The Effect of *meta* versus *para* Substitution on the Efficiency of Chemiexcitation in the Chemically Triggered Electron-Transfer-Initiated Decomposition of Spiroadamantyl Dioxetanes

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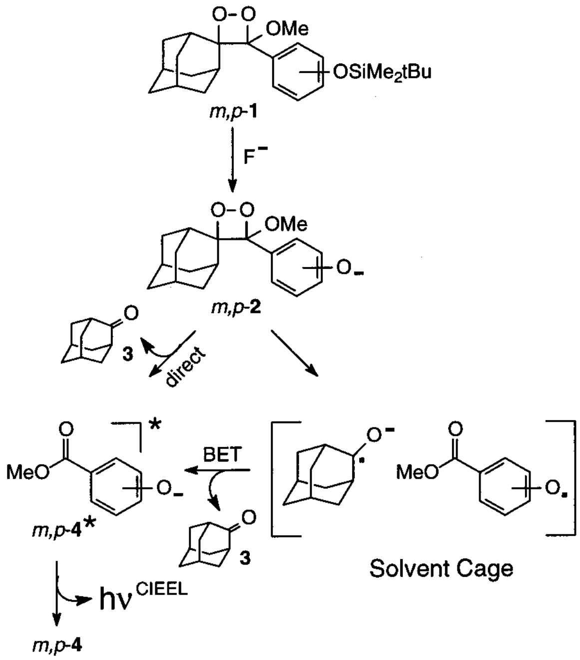
A similar viscosity dependence of the CIEEL efficiencies for the *para*- and *meta*-substituted spiroadamantyl dioxetanes **1** has been observed, which implies that an electron-transfer mechanism operates through solvent-caged species for both regioisomers. The pronounced difference in the chemiexcitation yields for the *meta*- and *para*-substituted dioxetanes is rationalized in terms of the much larger (ca. 200-fold) rate constant for the electron back-transfer (BET) step to afford the excited *meta* CIEEL emitter. An in-depth kinetic analysis of the viscosity effect on the excited-state generation for the *para* versus *meta* regioisomers supports this conclusion.

Introduction

Chemically initiated electron-exchange luminescence (CIEEL),[1] a phenomenon of light emission derived from electron-transfer chemistry,[2,3] is of particular interest for modern chemiluminescent bioassays[4,5] developed for clinical applications. The most popular clinical bioassays[6,7] utilize thermally persistent spiroadamantyl-substituted dioxetanes with a properly protected phenolate group. The CIEEL of these dioxetanes may be generated at will by treatment with an appropriate reagent (trigger) to release the phenolate ion **2**, cleavage of which leads to the electronically excited emitter **4** (Scheme 1). In the rational design of the CIEEL-active dioxetanes for bioanalytical applications, the dioxetane structure is of prime importance in regard to chemiluminescence efficiency, a question we have addressed in the present study.

Scheme 1



Dramatic differences in the CIEEL efficiences have been reported[4−6,8,9] for triggered dioxetanes, in which regioisomeric phenolate-type emitters are generated. A prominent example constitutes the pair of crossed-conjugated *meta*- and extended-conjugated *para*-oxybenzoates *m*-**4** and *p*-**4**, released from the respective dioxetanes *m,p*-**1**[4−8] (Scheme 1), of which the *meta* regioisomer is much more efficient. To rationalize the differences in the chemiluminescence efficiency for the *meta* versus *para* dioxetane regioisomers *m,p*-**1**, the mechanistic details of the CIEEL generation must be well understood. For this purpose, we have recently studied the viscosity

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dependence of the CIEEL process for the *meta*-regioisomeric dioxetane *m*-**1** to differentiate the mechanistic alternatives for the excited-state generation[10] shown in Scheme 1, namely, direct chemiexcitation versus the excited-state generation through the BET process in the solvent cage. It is difficult to expect a viscosity dependence for the direct chemiexcitation channel. Conversely, if the excited-state generation takes place through the BET process, its efficiency should be subject to a solvent-cage effect, and thereby, it should be viscosity dependent. The viscosity effect on CIEEL efficiency observed for the dioxetane *m*-**1** suggests that the BET process operates in the CIEEL mechanism.[10]

From the aforementioned, it should be evident that if the BET process operates also in the CIEEL triggered from the *para*-regioisomeric dioxetane *p*-**1**, its viscosity behavior should be similar to that observed for the *meta*-regioisomeric dioxetane *m*-**1**. Herein we report our studies of the viscosity behavior of the CIEEL efficiency for *para* (*p*-**1**) versus *meta* (*m*-**1**) regioisomer and rationalize our observations in terms of the mechanistic alternatives for the chemiexcitation process.

