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On the Mechanism of Reaction of Radicals with Tirapazamine

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Abstract: Ketyl radicals produced by photolysis of ketones or di-*tert*-butyl peroxide (DTBP) in alcohol solvents react rapidly with tirapazamine (TPZ). The acetone ketyl radical (ACOH) reacts with TPZ with an absolute second-order rate constant of $(9.7 \pm 0.4) \times 10^8$ M^{-1} s^{-1}. The reaction kinetics can be followed by monitoring the bleaching of TPZ absorption at 475 nm or the formation of a reaction product which absorbs at 320 and 410 nm. The ACOD radical reacts with TPZ in 2-propanol-OD with an absolute rate constant of $(6.7 \pm 0.5) \times 10^8$ M^{-1} s^{-1}, corresponding to a kinetic isotope effect (KIE) of 1.4. Deuteration of the radical on carbon (ACOH-d_6) retards the reaction of the radical with TPZ even further (absolute rate constant $= (4.8 \pm 0.04) \times 10^8$ M^{-1} s^{-1}). This result corresponds to a KIE of 2.0. Radicals derived from dioxane and diisopropyl ether by flash photolysis of DTBP in ethereal solvent react with TPZ more slowly than do ketyl radicals. It is concluded that ketyl radicals react, in part, with TPZ in organic solvents by transfer of a hydrogen atom from the OH and CH_3 groups of the ketyl radical to the oxygen atom at the N4 position of TPZ to form acetone or acetone enol and a radical derivative of TPZ (TPZH). The latter species absorbs at 320 and 405 nm, has a lifetime of hundreds of microseconds in alcohol solvents, and decays by disproportionation to form TPZ and a reduced heterocycle. The reduced heterocycle eventually forms a desoxytirapazamine by a polar mechanism. The results are supported by density functional theory calculations. It is proposed that dioxanyl radical will also react, in part, with TPZ by transfer of a hydrogen atom from the carbon adjacent to the radical center to the oxygen atom at the N4 position of TPZ. This produces the enol ether and the previously mentioned TPZH radical. It is further posited that ether radicals react a bit more slowly than ketyl radicals because they lack the second mode of hydrogen transfer (from the OH group) that is present in the ACOH radical. Our data are permissive of the possibility that ether radicals add to TPZ at a rate that is competitive with β-hydrogen atom transfer.

1. Introduction

Tirapazamine (TPZ) is currently in clinical trials for the treatment of tumors,[1] and its mechanism of action is something of a paradox. TPZ promotes free radical oxidative cleavage of DNA, yet it is more active in the *absence* than in the *presence* of molecular oxygen.[2] To explain these observations, it has been proposed that TPZ has a dual mechanistic role.[3−5] First, in a bioreductive process, TPZ generates radicals, perhaps hydroxyl radical or a drug-derived benzotriazine radical (BTA, Scheme 1), which then reacts with a DNA sugar moiety to produce DNA-centered radicals. Molecular oxygen quenches this process by accepting an electron from the TPZ radical anion, hence the greater activity of the drug in hypoxic environments. In a second step, DNA-centered sugar radicals react with TPZ to ultimately affect their oxidation. Thus, it is the drug, and not molecular oxygen, which oxygenates DNA-centered radicals.

Gates, Greenberg, and co-workers[3,4] have proposed that DNA-centered radicals add to TPZ to form an adduct which fragments to form desoxytirapazamine (dTPZ) and a DNA oxy radical (Scheme 2).

As shown in Scheme 3, a C1−nucleotide radical (C1N) can be generated by photolysis of a *tert*-butyl ketone precursor (K). When this radical is generated in a single-stranded nucleic acid,[4] it reacts with TPZ with a rate constant of 2×10^8 M^{-1} s^{-1}. When the radical is generated in double-stranded DNA,[4] the rate constant falls to 4×10^6 M^{-1} s^{-1}. Gates, Greenberg, and

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(1) (a) Kelson, A. B.; McNamara, J. P.; Pandey, A.; Ryan, K. J.; Dorie, M. J.; McAfee, P. A.; Menke, D. R.; Brown, J. M.; Tracey, M. *Anti-Cancer Drug Des.* **1998**, *13*, 575. (b) Lee, D. J.; Trotti, A.; Spencer, S.; Rostock, R.; Fisher, C.; von Romeling, R.; Harvey, E.; Groves, E. *Int. J. Radiat. Oncol., Biol., Phys.* **1998**, *4*, 811. (c) Del Rowe, J.; Scott, C.; Werner-Wasik, M.; Bahary, J. P.; Curran, W. J.; Urtasun, R. C.; Fisher, B. *J. Clin. Oncol.* **2000**, *18*, 1254. (d) von Pavel, J.; et al. *J. Clin. Oncol.* **2000**, *18*, *37*, 1487.

(2) Luderoute, K.; Wardman, P.; Rauth, A. M. *Biochem. Pharmacol.* **1998**, *37*, 1487.

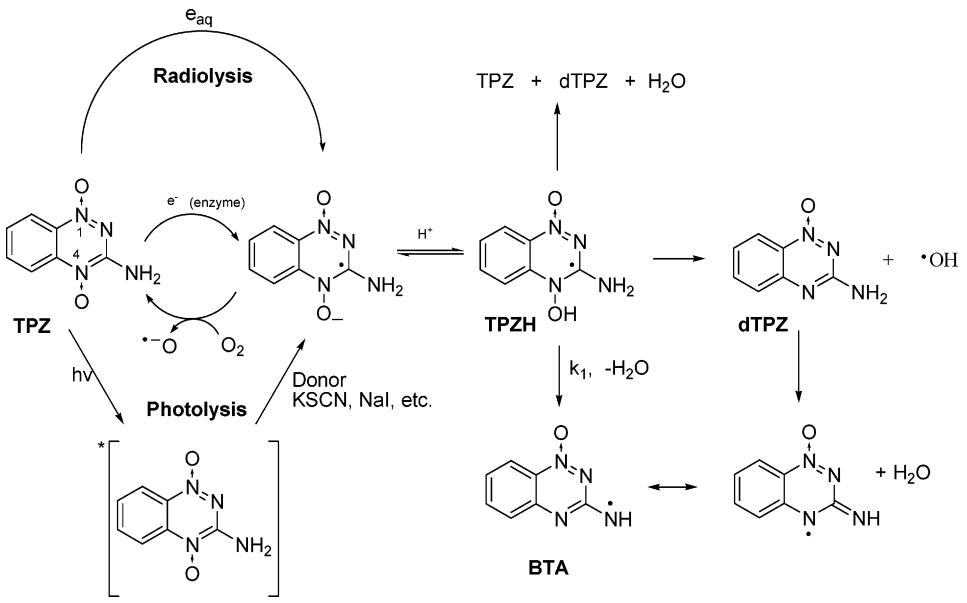
(3) (a) Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1996**, *118*, 3380.

