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Stereodifferentiation in the Photochemical Cycloreversion of Diastereomeric Methoxynaphthalene−Oxetane Dyads

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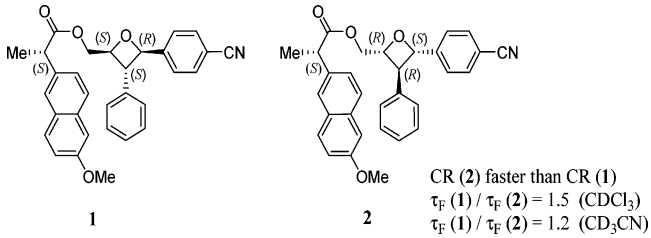
Received July 27, 2004



Intramolecular PET cycloreversion of oxetanes **1** and **2** has been achieved in acetonitrile and chloroform as solvents. Interestingly, a higher photoreactivity has been found in acetonitrile, while a significant stereodifferentiation has been found in chloroform. This stereodifferentiation can be attributed to the folded conformation which predominates in **2**, with the naphthalene ring directed toward the oxetane region, allowing for the intramolecular electron transfer. Accordingly, intramolecular fluorescence quenching is also more efficient in acetonitrile, whereas stereodifferentiation is markedly higher in chloroform. Thus, a good correlation can be established between the results from steady-state irradiations and fluorescence measurements.

Introduction

A variety of DNA lesions with known mutagenic, carcinogenic, and lethal effects can be formed by the exposure of cells to UV-radiation.[1,2] They include cyclobutane pyrimidine dimers and (6−4) photoproducts.[3,4] The latter (with higher mutagenic potential) are formed through a Paterno−Büchi photoreaction between two adjacent pyrimidines in the DNA duplex; the resulting oxetanes rearrange to the final (6−4) photoadducts.[5]

Repair of the (6−4) photoproducts in DNA follows a mechanism analogous to the reductive electron transfer established for cyclobutane pyrimidine DNA dimers.[6−8] This process is enzymatically achieved by photolyases,[9] whose mode of action involves photochemical transfer of one electron from a reduced and deprotonated flavin ($FADH^-$)[10,11] to an oxetane. Subsequently, the oxetane radical anion cleaves to provide one neutral pyrimidine plus one pyrimidine radical anion.

Despite its biological interest, only a few reports have appeared on the cycloreversion (CR) of oxetane radical anions generated by photoinduced electron transfer (PET).[12] Cycloadducts of 1,3-dimethylthymine with benzaldehyde and benzophenone have been used as model systems to study their PET reactions with a variety of electron-donor photosensitizers.[12−15] The radical anion of

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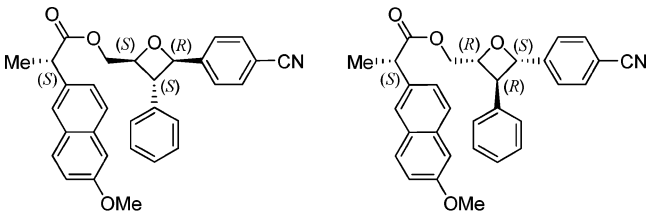
10.1021/jo048708+ CCC: $30.25
Published on Web 01/19/2005

CHART 1



1 **2**

the carbonyl fragment has been detected by means of laser flash photolysis (LFP), which supports an electron-transfer mechanism. Taking into account that the photosensitizer fluorescence is quenched by oxetanes, it has been proposed that the reaction occurs from the singlet excited state.

More recently, the mechanistic aspects of the reductive intermolecular CR of the model compound 2-(*p*-cyanophenyl)-4-methyl-3-phenyloxetane using 1-methoxynaphthalene (1-MN) as the electron-donor photosensitizer have been studied.[16] Clear evidence has been obtained confirming that CR of the oxetane takes place from the singlet excited state of the sensitizer. Ring splitting of the oxetane radical anion occurs with cleavage of the $O-C_2$ and C_3-C_4 bonds, leading to products (acetaldehyde and *p*-cyanostilbene) different from the reagents used in the Paterno–Büchi synthesis of the oxetane. Moreover, the radical anion of *p*-cyanostilbene involved in the PET process has been detected by LFP.

As 1-MN has been shown to be a good electron-donor photosensitizer for the CR of *p*-cyanophenyl-substituted oxetanes, it appeared interesting to study the analogous intramolecular process in oxetanes **1** and **2**, which contain a chiral methoxynaphthalene covalently linked to the oxetane ring (Chart 1).

