity of taDTGuo and Bis-MSB against the incident laser power at 556 nm is presented in Figure 3. By analyzing the plots with the polynomial function, the  $U^{(2)} / I^2$  value was obtained (Table 1). Substituting these values into eq. (2), the  $\sigma^{(2)}$  value for taDTGuo was successfully determined to be  $26 \pm 3$  GM at 556 nm. Some researchers reported two-photon absorption cross sections of purine derivatives in water; 0.7 GM (532 nm) for guanosine 5'-monophosphate,<sup>24</sup> 0.1 GM (612 nm) for 2-aminopurine,<sup>25</sup> and 1.8 GM (560 nm) for 7-methylguanosine.<sup>26</sup> It was obviously demonstrated that taDTGuo has much larger  $\sigma^{(2)}$  value than other purine derivatives.

**4 Simulation of two-photon absorption spectra.** To obtain deeper insight of two-photon

1  
2  
3 absorption properties of taDTGuo, the two-photon absorption spectra of three guanine  
4 derivatives were simulated; dithioguanine (DTG), 8-thioguanine (8TG), and 8-oxoguanine  
5 (8OG) (Figure 4 and Table S2 in Supporting Information). All guanine derivatives have two  
6 types of absorption bands; (i) weak two-photon absorption bands by the transition to the lowest  
7 two-photon allowed excited state, and (ii) intense two-photon absorption bands by the  
8 transition to the second and any higher two-photon allowed excited states, designated by green  
9 arrows. The intense two-photon absorption bands were assigned to the  $S_5 \leftarrow S_0$  transition for  
10 DTG, the  $S_8 \leftarrow S_0$  transition for 8TG, and the  $S_{12} \leftarrow S_0$  transition for 8OG (Figure 5 and Figure  
11 S5 in Supporting Information). The simulated two-photon absorption spectrum of DTG (Figure  
12 4(a)) shows an intense two-photon absorption band at 543 nm, which well agrees with the  
13 experimental result (556 nm). The  $\sigma^{(2)}$  values of intense absorption bands, shown by green  
14 arrows, are in the order of DTG > 8TG > 8OG, suggesting that the thiocarbonylation at 6-  
15 and/or 8-positions should increase the  $\sigma^{(2)}$  value.

16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33 The position effect of the thiocarbonyl group on the  $\sigma^{(2)}$  values was analyzed. The  $\sigma^{(2)}$   
34 value of DTG (130 GM), 8TG thiocarbonylated at 6-position, is 1.6 times larger than that of  
35 8TG (84 GM). On the other hand, the  $\sigma^{(2)}$  value of 8TG (84 GM), 8OG thiocarbonylated at  
36 8-position, is 3.4 times larger than that of 8OG (25 GM). These results indicates that  
37 thiocarbonylation at 8-position of guanine has a larger effect on increasing  $\sigma^{(2)}$  value than  
38 that at 6-position.

39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
The two-photon absorption cross section can be theoretically expressed as the sum of three  
terms; three-state, dipolar, and cross terms.<sup>18</sup> Simulated two-photon absorption spectra  
decomposed into three terms (see Figure S6 in Supporting Information) revealed that  
three-state term dominantly contributes to  $\sigma^{(2)}$  and the contributions from dipolar and cross  
terms are negligible for all guanine derivatives. Three-state term,  $T_{\text{three-state}}$ , is described as  
follows.<sup>18</sup>

$$T_{\text{three-state}} = \frac{4}{15\hbar^2} \sum_{\substack{k \neq g \\ k \neq f}} \left[ (1 + 2 \cos^2 \theta_k) \frac{|\mathbf{M}_{gk}|^2 |\mathbf{M}_{kf}|^2}{(\omega_{gk} - \omega)^2} \right] \quad (3)$$

Here,  $\mathbf{M}$  is the transition dipole moment between the states ( $g$ : ground state,  $k$ : intermediate state,  $f$ : final state),  $\hbar(\omega_{gk} - \omega)$  is the detuning energy (the difference between the vertical transition energy to the intermediate state and the photon energy of the incident light),  $\Delta E$ , and  $\theta_k$  is the angle between the two vectors of  $\mathbf{M}_{gk}$  and  $\mathbf{M}_{kf}$ . We calculated the  $T_{\text{three-state}}$  term, with the 20 excited singlet states as an intermediate state and the 20 excited singlet states as a final state. The decomposed two-photon absorption spectra via intermediate states are illustrated in Figure S7 in Supporting Information. The  $S_1$  state for DTG and 8TG, and the  $S_1$  and  $S_3$  states for 8OG have the major contribution to the three-state term.

