Identification of the Reactive Intermediates Produced upon Photolysis of *p*-Azidoacetophenone and Its Tetrafluoro Analogue in Aqueous and Organic Solvents: Implications for Photoaffinity Labeling[†]

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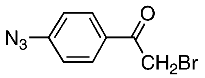
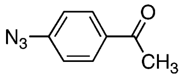
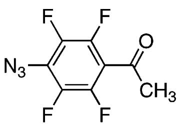
ABSTRACT: Photolysis of *p*-azidoacetophenone (**1a**) or 2,3,5,6-tetrafluoro-*p*-azidoacetophenone (**1b**) releases the corresponding singlet nitrenes **2a** and **2b**. In aqueous solutions singlet nitrenes relax (1.1 ps and 43 ns, respectively) to the lower energy triplet nitrenes **3a** and **3b**, intermediates which do not react to form cross-links or adducts with typical amino acids and nucleic acids. In a hydrophobic environment singlet nitrene **2a** partitions between forming triplet nitrene **3a** and an acyl-substituted didehydroazepine **4a**, which can be detected by LFP and time-resolved IR spectroscopy. The absolute rate constant of reaction of didehydroazepine **4a** with water, in acetonitrile, was determined (3.5×10^4 M^{-1} s^{-1}) by laser flash photolysis (LFP) techniques with IR detection at ambient temperature. Photolysis of tetrafluoro azide **1b** releases singlet nitrene **2b**, which has a lifetime of 172 ns in benzene and can readily be intercepted by pyridine to form ylide **10b** ($\lambda_{max} = 415$ nm). Singlet nitrene **2b** reacts with the unactivated CH bonds of cyclohexane to form adduct **8b** in 46% yield. Absolute rate constants of reaction of **1b** with *N*-methylimidazole, phenol, dibutyl sulfide, indole, methanol, and dimethyl sulfoxide were determined using the pyridine ylide probe method. It is concluded that photolysis of *p*-azidoacetophenone (**1a**) will not lead to cross-link formation but that tetrafluorinated azide **1b** can form useful singlet nitrene derived adducts upon photolysis.

Hixson and Hixson synthesized *p*-azidophenacyl bromide in 1975 (*1*), and it has since become a popular reagent for photoaffinity labeling studies and in photo cross-linking experiments (*2*). This reagent has several favorable design features; the phenacyl bromide moiety is labile and easily displaced by amino or thiol nucleophiles, leading to a robust covalent linkage in a dark reaction. The aromatic ketone functionality allows practitioners to use wavelengths greater than 320 nm to excite the probe, thereby allowing selective photolysis of the aryl azide moiety in a modified protein or nucleic acid. It is now clear that photolysis of *p*-azidoacetophenone (**1a**) (*3*) does release singlet nitrene **2a** (Scheme 1) but that this intermediate is too short-lived (1 ns) in aprotic organic solvents (*4*) to form useful adducts or cross-links. Polyfluorination of the azide (e.g., **1b**) can be expected to lengthen the lifetime of singlet nitrene **2b** in organic solvents (*5*) and favor the formation of adducts (*6*).





1a



1b

However, our present understanding of the basic photochemistry of **1a** and **1b** in aqueous solution is minimal due to a general lack of mechanistic photochemical studies of azides in water. To begin to understand the photochemistry of **1a**,**b** in water, we have studied these compounds in both aqueous and nonaqueous solution by chemical analysis of reaction mixtures and laser flash photolysis with UV−vis and IR detection of intermediates.

MATERIALS AND METHODS

All solvents and starting materials were used as received from the vendor without further purification unless specifically stated otherwise.

Synthesis of Azides. Azides **1a** (*7*) and **1b** (*8*) were synthesized as described in the literature.

Photoreactions of Azides. An approximately 0.001 or 0.01 M solution of the azide in the desired solvent was prepared. A sample was irradiated using 365 nm light in a Rayonet reactor fitted with low-intensity bulbs. The samples were irradiated for approximately 2 h. The product ratios were determined by injection onto a Beckmann Coulter System Gold 126 HPLC with a silica column from IBM Instrument Co. The products were eluted using a mixture of 2% ethyl acetate in hexanes. Detection of products was accomplished with a diode array UV−vis at 310 nm. The product ratios provided are corrected. Products were identified using authentic standards.

Synthesis of Standards. Several products were commercially available (**5a**, **6a**).

