Hydrogen-Bonding Effects on the Fluorescence versus Electron-Transfer-Initiated Chemiluminescence Spectra of the *m*-Oxybenzoate Ion Derived from a Bicyclic Dioxetane

Waldemar Adam,*[,†] Masakatsu Matsumoto,[‡] and Alexei V. Trofimov*[,†,§]

Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany, Department of Material Science, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa, 259-12, Japan, and Institute of Biochemical Physics, United Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow 117977, Russia

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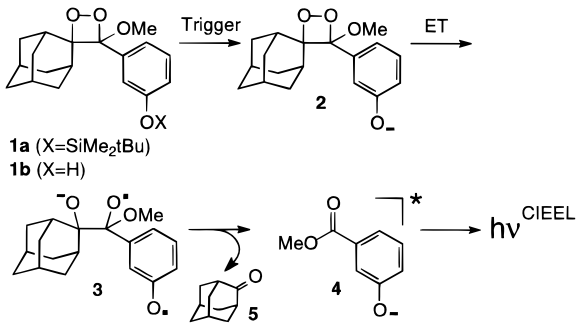
A comparative spectral study of the fluorescence of the *m*-oxybenzoate anion versus the electron-transfer-initiated chemiluminescence (CIEEL) of the same anion derived from the bicyclic dioxetanes in various solvents is reported. The present study reveals that the fluorescence of this oxyanion is blue-shifted in protic versus aprotic solvents, while the CIEEL-spectral maximum is independent of the medium. The same phenomenon has been recently observed for the *m*-oxybenzoate ion derived from CIEEL-active spiroadamantyl dioxetanes. The reported spectral differences between the fluorescence and chemiluminescence emissions cannot be attributed to exciplex formation in the CIEEL process, but result from the differences in hydrogen-bonding effects on the photo- and chemiexcited oxyanion species. The observed solvatochromism is qualitatively rationalized in terms of the semiempirical AM1 calculations.

Introduction

The phenomenon of light emission known as *c*hemically *i*nitiated *e*lectron-*e*xchange *l*uminescence (CIEEL)[1] constitutes a general process that involves electron-transfer chemistry.[2,3] Soon after it had been reported for high-energy organic peroxides,[4,5] the CIEEL mechanism was proposed for the firefly bioluminescence.[6] The latter process represents an example of intramolecular CIEEL, which has served as a basis to develop the most effective probes for modern chemiluminescence bioassays,[7,8] with the triggerable dioxetanes **1**[9,10] as the CIEEL-active species (Scheme 1). A generally accepted mechanism of the CIEEL phenomenon still needs to be established.[11] The CIEEL may be generated at will by treatment of the dioxetanes **1** with an appropriate reagent (trigger) to release the phenolate ion **2**. The formation of **2** is followed by the intramolecular electron transfer (ET) from the phenolate moiety to the antibonding σ^* orbital of the peroxide bond (Scheme 1), concomitant with O−O bond cleavage. The transitory species **3** is likely to yield a solvent-caged ion-radical pair; the chemiexcitation is envisaged to take place by electron back-transfer (BET) in such a pair.[12,13]

Scheme 1



An important advantage of the spiroadamantyl-substituted dioxetanes **1** for practical use is their thermal persistence, which makes them easy to handle in bioanalytical, clinical applications. Also the novel triggerable bicyclic dioxetanes **6** (Scheme 2) possess remarkable thermal persistence and appreciable CIEEL efficiency,[14] and thereby qualify for chemiluminescence bioassays.

* To whom correspondence should be addressed. Prof. Waldemar Adam: Tel +49 931 8885340/339. Fax: +49-931-8884756. e-mail: adam@chemie.uni-wuerzburg.de. Internet: http://www-organik.chemie.uni-wuerzburg.de.

† University of Würzburg.

‡ Kanagawa University.

§ Russian Academy of Sciences.

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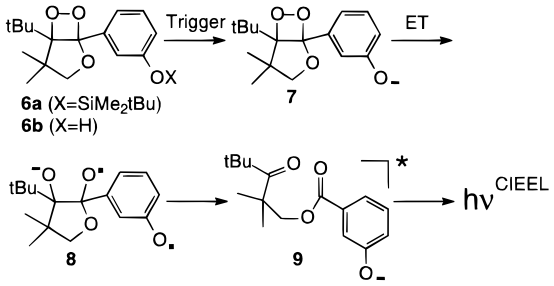
Scheme 2



The choice of the trigger and the reaction medium, which depends on the nature of the protective group (X), is of prime importance for the rational design of efficient CIEEL systems. For that reason, a detailed knowledge on the medium effects for the CIEEL generation is required. Since the chemiluminescence bioassays are conducted in aqueous media, the elucidation of the hydrogen-bonding effects on the CIEEL emission is particularly relevant.

