

Synthesis and Photolysis of a Series of Substituted Aroyl Nitrogen Ylides: Development of Photo-Cross-Linking and Photolabeling Reagents

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A series of substituted nitrogen ylides was prepared to study their suitability as photolabeling agents or as photo-cross-linking agents for biological systems. Two objectives were set in the design of these reagents: first, that they have a strong absorption beyond 320 nm, and second, that they be bifunctional so that they might be linked to the biological target before activation with light. Several systems were explored by analysis of their photochemical and photophysical properties. It was discovered that a biphenyl group-containing triazolium ylide satisfies these requirements.

Introduction

Light-initiated reactions offer special advantages in applications where high reactivity is required. In particular, the development of efficient and effective photolabeling and photo-cross-linking reagents for biological macromolecules is a long sought goal.¹ In these applications, a reagent is positioned at the targeted site of a biological macromolecule by a molecular recognition event or by a preliminary thermal reaction. The positioned reagent is activated by light to generate a highly reactive intermediate. In the ideal case, this intermediate possesses global reactivity, or, in other words, has the capacity to react rapidly and irreversibly with unactivated carbon-hydrogen bonds. In this circumstance, the labeling (or cross-linking) reaction will not be sensitive to its specific chemical environment since all sites are expected to contain these bonds.

Most of the photolabeling and photo-cross-linking reagents presently in widespread use are based on photolysis of an aryl azide.² Depending on the specific nature of the substituents on these reagents, their irradiation generates either an intermediate dehydroazepine or a triplet nitrene. The former reacts rapidly only with nucleophilic groups, the latter reacts slowly with unactivated hydrogen atoms.³ For these reasons, aryl azide labeling agents do not possess the global reactivity we seek. In an earlier stage of this research project, we discovered that photolyses of aroyl azides generally yield nearly equal amounts of the aroylnitrene by nitrogen loss and the isocyanate from photo-Curtius rearrangement of the excited singlet azide.⁴ The ground state of these aroyl nitrenes was assigned a singlet spin multiplicity on the basis of spectroscopic and chemical evidence. Most significantly, these intermediates appear to possess global reactivity—they insert rapidly into unactivated carbon-hydrogen bonds. Unfortunately, however, the simulta-

neous generation of the isocyanate, a weak electrophile that may migrate from the targeted site before its reaction, complicates their use. Attempts to circumvent this problem by accelerating intersystem crossing of the singlet azide met with only partial success since the carbonyl groups that enhance the intersystem crossing rate concomitantly accelerate the rate of nucleophilic attack on the ground-state azide rendering them unstable to the conditions of the labeling experiment.⁵ These limitations led us to seek alternative photochemical precursors to aroylnitrenes that would not generate an isocyanate and would be stable to the conditions of the labeling experiment.

Previous workers have shown that the irradiation of certain N-substituted pyridinium ylides leads to low yields of aroylnitrenes.⁶ This reaction is believed to originate with the triplet state of the ylide—their singlet states generally undergo a valence tautomerization reaction that gives 1*H*-1,2-diazepines. We recently reported that photolysis of *N*-(benzoylimino)-1,2,4-triazolium ylides leads to the exclusive generation of benzoylnitrene.⁷ Further, these ylides are stable under the conditions of a labeling experiment until they are activated with UV light. These properties seemed to make the ylides ideal candidates for development as photolabeling and photo-cross-linking reagents. Two issues remained to be investigated before they might prove useful in general applications. First, the triazolium ylides previously studied absorb strongly only in the deep UV spectral region. Since most biological macromolecules also absorb in this region, irradiation of these ylides would be inefficient, at best, and might cause the simultaneous photodestruction of the targeted substrate. Thus we sought modifications to the ylide structure that would result in stronger absorption at wavelengths greater than 320 nm. Second, to be useful as a labeling reagent, the ylide must first be attached either to a molecular recognition element or to one partner in a cross-