The main goal of this work was to detect a possible stereodifferentiation in the intramolecular PET process, either in the quenching of the reactive singlet excited state or in the kinetics of oxetane disappearance with formation of CR photoproducts.

For this purpose, compounds **1** and **2** have been submitted to preparative photolysis and fluorescence (steady-state as well as time-resolved) studies, in both acetonitrile and chloroform, to compare their photophysical and photochemical behavior.

Cycloreversion has been found to occur with cleavage of the $O-C_2$ and C_3-C_4 bonds. Interestingly, there is a significant stereodifferentiation in chloroform, while a higher photoreactivity is observed in acetonitrile. Accordingly, intramolecular fluorescence quenching is also more efficient in acetonitrile, whereas stereodifferentiation is markedly higher in chloroform. Thus, a good correlation can be established between the results from steady-state irradiations and fluorescence measurements.

Results and Discussion

Synthesis of Oxetanes 1 and 2. The target compounds **1** and **2** were prepared by esterification of (*S*)-naproxen (NPX) with racemic 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane, synthesized in turn by Paterno–Büchi photocycloaddition of *p*-cyanobenzaldehyde and *trans*-cinnamyl alcohol. The resulting mixture was separated by high performance liquid chromatrography (HPLC) leading to the pure diastereomers **1** and **2**.

The structural assignment was made on the basis of ^1H and ^{13}C NMR spectroscopy, using NOE data to clarify the encumbered aromatic region. This information was checked against the MOPAC (AM1) calculations, which allowed us to obtain the optimized folded and extended geometries for the two compounds, **1** and **2** (see Supporting Information, pages S18 and S19). NOESY experiments (Figures 1 and 2) were performed to establish the relative stereochemistry of the chiral centers; their results were consistent with a 2*R*, 3*S*, 4*S* configuration for isomer **1** and the opposite one (2*S*, 3*R*, 4*R*) for **2**. Some conclusions were drawn from NOESY data: In compound **1**, a clear interaction was observed between the methyl group (δ = 1.58 ppm) and H_3 (δ = 3.58 ppm). This was absent in compound **2**, where the methyl protons interacted instead with the methylene group (δ = 4.39 ppm) and with the *ortho* protons of the phenyl substituent at C_3. Besides, the methoxy group of **2** presented a small but clear interaction with the *p*-cyanophenyl protons that could not be observed for **1**.

A closer inspection of the aromatic region (see Figures 1b and 2b) showed a clear correlation between the naproxen protons and the *p*-cyanophenyl group in isomer **2**. A less clear situation resulted for **1**, where the chemical shifts of the relevant protons were too close to observe a hypothetical NOE effect.

To ensure that similar intensity NOE effects were being compared for **1** and **2**, a 50:50 mixture of both diastereomers in $CDCl_3$ was prepared. From its NOESY spectra, essentially the same conclusions were drawn (whenever possible). This allowed us to validate the observed NOE effects and to conclude that they were intramolecular in nature.

Taking into account the whole ensemble of NOE data and the optimized geometries according to MOPAC (AM1) calculations, it was concluded that a folded conformation predominates in **2**, with the naphthalene ring directed toward the oxetane region; by contrast, an extended conformation with the naphthalene ring pointing to the external region appears to be preferred in the case of **1**. This is in good agreement with the number and strength of the interactions found between the aromatic protons of the naphthalene ring and those of the *p*-cyanostilbene group, which were more important for **2** (see above).

Photosensitized Cycloreversion of 1 and 2. The two diastereomeric oxetanes **1** and **2** were separately irradiated at λ_{max} = 300 nm in acetonitrile, under nitrogen. As outlined in Scheme 1, the main photoproducts were *cis*-*p*-cyanostilbene (**3b**) and the naproxen ester of 2-hydroxyacetaldehyde (**4**). This result is analogous to that of the previously reported intermolecular process,[16] where cleavage of the $O-C_2$ and C_3-C_4 bonds was also observed.

