Significant effects of branches and bromine substitution of near-infrared two-photon photosensitizers on the generation of singlet oxygen

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This letter reports the influence of branches and bromine substitution of the photosensitizers on one- and two-photon absorption generation of singlet oxygen. Near-infrared femtosecond Ti:squassier laser was utilized to determine two-photon properties of the photosensitizers tuning wavelength from 700 to 880 nm at intervals of 20 nm. One- and two-photon optical and photophysical properties of the photosensitizers show significant dependence on the branches and substituted bromine atoms.

two-photon absorption, singlet oxygen, photosensitizers

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Numerous efforts have been devoted to singlet oxygen ($^1\text{O}_2$) since its discovery in the 1960s [1,2], which plays significantly important role in photodynamic therapy (PDT) due to its efficient damage to tumor cells [3–11]. Near-infrared photon has remarkable advantage, such as low energy, excellent penetration and negligible damage to normal biological tissues. Hence, the utilization of near-IR laser to irradiate two-photon triplet photosensitizers to generate singlet oxygen receives considerable attentions [12–18]. While unfortunately, near-infrared TPA photodynamic therapy has never reached its full potentials due to the absence of the criteria for the design of photosensitizers with large two-photon absorption cross sections and high singlet oxygen quantum yields. Bromine substitution is considered to be efficient approach to increase the quantum yields of singlet oxygen of TPA photosensitizers [16]. To our limited knowledge, it is unclear if bromine substitution has effects on the TPA properties of triplet photosensitizers so far (if yes, how big and why?). It is necessary to investigate the structure-activity interrelationship so that it would provide the

guidance to develop efficient TPA triplet photosensitizer for near-infrared PDT. Here, we present our recent efforts to clarify the effect of the branches and bromine substitution on one- and two-photon optical and photochemical properties of TPA photosensitizers. These photosensitizers divided by two groups (**G1** and **G2**) shown in Scheme 1 were obtained with satisfactory yields according to the routine procedures (Scheme S1), in which **C2–C4** and **C6–C8** were reported firstly herein.

Typical linear absorption and fluorescence spectra of the photosensitizers in tetrahydrofuran (THF) were presented in Figure 1. As shown in Figure 1(a) and (b), the ultraviolet/visible absorption spectra of these photosensitizers are affected by the bromine substitution, which plays more remarkable effects on the one-photon emission spectra of the photosensitizers. Figure 1(c) and (b) showed that one-photon emission of the photosensitizers of **G1** and **G2** were reduced gradually with the amount of substituted bromine atoms in THF respectively. It is accepted well that heavy atom effect of bromine atom in the excited states makes the extent of spin-orbit coupling of a molecule increased, and thus the intersystem cross constants of these photosensitizers

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Scheme 1 Chemical structures of two groups of the photosensitizers.

Figure 1 Typical UV/visible absorption spectra of some photosensitizers (a), (b) and representative emission spectra of the photosensitizers (c), (d) in THF. Performed under the same experimental condition, c : 1×10^{-5} mol L $^{-1}$.

are enhanced. Furthermore, the singlet crystal analysis of **C3** indicates that heavy weight bromine substitution could lower the coplanarity of these photosensitizers (Figure S1), which could be more remarkable for penta-bromine substitution of **C4** and **C8**. This has been confirmed by the molecular geometry optimization computation (Figure S2). The dipole moment changes between the excited states and the ground states of the photosensitizers in **G1** and **G2** are reduced with the substituted bromine atoms respectively (in the order as **C1**>**C2**>**C3**>**C4** and **C5**>**C6**>**C7**>**C8**, respectively (Table S1)), which in turn suggests that the extent of

intramolecular charge transfer (ICT) of the photosensitizers. These could be fundamental reasons to interpret the orderly reduction of emission intensity of the photosensitizers in **G1** and **G2** respectively. On the other hand, as shown in Figure 1(c) and (d), the photosensitizers in **G2** displayed stronger emission and larger fluorescence quantum yields (Φ) than those in **G1** respectively (for instance, Φ of **C2** is 0.062, Φ of **C6** is 0.309 in THF, ca. 6 times (Table 1), also can see Figures S3 and S4)), although twice amount of the bromine atoms are contained in the branches of the photosensitizers of **G2**. It looks abnormal but it is reasonable that as compared

