# Model Studies of the (6−4) Photoproduct Photolyase Enzyme: Laser Flash Photolysis Experiments Confirm Radical Ion Intermediates in the Sensitized Repair of Thymine Oxetane Adducts

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**Abstract:** The DNA (6−4) photoproduct photolyases are proteins that bind to UV-damaged DNA, specifically to sites that contain a (6−4) pyrimidine−pyrimidone lesion. Upon absorption of UV-A and visible light they catalyze the reversal of these lesions back to normal bases. It has been proposed that the photorepair occurs via an oxetane intermediate, which is formed from a ring-closing isomerization of the (6−4) photoproduct. Four model compounds for the oxetane intermediate have been prepared through photocycloaddition of carbonyl compounds (benzophenone, benzaldehyde, tolualdehyde, and anisaldehyde) with the 5,6 C═C of 1,3-dimethylthymine. The behavior of these compounds under sensitized photolysis conditions has been examined. On the basis of laser flash photolysis, fluorescence quenching, and product analysis experiments, it is demonstrated that these oxetane intermediates undergo a cycloreversion reaction upon photosensitized reductive electron-transfer reactions. The cycloreversion process yields the anion radicals of the carbonyl compounds. A lower limit on the rate constant of this anion radical splitting reaction is estimated to be $>5 \times 10^7$ $s^{-1}$. These results support the proposed mechanism for DNA (6−4) photoproduct photolyase.

## Introduction

The DNA molecule is damaged by ultraviolet light to give a variety of potentially mutagenic photoproducts.[1−3] The pyrimidine cyclobutane dimers pyr<>pyr are formed most abundantly. Consequently, most research on DNA photodamage, mutagenesis, and repair has focused on this particular type of damage. More recent studies have identified the (6−4) pyrimidine-pyrimidine photodimers, so-called because they include a bond between the 6-position of one pyrimidine base and the 4-position of the adjacent base (Scheme 1).[4−8] These adducts are considered to be formed from a photochemical cycloaddition involving the 5−6 C═C double bond of the 5′-pyrimidine with either the C4 carbonyl group (in the case of thymine) or C4 imino group (in the case of the high-energy tautomer of cytosine) of the 3′-pyrimidine. The presumed oxetane or azetidine intermediates are thought to rapidly isomerize through ring-opening to give the observed (6−4) photoproducts. When the C4 carbonyl group (C═O) is replaced with a thiocarbonyl group (C═S), the four-membered ring (in this case a thietane) is stable and can be photolytically reverted back to the free bases.[9,10] The (6−4) photoproducts are themselves mutagenic[6,7,11,12] and are further photoisomerized with UV-B light to give mutagenic Dewar photoproducts (not shown).[13,14]

Todo and co-workers discovered a single protein from *Drosophila melanogaster* that utilizes UV-A and blue light to mediate the reversal of (6−4) photoproducts.[15] Enzymes with similar function and sequence were subsequently discovered in other species including *Xenopus laevis*.[16,17] Overall, this photoreversal process is analogous to photoreversal of pyr<>pyr mediated by the cyclobutane pyrimidine dimer (CPD) photolyase enzyme.[18,19] In both cases the photolyases bind to a damaged site, absorb a photon, and then restore the normal bases (Scheme 2).[20,21] These similarities, however, are deeper than the overall

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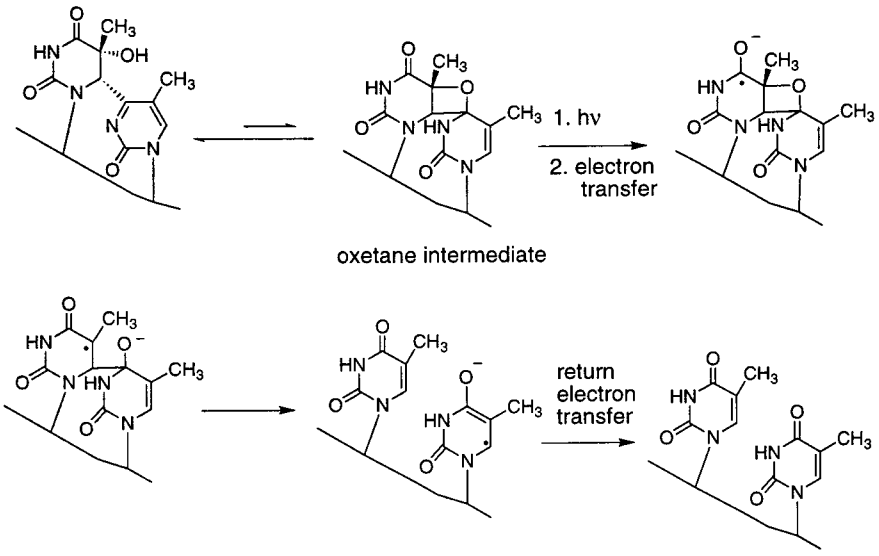
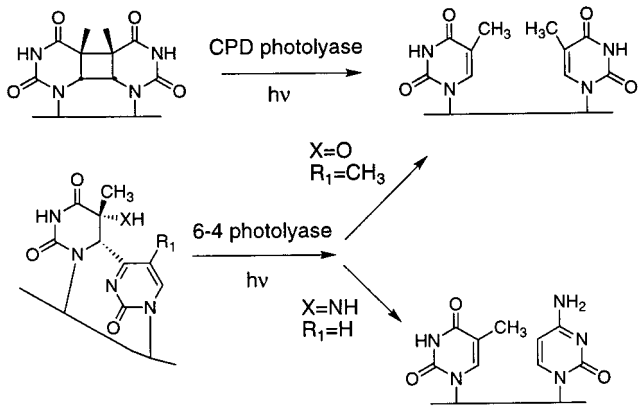
**Scheme 1.** Formation Mechanism of the 6−4 Photoproduct