(4) (a) Hwang, J. T.; Greenberg, M. M.; Fuchs, T.; Gates, K. S. *Biochemistry* **1999**, *38*, 14248. (b) Daniels, J. S.; Gates, K. S.; Tronche, C.; Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1254.

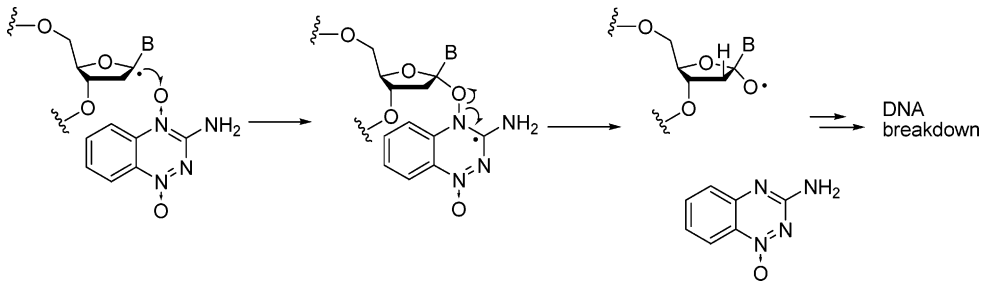
(5) (a) Anderson, R. F.; Shinde, S. S.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 748. (b) Shinde, S. S.; Anderson, R. F.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *J. Am. Chem. Soc.* **2004**, *126*, 7865. (c) Anderson, R. F.; Harris, T. A.; Hay, M. P.; Denny, W. A. *Chem. Res. Toxicol.* **2003**, *16*, 1477.

10.1021/ja0647405 CCC: $37.00

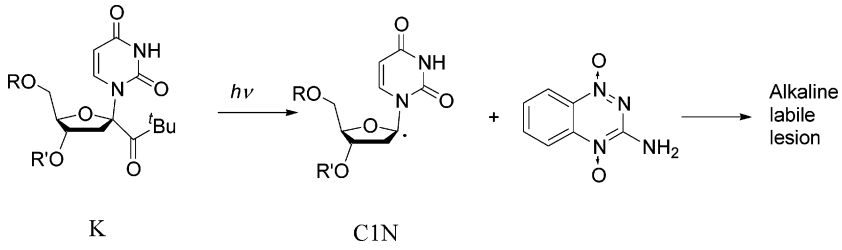
Scheme 1



Scheme 2



Scheme 3



co-workers concluded that the C1N radical adds to TPZ in aqueous solvent as shown in Scheme 2. This conclusion was buttressed by isotopic labeling experiments. Reaction of ^{16}O-labeled TPZ with the C1N radical in $H_2{}^{18}O$ formed an oxidized nucleotide product that was 83 ± 2% enriched in ^{16}O, as predicted by Scheme 2.[4b]

Anderson et al. reported[5] that a deoxyribose radical reacts with TPZ in water with a rate constant of 2.5×10^9 M^{-1} s^{-1} and proposed a different reaction mechanism on the basis of their kinetic data. They proposed that the sugar radical adds to the nitrogen atom to form an N4 adduct. It was postulated further that the N4 adduct fragments to form TPZH (Scheme 4), a radical intermediate also invoked in the initial bioreductive activation step of TPZ (Scheme 1). This radical is proposed to form either hydroxyl radical (Daniels and Gates[3]) or benzotriazinyl radical (BTA, Anderson et al.[5]). If these radicals can abstract hydrogen from a sugar moiety to form a DNA-centered radical, then the nucleoside oxidation can proceed by a chain reaction.

The cleavage reaction depicted in Scheme 4 is not possible with nucleotides (which lack the required hydroxyl hydrogen atom); thus, this cannot be the mechanism of TPZ induced DNA damage *in vivo*.

In this work, we report laser flash photolysis (LFP) data on the reactions of simple organic radicals with TPZ. We will conclude that, in organic solvents, ketyl radicals react rapidly with TPZ by a hydrogen atom transfer reaction, and that ether-derived radicals react with TPZ in organic solvents at a somewhat slower rate than do ketyl radicals. Our data do not rule out the possibility that ketyl and ether-derived radicals can add to TPZ at a rate competitive with β-hydrogen transfer.

Scheme 4

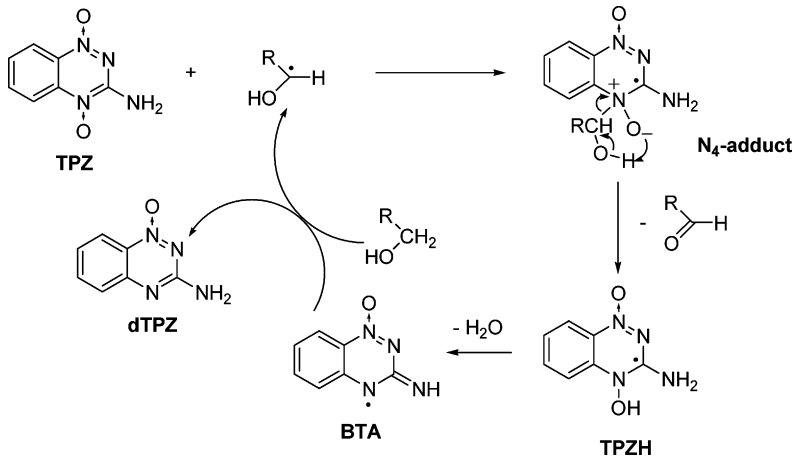
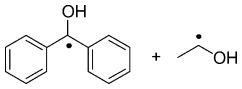
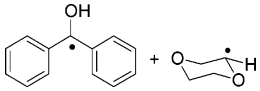
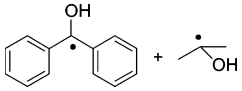
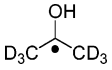