Amine **5b** was synthesized by refluxing 0.5 g (0.0024 mol) of 2,3,4,5,6-pentafluoroacetophenone in 5 mL of THF with

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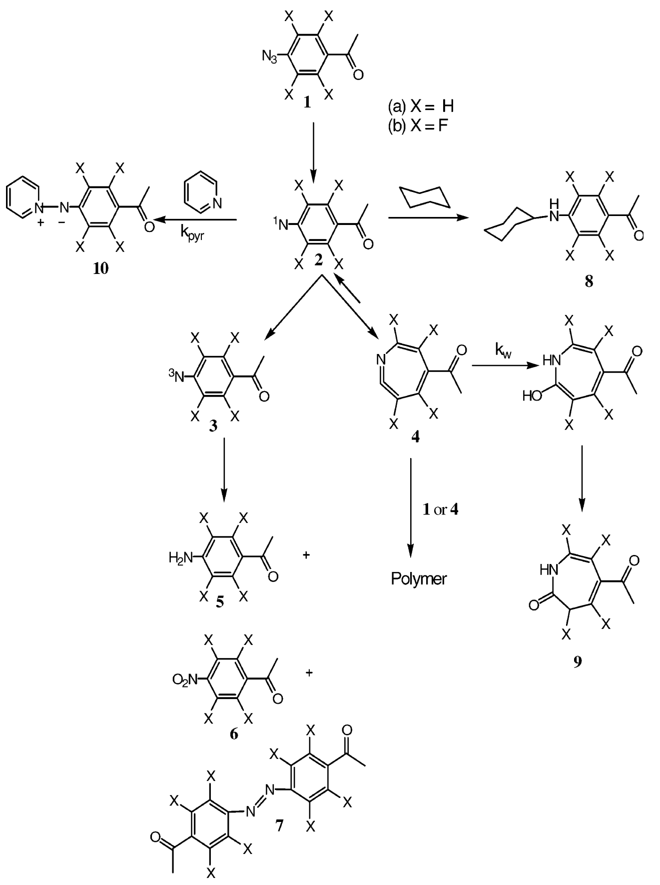
Scheme 1



10 mL of NH_4OH−water solution for 48 h. The reaction mixture was then extracted three times with ethyl acetate (50 mL). The organic layer was dried with $MgSO_4$, and the solvent was removed under reduced pressure to yield a white solid (0.200 g, 40% yield). ^{1}H NMR (500 MHz, $CDCl_3$): 2.6 (t, 3H, 1.5 Hz), 4.4 (s, 2H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): 33.1 (s), 106.7 (s), 129.8 (s), 136.8 (d, $J = 212$ Hz), 145.0 (d, $J = 200$ Hz), 192.0 (s) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): −163.22 (2F, d), −143.53 (2F, d) ppm. IR (NaCl, CCl_4): 3337, 3210, 1658, 1539, 1495, 1308 cm^{-1}. mp (ethyl acetate/pentane): 104−106 °C. MS: 207 (50%), 192 (100%), 164 (25%), 137 (25%).

Nitro compound **6b** was synthesized by oxidation of amine **5b**. To a solution of 250 mg (0.0011 mol) of amine **5b** in 2 mL of dichloromethane was slowly added 0.45 mL of trifluoroacetic anhydride and 0.25 mL of 50% H_2O_2 in water. The reaction was allowed to reflux for 1 h, and another addition of 0.45 mL of trifluoroacetic anhydride and 0.25 mL of 50% H_2O_2 was added. The reaction was then refluxed for 24 h until a green color was observed. The solution was then extracted with dichloromethane (3 × 25 mL), and the organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure, and the resulting pale green solid was purified by sublimation to yield pure **6b**. ^{19}F NMR (CD_2Cl_2, 376 MHz): −159.95 (2F, d, $J = 175.6$ Hz), −140.18 (2F, d, $J = 189.2$ Hz) ppm. ^{13}C NMR (125.7 MHz, CD_2Cl_2): 32.7, 124.8, 140.3 (d, $J = 377.1$ Hz), 144.6, 144.8 (d, $J = 75.2$ Hz), 191.32 ppm. mp: 24−25 °C. IR (CCl_4): 1719, 1646, 1401, 1356 cm^{-1}. MS: 237 (43%), 222 (60%), 192 (20%), 176 (42%), 148 (33%), 43 (100%).

Synthesis of adduct **8b** was accomplished by refluxing 0.5 g (0.0024 mol) of 2,3,4,5,6-pentafluoroacetophenone with 0.231 g (0.0024 mol) of cyclohexylamine in 10 mL of THF. The solution was refluxed overnight and then extracted with ethyl ether (3 × 50 mL). The organic layer was then dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude reaction mixture was then purified by column chromatography to yield the product **8b** (0.34 g, 50% yield). ^{19}F NMR ($CDCl_3$, 376 MHz): −162.36 (d, 2F, $J = 240.6$ Hz), 143.44 (2F, d, $J = 210.6$ Hz). ^{1}H NMR ($CDCl_3$, 500 MHz): 3.96 (s, 1H), 3.59 (m, 1H), 1.97 (2H, m), 1.69 (2H, m), 1.59 (1H, m), 1.25 (2H, m), 1.12 (4H, m) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): 24.65, 25.38, 32.47, 32.50, 53.54, 105.67, 130.46, 135.22 (d, $J = 238.8$ Hz), 144.71 (d, $J = 325.6$ Hz), 191.87 ppm. mp (ethyl acetate/hexanes): 128−132 °C. IR ($CHCl_3$): 3505, 3411, 1668, 1654 cm^{-1}. MS: 289 (50%), 246 (100%), 192 (50%).