The energy diagrams of guanine derivatives for the transition designated by green arrows are presented in Figure 5. DTG has larger transition dipole moments ( $|\mathbf{M}_{gk}|=7.94$  D,  $|\mathbf{M}_{kf}|=9.41$  D) and smaller detuning energy ( $\Delta E=1.09$  eV) than other guanine derivatives. Since the three-state term is proportional to  $|\mathbf{M}_{gk}|^2 |\mathbf{M}_{kf}|^2$  and inversely proportional to the square of the detuning energy, both the large transition dipole moments and the small detuning energy would be responsible for the large two-photon absorption cross-section for taDTGuo (26 GM at 556 nm). From the electronic configuration of the excited states (Table S3 in Supporting Information), it was found that the  $S_5 \leftarrow (S_1) \leftarrow S_0$  electronic transition should correspond to the LUMO  $\leftarrow$  HOMO and HOMO  $\leftarrow$  HOMO-3 transitions for DTG, the  $S_8 \leftarrow (S_1) \leftarrow S_0$  transition is the LUMO  $\leftarrow$  HOMO and LUMO+3  $\leftarrow$  LUMO transitions for 8TG, and the  $S_{12} \leftarrow (S_3) \leftarrow S_0$  transition is the LUMO+1  $\leftarrow$  HOMO and LUMO+8  $\leftarrow$  LUMO+1 transitions for 8OG. The molecular orbitals related to these transitions are presented in Figure 6. Although the shapes of MOs for the guanine derivatives were almost the same, the electron density at the sulfur atom

1  
2  
3 was higher than that of oxygen atom, resulting in increase of the electron density at the  
4 peripheral groups by thiocarbonylation at 6- and/or 8-position (for example see HOMOs of  
5 DTG, 8TG, and 8OG in Figure 6.). The larger difference of the electron density at the peripheral  
6 group between MOs relating to the transition for the thiolated guanosines would result in a  
7 larger transition dipole moment. The parameters involving three-state terms of DTG, 8TG, and  
8 8OG are summarized in Tables S4 and S5 in Supporting Information.  
9

10  
11 One-photon absorption spectra of guanosine derivatives are shown in Figure S1(b) (see also  
12 Table S1).<sup>9,12</sup> taDTGuo has an absorption maximum at 381 nm in acetonitrile, which is the most  
13 red-shifted absorption band among thionucleosides so far reported<sup>9,12,13</sup>, while the absorption  
14 maxima of ta8TGuo and ta8OGuo, which significantly contributes to the two-photon  
15 absorption as the intermediate states, were observed at 304 and 249 nm, respectively. As the  
16 photon energy of the incident light for two-photon absorption is much smaller than the vertical  
17 excitation energy to the intermediate states, the detuning energy decreases by the existence of  
18 the excited states with low vertical transition energy which mainly contribute as the  
19 intermediate states.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36

37 Among these thioguanine analogs, DTG was found to have the most red-shifted band and  
38 the largest  $\sigma^{(2)}$  value for the two-photon absorption band designated by the green arrow.  
39 Therefore, DTG would be the most promising agent for two-photon induced PDT.  
40  
41  
42  
43  
44  
45  
46

47 **5 Application of thioguanosine derivatives to two-photon induced PDT.** At present,  
48 one-photon activated PDT using porphyrin compounds such as Photofrin, Laserphyrin, and  
49 Visudyne has been applied to the treatments of cancers,<sup>27</sup> while two-photon induced PDT is  
50 still under research and development stages. Any potential drugs for two-photon induced PDT  
51 requires a large two-photon absorption cross section  $\sigma^{(2)}$  in the biological optical window and  
52 also a large singlet molecular oxygen quantum yield  $\Phi_{\Delta}$ . taDTGuo would be a good drug  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 candidate for two-photon induced PDT in terms of the singlet molecular oxygen quantum yield,  
4 the penetration depth into the biological tissues by the excitation light wavelength, and the  
5 two-photon absorption cross section.  
6  
7  
8

9  
10 As reported in our recent work,<sup>9–11</sup> taDTGuo can be excited by one-photon absorption of  
11 UVA light and will generate  $^1\text{O}_2^*$  under low-pH and low-oxygen conditions similar to the  
12 carcinoma cell microenvironment. The current paper demonstrated that taDTGuo is excited by  
13 two-photon absorption of visible light. Since the excited state following the two-photon  
14 absorption was the same as that following the one-photon absorption, taDTGuo excited by  
15 the two-photon absorption of visible light is expected to generate singlet molecular oxygen.  
16  
17  
18  
19  
20  
21  
22

23  
24 The penetration depth of the excitation light for the two-photon absorption ( $\lambda^{(2)}=556$  nm) is  
25 about 330  $\mu\text{m}$ , which is 8 times as high as that for one-photon absorption (40  $\mu\text{m}$  at  $\lambda^{(1)}=381$   
26 nm).<sup>28</sup> Since the excitation wavelength for the two-photon absorption of taDTGuo (556 nm) is  
27 shorter than that for the one-photon absorption of Photofrin (630 nm), porphyrin compounds  
28 remain more favorable for the treatments of deep-seated tumors. However, taDTGuo is a  
29 nucleoside analog which could be incorporated into target cellular DNA. Thus, the combination  
30 of thionucleoside and two-photon absorption would certainly offer a more specific approach to  
31 targeting the DNA in deep-seated tumors.  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