**Scheme 2.** Photoenzymatic Repair of the CPD and 6−4 Photoproducts




outline of the process. Both photolyases exhibit a high degree of sequence homology,[22] both have a noncovalently bound flavin chromophore,[16] and both exhibit similar action spectra.[23]

Such structural similarities suggest that the (6−4) photolyases and the CPD photolyases might also show similarities in their catalytic mechanisms. The CPD photolyase mechanism has been studied extensively. A variety of flash spectroscopic[24−29] and model studies[30−38] indicate that the primary photochemical step is one-electron transfer from the excited chromophore (a reduced flavin, $FADH^-$) to the pyr<>pyr group.[24,26−29,34,39,40] The pyr<>$pyr^{-\bullet}$ thus formed splits rapidly and spontaneously in solution.[29] Thus the photolyase protein increases the quantum efficiency of this process by holding the pyr<>pyr lesion in contact with the relevant chromophore prior to photon absorption. This allows for efficient conversion of the photon energy into charge separationn and ultimately chemical products. Some recent work has focused on computational modeling of the binding and electron-transfer events.[41−43]

Kim et al.[44] proposed an analogous electron-transfer mechanism for the (6−4) photolyase (Scheme 1). Binding of the (6−4) photoproduct to the photolyase active site causes it to revert back to the oxetane or azetidine intermediate. Absorption of a photon by this complex results in an electron transfer from the reduced flavin to the lesion. The anion radical of the oxetane or azetidine then fragments to give a neutral base and a base anion radical. The latter restores its electron to the flavin radical, resulting in the fully repaired bases.

A key premise of this mechanism is rapid fragmentation of the oxetane anion radical. At the time of the original proposal there was no definitive evidence suggesting that any oxetane adduct of a pyrimidine would fragment following one-electron transfer. One problem in testing this proposal is that the presumed oxetane intermediates in DNA are not stable, but spontaneously open to give the (6−4) photoproducts. In an earlier communication we synthesized model thymine-based

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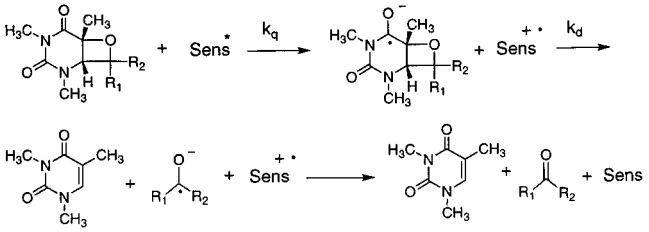
**Scheme 3.** Sensitized Splitting of the Oxetanes



oxetane adducts with benzaldehyde and benzophenone.[45] It was further demonstrated that both sensitized and direct irradiation of these model compounds resulted in their splitting to give the base and the corresponding carbonyl compound (Scheme 3). We asserted that the mechanism of this sensitized splitting was electron transfer from the sensitizer to the oxetane; this argument was based on somewhat indirect evidence. The sensitizers employed were known to be singlet excited-state electron donors, and the observation of fluorescence quenching was consistent with an electron-transfer mechanism.

To determine whether the observed splitting reactions occur via the proposed electron-transfer mechanism, we carried out laser flash photolysis (LFP) investigations. The detection of ion radical intermediates in the sensitized photolysis reactions confirms the proposed electron-transfer mechanism. The radical anion fragmentation is further shown to occur rapidly (<30 ns) following the electron-transfer reaction. This implies that such an enzymatic pathway is capable of showing very high efficiencies.

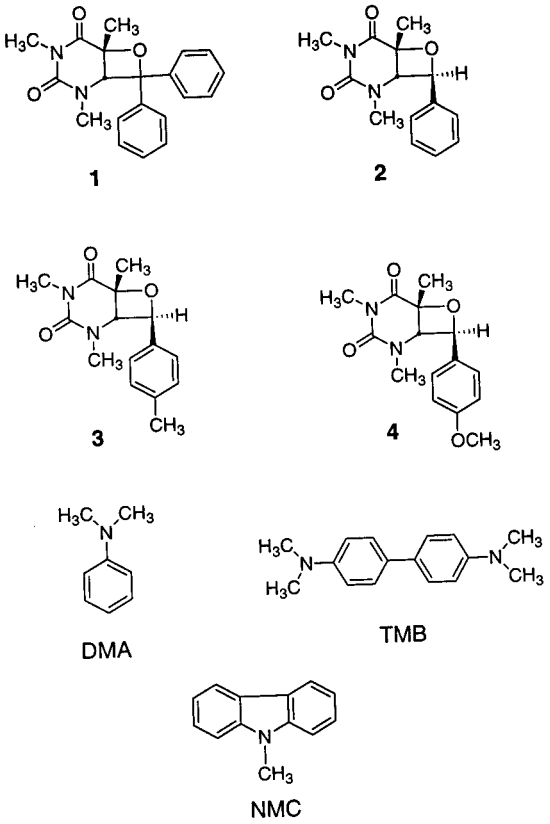
## Results and Discussion

The substrates and sensitizers used in this study are shown in Chart 1. Substrates include the oxetane adducts of 1,3-dimethythymine with benzophenone (**1**), benzaldehyde (**2**), 4-methylbenzaldehyde (**3**), and 4-methoxybenzaldehyde (**4**). The oxetane substrates were prepared by irradiating the carbonyl compound in the presence of 1,3-dimethylthymine (DMT) (Scheme 4). The resulting oxetane adducts are stable, crystalline materials and can be chromatographed over silica gel with no significant decomposition