Azo dimer **7a** was first synthesized by Binev (*9a*) and prepared in this study via the method of Barman et al. (*9b*). To a solution of 0.35 g of *p*-aminoacetophenone in 10 mL of dichloromethane was added a mixture of $KMnO_4$ (1 g) and $FeSO_4 \cdot 7H_2O$ (1 g) that had been ground together in a mortar and pestle. The heterogeneous reaction mixture was allowed to reflux for 8 h in dichloromethane. It was then filtered through Celite to yield an orange solution, which had the solvent removed under reduced pressure. The orange oil was then further purified by column chromatography on silica gel with 10% dichloromethane in hexanes as the eluant to yield orange crystals. mp (dichloromethane/hexanes): 192−194 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): 8.21 (2H, d), 7.89 (2H, d), 2.58 (3H, s) ppm. IR ($CHCl_3$): 1680, 1599 cm^{-1}. MS: 266 (50%), 119 (100%), 91 (35%), 76 (15%).

Azo dimer **7b** was isolated from the reaction mixture of a 0.01 M solution of azide **1b** in acetonitrile, which was photolyzed for 5 h with 365 nm light in a Rayonet reaction chamber. The acetonitrile was then removed under reduced pressure, and the resulting orange oil was purified by column chromatography on a silica gel column with 5% ether in hexanes as the eluant. ^{19}F NMR ($CDCl_3$, 376 MHz): 148.0 (2F, d, $J = 113$ Hz), 142.0 (2F, d, $J = 188$ Hz) ppm. ^{1}H NMR (500 MHz, $CDCl_3$): 2.7 ppm (d, 3H, $J = 1.5$ Hz). ^{13}C NMR (125.7 MHz, $CDCl_3$): 29.7, 31.9, 121.2, 133.5, 139.2 (d, $J = 414.8$ Hz), 143.8 (d, $J = 213.7$ Hz), 191.1 ppm. IR (NaCl, $CHCl_3$): 1709, 1684 cm^{-1}. mp (tetrahydrofuran/hexanes): 88−90 °C. MS: 410 (80%), 219,(75%) 191 (90%), 176 (100%), 148 (30%).

Laser Flash Photolysis Protocols. The laser flash photolysis set up at The Ohio State University has been described in detail elsewhere (*10*). Briefly, a sample was prepared to an absorption of approximately 1 at the excitation wavelength (308 nm, XeCl excimer laser, Lamba Physik LPX 100, 50 mJ/pulse, 30 ns pulse width). The ketenimine intermediates **4a**,**b** were probed at 415 nm, and the growth of the azo dimers **7a**,**b** was monitored at 330 nm. Briefly, all samples were dissolved in spectroscopic grade solvent with an absorption of ∼1 at 308 nm and placed in a 3 mL quartz cuvette. Samples were not purged with argon before use. Quenchers were present in solution at the desired concentrations.

Time-Resolved IR Spectroscopy Protocols. The Ohio State University TRIR[1] spectrometer has been described (*11*).

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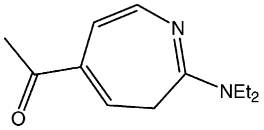
[1] Abbreviations: DFT, density functional theory; LFP, laser flash photolysis; TRIR, time-resolved infrared.

Briefly, all TRIR experiments were performed with a JASCO TRIR-1000 dispersive-type IR spectrometer, which has 16 cm^{-1} and 200 ns spectral and time resolution. A reservoir of 50 mL of sample solution contained a typical concentration of between 3 and 7 mM in terms of azide precursor concentration. The sample solution was circulated between two barium fluoride plates with a path length of 0.5 mm. The sample was excited with a 266 nm laser pulse from a Nd:YAG laser (50 Hz repetition rate, 0.5 mJ/pulse power, 17 ns pulse width), and the IR spectrum was obtained from the broad-band output of a $MoSi_2$ IR source.

Chemical Analysis of Reaction Mixtures. Li et al. (*12*) reported that photolysis of **1a** in cyclohexane containing 2.0 M diethylamine produces 3*H*-azepine **11** in 70% yield along with a 30% yield of aniline **5a**. The former product is associated with trapping ketenimine **4a**, and the latter product is typically thought to be formed from a triplet nitrene such as **3a**. In cyclohexane, however, an 87% yield of azo compound **7a** is produced upon photolysis of **1a** in the absence of diethylamine. This result indicates that singlet nitrene **2a** prefers to relax to ketenimine **4a** in cyclohexane but that this process must be reversible. Ketenimine−singlet nitrene equilibration has been previously postulated, as the parent cyclic ketenimine is predicted to be only 5 kcal/mol lower in energy than singlet nitrene (*5*, *13*, *14*). Singlet and triplet nitrenes do not equilibrate because of the large (17−18 kcal/mol) energy difference between the spin states (*15*−*17*).