Experimental Section

Materials. 3-(2′-Spiroadamantane)-4-methoxy-4-(3″-butyldimethylsilyloxy)phenyl-1,2-dioxetane (*m*-**1**) and 3-(2′-spiroadamantane)-4-methoxy-4-(4″-butyldimethylsilyloxy)phenyl-1,2-dioxetane (*p*-**1**) were prepared according to the patent procedures.[11] Methyl 3- and methyl 4-butyldimethylsilyloxybenzoates were kindly made available by Dr. M. Schulz.[12] As fluoride ion source, a 1 M stock solution of tetrabutylammonium fluoride in tetrahydrofuran (THF) [Aldrich] was used. The solvents benzene and diphenylmethane (Fluka) were distilled from EDTA (50 g/L) under a nitrogen gas atmosphere at reduced pressure.

CIEEL and Chemiexcitation Yields. The CIEEL intensity $\boldsymbol{i}^{\mathrm{CIEEL}}$ is expressed by eq 1, in which ν is the reaction rate,

$$i^{\mathrm{CIEEL}} = \Phi^{\mathrm{CIEEL}}\nu = \Phi^{\mathrm{fl}}\Phi_{\mathrm{S1}}\nu \tag{1}$$

$\Phi^{\mathrm{CIEEL}} = \Phi_{\mathrm{S1}}\Phi^{\mathrm{fl}}$ represents the CIEEL yield, and Φ_{S1} and Φ^{fl} are the singlet chemiexcitation yield and the fluorescence efficiency of the CIEEL emitter. Data on the CIEEL yield were made available through the measurement of the total amount of light (N_{photons}) emitted in the complete dioxetane decomposition. The expression for N_{photons} is given by the area under the CIEEL-intensity curves and assessed by integration of $i^{\mathrm{CIEEL}}(t)$ over the reaction time (eq 2), in which $\int_0^\infty \nu \, \mathrm{d}t$ constitutes the

$$N_{\mathrm{photons}} = \int_0^\infty i^{\mathrm{CIEEL}} \, \mathrm{d}t = \Phi^{\mathrm{CIEEL}} \int_0^\infty \nu \, \mathrm{d}t = \Phi^{\mathrm{CIEEL}}[\mathbf{1}] \tag{2}$$

total concentration [**1**] of the decomposed dioxetane; thus, Φ^{CIEEL} is experimentally defined by eq 3 . The data for Φ_{S1}

$$\Phi^{\mathrm{CIEEL}} = \frac{N_{\mathrm{photons}}}{[\mathbf{1}]} \tag{3}$$

versus viscosity (η) were obtained from eq 4

$$\Phi_{\mathrm{S1}} = \frac{\Phi^{\mathrm{CIEEL}}}{\Phi^{\mathrm{fl}}} \tag{4}$$

