



Synthesis of 3-Alkoxyethyl-4-aryl-3-*tert*-butyl-4-methoxy-1,2-dioxetanes as a Chemiluminescent Substrate with Short Half-life Emission

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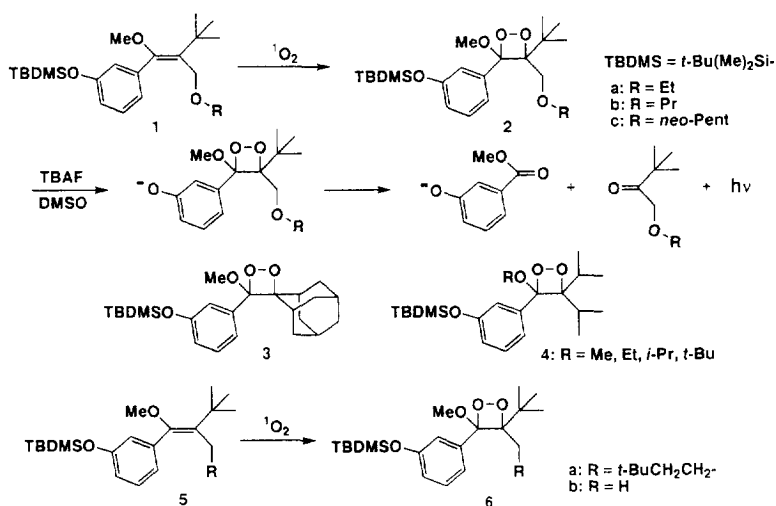
Abstract: Treatment of 3-alkoxymethyl-3-*tert*-butyl-4-(3-*tert*-butyldimethylsiloxy)phenyl-4-methoxy-1,2-dioxetanes (**2**) with TBAF in DMSO gives intensive blue light emission ($\lambda_{\text{max}} = 463 \text{ nm}$, $\Phi_{\text{CL}} > 0.1$) with short half-life ($t_{1/2} < 1 \text{ sec}$), whereas the methylene analogues (**6**) cause chemiluminescence with far longer half-life. A dioxetane (**1**) bearing a phosphoryloxyphenyl is cleaved enzymatically by alkaline phosphatase to generate light with high intensity. Copyright © 1996 Elsevier Science Ltd

Deprotonation of a thermally stable dioxetane bearing a phenolic substituent with a base can generate an unstable phenoxide-substituted dioxetane¹ which decomposes rapidly to emit light by intramolecular CIEEL (chemically initiated electron exchange luminescence) mechanism.² A dioxetane bearing a protected phenolic group, such as siloxyphenyl, can also give a CIEEL-active phenoxide by the deprotection.^{3,4,5} For the intramolecular CIEEL, an electron transfer from the phenoxide as a donor to the dioxetane induces decomposition of the dioxetane ring so that the rate of decomposition of the intermediary dioxetane, which is observed as the rate of emission or its half-life, depends directly on the ease of the electron transfer. The half-life of emission has been known to be varied by changing the phenolic substituent as an electron donor; a dioxetane bearing a *p*-phenoxy group emits light with far shorter half-life than the *m*-phenoxy analogue, though the chemiluminescent efficiency is extremely poor.^{6,7,8} However, there has been little known of thermally stable dioxetane to emit flash-like light with short half-life as well as with high efficiency by triggering. We report here that: a) 3-alkoxymethyl-3-*tert*-butyl-4-(3-*tert*-butyldimethylsiloxy)phenyl-4-methoxy-1,2-dioxetanes (**2**) possess sufficient thermal stability to enable handling at room temperature and emit flash-like intense light by triggering; b) the rapid decomposition of the intermediary dioxetane from **2** is likely attributed to an electrostatic interaction of the phenoxide with the vicinal alkoxyethyl moiety; and c) on the basis of the results, an efficient chemiluminescent substrate (**1**) was synthesized for the alkaline phosphatase-induced system.

