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4,5-Dimethylthio-4′-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene, a Highly Selective and Sensitive Chemiluminescence Probe for Singlet Oxygen

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Abstract: 4,5-Dimethylthio-4′-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene has been designed and synthesized as a highly selective and sensitive chemiluminescence (CL) probe for singlet oxygen ($^1O_2$). The design strategy for the probe is directed by the idea of photoinduced electron-transfer process and carried out through the incorporation of electron-rich tetrathiafulvalene unit into a reactive luminophore of anthracene specific for $^1O_2$. Upon reaction with reactive oxygen species (ROS), such as hydrogen peroxide, hypochlorite, superoxide, hydroxyl radical, or $^1O_2$, the probe exhibits both strong CL response to and high selectivity for $^1O_2$ only, rather than the other ROS. This remarkable CL property permits $^1O_2$ to be distinguished easily from the other ROS and makes the probe possible to be used widely for $^1O_2$ detection in many chemical and biological systems and even in light water (H_2O) environments. This applicability has been demonstrated by monitoring the $^1O_2$ generation in a metal-catalyzed decomposition system of *tert*-butyl hydroperoxide. Moreover, the CL reaction mechanism of the present system is also discussed, clearly confirming that the introduction of electron-rich tetrathiafulvalene into the 9-position of anthracene can greatly activate its reactivity toward $^1O_2$.

Introduction

Singlet oxygen ($^1O_2$), a nonradical reactive oxygen species, is an excited state of molecular oxygen that is generated by 94 kJ of energy transfer to ground-state triplet oxygen ($^3O_2$).[1] The chemical reactivity of $^1O_2$ is well characterized because of its extensive use in organic synthesis.[2,3] Like other ROS (reactive oxygen species), such as superoxide ion, hydroxyl radical, and hydrogen peroxide, $^1O_2$ is also believed to be an important species for oxidation in biological processes.[4] Several enzyme systems (e.g., lipoxygenase, peroxidase, and even eosinophil peroxidase) have been identified as biochemical sources of $^1O_2$,[5] and evidence has accumulated indicating that $^1O_2$ is implicated in the genotoxic effect of the ultra violet A (320−400 nm) component of solar radiation and likely plays an important role in the cell signaling cascade and in the induction of gene expression.[6] However, some results are still controversial, mainly because of the lack of a reliable detection method for $^1O_2$.[2,7] On one hand, to distinguish $^1O_2$ from a variety of other ROS, a technique with high selectivity is required; on the other hand, the technique should also possess high sensitivity due to low production and the short lifetime ($\sim 3\ \mu s$)[2] of $^1O_2$ in aqueous environments.

Monitoring the direct emission of $^1O_2$ at 1270 nm is a specific and noninvasive method, but its use in biological reactions is sometimes problematic because of the low efficiency for $^1O_2$ emission.[2] Chemical trapping by spectroscopic probes is also found to be specific and much more sensitive than the detection of the 1270 nm luminescence; therefore, most investigators employ chemical singlet oxygen traps to corroborate the involvement of $^1O_2$ in chemical and biological reactions.[7,8] The commonly used $^1O_2$ trap is 9,10-diphenylanthracene, which reacts specifically with $^1O_2$ to form an endoperoxide accompanied by the decrease in absorbance at 355 nm as a sign of $^1O_2$ production.[9] However, such detection is not very sensitive because it is based on the measurement of absorbance. To improve the sensitivity, Nagano et al. developed fluorescent probes by incorporating the reactive anthracene moiety into a xanthene ring. These probes react with $^1O_2$ to yield the corresponding endoperoxides giving a sensitive fluorescence response.[10]

Alternatively, chemiluminescent traps for $^1O_2$, not requiring excitation light sources, can be applied in certain cases (e.g.,

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10.1021/ja0481530 CCC: $27.50