by measurement of the Φ^{CIEEL} and Φ^{fl} values as a function of viscosity. The CIEEL-intensity measurements in benzene−Ph_2CH_2 solutions (reaction volume was 3.0 mL) were performed on a Mitchell−Hastings photometer.[13] Small portions (1.6 vol %) of the THF/DMSO solutions [1:1] of ammonium fluoride (0.5 M) (the fluoride ion source) were added to the benzene−Ph_2CH_2 mixtures to trigger the decomposition of the dioxetanes *m,p*-**1** (Scheme 1) in these media. The benzene−Ph_2CH_2 solutions of the dioxetanes *m,p*-**1** ([**1**] = 6.0×10^{-9} M) were prepared by addition of aliquots (1.6 vol %) of stock solutions in benzene (3.6×10^{-7} M) to the benzene−Ph_2CH_2 mixtures. The fluorescence quantum yields (Φ^{fl}) were obtained by the conventional procedure versus quinine bisulfate ([QBS] = 1.19×10^{-6} M) in 1 N H_2SO_4 as the fluorescence standard ($\Phi_{\mathrm{QBS}}{}^{\mathrm{fl}} = 0.55$).[14] The fluorescence measurements were conducted on a Perkin-Elmer LS 50 spectrofluorimeter. The solutions of the oxybenzoate ions *m,p*-**4** in benzene and Ph_2CH_2 for the fluorescence measurements were prepared by addition of small portions (0.8 vol %) of its stock solution in DMSO (1.2×10^{-3} M), obtained by the reaction of the methyl 3- and 4-butyldimethylsilyloxybenzoates with an excess of fluoride ions.

Choice of the Medium for the Viscosity Studies. As in the previous study,[10] variable compositions of mixtures of a fluid (benzene) and a viscous (diphenylmethane) solvents have been used in this work as medium for the viscosity variation. The major requirement in the choice of the solvent system for such a viscosity study[10] is conditioned by the Marcus free-energy relationship for the electron transfer. Since in the Marcus theory[15] the variation of the solvent may significantly affect the electron-transfer process through the changes of the solvent reorganization energy,[15,16] the $(1/n^2 - 1/\epsilon)$ factor (ϵ is the dielectric constant of the solvent and n its refractive index) should be as similar as possible for the low and high viscosity components of the reaction medium. This requisite is fulfilled for the benzene and diphenylmethane (Ph_2CH_2) pair, for which the $(1/n^2 - 1/\epsilon)$ value is ca. 0.004 each. This solvent combination was used originally with success for the viscosity studies on triplet−triplet energy transfer[17] and autoxidation kinetics[18] and was employed by us in our previous work[10] on the viscosity (η) dependence of the CIEEL process. The concentration dependence of the solvent viscosity for C_6H_6−Ph_2CH_2 mixture has been reported in our previous study.[10]

Computational Methods. The influence of the substitution pattern on the ground- and the excited-state energies of the methyl oxybenzoate anion **4**, the authentic CIEEL emitter, has been obtained by the semiempirical AM1 method[19] as implemented in the VAMP 5.0[20] software package and run on a Silicon Graphics Indigo workstation. The computations were performed by means of singles-plus-pair-excitation configuration interaction (PECI) and an active space of 10 molecular orbitals (MO).

Results and Discussion

The viscosity dependence of the CIEEL efficiency (Φ^{CIEEL}) for the *meta*- and *para*-substituted dioxetanes *m,p*-**1** in benzene−Ph_2CH_2 mixtures is displayed in Figure 1. As may be seen from Figure 1, whereas the viscosity profile of the Φ^{CIEEL} is rather similar for both regioisomers, the absolute values are dramatically different for *m*-**1** and *p*-**1**.

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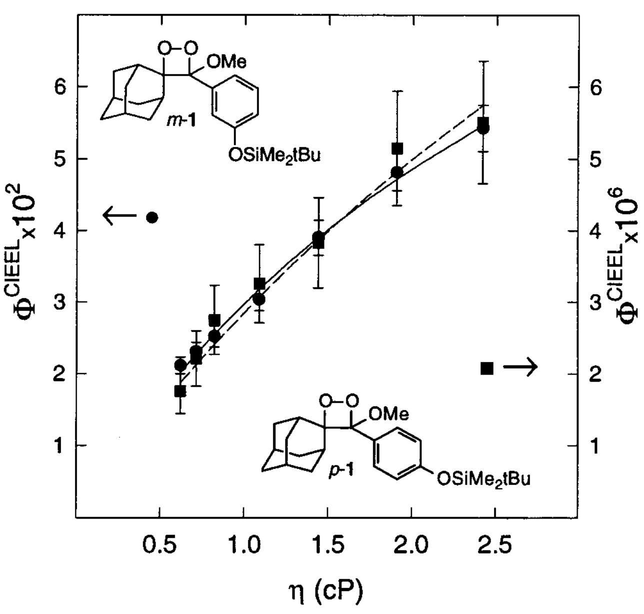

Figure 1. Viscosity dependence of the CIEEL yield (Φ^{CIEEL}) for the fluoride ion triggered ([n-Bu_4NF] = 8.33×10^{-3} M) dioxetanes m-**1** {●} and p-**1** {■} ([**1**] = 6.0×10^{-9} M) in aerated benzene–diphenylmethane mixtures at 26 °C; the error bars represent four measurements for each point.