To compare the photoreactivity of **1** and **2** under identical conditions and to follow the reaction by ^1H NMR, direct irradiation of a 1:1 diastereomeric mixture

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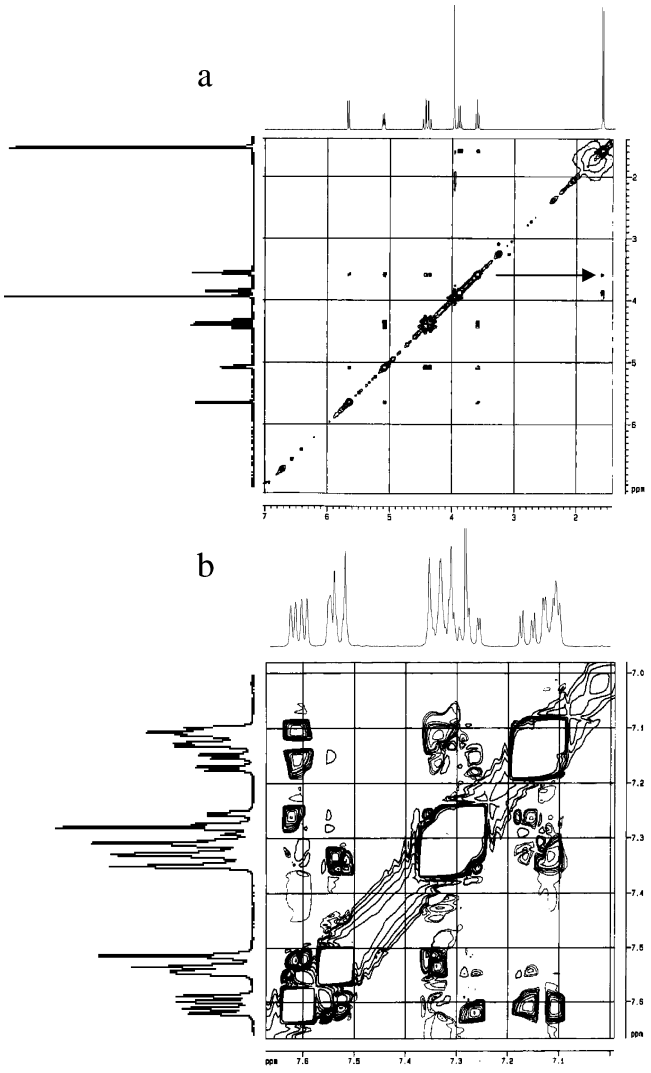


FIGURE 1. (a) Aliphatic and (b) aromatic part of the phase-sensitive 2D NOESY spectrum of **1** recorded in $CDCl_3$.

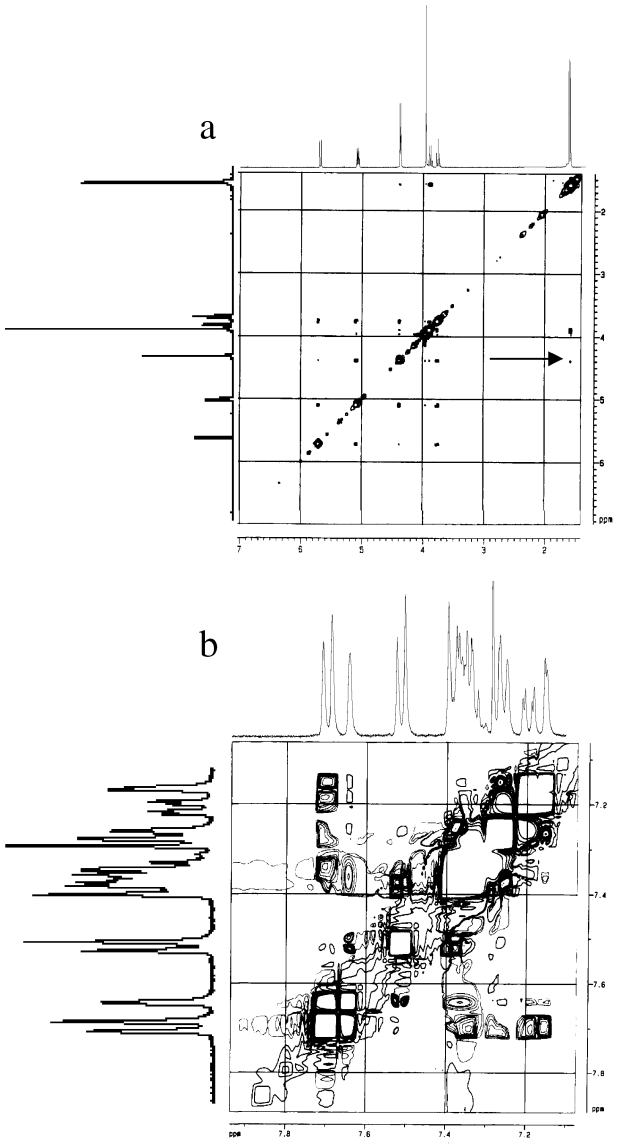


FIGURE 2. (a) Aliphatic and (b) aromatic part of the phase-sensitive 2D NOESY spectrum of **2** recorded in $CDCl_3$.