A solution of a *m*-(*tert*-butyldimethylsiloxy)phenylethylene (**1a**) (100 mg) and tetraphenylporphyrin (TPP) (1.5 mg) as a sensitizer in CH_2Cl_2 (5 ml) was irradiated with a Na lamp (180 W) at 0°C under an oxygen atmosphere for 3 h. The chromatographic purification (SiO_2) of the photolysate gave a dioxetane (**2a**) in a 75 % isolated yield.^{9,10} The dioxetane (**2a**) was quantitatively decomposed into methyl *m*-(*tert*-butyldimethylsiloxy)benzoate and *tert*-butyl ethoxymethyl ketone in hot toluene-d₈ (stability at 25 °C: $t_{1/2} = 0.94 \text{ y}$). Ethylenes (**1b** and **1c**) were similarly oxygenated with singlet oxygen to give the corresponding dioxetane (**2b**)(stability at 25 °C: $t_{1/2} =$

1.1 y) and (2c) (stability at 25 °C: $t_{1/2} = 1.2$ y). All the dioxetanes (2) emitted flash-like intense blue light ($\lambda_{\max} = 463$ nm, $\Phi_{\text{CL}} = 0.1 - 0.3$, $t_{1/2} < 1$ s, 2c: 0.4 s), when solutions of 2 in DMSO (1.0×10^{-5} mol dm $^{-3}$, 1 ml) were added to TBAF (tetrabutylammonium fluoride) solutions in DMSO (1.0×10^{-2} mol dm $^{-3}$, 2 ml) at 25 °C. It is the most characteristic of the chemiluminescent properties for the F $^{-}$ -induced reaction of 2 that all the dioxetanes (2) emit light with half-life far shorter than that of 3 ($\lambda_{\max} = 470$ nm (463 nm)⁵, $\Phi_{\text{CL}} = 0.25$, $t_{1/2} = 5$ s)³, and 4 ($\lambda_{\max} = 463$ nm, $\Phi_{\text{CL}} = 0.21 - 0.35$, $t_{1/2} = 6 - 38$ s).⁵ A dioxetane having a 4-isopropoxyl in place of a 4-methoxyl in 2c generated light also with half-life shorter than 1 s.

Next, we attempted to synthesize a dioxetane (6a: R = *tert*-BuCH $_2$ CH $_2$ -) bearing a methylene in place of an alkoxy O in 2c. The singlet oxygenation of an olefin (5a) afforded 6a (stability at 25 °C: $t_{1/2} = 0.92$ y) in a moderate yield (44 %) (isolated yield 37 %) together with an ene reaction product (56 %) ¹¹ under the conditions similar to 1. Treatment of 6a with TBAF in DMSO gave also intense blue light ($\lambda_{\max} = 462$ nm, $\Phi_{\text{CL}} = 0.3$) with half-life (4.8 s) as much as in the case of the alkyl dioxetanes (3) and (4). A dioxetane (6b: R = H), isolated in a yield of 18 % (stability at 25 °C: $t_{1/2} = 0.09$ y), gave also light ($\lambda_{\max} = 462$ nm, $\Phi_{\text{CL}} = 0.3$) with half-life (4.2 s) longer than 2. For 3-alkoxydioxetanes (4), the steric interaction between an aromatic ring and adjacent substituent(s) on the dioxetane ring has been suggested to affect significantly the half-life of the F $^{-}$ -induced chemiluminescence.⁵ However, it is unlikely that the difference in half-lives between 2 and the methylene analogue (6a) can be attributed to such a steric interaction, because the bulkiness of neopentylloxymethyl in 2c is similar to 4,4-dimethylpentan-1-yl in 6a, and even the dioxetane (6b), which bears the smallest alkyl substituent (methyl) at a *vicinal* position of the phenyl among the dioxetanes investigated here, has longer half-life of luminescence than 2. The difference is also improbably owing to an electronic or an electrostatic interaction between O-O and alkoxy O, because the parent dioxetanes (2) possess thermal stability equal or higher than 6. An electrostatic repulsion between an aromatic ring and an unshared electron pair on the oxygen of adjacent alkoxy methyl assists likely an electron transfer from the aromatic ring to the dioxetane; such a repulsion is stronger for an anionic intermediary dioxetane than for the neutral 2, and the electrostatic repulsion may change the rotational angle of the aromatic ring in respect to the plane of the dioxetane so that an electron transfer occurs easily, contrary to the case of 4.¹²