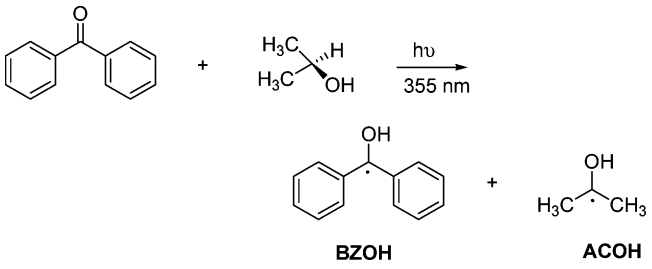
Table 1. Rate Constants for the Reaction of Benzophenone Ketyl and/or Acetone Ketyl Radicals with TPZ[a]

reactive radicals	generation	solvent	rate constant ($\times 10^8$ M^{-1} s^{-1}) 550/545 nm decay	475 nm bleaching	410/405 nm growth	350 nm growth	320 nm decay
	benzophenone sensitization	ethanol	3.2 ± 0.4	6.4 ± 0.4			
	benzophenone sensitization	dioxane	4.0 ± 0.2	4.5 ± 0.4			
	benzophenone sensitization	2-propanol	1.2 ± 0.4	6.5 ± 0.3	5.2 ± 0.2		1.4 ± 0.1
	*tert*-butoxy radical	2-propanol		10.1 ± 1.0	10.0 ± 1.0	8.9 ± 1.0	
	acetone sensitization	2-propanol		11.0 ± 1.0	9.7 ± 0.4		
	acetone sensitization	2-propanol-OD			6.7 ± 1.0		
	acetone sensitization	2-propanol			4.8 ± 1.2		

[a] Note that 550/545 and 320 nm are absorptions due to BZOH radical, 475 nm is absorption of TPZ, and 410/405 and 350 nm are absorptions of the newly generated transient species.

2. Results

2.1. Benzophenone Ketyl Radical. Photolysis of benzophenone in neat 2-propanol rapidly and efficiently forms benzophenone ketyl (BZOH) and acetone ketyl (ACOH) radicals.[6]



LFP (355 nm) of benzophenone in 2-propanol gives the familiar spectrum of BZOH with $\lambda_{max} = 320$ and 545 nm (Supporting Information (SI), Figure S1). The decay of transient absorption recorded at 550 nm can be fit to a single-exponential function and analyzed to yield an observed pseudo-first-order constant of decay, k_{obs}. A plot of k_{obs} (measured at 550 nm) versus [TPZ] is shown in Figure 1. The plot is linear, with a slope of $(1.2 \pm 0.4) \times 10^8$ M^{-1} s^{-1}, which corresponds to the absolute rate constant of reaction of BZOH with TPZ in 2-propanol.

It is interesting to note that the decay of BZOH (320, 545 nm) is accompanied by bleaching at 470 nm (Figure 2), a wavelength where TPZ absorbs strongly (SI, Figure S2) in 2-propanol. As shown in Figure 3, the bleaching of TPZ absorption proceeds exponentially. There is also a small growth at 410 nm. The observed pseudo-first-order decay rate constants (k_{obs}) recorded at 410 and 470 nm are similar, 1.1×10^5 and 1.8×10^5 M^{-1} s^{-1}, respectively (Figure 3). Plots of k_{obs} versus [TPZ] measured at 410 and 470 nm are linear, with the absolute rate constants given in Table 1 (Figure 4).

Kinetics monitored at 545 or 550 nm report the decay of BZOH, which is dominated by its reaction with TPZ. The

(6) Porter, G.; Wilkinson, F. W. *Trans. Faraday Soc.* **1963**, *59*, 1686.

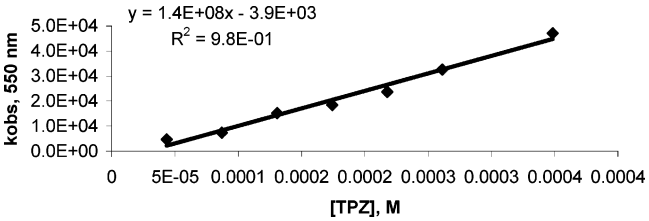


Figure 1. Plot of the observed rate constant of decay of transient absorbance at 550 nm versus [TPZ] produced by 355 nm LFP of benzophenone in 2-propanol containing TPZ.

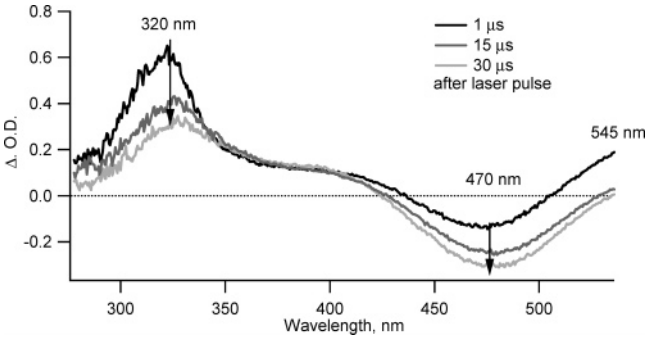


Figure 2. Transient spectrum produced by LFP of 8 mM benzophenone in 2-propanol, in the presence of 0.2 mM TPZ, at 355 nm.

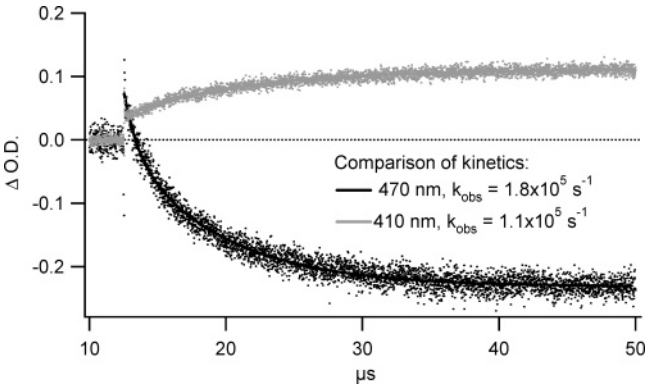


Figure 3. Kinetic curves, monitored at 470 and 410 nm, of transient species produced by 355 nm LFP of 7 mM benzophenone in 2-propanol containing 0.2 mM TPZ.

consumption of TPZ, recorded at 470 nm, proceeds by reaction of TPZ with *both* the BZOH and "invisible" ACOH radicals; thus, the second-order rate constants (the slopes of Figure 1 and 4) need not be identical. One expects that the rate constant of TPZ consumption will exceed that of BZOH, because both BZOH and ACOH react with TPZ. Indeed this is the case (Table 1). The data indicate that the consumption of TPZ produces a new species which absorbs at 410 nm and has a lifetime that is in excess of hundreds of microseconds.