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The stable products formed on photolysis of **1b** (0.001 M azide concentration) were investigated in acetonitrile and cyclohexane. In acetonitrile the major product observed was the nitro compound **6b**, which is attributed to oxygen quenching of the triplet nitrene **3a**. A trace amount of amine **5b** was also observed. In acetonitrile no products were observed which could be attributed to reaction of the singlet nitrene. In cyclohexane the major product observed was the insertion product **8b** (46% of consumed azide). Amine **5b** was also observed (8%). Product studies were also attempted in methanol and methanol−water solutions, but the only products observed were high molecular weight oligomers which we theorize arise from the oxidation of methanol and the formation of formaldehyde which then reacts with anilines.

There was no evidence for the formation of ketenimine adducts **9a**,**b** upon photolysis of **1a**,**b** in water−methanol solutions. This type of product is known to form upon photolysis of the parent phenyl azide in water, due to trapping of 1,2-didehydroazepine (*18*). High molecular weight products were observed in these systems. Polymer formation is often associated with ketenimines [such as **4a** and the parent 1,2,4,6-azacycloheptatetraene (*19*)]. In methanol solvent, polymer formation can also result from reaction of anilines and formaldehyde created from methanol oxidation by triplet nitrene **3a**.

Certain singlet arylnitrenes react with water or in acidic aqueous solution to form nitrenium ions (*18*). Phenol and quinone-type products are the eventual persistent products produced in this reaction. Such products were not observed in this study. This is not surprising as electron-donating substituents promote nitrenium ion formation and the acetyl group is electron withdrawing.

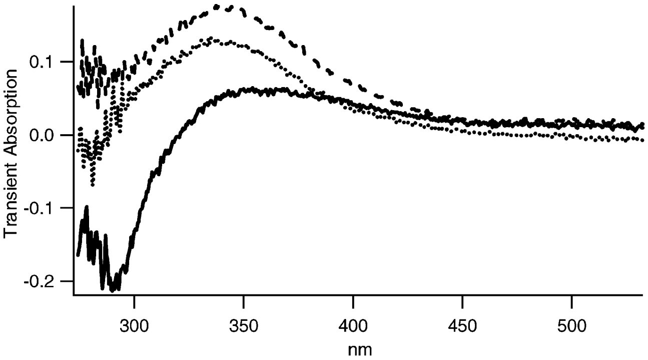

FIGURE 1: Transient absorption spectra produced by 308 nm LFP of azide **1a**. The spectra of triplet nitrene **3a** (solid line, water, 10 ns after the flash), didehydroazepine **4a** (dotted line, benzene, 10 ns after the laser flash), and the azo dimer **7a** (dashed line, benzene, 10 μs after the laser flash) were observed.

In summary, the trapping data argue against the formation of singlet nitrene−cyclohexane adducts upon photolysis of *p*-azidoacetophenone (**1a**). Photolysis of the tetrafluoro analogue **1b** in cyclohexane does lead to the formation of a nitrene adduct in 46% yield.

Laser Flash Photolysis (LFP) Studies. LFP (308 nm) of azide **1a** in benzene produces the transient spectra shown in Figure 1. Similar transient spectra were obtained in cyclohexane and acetonitrile. The carrier of transient absorption is assigned to didehydroazepine **4a** on the basis of the published work of Li et al. (*12*). The lifetime of this transient cannot be measured precisely because of the growth of the strong and persistent spectrum of the azo dimer **7a** in the same spectral regime of the transient intermediate (Figure 1) but is likely to be several hundred microseconds at dilute azide concentration on the basis of studies of related intermediates (*3*, *12*). No transient absorption was observed between 450 and 700 nm in either water or cyclohexane upon LFP of **1a**. The transient spectrum produced in water immediately after the laser pulse is slightly red shifted (Figure 1) relative to that observed in benzene and will be assigned to triplet nitrene **3a** on the basis of our kinetic studies (to be discussed) and its resemblance to the published spectra of other triplet arylnitrenes (*3*−*5*).

It was not possible to obtain the absolute kinetics of reaction of didehydroazepine **4a** with nucleophiles in benzene because of the severe overlap of the transient absorptions in this region.

The carrier of the transient spectrum in water does not react rapidly with water and therefore is likely not the same species detected by time-resolved IR spectroscopy upon LFP of **1a** in acetonitrile (as discussed later), a species which does react with water. The reactive intermediate observed in water does react with reducing agents: glutathione ($k = 2.8 \times 10^5$ M^{-1} s^{-1}) and sodium ascorbate ($k = 1.6 \times 10^6$ M^{-1} s^{-1}) (see Figure 2). This observation leads us to assign the carrier of the transient spectrum observed in water to triplet nitrene **3a**.

