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Accounts

Structural Aspects of 1,2-Dioxetanes Active toward Intramolecular Charge-Transfer-Induced Chemiluminescent Decomposition

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Simple 1,2-dioxetanes decompose thermally to produce mainly triplet-excited carbonyl fragments, whereas 1,2-dioxetanes bearing an aromatic electron donor display intramolecular charge-transfer-induced decomposition with accompanying effective emission of light. This type of 1,2-dioxetanes has been extensively studied with a view to understanding the mechanisms of bioluminescence and chemiluminescence and applying them to modern biochemical and biomedical analyses. In this account, recent developments of dioxetane-based chemiluminescent substrates are described from the viewpoint of relationships between the structure of dioxetanes and the properties of chemiluminescence, namely, chemiluminescent efficiency, singlet-chemiexcitation efficiency, color, and the rate of chemiluminescent decomposition.

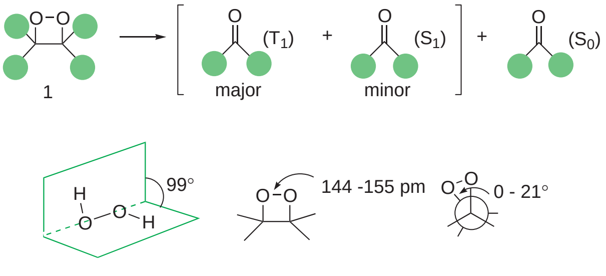
The high-energy molecules 1,2-dioxetanes have been studied intensively for more than 30 years from the viewpoints of synthetic chemistry and structural chemistry, and of understanding the bioluminescent and chemiluminescent mechanisms. During this period, hundreds of 1,2-dioxetanes have been synthesized.[1–5] However, in the early decades of the history of 1,2-dioxetanes, a puzzling problem was the crucial discrepancy in the efficiency of light emission for bioluminescence represented by the firefly, for which the luciferin/luciferase reaction has been believed to produce a dioxetanone intermediate. The clue leading to 1,2-dioxetanes with high light yield was the theory of chemically initiated electron exchange luminescence (CIEEL).[6] Nowadays, dioxetane-based chemiluminescent substrates have been designed that exhibit luminescent efficiencies as high as those of most bioluminescences.[7–10]

Although certain substrates are now being used for modern high-sensitivity biochemical and biomedical analyses, further development of dioxetane-based chemiluminescent substrates is eagerly expected. The subjects under study are how the chemiluminescent efficiency can be improved, for which knowledge of the detailed chemiexcitation mechanism is quite important, and how the color varies for chemiluminescence in relation to the bioluminescence mechanism of beetles and the design of multi-channel bioassay systems. We describe here our recent studies on dioxetane-based chemiluminescent substrates to explain these subjects.

Decomposition of 1,2-Dioxetanes Leading to the Chemiexcitation

The four-membered ring peroxides, namely 1,2-dioxetanes (dioxetanes), undergo a variety of reactions, such as reduction to epoxides, nucleophilic O–O bond cleavage, rearrangement, and decomposition.[4] Among them, the most characteristic is the decomposition of dioxetanes into two carbonyl fragments, one of which is produced more or less as an electronically excited species. Dioxetanes **1** involve high strain energy, as expected from the comparison of their bond angle (∠CCO) and dihedral angle (OCCO) with those of hydrogen peroxide and dialkyl peroxides.[11] Hence, dioxetanes are essentially unstable thermally and liberate much energy during the decomposition into two carbonyl fragments. A total of ca. 360 kJ mol^{-1} of energy, which is the heat of reaction (ΔH_0) plus activation enthalpy ($\Delta H^{\ddagger}$), has been estimated to be available in the transition state for thermal decomposition of dioxetanes.[1] They can thus provide the energy sufficient for the chemiexcitation of a carbonyl fragment in the transition state. The thermal decomposition of rather simple dioxetanes, however, affords predominantly a triplet-excited carbonyl compound along with a small amount of a singlet-excited carbonyl compound. Thus, little direct emission of bright light is expected (Scheme 1).

To account for the foregoing fact, a decomposition mechanism has been proposed as follows. The reaction proceeds through a twisted diradical-like transition state **2**, for which



Scheme 1.

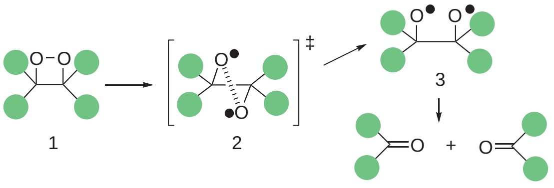
the C–C bond is still intact, and includes crossover of the diradical path to a triplet-excited product path, prior to reaching a true stable diradical intermediate **3** (Scheme 2).[1] Some recent theoretical studies[12–14] have suggested that the chemiexcitation of 1,2-dioxetanes is a chemical change from the lowest singlet potential surface (S_0) for a dioxetane to the lowest triplet surface (T_1), and the T_1 surface and the S_0 surface cross each other twice in the region of the diradical **3** (Fig. 1). The spin–orbit interaction should be effective at the first crossing point, while minimally effective near the second crossing point. Thus, a considerable amount of the initially formed singlet diradical transfers from the S_0 surface to the T_1 surface and then cleaves to give a triplet-excited carbonyl fragment. Here, a transfer of the singlet diradical from the S_0 surface to the higher S_1 surface is presumably far less likely, since the S_1 surface would lie in an energy level higher than the T_1 surface. Hence, triplet chemiexcitation but not singlet chemiexcitation occurs predominantly.

In contrast with the rather simple dioxetanes, dioxetanes bearing an aromatic electron donor have been found to undergo intramolecular charge-transfer (CT)-induced decomposition to two carbonyl fragments with accompanying effective light emission. Thus, stable dioxetane **4** substituted with a phenol or a protected phenol can be transformed on demand into unstable dioxetane **5** bearing a phenolate ion, from which intramolecular CT occurs to the O–O of dioxetane to induce its rapid decomposition into aromatic ester **6** with flash emission of blue light (Scheme 3).[15–20]

There have been two influential mechanisms proposed for the intramolecular CT-induced chemiluminescent decomposition of dioxetanes. The first is the intramolecular CIEEL mechanism. The "CIEEL" was originally proposed by Schuster for an intermolecular chemiluminescent decomposition of a peroxide induced by easily-oxidized polynuclear aromatic compounds,[6] though a crucial question has been posed mechanistically.[21] According to the theory of CIEEL, full single electron transfer (SET) occurs from an oxido anion of phenol to the O–O of dioxetane **7** to induce its decomposition through a transient species **8** as shown in Scheme 4. The thus-produced radical ion pair **9** is annihilated by transferring an electron backward (BET) to generate a singlet-excited aromatic carbonyl compound **10**.[22–26] However, whether the mechanism for the intramolecular CT-induced chemiluminescent decomposition of dioxetanes includes or does not include BET as a fundamental process is a serious problem which is being argued and still remains unclarified.

The second mechanistic alternative is that a transient species **11** produces a singlet-excited aromatic carbonyl compound **10** directly without discrete formation of the radical–ion pair **9** (Scheme 5).[27] It should be noted here that a mechanism including simultaneous cleavage of the O–O bond and the C–C bond in a dioxetane seems to be less plausible. Another point to be noted is that the transient species **11** does not necessarily form through a full single ET (SET), but a range of CT probably induces the O–O bond cleavage.

Adam and his co-workers have strongly supported the ET/BET (exact intramolecular CIEEL) based mainly on the studies of viscosity dependence of chemiluminescent efficiency for the CT-induced decomposition of dioxetanes such as **4a**.[22] According to the ET/BET mechanism, the efficiency of singlet-chemiexcitation depends on the efficiency of the BET step. On the other hand, a theoretical study (ab initio MO calculation) has suggested for the decomposition of dioxetane **7** that the intramolecular CT takes place to afford a transient diradical **11**, which decomposes directly into a singlet-excited carbonyl fragment **10** by the transfer of the singlet diradical from the S_0 surface to the excited singlet surface (S_1), in a manner



Scheme 2.

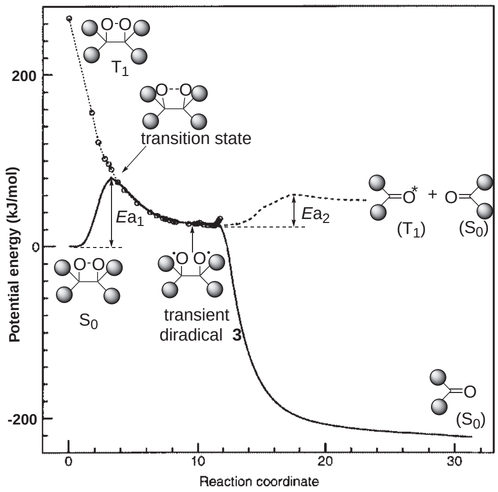
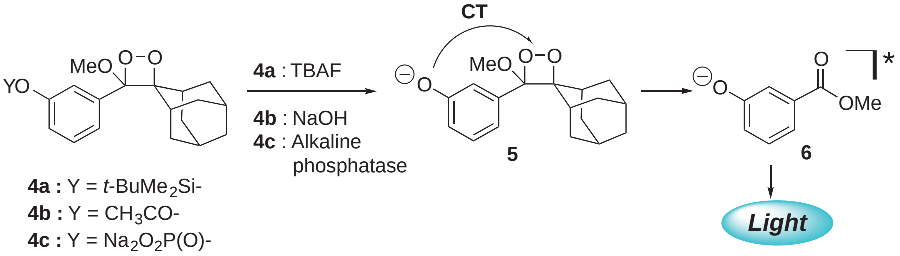
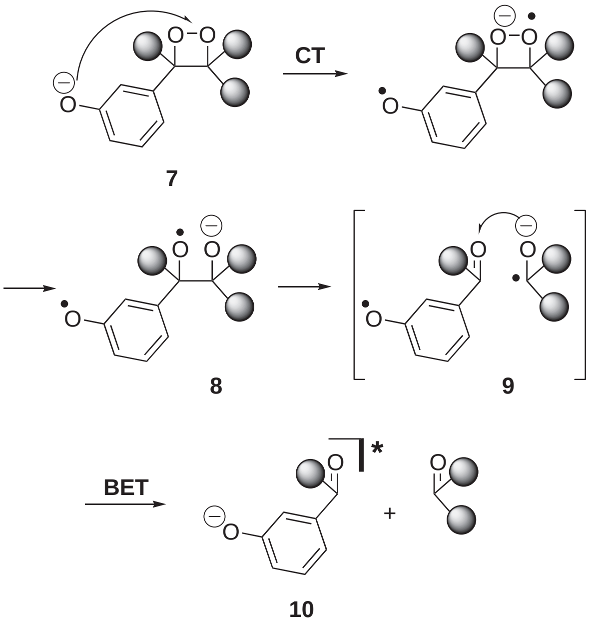


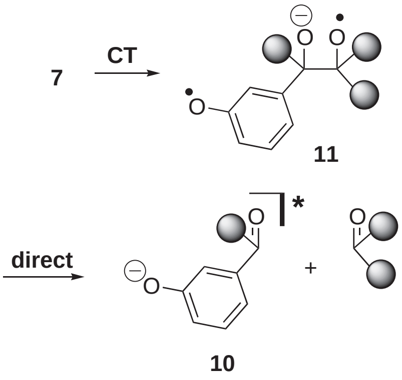
Fig. 1. Triplet chemiexcitation process for thermolysis of 1,2-dioxetane.



Scheme 3.



Scheme 4.



Scheme 5.

rather resembling the triplet-chemiexcitation process for the thermolysis of dioxetane (Fig. 2).[28] According to this study, the efficiency of singlet-chemiexcitation depends on the easiness of crossover of dioxetane in the ground state with the singlet state and on the magnitude of Ea_2.

The ET/BET mechanism and the CT/direct mechanism seem to be the limit features for the singlet chemiexcitation process for the intramolecular CT-induced chemiluminescent decomposition of dioxetanes. For the ET/BET mechanism, the BET must proceed so rapidly that the spin-conversion of the radical–ion pair in the singlet state into the triplet state has little chance, when a singlet-excited species is produced effectively, since such spin-conversion proceeds between sequential recontacts.[29] This means that the component parts of the radical–ion pair would closely interact with each other. Therefore, an ultimate feature would be the same as the direct mechanism, in which the C–C bond does not cleave, even if elongated, before the chemiexcitation. Eventually, a range of chemiexcitation mechanisms is probable for the intramolecular CT-induced chemiluminescent decomposition, in which the BET channel and the direct channel are involved as the ultimate features. In conclusion, the ET/BET channel or the CT/direct channel should be reflected in the singlet chemiexcitation process, depending on the structure of dioxetanes and even on the reaction conditions.

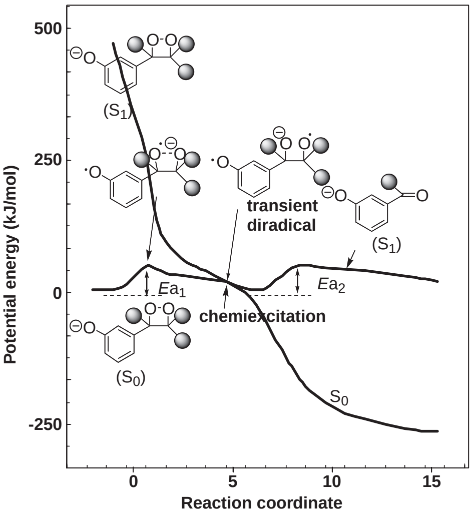
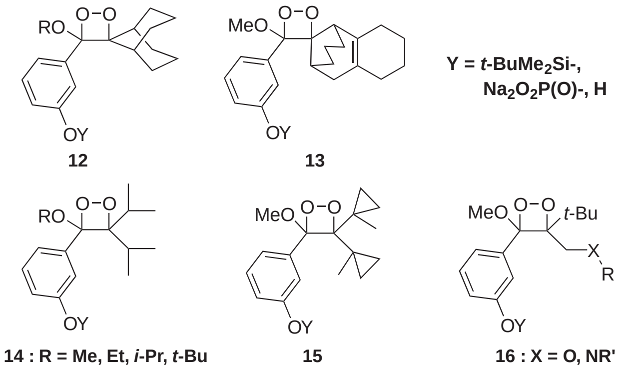


Fig. 2. Singlet chemiexcitation of a dioxetane bearing a 3-oxidophenyl group.