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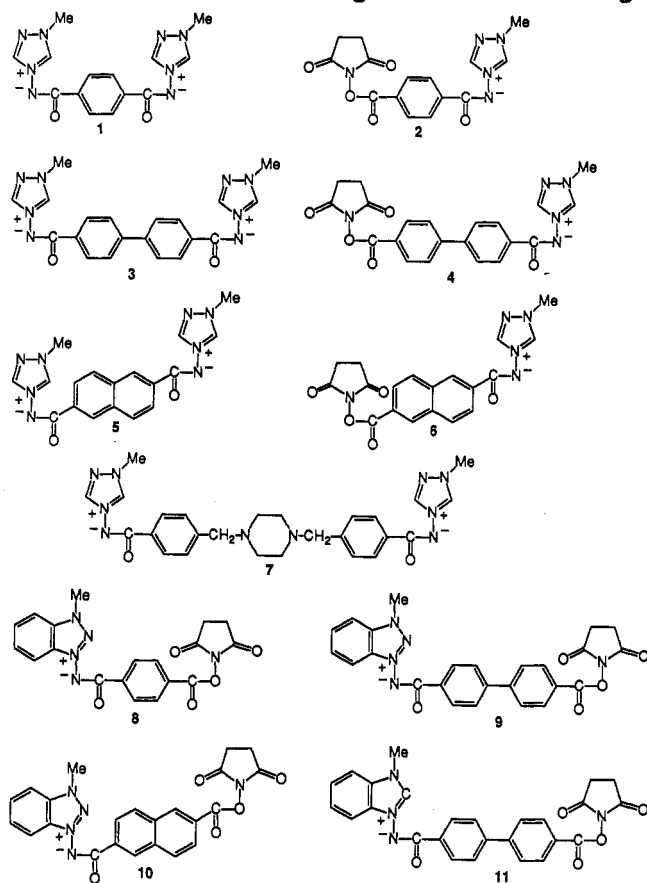
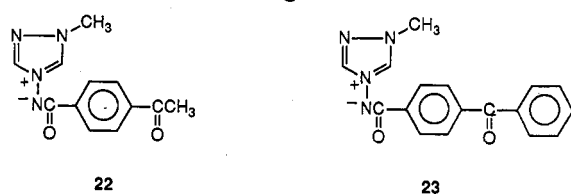
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Chart I. Bifunctional Reagents for Photolabeling**Chart II. Model Carbonyl Group-Containing Reagents**

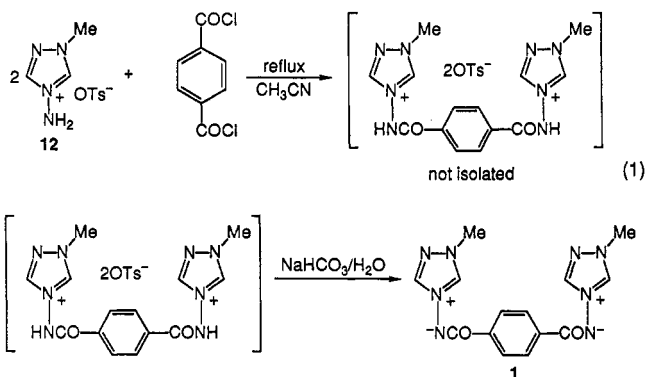
linking experiment. Consequently, a useful ylide must be bifunctional. That is, it must contain one group capable of a reaction that will bind it to the molecular recognition element for an affinity labeling experiment, or to one component of the pair of macromolecules to be cross-linked, in addition to the photoactive functional group.

We report herein the synthesis and investigation of three classes of bifunctional and long-wavelength-UV-absorbing reagents which incorporate ylides as potential arylnitrene precursors. The compounds shown in Chart I are bifunctional reagents containing an ylide or an aromatic group as the long wavelength absorber. The compounds shown in Chart II are model compounds where the absorbing group is an aryl-substituted ketone. These compounds were selected in the attempt to satisfy the criteria outlined above for a useful photolabeling reagent. Some of these materials show significant promise for this application.