Triplet nitrene and ketenimine have nondescript UV spectra, which lends doubt to the assignments of the carriers

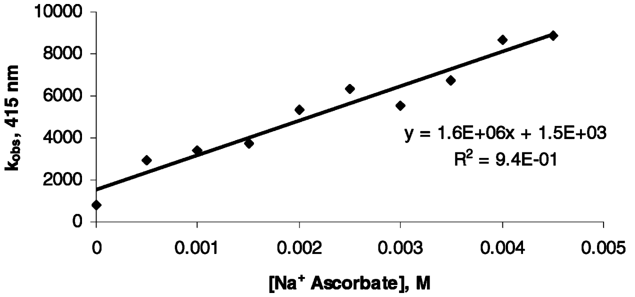

Figure 2: Plot of k_{obs} versus the concentration of sodium ascorbate, produced by LFP (308 nm) of **1a** in water.

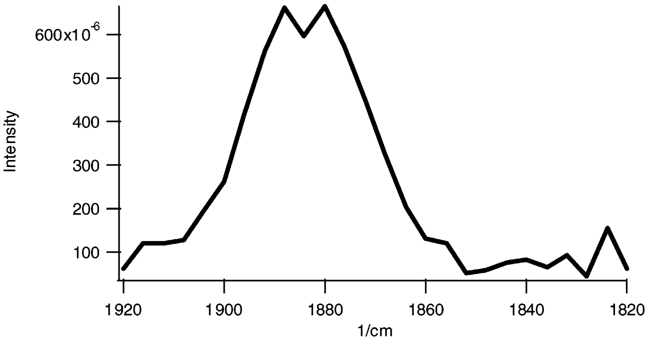

Figure 3: Transient IR spectrum obtained by LFP of **1a** in CH_3CN (266 nm excitation, 3.1 ms after the pulse, 3 mM azide concentration).

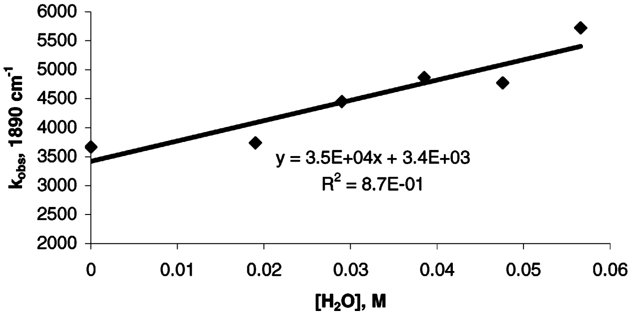

Figure 4: First-order quenching of **4a** with water in CH_3CN (266 nm LFP, 1890 cm^{-1} detection, 3 mM concentration of **1a**).

of the transient absorptions observed by LFP. However, the IR spectra of these species are distinct. 1,2-Didehydroazepines have intense bands around 1900 cm^{-1} (*20*), and triplet nitrenes (*21*) do not. Li et al. (*12*) demonstrated using TRIR spectroscopy that the rate of quenching of a ketenimine can be studied unambiguously. LFP of **1a** in acetonitrile produces the transient IR spectrum of Figure 3, which is characteristic of a cyclic ketenimine (*12*, *20*, *21*).

The lifetime of ketenimine **4a** is 29 μs in acetonitrile and is shortened in the presence of small amounts of water, unlike the species detected by transient UV spectroscopy in water. The lifetime of **4a** determined in TRIR experiments is shorter than that observed in LFP experiments using UV−vis detection because the TRIR experiments require a much greater concentration of azide to generate useful signals. The cyclic ketenimine can react with the azide precursor to form polymeric tar (*19*), which must shorten its lifetime in solution. A plot of k_{obs} ($1/\tau$) versus [H_2O] is linear with a slope of $k_w = 3.5 \times 10^4$ M^{-1} s^{-1} (Figure 4).

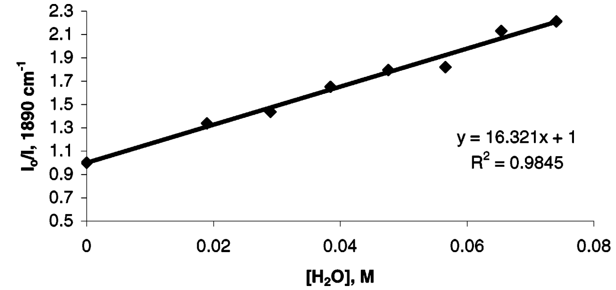

Figure 5: Stern−Volmer treatment of the quenching of the yield of **4a** with water in CH_3CN (266 nm excitation, 1890 cm^{-1} detection, 3 mM concentration of **1a**). I_0 and I are the transient absorptions of ketenimine recorded immediately after the laser pulse at 1890 cm^{-1}.

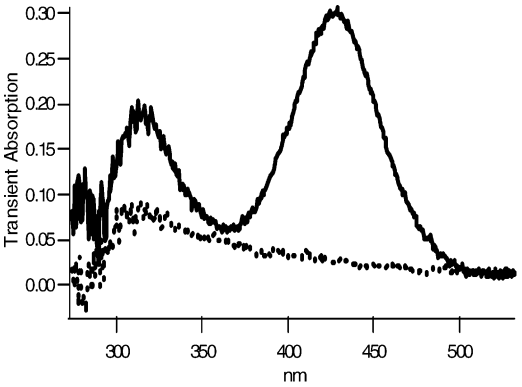

Figure 6: Transient absorption produced upon LFP of **1b** in benzene with 0 M pyridine 10 ns after the pulse (dashed line) and with 1 M pyridine 10 ns after the pulse (solid line).