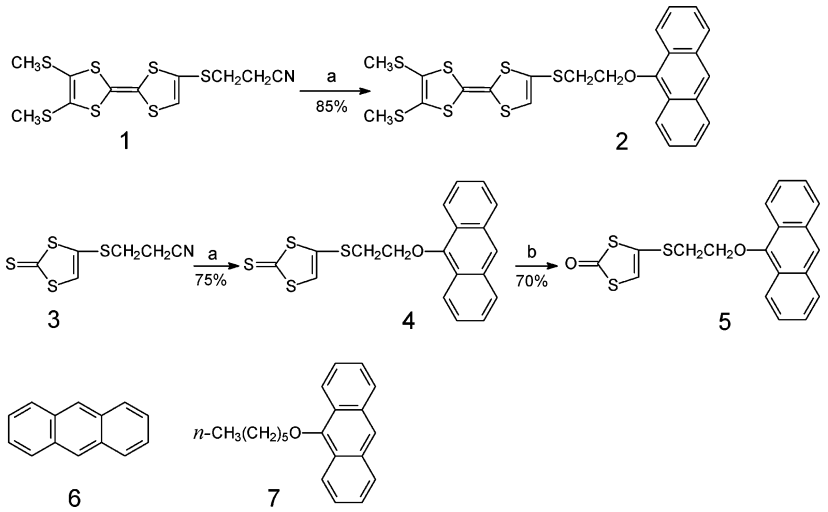

Figure 1. Syntheses of compounds **2** and **5**, and the structures of reference compounds. Reagents: (a) $CsOH·H_2O$, 9-(2-bromoethoxy)anthracene, THF; (b) $Hg(CH_3COO)_2$, CH_2Cl_2.

eliminating background fluorescence and various light scattering to improve signal-to-noise ratio). Furthermore, due to the high sensitivity of chemiluminescence (CL) detection, only relatively low probe concentrations are necessary, thereby decreasing the likelihood of artifactual interference of secondary reactions.[8] Nevertheless, only limited CL probes are available so far for $^1O_2$ assay,[11−13] particularly for $^1O_2$ selective detection.[14] Herein we report 4,5-dimethylthio-4′-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene **2** (Figure 1) as a highly selective and sensitive CL trap for this purpose.

Compound **2** is designed by incorporating a strong electron donor of tetrathiafulvalene (TTF)[15,16] into a reactive luminophore of anthracene specific for $^1O_2$.[9,10,17] The strong electron-releasing ability of TTF would facilitate the photoinduced electron transfer[18] between TTF and anthracene units,[15] leading to weak fluorescence, which is desired for a low background signal. Moreover, the electron-rich character of TTF would also promote the reactivity of anthracene unit toward $^1O_2$,[8,14,19] benefiting the $^1O_2$ trapping. Thus, we expect the designed molecule **2** might have some special properties upon reacting with $^1O_2$. In this paper, the results of detailed studies on the synthesis of the probe **2** and its CL properties for trapping $^1O_2$ are presented. Strong CL is found upon the reaction of **2** with $^1O_2$ only, but not with the other ROS such as hydrogen peroxide, hypochlorite, superoxide, and hydroxyl radical, showing an extremely high selectivity for $^1O_2$. The probe's applicability to the detection of $^1O_2$ has been demonstrated by analyzing $^1O_2$ production during the metal-catalyzed decomposition of *tert*-butyl hydroperoxide. The CL reaction mechanism of **2** with $^1O_2$ is also explored.

Experimental Section

Reagents and Materials. Anthrone and $CsOH·H_2O$ were purchased from Acros (Belgium). Hydrogen peroxide, sodium hypochlorite, *tert*-butyl hydroperoxide, ferrous ammonium sulfate, deuterium oxide, and tetrahydrofuran (THF) were obtained from Beijing Chemical Company. Syntheses and characterization of compounds **1**−**5** and **7** are presented in the Supporting Information. Prior to use, hydrogen peroxide was diluted immediately from a stabilized 30% solution, and was assayed by using 43.6 M^{-1} cm^{-1} as the molar absorptivity at 240 nm.[20] Hypochlorous acid was prepared by distillation from the 5% commercial sodium hypochlorite solution and stored, for periods less than one week, at 4 °C as a 300 mM solution with a pH of 11 adjusted by the addition of sodium hydroxide. Before use, sodium hypochlorite was assayed using a molar absorptivity of 391 $M^{-1}cm^{-1}$ at 292 nm.[21] *tert*-Butyl hydroperoxide (70% in water) was assayed by oxidation of iodide ion in acetic acid using hydrogen peroxide as a standard.[22] Deuterium oxide (99.8% purity) was used without further purification. THF was distilled from sodium/benzophenone. The stock solution of probe **2** (200 μM) was prepared in THF. All other chemicals were local products of analytical grade. Deionized and distilled water was used throughout.