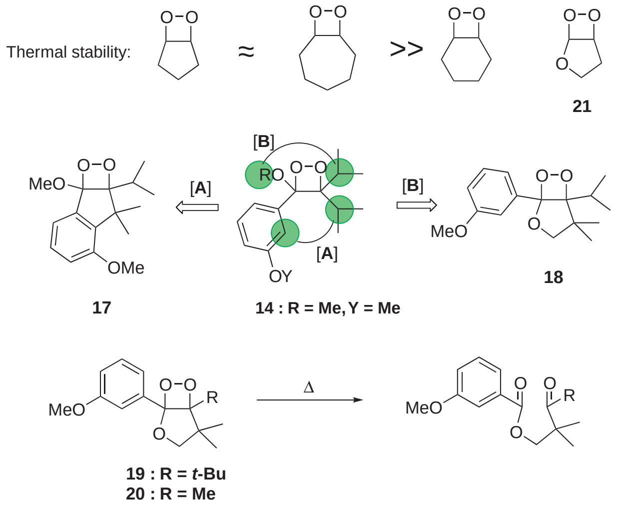
Design and Synthesis of 1,2-Dioxetanes with High Thermal Stability and Flexibility for Structural Modification

As described in the previous section, dioxetanes bearing an aromatic substituent, which is easily transformed into an electron donor causing the CT-induced decomposition, would become a high-performance chemiluminescent substrate. One requisite property of dioxetane to function as a chemiluminescent substrate is sufficient thermal stability to permit handling at room temperature and long-term storage, and its easy transformation (*triggering*) as desired to an unstable dioxetane, which undergoes easily the CT-induced decomposition. The thermal decomposition of dioxetane is initially caused by the stretching of the O–O bond, as mentioned already. Therefore, prevention of the twisting of the peroxidic four-membered ring should lead to improving the thermal stability of dioxetanes. For dioxetanes **4**, an adamantylidene moiety is called a "stabilizer," the steric interaction of which with phenolic and methoxy groups has been suggested to prevent the twisting of the dioxetane ring as in the case of diadamantylidene dioxetane.[8,30] As an extension of such idea, there have been synthesized spirodioxetanes bearing a bicyclic ring **12**, **13**,[31,32] and phenolic dioxetanes substituted with two bulky alkyl groups **14–16** (Scheme 6).[33–39] These dioxetanes including **4**, however, rather lack flexibility for further structural modification.

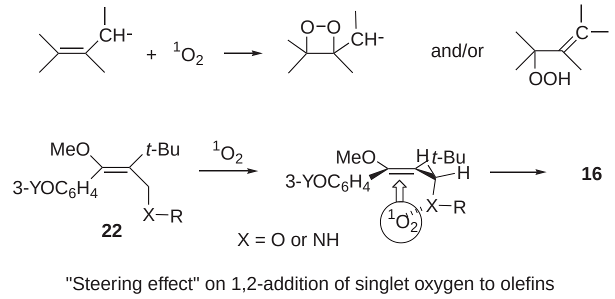
Construction of an annelated five-membered ring by joining two substituents at the 3- and 4-positions of dioxetane has been reported to be effective for the dioxetane ring to resist the necessary twisting action of decomposition.[40,41] Starting from dioxetane **14**, were designed two types of bicyclic dioxetanes. The first was a derivative of indane **17**, while the second was a derivative of tetrahydrofuran **18**. The indane derivative **17** was unstable and decomposed with a half-life of less than 3 h at room temperature.[42] In contrast, a derivative of tetrahydrofuran **18** was found to exhibit marked thermal stability.[43] Exchange of an isopropyl into a more bulky *t*-butyl at the 5-po-



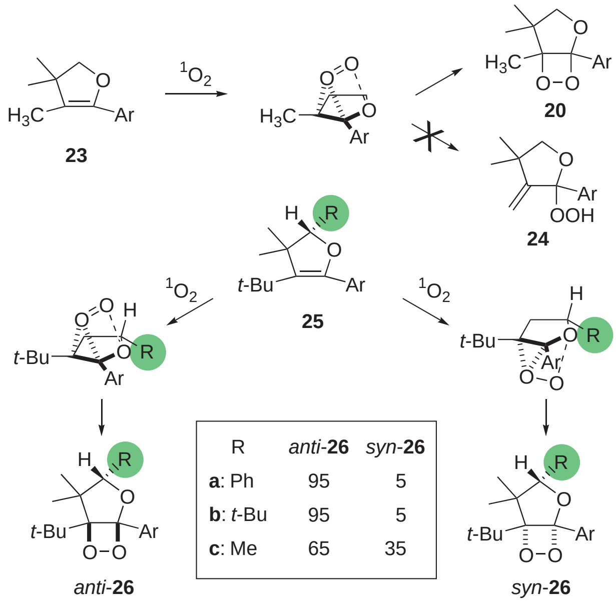
Scheme 6.



Scheme 7.



Scheme 8.



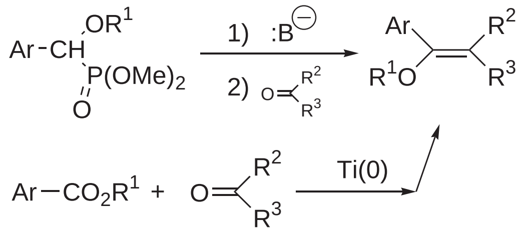
R	*anti*-**26**	*syn*-**26**
a: Ph	95	5
b: *t*-Bu	95	5
c: Me	65	35

Scheme 9. π-Face selective 1,2-addition of singlet oxygen.

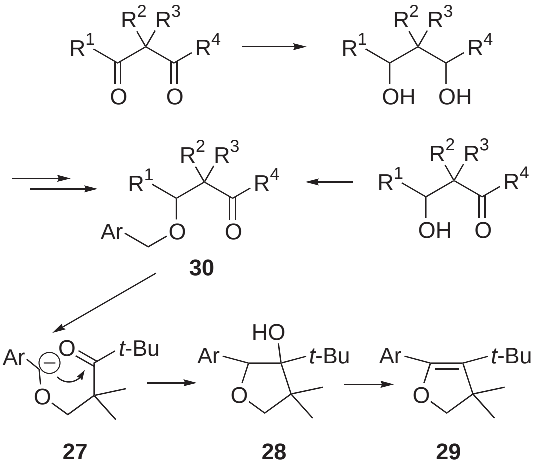
sition for **18** increased the thermal stability of dioxetane **19**, while the exchange into a small methyl decreased the stability of **20**: the activation free energies for thermolysis were estimated to be $\Delta G^{\ddagger} = 120$ kJ mol^{-1} for **18**, 124 kJ mol^{-1} for **19**, and 117 kJ mol^{-1} for **20** (Scheme 7). These results and the fact that unsubstituted bicyclic dioxetane **21** is rather unstable even at room temperature[44] suggest that the markedly increased stability can most likely be attributed to prevention of the twisting of the dioxetane ring, not only by the fused five-membered ring but also by steric interaction between the 1-aryl and 5-alkyl, the rotation of which is retarded considerably by methyl(s) at the 4-position.

Dioxetanes described here could be synthesized effectively by the 1,2-addition of singlet oxygen ($^1O_2$) to the corresponding enol ethers. The 1,2-addition of $^1O_2$ concurs generally with "ene" reaction for an olefin having allylic hydrogen(s), and, furthermore, concurs with the 1,4-cycloaddition for a conjugate diene. However, the 1,2-addition of $^1O_2$ becomes the preferential mode by contriving the arrangement of substituents on an olefin. For example, olefins bearing an oxygen or nitrogen at the allylic position **22** have been known to undergo selective 1,2-addition of $^1O_2$ by the "steering effect" of the heteroatom,[36–38,45] which controls the π-face and the side selectivity of attacking $^1O_2$ as illustrated in Scheme 8.[46] The heteroatom at the vinylic position was also effective to guide $^1O_2$. Thus, $^1O_2$ was apparently guided by the oxygen of the dihydrofuran ring **23** to the side opposite from a methyl at the 4-position, so that bicyclic dioxetane **20** was produced with no allylic hydroperoxide **24** due to the "ene" reaction. A bulky substituent at the 2-position of 5-aryl-2,3-dihydrofurans exhibited a steric effect to control π-face selectivity of 1,2-cycloaddition of $^1O_2$. Thus, dihydrofurans **25** bearing 2-*t*-butyl or 2-phenyl gave predominantly dioxetane (*anti*-**26**) in which $^1O_2$ adds to the less crowded π-face: *anti*:*syn* = 95:5 (Scheme 9).[47]

α-Alkoxystyrene derivatives as a key intermediate for aryl-substituted dioxetanes can be synthesized conveniently by the Wadsworth–Emmons reaction of dialkyl 1-aryl-1-alkoxymethylphosphonate with ketone or by the McMurry cross-coupling of aromatic ester with ketone using Ti(0) (Scheme 10). The latter reaction is especially convenient since it is applicable for the synthesis of cyclic enol ethers as well as acyclic ethers, though unsuitable for large-scale synthesis. Thus, a new method to synthesize 5-aryl-2,3-dihydrofurans has been developed. Carbanion of benzylic ether **27** cyclized effectively to give tetrahydrofuran **28**, dehydration of which produced dihydrofuran **29** (Scheme 11).[48] The usual demethylation of methoxyphenyl and successive protection of phenolic OH with an appropriate group afforded the desired dihydrofuran-type key intermediate. One notable advantage of this method is its flex-



Scheme 10.



Scheme 11.

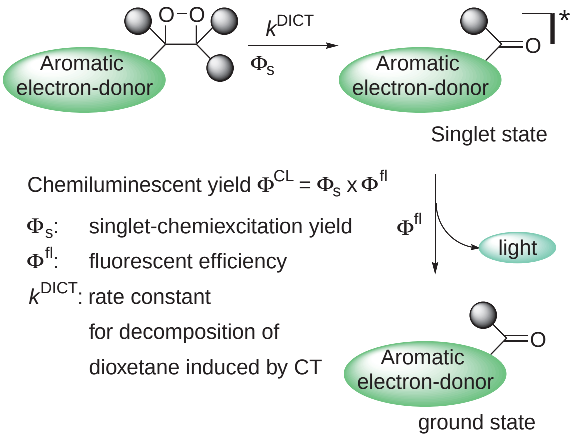


Fig. 3.

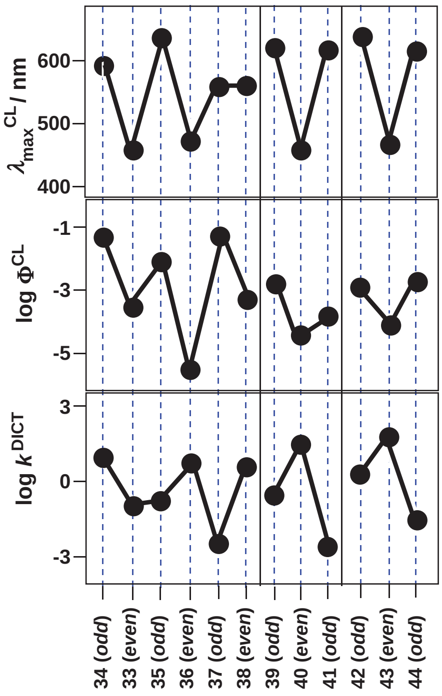


Fig. 4. "Odd/even" relationship between Φ^{CL}, k^{DICT}, and $\lambda_{max}{}^{CL}$ for dioxetanes.

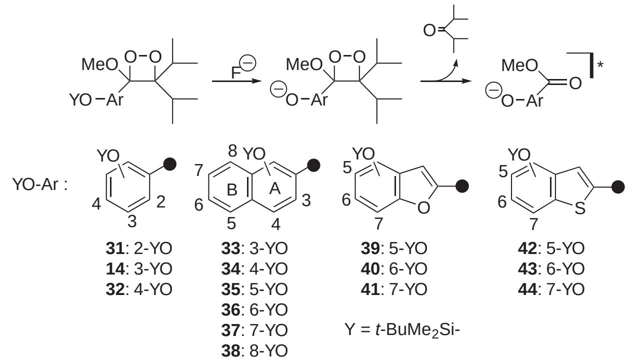
ibility for structural modification, since various analogs of benzylic ether **30** as a starting material can be easily prepared from β-keto esters or β-diketones with a variety of substituents, or by the use of aldol condensation.

"Odd/Even" Relationship between the Position of Phenolic Oxygen and Chemiluminescent Properties for 1,2-Dioxetanes Bearing a Siloxyarene Moiety

A hydroxyarene-type substituent on dioxetane acts as an electron-donor initially, and then as the most important part in a singlet-excited aromatic carbonyl fragment after the CT-induced decomposition. Therefore, it affects significantly the chemiexcitation efficiency (Φ_S), fluorescence efficiency of the emitter (Φ^{fl}), chemiluminescent efficiency (Φ^{CL}), which is expressed as the product of Φ_S by Φ^{fl}, the spectrum of chemiluminescence, which is represented by the maximum wavelength ($\lambda_{max}{}^{CL}$), and the decomposition rate of dioxetane induced by CT (rate constant: k^{DICT}) (Fig. 3).

Dioxetanes bearing a 3-siloxyphenyl (**14**: R = Me, Y = t-BuMe$_2$Si-) ("odd" pattern) decomposed to emit blue light ($\lambda_{max}{}^{CL} = 463$ nm) in high yield ($\Phi^{CL} = 0.21$) on treatment with TBAF (tetrabutylammonium fluoride) in DMSO.[33,34] On the other hand, 2- or 4-siloxyphenyl analogs **31** and **32** ("even" pattern) afforded far weaker light, though their decay-rates were faster than those for the "odd" analog: Φ^{CL} for the "even" pattern **31**, **32** was 1/250–1/400 of the Φ^{CL} for the "odd" pattern **14**.[49] This phenomenon has been observed for the chemiluminescent decomposition of **4a** and its p-oxyphenyl-analog and is described as "odd/even" relationship.[50] A similar phenomenon was observed for dioxetanes bearing a siloxy-substituted 2-naphthyl moiety **33**–**38**.[51,52] As shown in Fig. 4, isomers having a siloxy group (trigger) at the 5- or 7-position (**35** and **37**: "odd" pattern) of the B-ring of naphthalene decomposed more slowly to afford longer wavelength light in higher chemiluminescent efficiency than isomers having a trigger at the 6- or 8-position (**36** and **38**: "even" pattern) of the naphthalene ring. The "odd/even" relationship fitted also for 3-(2-naphthyl)dioxetanes **33** and **34** having a trigger on the A-ring, except for the CT-induced decomposition rate. The CT-induced decomposition by triggering with an alkaline phosphatase has also been reported to exhibit the "odd/even" relationship as exemplified by dioxetanes bearing a phosphate-substituted naphthyl moiety (Scheme 12).[50]

Dioxetanes bearing a siloxybenzo[b]furan-2-yl **39**–**41** or siloxybenzo[b]thiophen-2-yl group **42**–**44** also exhibited the "odd/even" relationship between the trigger position and



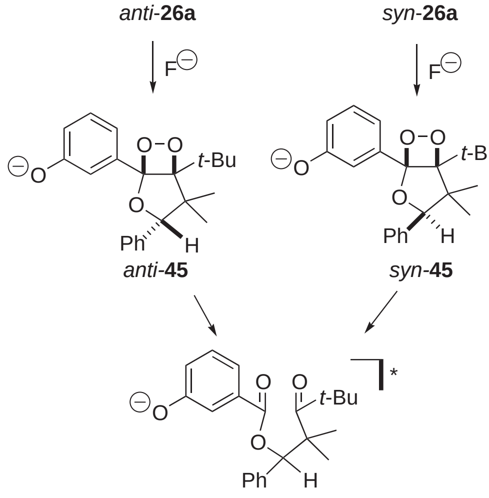
Scheme 12.

chemiluminescent properties as shown in Fig. 4.[53] Here, we should be aware of the fact that the oxidation potentials should be little different between "odd" (*meta*-substitution) and "even" (*para*- or *ortho*-substitution) for phenol, whereas they are considerably different between 1-naphthol and 2-naphthol which include both "odd" and "even" pattern dependent on the position of the naphthyl attached to a dioxetane ring. In fact, the CT-induced decomposition rate (k^{DICT}) for **34** was so rapid that it deviated apparently from the "odd/even" relationship. This is attributed presumably to the oxidation potential of an oxide anion of 1-naphthol, which is considerably lower than that of 2-naphthol: formal oxidation potential vs Ag/Ag^+ in DMSO was estimated to be −498 mV for 1-naphtholate, and −369 mV for 2-naphtholate.[51] Similar attention should be paid to the heteroaromatics **39**–**44** described above.