It is interesting to note that water also quenches the yield of ketenimine **4a**. A Stern−Volmer plot (Figure 5) is linear with slope $k_q\tau = 16$, where τ is the lifetime of singlet nitrene **2a** in acetonitrile [1 ns (*3*, *4*)]. It is possible that water could reduce the molar absorptivity of the ketenimine or could retard the rate of isomerization of singlet nitrene to ketenimine. However, given the results of LFP with UV−vis detection, we deduce that water "reacts" with singlet nitrene **2a** with $k_q = 1.6 \times 10^{10}$ M^{-1} s^{-1}. On the basis of LFP (UV detection) studies we conclude that water catalyzes intersystem crossing of singlet nitrene **2a** at a diffusion-controlled rate. This is consistent with previous reports from our group that protic solvents can influence singlet nitrene intersystem crossing rates (*6a*). If these results can be extrapolated to neat water, then one predicts that the lifetime of singlet nitrene **2a** will be 1.1 ps in water. Thus, photolysis of probes derived from Hixson and Hixson's reagent in hydrophilic environments will produce long-lived triplet nitrenes which will form anilines and nitro aromatic compounds rather than cross-links. In hydrophobic environments derivatives of ketenimines **4a** will be formed which will react with nucleophiles. In the absence of nucleophiles they will equilibrate with the singlet and then ultimately relax irreversibly to triplet nitrenes.

*Tetrafluorinated Azide **1b**.* LFP of **1b** in benzene produces the transient spectrum of Figure 8. In the presence of pyridine, an intense new absorption band is observed at 415 nm (Figure 6). The growth of transient absorption at 425 nm is exponential and can be analyzed to yield k_{obs}, the pseudo-first-order rate constant of formation of the transient species. A plot of k_{obs} versus [pyridine] is linear (Figure 7). The carrier of the 415 nm absorbing species is assigned to ylide **10b**. The slope of the plot is k_{pyr} (Scheme 1), and the

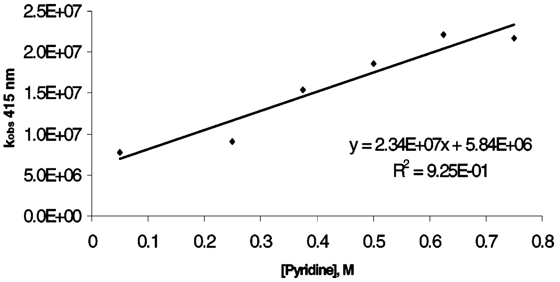

FIGURE 7: Plot of k_{obs} versus [pyridine] in benzene at ambient temperature (308 nm excitation, 415 nm detection).

Table 1: Kinetic Parameters to Nitrene Pyridine Ylide **10b** Formation

solvent	k_{pyr} (M^{-1} s^{-1})	τ *(ns)*
C_6H_6	2.3×10^7	172
CH_2Cl_2	2.5×10^7	196
CH_3CN	1.8×10^7	185

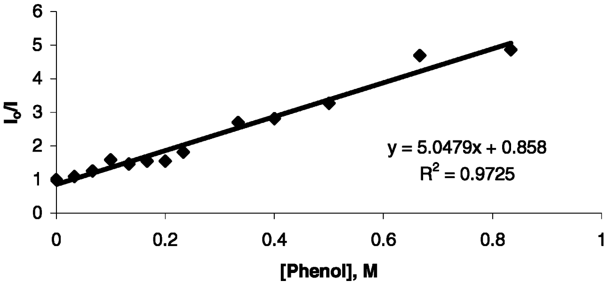

FIGURE 8: Stern−Volmer analysis of quenching of the yield of pyridine ylide **10b** by phenol in benzene (0.5 M pyridine concentration, 308 nm excitation, 415 nm detection). I_0 and I are the transient absorptions of ylide recorded immediately after the laser pulse at 415 nm.

intercept is $1/\tau$, where τ is the lifetime of singlet nitrene in benzene in the absence of pyridine (*6*).

The data are collected in Table 1 and resemble other pyridine-trapping rate constants and lifetimes reported with related polyfluorinated singlet arylnitrenes (*3*, *6*, *22*).

Similar results were obtained in acetonitrile and dichloromethane. Reproducible results were not obtained in cyclohexane because of the formation of precipitates and the fact that secondary photolysis of photoproduct **8b** produced a transient absorbing at 415 nm. The transient spectrum of the pyridine ylide was very weak in methanol, a protic solvent, and absolute kinetic data could not be obtained in this solvent for that reason.

