

# Synthesis and Characterization of New Fluorene-Based Singlet Oxygen Sensitizers

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The synthesis, photophysical characterization, and determination of singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) for a class of fluorene derivatives with potential application in two-photon photodynamic therapy (PDT) is reported. It has been demonstrated that these compounds possess the ability to generate singlet oxygen ( $^1\text{O}_2$ ) upon excitation. A photochemical method, using 1,3-diphenylisobenzofuran (DPBF) as  $^1\text{O}_2$  chemical quencher, was employed to determine the singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) of the fluorene-based photosensitizers in ethanol.  $\Phi_{\Delta}$  values ranged from 0.35 to 0.75. These derivatives may have potential application as two-photon photosensitizers when pumped via two-photon excitation in the near-IR spectral region.

**KEY WORDS:** photosensitizer; photodynamic therapy; singlet oxygen; two-photon absorption .

## INTRODUCTION

Photodynamic therapy (PDT) has been investigated over the past three decades and is currently an approved therapeutic modality for skin cancer (Levulan Kerastick<sup>®</sup>), the treatment of superficial bladder, early lung and advanced oesophageal cancers (Photofrin<sup>®</sup> II), and age-related macular degeneration (Visudyne<sup>®</sup>). In PDT, the absorption of light by a chromophore generates cytotoxic species such as reactive singlet oxygen  $\text{O}_2$  ( $^1\Delta_g$ ), leading to irreversible destruction of the treated tissue. Since it is well established that  $\text{O}_2$  ( $^1\Delta_g$ ) plays a dominant role in many photosensitization processes, great interest is focused on the determination of the singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) of organic molecules and the mechanism of singlet oxygen sensitization [1–3]. The quantum yield of sensitized production of singlet oxygen,  $\Phi_{\Delta}$ , is given by the sum of the contributions arising from

oxygen quenching of the lowest excited singlet state ( $S_1$ ) and the lowest excited triplet state ( $T_1$ ) of the sensitizer, i.e.

$$\Phi_{\Delta} = \Phi_{\Delta}(S_1) + \Phi_{\Delta}(T_1) \quad (1)$$

The quenching of excited singlet  $S_1$  and triplet states  $T_1$  of the sensitizer by molecular oxygen  $\text{O}_2$  ( $^3\Sigma_g^-$ ) leads to the formation of the excited states  $\text{O}_2$  ( $^1\Sigma_g^+$ ) and  $\text{O}_2$  ( $^1\Delta_g$ ) (which lie 157 and 94  $\text{kJ mol}^{-1}$ , respectively, above the  $\text{O}_2$  ( $^3\Sigma_g^-$ ) ground state.

Wilkinson *et al.* compiled the singlet oxygen quantum yields of 755 compounds, some values for the fraction of  $S_1$  and  $T_1$  states that are quenched by oxygen providing  $\text{O}_2$  ( $^1\Delta_g$ ) ( $f^S_{\Delta}$  and  $f^T_{\Delta}$ , respectively), and rate constants for singlet oxygen deactivation in solution ( $k_d$ ) [1, 2]. Schweitzer and Schmidt recently reviewed the physical mechanisms of generation and deactivation of singlet oxygen, including the parameters influencing the generation of singlet oxygen [3]. Ogilby *et al.* reported the two-photon sensitized generation of singlet oxygen via near-IR short-pulse excitation, providing impetus for the development of near-IR two-photon excitable singlet oxygen photosensitizers for PDT [4, 5].

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Our laboratory has been involved in the study of the photophysical properties of new two-photon absorbing organic materials due to a number of important applications such as fluorescence imaging, two-photon microfabrication, and optical power-limiting devices. Some aspects of the photophysical properties studied include one- and two-photon photostability of several new fluorene-based compounds and their nonlinear optical spectroscopic characterization [6–9]. Systematic variation of molecular structure has been accomplished, providing derivatives of varying electronic character. Some of these derivatives incorporate heavy atoms and are particularly interesting for PDT due to the expected increase in the rate of intersystem crossing (ISC) to the triplet excited state. In this paper, we report the results of the evaluation of the ability of several fluorene derivatives to produce  $O_2$  ( $^1\Delta_g$ ) upon one-photon excitation. The particular interest in these compounds is in their potential use as two-photon photosensitizers in the near-IR spectral region in biological and medical applications.