Singlet-Chemiexcitation Efficiency Affected by the Structure of 1,2-Dioxetane Other than the Hydroxyarene Moiety

Although dioxetanes **4a**, **14** (R = Me), **15**, and **16** give the same oxide anion of methyl 3-hydroxybenzoate (**6**) as an emitter, when they are treated with a base, their chemiluminescent efficiencies are revealed to vary ranging from 0.12 to 0.29 under the same conditions (TBAF/DMSO at 25 °C). These facts suggest that the substituents, other than the aromatic moiety attached to a dioxetane ring, should influence the formation of a singlet-excited aromatic ester fragment; thereby, chemiluminescent efficiency varies. Thus, studies of relationships between structures of these dioxetanes and their Φ^{CL} are important to design the more efficient chemiluminescent substrates. However, one difficulty hampering such studies is development of a method to evaluate the difference in the participation of another carbonyl fragment concomitantly formed with an emitter.

Examination of the CT-induced decomposition of a pair of stereoisomeric dioxetanes affording the very same couple of two carbonyl fragments can avoid such problems. Synthesized were the *anti*-form of dioxetane (*anti*-**26a**, R = Ph) and its *syn*-isomer (*syn*-**26a**, R = Ph).[47] *anti*-Dioxetane (*anti*-**26a**, R = Ph) and its stereoisomer (*syn*-**26a**, R = Ph) were transformed by desilylation into the corresponding unstable dioxetanes (*anti*-**45**) and (*syn*-**45**) bearing a phenoxide anion, both of which produced the very same keto ester **46** as an emitter (Scheme 13). However, the *anti*-isomer (*anti*-**45**) gave light in yield ca. 1.5 times higher than that from the *syn*-isomer (*syn*-**45**): chemiluminescent efficiencies (Φ^{CL}) and singlet-chemiexcitation yields (Φ_{S}) were estimated to be $\Phi^{\mathrm{CL}} = 0.18$ and $\Phi_{\mathrm{S}} = 0.58$ for the *anti*-isomer, while $\Phi^{\mathrm{CL}} = 0.12$ and $\Phi_{\mathrm{S}} = 0.39$ for the *syn*-isomer in DMSO. These facts show that the dioxetane skeleton itself undoubtedly influences the singlet-chemiexcitation process for the CT-induced decomposition of dioxetanes. It should be pointed out that *anti*-dioxetane (*anti*-**26a**, R = Ph), requiring higher activation enthalpy ($\Delta H^{\ddagger}$) for thermolysis, attained the singlet-chemiexcitation in higher yield than its *syn*-isomer (*syn*-**26a**, R = Ph) for the CT-induced decomposition: $\Delta H^{\ddagger}$ was estimated to be 131 kJ mol^{-1} for the *anti*-isomer and 120 kJ mol^{-1} for the *syn*-isomer. These tendencies were observed also for a methyl-analog (**26c**, R = Me).



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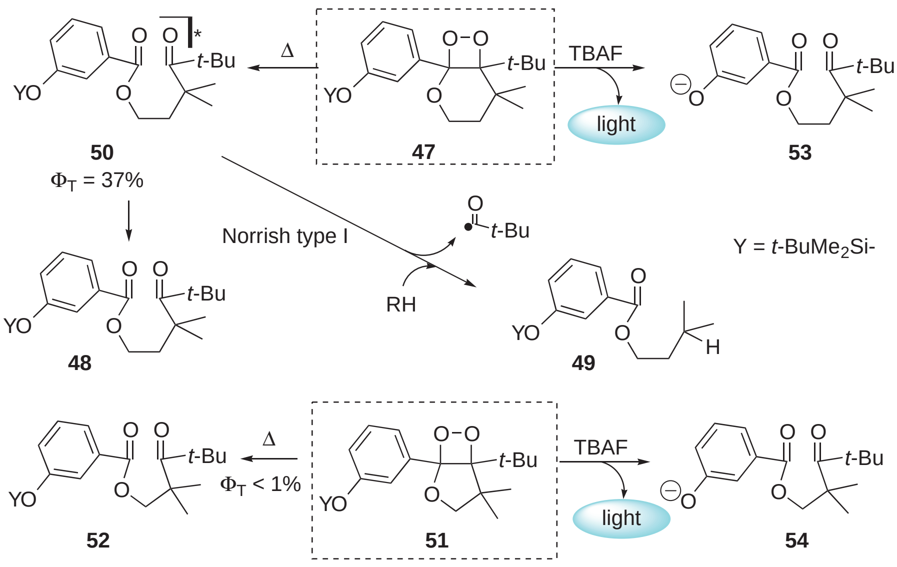
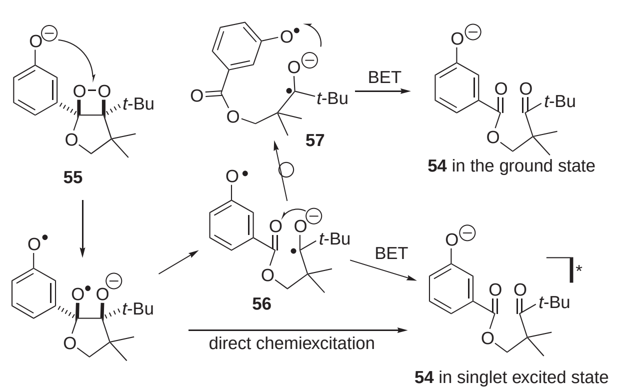
Scheme 13.

Dioxetane with an annelated six-membered ring **47** was found to possess high thermal persistency beyond expectation and to exhibit intriguing features for the thermolysis as well as for the base-induced chemiluminescent decomposition.[54] The thermolysis of **47** gave a normal decomposition product **48** along with a considerable amount of an unexpected product **49**, which was derived from the Norrish type I reaction of triplet-excited keto ester **50**. Thus, the triplet-chemiexcitation was estimated to occur in ca. 37% yield for the themolysis of **47**. On the other hand, the related dioxetane with annelated five-membered ring **51** decomposed on heating to give normal keto ester **52** exclusively. The result reveals that little triplet-chemiexcitation takes place for the thermolysis of **51**, since keto ester **52** bearing a di-*t*-alkyl ketone moiety in the excited state would cause the Norrish type I reaction, similarly to **50**. The structural difference between a dioxetane with an annelated six-membered ring **47** and one with an annelated five-membered ring **51** is that the former has a fairly puckered four-membered ring, while the latter has a nearly planar four-membered ring (torsion angle = 6.6° by a single-crystal X-ray analysis).[55] On treatment with TBAF in DMSO, dioxetane with an annelated six-membered ring **47** afforded an ester **53** with singlet-chemiexcitation efficiency ($\Phi_{\mathrm{S}} = 0.72$), which was considerably higher than that for dioxetane with an annelated five-membered ring **51** giving a keto ester **54** ($\Phi_{\mathrm{S}} = 0.63$) (Scheme 14). These results suggest, as in the case



Scheme 14.



Scheme 15.

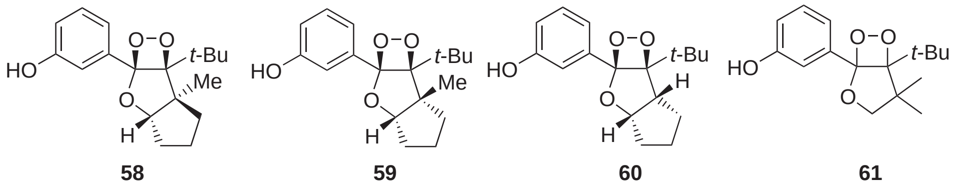
of stereoisomeric dioxetanes **26**, that the singlet-chemiexcitation process for the CT-induced decomposition of dioxetanes correlates essentially with the triplet-chemiexcitation process in thermolysis.

According to the ET/BET mechanism, the efficiency of singlet-chemiexcitation (Φ_S) depends on the efficiency of BET, which occurs in the solvent cage, thereby, diffusion of radical ions would cause the decrease of Φ_S. In addition, it has been proposed that another BET process operates, in which the radical–ion pair leads directly to the ground state, based on the study of viscosity dependence for the CT-induced decomposition of bicyclic dioxetane **51**.[25] Scheme 15 illustrates that the initial conformation of the intramolecular diradical ion-pair **56** formed on dioxetane cleavage has the ketyl radical functionality in proximity to the ester carbonyl group. This geometrical arrangement is predestined for BET, affording the excited phenolate **54**. Competing with this BET process, rotation about the methylene bonds in the diradical **56** occurs to separate spatially the ketyl radical and the ester carbonyl functionality. During such conformational motion, the ketyl-radical fragment approaches the phenoxyl group, which corresponds to the conformation **57**. BET from the ketyl radical to the phenoxy moiety in **57** should generate preferably the ground state phenolate **54** (Scheme 15).

The difference in singlet-chemiexcitation efficiency between two stereoisomeric dioxetanes (*anti*-**26** and *syn*-**26**) can be explained by the above proposal. Thus, the conformational motion unfavorable to the singlet-chemiexcitation might occur more easily for the *syn*-isomer than for the *anti*-isomer, since the steric repulsion between the phenyl and two carbonyl oxygens is presumably larger for the *syn*-isomer. On the other hand, the fact that dioxetane with an annelated six-membered ring **47** exhibits higher efficiency than a five-membered ring analog **51** may not be persuasively accounted for by the ET/BET mechanism including two BET channels. However, the CT/direct mechanism can also not account for the structural relationship of dioxetanes with singlet-chemiexcitation efficiency with convincing evidence, though the singlet-chemiexcitation process in the CT-induced decomposition of dioxetanes seems to resemble fundamentally the triplet-chemiexcitation process in the thermolysis.

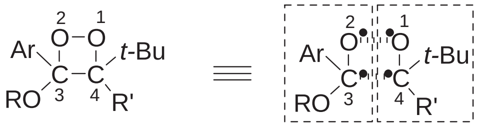
As part of a further attempt to explain the structural relationship with singlet-chemiexcitation efficiency, several tricyclic dioxetanes **58**–**60** were synthesized. Dioxetane **58** possesses a structure in which the second five-membered ring fuses to bicyclic dioxetane **61** with *trans*-junction, while dioxetane **59** has a new *cis*-fused five-membered ring (Scheme 16). Dioxetane **60** is a demethylated analog of **59**. All these dioxetanes decomposed to afford the corresponding ester of *m*-oxybenzoic acid with accompanying flash emission of blue light, on treatment with TBAF/DMSO.[55] The results summarized in Table 1 reveal that a) dioxetane **58** displays chemiluminescence with the efficiency (Φ^{CL}), the highest among those for hitherto-known dioxetanes, producing an ester of *m*-oxybenzoic acid as an emitter, and b) dioxetane **60** gives light, the yield of which is only $<1/4$ for the case of dioxetane **59**, though both structures should quite resemble each other except that a methyl at the bridge head exists or is abscent.

The intramolecular radical–ion pair **62**, if produced from **58**, in which ketyl radical and ester carbonyl functionalities are joined in a *trans*-configuration to the cyclopentane ring, should



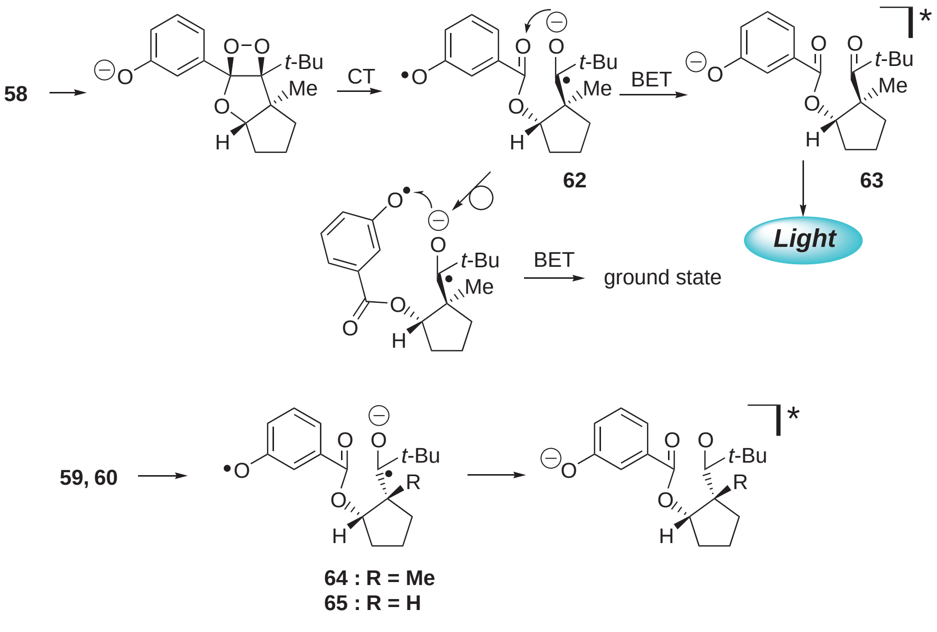
Scheme 16.