42  
43 Taken together, these results indicate that two-photon induced PDT would be possible by  
44 the enhancement of the  $\sigma^{(2)}$  value of the transition to any two-photon allowed excited states  
45 and the red-shift of the two-photon absorption peak. Based on the results of our simulated  
46 two-photon absorption spectra of guanine derivatives, the modification at 8-position to  
47 increase the electron distribution should be effective to enhance the  $\sigma^{(2)}$  value.  
48  
49  
50  
51  
52  
53

54  
55 In this study, we successfully obtained the two-photon absorption spectrum and the  $\sigma^{(2)}$   
56 value of taDTGuo. This is the first experimental report on the two-photon absorption spectrum  
57 and cross section of a thionucleoside analog, and offers the possibility to develop a more  
58  
59  
60

1  
2  
3 specific PDT for deep-seated tumors by using nucleoside derivatives and simultaneous  
4 two-photon absorption.  
5  
6  
7  
8  
9

## 10 ■ CONCLUSIONS

11  
12 Two-photon absorption properties such as excitation wavelength and two-photon absorption  
13 cross section of taDTGuo were investigated by OPPAS and quantum chemical calculations.  
14 The two-photon absorption band for the  $S_5 \leftarrow S_0$  transition was observed at 556 nm. The  
15 two-photon absorption cross section of taDTGuo was estimated to be  $26 \pm 3$  GM at 556 nm,  
16 which is much larger than those of other nucleobases and nucleoside derivatives reported so  
17 far. Simulation of the two-photon absorption spectra explained that the large  $\sigma^{(2)}$  value of  
18 taDTGuo is responsible for large transition dipole moments and small detuning energy.  
19  
20  
21  
22  
23  
24  
25  
26  
27

28 Thionucleosides can be incorporated specifically into cancerous DNA, and longer  
29 wavelength light can substantially enhance the light penetration into the tissue. Using  
30 thionucleosides and two-photon absorption would offer a more specific approach to targeting  
31 cancerous DNA than the current non-nucleoside-based PDT.  
32  
33  
34  
35  
36  
37  
38  
39

## 40 ■ ASSOCIATED CONTENT

### 41 42 43 44 **Supporting Information**

45 The Supporting Information is available free of charge at

46  
47  
48 <https://pubs.acs.org/doi/10.1021/>.  
49  
50  
51

52  
53 A: One-photon absorption spectra of taDTGuo, ta8TGuo, ta8OGuo, and ta6TGuo, B:  
54 Two-photon absorption spectrum of taDTGuo and laser power, C: The procedure for  
55 determination of two-photon absorption cross section, and D: Simulation of two-photon  
56 absorption of guanine derivatives.  
57  
58  
59  
60

## ■ AUTHOR INFORMATION

Saki Ando and Tasuku Isozaki are Co-first author.

## ORCID

Tasuku Isozaki: 0000–0003–0070–8335

Yao–Zhong Xu: 0000–0002–5713–8030

Tadashi Suzuki: 0000–0002–9145–8601

## Notes

The authors declare no competing financial interest.

## ■ REFERENCES

- (1) Karran, P.; Attard, N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy–related cancer. *Nature Reviews Cancer* **2008**, *8*, 24–36.
- (2) Karran, P. Thiopurines, DNA damage, DNA repair and therapy–related cancer. *Br. Med. Bull.* **2006**, *79–80*, 153–170.
- (3) O’Donovan, P.; Perrett, C. M.; Zhang, X.; Montaner, B.; Xu, Y.–Z.; Harwood, C. A.; McGregor, J. M.; Walker, S. L.; Hanaoka, F.; Karran, P. Azathioprine and UVA Light Generate Mutagenic Oxidative DNA Damage. *Science* **2005**, *309*, 1871–1874.
- (4) Lepage, G. A. Incorporation of 6–thioguanine into nucleic acids. *Cancer Res.* **1960**, *20*, 403–408.
- (5) Lepage, G. A.; Jones, M. Further studies on the mechanism of action of 6–thioguanine. *Cancer Res.* **1961**, *21*, 1590–1594.
- (6) Bohon, J.; de los Santos, C. R. Structural effect of the anticancer agent 6–thioguanine on