A phosphoryloxy analogue (7) of 3 has recently been reported to emit light by deprotection with alkaline phosphatase and is now used in an enzyme immunoassay system,^{7,13,14} where the intensity of light emission is one of important factors to attain high sensitivity. For the alkaline phosphatase-induced chemiluminescence of 7, the overall kinetics of the light emission is substantially a two-step process comprising of the dephosphorylation of 7 (step a) and the decomposition of the resulting dioxetane anion (8) (step b), though the process includes rapid equilibrium between 8 and its protonated form (9), and the rate limiting step is apparently not step a but step b.⁷ Accordingly, the rate of decomposition of the dioxetane anion (8) as well as the chemiluminescent efficiency (Φ_{CL}) affects directly the intensity of chemiluminescence, and a phosphoryloxy analogue of dioxetanes 2 was expected to become an efficient chemiluminescent substrate for the alkaline phosphatase system.

Thus, we synthesized a dioxetane (11) as a representative of the phosphate analogues of (2) to examine chemiluminescent properties in the alkaline phosphatase system. An olefin (10) (200 mg) was oxygenated similarly to the case of 1 except for the solvent used (CH_2Cl_2 -MeOH, 10 : 1). The crude photolysate was purified by HPLC (column: YMC-Pack polymer C18, eluate: $\text{CH}_3\text{CN}/0.1\%$ aq NaHCO_3 , 10 to 45 % gradient) and in turn by desalination using aq CH_3CN and by lyophilization to yield a dioxetane (11)¹⁵ as an amorphous colorless solid in 38 % yield. The phosphate triggering experiments were carried out by the use of a modification of the method reported by Schaap¹⁶ and Bronstein;¹³ 11: 0.2 mg/ml, buffer solution: 0.1 M diethanol amine /

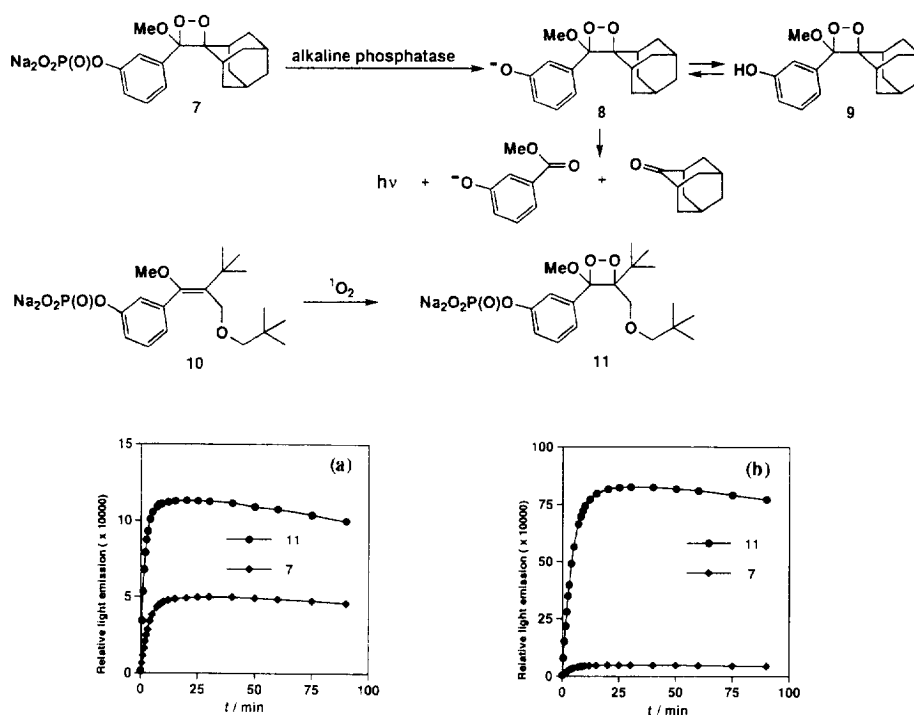


Fig 1 Alkaline phosphatase-induced chemiluminescence of dioxetanes 7 and 11

- (a) Dioxetane: 0.2 mg / ml, buffer solution: 0.1 M diethanol amine / HCl (pH 10.0) + MgCl_2 (1mM) + NaN_3 (0.05 %), alkaline phosphatase: 2.9×10^{-12} M, temperature: 37 °C.
 (b) TBQ (*N,N*-tributylammonium chloride of poly(aminomethylstyrene)) (0.4 mg / ml) was added as an enhancer to the system (a).