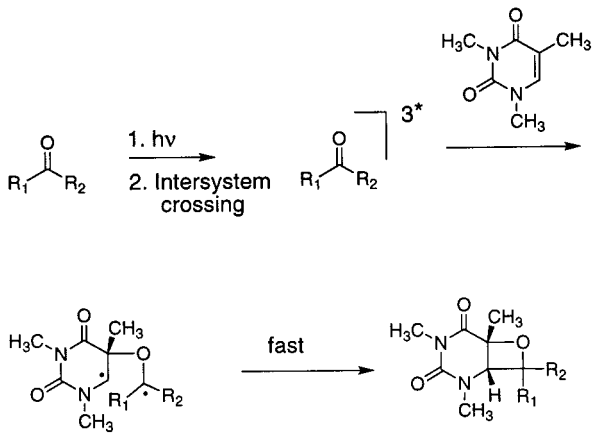
For the aldehyde adducts **2**, **3**, and **4** there are two possible isomers that might form. The *endo* form has the two hydrogens *cis* and the *exo* form has the two hydrogens *trans*. The products **3** and **4** are assigned to the *exo* forms on the basis of the $J_{H-H}$ coupling constants between the C6 and C1′ protons on the cyclobutyl ring. The observed coupling constants are 6.4 Hz for **3** and 6.3 Hz for **4**. We considered the values to be closer to those typical for *trans* 1,2-cyclobutyl protons than for *cis*. The latter tend to be closer to 10 Hz.[46] Both of these are similar to that of **2** (6.2 Hz), whose *exo* stereochemistry was confirmed by X-ray crystallography.[47] This reaction is stereoselective. Analysis of the crude reaction mixtures by $^1$H NMR showed additional minor product peaks that would be consistent with the *endo* diastereomers. However, the low yields precluded their isolation.

The formation of the oxetanes occurs through the excited triplet state of the carbonyl compound. This was confirmed through laser flash photolysis experiments. Pulsed laser excitation (308 nm) of benzophenone yields the well-characterized

**Chart 1.** Substrates and Sensitizers




**Scheme 4.** Synthesis of Oxetanes




spectrum of the latter's excited triplet state.[48] Adding varying concentrations of DMT to the solution leads to the quenching of the triplet spectrum. A pseudo-first-order analysis of these data provides a rate constant of $3.5 \times 10^8$ M$^{-1}$ s$^{-1}$ for the photocycloaddition. LFP experiments carried out with 4-methoxybenzaldehyde gave very similar results. The excited triplet state ($\lambda_{max} = 390$ nm, Figure 1) of this aldehyde is quenched by DMT with a rate constant of $3.2 \times 10^8$ M$^{-1}$ s$^{-1}$.

Paterno−Büchi type cycloadditions are generally considered to proceed via 1,4-diradical intermediates, with the more stable diradical leading to the major products.[49−51] Clearly, the products observed here are consistent with such a mechanism. Addition

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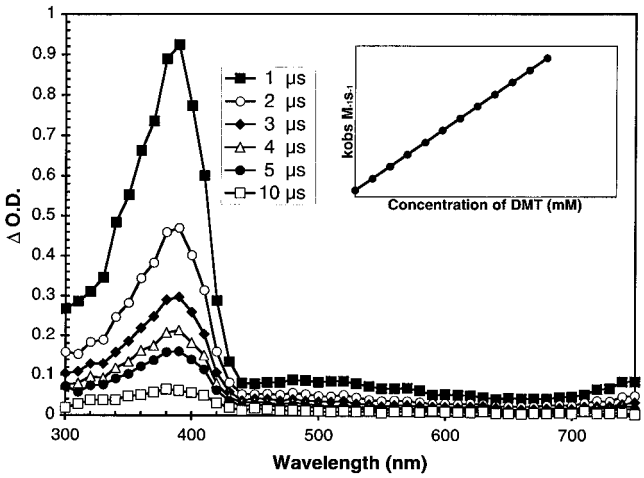


**Figure 1.** Transient absorption spectrum from LFP (308 nm) of 4-methoxybenzaldehyde. Inset: dependence of the decay rate constant on [DMT].

**Table 1.** Fluorescence Quenching and Product Yields from Sensitized Photolysis of Oxetanes **1**−**4**

| | | | | yield (%) | | |
|---|---|---|---|---|---|---|
| oxetane | sensitizer | $k_q \times 10^9$ ($M^{-1}$ $s^{-1}$) | photolysis time (min) | conver. (%) | DMT | carbonyl cmpd |
| 1 | DMA | 17.9(± 1.40) | 15[c] | 5.6 | 69 | 50.4 |
| | TMB | 9.04(± 1.38) | 5[b] | 52.7 | 100 | 98.3 |
| | NMC | 2.02(± 0.41) | 5[b] | 47.9 | 100 | 91.7 |
| 2 | TMB | 22.0(± 0.88) | 10[a] | 80.9 | 86.3 | 78.4 |
| | NMC | 1.56(± 0.12) | 5[b] | 29.7 | 100 | 77.1 |
| 3 | TMB | 12.0(± 0.21) | 10[a] | 31.3 | 96.7 | 100 |
| | NMC | 2.73(± 0.61) | 5[b] | 41.4 | 100 | 91.6 |
| 4 | DMA | 45.7(± 2.50) | 10[a] | 37.5 | 42.9 | 33.3 |
| | TMB | 27.0(± 2.20) | 10[a] | 65.8 | 100.0 | 68.0 |
| | NMC | 3.89(± 0.56) | 5[b] | 22.5 | 95.9 | 93.9 |

[a] Photolysis was conducted using a 150 W Hg−Xe lamp fitted with a 295 nm cutoff filter. [b] Photolysis was conducted using a 355 nm Nd:YAG laser. [c] Photolysis was conducted using a 350 W Xe arc lamp.

of the O atom of the carbonyl to the C-5 position of the thymine would generate a diradical with one of the radical centers stabilized by conjugation with the phenyl ring and the other radical center stabilized by conjugation with N-1 (Scheme 4). We attempted to detect this putative intermediate. The transient spectrum was examined for a sample containing benzophenone and 15 mM DMT, a concentration sufficient to quench the excited triplet state in <20 ns. Under these conditions, no new transient bands were detected in the range 300−800 nm. If such intermediates are formed, they must decay in less than 20 ns.