Recently, we have reported a detailed comparative spectral study of the hydrogen-bonding effects on the CIEEL process of the spiroadamantyl-substituted dioxetanes **1** (Scheme 1) and the fluorescence emission of the methyl *m*-oxybenzoate ion (**4**) in protic versus aprotic media.[12] Comparison of the CIEEL spectra of the dioxetanes **1**, which have been triggered in protic (H_2O, D_2O, and MeOH) and aprotic (MeCN, DMSO) solvents (Scheme 1), with the fluorescence spectra of the *m*-oxybenzoate ion **4**, the authentic CIEEL emitter, has revealed the following intriguing facts: The fluorescence of the **4** species is blue-shifted ($\Delta\lambda_{max}^{fl} = 51$ nm) in protic versus aprotic solvents, while the CIEEL-spectral maxima ($\lambda_{max}^{CIEEL} = 466$ nm) are independent of the reaction medium and the type of trigger![12] In other words, the fluorescence of **4** is blue-shifted in protic media relative to the CIEEL emission, whereas in aprotic solvents the CIEEL and the fluorescence spectra coincide. These divergent spectral observations have been rationalized in terms of different hydrogen-bonding effects on the photo- and chemiexcited *m*-oxybenzoate ion **4**.[12]

It was supposed that the second dioxetane cleavage fragment, namely adamantanone (**5**), does not play any significant role in the spectral difference between the chemiluminescence (CIEEL) and fluorescence emissions observed in protic media.[12] However, two possible reasons for the involvement of the adamantanone (**5**) may be considered, which may cause the above-mentioned spectral shift for the photoexcited emitter **4**: (i) The adamantanone (**5**) fragment, generated in the immediate proximity of the CIEEL emitter **4** (Scheme 1), may protect the latter from hydrogen bonding through aggregation in the solvent cage; (ii) the oxybenzoate ion **4** may form an exciplex with the adamantanone (**5**). In our previous analysis of the CIEEL-spectral behavior,[12] we had only examined the latter possibility. Such exciplex involvement was ruled out, since the observed shift between the CIEEL and the fluorescence spectra did not correlate with the solvent polarity as would be expected for an exciplex,[15] but rather it depended on whether the solvent is protic or not.[12] Moreover, the coincidence of the CIEEL-spectral maxima (466 nm) in all the solvents we have used also renders improbable exciplex formation in the CIEEL process shown in Scheme 1. Nevertheless, a more rigorous scrutiny of exciplex involvement appears necessary. For this purpose, we have chosen in the present spectral study the bicyclic dioxetanes **6** (Scheme 2) as precursors to the CIEEL emitter, namely the *m*-oxybenzoate ion **9**, which is akin to the *m*-oxybenzoate ion **4** produced from the adamantyl-substituted dioxetanes **1** (Scheme 1). The incentive and the relevance of such a study are the following: First, the CIEEL emitters **4** and **9** possess the same chromophore, i.e, the *m*-oxybenzoate-ion moiety, and one may expect similar spectral characteristics and solvatochromism of the fluorescence for both anions **4** and **9**, provided that for the latter no intramolecular exciplex intervenes. Second, contrary to the *m*-oxybenzoate ion **4**, which is formed from the dioxetanes **1** along with the ketone fragment **5** as separate species (Scheme 1), the *m*-oxybenzoate ion **9** is a *single* reaction product in the CIEEL-triggering process of the dioxetanes **6** (Scheme 2); the latter fact should facilitate exciplex formation in view of intramolecularity and, moreover, escape from the solvent cage is prevented.

Indeed, the only reported example of an exciplex formed in the CIEEL cleavage of dioxetanes, namely from a bicyclic indole-derived dioxetane, is of the intramolecular type, whose spectral emission was strongly dependent on solvents.[16] Thus, the bicyclic dioxetanes **6** may serve as a probe for intramolecular exciplex involvement in the triggered CIEEL generation, by elucidation of chemiluminescence and fluorescence spectral properties of the CIEEL emitter **9** in protic and aprotic solvents. Such an investigation of the solvatochromic effects on the *m*-oxybenzoate ion **9** in protic versus aprotic media is of particular relevance since the bicyclic dioxetane **6**, from which the CIEEL emitter is released by triggering, should be useful for chemiluminescence bioassays.