Table 1. Chemiluminescent Efficiencies and X-ray Structural Parameters of Dioxetanes **58**–**61** Bearing a 3-Hydroxyphenyl Group



Dioxetane	Φ^{CL} a)	$\Delta\Theta_A$ b) /deg	$\Delta\Theta_B$ c) /deg	Bond distances of dioxetane ring/pm $r(O_1–O_2)$	$r(C_3–C_4)$	$r(O_1–C_4)$	$r(O_2–C_3)$
58	0.32	1.7	9.8	149.8 d)	158.5 d)	148.3 d)	144.9 d)
59	0.26	1.5	9.3	153.1	158.6	145.7	146.7
60	0.063	2.6	7.1	149.7	156.2	148.9	145.9
61	0.20	1.1	8.8	149.6	157.2	148.4	146.7

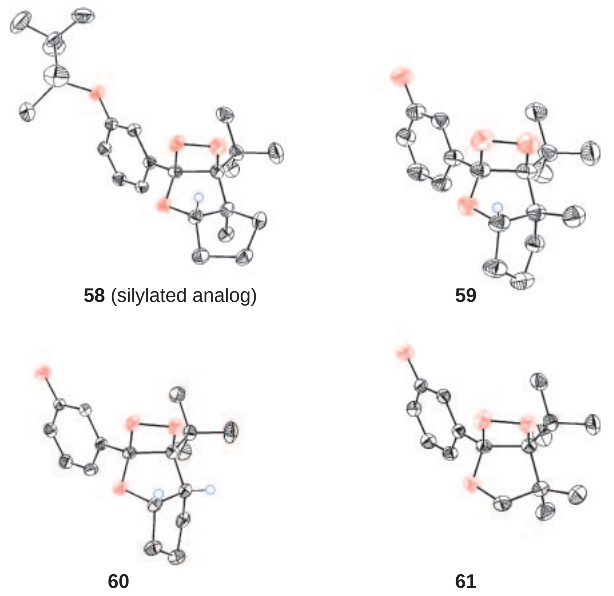
a) Chemiluminescent efficiency in TBAF/DMSO at 25 °C. b) $\Delta\Theta_A = \Theta_A - 328.5$. $\Theta_A = \Sigma(\angle\mathrm{Ar}–C_3–O_2 + \angle\mathrm{Ar}–C_3–\mathrm{OR} + \angle\mathrm{RO}–C_3–O_2)$. c) $\Delta\Theta_B = \Theta_B - 328.5$. $\Theta_B = \Sigma(\angle t\text{-Bu}–C_4–O_1 + \angle t\text{–Bu}–C_4–R' + \angle R'–C_4–O_1)$. d) Data for 3-(*t*-butyldimethylsiloxy)phenyl-analog of **58**.



Scheme 17.

change its initial conformation as easily as or more easily than in the case of the related bicyclic dioxetane **61** and *cis*-isomer **59**. According to the ET/BET mechanism, this conformational motion is presumably unfavorable for the singlet-chemiexcitation, and thereby an increased efficiency may not be expected for **58**, contrary to the fact. Furthermore, a radical–ion pair **65** derived from **60** should possess a very similar skeleton to that of **64** derived from **59** except that the 4-methyl group is removed, so that the chemiluminescent efficiency (Φ^{CL}) for **60** is expected to be as high as that for **59**, according to the ET/BET mechanism. However, dioxetane **60** gave light in very poor yield (Scheme 17).

Dioxetanes **58**–**61** compose formally of two elements facing each other, namely Ar(RO)C$^\bullet$–O$^\bullet$ and *t*-Bu(R′)C$^\bullet$–O$^\bullet$, of which the former becomes an excited aromatic ester and the latter becomes a ketone in the ground state, after the CT-induced decomposition: **58**–**61** are regarded here as 1,2-dioxetanes bearing Ar and RO at the 3-position, and *t*-Bu and R′ at the 4-position. These elements, especially *t*-Bu(R′)C$^\bullet$–O$^\bullet$, apparently possess tetrahedral structure distorted considerably



58 (silylated analog) **59**

60 **61**

Fig. 5. ORTEP views of tricyclic dioxetanes **58–60** and parent dioxetane **61**.

into the trigonal frame of carbonyl, referring to X-ray structural parameters of these dioxetanes as shown in Fig. 5 and Table 1. The sum of bond angles, $\Theta_A = \Sigma(\angle\text{Ar–C}_3\text{–O}_2 + \angle\text{Ar–C}_3\text{–OR} + \angle\text{RO–C}_3\text{–O}_2)$, $\Theta_B = \Sigma(\angle t\text{-Bu–C}_4\text{–O}_1 + \angle t\text{-Bu–C}_4\text{–R}' + \angle\text{R}'\text{–C}_4\text{–O}_1)$, and bond lengths, r(C–O) of dioxetane, may become indices to examine relationship between the structures of dioxetanes and Φ^{CL} (Φ_S). Table 1 shows that the deviation ($\Delta\Theta_B$) of Θ_B from three times of tetrahedral bond angles (328.5°) was the largest for dioxetane **58** performing the highest Φ^{CL}, while the smallest was for dioxetane **60** with the lowest Φ^{CL} among those for dioxetanes **58–61**, and the order of $\Delta\Theta_B$ agreed with the order of Φ^{CL}, **58** > **59** > **61** ≫ **60**, though deviations, $\Delta\Theta_A$ were small. The bond length r(C–O) (Ar side) was the shortest and $\Delta r = r(\text{C–C}) - r(\text{O–O})$ was the largest for **58** among those for dioxetanes **58–61**. Although much more data for X-ray single crystallographic analysis of dioxetanes and their chemiluminescent properties are required to clarify the relationships between the structures of dioxetanes and their chemiluminescent efficiency, the data summarized in Table 1 show that these dioxetanes appear to conceal a pre-existing structure more or less comprised of two carbonyl elements. Thus, among **58–61**, a dioxetane exhibiting higher chemiluminescent efficiency might possess t-Bu(R′)C$^\bullet$–O$^\bullet$ element closer to t-Bu(R′)C=O, which is predestined to form in the ground state with trigonal frame after the decomposition of dioxetane.

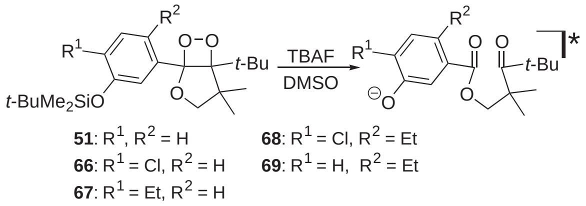
Rate of the CT-Induced Decomposition Affected by the Conformation of Hydroxyarene Moiety Attached to 1,2-Dioxetane Ring

The position of oxido anion on the aromatic ring attached to dioxetane as well the easiness of oxidation of an oxidoarene anion as an electron donor have already been described to affect significantly the CT-induced decomposition rate of dioxetane (k^{DICT}). In addition to these electronic factors of the aromatic electron donor, the decomposition rate has been already observed to change considerably in response to the structure of dioxetanes bearing a m-(t-butyldimethylsiloxy)phenyl **4**, **14–16** under the same conditions. Thus, the study of dioxetanes **14** has suggested that the CT-induced decomposition of a dioxetane bearing a phenoxide anion proceeds preferentially when the aromatic electron donor lies in a specific conformation relative to the dioxetane ring.[33,34] Such conformational effect appears to be caused by restricted rotation of the aromatic ring, though it can formally rotate freely around the bond joining it to the dioxetane ring. A similar effect has been typically observed for the thermal stability of bicyclic dioxetanes bearing a methoxyphenyl, among which the *ortho*-isomer of **19** is surprisingly stable, though the *para*-isomer is less stable than **19**, as expected.[56]

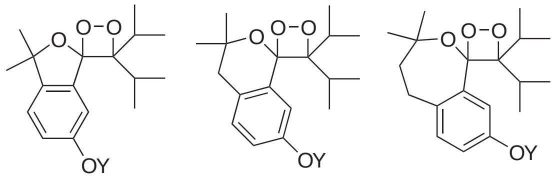
We attempted here to examine whether the *ortho*-substituent on the aromatic ring affects sterically also the CT-induced decomposition rate of dioxetanes **66–69** bearing a m-siloxyphenyl group substituted with ethyl and/or Cl.[57,58] The results in Table 2 reveal that the introduction of an ethyl at the *para*-position effected increase in the CT-induced decomposition rate (case **67**), whereas the *ortho*-substitution of ethyl tended to decrease the decay rate (cases **68** and **69**), though its effect should be similar to that of the *para*-substitution in respect of an electronic factor.

For spirodioxetanes **70–72**, a phenolic group is unable to rotate around the C–C bond joining it to the dioxetane ring (Scheme 18). When **70–72** were treated with TBAF/DMSO at 25 °C, the CT-induced decomposition occurred immediately to emit light with significantly different rates. The half-life of dioxetanes for the CT-induced decomposition increased in

Table 2. Substituent Effect on TBAF-Induced Chemiluminescent Decomposition of Bicyclic Dioxetanes Bearing a 3-Siloxyphenyl Group



	R^1	R^2	$\Delta G^\ddagger$/kJ mol^{-1}	k^{DICT} at 25 °C/s^{-1}
51	H	H	78.2	0.151
66	Cl	H	87.9	2.26×10^{-3}
67	Et	H	66.9	1.78
68	Cl	Et	90.0	1.14×10^{-3}
69	H	Et	82.0	2.40×10^{-3}



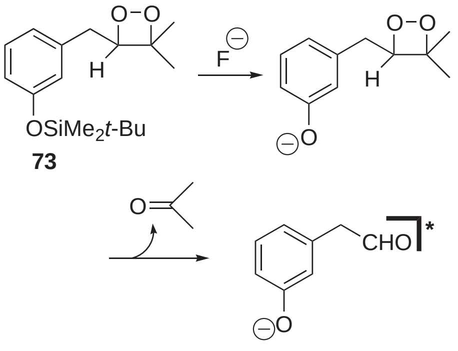
70 **71** **72**

Y = -SiMe$_2$$t$-Bu

Scheme 18.

the order of a five-membered ring analog **70** ($t_{1/2}^{\mathrm{DICT}} = \log_e 2/k^{\mathrm{DICT}} = 150$ s) to a six-membered ring one **71** ($t_{1/2}^{\mathrm{DICT}} = 4500$ s), while that for a seven-membered ring analog **72** became again as short ($t_{1/2}^{\mathrm{DICT}} = 9.4$ s) as that for the parent dioxetane **14** (R = Me) ($t_{1/2}^{\mathrm{DICT}} = 6.3$ s).[59] These facts are most likely rationalized also by the suggestion that there exists a specific conformation of an aromatic electron donor preferable for the intramolecular CT-induced decomposition for dioxetanes bearing an aromatic electron donor.

The *ortho*-substitutent effect and the results of chemiluminescent decomposition of spirodioxetanes described above suggest that the intramolecular CT from an aromatic electron donor to O–O should occur "through bond" rather than by "jump through space." This suggestion is consistent with the "odd/even" relationship regarding the intramolecular CT-induced decay rates for dioxetanes bearing a phenolic moiety **14**, **31**, and **32**. That the CT might operate "through bond" for the decomposition of dioxetane has been reported also for the TBAF-induced reaction of dioxetane **73**, in which a phenoxide anion is joined through a methylene, so that CT occurs likely through the σ-bonds, based on the kinetic analysis (Scheme 19).[60,61] Dioxetanes **74** and **75** are another example of dioxetane bearing a phenoxide separated through several σ-bonds.[62,63] However, on treatment with TBAF/DMSO, dioxetane **74** underwent an unexpected reaction that yielded predominantly acyloin **76**, but only a small amount of the product **77** derived from the CT-induced decomposition with accompanying very weak light. Similar base-induced decomposition of dioxetane **75** gave an intramolecular redox product **78** exclusively (Scheme 20).

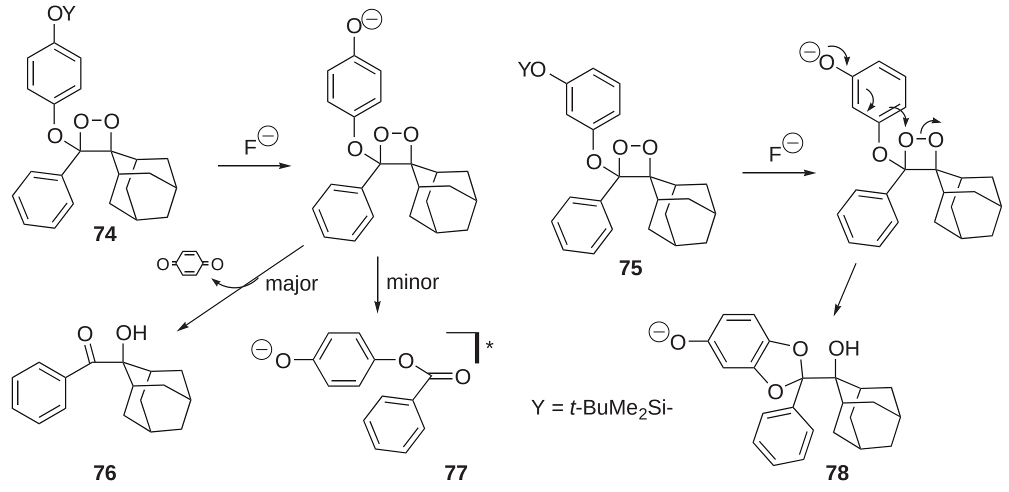


Scheme 19.

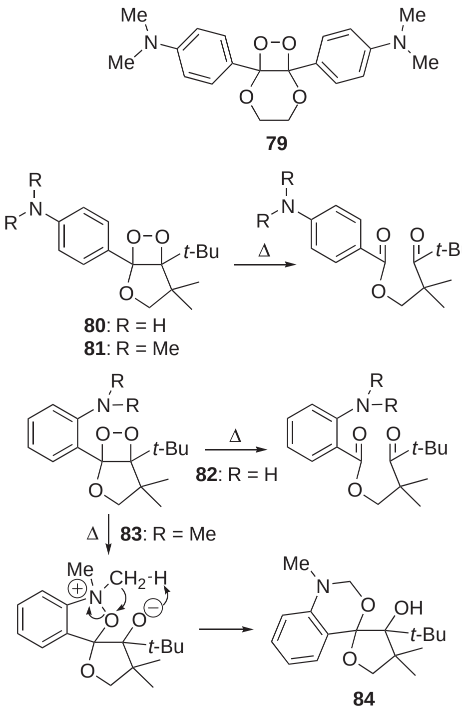
Dioxetanes substituted with a *p*-aminophenyl group, represented by bicyclic dioxetane **79**, have been reported to undergo thermally the intramolecular CT-induced decomposition without any base-triggering.[64] A similar phenomenon was also observed for bicyclic dioxetanes bearing a *p*-aminophenyl **80** or *p*-(dimethylamino)phenyl **81**.[56] Dioxetanes **80** and **81** decomposed, on heating, far more rapidly than their methoxyphenyl-analog (*p*-isomer of **19**). On the other hand, their *ortho*-analogs exhibited a significantly different manner on thermolysis. Dioxetane **82** bearing an *o*-aminophenyl was far more stable than the *p*-isomer **80**, as observed for methoxyphenyl-substituted dioxetane **19** and its *ortho*-isomer. However, dioxetane bearing an *o*-(dimethylamino)phenyl **83** was very unstable thermally and underwent intramolecular nucleophilic reaction of the amino group onto peroxide to afford predominantly a cyclic compound **84** (Scheme 21).[56] Unusual decomposition occurred as a predominant mode similarly for an *N*-methyl-analog of **82** and **83**. The "through space" CT from the amino group to O–O may participate initially for these unusual decompositions, as often pointed out for the oxidation of amines.