Product studies show that irradiation of the oxetanes **1**−**4** with the electron donor sensitizers (see Chart 1) results in the net reversal of the cycloaddition process. (Scheme 3) The sensitizers were chosen for their low excited-state oxidation potentials ($E_{ox}$*) and ability to absorb light at wavelengths higher than the oxetanes. These include *N*-methylcarbazole (NMC, $E_{ox}$* = − 2.45 V, $\lambda_{max}$ = 345 nm) *N*,*N*,*N*′,*N*′-tetramethylbenzidine (TMB, $E_{ox}$* = −3.25 V, $\lambda_{max}$ = 345 nm), and *N*,*N*-dimethylaniline (DMA, $E_{ox}$* = −3.32 V, $\lambda_{max}$ = 302 nm).[34] As indicated in Table 1 the oxetane substrates **1**−**4** were subjected to sensitized photolysis with the indicated electron donors. In these experiments, the photolysis light was absorbed by the sensitizers but not the oxetanes. This was assured through the use of appropriate cutoff filters or through the use of monochromatic (355 nm) laser light. The photolysis mixtures were analyzed

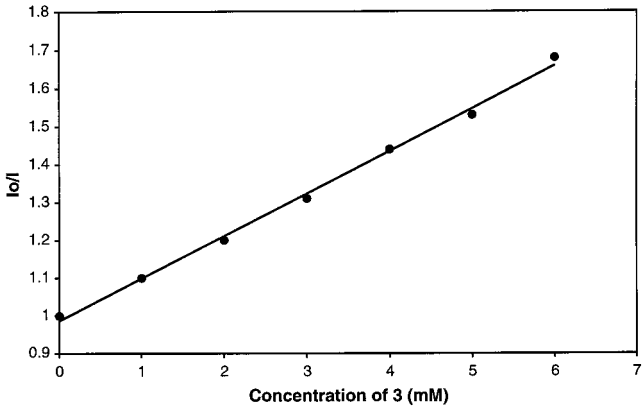


**Figure 2.** Stern−Volmer analysis of the fluorescence-quenching of the sensitizer TMB by oxetane **3**.

using HPLC. As shown in Table 1, good chemical yields of the DMT and the carbonyl compound are detected. Unfortunately, DMT and the corresponding carbonyl compound are not indefinitely stable to the sensitized photolysis conditions. The deviations from quantitative yields are attributed to secondary photoprocesses that have not been completely characterized.

The sensitized photolysis proceeds via the singlet state of the sensitizers (Scheme 3). This was established via fluorescence-quenching experiments. Fluorescence from each of the sensitizers was quenched upon addition of any of the oxetanes. The dependence of quenching on the concentration of the oxetane fit well to the Stern−Volmer relationship as illustrated in Figure 2, which shows the quenching of the sensitizer TMB by oxetane **3**. Using the slopes from the Stern−Volmer fits and literature values for the lifetimes[52] gives the quenching rate constants ($k_q$) listed in Table 1. These values are at or near the diffusion limit, ranging from 1.6 to 27 × $10^9$ $M^{-1}$ $s^{-1}$. We note that the more potent excited-state electron donor, TMB, generally gives the highest $k_q$ values for a given oxetane.

The reduction potentials for the oxetanes are not known. However, earlier studies of pyrimidines and 5,6-dihydropyrimidine derivatives showed that such compounds have reduction potentials ($E_{red}$) in the range −1.8 to −2.0 V vs SCE.[53] It is expected that the pyrimidine residue on the oxetanes should show similar values (if slightly less negative due to the inductive effect of the oxetane group). The observed $k_q$ values are consistent with this expectation, although the limited data set does not make it possible to independently derive $E_{red}$ for these oxetanes. The other parts of the oxetane consist of a benzene, toluene, or anisole ring, and these are expected to be reduced at much more negative potentials.[54]

While the fluorescence quenching experiments are consistent with the proposed electron-transfer mechanism, it was desirable to obtain more direct evidence. This was accomplished via laser flash photolysis (LFP) experiments. For these experiments solutions containing both the sensitizer and a high concentration of the oxetanes were subjected to pulsed laser irradiation at 355 nm (308 nm in the case of DMA) where the sensitizers, but not the oxetanes, absorb. The excited sensitizer is quenched by the oxetane. The mechanism in Scheme 3 predicts that this will initially create the cation radical of the sensitizer along with the anion radical of the oxetane. The latter is predicted to cleave