## EXPERIMENTAL

### Fluorene Derivatives

The structures of the compounds studied are shown in Fig. 1. The synthesis and characterization of 7-iodo-9,9-didecyl-2-nitrofluorene (**1**) and 2-(9,9-didecyl-7-nitrofluoren-2-yl)benzothiazole (**2**) have been reported [8]. 2-Iodo-7-nitro-9H-fluorene and 2-bromo-9,9-didecyl-7-iodofluorene were synthesized according to a literature procedure [10]. All other chemicals were used as received.

#### *Synthesis of 9,9-didecyl-2-nitro-7-vinylfluorene (3)*

7-Iodo-9,9-didecyl-2-nitrofluorene (2.6 g, 4.20 mmol), tributyl (vinyl)tin (2.04 g, 6.43 mmol), Pd(dppf)  $Cl_2 \cdot CH_2Cl_2$  (0.059 g, 0.062 mmol) and CuI (0.028 g, 0.096 mmol) were dissolved in 25 mL of dry THF at room temperature under Ar. The reaction mixture was heated at reflux for 82 hr. The reaction progress was followed by  $^1H$  NMR in  $CDCl_3$  until 7-iodo-9,9-didecyl-2-nitrofluorene could not be detected. Upon completion, the dark brown mixture was cooled to room temperature, filtered, and concentrated under reduced pressure. The product was poured into water and extracted with  $CH_2Cl_2$ . The organic solution obtained was then dried over anhydrous  $MgSO_4$ . A brown oil was obtained after evaporating the solvent. The oil was purified by a silica gel column using first a mixture of hexane and ethyl acetate (9:1), followed by hexane as eluent, resulting in 2.12 g (97%) of yellow oil. Anal. Calcd. for  $C_{35}H_{51}NO_2$ : C, 81.19; H, 9.93;

N, 2.71; Found: C, 81.14; H, 10.00; N, 2.74;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$ : 8.26 (d, 1H,  $J = 6.6$  Hz), 8.19 (s, 1H), 7.75 (t, 2H,  $J = 8.7$  Hz), 7.45 (d, 1H,  $J = 7.8$  Hz), 7.40 (s, 1H), 6.81 (q, 1H,  $J = 17.7$  Hz), 5.84 (d, 1H,  $J = 17.4$  Hz), 5.33 (d, 1H,  $J = 10.8$  Hz), 2.02 (m, 4H), 1.16 (bm, 28H), 0.84 (t, 6H), and 0.57 (bs, 6H).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$ : 153.0, 152.3, 147.5, 147.2, 138.8, 138.7, 137.1, 125.9, 123.5, 121.5, 121.0, 119.9, 118.4, 114.9, 55.8, 40.3, 32.0, 30.0, 29.7, 29.4, 29.4, 26.8, 23.9, 22.8, 14.3. FT-IR (KBr,  $cm^{-1}$ ): 3063 (ArCH), 2925, 2854 (alCH), 1629 (C=C), 1521 ( $NO_2$  as), 1337 ( $NO_2$  sy), 906 ( $=CH_2$ ).

#### *Synthesis of 2-bromo-9,9-didecyl-7-iodofluorene (4) [10]*

2-Bromo-9,9-didecylfluorene (2.44 g, 4.64 mmol) was dissolved in acetic acid (75 mL) with stirring at room temperature under  $N_2$ .  $I_2$  (0.588 g, 4.63 mmol) was then added, and stirred at room temperature for 30 min. Concentrated  $H_2SO_4$  (8 mL) and  $NaNO_2$  (0.325 g, 4.71 mmol) were then added to the reaction mixture, turning the clear orange-brown liquid cloudy. The reaction mixture was heated to 80 °C and maintained for 2 hr, cooled to room temperature, and diluted in water. The reaction mixture was extracted into hexanes, washed with 5%  $NaHSO_3$ , and dried over  $MgSO_4$ . The product was isolated using a column of silica gel with hexanes as the eluent, producing a clear oil (2.15 g, 71% yield) that crystallized on standing 2–3 days.  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$ : 7.63 (d, 2H,  $J = 6$  Hz), 7.44 (m, 4H), 1.89 (t, 4H,  $J = 9$  Hz), 1.12 (bm, 28H), 0.85 (t, 6H), 0.57 (bs, 4H).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$ : 152.8, 152.4, 139.8, 139.2, 136.1, 132.2, 130.2, 126.2, 121.7, 121.6, 121.3, 93.2, 56.0, 40.5, 32.3, 30.3, 29.9, 29.8, 29.7, 29.6, 24.1, 23.1, 14.6.