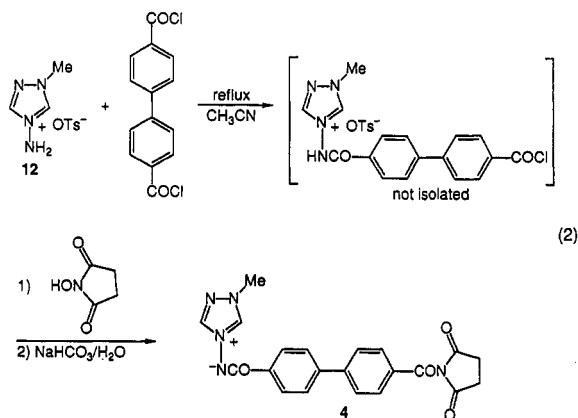
Results and Discussion

(1) Synthesis and Spectral Characterization of the Triazolium Ylides. The synthesis of the bifunctional photoprobes 1–11 generally follows one of two patterns depending upon whether the reagent is intended to be activated exclusively with light (photo-photo probe, 1, 3,

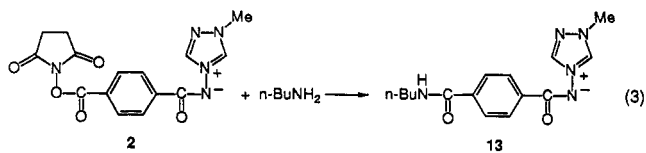
5, 7) or if it contains a thermally reactive electrophilic component in addition to a photoreactive ylide (photo-thermal probe, 2, 4, 6, 8, 9, 10, 11). The preparation of the photo-photo probes is effected simply, and generally in good yield, by reaction of the triazolium tosylate salt (12) with a bis-acid chloride and then deprotonation of the unisolated bis-tosylate salt with NaHCO_3 as is shown in eq 1 for the preparation of 1. The synthesis of the photo-



thermal probes starts similarly except that only 1 equiv of the triazolium tosylate salt is combined with the bis-acid chloride. The resulting mono-acid chloride derivative is then combined with *N*-hydroxysuccinimide, and the unisolated mono-tosylate salt is deprotonated with NaHCO_3 to give the ylide, as is shown in eq 2 for the synthesis of 4.

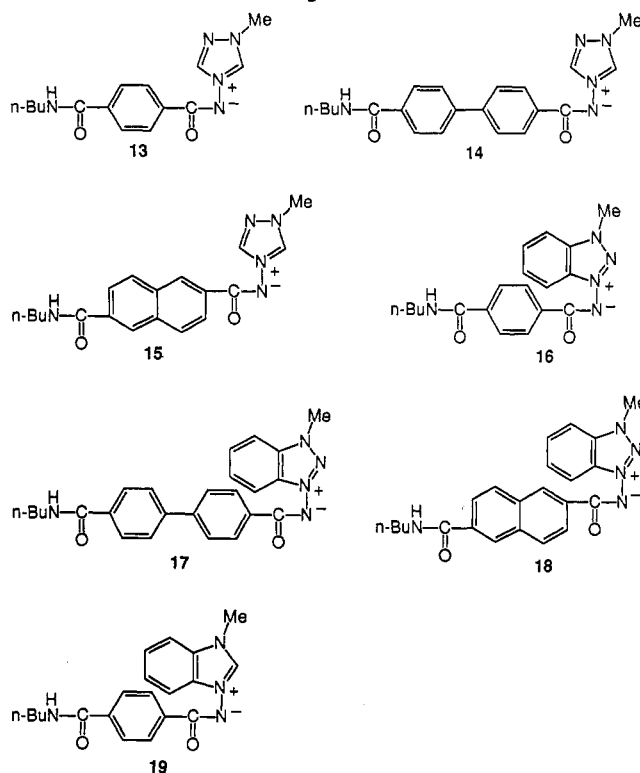


The photo-thermal probes are designed to react rapidly with a nucleophilic component (particularly an amine) of the molecular recognition element or one member of the pair of molecules to be cross-linked. This process was simulated by their reaction with *n*-butylamine to form the corresponding amide as exemplified in eq 3 for 2. This



reaction proceeds rapidly and in nearly quantitative yield. Since the light-activated reaction of the ylide will occur after the thermal reaction for these reagents, we prepared the amides shown in Chart III as models of the products of the thermal reaction so that their spectroscopic and photochemical properties could be studied. The syntheses of these compounds are described in the Experimental Section.