Similar results were obtained in ethanol. In this solvent, BZOH and acetaldehyde ketyl (AAOH) are present. In ethanol, we find that the rate constant of reaction of BZOH with TPZ is more than twice that observed in 2-propanol. We also find that the rate constant of reaction of BZOH with TPZ is half the rate constant of consumption of TPZ (SI, Figures S3 and S4) in ethanol.

In methanol solvent, the absolute rate constant of reaction of the BZOH radical with TPZ is $(7.7 \pm 0.2) \times 10^8$ M^{-1} s^{-1}, and in the more acidic solvent 2,2,2-trifluoroethanol, the rate constant of reaction of BZOH with TPZ is $(1.4 \pm 0.2) \times 10^9$ M^{-1} s^{-1}.

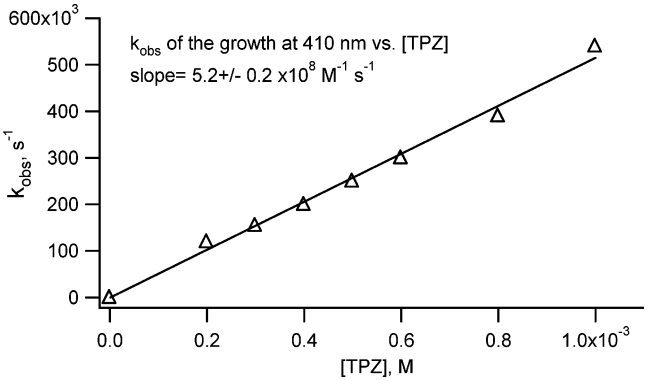
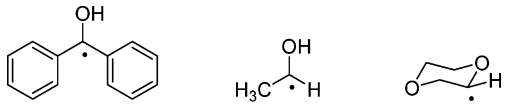


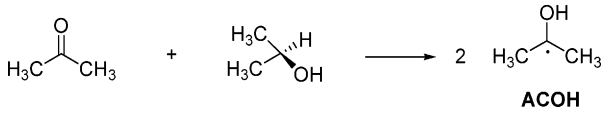
Figure 4. Plot of the observed rate constant of the growth of the transient absorbance at 410 nm versus [TPZ] produced by 355 nm LFP of benzophenone in 2-propanol containing TPZ.

In fact, the more acidic the solvent, the greater the reactivity of BZOH radical with TPZ (Figure S5).



LFP of benzophenone in dioxane produces BZOH and dioxanyl radical (DX).[7] In this system (Table 1), the rate constant of reaction of BZOH with TPZ is equal, within experimental error, to the rate constant of consumption of TPZ. This result implies that the BZOH radical reacts more rapidly with TPZ than does the dioxanyl radical.

2.2. Acetone Ketyl Radical. The benzophenone ketyl system suffers from two complications. First, two different radicals are simultaneously generated, and several UV−vis-active species are derived from its reactions. This leads to overlapping transient spectra and a complex kinetic analysis. Both of these concerns are alleviated by LFP of acetone in 2-propanol, which produces two UV−vis-inactive ACOH ketyl radicals.[7]



A 7.5% (v:v) solution of acetone in 2-propanol containing 0.1 mM TPZ was studied by LFP (308 nm, Figure 5) techniques. The absorption of TPZ at 475 nm is bleached on the microsecond time scale, and new absorption grows at 350 and 405 nm on the same time scale (SI, Figure S6). Plots of k_{obs} versus [TPZ], recorded at both 405 and 475 nm, are linear and have the same slope, within experimental error (SI, Figures S7 and S8, and Table 1).

In 2-propanol-OD, the ACOH radical is again formed, but it should undergo rapid isotopic exchange with solvent to form the ACOD radical. The ACOD radical reacts with TPZ with an absolute rate constant of $(6.7 \pm 1.0) \times 10^8$ M^{-1} s^{-1}, corresponding to a kinetic isotope effect (KIE) of 1.4. LFP of acetone-d_6 in 2-propanol-d_7 produces a different isotopically modified radical, ACOH-d_6. Deuteration of the radical on carbon (ACOH-d_6) retards the reaction of the radical with TPZ even further

(7) See: Charney, D. R.; Dalton, J. C.; Hautala, R. R.; Snyder, J. J.; Turro, N. J. *J. Am. Chem. Soc.* **1974**, *96*, 1407 and references therein.

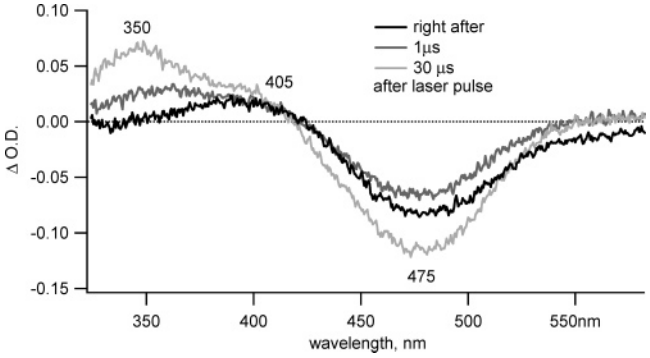
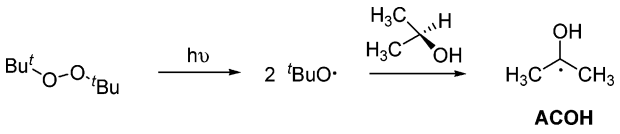


Figure 5. Transient spectra produced upon 308 nm LFP of 7.5% acetone in 2-propanol, in the presence of 0.1 mM TPZ.

(absolute rate constant = $(4.8 \pm 0.2) \times 10^8$ M^{-1} s^{-1}). This result corresponds to a KIE of 2.0 (SI, Figure S9).

ACOD **ACOH-*d6***

LFP (308 nm) of a 17% (v:v) solution of di-*tert*-butyl peroxide (DTBP) in 2-propanol efficiently produces ACOH.[8]



ACOH

In the presence of 0.2 mM TPZ, the absorption of TPZ is bleached and new absorption bands at 350 and 400 nm are formed within 15 μs of the laser pulse (Figure 6). The absorption at 350 nm decays in a millisecond, the absorption at 400 shifts to 410 nm, and the absorption of TPZ recovers slightly over the same time scale (SI, Figures S10 and S11). Plots of changes in absorbance recorded at 350 (growth), 405 (decay), and 475 nm (decay) versus [TPZ] are linear and have the same slopes within experimental error (Table 1).