### Materials

1,4-Diazabicyclo[2,2,2]octane (DABCO, Alfa Aesar 99%),  $NaN_3$  (Aldrich 99%), and 2,2,6,6-tetramethyl-1-piperidinyloxy- (9CI) (TEMPO, Aldrich) were used as received. The singlet oxygen acceptor 1,3-diphenylisobenzofuran (DPBF) was purchased from Acros Organics (99+%). The sensitizers, rose bengal (Kodak laser grade) and methylene blue (Matheson & Co.), were used as received.

### Quantum Yields of Singlet Oxygen Generation in Ethanol

Though singlet oxygen quantum yields are often now determined from singlet oxygen luminescence at 1270 nm, using cooled Ge and InGaAs detectors, we employed a photochemical method, using 1,3-diphenylisobenzofuran

(DPBF) as  $^1\text{O}_2$  chemical quencher to determine the singlet oxygen quantum yields ( $\Phi_\Delta$ ) of the fluorene-based photosensitizers in ethanol. Solutions containing different concentrations of DPBF ( $\sim 5 \times 10^{-7}$  to  $\sim 2 \times 10^{-6}$  M) and the sensitizer (RB  $9.7 \times 10^{-7}$  M for rose bengal,  $1 \times 10^{-6}$  M for methylene blue,  $8 \times 10^{-7}$  M for haemtoporphyrin, and  $\sim 8 \times 10^{-7}$  to  $1.5 \times 10^{-6}$  M for the fluorene-based compounds) were prepared in ethanol on the day of use and stored in the dark. Solutions were saturated with air and placed in a  $12 \mu\text{L}$  quartz microcuvette. The photosensitizers were excited at their  $\lambda_{\text{max}}$ . The fluorescence intensity of DPBF was measured in the photon-counting regime using a weak Xe lamp as a probe source, monitored at 450 nm. Excitation of the DPBF alone did not significantly change its initial concentration during the time of exposure in this experiment. To simplify the kinetic analysis, DPBF was irradiated to low conversions ( $\leq 10\%$ ), such that its concentration may be assumed to be fixed at the initial value.

The relative singlet oxygen quantum yields ( $\Phi_\Delta$ ) were calculated from the DPBF-photooxidative product quantum yield ( $\Phi_D$ ) according to [11, 12]

$$\frac{1}{\Phi_D} = \frac{1}{\Phi_\Delta} + \frac{1}{\Phi_\Delta} \frac{k_d}{k_a} \frac{1}{C_{\text{DPBF}}} \quad (2)$$

where  $k_d/k_a$  is constant for the DPBF decomposition in certain solvent [12]. Equation (1) was used to obtain the value of  $\Phi_\Delta$  from the Stern–Volmer plot, ( $1/\Phi_D$ ) versus ( $1/C_{\text{DPBF}}$ ). The determination of  $\Phi_D$  was based on the following assumptions. The entire volume of the sensitizer solution was irradiated in the microcuvette simultaneously. The absorbance before irradiation of the solutions in the microcuvette in the direction of the excitation beam (1.5 mm pass) did not exceed 0.1 and the irradiation intensity,  $I_0$ , was approximately constant in the entire volume of the microcuvette. The absorbance and fluorescence of the photochemical products of the reaction of singlet oxygen with DPBF are negligible in the spectral region of the observed fluorescence and did not effect the fluorescence intensity of DPBF [12]. For these conditions, the expression for the photochemical bleaching of DPBF in the presence of singlet oxygen is

$$\Phi_D = \frac{C_0 - C_t}{I_0(1 - 10^{-A}) \frac{St}{V}} \quad (3)$$

$C_0$  and  $C_t$  ( $\text{mol}/\text{m}^3$ ) are the initial (before irradiation) and final (after irradiation) concentrations of the DPBF solution.  $I_0$  is the intensity of the beam at the irradiation wavelength (adjusted to be  $\sim 1 \times 10^{18}$  photons/s  $\text{m}^2$ ) measured with a Laserstar powermeter (Ophir Optronics Inc.) with sensitivity in nW range.  $V$  is the irradiation volume in  $\text{m}^3$ ,  $A$  is the absorbance of the sensitizer at the irradiation wavelength,  $S$  is the irradiation area in  $\text{m}^2$ , and  $t$  is time in s.