The yield (I) of ylide **10b** in benzene is reduced in the presence of competitive quenchers of singlet nitrene **2b**. A Stern−Volmer (SV) analysis of the data is presented in Figure 8, where the concentration of pyridine is a constant 0.5 M and I_0 is the intensity of the ylide signal in the absence of quencher. The slopes of the SV plots are $k_q\tau'$, where $1/\tau' = 1/\tau + k_{pyr}[pyr]$, where τ' is the lifetime of singlet nitrene **2a** in benzene in the presence of 0.5 M pyridine but in the absence of quencher. The data are collected in Table 2.

The yield of pyridine ylide **10b** is very low in methanol, even at very large concentrations of pyridine. Methanol and water both quench the yield of pyridine ylide in aprotic solvents. This is due to the acceleration of intersystem crossing to triplet nitrene as observed with the nonfluorinated arylnitrene **2a** and in pentafluorophenylnitrene (*6*). However, protic catalysis of intersystem crossing of singlet nitrene **2b** is much less dramatic than that of the nonfluorinated singlet acylnitrene **2a**. Nevertheless, if the Stern−Volmer results can be extrapolated to water, we deduce that the singlet nitrene lifetime will be 43 ns in water as compared to 185 ns in acetonitrile. This lifetime is much longer than that of singlet acetylphenylnitrene **2a** in water (1.1 ps) and thereby increases the likelihood that singlet nitrene **2b** can undergo useful cross-linking reactions, relative to its nonfluorinated counterpart.

Table 2: Stern−Volmer Quenching Rate Constants with [Pyridine] = 0.5 M

quencher	$k_q\tau$ (M^{-1})	k_q (M^{-1} s^{-1})	solvent
water	0.047	4.2×10^5	CH_3CN
N-methylimidazole	1.8	1.5×10^7	C_6H_6
phenol	5.0	2.4×10^7	C_6H_6
dibutyl sulfide	33	4.1×10^8	C_6H_6
indole	65	7.8×10^8	C_6H_6
methanol	0.11	1.4×10^6	C_6H_6
dimethyl sulfoxide	8.2	7.4×10^7	CH_3CN

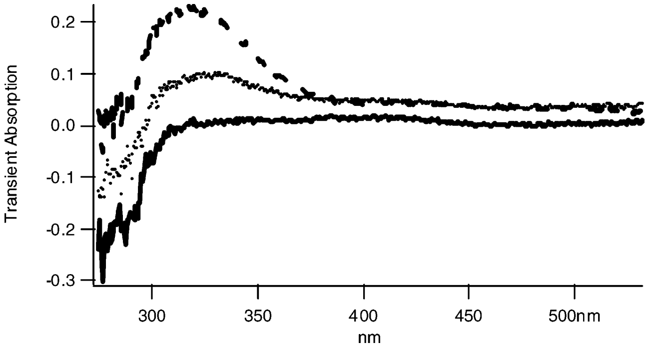

FIGURE 9: Transient UV spectra of **1b** in water at 10 ns delay (solid line), 1 ms (dotted line), and 10 ms (dashed line), 308 nm excitation.

LFP of **1b** in water does not produce significant transient absorption above 300 nm immediately after the laser pulse. The transient spectrum of the triplet nitrene is not observed due to a poor chromophore. Its transient absorption must be very weak in aqueous solution. As shown in Figure 9 transient absorption assigned to azo dimer **7b** grows in slowly. The rate of appearance of the transient absorption in water is not shortened in the presence of ketenimine quenchers such as adenosine, tryptophan, or tyrosine ethyl ester hydrochloride. Sodium ascorbate, a quencher of triplet nitrene **3b**, quenched the yield of azo dimer **7b** and increased the rate of its formation. Plots of the observed rate constant of azo formation versus the concentration of either sodium ascorbate or glutathione were linear (Supporting Information, Figures S3 and S4). The slopes of these plots are the absolute rate constants of triplet nitrene **3b** with the reducing agents (sodium ascorbate, 1.2×10^5 M^{-1} s^{-1}, and glutathione, 2.8×10^5 M^{-1} s^{-1}).

We could not use the pyridine ylide method in methanol or water to trap singlet nitrene **2b** because of protic catalysis of intersystem crossing.

Due to overlapping and weak chromophores in the UV spectra, transient UV spectroscopy was not a useful method for directly probing the nitrene intermediates created from irradiation of **1b**. To gain direct insight into the lifetime of these intermediates, TRIR spectroscopy was employed again. The pyridine ylide transient UV experiment indicates that

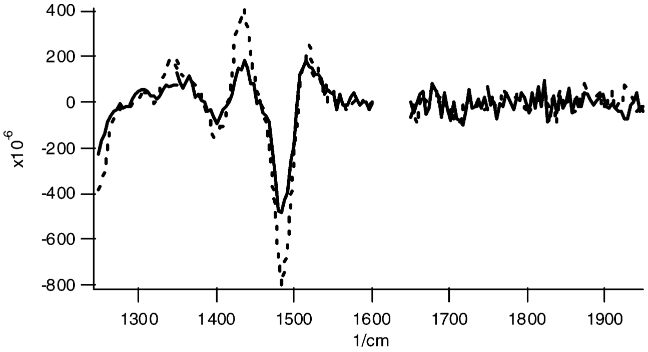

FIGURE 10: Transient IR spectrum produced by LFP of azide **1b** in CD_3CN (3 mM concentration of **1b**, 266 nm excitation). The spectrum was collected 0−0.5 μs after the laser pulse (solid line) and 1.0 μs after the laser pulse (dashed line).

the lifetime of singlet nitrene **2b** is 185 ns in acetonitrile, which is shorter than the time resolution of the TRIR spectrometer (*11*, *23*). Excitation of **1b** with 266 nm light gave rise to the transient IR spectrum shown in Figure 10.