Instruments. Lumat LB 9507 (EG & G BERTHOLD, Bad Wildbad, Germany) was used for CL measurements. This apparatus equipped with a variable automatic volume injector has a function of monitoring kinetic behavior of light emission; the emitted light is measured with a selected high sensitivity, low noise photo multiplier. Its spectral sensitivity covers a range of 390−620 nm. CL and fluorescence spectra were recorded with a Hitachi F-2500 spectrofluorimeter; in the measurement of CL spectrum, the excitation light source was switched off. Absorption spectra were obtained with Techcomp UV-8500 spectrophotometer (Shanghai, China). A model 25 pH-meter was used for pH measurements.

CL Reaction and Detection. All experiments were run at 25 °C in 50 mM sodium phosphate buffer (pH 7) containing 50% (v/v) THF as

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a cosolvent unless otherwise noted. Typically, a 1 mL portion of the phosphate buffer containing 20 μM of the probe **2** and an appropriate concentration of the reactant (e.g., 1 mM hydrogen peroxide or other ROS) was placed in a test tube in the CL detector. The reaction was initiated by rapid automatic injection of 0.1 mL sodium hypochlorite, and CL was measured as the integral of the CL intensity in RLU (relative light units) over the total reaction period (typically 5 s) with Lumat LB 9507 luminometer. Each of the data was expressed as the mean of three determinations with a relative error of less than ±5%.

ROS Production and $^1O_2$ Detection. Superoxide solution ($O_2^{-\bullet}$) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min.[23] Hydroxyl radical ($^\bullet$OH) was generated through the Fenton reaction of ferrous ammonium sulfate and hydrogen peroxide.[24] $^1O_2$ was chemically generated from the H_2O_2/MoO_4^{2-} system in alkaline media[10] or from the H_2O_2/NaOCl system in neutral and alkaline media.[25]

Quantitative measurements of $^1O_2$ generated were made according to the Kanofsky's method.[5] Briefly, the H_2O_2/NaOCl reaction at pH 7 was used as standard, since the yield of singlet oxygen is near 100% under this condition. In our experiment, the calibration curve for $^1O_2$ was derived from the integrated emission intensity of the H_2O_2/NaOCl/**2** reaction in phosphate buffers with 20 μM of **2**, 10 mM of NaOCl and a series of H_2O_2 concentrations of 1 mM or less. The production of $^1O_2$ in a metal-catalyzed decomposition system of *tert*-butyl hydroperoxide was quantified by comparing the time integral of the CL intensity from this system with the above calibration curve. Effects of D_2O and sodium azide on this CL reaction were also examined.

Results and Discussion

Design of Probe 2 and Its Spectroscopic Properties. There are two separate basic reaction processes to be considered for designing a CL probe for $^1O_2$. One is the chemically selective trapping and the other is the efficient CL generation. As is known, $^1O_2$ manifests substantial reactivity toward electron-rich organic molecules,[4] and the anthracene skeleton reacts specifically with $^1O_2$ producing CL.[9,10,17] Therefore, the introduction of electron-donating groups into the anthracene moiety may enhance its reactivity toward $^1O_2$, and a stronger CL might be expected to occur during oxidation. Toward this end, we take advantage of the strong electron donating property of TTF in making a probe molecule for $^1O_2$, and such a probe was synthesized by coupling a TTF motif to the well-known luminophore of anthracene, as shown in Figure 1.

The probe **2** has a fluorescence excitation maximum at 370 nm and an emission one at 420 nm (Figure 2a) with a low quantum yield of $\Phi = 0.0039$ (quinine sulfate as a reference, $\Phi = 0.55$ in 0.05 M H_2SO_4[26]). This largely decreased quantum yield, compared to that ($\Phi = 0.25$) of anthracene under the same condition, may be ascribed to the effective photoinduced electron transfer between TTF and anthracene units,[15,18] which is favorable to affording a low background signal. Upon reaction with $^1O_2$, the excitation maximum was changed to 390 nm, and a 54-fold increase in fluorescence ($\Phi = 0.21$) and a strong CL (vide infra) were observed, concomitant with a dramatic decrease of the three absorption bands characteristic of its anthracene core (Figure 2b). This fluorescence enhancement, resulting from