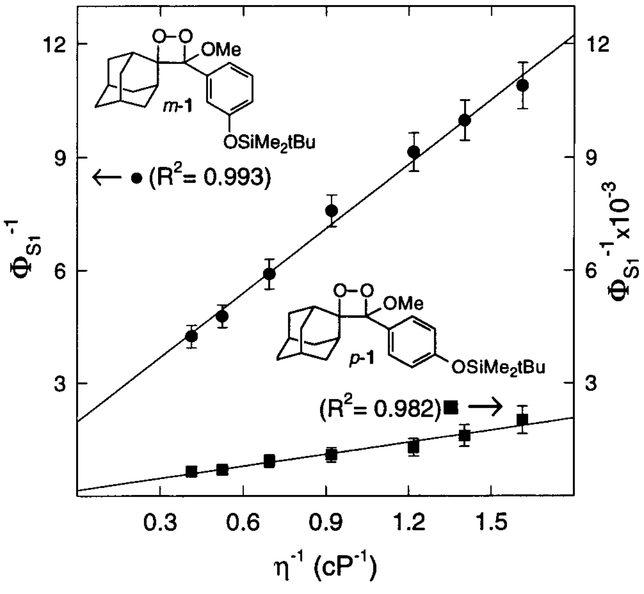

Figure 2. Double-reciprocal plot according to eq 7 for the viscosity dependence of the singlet chemiexcitation yield (Φ_{S1}) in the fluoride ion triggered CIEEL cleavage of dioxetanes m-**1** {●} and p-**1** {■} at the experimental conditions of Figure 1.

The experimental data in Figure 2 show that for both dioxetanes m-**1** and p-**1** the CIEEL efficiency ($\Phi^{CIEEL} = \Phi_{S1}\Phi^{fl}$) is enhanced with increasing viscosity. To rationalize such a behavior, the chemiexcitation (Φ_{S1}) and the fluorescence (Φ^{fl}) yields should be considered as a function of viscosity (η).

As we have previously established,[10] the fluorescence quantum yield (Φ^{fl}) of the methyl m-oxybenzoate anion (m-**4**) does not depend on the solvent viscosity since it takes the values $\Phi^{fl} = 0.230 \pm 0.015$ over the entire concentration range of Ph_2CH_2 in C_6H_6–Ph_2CH_2 mixtures. Consequently, the observed viscosity influence on the chemiluminescence efficiency ($\Phi^{CIEEL} = \Phi_{S1}\Phi^{fl}$) is accounted for exclusively by the viscosity dependence of the chemiexcitation yield (Φ_{S1}).

Unfortunately, for the *para* emitter p-**4** it is difficult to acquire reliable data on Φ^{fl} versus η because of the very low fluorescence yield of p-**4** [$(3.6 \pm 0.3) \times 10^{-3}$ in pure benzene] and appreciable background light emission at [Ph_2CH_2] > 15 vol % under photoexcitation. However, in view of the lack of the viscosity effect on the fluorescence yield of the *meta*-substituted emitter m-**4**, it seems likely that the fluorescence yield of its *para* isomer p-**4** is also insensitive to viscosity changes. With this assumption, the similar viscosity profiles of the chemiluminescence yield (Φ^{CIEEL}) for the *meta* and *para* regioisomers m,p-**1** (Figure 2) suggest that the viscosity influence is manifested during the step of excited-state generation and therewith reflected in the chemiexcitation efficiency $\Phi_{S1}(\eta)$. Thus, the viscosity dependence of the chemiexcitation yield $\Phi_{S1}(\eta)$ for the *para*-substituted dioxetane p-**1** is accessible through eq 4 by employing the experimental data on Φ^{CIEEL} versus η (Figure 1) and the fluorescence quantum yield $\Phi^{fl} = (3.6 \pm 0.3) \times 10^{-3}$ of p-**4**, measured in benzene. From the viscosity dependence (Figure 1) of the chemiexcitation yield $\Phi_{S1}(\eta)$ for the m-**4** and p-**4** regioisomers, in analogy to the *meta* case, we infer that also for the *para* one the BET process operates in the CIEEL mechanism.