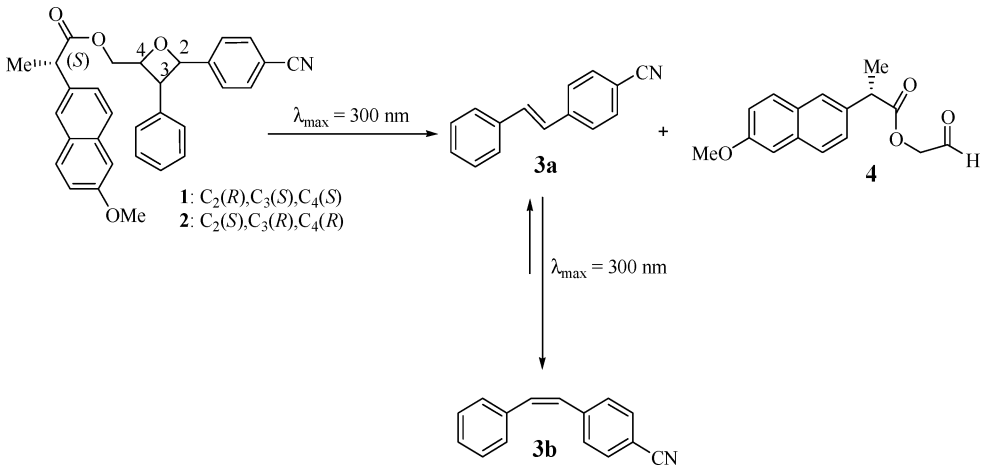
was performed using deuterated chloroform and acetonitrile as solvents. At the beginning of the reaction, the two doublets in the region $\delta = 5.60-5.80$ ppm, corresponding to the H_2 protons of the oxetane rings of **1** and **2**, were of the same intensity. These signals decreased with increasing irradiation time, giving rise to a new AB system centered at $\delta = 6.65$ ppm in chloroform and $\delta = 6.80$ ppm in acetonitrile, corresponding to the olefinic protons of *cis*-*p*-cyanostilbene.

The relevant part of the ^1H NMR spectra is shown in the Supporting Information, page S25. It is remarkable that a significant stereodifferentiation was found in chloroform, where compound **2** (monitored through its more deshielded doublet) reacted faster than its diastereomer **1**. On the other hand, higher conversions were achieved in acetonitrile after shorter irradiation times; however, a lower stereodifferentiation was observed in this solvent. This is in agreement with the fact that reactivity differences are usually better observable when the reaction is not too fast.

Intramolecular Interactions in the Ground and Excited States. To gain some insight into the mechanistic aspects of the reaction, experimental studies were carried out looking for intramolecular interactions in the ground and the excited states. Thus, the ultraviolet absorption spectra of **1**, **2**, NPX, and 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane were recorded in acetonitrile and chloroform, and then difference spectra [**1** − (NPX + oxetane)] and [**2** − (NPX + oxetane] were obtained. Figure 3 shows the results in acetonitrile. Further material can be found in the Supporting Information, pages S20−S24. Interestingly, a new band was clearly observed at 336−337 nm in both compounds that can be attributed to a CT ground-state complex.

Previously, the fluorescence emission as well as the triplet−triplet (T−T) absorption of 1-methoxynaphthalene (1-MN) has been studied in the presence of increasing amounts of 2-(*p*-cyanophenyl)-4-methyl-3-phenyloxetane, to establish the nature of the excited state involved in the intermolecular PET process.[16] Although both singlet and triplet quenching were observed, product studies in the presence of triplet quenchers, together with the calculated free energy changes, provided a clear