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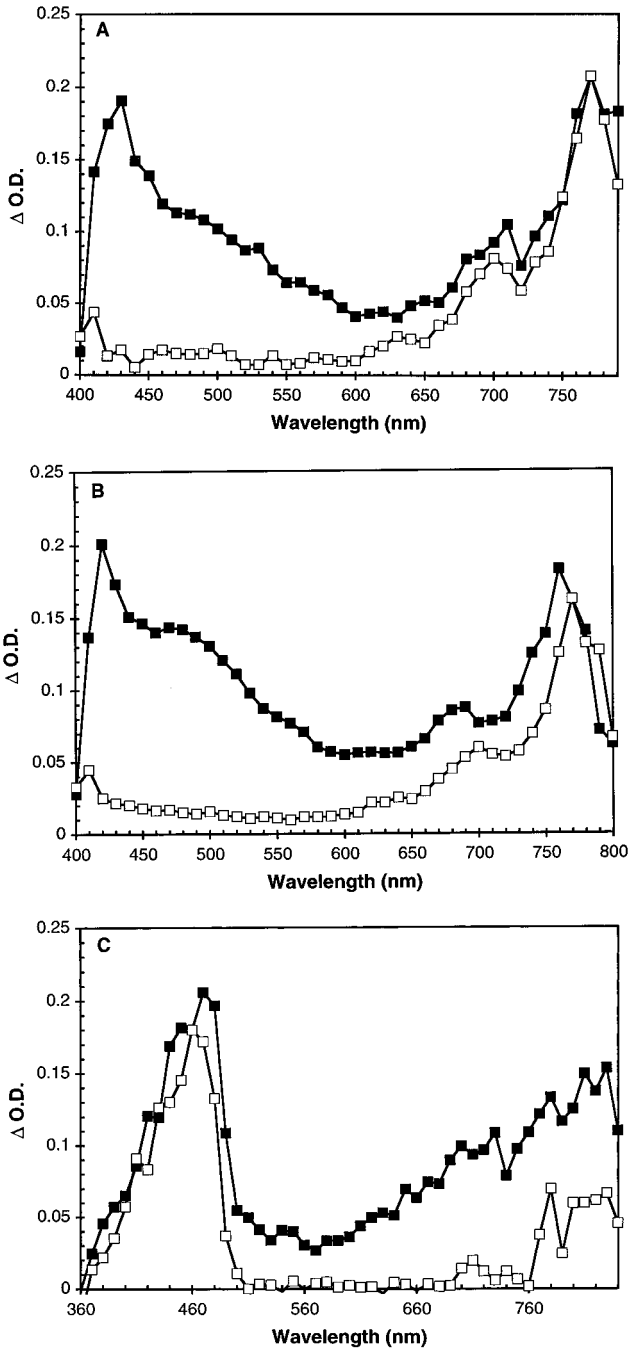


**Figure 3.** Transient spectra generated from LFP of sensitizers in the presence of the oxetanes under $N_2$-purged conditions (filled symbols) and $O_2$-purged conditions (open symbols). (a) benzaldehyde oxetane **2** + NMC; (b) anisaldehyde oxetane **4** + NMC; (c) benzophonone **1**+ TMB; (d) benzophenone oxetane **1**+ DMA.

to give the neutral DMT along with the anion radical of the corresponding aldehyde or ketone. These LFP experiments were carried out on all of the sensitizer/oxetane combinations indicated in Table 1. In each case, the predicted sensitizer cation radical and carbonyl anion radical were detected immediately after the laser pulse.

For example the LFP spectrum taken with NMC and benzaldehyde oxetane **2** in $N_2$ purged $CH_3CN$ is shown in Figure 3a. There are distinct, sharp peaks at 410, 690, and 770 nm, which we assign to the cation radical of NMC. Additionally there is a broader peak at ∼490 nm. The latter is assigned to the anion radical of benzaldehyde, the carbonyl product of fragmentation. This assignment is supported by a previously

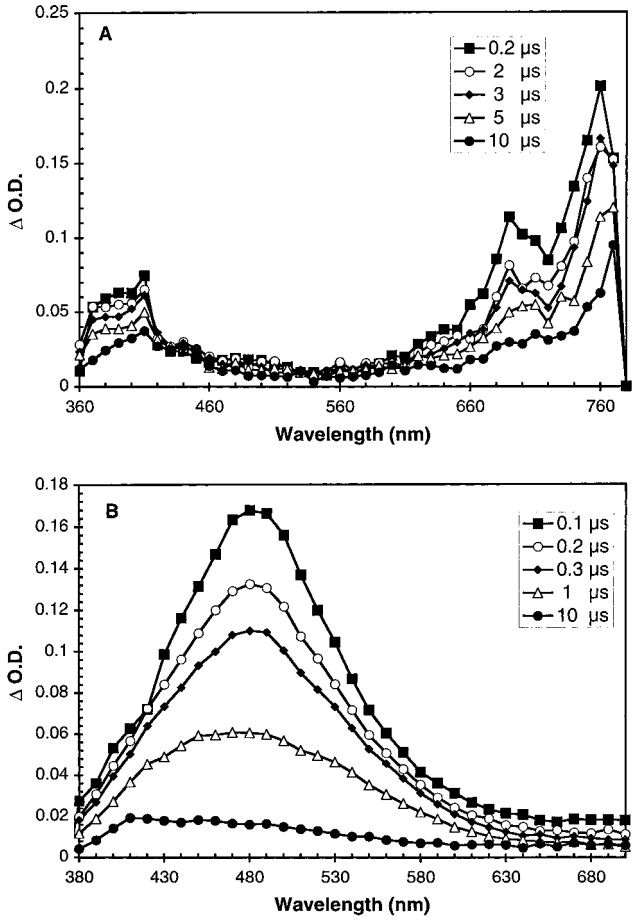


**Figure 4.** Authentic spectra of ion radicals from independent LFP generation. (a) NMC excited at 355 nm in the presence of 1,2-dicyanobenzene (30 mM in $CH_3CN$). (b) *p*-Anisaldehyde excited at 266 nm in the presence of 1,4-diaza-[2,2,2]-bicyclooctane (30 mM in $CH_3CN$).

published[55] spectrum of the benzaldehyde anion radical as well as the observation that it, but not the NMC cation radical peaks, are abolished when the LFP experiment is repeated on an $O_2$-purged solution ($O_2$ reacts rapidly with anion radicals, but is much less reactive toward cation radicals). An independent LFP generation of the NMC cation radical confirms the assignment of the 410, 690, and 770 nm peaks. In this experiment a solution containing NMC and the electron acceptor, 1,2-dicyanobenzene (DCB), was irradiated. The resulting transient spectra (Figure 4a) show the same peaks, as well as some additional absorption below 380 nm due to the anion radical of DCB.[56]

Similar behavior is seen with oxetane **4**. The expected fragments from this compound correspond to the anion radical of anisaldehyde. The transient spectra obtained from LFP of NMC with **4** (Figure 3b) taken 250 ns after the laser pulse shows the aforementioned NMC cation radical peaks at 690 and 770 nm along with a broader peak near 465 nm. The latter corresponds to the anion radical of the anisaldehyde. This assignment was confirmed by generating the same anion radical through an alternative pathway. Specifically, anisaldehyde was subject to LFP (266 nm) in the presence of DABCO, a ground-state electron donor.[57] This also produces a transient species with a similar broad peak at 465 nm (Figure 4b).