2.3. Radicals Derived from Ethers and Amines. LFP (308 nm) of 17% DTBP in dioxane produces DX.[9] Ethereal radicals will also be formed in trioxane and diisopropyl ether in analogous experiments. Figure S11 (Supporting Information) demonstrates that LFP (308 nm) of 17% DTBP in dioxane containing 0.2 mM TPZ (the same conditions used with 2-propanol) fails to generate transient absorption or bleaching of TPZ.

Similar results were obtained in other ether solvents. Only weak transient spectra are obtained upon LFP (308 nm) of 17% DTBP and 2 mM TPZ in neat diisopropyl ether, in neat tetrahydrofuran, in 1 M 1,3,5-trioxane in acetonitrile (SI, Figure S12), and in triethylamine (SI, Figure S13). It was not possible to perform accurate kinetic studies in these solvents. We conclude that the transient signals obtained by LFP of peroxide and ether are weaker than those obtained with acetone or DTBP in 2-propanol because ketyl radicals react more rapidly with TPZ than do ether-derived radicals.

(8) Paul, H.; Small, R. D., Jr.; Scaiano, J. C. *J. Am. Chem. Soc.* **1979**, *101*, 3780.
(9) Malatesta, V.; Scaiano, J. C. *J. Org. Chem.* **1982**, *47*, 1455.

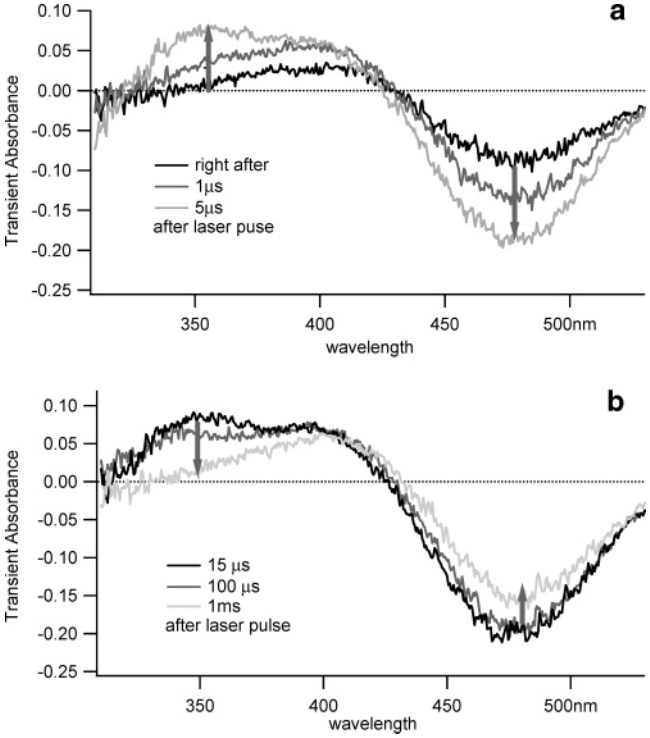


Figure 6. (a) Transient spectra produced by 308 nm LFP of 17% *tert*-butyl peroxide in 2-propanol, in the presence of 0.2 mM TPZ. There is no transient absorption in the 480−720 nm region (SI, Figure S10). (b) Transient spectra produced by 355 nm LFP of 17% *tert*-butyl peroxide in 2-propanol, in the presence of 0.2 mM TPZ, at longer time scales.

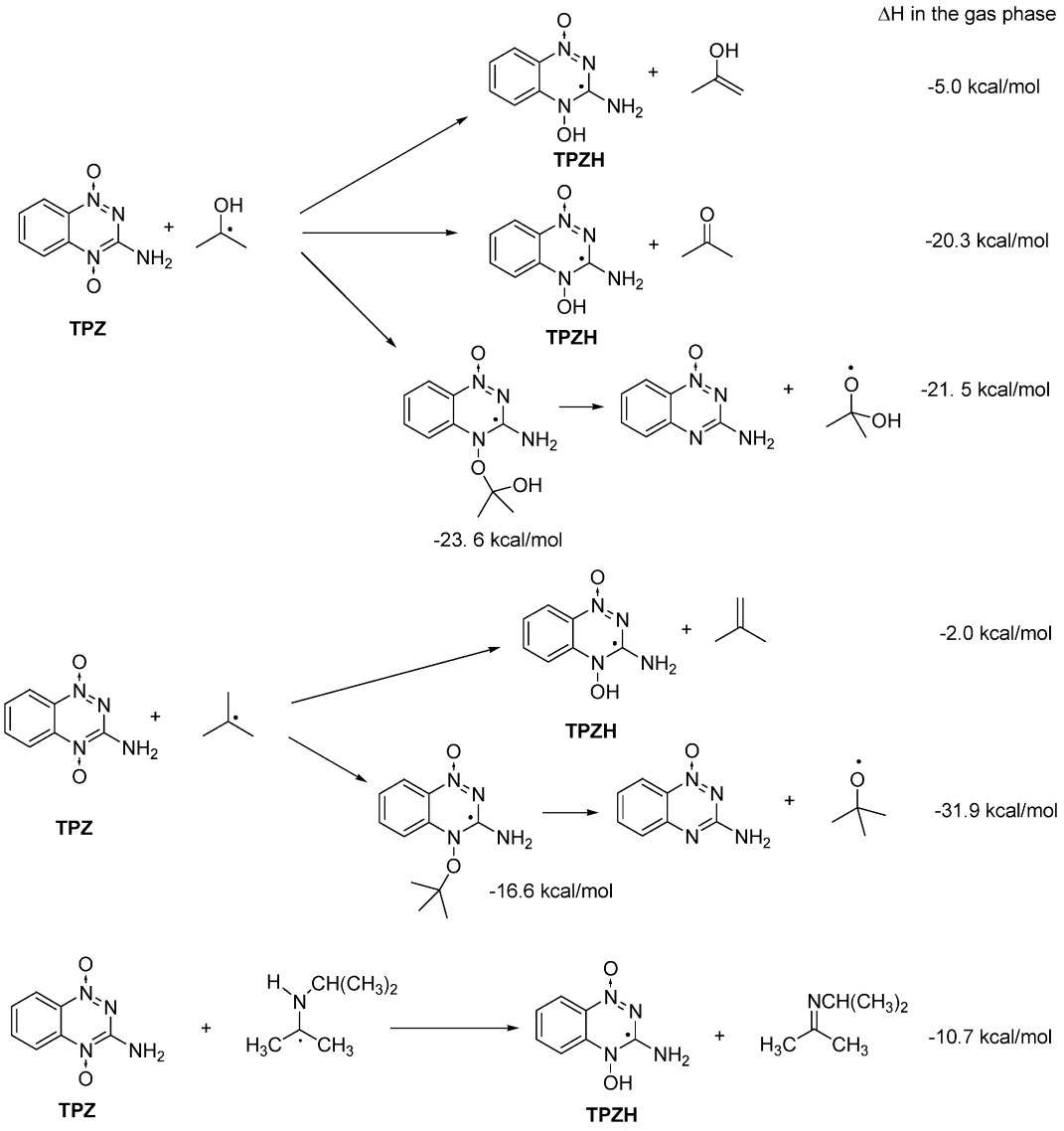
However, a strong change in transient absorption was observed in diisopropylamine containing 17% DTBP and 0.2 mM TPZ. The absolute rate constant of reaction of the diisopropylamino radical with TPZ was measured at 500 nm and found to be $(4.1 \pm 0.3) \times 10^9$ M^{-1} s^{-1}, from a plot of k_{obs} versus the concentration of TPZ. As shown in Figure S14 (SI), the absorption maximum of TPZ is observed at 500 nm in diisopropylamine; hence, the bleaching in diisopropylamine is red-shifted relative to that observed in 2-propanol.