A quantitative analysis of the viscosity dependence of the chemiexcitation yield for the BET process has been conducted for the *meta* dioxetane m-**1** in terms of the probabilistic model for the solvent-cage effect,[21] based on the conceptual distinction between a *collision* and an *encounter* of the reacting species.[21,22] This approach, which is similar to that previously developed for triplet–triplet energy transfer,[17] was described in detail as Supplementary Information in our previous work.[10] Herein, we apply this method to the *para* substrate p-**1** to estimate the quantitative characteristics of the BET process for the *para*-regioisomeric CIEEL system.

As we have previously derived (see Supplementary Information in ref 10), the viscosity dependence of the chemiexcitation yield Φ_{S1} (η) is given by eq 5, in which

$$\Phi_{S1}(\eta) = \frac{p_{S1}\eta}{(p_{S1} + p_{T1} + p_{S0})\eta + A_{\eta}} \qquad (5)$$

$$\eta = A_{\eta} e^{E_{\eta}/RT} \qquad (6)$$

$$\Phi_{S1}^{-1}(\eta) = \frac{(p_{S1} + p_{T1} + p_{S0})}{p_{S1}} + \frac{A_{\eta}}{p_{S1}}\eta^{-1} \qquad (7)$$

p_{S0}, p_{S1}, and p_{T1} constitute the partial probabilities of the BET process at the *collision* moment of the solvent-caged partners (**5**) (Scheme 1) to yield the *ground*, excited *singlet*, and *triplet* states of the CIEEL emitters m,p-**4**. The sum $p_{S0} + p_{S1} + p_{T1}$ represents the total probability of the BET (p_{BET}) per collision in the solvent cage. Parameter A_{η} is the preexponential factor for the viscosity in its Arrhenius form, expressed by eq 6 (E_{η} in eq 6 represents the activation energy of fluidity, η^{-1}).

The relation between the probabilistic approach and kinetic description in terms of rate constants of the chemiexcitation ($\boldsymbol{k}_{S1}$) and the total ($\boldsymbol{k}_{BET}$) BET processes

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Table 1. BET Parameters for the *meta*- versus *para*-Substituted Dioxetanes 1

substitution	p_{S1}[a] (× 10^6)	p_{BET}[a] (× 10^3)	k_{S1} (s^{-1})[b] ($\tau_C^{-1}p_{S1}$ × 10^{-10})	k_{BET} (s^{-1})[b] ($\tau_C^{-1}p_{BET}^{-1}$ × 10^{-10})
meta	1700 ± 100	3.4 ± 0.1	ca. 1.7	3.4
para	9.2 ± 1.5	1.4 ± 0.3	ca. 0.009	1.4

[a] Obtained from the double-reciprocal plot of Figure 2. [b] Estimated for τ_C^{-1} ca. 10^{13} s^{-1} (ref 23).

in the solvent cage is given by eqs 8 and 9 , in which τ_C^{-1}

$$k_{S1} = \tau_C^{-1}\, p_{S1} \tag{8}$$

$$k_{BET} = \tau_C^{-1}\, p_{BET} \tag{9}$$

is the mean collision frequency (reciprocal time between collisions) in the solvent cage.

As we have substantiated recently,[10] when the BET channel dominates in the CIEEL process (Scheme 1), eq 5 should apply. Its double-reciprocal relation in eq 7 predicts a linear dependence between viscosity and the experimental singlet chemiexcitation yield, as demonstrated in Figure 2 for both dioxetanes *m*-**1** ($R^2 = 0.993$) and *p*-**1** ($R^2 = 0.982$).