Presently we report the results of the solvatochromic effects on the CIEEL of the dioxetanes **6** and the fluorescence of the authentic CIEEL emitter **9** (Scheme 2) and compare these spectral data with those previously obtained for the CIEEL of the dioxetanes **1** and the fluorescence of CIEEL emitter **4** (Scheme 1) in the same solvents. The experimental observations are qualitatively rationalized in terms of semiempirical (AM1) calculations.

Results and Discussion

Comparison of the CIEEL spectra of the dioxetanes **6a**,**b** (Scheme 2), triggered in protic (H_2O, D_2O, and MeOH) and aprotic (MeCN, DMSO) solvents, with the fluorescence spectra of the *m*-oxybenzoate ion **9**, the authentic CIEEL emitter (Figure 1), has revealed the same phenomenon as we have observed before for dioxetanes **1**: The fluorescence (Figure 1a; Table 1) of the emitter **9** species is blue-shifted ($\Delta\lambda_{max}^{fl} \approx 50$ nm) in protic versus aprotic media, while the CIEEL-spectral maxima ($\lambda_{max}^{CIEEL} = 467$ nm) remain the same (Figure 1; Table 1) in all the solvents. In other words, the solvatochromic behavior of the oxyanion **9** matches that which we have observed previously for the anion **4**.[12]

Besides the qualitatively similar spectra for both oxyanions **4** and **9**, also the quantitative data (Table 1)

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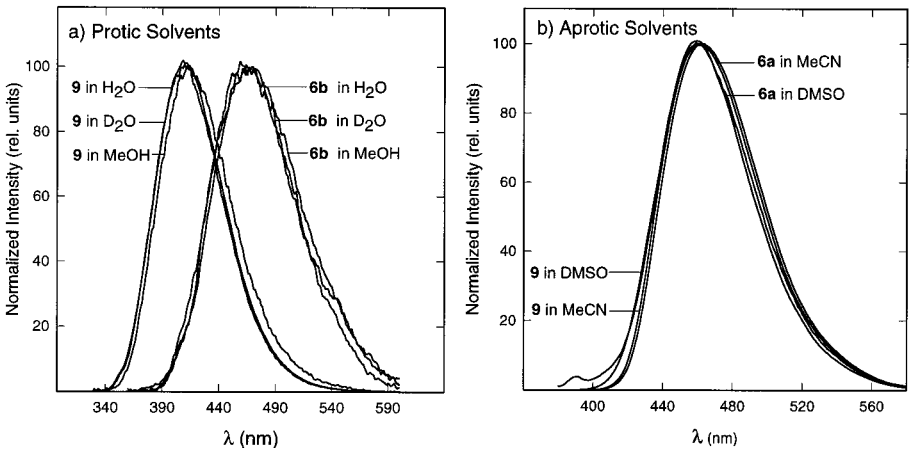


Figure 1. Normalized CIEEL and fluorescence spectra in protic (a) and aprotic (b) media. (a) CIEEL emissions (at the right) of the NaOH-triggered (pH 12.6) hydroxy-substituted dioxetane **6b** (3.0×10^{-3} M) in H_2O, D_2O and MeOH at room temperature (ca. 20 °C) and the fluorescence emission (at the left, $\lambda_{ex} = 330$ nm) of the authentic *m*-oxybenzoate ion ([**9**] $= 4.5 \times 10^{-5}$ M) under the same conditions. (b) CIEEL emissions in the fluoride-ion-triggered ([*n*-Bu_4NF] $= 6.3 \times 10^{-4}$ M) decomposition of the dioxetane **6a** (1.1×10^{-4} M) at room temperature (ca. 20 °C) in MeCN and DMSO and the fluorescence emission ($\lambda_{ex} = 330$ nm) of the *m*-oxybenzoate ([**9**] $= 1.2 \times 10^{-5}$ M) under the same conditions.