Chart III. Model Reagents for Amine-Linked Compounds



One of the primary goals of this work is to discover a photoreactive ylide that will generate an aroylnitrene when irradiated at wavelengths greater than 320 nm. Three general approaches were examined in the attempt to meet this objective. In the first, the aryl group linking the two reactive components of the reagent was modified. In particular, reagents 1, 3, and 5 incorporate phenyl, 4,4'-biphenyl, and 2,7-naphthyl groups, respectively, as the linker. In the second approach, the nature of the ylide itself is modified to extend the conjugation of its π system. For example, reagents 2, 8 and 11 incorporate a 1,2,4-triazolium, a benzo-1,2,3-triazolium, and a benzimidazolium group, respectively, in the ylide structure. Reagents 9 and 11 incorporate modifications to both the linking aryl group and the ylide in an attempt to simultaneously adjust their absorption spectra and to match the triplet energies of the relevant functional groups. In the third approach, an aryl-substituted carbonyl group, reagents 22 and 23, was included in the structure to function both as a long wavelength absorber and as a tool to enhance triplet formation due to rapid intersystem crossing that is typical of carbonyl groups.

The UV absorption spectra of ylides 1, 3, 5, and 7 in water solution are shown in Figure 1. Their extinction coefficients at 320 nm (ϵ_{320}) were monitored as an index of the pertinent UV absorption properties of these reagents. This value increases ca. 7-fold (from 600 to ca. 4600 $M^{-1} cm^{-1}$) for 3 and 5 compared with 1. This trend is reproduced in the spectra of the related amides 13, 14, and 15 where ϵ_{320} are 1300, 7700, and 6000 $M^{-1} cm^{-1}$, respectively.

The UV absorption spectra of model amides 16, 17, 18, and 19 are shown in Figure 2. For each compound in this group the absorption at wavelengths greater than 320 nm is increased significantly ($\epsilon_{320} \approx 10000 M^{-1} cm^{-1}$) compared with that for 13. The UV absorption spectra of 22 and 23

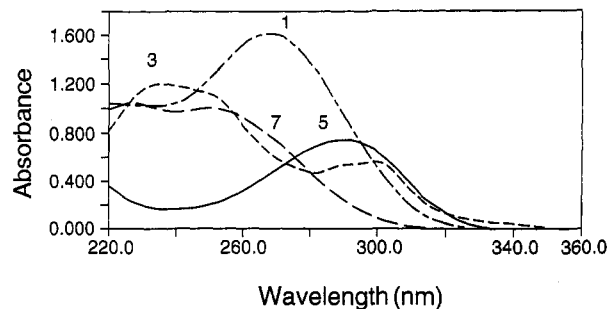


Figure 1. Absorption spectra of ylides 1 ($8.58 \times 10^{-5} M$), 3 ($3.18 \times 10^{-5} M$), 5 ($2.98 \times 10^{-5} M$), and 7 ($4.27 \times 10^{-5} M$) in water solution at room temperature.

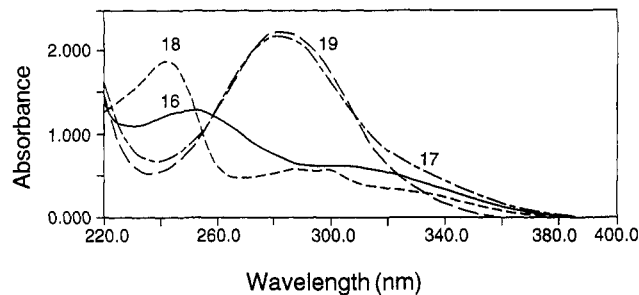


Figure 2. Absorption spectra of ylides 16 ($7.96 \times 10^{-5} M$), 17 ($7.48 \times 10^{-5} M$), 18 ($3.55 \times 10^{-5} M$), and 19 ($6.56 \times 10^{-5} M$) in methanol solution at room temperature.