It was previously established for C_6H_6−Ph_2CH_2 mixtures that the viscosity preexponential factor A_η is independent of the [Ph_2CH_2] variation.[10,17] This happenstance is fortunate for our subsequent analysis of the viscosity dependence of Φ_{S1} because A_η is constant in eqs 5 and 7.

From the double-reciprocal plots of Figure 2 according to eq 7, one may obtain the BET probabilities p_{S1} and $p_{BET} = p_{S0} + p_{S1} + p_{T1}$ and from them the rate constants $\boldsymbol{k}_{S1}$ and $\boldsymbol{k}_{BET}$. With the value of $A_\eta = (9.9 \pm 0.6) \times 10^{-3}$ cP,[10] from the slopes A_η/p_{S1} of these plots the data for p_{S1} are obtained; multiplication of the intercepts $(p_{S0} + p_{S1} + p_{T1})/p_{S1}$ for each plot by p_{S1} gives $p_{BET} = p_{S0} + p_{S1} + p_{T1}$. To estimate the rate constants of the chemiexcitation ($\boldsymbol{k}_{S1}$) and the total ($\boldsymbol{k}_{BET}$) BET processes in the solvent cage (Scheme 1) according to eqs 8 and 9, one may assume the collision frequency (τ_C^{-1}) in the cage to be ca. 10^{13} s^{-1}.[23] The data on p_{S1}, p_{BET}, $\boldsymbol{k}_{S1}$, and $\boldsymbol{k}_{BET}$ are shown in Table 1.

The comparison of the BET parameters (Table 1) for the *meta* and *para* regioisomers shows the profound difference (ca. 200-fold!) in the quantitative characteristics of the chemiexcitation BET process (p_{S1}, $\boldsymbol{k}_{S1}$); however, the difference in the parameters for the total BET process (p_{BET}, $\boldsymbol{k}_{BET}$) of the crossed- and extended-conjugated cases *m*-**1** and *p*-**1** is not dramatic. Thus, the singlet-excitation BET channel ($\boldsymbol{k}_{S1}$) depends mainly on the substitution pattern.

What is the reason for the observed regioisomeric dependence of the excited-state generation? Figure 3 displays the effect of crossed versus extended conjugation on the ground- and excited-state energies of the CIEEL emitter (*p*-**4** versus *m*-**4**). From Figure 3 it is evident that the excited state of the crossed-conjugated oxybenzoate ion *m*-**4** is of lower energy (by ca. 12 kcal/mol in a vacuum) than its extended-conjugated regioisomer *p*-**4**. This computational result is consistent with the our recent spectral study,[24] which established that the emission of the *para*-patterned CIEEL emitter (*p*-**4**) occurs at a shorter wavelength in all media (by 70−115 nm depending on the solvent) compared to the *meta* regioisomer (*m*-**4**). A similar observation has been reported for the *para* versus *meta* nitrophenoxide ions.[25] Thus, this spectral phenomenon appears to be general for the *para*- versus *meta*-substituted phenoxides. The ground states do not display such an energy differentiation; in fact, the crossed-conjugated regioisomer is slightly (by ca. 1 kcal) higher in energy. Since the energy for the singlet-excited state of the extended-conjugated emitter (*p*-**4**) is significantly higher than that of its crossed-conjugated isomer *m*-**4**, the BET process in the solvent cage (Scheme 1) to generate the excited *p*-**4** emitter should be more endothermic. Presumably, this may be the reason for the low p_{S1} and $\boldsymbol{k}_{S1}$ values of the *para* regioisomer when compared to the *meta* one (Table 1).

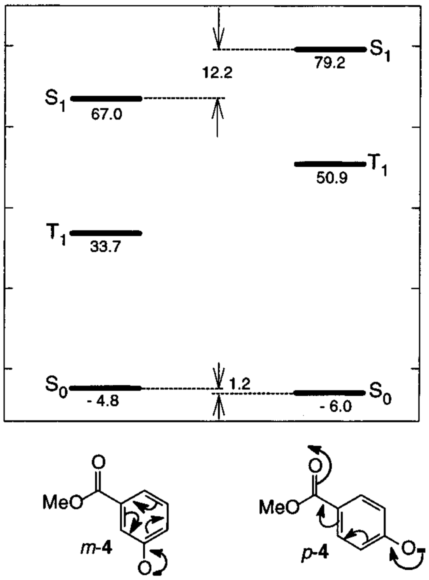

Figure 3. Energies of the ground (S_0) and excited (S_1, T_1) states for the *meta* (*m*-**4**)- and *para* (*p*-**4**)-substituted methyl oxybenzoate ions as calculated by the AM1 method.