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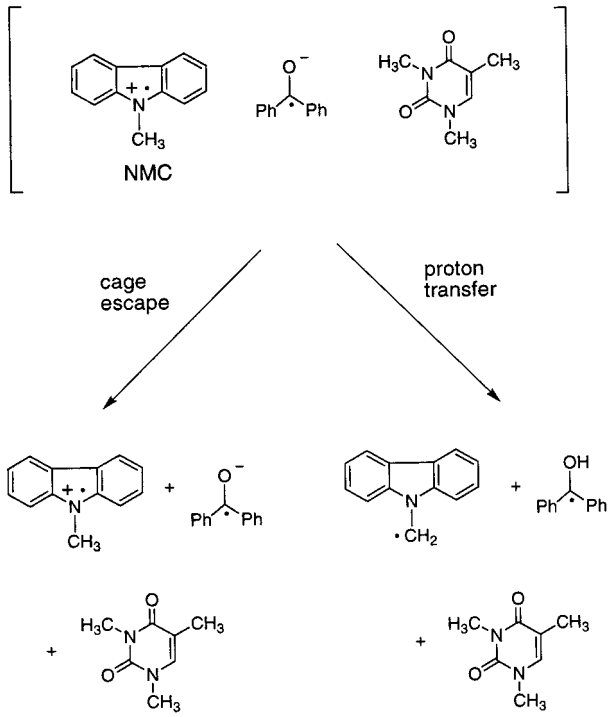
**Scheme 5**




When oxetane **1** is subjected to TMB-sensitized LFP, the anticipated benzophenone anion radical ($\lambda_{max}$ = 680 nm) is observed (Figure 3c). This signal does not appear as a distinct peak, but it is superimposed on the high wavelength absorption bands for the TMB cation radical ($\lambda_{max}$ = 820, 925 nm).[56] As with the other oxetanes, running the LFP experiment on $O_2$ -purged solutions gives transient spectra for only the TMB cation radical. When DMA and NMC are used as the sensitizers, both the benzophenone anion radical[58] as well as its conjugate base (aka diphenylhydroxymethyl radical, $\lambda_{max}$ = 540 nm)[59,60] are detected. This is illustrated in Figure 3d for the case of **1** with DMA. Both of these species are present immediately following the laser pulse. The formation of the diphenylhydroxymethyl radical is attributed to a proton-transfer reaction between the cation radical and the benzophenone anion radical, which competes with solvent cage escape of the latter (Scheme 5). Such behavior is well-known for benzophenone/amine systems.[49,61]

The remaining sensitizer/oxetane pairs show similar behavior. Thus, LFP of TMB in the presence of oxetanes **2**, **3**, or **4** gives spectra that correspond to the sum of the anion radicals of the corresponding aldehyde and TMB cation radical. In these cases, both of the ion radicals have overlapping absorption bands in the region 400−500 nm. Indeed, the transient spectra immediately following the laser pulse are dominated by the TMB cation radical absorption band with its $\lambda_{max}$ at 470 nm.[56] The aldehyde anion radicals do not show distinct peaks. Instead, each appears as an absorption tail that extends from 500 to approximately 580 nm. As with the NMC experiments, this tail is abolished when the experiment is repeated under $O_2$-purged conditions. Likewise, excitation of NMC in the presence of oxetane **3** gives a spectrum that is qualitatively similar to that in Figure 3b, consisting of the tolualdehyde anion radical[55] and the NMC cation radical.

All photoinduced electron transfer-initiated chemical reactions compete with an exothermic back electron-transfer process. Quantum yields approaching unity are realized only when the rate of chemical process is fast relative to the rate of back electron transfer. The detection of the carbonyl anion radicals in the sensitized LFP experiments make it possible to assess the rate of splitting ($k_d$) for the anion radicals of oxetanes **1**−**4**. In each case the carbonyl anion radicals are observed to appear within the excitation laser pulse (6 ns). In some cases (**2** and **4**) there appear to be a ~20 ns rise. However, control experiments suggest that this apparent rise is an artifact due to fluorescence and scattered laser light. We thus set a conservative lower limit on the bond dissociation step as $k_d > 5 \times 10^7$ s$^{-1}$ for all four oxetanes. Thus, these anion radicals split at least 10 times faster than thymine cyclobutane dimer anion radicals.[29] The corresponding rate constants for the naturally occurring oxetanes (Scheme 1) are not known. However, given the apparent insensitivity of $k_d$ to changes in the structure of the carbonyl compound, it is not unreasonable to assume that the natural oxetanes cleave with a similar rate constant.

## Conclusions

The results presented above provide further support for the feasibility of the oxetane reversal mechanism originally postulated by Kim et al.[44] First, it appears that cycloreversion is a general behavior of pyrimidine oxetane anion radicals. The reactions proceed cleanly with a number of substrates and give little, if any, primary side products. Second, the cycloreversion reactions are extremely rapid, occurring in nanosecond or subnanosecond time scales. Thus, this pathway meets one crucial criterion for efficient photoinduced electron transfer-initiated reactions: the chemical step must be very rapid to compete with return electron transfer. The electron-transfer mechanism is also attractive because it accounts for the sequence homology between the (6−4) and CPD photolyases as well as the fact that the active forms of both photolyases have a reduced flavin chromophore.