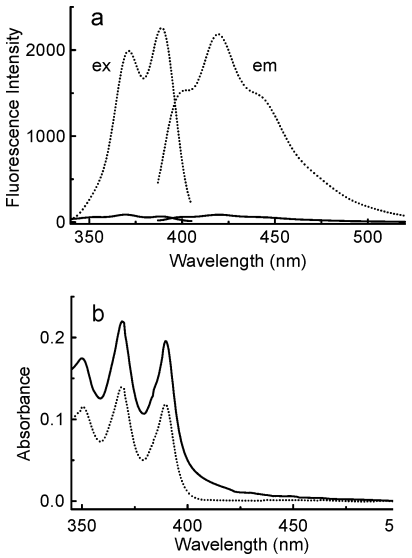

Figure 2. Fluorescence (a) and absorption (b) spectra of the probe **2** before (—) and after (···) reaction with $^1O_2$. Fluorescence spectra ($\lambda_{ex/em}$ = 370/420 nm before reaction, and $\lambda_{ex/em}$ = 390/420 nm after reaction) and absorption spectra were measured in 50 mM sodium phosphate buffer of pH 7 (50% THF as a cosolvent, v/v) with a final concentration of 20 μM of **2**. $^1O_2$ was produced by the reaction of 10 mM NaOCl with 1 mM H_2O_2.

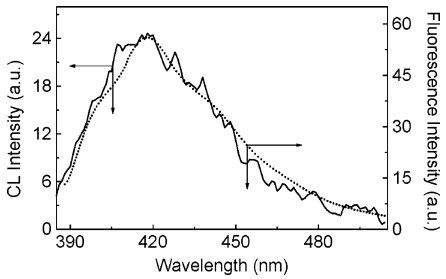

Figure 3. CL spectrum (—) from **2** and its fluorescence spectrum (···) after reaction with $^1O_2$. The concentrations of reactants were 200 μM of **2**, 10 mM of H_2O_2 and 30 mM of NaOCl. After oxidation and an appropriate dilution the reaction solution was then used to measure the fluorescence spectrum.

the oxidation of TTF unit into a cation species[15] that prohibits the photoinduced electron-transfer action, is however desirable for strong CL production. Figure 3 depicts the $^1O_2$-initiated CL spectrum of **2** along with its fluorescence spectrum after reaction. The CL spectrum is identical not only with the fluorescence spectrum of the reaction product but also with that of the unreacted **2**, implying that the emitting species should hold a complete anthracene core.

CL Reaction of 2 with ROS. The CL reaction of **2** with different ROS ($O_2^{-\bullet}$, H_2O_2, $^\bullet$OH, OCl^-, and $^1O_2$) was investigated to examine its selectivity. As shown in Table 1, the reaction of **2** with either H_2O_2, OCl^-, $^\bullet$OH, or $O_2^{-\bullet}$ does not give noticeable CL, whereas a very strong CL is produced upon addition of OCl^- or MoO_4^{2-} in the presence of H_2O_2, indicating that the probe exhibits a highly selective CL response to $^1O_2$ instead of the other ROS. This may be attributed to the specific reactivity of anthracene unit toward $^1O_2$,[9,10,17] as expected. Although a stronger CL was observed in more basic solutions (Table 1), a neutral medium of pH 7 was chosen for the present H_2O_2/NaOCl/**2** system and maintained with 50 mM sodium phosphate buffer because the yield of $^1O_2$ is about 100% under this condition.[5] The CL intensity was increased with increasing the probe concentration. Considering the rather low solubility

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Table 1. Comparison of Relative CL Intensities from the Reaction of **2** with Different ROS[a]

	reagent blank (control)	$^1O_2$ [b]	$^1O_2$ [c]	H_2O_2 [d]	OCl^- [e]	·OH [f]	$O_2^{-}\cdot$ [g]
pH 7	1.7×10^{-5}	1.0		3.0×10^{-5}	0.0054	2.9×10^{-4}	1.1×10^{-4}
pH 10	2.0×10^{-5}	1.3	1.6	2.6×10^{-4}	0.0037	6.0×10^{-4}	4.5×10^{-4}

[a] The CL intensity (1.15×10^6 RLU) from the reaction of **2** with $^1O_2$ at pH 7 was defined as 1.0. CL reaction was initiated by injecting appropriate amount of ROS into 50 mM sodium phosphate buffer of pH 7 or pH 10 containing 20 μM of **2** and 50% (v/v) THF as a cosolvent at 25 °C. [b] 1 mM H_2O_2 + 10 mM NaOCl. [c] 1 mM H_2O_2 + 1 mM MoO_4^{2-} (working only in basic solutions). [d] 1 mM H_2O_2. [e] 10 mM NaOCl. [f] 1 mM H_2O_2 + 0.1 mM ferrous ammonium sulfate. [g] 0.1 mL of superoxide solution (1 mg KO_2/mL dimethyl sulfoxide).