## RESULTS AND DISCUSSION

### Photophysical Characterization and $\text{O}_2$ ( $^1\Delta_g$ ) Sensitization

Structures for fluorene derivatives used in this study are presented in Fig. 1.

The absorption spectra of the derivatives in ethanol are shown in Fig. 2. Absorption spectra were obtained with a Cary-3 UV-Vis spectrophotometer.

Steady-state fluorescence spectra were measured in the photon counting regime for dilute solutions (concentrations  $\leq 2 \times 10^{-6}$  M) in a 10 mm quartz cuvette with a PTI QuantaMaster spectrofluorimeter. The fluorescence of each compound in ethanol was negligible (fluorescence quantum yields are  $< 1 \times 10^{-2}$  for derivatives 1–4).

Previous studies have demonstrated that fluorene and some of its derivatives produce  $\text{O}_2$  ( $^1\Delta_g$ ) upon quenching of the triplet excited state by ground state  $\text{O}_2$  ( $^3\Sigma_g^-$ ). In 1970, Gollnick *et al.* reported a  $\Phi_\Delta$  for fluorene in methanol of 0.1 [13]. Gorman *et al.* determined the overall efficiency of singlet oxygen sensitization during  $\text{O}_2$  quenching of  $T_1$ ,  $S_\Delta$ , of fluorene and other aromatic hydrocarbons by time-resolved luminescent measurements supported by 1,3-diphenylisobenzofuran bleaching experiments [14]. In nonaromatic solvents,  $S_\Delta$  was found to be unity. In contrast,  $S_\Delta$  determined in benzene and toluene varies significantly from one hydrocarbon to another.  $S_\Delta$  evaluated for fluorene in benzene was 0.66. Recently, Schmidt *et al.* measured the triplet energies  $E_T$  and free energies  $\Delta G_{\text{CET}}$  for a complete electron transfer and rate constants  $k_T^{1\Sigma}$ ,  $k_T^{1\Delta}$ , and  $k_T^{3\Sigma}$  of formation of  $\text{O}_2$  ( $^1\Sigma_g^+$ ),  $\text{O}_2$  ( $^1\Delta_g$ ), and  $\text{O}_2$  ( $^3\Sigma_g^-$ ) during  $\text{O}_2$  quenching of  $T_1$  for a series of fluorene derivatives (1-methylfluorene, 2-methylfluorene, 2-bromofluorene, 2,7-dibromofluorene, and 2,4,7-trichlorofluorene) [15, 16].

To determine the ability of the fluorene derivatives 1–4 to sensitize the formation of  $\Phi_\Delta$ , the kinetics of the reaction of DPBF with  $\text{O}_2$  ( $^1\Delta_g$ ) in the presence of the

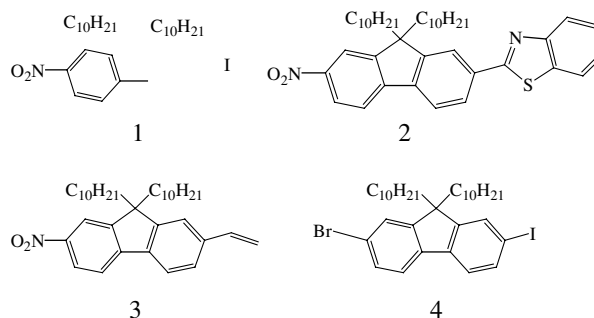


Fig. 1. Structures of fluorene derivatives.

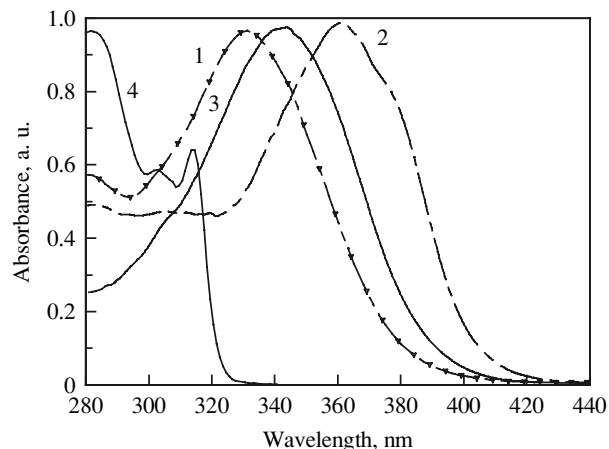


Fig. 2. Absorption spectra of 1–4 in ethanol.

fluorene-based photosensitizers and in the presence of known  $O_2$  ( $^1\Delta_g$ ) quenchers such as sodium azide and 1,4-diazabicyclo[2,2,2]octane (DABCO) [11,17] were determined.