Table 1 One-photon spectral parameters of the photosensitizers^{a)}

Photosensitizers	$\lambda_{\text{abs, max}}$ (nm)		ε (1×10 ⁵ L mol ^{−1} cm ^{−1})		$\lambda_{\text{em, max}}$ (nm)		Φ		
	Benzene	THF	Benzene	THF	Benzene	THF	Benzene	THF	
G1	C1	360	366	0.127	0.146	392	513	0.0055	0.097
	C2	357	364	0.112	0.136	391	508	0.0016	0.062
	C3	356	356	0.121	0.159	391	506	0.0010	0.039
	C4	354	355	0.099	0.125	390	501	0.0004	0.034
G2	C5	373	379	0.124	0.216	494	531	0.062	0.390
	C6	372	378	0.104	0.208	492	526	0.017	0.309
	C7	369	371	0.131	0.172	483	523	0.002	0.177
	C8	369	370	0.109	0.139	472	517	0.001	0.102

a) $\lambda_{\text{abs, max}}$, the maximal absorption wavelength; ε , molar extinction coefficient; $\lambda_{\text{em, max}}$, the maximal emission wavelength.

with the photosensitizers in **G1**, the molecular twist could be reduced by much heavier branches of the photosensitizers in **G2** due to more substituted bromine atoms, and the collision possibility of solute-solute in solution could be lowered by the larger molecule size that leads to diminish photo-induced solute-solute interaction (energy and electron transfer). As a result, the singlet internal conversion (i.e. non-radiative transition) for the photosensitizers of **G2** is reduced.

We utilized femtosecond Ti: sapphire laser to determine two-photon optical properties of the photosensitizers tuning wavelength from 700 to 880 nm at intervals of 20 nm (see the Supporting Information). TPA emission maxima of the photosensitizers under various frequencies near-IR laser are almost identical to those of one-photon, indicating one- and two-photon emission is from the same or similar excited states. Figure 2(a) and (b) presented typical TPA fluorescence emission spectra of the photosensitizers in THF excited by 760 nm. The photosensitizers in **G2** display stronger TPA fluorescence emission than those in **G1** respectively. We further measured TPA cross sections of the photosensitizers with the two-photon fluorescence excitation method (see the Supporting Information). TPA cross sections (σ_{TPA}) of these photosensitizers and TPA emission maxima excited by 760 nm laser were shown in Table 2. The data suggest

that the branched photosensitizers exhibit larger TPA cross sections than the linear ones, for example TPA cross section of **C2** is 42.9 GM, while TPA cross section of **C6** is 153 GM in THF. These could be ascribed to the larger transition dipole moment in the excited state of **C5–C8** because the molecular three-dimensional structures in the space could be extended significantly by the branches. We shall point out that TPA cross sections of the photosensitizers were reduced by the substituted bromine atoms under various laser frequencies, as shown in Figure 3(a) and (b). Owing to electron-withdrawing properties of the substituted bromine atoms, electron-donating nature of O-CH₂-Ar group could be lowered, and thus the extent of intramolecular charge transfer (ICT) of the compounds could be reduced by the substituted bromine atoms, and the transition dipole moment in the excited state of the photosensitizers could be diminished. This could lead to decrease TPA cross sections of the photosensitizers gradually with the substitution of bromine atoms. While even the smallest TPA cross sections of **C4** and **C8** in **G1** and **G2** (ca. 20 GM) are close to those of typical fluorene-based two-photon photosensitizers [19] suggesting that these photosensitizers have great potential for near-infrared PDT. Tables 1 and 2 also showed that the absorption maxima, one- and two-photon emission maxima of the branched photosensitizers were red-shifted with

Figure 2 Typical TPA fluorescence emission of the photosensitizers excited by 760 nm laser, $c: 1 \times 10^{-4} \text{ mol L}^{-1}$.

Figure 3 TPA cross sections of some typical sensitizers under various near infrared laser wavelength.