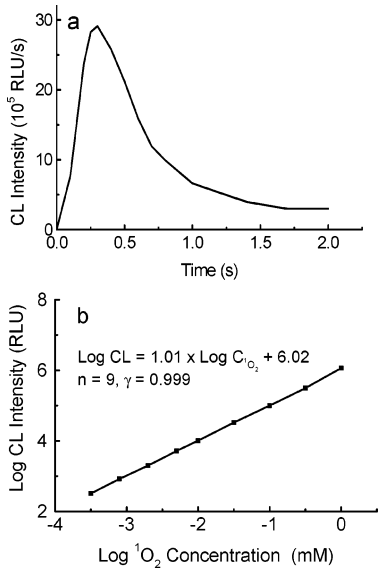

Figure 4. (a) CL kinetic curve of the system with 1 mM of H_2O_2, 10 mM of NaOCl, and 20 μM of **2**, and (b) the dependence of CL intensity of **2** (20 μM) on $^1O_2$ generated from the reaction of 10 mM of NaOCl with a series of H_2O_2 concentrations of 1 mM or less. The calibration curve was derived from the integrated CL signal of the H_2O_2/NaOCl/**2** system over a 5 s period minus that of the corresponding reagent blank (NaOCl/**2**) without H_2O_2. The reaction was carried out in 50 mM sodium phosphate buffer (pH 7) containing 50% (v/v) THF as a cosolvent.

of **2** in water, however, a concentration of 20 μM of the probe producing sufficient CL was used, and the presence of 50% THF (v/v) in the phosphate buffer was required to avoid its precipitation.

Detection of $^1O_2$. We used the reaction of H_2O_2 with NaOCl at pH 7 as a standard. As shown in Figure 4a, the CL rate of the present H_2O_2/NaOCl/**2** system is very quick, and a measuring time of 5 s for recording the CL signal of this system may be used. Figure 4b depicts the dependence of CL intensity of **2** on $^1O_2$ generated from H_2O_2/NaOCl system, and a good linearity between the CL intensity and the amount of $^1O_2$ produced is observed. The detection limit for $^1O_2$ is 76 nM based on 11 blank determinations ($k = 3$), also showing a highly sensitive character.

Recently, much interest has been shown in alkylhydroperoxide decomposition as a potential source of $^1O_2$ in biological systems,[27] and the generation of $^1O_2$ from the metal-catalyzed decomposition of several alkylhydroperoxides into peroxy radicals has been confirmed,[27] but it was unsuccessful in the case of *tert*-butyl hydroperoxide.[28] By using deuterium oxide as solvent to lengthen the $^1O_2$ lifetime, Kanofsky obtained unequivocal evidence for $^1O_2$ production from this alkylhydroperoxide.[22] In view of the high sensitivity and high specificity of **2** for $^1O_2$ in light water (H_2O) environments, here using the probe **2** as a CL trap, we made an attempt to monitor the generation of $^1O_2$ during the decomposition of *tert*-butyl hydroperoxide in the presence of Fe^{2+}.

Table 2. Relative CL Intensities from Different Reaction Systems[a]

system	relative CL intensity
1. probe **2** + Fe^{2+}	1.0
2. *tert*-butyl hydroperoxide + Fe^{2+}	0.57
3. probe **2** + *tert*-butyl hydroperoxide + Fe^{2+}	8.5 (210)[b]
4. system 3 containing 50% D_2O (v/v)	18
5. system 3 containing 5 mM sodium azide	5.9

[a] The CL intensity (214 RLU) from the reagent blank of system 1 was defined as 1.0. All reactions were initiated by injecting appropriate amount of $FeSO_4$ solution into 50 mM sodium phosphate buffer (pH 7) containing 50% THF (v/v) at 25 °C. The integrated CL intensity was measured in RLU over a 5 s period. Each of the data was expressed as the mean of three determinations with a relative error of less than ±5%. The reactant concentration was 20 μM of **2**, 1 mM of Fe^{2+}, and 1 mM of *tert*-butyl hydroperoxide, respectively. [b] This value was obtained with a measuring time of 200 s.