In the presence of  $O_2$  ( $^1\Delta_g$ ) (photosensitized by fluorene **2**), photooxidation of DPBF produces a non-fluorescent product, and the reaction of DPBF with  $O_2$  ( $^1\Delta_g$ ) can be monitored by measuring the decrease in the fluorescence intensity of DPBF with time, as shown in Fig. 3(1), in the absence and presence of singlet oxygen quenchers.

Both DPBF and DABCO are  $^1O_2$  quenchers. The competition for  $^1O_2$  to react with DPBF versus DABCO was manifested in a slower decrease in the fluorescence intensity of DPBF with time, as shown in Fig. 3(2). The same effect was observed when DPBF was irradiated in the presence of  $NaN_3$ , as shown in Fig. 3(3). Since both DABCO and  $NaN_3$  quench the  $^1O_2$  photosensitized by fluorene **2**, the net effect observed was the decrease in the decay of the fluorescence of DPBF, representing a decrease in the formation of the DPBF photooxidative product.

1-Piperidinyloxy, 2,2,6,6-tetramethylradical (TEMPO), a radical scavenger, was added to the reaction solution, to evaluate if radicals were present. Upon illumination, in the presence of oxygen at concentrations up to  $1 \times 10^{-5}$  M, no influence on the reaction of the  $^1O_2$  (photosensitized by fluorene **3**) with DPBF was observed (Fig. 4). Therefore, the participation of radicals such as  $\bullet OH$ ,  $HO_2\bullet$  or  $O_2\bullet^-$  was unlikely.

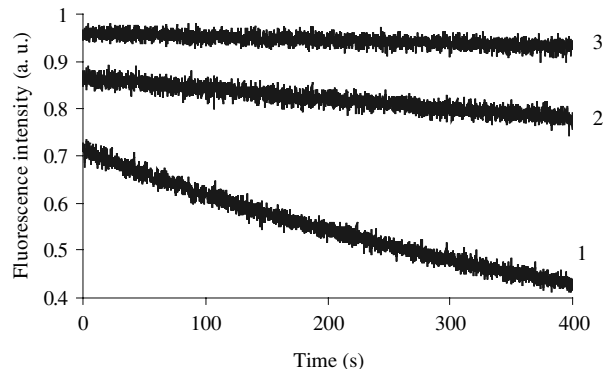


Fig. 3. Time-based fluorescence emission of DPBF, monitored at 450 nm. (1) **2** and DPBF; (2) **2**, DPBF, and DABCO; (3) **2**, DPBF, and  $NaN_3$  during irradiation.

### Singlet Oxygen Quantum Yield of Fluorene-Based PS

The photochemical method using DPBF as a trap was implemented as described in the experimental section. Values of  $\Phi_{\Delta}$  measured for rose bengal, methylene blue, and haematoporphyrin were in good agreement with photophysically determined values reported in the literature (direct detection of the singlet oxygen luminescence at 1270 nm) and the values determined by a photochemical method using DPBF, published by Spiller (Table I) [12]. Values for  $\Phi_{\Delta}$ , calculated for the compounds studied here, are summarized in Table I.

Figure 5 shows the Stern–Volmer plot ( $1/\Phi_D$ ) versus ( $1/C_{DPBF}$ ) for compound **2**. From the value of the intercept (1.87), a  $\Phi_{\Delta}$  of 0.53 was obtained. From the value of the slope ( $1.41 \times 10^{-4}$ ), a  $k_d/k_a$  (or  $\beta$ ) value of  $7.54 \times 10^{-5}$  mol L $^{-1}$  was obtained, similar to the one reported by Spiller ( $7.57 \times 10^{-5}$  mol L $^{-1}$ ) [12]. As can be seen in

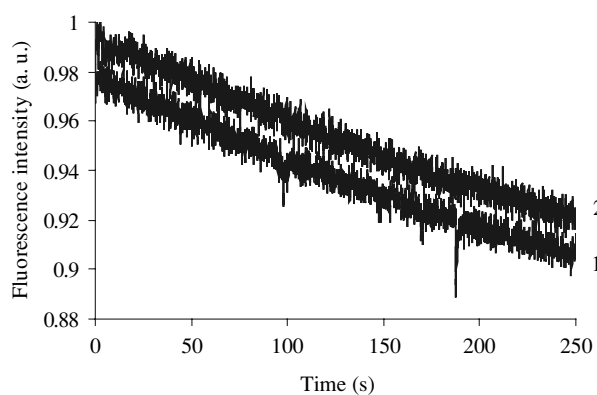


Fig. 4. Lack of quenching of fluorene **2** sensitized photooxidation of DPBF by TEMPO. (1) DPBF and **2**, (2) DPBF, **2**, and TEMPO.