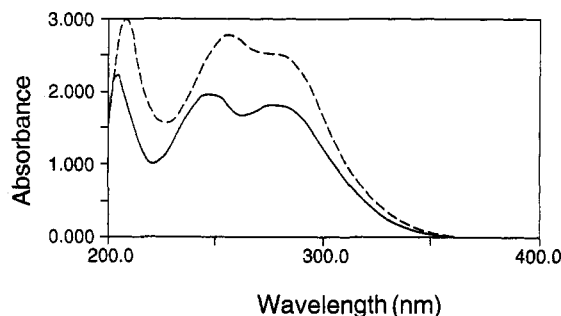


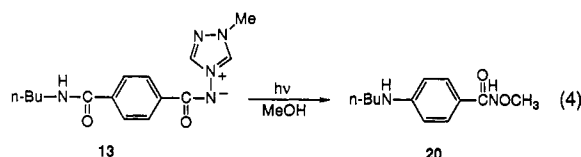
Figure 3. Absorption spectra of ylides 22 (solid line, $1.37 \times 10^{-4} M$) and 23 (broken line, $1.39 \times 10^{-4} M$) in methanol solution at room temperature.

are shown in Figure 3. As expected, they absorb significantly in the long wavelength UV region. For 22, $\epsilon_{320} = 3300 M^{-1} cm^{-1}$ and for 23 this value is 4600 $M^{-1} cm^{-1}$. These findings show that the bifunctional reagents containing a linking biphenyl or naphthyl group, a benzimidazolium or benzo-1,2,3-triazolium ylide, or an aryl-substituted carbonyl group will satisfy the spectroscopic criteria required of a useful photolabeling or photo-cross-linking reagent.

(2) **Photochemistry of the *N*-Aroyl Ylides.** In a previous study we showed that irradiation of *N*-(benzoylimino)-1-methyl-1,2,4-triazolium ylide in methanol solution gives first the methyl hydroxamate of benzoic acid and then, by secondary irradiation of the hydroxamate, benzamide.^{7,8} Generation of the hydroxamate in this reaction shows clearly that singlet benzoylnitrene is an intermediate formed from the photolysis of this ylide. Similarly, irradiation of the model triazolium ylide 13 in methanol solution at 300 nm converts it exclusively to the

(8) Johnson, J. E.; Arfan, M.; Hodzi, R.; Caswell, L. R.; Rasmussen, S. *Photochem. Photobiol.* 1990, 139.

substituted hydroxamate 20 as is shown in eq 4. Prolonged



irradiation of 13 at 300 nm results in the formation of some of the primary amide, presumably by secondary reaction, but irradiation at 350 nm, though slower, gives the hydroxamate exclusively. Significantly, comparable results are obtained from the photolysis of ylide 14 which contains a biphenyl group as a long wavelength absorber. Its irradiation leads to nitrogen–nitrogen bond scission with formation of the nitrene. When a methanol solution of this ylide is irradiated in a Rayonet reactor at 350 nm, the exclusive product is the expected methyl hydroxamate. However irradiation of the naphthalene containing ylide 15 or benzimidazole ylide 19 at 350 nm in N_2 -purged methanol solution does not lead to any observable reaction. And irradiation of the benzotriazole ylide 16 at 300 or 350 nm leads to its destruction without formation of a detectable amount of the hydroxamate that would signal intermediate nitrene formation. The photolysis of carbonyl group-containing ylides 22 and 23 in methanol solution slowly give the hoped-for hydroxamates at low conversion, ca. 10%, but this product is consumed by secondary photolysis as the reaction proceeds. Thus only the biphenyl-containing triazolium ylides of those investigated have both an extended absorption spectrum and the requisite reactivity to function as useful photolabeling or photo-cross-linking agents.

(3) Fluorescence and Time-Resolved Absorption Spectroscopy. The photophysical properties of ylides 13, 14, and 16 were investigated in the attempt to appraise the reasons for the failure of some of these compounds to yield nitrenes when they are irradiated. Previous work has shown that nitrene formation is predominantly a reaction of the triplet excited ylide,^{6,7} so our effort was focused first on estimating the efficiency for triplet formation and in analyzing some properties of this state.