Color for Chemiluminescence from Hydroxyarene-Substituted Dioxetanes

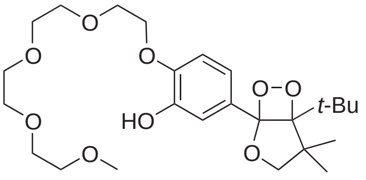
As described partly in the previous section, various dioxetanes bearing a *meta*-phenoxide anion give intense blue light ($\lambda_{\max}^{\mathrm{CL}} = 463$–$470$ nm), while related dioxetanes **31** and **32** bearing an *ortho*- or a *para*-phenoxide anion afford light with $\lambda_{\max}^{\mathrm{CL}}$ shorter than that of the *meta*-isomer, though their chemiluminescent efficiency decreases significantly. Introduction of a chlorine or an alkoxy group at the 4-position of 3-hydroxyphenyl caused the blue-shift of $\lambda_{\max}^{\mathrm{CL}}$ of chemiluminescence without decreasing of efficiency (Φ^{CL}), as observed for dioxetanes **66** and **85**: $\lambda_{\max}^{\mathrm{CL}} = 453$ nm for **66** and **85** (Scheme 22).[57,65] It is worth pointing out that spirodioxetane with a five-membered ring **70** gave violet light ($\lambda_{\max}^{\mathrm{CL}} = 456$ nm), while its six-membered ring analog **71** gave light with rather longer wavelength ($\lambda_{\max}^{\mathrm{CL}} = 476$ nm), and a seven-membered ring analog **72** emitted light again



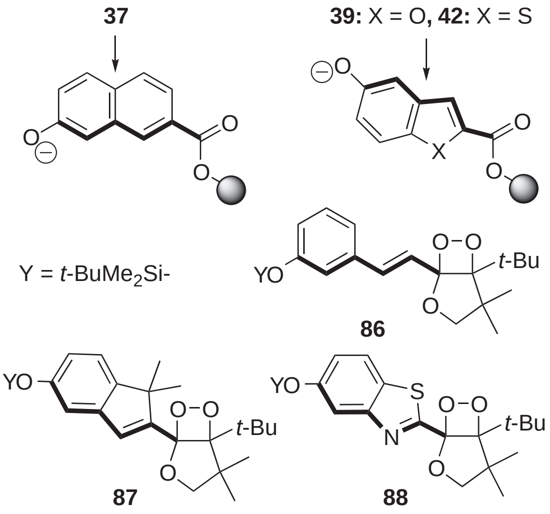
Scheme 20.



Scheme 21.



Scheme 22.



Scheme 23.

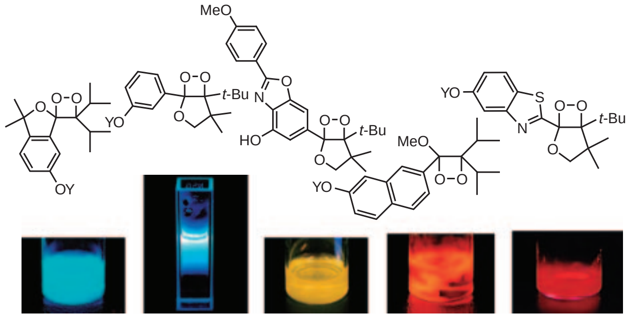
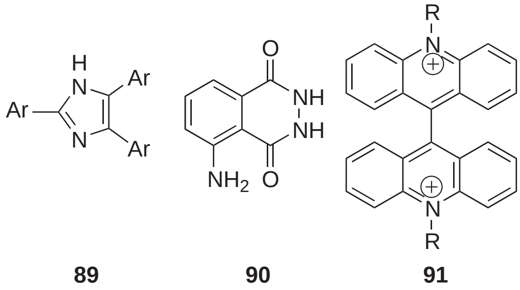


Fig. 6.

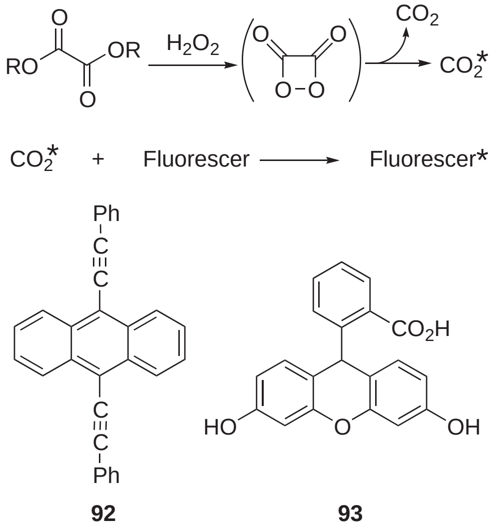


Scheme 24.

at the shorter wavelength region ($\lambda_{\rm max}{}^{\rm CL} = 464$ nm).[59]

On the other hand, the results in Fig. 4 show that three types of dioxetanes bearing 5-oxidonaphthalen-2-yl **35**, 5-oxidobenzofuran-2-yl **39**, or 5-oxidobenzothiophen-2-yl **42** exhibit emission of similar red light: $\lambda_{\rm max}{}^{\rm CL} = 620$–$628$ nm. For these cases, a cinnamate-like π-conjugation system fixed in a plane appears to play an important role to emit red light rather with high efficiency. In fact, dioxetanes **86** and **87** giving a cinnamate or sterically fixed cinnamate as an emitter were found to emit similarly red light ($\lambda_{\rm max}{}^{\rm CL} = 644$ and 637 nm), though the efficiency ($\Phi^{\rm CL}$) was considerably lower than that of **37**, **39**, and **42**.[66,67] Further longer-wavelength chemiluminescence was realized by the exchange of the cinnamate-like π-conjugation system to a benzylideneamine-like system.[67] Thus, colorless dioxetane **88** bearing a 5-siloxybenzothiazol-2-yl group decomposed to emit flash crimson light ($\lambda_{\rm max}{}^{\rm CL} = 725$ nm) in TBAF/DMSO (Scheme 23).

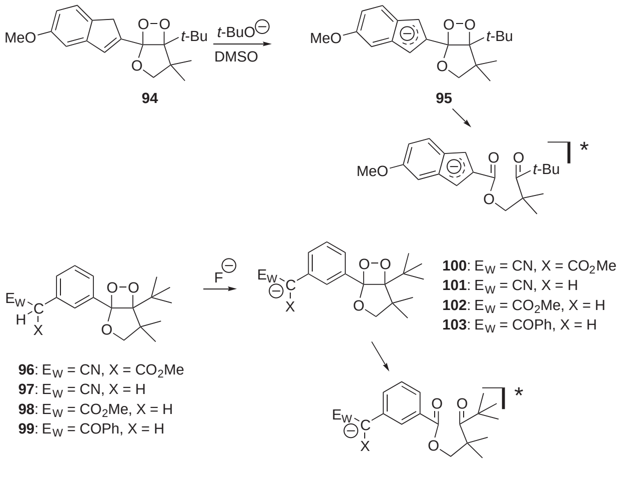
The color modulation from violet to crimson has now become feasible for dioxetane-based chemiluminescence by design of a substituent(s) and its position on aromatic electron donor as well as an aromatic ring itself (Fig. 6). This would be one strong advantage of dioxetane-based substrates comparing with synthetic chemiluminescent substrates having a rather old history, namely, derivatives of lophine (**89**), luminol (**90**), and lucigenin (**91**) (Scheme 24).[68–70] On the other hand, as typically known for cyalume®-type (oxalate/hydrogen peroxide) chemiluminescence, the color of luminescence can be modulated by adding a fluorescer such as 9,10-bis(phenylethynyl)anthracene (**92**) (blue light) in the chemiluminescent system.[71] Addition of a fluorescer can be applied also to the dioxetane-based chemiluminescence. For example, yellow light emission was observed when dioxetane **51** was triggered in the presence of fluorescein (**93**) (Scheme 25). Since this type of color modulation is based on the energy transfer from the initially formed excited species to the fluorescer, tethering of a fluorescer to dioxetane should become a method to improve chemiluminescent efficiency as well as the color modulation. Such design of dioxetanes has been partly realized.[72]



Scheme 25.

Color for Chemiluminescence from Dioxetanes Bearing an Electron-Donor Other than an Anion of Arenoxide

Dioxetanes active toward the intramolecular CT-induced decomposition do not require necessarily arenoxide anion as an electron donor but an easily-oxidized aromatic substituent. Thus, we attempted to develop a new type of dioxetanes, an aromatic substituent attached to which can be transformed into an easily-oxidized anion, such as phenylmethanide anion, benzenethiolate anion, and phenylamido anion. On treatment with *t*-BuOK in DMSO, dioxetane **94** related to **87** decomposed to give flash crimson light.[73] In this case, a benzocyclopentenide anion intermediate **95** might cause the CT-induced decomposition of dioxetane **94**. Rather simple dioxetanes **96–99** designed for generating a benzilic anion were those which had a phenyl group substituted with a methyl bearing electron-withdrawing group(s) at the *meta*-position.[74] When dioxetane **96** bearing a cyano(methoxycarbonyl)methyl group was treated with TBAF in DMSO, it decomposed to afford intense yellow light ($\lambda_{\max}{}^{\mathrm{CL}} = 530$ nm, $\Phi^{\mathrm{CL}} = 5.7 \times 10^{-3}$). On similar treatment with TBAF, dioxetanes bearing a cyano **97**, methoxycarbonyl **98**, or benzoyl group **99** exhibited emission of flash red light: $\lambda_{\max}{}^{\mathrm{CL}} = 702$ nm for **97**, 666 nm for **98**, and 622 nm for **99**. It should be noted here that the maximum wavelength of chemiluminescence ($\lambda_{\max}{}^{\mathrm{CL}}$) became shorter, as the acidity of the parent carbon acid, corresponding to the aromatic moieties on dioxetanes **96–99**, increased: CH_3CN ($pK_a = 25$) for **97** < CH_3CO_2Me ($pK_a = 24.5$) for **98** < CH_3COPh ($pK_a = 19$) for **99** < $CH_2(CN)CO_2Me$ ($pK_a = 9$) for **96**. The CT-induced decomposition rate was found also to decrease in a similar order: $k^{\mathrm{DICT}} > 35$ s^{-1} for **97**, $k^{\mathrm{DICT}} = 1.7$ s^{-1} for **98**, $k^{\mathrm{DICT}} = 1.6$ s^{-1} for **99**, and $k^{\mathrm{DICT}} = 3.0 \times 10^{-4}$ s^{-1} for **96**. These results show that the CT-induced decomposition of dioxetanes **100–103** bearing a phenylmethanide anion produced from the corresponding dioxetanes **96–99** occurs more easily to give light with the longer $\lambda_{\max}{}^{\mathrm{CL}}$, as the phenylmethanide anions become less stable and are more easily oxidized (Scheme 26).

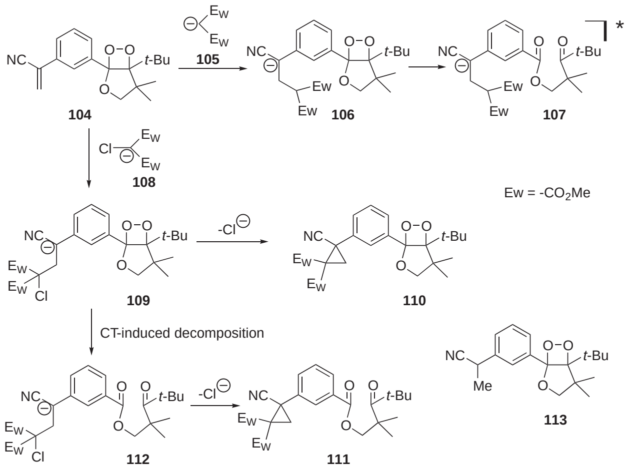


Scheme 26.

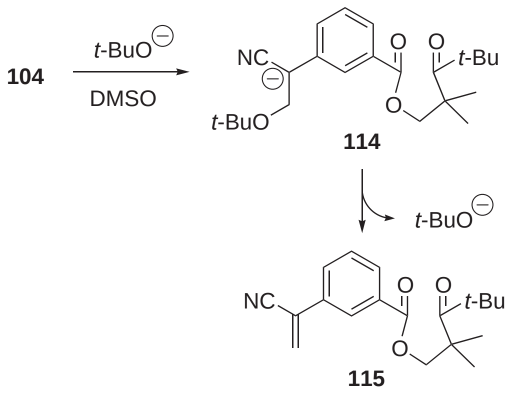
A benzylic carbanion like that as described above would be generated by the Michael (conjugate) addition of a nucleophile to a styrene skeleton bearing an electron-withdrawing group at the α-position. Dioxetane **104** was one in which such idea was incorporated into the design of new chemiluminescent substrates. When dioxetane **104** was treated with a stable anion of bis(methoxycarbonyl)methanide (**105**) complexed with 18-crown-6 ether, $[\mathrm{K}\subset(18\text{-crown-6})]^{+}\cdot(\mathbf{105})^{-}$, in benzene, **105** added to dioxetane **104** to give an intermediary dioxetane **106** bearing a benzylic carbanion, which rapidly decomposed into a keto ester (**107**) with accompanying emission of crimson light ($\lambda_{\max}{}^{\mathrm{CL}} = 737$ nm).[75] When an anion of chlorobis(methoxycarbonyl)methanide (**108**) was used as a reagent for triggering instead of **105**, the CT-induced chemiluminescent decomposition occurred to give a keto ester **111** with accompanying emission of light, though the true emitter was presumably a carbanion **112**. In this case, the chemiluminescent decomposition contended with the intramolecular cyclopropanation which extinguished the intermediary phenylmethanide anion **109** to give dioxetane bearing a cyclopropylphenyl group **110**. The decrease of light emission from **104** coincided with the degree of formation of dioxetane **110**. Thus, the rate of intramolecular cyclopropanation was roughly estimated from these results and the rate of CT-induced decomposition for closely related dioxetane **113**. The rate constant estimated was 3.2 s^{-1} for CT-induced decomposition of **109**, while it was 1.3 s^{-1} for the intramolecular cyclopropanation of **109** to **110** (Scheme 27).

The results described above reveal that dioxetanes bearing a phenyl substituted with an E_w–CH_2–, E_w–CH(R)–, or $E_w(E_w{}')$–CH– group tend to afford chemiluminescence with far longer $\lambda_{\max}{}^{\mathrm{CL}}$ than those for dioxetanes bearing a phenolic group, and the modulation of color ranging from yellow to crimson can be attained by the choice of phenylmethanide anion generated. In addition, the results show that the chemiluminescent decomposition of dioxetane induced by the Michael-type reaction of a nucleophile provides possibly a new probe to know the feature of intramolecular reaction, which occurs concurrently with the CT-induced decomposition of an intermediary dioxetane bearing a phenylmethanide anion.

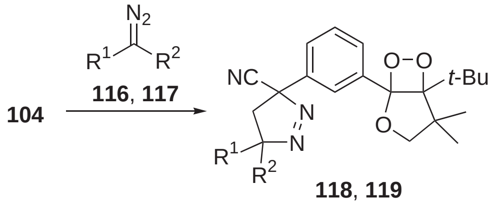
The attack of *t*-butoxide anion to **104** caused its decomposition to emit crimson light ($\lambda_{\max}{}^{\mathrm{CL}} = 706$ nm), but observed was not a Michael addition/decomposition product (neutral form of **114**) but a keto ester **115** bearing a 3-(1-cyanovinyl)-



Scheme 27.