- duplex DNA. *Nucleic Acids Res.* **2003**, *31*, 1331–1338.
- (7) Bohon, J.; de los Santos, C. R. Effect of 6–thioguanine on the stability of duplex DNA. *Nucleic Acids Res.* **2005**, *33*, 2880–2886.
- (8) Pollum, M.; Ortiz–Rodríguez, L. A.; Jockusch, S.; Crespo–Hernández, C. E. The triplet state of 6–thio–2’–deoxyguanosine: intrinsic properties and reactivity toward molecular oxygen. *Photochem. Photobiol.* **2016**, *92*, 286–292.
- (9) Miyata, S.; Yamada, T.; Isozaki, T.; Sugimura, H.; Xu, Y. Z.; Suzuki, T. Absorption characteristics and quantum yields of singlet oxygen generation of thioguanosine derivatives. *Photochem. Photobiol.* **2018**, *94*, 677–684.
- (10) Miyata, S.; Hoshino, M.; Isozaki, T.; Yamada, T.; Sugimura, H.; Xu, Y. Z.; Suzuki, T. Acid dissociation equilibrium and singlet molecular oxygen quantum yield of acetylated 6,8–dithioguanosine in aqueous buffer solution. *J. Phys. Chem. B* **2018**, *122*, 2912–2921.
- (11) Miyata, S.; Tanabe, S.; Isozaki, T.; Xu, Y. Z.; Suzuki, T. Characteristics of the excited triplet states of thiolated guanosine derivatives and singlet oxygen generation. *Photochem. Photobiol.* **2018**, *17*, 1469–1476.
- (12) Suzuki, T.; Miyata, S.; Isozaki, T. Absorption spectra of 8–oxoguanosine and 8–thioguanosine. *Photomed. Photobiol.* **2015**, *37*, 21–22.
- (13) Ashwood, B.; Pollum, M.; Crespo–Hernández C. E. Photochemical and Photodynamical Properties of Sulfur–Substituted Nucleic Acid Bases. *Photochem. Photobiol.* **2019**, *95*, 33–58.
- (14) Khurana, M.; Collins, H. A.; Karotki, A. H.; Anderson, L.; Cramb, D. T.; Wilson, B. C. Quantitative in vitro demonstration of two–photon photodynamic therapy using Photofrin and Visudyne. *Photochem. Photobiol.* **2007**, *83*, 1441–1448.
- (15) Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L. Two–photon absorption and the design of two–photon dyes. *Angew. Chem.* **2009**, *48*, 3244–3266.

- 1  
2  
3 (16) Suzuki, T.; Nakamura, M.; Isozaki, T.; Ikoma, T. ‘Dark’ excited states of  
4 diphenylacetylene studied by nonresonant two-photon excitation optical-probing  
5 photoacoustic spectroscopy. *Int. J. Thermophys.* **2012**, *33*, 2046–2054.  
6  
7  
8  
9  
10 (17) Isozaki, T.; Oba, H.; Ikoma, T.; Suzuki, T. Simultaneous two-photon absorption to grade  
11 excited singlet states of diphenylacetylene and diphenylbutadiyne using optical-probing  
12 photoacoustic spectroscopy. *J. Phys. Chem. A* **2016**, *120*, 6137–6145.  
13  
14  
15  
16 (18) Ishii, T.; Ando, S.; Isozaki, T.; Takeuchi, R.; Kashihara, W.; Suzuki, T. Two-photon  
17 absorption property of Cl-substituted diphenylacetylenes by optical-probing  
18 photoacoustic spectroscopy. *J. Chem. Phys.* **2019**, *151*, 134304–1–9.  
19  
20  
21  
22  
23 (19) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman,  
24 J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson G. A. *et al. Gaussian 09 D.01*  
25 (Gaussian, Inc., 2013).  
26  
27  
28  
29  
30 (20) Hirata, S.; Head-Gordo, M. Time-dependent density functional theory within the  
31 Tamm-Dancoff approximation. *Chem. Phys. Lett.* **1999**, *314*, 291–299.  
32  
33  
34  
35 (21) Ohta, K.; Yamada, S.; Kamada, K.; Slepko, A. D.; Hegmann, F. A.; Tykwinski, R. R.;  
36 Shirtcliff, L. D.; Haley, M. M.; Salek, P.; Gel’mukhanov, F.; Agren, H. Two-photon  
37 absorption properties of two-dimensional  $\pi$ -conjugated chromophores: combined  
38 experimental and theoretical study. *J. Phys. Chem. A* **2011**, *115*, 105–117.  
39  
40  
41  
42  
43 (22) Kennedy, S. M.; Lytle, F. E. *p*-Bis(*o*-methylstyryl)benzene as a power-squared sensor  
44 for two-photon absorption measurements between 537 and 694 nm. *Anal. Chem.* **1986**,  
45 *58*, 2643–2647.  
46  
47  
48  
49  
50 (23) van Haver, Ph.; Viaene, L.; van der Auweraer, M.; de Schryver, F. C. References for  
51 laser-induced optoacoustic spectroscopy using UV excitation. *J. Photochem. Photobiol.*  
52 *A:Chemistry* **1992**, *63*, 265–277.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 (24) Gut, I. G.; Hefetz, Y.; Kochevar, I. E.; Hillenkamp, F. Two-photon absorption cross  
4 sections of guanosine 5'-monophosphate and uridine 5'-monophosphate at 532 nm. *J.*  
5  
6  
7 *Phys. Chem.* **1993**, *97*, 5171–5176.  
8  
9  
10 (25) Lane, R. S. K.; Magennis, S. W. Two-photon excitation of the fluorescent nucleobase  
11 analogues 2-AP and tC. *RSC Advances* **2012**, *2*, 11397–11403.  
12  
13  
14 (26) Mikhaylov, A.; de Reguardati, S.; Pahapill, J.; Callis, P. R.; Kohler, B.; Rebane, A.  
15 Two-photon absorption spectra of fluorescent isomorphous DNA base analogs. *Biomed.*  
16  
17 *Opt. Express* **2018**, *9*, 447–452.  
18  
19  
20 (27) dos Santos, A. F.; de Almeida, D. R. Q.; Terra, L. F.; Baptista, M. S.; Labriola, L.  
21 Photodynamic therapy in cancer treatment – an update review. *J. Cancer Metastasis Treat*  
22  
23  
24 **2019**, *5*, 25.  
25  
26  
27  
28 (28) Tamura, M. *Medical Diagnoses by light* (Kyoritsu Shuppan, 2001).  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Table 1. Concentration,  $U^{(2)} / I^2$ , the heat conversion efficiency at 355 nm, and two-photon absorption cross section of taDTGuo and Bis-MSB at 556 nm.