3. Discussion

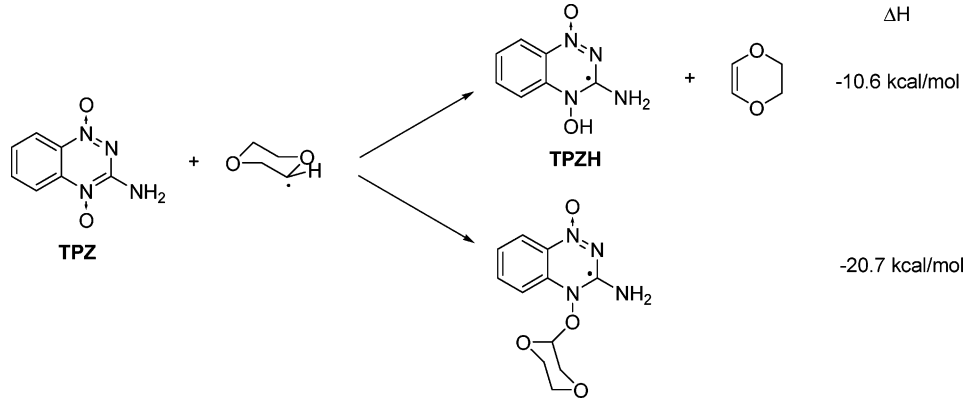
Our data indicate that ACOH radical reacts somewhat more rapidly with TPZ than do DX and other ethereal radicals. If the mode of radical reaction was addition to nitrogen (Scheme 4), then on steric grounds one would expect that DX radical would be more reactive than ACOH, exactly opposite to what is observed. If the mode of reaction is addition to oxygen (Scheme 2), then the steric effect would be smaller and the rates of reaction of ACOH and DX would be comparable, but again this is contrary to our observations.

We propose that the data are best explained by a component of hydrogen atom transfer reactions of ACOH and ether and amine-derived radicals in their reactions with TPZ (Schemes 5−7) to form the TPZH radical. The ACOH radical is simultaneously converted to acetone (major) and the enol of acetone (minor) in this reaction. Density functional theoretical (DFT) calculations indicate that the addition of radicals to the N4 oxygen atom of TPZ is quite exothermic (23.6 kcal/mol). However, transfer of an OH hydrogen atom from ACOH to TPZ with the formation of acetone (20.3 kcal/mol) is almost as exothermic as the addition process and surely is less sterically

Scheme 5. DFT-Calculated Enthalpies of Reaction of TPZ with the ACOH, *tert*-Butyl, and Diisopropylamine-Derived Radicals



Scheme 6. DFT-Calculated Enthalpies of Reaction of TPZ with the DX Radical



demanding. Furthermore, quantum mechanical tunneling can contribute to the hydrogen atom transfer process, providing this process with additional acceleration. Thus, we think it is reasonable that this reaction can compete with the addition reaction.

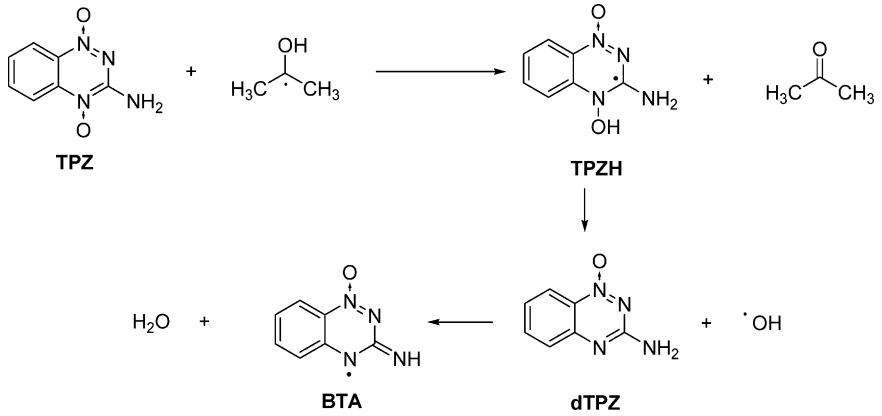
Hydrogen transfer from a C−H bond of ACOH to TPZ is still exothermic (5 kcal/mol) but is not as exothermic as transfer of an OH hydrogen. Thus, despite the statistical advantage of CH transfer, we think it is reasonable to propose that this reaction is somewhat slower than the OH hydrogen transfer. As ethers can only transfer CH hydrogens, whereas ketyl radicals can transfer both OH and CH hydrogens, we think that ether radicals will likely react a bit more slowly than ketyl radicals.