Of course as with any model study, these results can only test the feasibility of one step in the overall mechanism. The anion radical mechanism also requires that the (6−4) photoproduct, upon binding to photolyase, be converted into its oxetane isomer. Recent calculations by Heelis and Liu[62] have suggested that the energy gap between the (6−4) photoproduct and its oxetane isomer might be too large to be overcome by binding to the substrate. However, Zhao et al.[20] have pointed out that these same calculations overestimate the energy gap for the analogous thietane rings. Second, the calculations were carried out on free bases. Constraints of the DNA backbone have been argued to further destabilize the (6−4) photoproduct relative to the oxetane.

## Experimental Section

***Z*-2,4,6-Trimethyl-8,8-diphenyl-7-oxa-2,4-diazabicyclo[4.2.0]octane-3,5-dione (1).**[63] Benzophenone (1.46 g, $8 \times 10^{-3}$ mol) was added to 1,3-dimethylthymine (0.616 g, $4 \times 10^{-3}$ mol) in $CH_3CN$ (40 mL). This solution was placed in a quartz tube, sealed, purged for 30 min with $N_2$, and irradiated using the unfiltered output of a 150 W Hg−Xe lamp for 10 h. The solvent was removed by rotary evaporation, and the oxetane was purified by elution over silica gel using 20:80 EtOAc/hexane. The fractions containing the oxetane were collected and pooled,

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and the solvent was removed by rotary evaporation. Yield 0.55 g, 41%: mp 147−148 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.32 (m, 10H), 4.59 (s, 1H), 3.12 (s, 3H), 2.86 (s, 3H), 1.73 (s, 3H); $^{13}$C NMR ($CDCl_3$, 55.1 MHz) $\delta$ 169.84, 151.61, 144.16, 138.74, 128.58, 128.26, 128.00, 127.88, 125.54, 124.91, 91.62, 66.62, 35.69, 27.31, 23.92. HRMS (EI) calcd for ($M^+$) $C_{20}H_{20}O_3N_2$: 336.1474, found: 336.1469.

**(6*Z*,8*Z*)-2,4,6-Trimethyl-8-phenyl-7-oxa-2,4-diazabicyclo[4.2.0]-octane-3,5-dione (2).** Benzaldehyde (0.848 g, $8 \times 10^{-3}$ mol) was added to 1,3-dimethylthymine (0.616 g, $4 \times 10^{-3}$ mol) in $CH_3CN$ (40 mL). This solution was placed in a quartz tube, sealed, purged for 30 min with $N_2$, and irradiated using the unfiltered output of a 150 W Hg−Xe lamp for 10 h. The solvent was removed by rotary evaporation, and the oxetane was purified by elution over silica gel using 20:80 EtOAc/hexane. The fractions containing the oxetane were collected and pooled, and the solvent was removed by rotary evaporation. Yield 0.33 g, 32%: mp 128−129 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.36 (bs, 5H), 5.43 (d, $J = 6.2$ Hz, 1H), 3.85 (d, $J = 6.2$ Hz, 1H), 3.27 (s, 3H), 2.93 (s, 3H), 1.75 (s, 3H); $^{13}$C NMR ($CDCl_3$, 55.1 MHz) $\delta$ 169.73, 151.70, 139.13, 133.27, 129.94, 128.87, 127.93, 124.99, 86.46, 76.34, 64.73, 34.51, 28.13, 23.83; IR 1712, 1681 cm$^{-1}$. HRMS (EI) calcd for ($M^+$) $C_{14}H_{16}O_3N_2$: 260.1160, found: 260.1153.

**(6*Z*,8*Z*)-2,4,6-Trimethyl-8-(4-methylphenyl)-7-oxa-2,4-diazabicyclo-[4.2.0]octane-3,5-dione (3).** *p*-Tolualdehyde (0.961 g, $8 \times 10^{-3}$ mol) was added to 1,3-dimethylthymine (0.616 g, $4 \times 10^{-3}$ mol) in $CH_3CN$ (40 mL). This solution was placed in a quartz tube, sealed, purged for 30 min with $N_2$, and irradiated using the unfiltered output of a 150 W Hg−Xe lamp for 10 h. The solvent was removed by rotary evaporation, and the oxetane was purified by elution over silica gel using 20:80 EtOAc/hexane. The fractions containing the oxetane were collected and pooled, and the solvent was removed by rotary evaporation. Yield 0.75 g, 68%: mp 88−90 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.25 (m, 4H), 5.43 (d, $J = 6.3$ Hz, 1H), 3.87 (d, $J = 6.3$ Hz, 1H), 3.32 (s, 3H), 2.97 (s, 3H), 2.37 (s, 3H), 1.79 (s, 3H); $^{13}$C NMR ($CDCl_3$, 55.1 MHz) $\delta$ 169.95, 138.93, 136.25, 129.63, 125.21, 86.58, 64.88, 34.59, 28.25, 23.94, 21.22. HRMS (EI) calcd for $C_{15}H_{18}O_3N_2$: 274.1317, found 274.1362.

**(6*Z*,8*Z*)-2,4,6-Trimethyl-8-(4-methoxyphenyl)-7-oxa-2,4-diazabicyclo[4.2.0]octane-3,5-dione (4).** *p*-Anisaldehyde (1.09 g, $8 \times 10^{-3}$ mol) was added to 1,3-dimethylthymine (0.616 g, $4 \times 10^{-3}$ mol) in $CH_3CN$ (40 mL). This solution was placed in a quartz tube, sealed, purged for 30 min with $N_2$, and irradiated using the unfiltered output of a 150 W Hg−Xe lamp for 10 h. The solvent was removed by rotary evaporation, and the oxetane was purified by elution over silica gel using 20:80 EtOAc/hexane. The fractions containing the oxetane were collected and pooled, and the solvent was removed by rotary evaporation. Yield 0.89 g, 77%: mp 129−130 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.30 (d, $J = 8.7$ Hz, 2H), 6.92 (d, $J = 8.7$ Hz, 2H), 5.38 (d, $J = 6.4$ Hz, 1H), 3.85 (d, $J = 6.4$ Hz, 1H), 3.81 (s, 3H), 3.31 (s, 3H), 2.94 (s, 3H), 1.77 (s, 3H); $^{13}$C NMR ($CDCl_3$, 55.1 MHz) $\delta$ 170.00, 160.27, 154.00, 131.33, 126.87, 114.39, 86.45, 65.12, 55.36, 34.59, 28.26, 23.98. HRMS (EI) calcd for $C_{15}H_{18}O_4N_2$: 290.1266, found: 290.1256.