**Table I.** Singlet Oxygen Quantum Yields ( $\Phi_{\Delta}$ ) of Different PS in Ethanol Obtained by Chemical Quenching of DPBF and Published Values

Compound	$\lambda$ (nm)	$\Phi_{\Delta}$ measured (DPBF) <sup>a</sup>	Published values of $\Phi_{\Delta}$ obtained by		
			DPBF method	SOLM <sup>b</sup> ( $\pm 0.05$ )	Other photochemical methods
Rose bengal	560	$0.80 \pm 0.08$	$0.79 \pm 0.06^c$ $0.86^c$	$0.79 \pm 0.05^c$	$0.68^c$
Methylene blue	660	$0.50 \pm 0.05$	$0.49^c$ $0.49^c$	$0.42^c$	$0.52^c$ $0.50^c$
HP	400	$0.55 \pm 0.05$			$0.57 \pm 0.04^d$
<b>1</b>	344	$0.35 \pm 0.03$			
<b>2</b>	362	$0.53 \pm 0.05$			
<b>3</b>	357	$0.53 \pm 0.05$			
<b>4</b>	314	$0.75 \pm 0.08$			

<sup>a</sup>Our experimental data.

<sup>b</sup>Singlet oxygen luminescent method.

<sup>c</sup>Data published by Spiller [12].

<sup>d</sup>Data published by Roitman [18].  $\Phi_{\Delta}$ : singlet oxygen quantum yield.

Table I, the fluorene derivatives exhibited  $^1\text{O}_2$  quantum yields of 0.35–0.75, equivalent to or greater than that reported for good  $^1\text{O}_2$  sensitizers (rose bengal, methylene blue, and HP).

## CONCLUSIONS

The photophysical characterization of fluorene derivatives incorporating heavy atoms, and the determination of their singlet oxygen quantum yields ( $\Phi_{\Delta}$ ), facilitated the identification of lead compounds with efficient

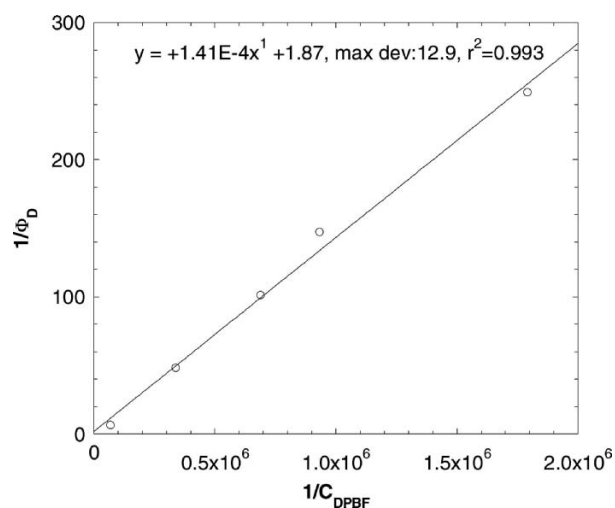
singlet oxygen sensitization, with  $\Phi_{\Delta}$  ranging from 0.35 to 0.75. Singlet oxygen was determined to be the oxidant under photooxidation conditions with the fluorene-based photosensitizers. No effect was observed in the efficiency of  $^1\text{O}_2$  scavenging by DPBF in the presence of TEMPO, demonstrating that formation of radicals is unlikely. Although the absorption spectra of these derivatives are blue-shifted relative to phthalocyanines and porphyrins with high values of  $\Phi_{\Delta}$ , these derivatives are well suited for two-photon excitation and have potential applications as two-photon photosensitizers when excited in the near-IR spectral region. Preliminary studies of similar fluorene derivatives have indicated very low cytotoxicity, a subject for further investigation [19].

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**Fig. 5.** Linear fit for the photo-oxidation of DPBF using compound **2** in ethanol.

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