When Fe^{2+} was introduced into the solution of **2** or *tert*-butyl hydroperoxide, only a rather low level of CL was detected (Table 2), and no CL signal was produced upon mixing **2** with *tert*-butyl hydroperoxide (data not shown). As shown in Table 2, however, a much more intense CL signal is produced from the system 3 compared to the reagent blank systems 1−2, which should be ascribed to the reaction of **2** with $^1O_2$ generated from the metal-catalyzed decomposition of *tert*-butyl hydroperoxide. Large enhancement (200%) of the CL intensity from system 3 by deuterium oxide (system 4) and significant quenching (30%) by azide ion (system 5) provided strong evidence for the production and involvement of $^1O_2$. After subtracting the background signal (system 1), a $^1O_2$ yield of 1.54 μM was thus obtained for the system 3 over a 5-s reaction period based on the above calibration curve constructed with the H_2O_2/NaOCl/**2** system. Consistent with the previous observation of Kanofsky,[22] the metal-catalyzed decomposition of *tert*-butyl hydroperoxide is a slow reaction. Even with a measuring time of 200 s, which gave a $^1O_2$ yield of 45 μM, considerable CL signal still remained (Figure S1, Supporting Information), suggesting that the reaction has not yet ceased completely. These results indicate that the generation of $^1O_2$ during the decomposition of an alkylhydroperoxide could be effectively traced with the present CL system.

CL Reaction Mechanism. To investigate the CL mechanism of the present system, the reactions of **2** and its reference compounds (**1** and **6**) with $^1O_2$ were first compared. As shown in Table 3, the introduction of H_2O_2 into the solution of any compound scarcely generates CL, while a much stronger CL signal can be yielded upon reacting with OCl^-, and particularly with $^1O_2$. Instead of its reference compounds, the probe **2**

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Table 3. Comparison of CL Reactions and Fluorescence Quantum Yields of Compounds **1**, **2**, **6**, and **7** in the H_2O_2/NaOCl System[a]

	relative CL intensity[b]			quantum yield[c]	
compounds	H_2O_2	OCl^-	$^1O_2$	without $^1O_2$	with $^1O_2$
1	3.7×10^{-5}	0.0018	0.068	nd[d]	nd[d]
2	3.0×10^{-5}	0.0054	1.0	0.0039	0.21
6	3.0×10^{-5}	8.7×10^{-4}	0.011	0.25	0.10
1 + **6**	4.2×10^{-5}	0.0019	0.072	0.22	0.10
7	5.0×10^{-5}	0.0023	0.031	0.36	0.25

[a] All reactions were initiated by injecting appropriate amount of oxidant into 50 mM sodium phosphate buffer (pH 7) containing 50% THF (v/v) at 25 °C. The concentration of the tested compounds was each 20 μM, and the final concentrations of H_2O_2 and OCl^- were 1 and 10 mM, respectively. $^1O_2$ was produced with the H_2O_2/NaOCl system. [b] The CL intensity (1.15×10^6 RLU) from the reaction of **2** with $^1O_2$ was defined as 1.0. [c] Fluorescence quantum yield was determined by using quinine sulfate as a standard with $\Phi = 0.55$ in 0.05 M H_2SO_4 (ref 26). [d] Not determined.

reacting with $^1O_2$ produces the strongest CL, about 185-fold stronger CL than that from the blank (in the case of OCl^- in Table 3) and 91-fold stronger than that from the reaction of anthracene with $^1O_2$. Moreover, this oxidation also led to a large increase in the probe's fluorescence quantum yield as mentioned above, which is very conducive to the generation of strong CL. In contrast, however, such an electron transfer process did not take place effectively in the simple donor/acceptor mixture system of the reference compounds **1** and **6**, and the quantum yield of anthracene (**6**) was markedly lowered in the presence of $^1O_2$ (Table 3), presumably resulting from the fluorescence quenching by $^1O_2$.[2] This behavior of **2** in the change of fluorescence property is quite different from that of anthracene, suggesting that the electron-rich TTF unit in **2** must play an unusually important role in the CL reaction.

To get an insight into the role of TTF moiety in activating the anthracene reactivity toward $^1O_2$, the CL reaction with $^1O_2$ of compound **7** that has an electron-donating alkoxyl group in the 9 position was then examined under the same condition. The CL intensity from this molecule was far weaker than that from **2** by a factor of about 32 (Table 3), and the CL intensities from the tested compounds containing anthracene skeleton were increased in the following order: compound **2** $\gg$ compound **7** $>$ compound **6** (Table 3), clearly reflecting that the presence of TTF unit in **2** is crucial for the production of strong CL.