Table 1. CIEEL (λ_{max}^{CIEEL}), Fluorescence (λ_{max}^{fl}), and Absorption (λ_{max}^{abs}) Maxima of the Oxyanion 9 and Spectral Shifts of the Fluorescence ($\Delta\lambda_{max}^{fl}$) and the Absorption ($\Delta\lambda_{max}^{abs}$) Maxima in a Variety of Solvents Relative to Water and the Fluorescence Quantum Yields (Φ^{fl})

solvent	λ_{max}^{CIEEL} (nm)	λ_{max}^{fl} (nm)	$\Delta\lambda_{max}^{fl}$ (nm)	λ_{max}^{abs} (nm)	$\Delta\lambda_{max}^{abs}$ (nm)	Φ^{fl} [b,c]
D_2O	467	416	–	312	–	0.110 ± 0.010[d]
H_2O	467	416	–	312	–	0.066 ± 0.004[d]
MeOH	467	418	2	315	3	0.046 ± 0.003[d]
MeCN	467	467	51	366	54	0.240 ± 0.010[e]
DMSO	467 [469[b]]	467	51	389	77	0.320 ± 0.010[e]

[a] Reference 14. [b] Measured versus quinine bisulfate as the fluorescence standard ([QBS] $= 10^{-5}$ M) in 1 N H_2SO_4. [c] Each value is an average of at least four measurements. [d] NaOH was used as base (pH 12.6). [e] [*n*-Bu_4NF] $= 6.3 \times 10^{-4}$ M.

match well. Consequently, only the *common m*-oxybenzoate chromophore of the anions **4** and **9** is responsible for the solvatochromism in protic versus aprotic solvents. This spectral agreement not only rules out exciplex formation in the CIEEL process of the dioxetanes **6** (Scheme 2), but provides experimental evidence for our supposition[12] that adamantanone (**5**) is also not involved in the CIEEL process of the dioxetanes **1** (Scheme 1), neither through aggregation with the CIEEL emitter **4** nor by exciplex formation with the latter species. Moreover, the coincidence of the CIEEL-spectral maxima in protic and aprotic media, contrary to the fluorescence emission, suggests that hydrogen bonding does not operate in the CIEEL emitters **4** and **9**, generated correspondingly from the dioxetanes **1b** and **6b** by triggered chemiexcitation (Schemes 1 and 2).

Recently, we have established[12] that the solvatochromic effect on aromatic oxyanions caused by hydrogen bonding in protic solvents depends on the anion structure: The anions with an *extended-conjugated* substitution pattern are insensitive to solvatochromic effects, while the *crossed-conjugated*[17] anions are subject to considerable solvatochromism, as manifested by a strong spectral blue shifts

(17) For discussion on extended and crossed conjugation, see: (a) March, J. *Advanced Organic Chemistry*, 4th ed.; John Wiley & Sons: New York, 1992. (b) Phelan, N. F.; Orchin, M. *J. Chem. Educ.* **1968**, *45*, 633−637.

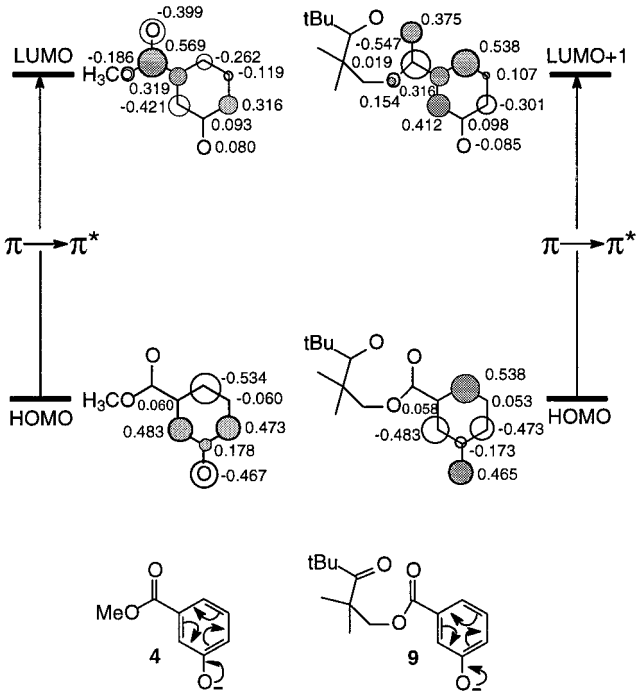


Figure 2. Pertinent MOs for the electronic excitation of the crossed-conjugated oxyanions **4** versus **9**, as calculated by AM1 method.

in protic versus aprotic media. Both *m*-oxybenzoate ions **4** and **9** belong to the *crossed-conjugated* category (Figure 2) and thereby reveal a strong hypsochromic shift in protic solvents.