Table 2. Wavelengths and *f* Factors of Three Radicals Derived from TPZ, Including Nine Transitions with Lowest Energies[a]

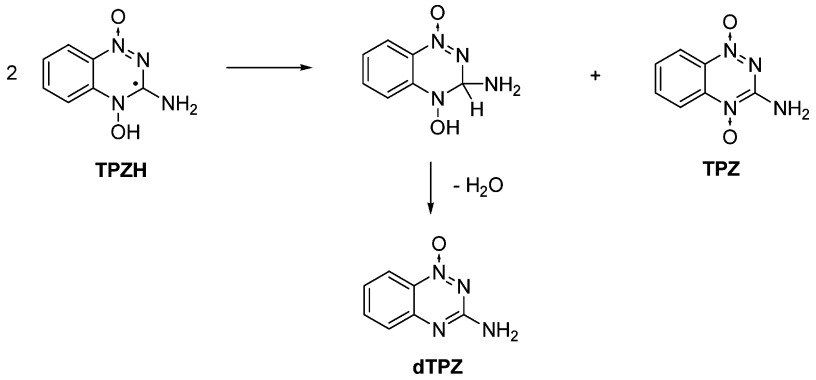
		transition								
radical		1	2	3	4	5	6	7	8	9
TPZ-H4	λ,nm	*472.13*	424.45	*413.29*	*361.09*	*326.04*	*304.85*	*287.13*	277.63	273.95
	f	0.0113	0.0000	0.0231	0.0133	0.0168	0.0368	0.0150	0.0607	0.0537
TPZ-H1	λ,nm	*546.41*	452.09	434.34	370.43	354.94	*337.55*	*309.45*	287.89	269.51
	f	0.0435	0.0003	0.0000	0.0077	0.0021	0.0352	0.0703	0.0088	0.0349
BTA	λ,nm	686.21	*622.81*	551.90	526.76	418.32	415.88	*378.07*	374.99	*362.23*
	f	0.0004	0.0291	0.0007	0.0012	0.0011	0.0000	0.0238	0.0000	0.0427

[a] Wavelengths with significant *f* factors are shown in italics.

Scheme 7. Overall Sequence of the Reaction of Ketyl Radical ACOH with TPZ



Scheme 8. Disproportionation Reactions of the TPZH Radical



The KIEs on the reaction of acetone ketyl radical with TPZ are consistent with primary isotope effects associated with a hydrogen atom transfer reaction. In fact, two such KIEs on ketyl radicals have been reported. Huyser and Kahl[10] have studied isotope effects on the rate of induced decomposition of DTBP in 2-butanol. It is believed that the 2-butanone ketyl radical (formed by reaction of *tert*-butoxy radical with 2-butanol) transfers a hydrogen atom to DTBP as part of a chain reaction. At 125 °C, the relative rate of peroxide decomposition in 2-butanol to 2-butanol-OD is 1.6. Tatikolov, Sklyarenko, and Kuz'min[11] studied the reaction of benzophenone ketyl radical (BZOH/BZOD) with a nitroxyl radical. They reported an isotope effect of 1.4−1.5 in methanol/methanol-OD at 20 °C. Thus, the KIEs observed in this work seem reasonable for a hydrogen/deuterium atom transfer from a ketyl OH(D) to an oxygen atom of an aryl *N*-oxide.

The product of reaction of ACOH with TPZ has absorption maxima at 350 and 400 nm (Figure 6). Although dTPZ has an absorption maximum at 400 nm, this molecule lacks significant absorption at 350 nm, nor will it decay over hundreds of milliseconds. Two other possible reaction products are radicals TPZH and BTA (Scheme 7).

Assigning the new species as BTA requires that the fragmentation of TPZH is a fast (nanoseconds) process. This would conflict with pulse radiolysis studies,[5] indicating that TPZH has a long lifetime and that TPZH undergoes second-order decay on the microsecond time scale, as shown in Scheme 7.

Instead, it seems likely that TPZH will decay in part by disproportionation, as shown in Scheme 8. This reaction produces TPZ, which explains the recovery of TPZ absorption at 470 nm and the formation of the stable reaction product dTPZ.

The final evidence in support of this spectroscopic assignment is provided by time-dependent DFT calculations of the absorption spectra of BTA, TPZH, and the isomeric radical TPZH1 (Table 2). The transient spectrum of the product of reaction of ketyl radicals with TPZ (Figure 6) is in poor agreement with the calculated spectra of BTA and TPZH but agrees well with the predicted spectrum (major peaks at 413, 361, and 326 nm) of TPZH. Note that both dTPZ and TPZH absorb at 400 nm,

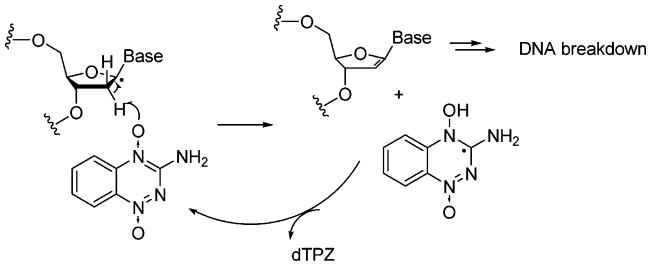
(10) Huyser, E. S.; Kahl, A. A. *J. Chem. Soc. D: Chem. Commun.* **1969**, *21*, 1238.
(11) Tatikolov, A. S.; Sklyarenko, V. I.; Kuz'min, V. A. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1990**, *5*, 999.

which explains the reshaping of the band in this region observed in Figure 6. Our assignment of the transient absorption growing at 350 and 400 nm is also consistent with the previous pulse radiolysis−transient spectroscopy work of Anderson and co-workers.[5]

The putative H atom transfer reaction of ketyl radical from the OH moiety is significantly more exothermic (9.7 kcal/mol) than the corresponding reaction of *tert*-butyl and dioxanyl radicals from a C−H bond adjacent to the radical center. The same idea explains the large difference in reactivity between the radicals derived from triethylamine and diisopropylamine. In the latter case, the radical can undergo a hydrogen atom transfer, similar to that possible with a ketyl radical, and has 3 times as many β-hydrogen atoms as the triethylamine-derived radical which are able to react with TPZ.