Table 2 TPA properties and quantum yields of singlet oxygen of the photosensitizers in various solvents

Photosensitizers		λ_{TPA} (nm)	σ_{TPA} (GM) ^{a)}	$\Phi(^1\text{O}_2)$	
		THF	THF	Benzene	THF
G1	C1	512	61.1	0.261	0.354
	C2	505	42.9	0.293	0.395
	C3	505	26	0.308	0.433
	C4	501	20	0.369	0.492
G2	C5	544	302	0.334	0.432
	C6	536	153	0.387	0.493
	C7	532	31	0.401	0.576
	C8	532	22	0.422	0.591

a) $1 \text{ GM} = 1 \times 10^{-50} \text{ cm}^4 \text{ s photo}^{-1} \text{ molecule}^{-1}$, under 760 nm laser.

respect to the linear ones respectively, which could be ascribed to the larger dipole moment changes and the lower HOMO-LUMO gaps of the branched photosensitizers in **G2** as well (Table S1).

According to Kasha's rule, the production of singlet oxygen is irrespective of the method by which initial excitation is realized, and thus the quantum yield of singlet oxygen generated upon two-photon excitation is considered to be the same as that produced upon one-photon excitation [16,17,20]. One-photon singlet oxygen quantum yields of the photosensitizers were determined by the photobleaching method of 9,10-diphenylanthracene (DPA) in this experiment (see the Supporting Information), and the data were also listed in Table 2. The singlet oxygen quantum yield quantifies the efficiency of singlet oxygen production by the excited-state photosensitizer. The results show that the singlet oxygen quantum yields of the photosensitizers are increased gradually with substituted bromine atoms, which reflects heavy atom effects on the excited states of the photosensitizers, for instance the quantum yield of singlet oxygen of **C1** is 0.354, while the quantum yield of singlet oxygen of **C4** reaches 0.492 in THF (ca. 1.4 times). It is accepted well that the generation of singlet oxygen is achieved by the energy transfer from the triple state of the photosensitizers and the ground state of molecular oxygen, meaning the quantum yields of singlet oxygen could be affected by multi-factors, in which bromine substitution does make the generation ability of singlet oxygen increased. Furthermore,

the branched photosensitizers have higher singlet oxygen quantum yields than the linear ones respectively (ca. 1.2 times). This suggests the branches carrying bromine atoms not only are able to inhibit singlet internal conversion of photosensitizers, but can facilitate intersystem cross from singlet state to triplet state.

We further employed photochemical method to determine the generation of singlet oxygen under one- and two-photon excitation (see the Supporting Information). We utilized regular visible light and near-IR laser to irradiate the oxygen-saturated solutions containing the photosensitizer and the substrates in quartz cells (Figure 4). The generated singlet oxygen could react with various substrates (such as 2,3-dihydro-2H-pyran, 2,3-dimethyl-butenen) to yield typical $^1\text{O}_2$ -photooxidation products. While as DBACO (an efficient singlet oxygen quencher) was added into the above photochemical systems, $^1\text{O}_2$ -photooxidation products could not be detected. These results demonstrate further that singlet oxygen could be generated by these photosensitizers via one- and two-photon process.

In conclusion, this brief report provides strong evidences that the branches and the amount of substituted bromine atoms show significant effect on one- and two-photon optical and photochemical properties of these photosensitizers. Although singlet oxygen quantum yields of the photosensitizers are enhanced, TPA cross sections are reduced by the substitution of multi bromine atoms more remarkably. Consequently, the substitution of multi bromine atoms

Figure 4 Photochemical approach for the detection of generated singlet oxygen via one- and two-photon process.

could be not right approach to design and synthesize new similar derivatives as ideal near-infrared TPA photosensitizers. The quantum yields of singlet oxygen and TPA cross sections of the photosensitizers are increased simultaneously with the introduction of one more same branch. We are working on the further enhancement of singlet oxygen and TPA cross sections by the construction of dendric or hyper-branched derivatives with substituted small amount of bromine atoms as TPA singlet oxygen photosensitizers, and we are going to develop water-soluble photosensitizers so that near-infrared laser photodynamic therapy of the photosensitizers will be evaluated in the near future.

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Supporting Information

Scheme S1 Synthesis route of the photosensitizers.

Figure S1 Single crystal analysis of **C3** (CCDC#821856).

Figure S2 Typical geometry optimization structures of **C4** and **C8**.

Table S1 Dipole moment difference and H-L gaps of the photosensitizers

Figure S3 Typical comparisons of one- and two-photon emission spectra of **C2** and **C6** in THF, performed at the same experimental condition.

Figure S4 Typical comparisons of normalized linear absorption and emission spectra of **C2** and **C6** in THF.

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