SCHEME 1



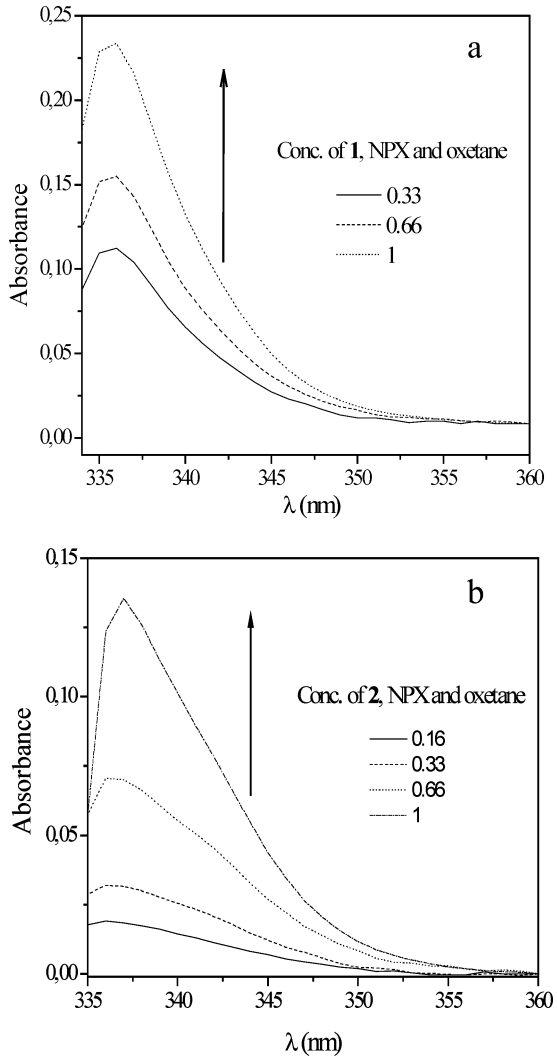


FIGURE 3. Difference UV-spectra in acetonitrile: (a) **1** − [(NPX + 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane)] and (b) **2** − [(NPX + 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane)], in the long wavelength region.

support for the reaction taking place from the singlet excited state of the sensitizer 1-MN. The redox properties of 2-methoxynaphthalene (2-MN) in the excited states are expected to be very similar to those of 1-MN. They only depend on the singlet energies (89 kcal/mol for 1-MN vs 86 kcal/mol for 2-MN) and on the oxidation potentials (1.4 V vs SCE for 1-MN vs 1.5 V vs SCE for 2-MN). On this basis, taking into account the presence of a chiral 2-methoxynaphthalene as donor in compounds **1** and **2**, singlet excited-state involvement in the intramolecular reaction was also expected.

The required information on intramolecular excited-state quenching was obtained by fluorescence (steady-state and time-resolved) and LFP of **1** and **2**, in both acetonitrile and chloroform. The results were compared with those obtained for NPX, the corresponding isolated chromophore.

In acetonitrile, the emission maxima of **1** and **2** appeared at ~350 nm, as in the case of NPX. However, the fluorescence intensity became strongly reduced in the two diastereomers (Figure 4a), indicating significant quenching attributable to electron transfer from the excited naphthalene moiety to the *p*-cyanophenyl group. The quenching rate constants $k_q(S_1)$ were calculated from eq 1 using the experimental fluorescence lifetimes (τ_s) of

$$1/\tau_s - 1/\tau_{s,0} = k_q(S_1) \qquad (1)$$

1 and **2** (2.6 and 2.2 ns, respectively). As the lifetime of NPX is known ($\tau_{s,0}$ = 10.8 ns),[17] the values of $k_q(S_1)$ for singlet quenching can be estimated as 2.9×10^8 s^{-1} (for **1**) and 3.6×10^8 s^{-1} (for **2**).

The fluorescence quantum yields (Φ_f) of the diastereomeric oxetanes were obtained from the integrated emission spectra, using NPX as the reference (Φ_f = 0.47).[17] They were found to be Φ_f = 0.075 (**1**) and 0.055 (**2**). The decrease of the Φ_f values as compared with those of NPX and the differences found between the two isomers are in good qualitative agreement with the trends observed in the lifetime measurements. This confirms that (apart from ET) the radiative and radiationless transitions in NPX and compounds **1** and **2** are similar.