To rationalize qualitatively the observed solvatochromism, it is essential to consider the MO's involved in the electronic excitation of the anions of interest.[12] In Figure 2 are displayed the pertinent MO's for the excitation of the *m*-oxybenzoate anions **4** and **9**, as calculated by the semiempirical AM1 method. As one can see from Figure 2, the orbital coefficients for both **4** and **9** look very similar, except that for the anion **4** the $\pi \rightarrow \pi^*$ excitation constitutes a transition between HOMO and LUMO, while for the anion **9** it is between HOMO and LUMO+1. Noteworthy is the pronounced difference

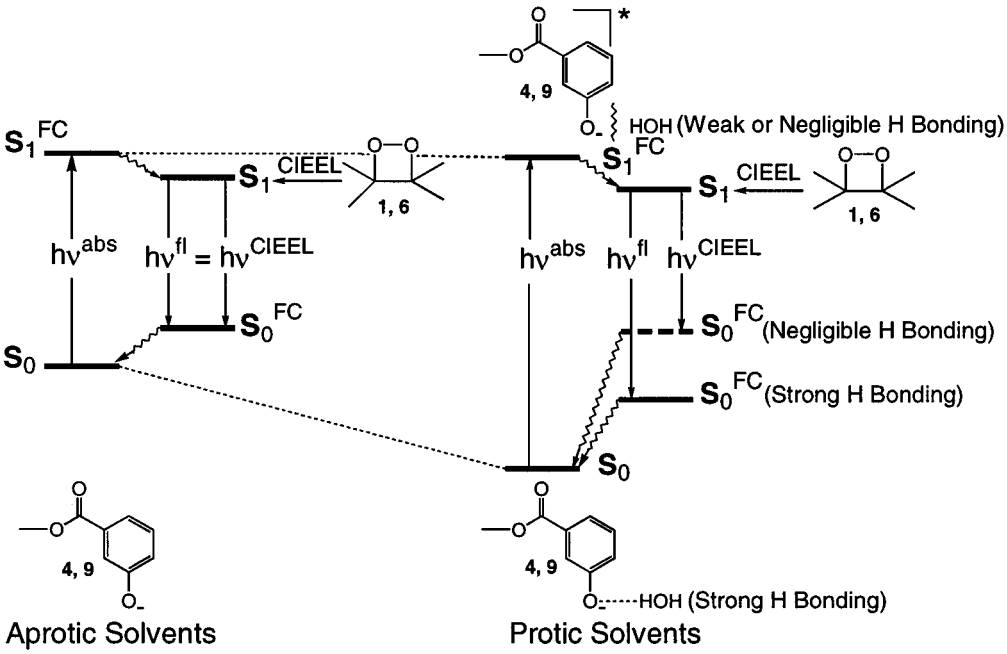
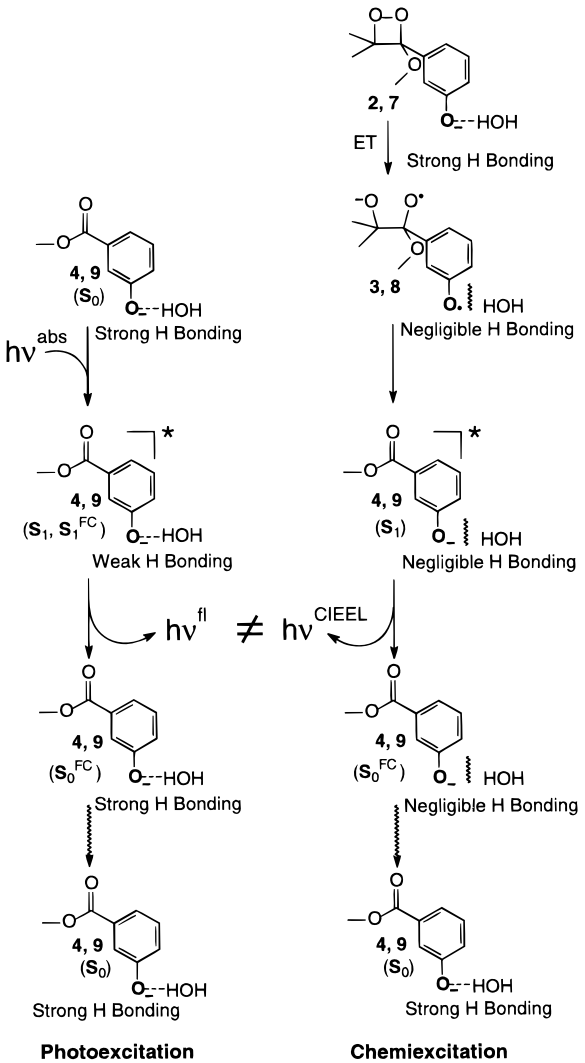