In addition to the bleaching of precursor azide **1b**, three new transient absorption bands are observed immediately (0−500 ns) after the laser pulse. The more persistent peaks at 1438 and 1348 cm^{-1} are attributed to triplet nitrene and have a lifetime of approximately 3 μs in CD_3CN under air. Triplet arylnitrenes react slowly with oxygen; absolute reaction rate constants are typically 1×10^6 M^{-1} s^{-1} (*24*). Given the low concentration of molecular oxygen present in aerated organic solvent, triplet nitrene will react with oxygen in hundreds of microseconds. Thus, it is unlikely that the transients we detect are products formed from the reaction of triplet nitrene with oxygen.

Interestingly, there is no evidence by TRIR spectroscopy for the formation of ketenimine **4b** in acetonitrile-d_3, which should have characteristic stretches at 1856 cm^{-1} (*12*, *20*, *21*) and 1562 cm^{-1} as determined by DFT at the 6-31G* level of theory using the PCM model of acetonitrile. No peak was observed at 1856 or at 1550 cm^{-1}. These findings are in contrast to our reported study of the photochemistry of 2,6-difluorophenyl azide, where the corresponding ketenimine was detected by TRIR spectroscopy in acetonitrile (*23*). We note that the rate constant of intersystem crossing (ISC) of singlet 2,6-difluorophenylnitrene in hexane is 2.4×10^6 s^{-1}. This is slightly smaller than that of parent singlet phenylnitrene which has a rate constant of 3.2×10^6 s^{-1} in pentane. Polar substituents can increase the rate constant of ISC, however (*3*−*6*), and a *p*-acetyl group increases the rate constant of ISC of a simple phenylnitrene by a factor of 2.5. A similar increase in the ISC rate of **2b**, relative to 2,6-difluorophenylnitrene, explains our failure to detect didehydroazepine **4b** by TRIR spectroscopy.

This supports the assignment of the transient UV spectra produced by LFP of **1b** in polar solvents to triplet nitrene **2b**. The data indicate that singlet nitrene **2b** prefers to relax to triplet nitrene **3b** in acetonitrile, rather than to rearrange to a ketenimine in contrast to the behavior of nonfluorinated singlet nitrene **2a** in the same solvent.

CONCLUSIONS

Photolysis of *p*-azidoacetophenone and its tetrafluorinated analogue releases the corresponding singlet nitrenes. In water the *p*-acetylphenylnitrene has a lifetime of only 1.1 ps due to very rapid intersystem crossing to the lower energy triplet nitrene. Typical bimolecular reaction rate constants of singlet arylnitrenes are 10^{7-10} M^{-1} s^{-1} (*3*). Thus, even a large local concentration of the singlet nitrene trap (~1 M) leads to a trapping rate of 10^{7-10} s^{-1} (100 ps−100 ns) which simply cannot compete with intersystem crossing. Thus, we believe that 1.1 ps is too short a lifetime, by this criterion, to allow cross-linking reactions of a singlet nitrene to compete with spin relaxation and that the ideal lifetime of a cross-linking intermediate will be 100 ps−100 ns. Triplet nitrene **3a** is long-lived and reacts with oxygen to form nitro compounds or with reducing agents to form anilines. It is not a useful intermediate for forming cross-links. Singlet *p*-acetylphenylnitrene rearranges to form a ketenimine in aprotic organic solvent. In the absence of nucleophiles ketenimine equilibrates with singlet nitrene and eventually relaxes to a triplet nitrene, a species which does not form cross-links.

Tetrafluorinated singlet acetylphenylnitrene has a lifetime of 172 ns in benzene and reacts even with the unactivated C−H bonds of cyclohexane to form a robust adduct in 46% yield. This singlet nitrene does not rearrange to a ketenimine in aprotic solvent, but rather it eventually relaxes to triplet nitrene which again forms anilines and nitro compound products. In water, singlet tetrafluoro-*p*-acetylphenylnitrene relaxes in 43 ns to form the lower energy triplet nitrene. The lifetime of tetrafluorinated singlet nitrene **2b** in water is long enough to allow the formation of robust cross-links.

SUPPORTING INFORMATION AVAILABLE

Figures depicting the UV spectra of azo dimers **7a** and **7b** and plots of the quenching of the growth of azo dimer **7b** versus sodium ascorbate and glutathione. This material is available free of charge via the Internet at http://pubs.acs.org.

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