As regards the LFP experiments, the expected T−T absorption of NPX at 440 nm was detected for **1** and **2**. The reduced intensity of this signal observed for the two diastereomers right after the laser pulse (Figure 4b)

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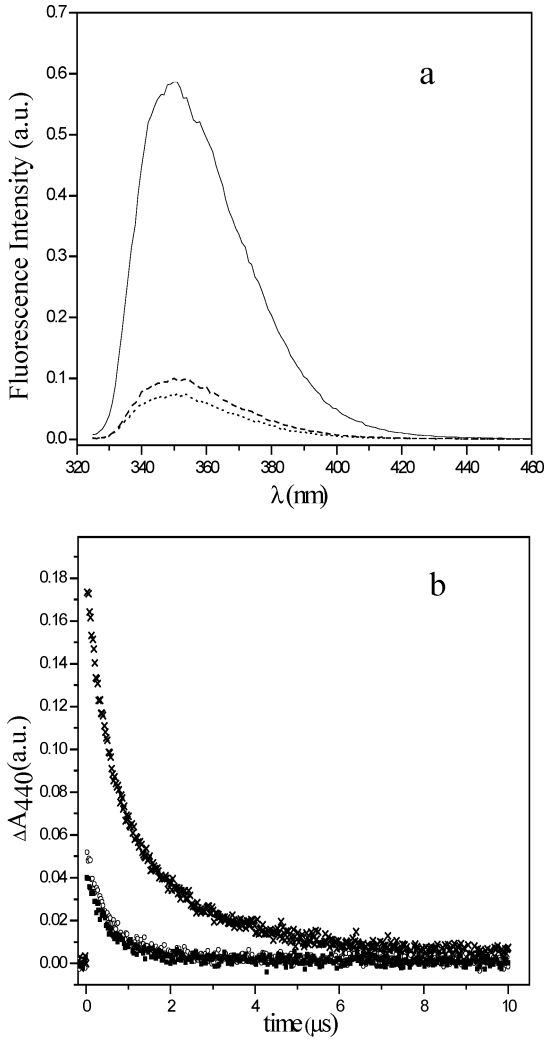

FIGURE 4. (a) Emission spectra of NPX (—), **1** (- - -), and **2** (···) in acetonitrile, recorded after excitation at 320 nm under nitrogen. (b) Decay traces of the T−T absorption of NPX (×), **1** (○), and **2** (■) measured at 440 nm in acetonitrile after LFP at 308 nm. All concentrations were fixed, adjusting the absorbance of the solution at an arbitrary value between 0.3 and 0.32 at the excitation wavelength, under nitrogen.

simply reflects the above-mentioned singlet excited-state quenching. Besides, there was a significant shortening of the triplet lifetimes τ_t of **1** and **2** (560 and 550 ns, respectively) as compared with those of NPX ($\tau_{t,0} \sim 1$ μs under the same conditions). Hence, the triplet quenching rate constants $k_q(T_1)$ were estimated using eq 2 and found to be 7.8×10^5 s^{-1} (for **1**) and 8.1×10^5 s^{-1} (for **2**).

$$1/\tau_t - 1/\tau_{t,0} = k_q(T_1) \quad (2)$$

Free energy changes associated with electron transfer from both the singlet and triplet excited states were estimated using the Weller[18] equation (eq 3):

$$\Delta G_{ET}\,(\text{kcal/mol}) = 23.06[E_D{}^{\bullet+}{}_{/D} - E_{A/A}{}^{\bullet-} + (2.6/\epsilon) - 0.13] - E^*(S_1 \text{ or } T_1) \quad (3)$$

The reduction potential ($E_{A/A^{\bullet-}}$) was taken to be −1.52 V versus SCE, as in the model oxetane previously used.[16] The oxidation potential of NPX ($E_{D^{\bullet+}/D}$ = 1.45 V vs SCE) and its singlet and triplet energies [$E^*(S_1)$ = 85 kcal/mol, $E^*(T_1)$ = 62 kcal/mol] have already been reported.[19] Finally, the value of the dielectric constant in acetonitrile is 35.9.[20] By fitting the above data in eq 3, the following free energy changes resulted: $\Delta G_{ET}(S_1) = -17.8$ kcal/mol and $\Delta G_{ET}(T_1) = +5.1$ kcal/mol. As expected, the intramolecular CR from the singlet excited state would be an exergonic process, whereas triplet involvement does not appear to be thermodynamically possible.