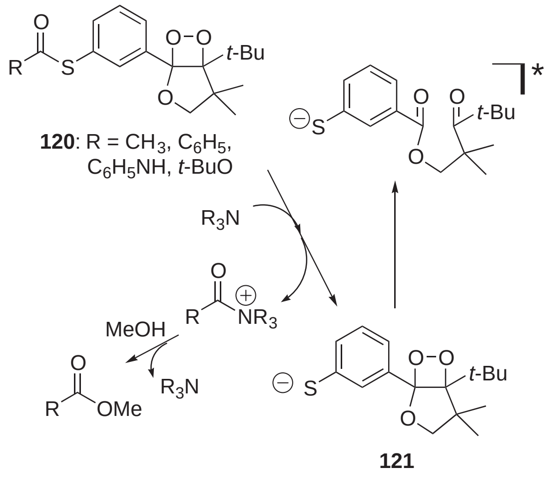
Scheme 28.



116, **118**: R^1, R^2 = Ph
117, **119**: R^1 = Me_3Si, R^2 = H

Scheme 29.

phenyl, which was most likely produced by elimination of the *t*-butoxide anion from **114** (Scheme 28). This result shows that the Michael addition of *t*-butoxide to **104** and the successive CT-induced decomposition occur to give intermediary phenylmethanide anion **114**, which loses reversibly the *t*-butoxide anion. Therefore, the rate of elimination of RO^- may be estimated from the decrease of the formal rate of the RO^--induced decomposition of **104**. On the other hand, diphenyldiazomethane (**116**) and trimethylsilyldiazomethane (**117**) underwent 1,3-dipolar cycloaddition onto the 1-cyanovinyl moiety of dioxetane **104** to afford the corresponding pyrazoline derivatives **118** and **119** with little accompanying decomposition of the dioxetane ring (Scheme 29).[76] The result agrees with the knowledge that 1,3-dipolar cycloaddition proceeds generally by the concerted mechanism except in special cases.

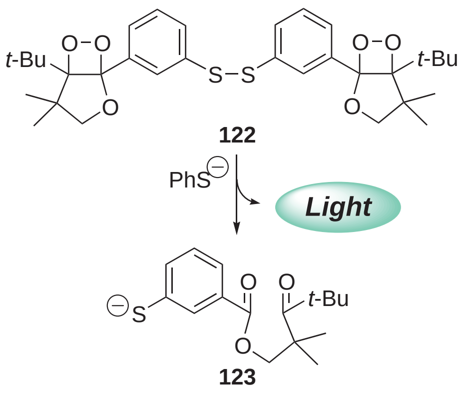


Scheme 30.

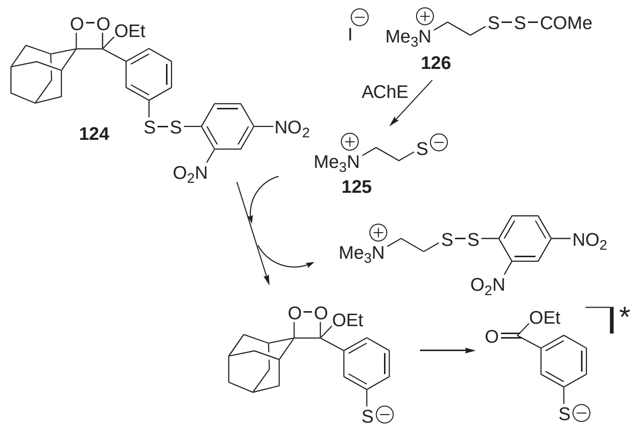
Benzenethiol is a stronger acid than phenol so that a conjugate base, namely, the benzenethiolate anion, is generated more easily than a phenolate anion. Therefore, benzenethiol can become also a new triggering system for the dioxetane-based chemiluminescence. Although benzenethiol and related sulfides are known to be unstable under various oxidation conditions, phenyl sulfide and the ester of benzenethiol were satisfactorily stable against singlet oxygenation and dioxetanes. Thermally stable dioxetanes **120** bearing an acylthiophenyl group were triggered with base to emit yellow light ($\lambda_{\max}{}^{CL} = 535$ nm) with chemiluminescent efficiency ($\Phi^{CL} = 1.5 \times 10^{-4}$–$2.4 \times 10^{-4}$) in DMSO or acetonitrile.[77] The triggering of **120** was able to be attained even by the use of a *tert*-amine to afford a thiolate anion **121** together with an intermediary acylammonium, which gave an ester by addition of methanol after the emission of light ceased (Scheme 30). Another characteristic feature of the dioxetane **120** was that the chemiluminescent efficiency (Φ^{CL}) decreased not so significantly even in an aqueous system, comparing with the case of dioxetanes **5** and **55** bearing a phenolic group, for which Φ^{CL} in H_2O is only 1/10000–1/40000 of Φ^{CL} in DMSO (vide infra).

Since a diaryl disulfide is generally far more persistent than a sulfide against oxidation, and is known to react with a thiolate anion to produce an arenethiolate anion, it should be used as a new triggering system for the dioxetane-based chemiluminescence. Thus, decomposition of dimeric dioxetane **122** was induced by the benzenethiolate anion to give keto ester **123** with accompanying yellow light (Scheme 31).[78] This type of chemiluminescence has been applied to estimate the activity of acetylcholine esterase by the use of a substrate **124**, which is triggered with the thiolate anion **125** generated from acetylthiocholine iodide (**126**), as shown in Scheme 32.[79]

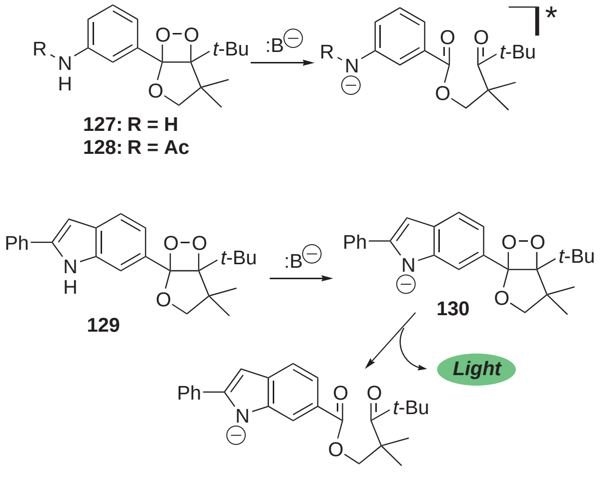
A proton of N–H in aniline can be removed with a strong base to afford a phenylamido anion, which acts also as an electron donor. As illustrated in Scheme 33, dioxetane **127** decomposed to emit red light ($\lambda_{\max}{}^{CL} = 642$ nm), though very weak, on treatment with *t*-BuOK in DMSO.[80] Although a proton of NH in N-acylated aniline should be far more easily abstracted,



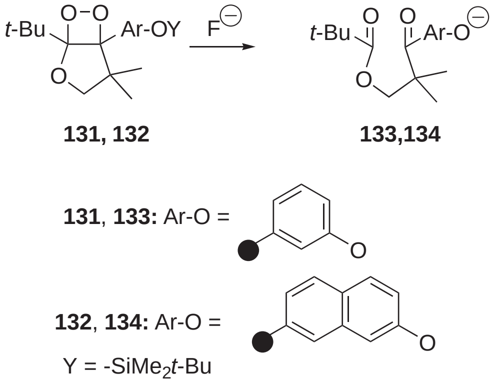
Scheme 31.



Scheme 32.



Scheme 33.



Scheme 34.

dioxetane **128** bearing an acetanilide decomposed sluggishly on treatment with *t*-BuOK. The N–H of the indole ring can be easily deprotonated with a base. Thus, dioxetane **129** bearing a 2-phenylindol-6-yl group was deprotonated to give unstable dioxetane **130**, which decomposed with accompanying flash emission of blue light ($\lambda_{max}{}^{CL}$ = 452 nm).[81]

Most dioxetane-based chemiluminescent substrates described produce an aromatic or α,β-unsaturated ester as an emitter. The main reasons are that aromatic esters are expected to produce the lowest excited state as singlet, and that 1-alkoxyvinylbenzenes but not 2-alkoxyvinylbenzenes undergo preferentially 1,2-addition of singlet oxygen.[82] However, there has been little known inspection based on experimental results. Thus, dioxetanes **131** and **132** were synthesized. These dioxetanes decomposed on treatment with TBAF in DMSO to give the corresponding aromatic ketones **133** and **134** (Scheme 34).[83] Characteristic features of chemiluminescence from **131** and **132** were that the maximum wavelength of emission was shifted to longer region ($\lambda_{max}{}^{CL}$ = 549 nm for **131** and 642 nm for **132**), comparing with those from the related dioxetanes **51** and **37** giving an aromatic ester, though the decomposition rates were significantly prolonged, and chemiluminescent efficiencies decreased considerably.

Dioxetanes **135–137** bearing an *N*-(substituted)methyl-2-pyridon-4-yl group were rather special examples, which were synthesized in the course of our investigation of heteroarene-substituted dioxetanes. Dioxetane **135** with an *N*-benzyl-2-pyridone moiety decomposed thermally to the expected keto ester **138** with emission of rather intense violet light ($\lambda_{max}{}^{CL}$ = 411 nm) in hot toluene,[84] while it exhibited flash emission of yellow light on treatment with $[K\subset(18\text{-crown-6})]^+ \cdot t\text{-BuO}^-$ in THF. Dioxetane **136** bearing an *N*-(*t*-butoxycarbonylmethyl)-2-pyridone moiety decomposed into keto ester **139** to give flash yellow light similarly to the case of **135**.[85] The t-BuO$^-$-induced chemiluminescent decomposition of **135** and **136** is most likely attributed to the formation of intermediary *N*-methanide anions **140** and **141**, since t-BuO$^-$ induced hardly the decomposition of dioxetane **137** bearing *N*-(1-*t*-butoxycarbonyl-1-methylethyl)-2-pyridone under similar conditions (Scheme 35).

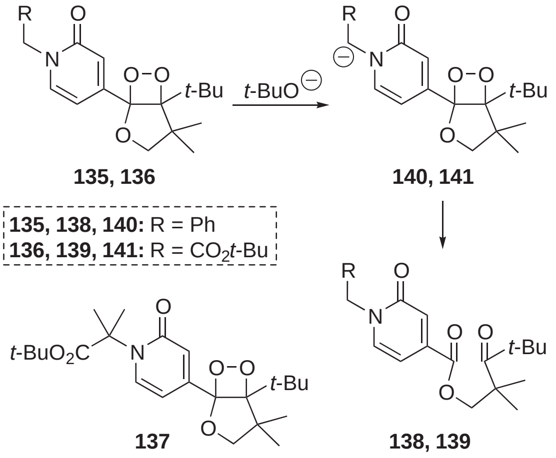
Chemiluminescence of Dioxetanes Bearing a Biaryl-Type Moiety

Dioxetanes **4c** and **142** are now being applied to modern biological and biomedical analyses, though their chemiluminescent efficiencies (Φ^{CL}) decrease into only 1.1×10^{-5}–7.5×10^{-6} in an aqueous system. One important purpose of our investigation has been to realize dioxetane-based chemiluminescent substrates with high-performance even in an aqueous system. In the course of such investigation, we have designed several models based on working hypotheses to decrease unfavorable participation of water. Among them, a successful model was to introduce a substituent, able to participate in the intramolecular hydrogen bonding, at the 4-position of the phenol in dioxetanes bearing a 3-hydroxyphenyl moiety.[86–90] Dioxetanes bearing a 3-hydroxyphenyl moiety substituted with isoxazol-3-yl (**143**),[89] benzoxazol-2-yl (**144**),[90] or benzothiazol-2-yl (**145**)[90] at the 4-position exhibited highly effective chemiluminescence even in an aqueous system: Φ^{CL} in TBAF/acetonitrile = 0.44 for **143**, 0.46 for **144**, and 0.39 for **145**, while Φ^{CL} in NaOH/H_2O = 0.24 for **143**, 0.18 for **144**, and 0.12 for **145**) (Scheme 36). The marked increase of Φ^{CL} in an aqueous system is most likely attributed to the synergetic effect of increased chemiexcitation efficiency (Φ_S) and increased fluorescence efficiency (Φ^{fl}) of the emitter.[88]

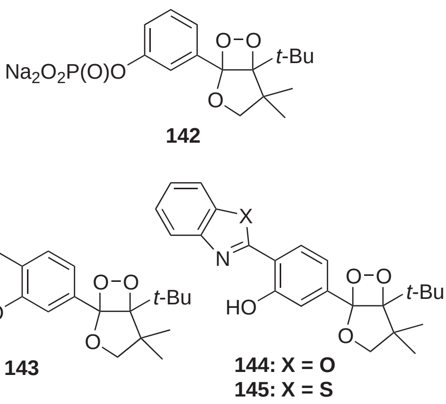
A semi-empirical AM1 MO calculation is suggestive in rationalizing the effect of water on the fluorescence efficiency of the emitter. HOMO and LUMO coefficients for **6** and **146** (a model for the emitter produced from **143**) are shown in Fig. 7. The HOMO coefficients distribute mainly on the phenoxide ring both for **6** and **146**, and the distribution patterns are similar. On the other hand, the LUMO coefficients distribute predominantly on the ester carbonyl moiety and the neighborhood carbons of phenoxide for **6**, whereas they distribute little there but are localized on the isoxazole ring for **146**. These results reveal that the hydrogen bonding of water molecule(s) onto the ester carbonyl moiety rather than onto the phenoxide oxygen most likely decreases Φ^{fl} for the parent emitter **6**. In other words, the effect of introducing an isoxazole ring is presumably that the feature of the excited state for the parent emitter **6** changes into a charge-separated excited state **146** in which the isoxazole part acts as the acceptor of an electron jumping to LUMO, which is initially distributed on the phenoxide in HOMO.

One important question arising from the results of an MO calculation was whether the initial hypothesis "intramolecular hydrogen bonding" was correct to improve Φ^{fl} for hydroxybenzoate-type emitters. Thus, we attempted to introduce isoxazole or benzothiazole at the 5-positon of the aromatic ring in dioxetane bearing a 3-hydroxyphenyl. Dioxetanes **147**, **148**, and **149** exhibited considerable increase of Φ^{CL} (3×10^{-4}–1×10^{-2}), though not comparable with those for the 4-isomers **143**, **144**, and **145** in an aqueous system (Scheme 37).[91]

Our next attempt was to introduce a larger aromatic substituent such as carbazol-9-yl into the 3-hydroxyphenyl moiety attached to dioxetane. The introduction of carbazol-9-yl at the less crowded 5-position of the 3-hydroxyphenyl moiety was successfully attained to afford dioxetane **150**.[92] Dioxetane **150** displayed emission of blue light with high efficiency



Scheme 35.



Scheme 36.