**Photoproduct Analysis.** Two different photolysis methods were used in this study, lamp photolysis and laser photolysis. Lamp photolysis: A solution of the oxetane in $CH_3CN$ (2.5 mL, 5 mM − 10 mM) is placed in a cuvette fitted with a stir-bar. To it, a sufficient amount of the respective sensitizer is added until the sensitizer's absorbance is >3 at 300 nm. This ensures that the vast majority of the light is absorbed by the sensitizer, and not directly by the oxetane. The cuvette is then sealed, purged with $N_2$ for 20 min, and irradiated, while stirring using the output of a 150 W Hg−Xe lamp fitted with a 295 nm cutoff filter. Aliquots (75 $\mu$L) are taken at various time points and analyzed by HPLC. The products and reactants were identified by co-injections and separated, and their peak areas quantified. From these data, the product yields and the percentage of reaction progress can be determined. Laser photolysis: A solution of the oxetane in $CH_3CN$ (2.5 mL, 2 mM) is prepared in a quartz cuvette to which sufficient amount of the sensitizer is added to give an absorbance value >2 at 355 nm. The cuvette is then sealed and purged with $N_2$ for 20 min, and irradiated with the output of a 355 nm Nd:YAG laser with a laser power of 15 mW and a frequency of 10 Hz. After irradiating for 5 min, 20 $\mu$L aliquots of the photolyzed solutions were analyzed by HPLC using a normal phase silica column. The peaks were identified and their areas quantified to determine the yield of the products in the photolysis. High performance liquid chromatography (HPLC) was performed on a Gradient HPLC System with an UV/vis absorption detector set at 254 nm. Analytical work was performed with a reverse phase 5 $\mu$m C-18 column or a normal phase 5 $\mu$m silica column. The solvent system was $H_2O/CH_3CN$ for reverse phase and hexane/*i*-PrOH for normal phase. The flow rate in all cases was 1 mL/min.

**Fluorescence Quenching Experiments.** The rate constant for quenching ($k_q$) of the sensitizer by the DMT−oxetanes was determined through Stern−Volmer analyses for each oxetane/sensitizer. To do so, a stock solution of the fluorescent sensitizer was prepared by sonicating the latter (1−3 mg) in spectroscopic grade $CH_3CN$ (100 mL) for 30 min. This process resulted in a sensitizer concentration of $\sim 10^{-2}$ M. This solution (2.5 mL) was placed in a quartz cuvette, sealed with a septum, and purged with $N_2$ for 20 min. A stock solution of the respective oxetane (200 mM) wass prepared by dissolving the appropriate amount in spectroscopic grade $CH_3CN$, and sonicating the resulting solution for 30 min. Aliquots (12.5 $\mu$L) of the oxetane (quencher) solution were injected into the sealed cuvette containing the sensitizer. This resulted in the increase of the oxetane concentration in the cuvette by 1 mM steps. The fluorescence scan of the sensitizer was recorded at each step (0−6 mM). The excitation wavelength of the sensitizer (310 nm) was chosen to ensure that none of the light was absorbed by the oxetane. The results of the fluorescence scans were analyzed by the Stern−Volmer equation, from which the $k_q$ was obtained.

**Laser Flash Photolysis (LFP) Experiments. General Procedures.** Two different laser flash photolysis (LFP) instruments were used in this study. LFP experiments on the dimethylaniline (DMA)-sensitized splitting of DMT−benzophenone and DMT−anisaldehyde oxetanes were performed using an excimer laser with XeCl as the source gas, emitting a 10 ns pulse of 308 nm light. LFP experiments on the *N*-methylcarbazole (NMC)- and *N*,*N*,*N*`,*N*`-tetramethylbenzidine (TMB)-sensitized splitting of the oxetanes were carried out using a Nd:YAG laser, emitting a 10 ns pulse of 355 nm light. The probe beam was supplied by a 350 W Xe continuous wave source. The light transmitted through the sample was detected with a photomultiplier tube.

The procedure used for the LFP experiments performed using the 355 nm Nd:YAG laser is elaborated first followed by the procedure used for the LFP experiments using the 308 nm XeCl excimer laser. Solutions of the sensitizer in $CH_3CN$ were prepared in a quartz cuvette by dissolving an appropriate amount of the sensitizer to have an absorbance of between 1.6 and 2.5 at 355 nm. To this solution a sufficient amount of the oxetane was added to quench at least 50% of the fluorescence of the sensitizer. The quartz cuvette was equipped with a magnetic stirrer, sealed, and purged with $N_2$ for 20 min. This sample was then photolyzed with the 355 nm Nd:YAG laser and the intermediates detected by transient absorbance. After the completion of the LFP experiment, the sample was then purged with $O_2$ for 15 min and the LFP experiment repeated. The LFP experiments at 308 nm were performed by dissolving the oxetane in $CH_3CN$ and adding sufficient amount of the sensitizer, DMA, to give an absorbance of 1.8 absorbance units. The quartz cuvette was equipped with a magnetic stirrer, sealed, and purged with $N_2$ for 20 min. This sample was then photolyzed with the 308 nm XeCl excimer laser and the intermediates detected by transient absorbance. After the completion of the LFP experiment, the sample was then purged with $O_2$ for 15 min and the LFP experiment repeated.

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