Sample	$C$ / mM	$U^{(2)} / I^2$ / V <sup>-1</sup>	$\alpha_{355}^{(1)}$	$\sigma^{(2)}$ / GM
taDTGuo	10	0.45 ± 0.01	0.60 ± 0.02	26 ± 3
Bis-MSB <sup>a</sup>	15	2.13 ± 0.03	0.18 ± 0.02	68 <sup>a</sup>

<sup>a</sup> Reference 22.

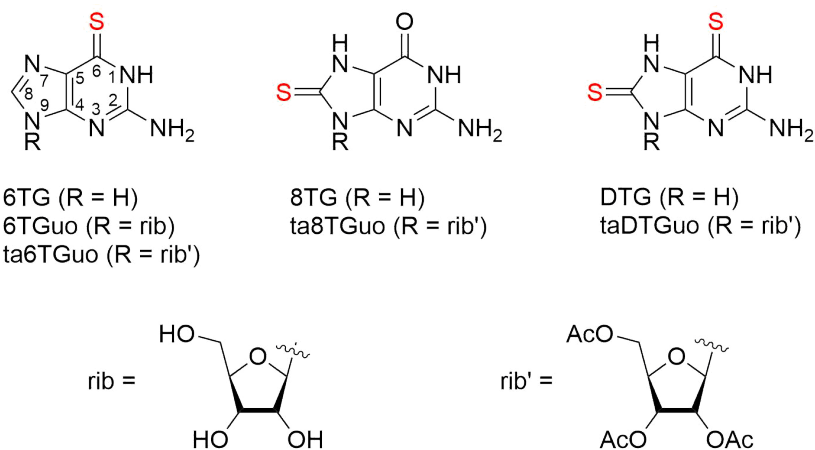


Chart 1. Structures of thioguanines and their nucleosides.

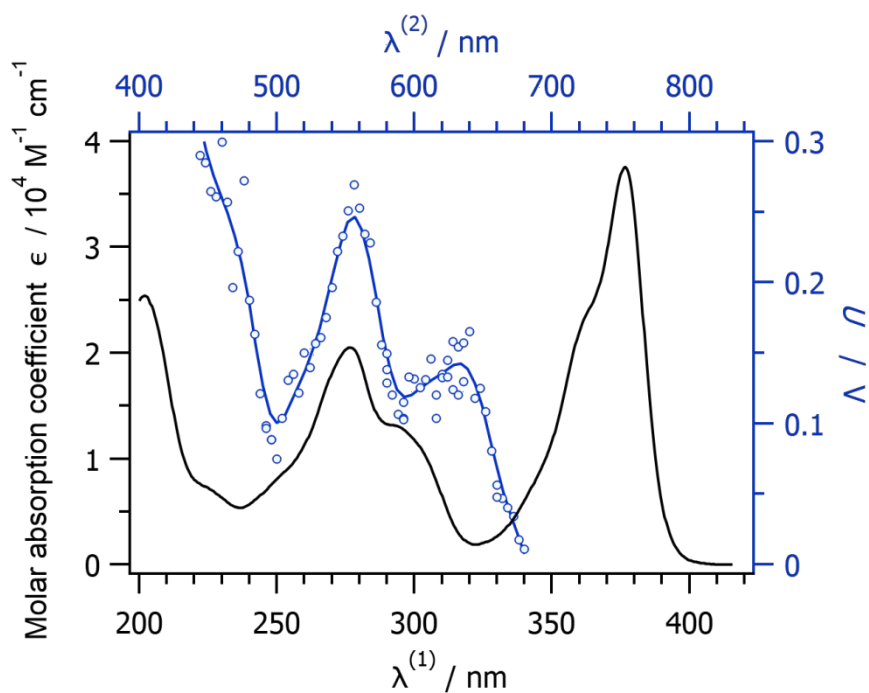


Figure 1. One- and two-photon absorption spectra of taDTGuo in water/acetonitrile [7:3(v:v)] (one-photon: black line, bottom and left axes and two-photon: blue line and circles, top and right axes).