The comparison of the absorption spectra before and after oxidation also clearly revealed the unequivocal action of TTF unit on promoting the probe's trapping ability for $^1O_2$. For example, upon addition of $^1O_2$, the absorption spectrum of anthracene was hardly altered (Figure S2, Supporting Information), showing that its activity[29] is not high enough to react with $^1O_2$ under the present condition. In contrast, a noticeable decrease in the absorption representing anthracene moiety did occur for the compound **7** that bears an electron-donating alkoxyl group in the 9 position. It is apparent that **7** lacks any reactive unit except the anthracene core. Based on previous studies,[19] in this molecule the most active sites for $^1O_2$ addition are the electron-rich carbons in the 9,10-positions; therefore, the decrease of the absorption bands indicative of the anthracene unit can be explained by the possible formation of endoperoxides, which consumes some anthracene core or reduces the conjugation of the polycyclic aromatic molecule. For probe **2**,

(29) Foote, C. S. *Acc. Chem. Res.* **1968**, *1*, 104−110.

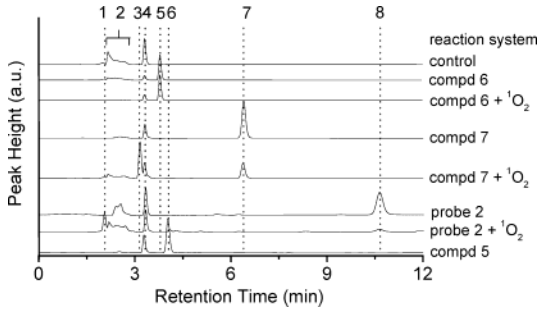


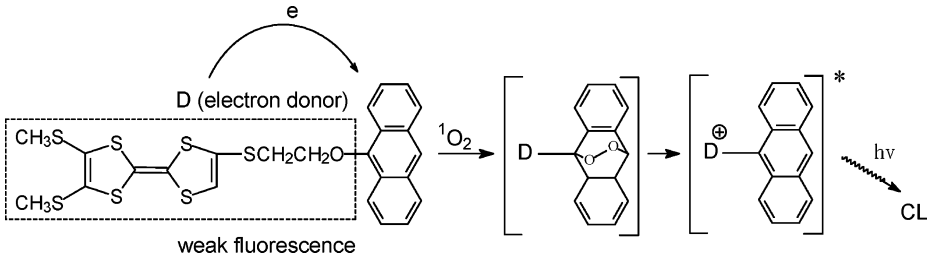
Figure 5. HPLC chromatograms of compounds **2**, **6**, and **7** before and after reaction with $^1O_2$. The compound **5** in the absence of $^1O_2$ was also tested. The peaks eluting from the column were monitored by the UV at 220 nm. The assignment of the peaks: (1) 2.04 min, reaction product of **2**; (2) 2.17−2.85 min (unstable peaks), H_2O_2/OCl^- from control; (3) 3.17 min, reaction product of **7**; (4) 3.33 min, THF from control; (5) 3.80 min, compound **6** (anthracene); (6) 4.04 min, compound **5**; (7) 6.40 min, compound **7**; (8) 10.62 min, probe **2**.

however, the absorption spectrum in the same wavelength range showed a much larger decrease (Figure 2b), which may result from the sum of the decreased absorbances caused by the reaction of both the TTF and anthracene units. Control experiment with **1** under the same condition (Figure S2, Supporting Information) showed that **1** can react with $^1O_2$. Therefore, it can be concluded that the observed strong CL may be associated with the reaction of the two units. Analogues of **2** were investigated, and it was found that the usage of a much longer linker such as hendecylthio between these two units led to a 7-fold weaker CL (data not shown) under the same conditions. In addition, it was noted that the substitution of anthracene at the 9 position by the investigated alkoxyl or TTF unit gave rise to a considerable redshift of its longest absorption band from 376 to 390 nm, similar to the known observations.[30]

To further confirm the above postulations, the reaction products with $^1O_2$ of compounds **2**, **6,** and **7** were also studied through HPLC analysis. As shown in Figure 5, upon addition of $^1O_2$, the peak at 3.80 min corresponding to anthracene (**6**) was unaltered and no new product peak was detected, consistent with the above result that anthracene is unreactive under the present condition. In contrast, however, the peak at 6.40 min for compound **7** decreased markedly after reaction, concomitant with the emergence of a new one at 3.17 min, which was confirmed to be the predicted corresponding endoperoxide ($[M+H]^+ = m/z$ 311, and $[M+Na]^+ = m/z$ 333) by MS analysis (Figure S3, Supporting Information). This result shows that the electron-donating group introduced into the 9 position of anthracene does activate the trapping ability of anthracene core for $^1O_2$. For probe **2**, its chromatographic peak at 10.62 min almost vanished after reacting with $^1O_2$, and a new one at 2.04 min (Figure 5) accompanying occasionally a small and rather unstable one (probably being the corresponding endoperoxide) at 3.44 min (data not shown) appeared. Efforts to isolate the predicted endoperoxide by varying temperature and reactant concentration were unsuccessful. Obviously, this unstability of the endoperoxide of **2**, different from that of **7**, is associated with the presence of TTF unit and is responsible for the strong CL production. The reaction product with the retention time of 2.04 min was stable, which however, when subjected to MS