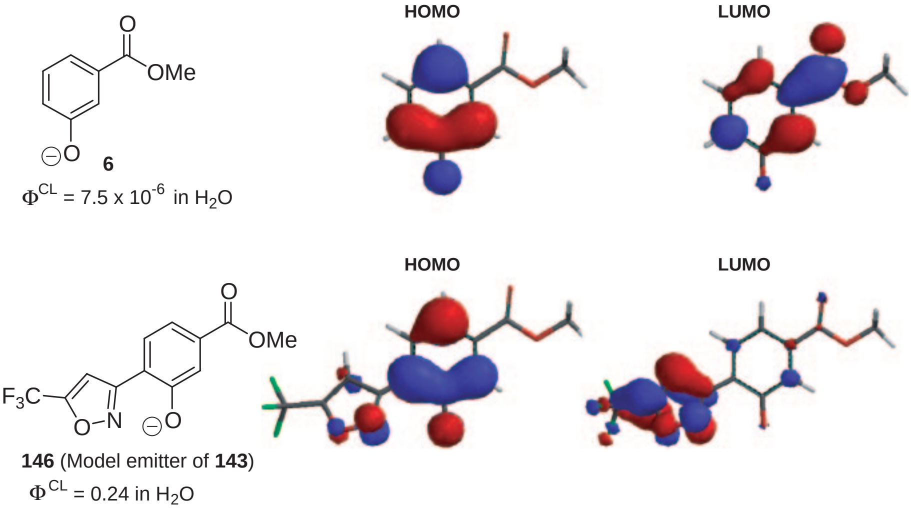
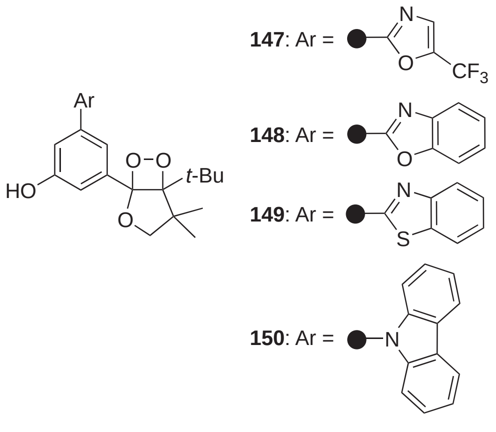


Fig. 7. HOMO and LUMO coefficients for oxido anions of methyl oxidoarenecarboxylates.



Scheme 37.

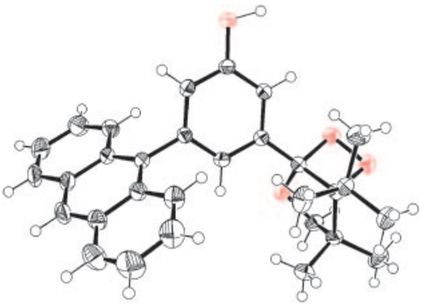
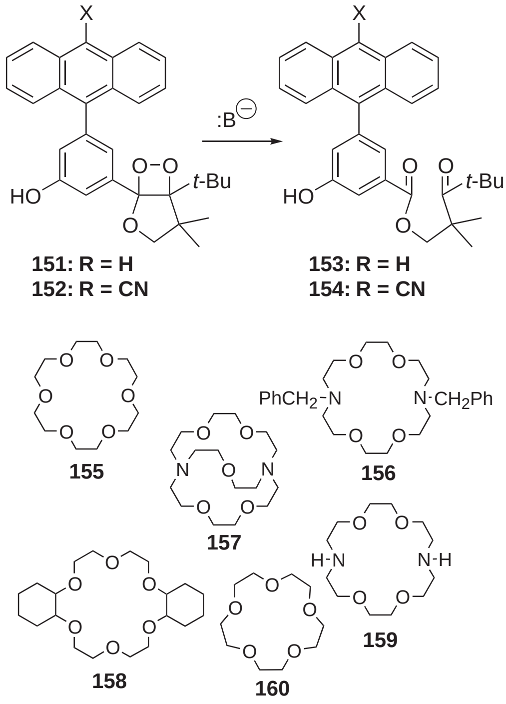


Fig. 8. ORTEP view of dioxetane **151**.

($\Phi^{\mathrm{CL}} = 0.37$) in TBAF/DMSO system, and with moderate Φ^{CL} in an aqueous system.

Polynuclear aromatic hydrocarbon skeletons such as 1-naphthyl-, 2-naphthyl, and 9-anthryl were introduced at the 5-position of the 3-hydroxyphenyl moiety of dioxetane **61**, similarly to the case of **150**. Dioxetane **151** possesses a 3-(9-anthryl)-5-hydroxyphenyl moiety, in which the twisted angle of two aromatic rings can vary considerably depending on the circumstances, though these two aromatic rings lie in twisted angle $\theta \fallingdotseq 66°$ for the stable conformation, as clarified by X-ray single crystallographic analysis illustrated in Fig. 8. Dioxetane **151** decomposed rapidly to afford crimson light ($\lambda_{\mathrm{max}}{}^{\mathrm{CL}} = 709$ nm) on treatment with tetrabutylammonium *t*-butoxide ($\mathrm{TBA}^{+}\cdot t\text{-}\mathrm{BuO}^{-}$) in benzene. The chemiluminescent decomposition of **151** triggered with TBAF/DMSO gave a similar result ($\lambda_{\mathrm{max}}{}^{\mathrm{CL}} = 709$ nm). For these cases, the maximum wavelength of chemiluminescence ($\lambda_{\mathrm{max}}{}^{\mathrm{CL}}$) coincided with the maximum wavelength of fluorescence ($\lambda_{\mathrm{max}}{}^{\mathrm{fl}}$) for oxido anion of an authentic keto ester **153**. When the decomposition of **151** was carried out by the use of various complexes of crown ether with *t*-BuOK, $[\mathrm{K}\subset(\text{crown ether})]^{+}\cdot t\text{-}\mathrm{BuO}^{-}$, in benzene, the color of chemiluminescence from **151** was observed to change significantly depending on the crown ether used as a ligand (Scheme 38).[93]

Thus, $\lambda_{\mathrm{max}}{}^{\mathrm{CL}}$ from **151** was 25 nm shorter for triggering with a complex of 18-crown-6 ether (**155**) ($[\mathrm{K}\subset(\mathbf{155})]^{+}$) ($\lambda_{\mathrm{max}}{}^{\mathrm{CL}} = 684$ nm) than $\lambda_{\mathrm{max}}{}^{\mathrm{CL}}$ with $\mathrm{TBA}^{+}\cdot t\text{-}\mathrm{BuO}^{-}$, while it became far shorter (>100 nm) with a complex of *N*,*N′*-dibenzyl-4,13-diaza-18-crown-6 ether (**156**) ($[\mathrm{K}\subset(\mathbf{156})]^{+}$) ($\lambda_{\mathrm{max}}{}^{\mathrm{CL}} = 606$ nm). These results are illustrated in Fig. 9 together with those for cryptand[2.2.1] (**157**), dicyclohexano-18-crown-6 ether (**158**), and 4,13-diaza-18-crown-6 ether (**159**). Chemiluminescent decomposition of a cyanoanthracene analog **152** exhibited a tendency similar to the case of **151**, though the emissions from **152** were observed in longer wavelength region as shown in Fig. 9. It is noteworthy that **152** is presumably the first dioxetane that emits near-infrared light for the intramolecular CT-induced decomposition.



Scheme 38.

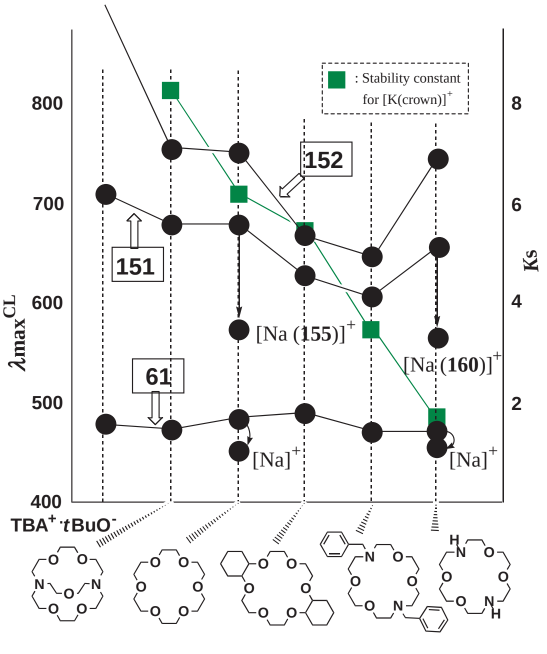


Fig. 9. Color change for chemiluminescence from dioxetanes **151** and **152** induced by a complex of crown ether with *t*-BuOK.

The stability of [K⊂(crown ether)]$^+$ is well known to vary dramatically with the ligand.[94] Since the interaction between the ligand and the potassium ion can be considered to be purely electrostatic in nature for complexes [K⊂(crown ether)]$^+$, the ionic attraction of potassium for phenoxide was assumed to vary with their stability, thereby the color change of chemiluminescence from **151** relates to the stability of [K⊂(crown ether)]$^+$. However, little obvious correlation was noted between the stability of [K⊂(crown ether)]$^+$ and the color change for chemiluminescence of **151**. Moreover, the variation of ligand for [K⊂(crown ether)]$^+$ caused only a little change of emission color from dioxetane bearing a 3-hydroxyphenyl group **61** as shown in Fig. 9. These results suggest that the ionic interaction between a phenoxide ion and the potassium ion of [K⊂(crown ether)]$^+$ varies with the ligand not so much as to cause the color change of chemiluminescence. This suggestion was supported by an experiment using complexes of crown ether with a sodium ion, which should attract a phenoxide anion far more strongly than a potassium ion, as the ionic radius of sodium ion is fairly smaller than that of the potassium ion. In fact, when [Na⊂(**155**)]$^+$•t-BuO$^-$ and a complex of 15-crown-5 (**160**), [Na⊂(**160**)]$^+$•t-BuO$^-$, were used in benzene, the maximum wavelength ($\lambda_{\max}{}^{\mathrm{CL}}$) of chemiluminescence from **61** shifted to shorter wavelength ($\Delta\lambda = 18$–27 nm), and, furthermore, marked blue shift was observed for **151** ($\Delta\lambda = 130$–140 nm) and **152** ($\Delta\lambda = 182$–223 nm), as shown in Fig. 9.

Hence, the color change of chemiluminescence observed for **151** is most likely attributed to the steric interaction of an emitter (oxido anion of **153**) with [K⊂(crown ether)]$^+$. An anthracene ring and a phenolic ring in a stable conformer of **151** lie presumably with large twisted angle (θ) also in a solution. If the conformation with large θ is maintained even for an oxido anion of **153** coordinated with [K⊂(crown ether)]$^+$, a wing (or terminus) of the 9-anthryl ring attached to the *meta*-position of a phenoxide is assumed to come near or into contact with a plane of the coordinated crown ether. For such case, the twisted angle (θ) would become smaller to avoid the steric repulsion of the anthracene ring with a plane of crown ether, and the change of θ affects presumably MO energies in a π-electron system of anionic **153**, as illustrated in Scheme 39. This type of steric interaction is little expected for emitter **54** bearing a simple 3-oxidophenyl. An AM1 MO calculation illustrates suggestive though qualitative features for the relationship between θ and LUMO/HOMO energies for emitter **153**. Thus, the calculation suggests that, as θ decreases from ca. 70° to 30°, the energy gap ($\Delta E = E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}}$) increases, thereby $\lambda_{\max}{}^{\mathrm{CL}}$ shifts to blue, as shown in Fig. 10. Among the complexes of crown ether with potassium, **156** and **158**, having fairly bulky substituents, are easily supposed to be conspicuously large sterically, and thus are influential toward conformational change of a biaryl system of an oxido anion of **153**, so that they should cause color change of chemiluminescence from **151** effectively. In fact, blue-shift of $\lambda_{\max}{}^{\mathrm{CL}}$ was far larger for [K⊂(**156**)]$^+$ and [K⊂(**158**)]$^+$ than the others as shown in Fig. 9.

Color modulation observed in the bioluminescence of beetles such as the firefly is a marvelous phenomenon in luminous organisms. Beetle luciferases use the same luciferin **161** to display light ranging in color from green to red. It has been pro-

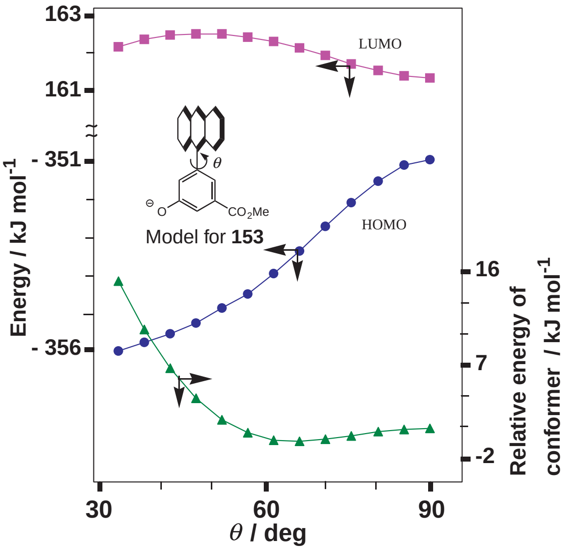
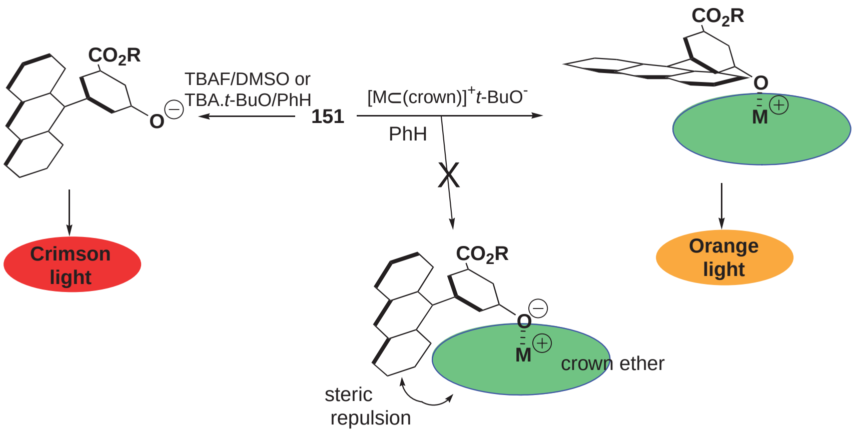
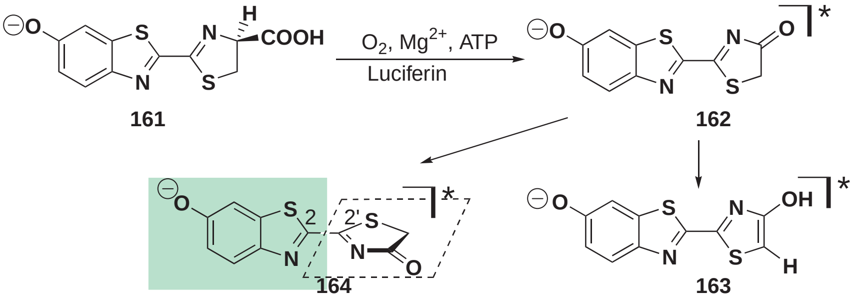


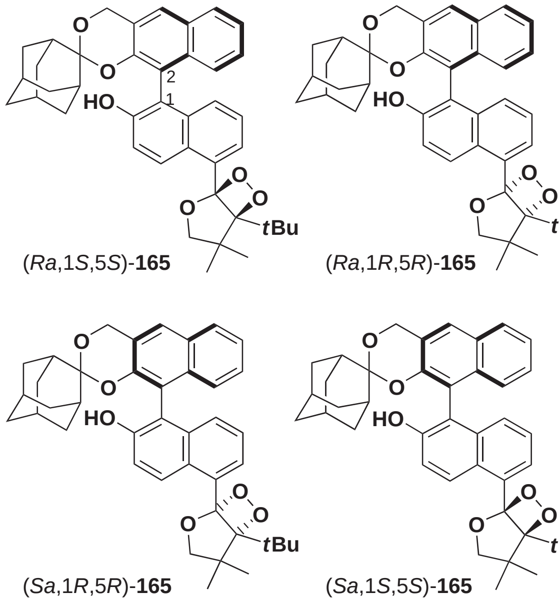
Fig. 10. Relationship of twisted angle (θ) with relative energy of conformer (triangle), the corresponding HOMO energy (circle), and LUMO energy (cube) for a model emitter (methyl ester) of **153**.