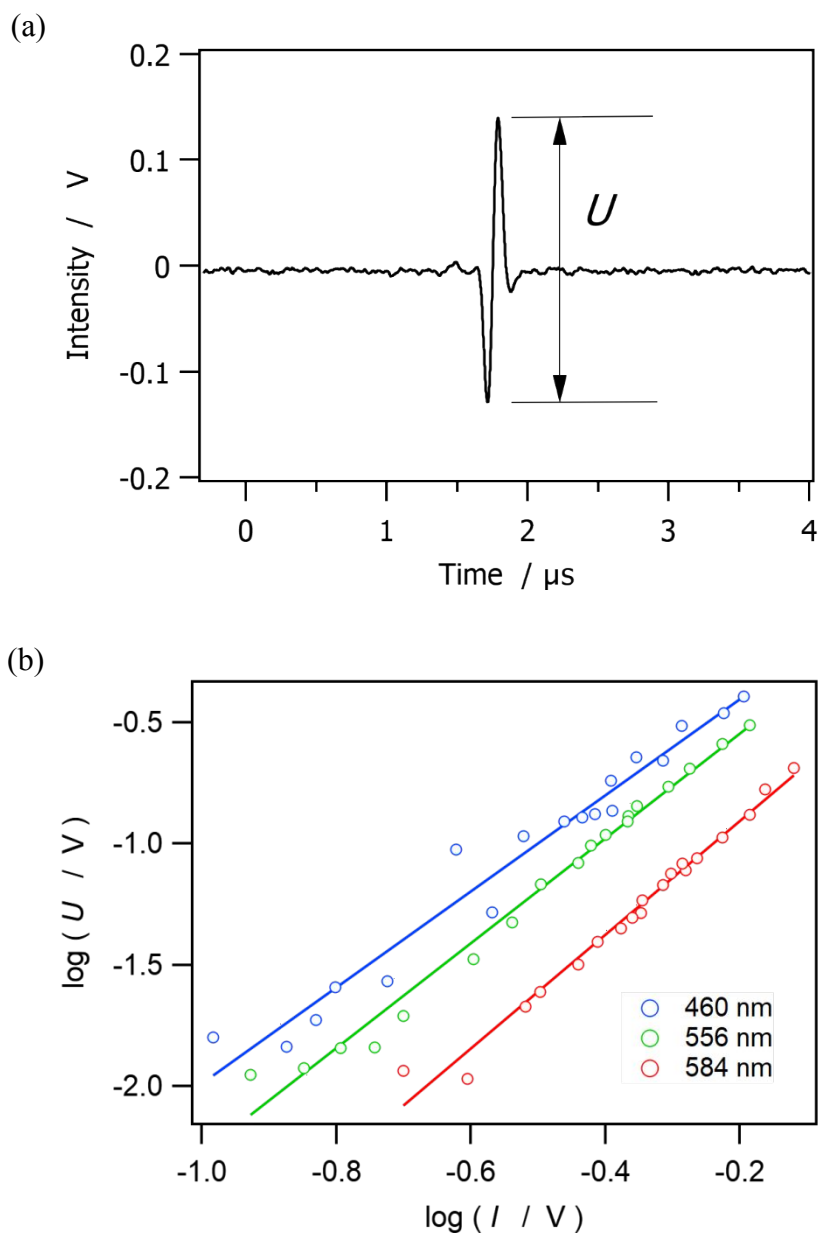


Figure 2. (a) Temporal profile of the OPPAS signal for taDTGuo (10 mM) in water/acetonitrile [7:3 (v:v)] excited at 556 nm, and (b) log-log plots of the laser power dependence of the OPPAS signal intensity at 460, 556, and 584 nm. Solid lines denote the best-fitting curves obtained by the least square's method.