In chloroform, a different tendency was observed when fluorescence (steady-state as well as time-resolved) and LFP studies were performed. Although the emission maxima of **1**, **2,** and NPX appeared at ∼352 nm, fluorescence quenching was clearly detected in the case of **2**, while the emission intensity of **1** was very similar to that of NPX (Figure 5a). As a matter of fact, the fluorescence lifetimes of NPX and **1** were in the same range (∼3.5 ns), whereas, in the case of **2**, τ_s was found to be 2.5 ns. From these data the quenching rate constant was only calculated for **2**, and its value was estimated as $k_q(S_1) = 1.1 \times 10^8$ s^{-1}.

On the other hand, the transient T−T absorption of NPX and the two oxetanes was also observed in chloroform at 440 nm by means of LFP. Again, the reduced intensity of the signal observed for compound **2** right after the laser pulse (Figure 5b) is simply a consequence of fluorescence quenching. The triplet lifetimes were determined and found to be 1.1 μs (**1**), 0.7 μs (**2**), and 0.7 μs (NPX). Hence, intramolecular triplet quenching does not appear to occur in oxetanes **1** and **2**.

The free energy changes associated with intramolecular PET in chloroform were estimated with eq 3, taking into account that the dielectric constant in this solvent is 4.8.[20] The obtained values were $\Delta G_{ET}(S_1) = -7$ kcal/mol and $\Delta G_{ET}(T_1) = +16$ kcal/mol. Thus, the process would be thermodynamically possible from the singlet excited state, but it would be disfavored from the triplet.

A possible reaction mechanism that is in agreement with the above data is shown in Scheme 2. It includes both the radical ion pair **3a**$^{\bullet-}$/**4**$^{\bullet+}$ and the biradical resulting from back electron transfer. The former pathway would be analogous to the known intermolecular process;[16] however, the radical anion of *p*-cyanostilbene (λ_{max} = 500 nm)[16] was not detected as a transient intermediate. Although formation of this species cannot be completely ruled out (it could be masked by the intense naphthalene T−T absorption between 350 and 550 nm; see Supporting Information, pages S12−S17), it appears more likely that fast intramolecular BET occurs in the biradical zwitterionic intermediate, to give a biradical as immediate precursor of the final products.

Conclusions

Overall, the above results support the view that the PET CR of **1** and **2** occurs from the singlet excited state

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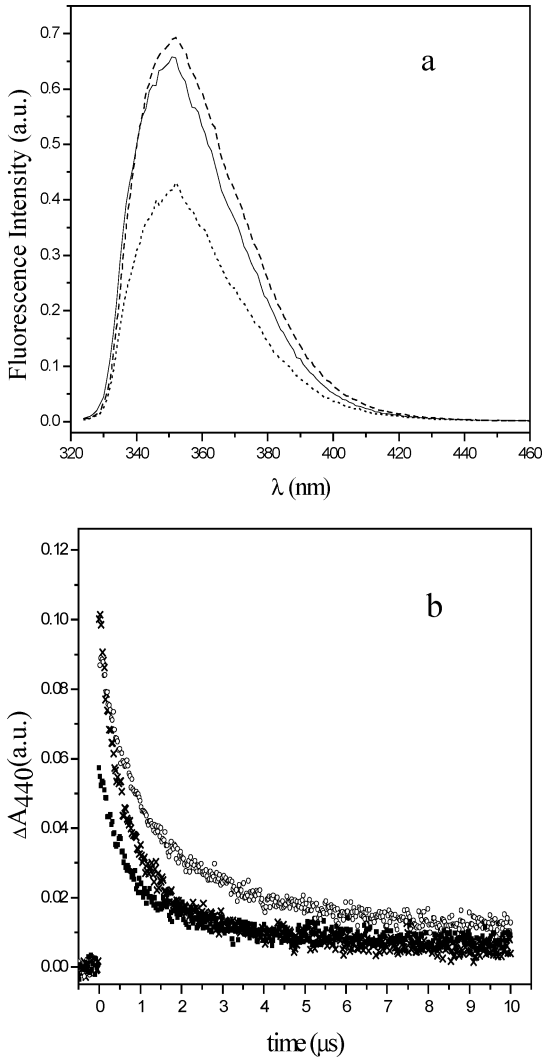
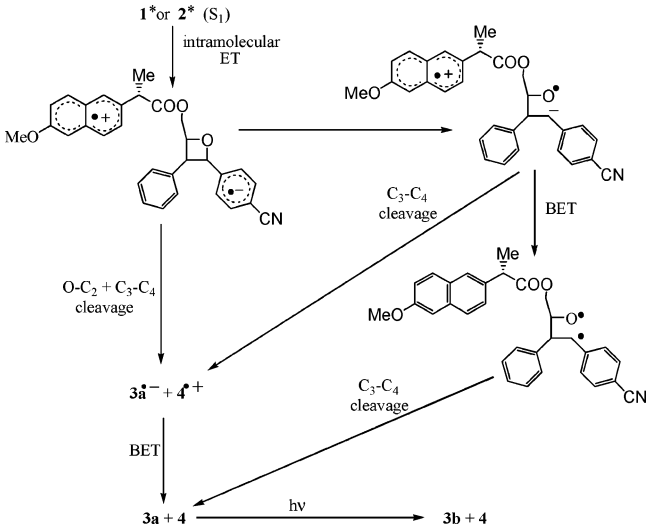