Figure 3. Schematic representation of the hydrogen-bonding effects on the photo- and chemiexcited oxyanions **4** and **9**.

between the HOMO and the LUMO (LUMO+1) coefficients centered on the phenolate oxygen atom.

What is the reason for the different hydrogen-bonding effects on the photo- and chemiexcited *m*-oxybenzoates **4** and **9**? Negligible LUMO (LUMO+1) versus significant HOMO coefficients on the phenolate oxygen atom of **4** and **9** (Figure 2) imply that the electron density on this oxygen atom is dramatically reduced on $\pi \rightarrow \pi^*$ excitation, with the consequence that the hydrogen bonding at this site is significantly diminished in the excited versus the ground state (cf. Figure 3 and Scheme 3). The greater stabilization of the ground versus excited state by hydrogen bonding accounts for the strong blue shift of the absorption, which is the optical transition between the relaxed ground and the Franck−Condon excited states ($S_0 + h\nu^{abs} \rightarrow S_1^{FC}$) (Figure 3). Conversely, the blue shift of the fluorescence emission, i.e., the transition between the relaxed excited state and the Franck−Condon ground state ($S_1 \rightarrow S_0^{FC} + h\nu^{fl}$), suggests stronger stabilization of the S_0^{FC} versus S_1 state in protic solvents by hydrogen bonding (Figure 3, Scheme 3). Appreciable hydrogen bonding in the S_0^{FC} state prior to its relaxation to S_0 means that during photoexcitation, despite the low electron density on the O^- site, enough solvent molecules are still associated with the singlet-excited anions **4** and **9** to be effective for weak hydrogen bonding (Scheme 3). The following reason may be suggested for this "memory effect":[12] The strong hydrogen bonding with the O^- site in the ground state of the **4** and **9** anions effectively localizes the charge so that in the photoexcited state more electron density (higher LUMO or LUMO+1 coefficients) is retained on the O^- site compared to that calculated for vacuum (Figure 2). This is not the case for the chemiexcited **4** and **9** anions formed in the triggering of the dioxetanes **1** and **6**. Indeed, the precursors to the chemiexcited CIEEL emitters **4** and **9**, namely the diradical species **3** and **8** formed on ET from the O^- site to the peroxide bond (Schemes 1 and 2), are negligibly hydrogen-bonded at their phenoxyl-radical site (Scheme 3). The lack of appreciable hydrogen bonding on this radical oxygen atom prior to chemiexcitation means that

Scheme 3



the CIEEL emitters **4** and **9**, formed *directly* in the S_1 state, are also not subject to hydrogen bonding on the

O^- site. Consequently, in the CIEEL process both S_1 and S_0^{FC} states are negligibly stabilized by hydrogen bonding and, contrary to photoexcitation, no spectral shift in the CIEEL emission is observed in protic media.

In conclusion, since the CIEEL spectra of both dioxetane phenolates **2** and **7**, released on triggering from the dioxetanes **1** and **6**, match well in all the solvents used, this observation rules out exciplex formation in the CIEEL process of the bicyclic dioxetane **6** (Scheme 2) and justifies our previous supposition that in the CIEEL process of the dioxetanes **1** (Scheme 1), the adamantanone fragment (**5**) is in no way involved, neither in aprotic nor in protic media.[12] Therefore, the spectral difference between the CIEEL and fluorescence emissions in protic solvents is rationalized in terms of different hydrogen-bonding effects on photo- and chemiexcited *m*-oxybenzoate-ion fragment, the common chromophore for the **4** and **9** emitters. Finally, as for the phenolate **2** derived from the dioxetanes **1** (Scheme 1), the CIEEL-spectral maximum of the dioxetane phenolate **7** obtained from the dioxetanes **6** (Scheme 2) is also independent of the medium. This appears to be a general phenomenon for the phenolate-initiated CIEEL processes.

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Supporting Information Available: Experimental section. This material is available free of charge from the Internet at http://pubs.acs.org.

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