HCl (pH 10.0) + MgCl₂ (1mM) + NaN₃ (0.05 %), alkaline phosphatase: 2.9 x 10⁻¹² M, temperature: 37 °C. The time course of the emission from the dioxetane (1) was measured with a luminometer (Berthold) and the results are shown together with those of 7 in Fig. 1, which discloses that the dioxetane (1) emits light efficiently (total emission in system (a) using 2.9 x 10⁻⁹ M alkaline phosphatase: 7 : 1 = 1 : 2.2).

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References and Notes

- Schaap, A. P.; Gagnon, S. D. *J. Amer. Chem. Soc.* **1982**, *104*, 3504 - 3506.
- Schuster, G. B. *Acc. Chem. Res.* **1979**, *12*, 366 - 373. See also Catalani, L. H.; Wilson, T. J. *Amer. Chem. Soc.* **1989**, *111*, 2633 - 2639.
- Schaap, A. P.; Chen, T.-S.; Handley, R. S.; DeSilva, R.; Giri, B. P. *Tetrahedron Lett.* **1987**, *28*, 1155-1158.
- Adam, W.; Fell, R.; Schulz, M. H. *Tetrahedron* **1993**, *49*, 2227-2238.
- Matsumoto, M.; Suganuma, H.; Katao, Y.; Mutoh, H. *J. Chem. Soc. Chem. Commun.* **1995**, 431 -432.
- McCapra, F. *Tetrahedron Lett.* **1993**, *34*, 6941-6944. See also ref. therein.
- Beck, S.; Köster, H. *Anal. Chem.* **1990**, *62*, 2258 -2270.
- Matsumoto, M.; Suganuma, H.; Azami, M.; Aoshima, N.; Mutoh, H. *Heterocycles* **1995**, *41*, 2419-2422.
- All the dioxetanes synthesized here gave satisfactory analytical data.
- ¹H-NMR spectrum of the photolysate showed that 2a formed selectively.
- A similar tendency in selectivity of singlet oxygenation of 1 and 5 has been observed for methoxyphenyl analogues; Matsumoto, M.; Kobayashi, H.; Matsubara, J.; Watanabe, N.; Yamashita, S.; Oguma, D.; Kitano, Y.; Ikawa, H. *Tetrahedron Lett.* **1996**, *37*, 397-400.
- Especially for a *tert*-butoxydioxetane (4), an aromatic ring can not rotate freely to the conformation favorable for the electron transfer.⁵ An electron transfer seems to occur when an aromatic ring lies nearly perpendicular to the plane comprising of 2-O, 3-C, and an aromatic carbon attached to the dioxetane for 3-aryldioxetane.
- a) Bronstein, I.; Voyta, J. C.; Thorpe, G. H. G.; Kricka, L. J.; Armstrong, G. *Clin. Chem.* **1989**, *35*, 1441-1446; b) Thorpe, G. H. G.; Bronstein, I.; Kricka, L. J.; Edwards, B.; Voyta, J. C.; *Clin. Chem.* **1989**, *35*, 2319 - 2321; c) Schaap, A. P.; Akhavan, H.; Romano, L. J.; *Clin. Chem.* **1989**, *35*, 1863-1864. See also a) Bronstein, I.; Voyta, J. C.; Edwards, B. *Anal. Biochem.* **1989**, *180*, 95-98; b) Bronstein, I.; Fortin, J.; Stanley, P. E.; Stewart, G. S. A. B.; Kricka, L. J. *Anal. Biochem.* **1994**, *219*, 169-181; c) Bronstein, I.; Olesen, C. E. M. *Detection Methods Using Chemiluminescence In Molecular Methods for Virus Detection*; Wiedbrauk, D. L.; Farkas, D. H. Ed.; Academic, NY, 1995, pp149-173.
- Mayer, A.; Neuenhofer, S. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1044 - 1072.
- Little decomposition of 11 was observed after >1 y at -20 °C, though 11 decomposed with t_{1/2} = 19 h in CD₃OD at 60 °C.
- Schaap, A. P.; Sandison, M. D.; Handley, R. S. *Tetrahedron Lett.* **1987**, *28*, 1159-1162.

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