(30) (a) Marsh, N. D.; Mikolajczak, C. J.; Wornat, M. J. *Spectrochim. Acta Part A* **2000**, *56*, 1499−1511. (b) Shin, E. J.; Lee, S. H. *Bull. Korean Chem. Soc.* **2002**, *23*, 1309−1314.

Scheme 1. Possible CL Mechanism of Probe **2** in the Presence of $^1O_2$



analysis, surprisingly gave the same molecular ion peak (*m*/*z* 548) as the probe **2** (Figure S4, Supporting Information). Moreover, this product retains the characteristic absorption bands of anthracene (Figure S5, Supporting Information), indicating that it may be the cation form of the probe **2**, which can be generated from the decomposition of the formed unstable endoperoxide accompanying the CL.[19,31] Compared to **2**, the reaction product with the retention time of 2.04 min has stronger polarity because its retention time becomes much shorter, also supporting the assumption that the final product exists as a cation species. Cyclic voltammetry analysis shows that the probe **2** has three oxidation waves (Figure S6, Supporting Information) located at about 0.47, 0.80, and 1.3 V (vs SCE), respectively, and $^1O_2$ has the ability to oxidize the TTF unit probably to be a cation radical because its oxidation potential (about 0.5 V) is higher than the first one but lower than the second one of the probe.[32] Besides, although the decomposition of a dioxetane intermediate generated by cycloaddition of $^1O_2$ to C═C double bonds is also a common CL mechanism,[8,14] such a reaction in the present system (e.g., cycloaddition of $^1O_2$ to the C═C double bond of TTF unit) may be ruled out because the corresponding degraded product (compound **5**) was not found (Figure 5).

Based on the above results, we propose that the CL reaction in the present system might proceed through the following way (Scheme 1): the anthracene moiety activated by TTF unit predominantly traps $^1O_2$ to yield an unstable endoperoxide, whose decomposition causes not only the excitation of the anthracene core which in turn emits light through radiative deactivation, but also the oxidation of the electron-rich TTF moiety to turn into a cation species. In such a tandem reaction, the formation of the final cation species of **2** promotes the proceeding of CL reaction forward and meanwhile prohibits the photoinduced electron-transfer process between TTF and excited anthracene units, thus enhancing CL. In addition, it cannot be ruled out that the oxidation of TTF unit by $^1O_2$ provides chemical energy to excite directly the anthracene core which then emits light, but this pathway, if existed, would be a minor one since the probe displayed a specific CL response only to $^1O_2$ rather than to the other ROS investigated here (Table 1). Otherwise, unselective reactions would be produced and stronger CL would be observed with stronger oxidants such as •OH.[32]

Conclusions

In summary, the unique feature of the electron-rich TTF moiety has been utilized for the first time as an important molecular scaffold for designing a CL probe for $^1O_2$. The results described here clearly demonstrate that the synthesized trap **2** exhibits both high selectivity for and high sensitivity to $^1O_2$, which makes it possible to be used widely for $^1O_2$ detection in many chemical and biological systems and even in light water environments. The application of this kind has been exemplified by monitoring the $^1O_2$ generation in a metal-catalyzed decomposition system of *tert*-butyl hydroperoxide. Moreover, the highly selective CL response of the present system allows $^1O_2$ to be determined in the presence of other ROS, and the proposed CL mechanism may be useful to developing highly chemiluminescent traps for $^1O_2$ based on anthracene.

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Supporting Information Available: Syntheses and characterization of compounds **1**−**5** and **7**; analysis of reaction products; measurement of oxidation potential; CL kinetic curve from the *tert*-butyl hydroperoxide/Fe^{2+}/**2** system; fluorescence and absorption spectra of compounds **1**, **6**, and **7** in the absence and presence of $^1O_2$; mass spectrum of reaction product from **7**; mass spectrum and absorption spectrum of reaction product from **2**; cyclic voltammogram of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0481530

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