FIGURE 5. (a) Emission spectra of NPX (—), **1** (- - -), and **2** (···) in chloroform, recorded after excitation at 320 nm under nitrogen. (b) Decay traces of the T−T absorption of NPX (×), **1** (○), and **2** (■) measured at 440 nm in chloroform, after LFP at 308 nm. All concentrations were fixed, adjusting the absorbance of the solution at an arbitrary value between 0.3 and 0.32 at the excitation wavelength, under nitrogen.

of the naphthalene-like chromophore. The efficiency of the photoreaction was higher in acetonitrile, which is consistent with the higher values of the fluorescence quenching rate constants found in this solvent, where the free energy changes associated with the process are more negative. Remarkably, the stereodifferentiation observed in the preparative photolysis (with oxetane **2** reacting significantly faster) agrees well with the relative $k_q(S_1)$ values obtained from fluorescence measurements.

It is interesting to note that stereodifferentiation is higher in chloroform, which can be explained in terms of the well-known reactivity/selectivity principle. Finally, it is also worth mentioning that the higher reactivity of oxetane **2** must be related to the contribution of folded conformations, with a significant interaction between the electron-donating methoxynaphthalene chromophore and the electron-accepting *p*-cyanophenyl group; this is in good agreement with the NOESY data.

SCHEME 2



Experimental Section

Cycloreversion Reaction. A solution of the isomer mixture (10^{-2} M) in CD_3CN and $CDCl_3$ (0.75 mL) was placed in NMR tubes and bubbled with nitrogen. Then, the solution was irradiated for 60 min in a multilamp photoreactor, using 8 W lamps (4×) with emission maxima at $\lambda = 300$ nm. The reaction was followed by ^{1}H NMR, which was recorded before and after the irradiation. A control experiment showed that the photocycloreversion does not take place in the dark.

Fluorescence Spectroscopy. The steady-state fluorescence spectra were obtained with a conventional fluorimeter, equipped with a 450 W xenon lamp. The time-resolved fluorescence determinations were performed using a hydrogen−nitrogen flash lamp (2 ns pulse width). The samples were placed into quartz cells of 1 cm path length. The concentrations of substrates **1** and **2**, as well as that of NPX, were fixed by adjusting the absorbance of the solution at an arbitrary value between 0.3 and 0.4, and deoxygenation was performed by bubbling nitrogen.

Time-Resolved Absorption Spectroscopy. The laser flash photolysis system has been described elsewhere.[16] Briefly, it was based on a pulsed XeCl Excimer laser, using 308 nm as the excitation wavelength. The single pulses were ∼17 ns duration, and the energy was ∼100 mJ/pulse. A xenon lamp was employed as the detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, a monochromator, a photomultiplier (PMT) system, and an oscilloscope. The output signal from the oscilloscope was transferred to a personal computer for study.

Acknowledgment. Financial support by the MCYT (Grant No. BQU2001-2725) and by Generalitat Valenciana (Grups 03/082) is gratefully acknowledged.

Supporting Information Available: Additional experimental details; ^{1}H, ^{13}C NMR of compounds **1**, **2**, and **4** and 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane; ^{1}H, ^{13}C correlation spectra of compounds **1** and **2**; transient absorption spectra of NPX, **1**, and **2**; MOPAC (AM1) optimized geometries for **1** and **2**; absorption spectra of NPX, 2-(*p*-cyanophenyl)-4-hydroxymethyl-3-phenyloxetane, **1**, and **2**, as well as difference spectra; ^{1}H NMR monitoring of the **1**:**2** mixtures (28 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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