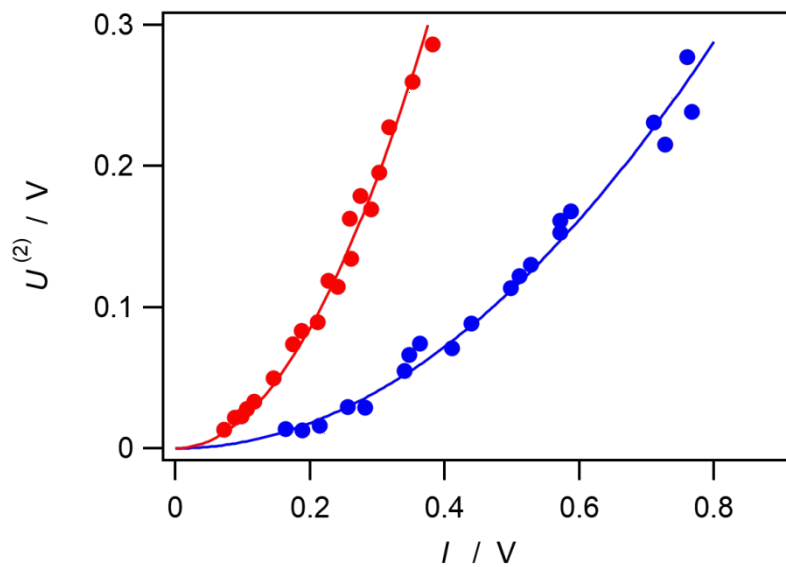


Figure 3. Plots of  $U^{(2)}$  for taDTGuo (10 mM) in water/acetonitrile (blue) and Bis-MSB (15 mM) in 1,4-dioxane (red) against the incident laser power at 556 nm.



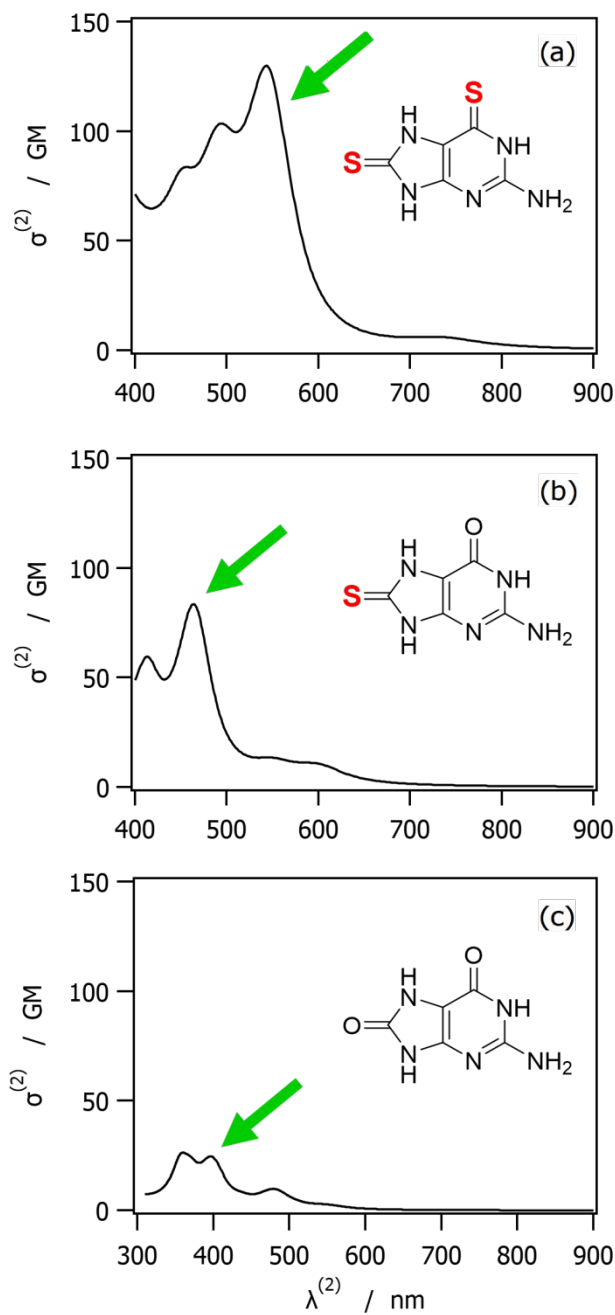


Figure 4. Simulated two-photon absorption spectra of (a) DTG, (b) 8TG, and (c) 8OG.