Scheme 1 constitutes a simplified description of the CIEEL process, which is convenient to discuss the experimental data. However, the mechanistic alternatives for the CIEEL generation require a more detailed analysis, which is presented in Schemes 2 and 3. In both schemes, the direct ET path constitutes the concerted chemiexcitation of the CIEEL emitters *m*,*p*-**4** without intermediates in the cleavage of the dioxetane phenolates *m*,*p*-**2**, analogous to the intermolecular case.[26] For the stepwise BET channel there are two possibilities to be considered, which differ in the distribution of the electrons among the two oxygen atoms of the ring-opened dioxetane after the electron transfer from the phenolate functionality. In path **A** the unpaired electron is localized on the dioxetane oxygen atom proximate to the phenolate moiety (structures *m*-**A** and *p*-**A** in Schemes 2 and 3), while in path **B** it is placed onto the remote dioxetane oxygen atom (structures *m*-**B** and *p*-**B**). Subsequent

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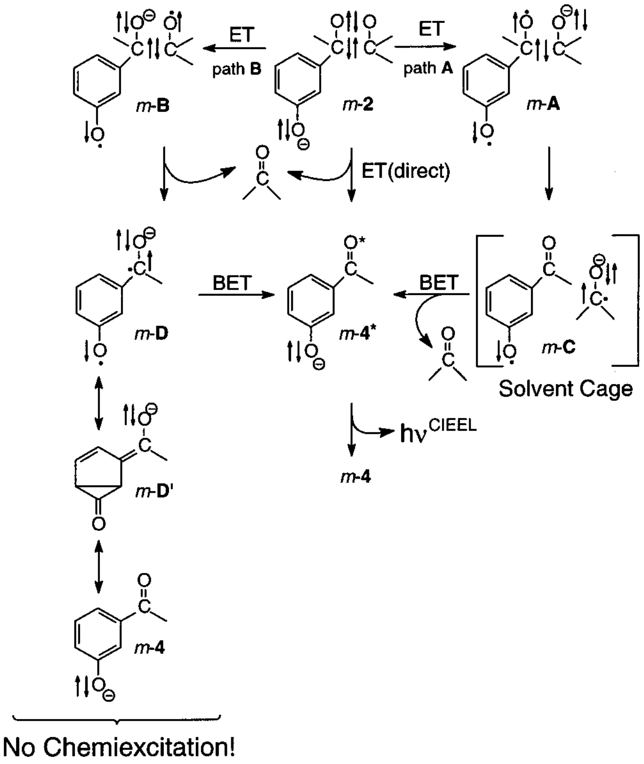
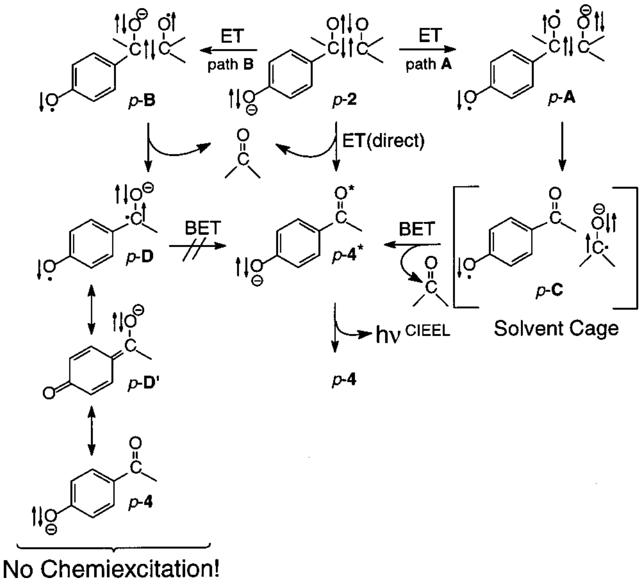
Scheme 2



Scheme 3



cleavage of the dioxetane CC bond in the diradical anions *m*-**A** and *p*-**A** affords the caged radical pairs *m*-**C** and *p*-**C**, while that in *m*-**B** and *p*-**B** generates the diradical ketyl structures *m*-**D** and *p*-**D** (cf. Schemes 2 and 3).