The results obtained in this study are completely consistent with the quantitative data previously reported. Greenberg, Gates, and co-workers reported that a single-stranded DNA C1− nucleotide radical (C1N, Scheme 3) reacts with TPZ with a rate constant of 2×10^8 M^{-1} s^{-1} in aqueous solution. This is the same order of magnitude as the rate constants of this study. When the radical is generated in double-stranded DNA,[4] the rate constant falls to 4×10^6 M^{-1} s^{-1}. Anderson and Denny[5] and co-workers also reported kinetic evidence that ribose-derived radicals react rapidly with TPZ. Both of these two groups favored the addition of sugar radicals to TPZ. On the basis of isotopic labeling studies, Gates and Greenberg et al.[4b] demonstrated that 83 ± 2% of the reaction of TPZ with a C1N radical proceeds via addion to an *N*-oxide oxygen atom. We posit that the remaining 17 ± 2% of the reaction may proceed by β-hydrogen atom transfer. The β-hydrogen transfer mechanism may be less important in a C1N radical than in the radical derived from diisopropyl ether because the C1N radical has fewer β-hydrogens available for atom transfer and the rigid framework of the ribose ring might introduce steric and stereoelectronic effects that retard the hydrogen atom transfer reaction.

Although we conclude that hydrogen transfer is an important mode of reaction, it is possible that radical addition chemistry proceeds at a rate competitive with hydrogen transfer from a CH group adjacent to a radical center in an ether. The hydrogen transfer reaction leads to dTPZ as required in any mechanism, but we posit it can also be formed by a polar mechanism which follows a disproportionation reaction of TPZH. The proposed reaction sequence converts the sugar radical to enol ethers, which can open and fragment to produce DNA strand breaks by polar mechanisms.



4. Conclusions

Ketyl radicals were generated by laser flash photolysis (LFP) of benzophenone or acetone in alcohols or by LFP of di-*tert*-butyl peroxide (DTBP) in the same solvents. The ketyl radicals react rapidly with tirapazamine (TPZ). The kinetics of this reaction are conveniently followed by monitoring the pseudo-first-order rate constant of disappearance of TPZ at 470 nm as a function of TPZ concentration. The reaction kinetics can also be followed by monitoring the rate of formation of the reaction product at 350 and 400 nm. The absolute second-order rate constant for reaction of acetone ketyl radical with TPZ in 2-propanol is 9.7×10^9 M^{-1} s^{-1}. Similar LFP experiments with DTBP were performed in dioxane, 1,3,5 trioxane in acetonitrile, and diethylamine solvents. The radicals formed in these solvents react somewhat more slowly with TPZ. It is concluded that ketyl radicals react, in part, with TPZ by transferring OH and CH hydrogen atoms from the ketyl radical to the *N*-oxide oxygen atom at N4 of TPZ. These reactions form ketone (major) and enol (minor) and a radical, TPZH, that has been previously postulated in TPZ oxygenation chemistry.[3−5] Radical TPZH has a lifetime of about 1 ms in alcohol solvent and decays by disproportionation to form TPZ and a reduced heterocyle, which subsequently extrudes water by a polar mechanism to form desoxytirapazamine (dTPZ). In ether-derived radicals, the reaction proceeds by transfer of a CH hydrogen to form TPZH and an enol ether. Hydrogen atom transfer reactions proceed at a rate that is competitive with addition reactions. The hydrogen atom transfer reaction likely contributes to the fast reaction of deoxyribose radicals with TPZ in aqueous solution. The resulting enol ethers can open and fragment by polar reactions.

5. Experimental Section

5.1. Laser Flash Photolysis Studies. The instrument used for LFP experiments and the protocols used in these experiments are described in detail in the literature.[12] The nanosecond laser flash system used an excimer laser (Lambda Physik LPX105EMC, 308 nm, 15 ns), or a Nd:YAG laser (Spectra Physics LAB-150-10, ~5 ns, 355 or 266 nm) was used as the excitation light source. The measurement beam was supplied by a 150 W xenon arc lamp (Applied Photophysics) used in pulsed mode (0.5 ms in duration) with a 1 Hz repetition rate. The pulse of light from the xenon lamp was focused onto a single ARC SP-308 monochromator/spectrograph, with a 1-015-300 grating. This model features dual ports, one with a slit and a photomultiplier for kinetic measurements and the other with a flat field and a Roper ICCD-Max 512T digital intensified charge-coupled device (ICCD) camera for spectroscopic measurements with up to 2 ns temporal resolution. The single monochromator/spectrograph negates the need for separate optimization of kinetic or spectral measurement system alignment, thus facilitating the usage of both types of measurements. The ICCD controller is directly interfaced to the computer using the Roper WinView software and ST-133A controller. Kinetic data acquisition uses a Tektronix TDS 680C 5Gs/s 1 GHz oscilloscope directly interfaced via a National Instruments PCI-GPIB to a computer running a custom LabView control and acquisition program. Laser, arc lamp, shutter, and other timing and control signals are routed through a National Instruments PCI-6602 DAQ interface. The measurement beam is supplied by a PTI 150 W xenon arc lamp with an LPS 210 power supply, LPS 221 stand-alone igniter, A-500 compact arc lamp housing, and MCP-2010 pulser, which allows for controlled pulsing of the arc lamp with pulses 0.5−2.0 ms in duration and up to 160 A in amplitude. Samples were placed in 10 × 10 mm^2 spectrasil quartz cuvettes and were deoxygenated by purging with a stream of argon for 5 min.

(12) (a) Gritsan, N. P.; Zhai, H. B.; Yuzawa, T.; Karweik, D.; Brooke, J.; Platz, M. S. *J. Phys. Chem.* **1997**, *101*, 2833. (b) Martin, C. B.; Shi, X.; Tsao, M.-L.; Karweik, D.; Brooke, J.; Hadad, C. M.; Platz, M. S. *J. Phys. Chem. B* **2002**, *106*, 10263−10271.

5.2. Materials. Benzophenone and *tert*-butyl peroxide were purchased from Aldrich. Solvents such as 2-propanol, acetonitrile, dioxane, benzene, and acetone were purchased from Aldrich. TPZ and dTPZ were synthesized as described in the literature.[13,14]

5.3. Computational Chemistry. In this study, we completely optimized all structures of interest at the B3LYP/6-31G* level. Analytical vibrational frequencies were calculated at the same level for each stationary point to verify a minimum energy structure and to provide zero-point vibrational energy corrections, which were scaled by a factor of 0.9806. Single-point energy calculations of all stationary points were performed at the B3LYP/6-311+G** level using the corresponding B3LYP/6-31G* geometries. All geometry optimizations, vibrational frequency calculations, and single-point energy calculations were performed using Gaussian 98[15] at the Ohio Supercomputer Center.

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Supporting Information Available: Additional transient spectra, various kinetic plots, the Cartesian coordinates of all species studied by computational methods, and complete ref 15. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Frisch, M. J.; et al. *SGI64-G98*, revision A9; Gaussian, Inc.: Pittsburgh, PA, 1998.