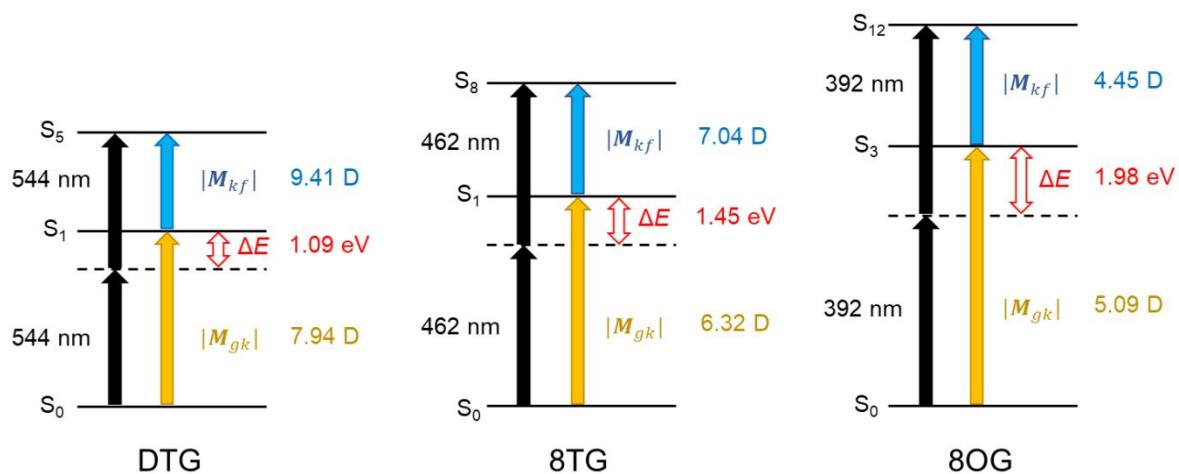


Figure 5. Energy diagrams for the intense two-photon absorption bands of guanine derivatives.

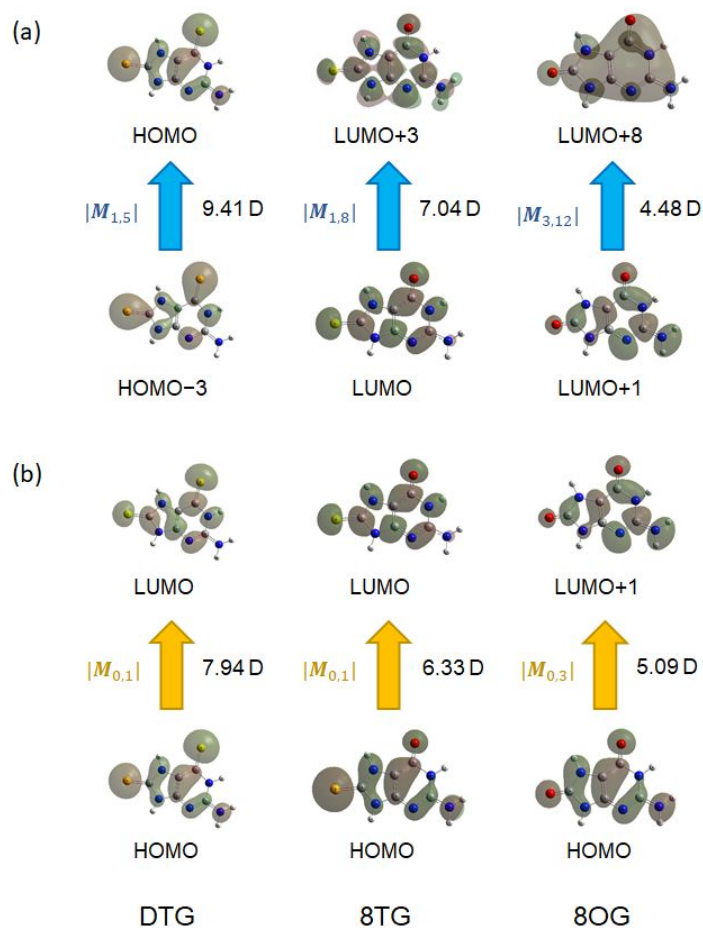
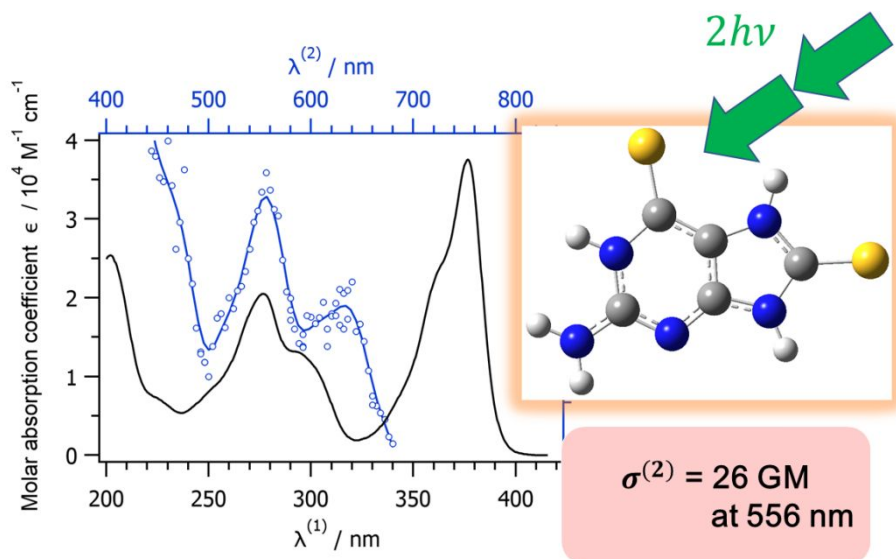


Figure 6. Molecular orbitals and transition dipole moments related to (a) the transition from the intermediate to the final states of guanine derivatives, and (b) the transition from the ground to the intermediate states.



TOC graphic