The essential difference between paths **A** and **B** relates to the intermolecular versus intramolecular character of the subsequent BET process for the chemiexcitation route. Thus, whereas path **A** represents the BET process between *two* solvent-caged cleavage fragments, i.e., *m,p*-**C** to *m,p*-**4***, the BET in route **B** takes place within *one* single diradical intermediate, i.e., *m,p*-**D** to *m,p*-**4*** (Schemes 2 and 3). The observed functionally similar viscosity dependence for both regioisomers *m,p*-**1** (Figure 1) resides in the intermolecular BET process of path **A**, because competitive diffusion out of the solvent cage expectedly reduces the efficiency of chemiexcitation. Such diffusion is more facile in less viscous media, as substantiated experimentally. But what is the origin of the dramatically different (ca. 10 000-fold!) chemiluminescence yields $\Phi^{CIEEL} = \Phi^{fl}\Phi_{S1}$ for these regioisomers, with the *meta* regioisomer *m*-**1** much more effective than the *para* one *p*-**1** (Figure 1)? The difference in the fluorescence quantum yields Φ^{fl} for the *meta*- versus *para*-substituted CIEEL emitters (*m*-**4** versus *p*-**4**) accounts for the factor of only 64. Thus, the chemiexcitation efficiency Φ_{S1} for the *meta* regioisomer is more than 2 orders of magnitude higher than for the *para* one, a difference which needs to be rationalized.

Closer inspection of Schemes 2 and 3 reveals a significant mechanistic distinction between the two emitters, namely, the fact that *m*-**4** is a crossed- and *p*-**4** an extended-conjugated system. The consequence of this may be found in path **B**: from the extended-conjugated *para*-patterned anion-diradical intermediate *p*-**D** (Scheme 3), spin annihilation leads directly to the resonance-stabilized mesomeric structure *p*-**D′**, which is equivalent to the *ground state* of the methyl oxybenzoate ion *p*-**4**. For the crossed-conjugated *meta*-patterned anion-diradical intermediate *m*-**D** (Scheme 2), the spin-coupled *m*-**D′** resonance structure is required to connect with the *m*-**4** ground state, which contributes minimally in view of its high energy. It is expected that the *intramolecular* BET process in path **B** to afford the electronically excited *m*-**4*** emitter should compete efficiently with the deactivation step *m*-**D** → *m*-**D′** → *m*-**4** compared to that of the *para* regioisomer *p*-**1**, namely, *p*-**D** → *p*-**D′** → *p*-**4**. Thus, chemiexcitation should be more effective for the *m*-**D** than for the *p*-**D** species. Although this rationale would account for the higher CIEEL efficiency of the *meta* versus *para* regioisomer, such an intramolecular BET process should not exhibit any significant viscosity dependence on the CIEEL generation (Figures 1 and 2). Therefore, the large difference in the chemiexcitation efficiencies must have another origin. Since the singlet excitation energy for the *m*-**4*** CIEEL emitter is substantially lower (by ca. 12 kcal/mol) than for *p*-**4*** (Figure 3), it is expected that the *meta* regioisomer is more efficiently chemiexcited. This is consistent with the higher (ca. 200-fold!) p_{S1} and $\boldsymbol{k}_{S1}$ values for the *meta* case (Table 1).

From the detailed mechanistic analysis presented herein, we conclude that cross-conjugated emitters are essential for the efficient generation of the CIEEL required in chemiluminescence bioassays, as manifested for the *meta* regioisomer *m*-**1**.[4] Moreover, the viscosity behavior of the CIEEL efficiency (Φ^{CIEEL}) for both the *meta*- and *para*-regioisomeric dioxetanes *m,p*-**1** provides evidence that the BET process operates in triggered CIEEL generation.

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