Scheme 39.



Scheme 40.



Scheme 41.

posed to elucidate the phenomenon that a luciferase-assisted tautomerization of excited-state oxyluciferin **162** to an enol **163** causes color change of luminescence.[95] Another persuasive elucidation proposed is that color variation relates to TICT (twisted intramolecular charge-transfer) excited state due to conformational changes of the keto form of excited-state oxyluciferin **162** to a twisted form **164**,[96] which occurs by rotation about the C2–C2′ bond (Scheme 40). A very recent experimental study using luciferase mutants has supported the latter elucidation including conformational change of **164**,[97] whereas a theoretical study reported recently has supported rather the former proposal.[98] The results of color change for chemiluminescence from **151** appears to agree with the elucidation that interactions between luciferase and emitter oxyluciferin **162** maintain specific conformers of the excited state thus influencing color variation. However, such results do not rule out other elucidations. In fact, triggering of **61** as well as **151** and **152** with $[\mathrm{Na}\subset(\mathbf{155})]^+\cdot t\text{-BuO}^-$ or $[\mathrm{Na}\subset(\mathbf{160})]^+\cdot t\text{-BuO}^-$ caused blue-shift of chemiluminescence as described already. This phenomenon should be rationalized by the ionic attraction of the sodium ion for a phenoxide stronger than that of potassium.

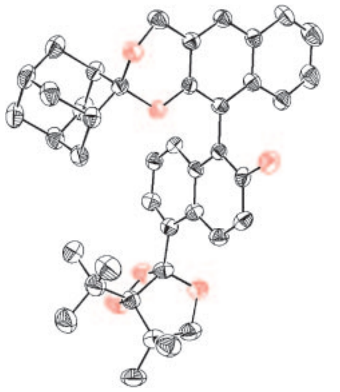


Fig. 11. ORTEP view of dioxetane **165**.

Bicyclic dioxetanes **165** bear a substituted binaphthyl moiety, for which axial chirality around the C1–C2 bond in addition to chirality of carbon in the dioxetane ring give rise to four optical isomers, namely (*Ra*,1*R*,5*R*), (*Ra*,1*S*,5*S*), (*Sa*,1*S*,5*S*), and (*Sa*,1*R*,5*R*) (Scheme 41 and Fig. 11).[99] These stereoisomeric dioxetanes **165** decomposed rapidly to the corresponding keto esters **166** with accompanying flash emission of cinnabar light (maximum wavelength $\lambda_{\max}{}^{\mathrm{CL}} = 600$ nm), on treatment with TBAF in DMSO. As shown in Table 3, the rate of chemiluminescent decomposition (rate constant: k^{DICT}) was different to some extent between a pair of [(*Ra*,1*R*,5*R*)-**165** and (*Sa*,1*S*,5*S*)-**165**] and a pair of [(*Ra*,1*S*,5*S*)-**165** and (*Sa*,1*R*,5*R*)-**165**], though little change in $\lambda_{\max}{}^{\mathrm{CL}}$ was observed among these four

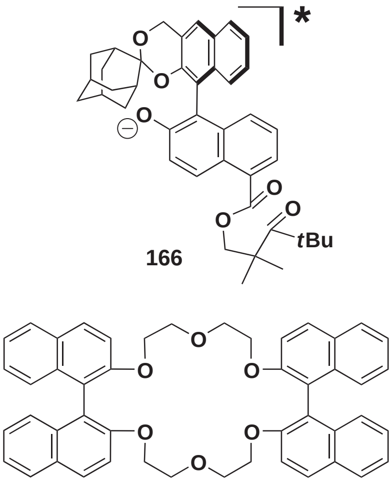
Table 3. Base-Induced Chemiluminescent Decomposition of Stereoisomeric Dioxetanes **165**[a)]

Dioxetane (**4**)	TBAF		$[\mathrm{K}\subset(18\mathrm{C}6)]^+\cdot t\text{-BuO}^-$		$\{\mathrm{K}\subset[(Ra,Ra)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$		$\{\mathrm{K}\subset[(Sa,Sa)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$	
	$\lambda_{\max}$/nm	k^{DICT}/s^{-1}	$\lambda_{\max}$/nm	k^{DICT}/s^{-1}	$\lambda_{\max}$/nm	k^{DICT}/s^{-1}	$\lambda_{\max}$/nm	k^{DICT}/s^{-1}
(*Ra*,1*R*,5*R*)	600	0.10	595	0.075	615	1.4×10^{-3}	646	5.9×10^{-3}
(*Ra*,1*S*,5*S*)	600	0.18	595	0.046	633	2.1×10^{-3}	605	1.1×10^{-3}
(*Sa*,1*S*,5*S*)	600	0.10	595	0.074	646	5.4×10^{-3}	615	1.4×10^{-3}
(*Sa*,1*R*,5*R*)	600	0.19	595	0.046	605	1.1×10^{-3}	633	2.1×10^{-3}

a) Solvent used were DMSO for TBAF, benzene–THF for $[\mathrm{K}\subset(18\mathrm{C}6)]^+\cdot t\text{-BuO}^-$, $\{\mathrm{K}\subset[(Ra,Ra)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$, and $\{\mathrm{K}\subset[(Sa,Sa)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$.

isomers. A similar tendency was also observed for the chemiluminescent decomposition in benzene–THF (1:1) using a complex of 18-crown-6 ether with *t*-BuOK, $[K\subset(18C6)]^+\cdot t\text{-BuO}^-$ as a base, as shown in Table 3.

The use of a complex of optically active crown ethers (*Ra*,*Ra*)-**167** and (*Sa*,*Sa*)-**167** (Scheme 42) with *t*-BuOK as a base exhibited completely different results. When a dioxetane (*Ra*,1*R*,5*R*)-**165** was treated with a complex of (*Ra*,*Ra*)-**167**, $[K\subset(Ra,Ra)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$, chemiluminescence was observed with $\lambda_{max}{}^{CL} = 615$ nm. Similar treatment of the other stereoisomers with $[K\subset(Ra,Ra)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$ gave also chemiluminescence, properties of which were different from each other, though the difference in their Φ^{CL} was minimal. Especially, it was remarkable that each of the four stereoisomers of **165** exhibited different shapes of the chemiluminescent spectrum as well as different maximum wavelength ($\lambda_{max}{}^{CL}$) as shown in Fig. 12 and Table 3.


166

(*Ra,Ra*)-**167**
(*Sa,Sa*)-**167**

Scheme 42.

When a complex of another enantiomeric crown ether, $[K\subset(Sa,Sa)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$, was used for base-induced decomposition of **165**, each of the four stereoisomers gave light with properties exchanged completely with those for the case using $[K\subset(Ra,Ra)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$ between enantiomers, namely, between (*Ra*,1*R*,5*R*)-**165** and (*Sa*,1*S*,5*S*)-**165**, and between (*Ra*,1*S*,5*S*)-**165** and (*Sa*,1*R*,5*R*)-**165** as shown in Fig. 12 and Table 3. These results reveal that each of the four stereoisomeric dioxetanes **165** are recognized for coordination with each of the optically active crown ether complexes $[K\subset(Ra,Ra)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$ and $[K\subset(Sa,Sa)\text{-}\mathbf{167}]^+\cdot t\text{-BuO}^-$, so that their features of coordination are reflected in their chemiluminescent properties different from each other.

The color change of chemiluminescence depending on the combination of stereoisomeric dioxetane **165** and a stereoisomeric complex, $[K\subset\mathbf{167}]^+\cdot t\text{-BuO}^-$ is an unprecedented phenomenon. This phenomenon should be accounted for by the idea that the twisted angle around an axis joining two naphthalene rings varies most likely with steric interaction between $[K\subset\mathbf{167}]^+$ and an emitter **166** produced from **165**, so that features of the π-electronic system vary for the excited emitter, as observed for **151**. The twisted angle of a binaphthyl system presumably differs also for an intermediary dioxetane bearing an oxido anion of naphthol coordinated with $[K\subset\mathbf{167}]^+$. Hence, it is not surprising that the rate of CT-induced decomposition as well as chemiluminescent yield vary more or less with combination of stereoisomeric dioxetane **165** and a stereoisomeric complex, $[K\subset\mathbf{167}]^+\cdot t\text{-BuO}^-$. Another important but rather unnoticed fact observed is that the color of chemiluminescence was different between diastereoisomers, namely, between (*Ra*,1*R*,5*R*)-**165** and (*Ra*,1*S*,5*S*)-**165** and between (*Sa*,1*S*,5*S*)-**165** and (*Sa*,1*R*,5*R*)-**165**, though chiral carbons at the 1- and 5-position in a dioxetane ring exist no longer in an emitter (**166**). This fact suggests that steric hysteresis of

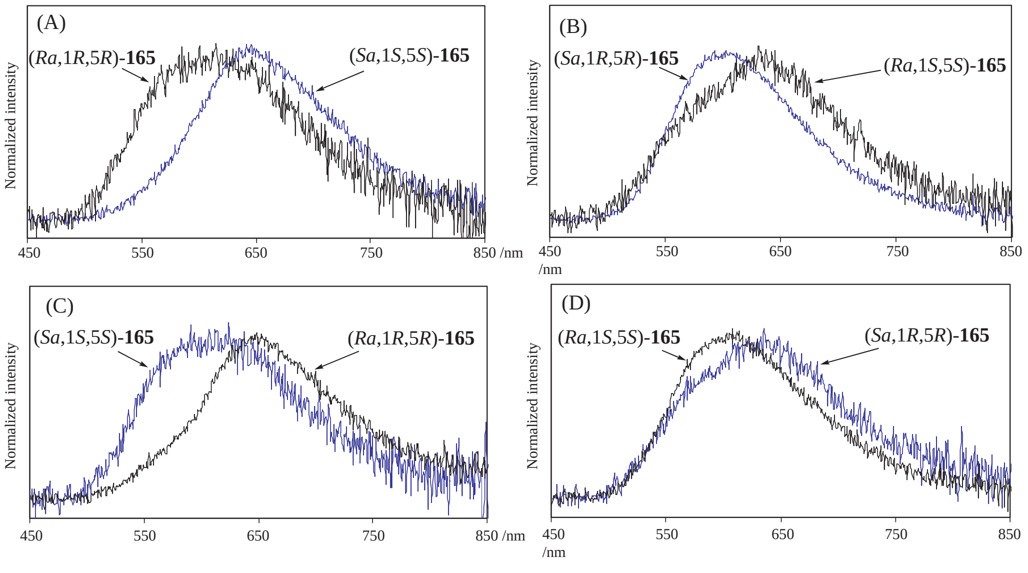
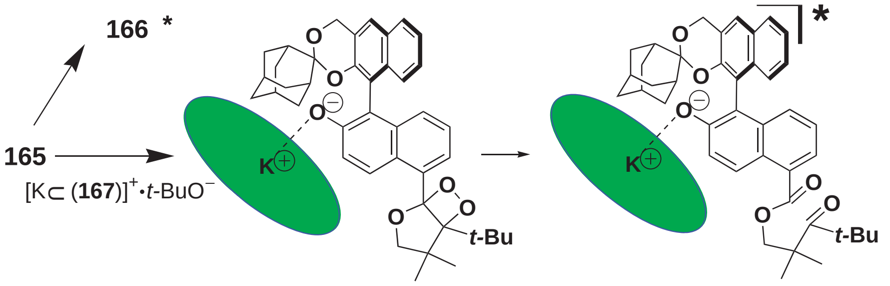


Fig. 12. Chemiluminescent spectra for decomposition of optically active dioxetanes **165** induced by a complex of optically active crown ether **167** with potassium ion. (A), (B): $\{K\subset[(Ra,Ra)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$/benzene–THF system, (C), (D): $\{K\subset[(Sa,Sa)\text{-}\mathbf{167}]\}^+\cdot t\text{-BuO}^-$/benzene–THF system.



Scheme 43.

the dioxetane part for **165** coordinated to [K⊂**167**]$^+$ is retained to some extent after the CT-induced decomposition into an emitter **166**, though the details are obscure at present (Scheme 43).

Conclusion

Bicyclic dioxetane, 5-*t*-butyl-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane bearing a 3-siloxyphenyl, was found to become a basic skeleton satisfying the fundamental properties requisite as a high-performance dioxetane-based chemiluminescent substrate, that is, high thermal stability, and ability to decompose easily on demand with accompanying efficient emission of light. One important advantage of this skeleton is that it possesses flexibility for further structural modification to develop dioxetanes exhibiting high-performance chemiluminescence with various distinctive features. The studies of relationship between chemiluminescent efficiency and structures of dioxetanes provided the suggestion that the singlet-chemiexcitaion process for the intramolecular CT-induced decomposition might resemble fundamentally the triplet-chemiexcitaion process for the thermal decomposition of dioxetanes. "Odd/even" relationship regarding the rate of the CT-induced decay as well as the color change of emission and chemiluminescent effciency was observed for various dioxetanes bearing an anion of areneoxide. In addition, the intramolecular CT-induced decomposition was strongly suggested to take place favorably when an aromatic electron donor lies in a certain conformation, even if it can rotate freely around a bond joining it to the dioxetane ring. The chemiluminescence was found to vary in color ranging from violet to crimson depending on the structure of the aromatic electron donor which constructs the important moiety of the emitter after the CT-induced decomposition of dioxetanes. The color of chemiluminescence was controlled also by the microenvironment. Thus, chemiluminescence from dioxetanes bearing a bulky aryl-substituted phenol changes its color depending on the crown ether used as a ligand complexed with potassium *t*-butoxide. Furthermore, optically active dioxetanes bearing a bisnaphthol-type electron donor were found to recognize optically active bases for the CT-induced chemiluminescent decomposition.

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