Generally, intersystem crossing to form a triplet state competes with fluorescence and nonradiative relaxation processes, such as chemical reaction, originating from the excited singlet state. As one assessment of the photophysical properties, the fluorescence yield (Φ_f) of the ylides was measured in acetonitrile solution at room temperature. Interestingly, Φ_f for 13, which does form the nitrene, is 1.1×10^{-4} and for 14 and 16, this value is 1.1×10^{-2} and 1.3×10^{-2} , respectively. Thus, in all cases examined, fluorescence accounts for no more than ca. 1% of the excited singlet state decay and is approximately equally efficient in a ylide that yields a nitrene (14) and one that does not (16). Time-resolved triplet–triplet absorption spectroscopy is generally a reliable means for detecting and characterizing triplet states. Laser flash photolysis of ylide 14 in N_2 -purged methanol solution gives a readily detected transient absorption signal with an apparent maximum at 435 nm, see Figure 4. This absorption is attributed to a triplet state since it has a first half-life for decay of ca. 7 μ s and is quenched rapidly by O_2 . For comparison purposes, we examined the triplet–triplet absorption spectrum of model chromophore 4'-biphenyl *n*-butylcarboxamide (21), see Figure 4. It pulsed irradiation in a

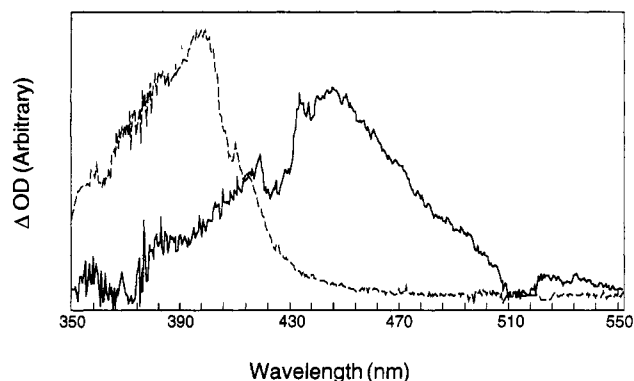


Figure 4. Transient spectra recorded 100 ns after pulsed irradiation (308 nm, 20 ns) of ylide 14 in N_2 -purged methanol solution (solid line) and model compound 21 recorded under the same conditions (broken line).

N_2 -purged methanol solution generates the triplet which absorbs at ca. 400 nm and decays with a first half-life of ca. 10 μ s. These findings reveal a potential problem with the application of biphenyl-containing reagents 3, 4, 9, or 11 as a photolabeling linking agent. The long triplet lifetime suggests low reactivity for the triplet state of the ylide. Consequently, other chemical or physical quenching processes that originate from the triplet state will compete effectively with formation of the desired nitrene.

(4) Time-Resolved Spectroscopy of the Carbonyl Group-Containing Ylides. It is well known that carbonyl groups incorporated in benzophenone or acetophenone-like chromophores intersystem cross rapidly to form triplet states. We sought to take advantage of these features in the design of a photolabeling agent based on the conversion of the triazolium ylide to a nitrene. Ylides 22 and 23 were prepared to test the feasibility of this approach. However, their irradiation in methanol solution gives nitrenes with low efficiency. We sought an explanation for this result by studying the time-resolved spectroscopy of 23.

Laser flash photolysis of 23 with a 20-ns pulse at 308 nm does not give a detectable transient species. However irradiation of 23 in acetonitrile solution with a 20-ps pulse at 355 nm gives the transient spectrum shown in Figure 5 which was recorded 90 ps after the pulse. This transient absorption decays with a lifetime of 8.0 ns under these conditions. Similar results are obtained from the irradiation of 23 in methanol solution except that the transient species maximum absorption occurs at ca. 700 nm and it decays with a lifetime of 6.5 ns.

The spectrum of the transient formed from 23 is similar to that observed for benzophenone radical anion under similar conditions.⁹ When the benzophenone radical anion is generated in an acid-containing solution, its protonation leads to the benzophenone ketyl radical and a characteristic shift in the absorption spectrum. A corresponding change is observed when a solution of 23 in acetonitrile containing 10% acetic acid is irradiated. The decay of the initial transient species absorbing at ca. 710 nm is accelerated ($\tau = 4.0$ ns) and the absorption spectrum recorded 10 ns after the pulse has a maximum at 585 nm (see Figure 5). This spectrum resembles that of the benzophenone ketyl radical.⁸ On the basis of these experiments, we assign the intermediate formed from irradiation of 23 as 24 formed by intramolecular electron transfer from the ylide group

(9) Shida, T. *Electronic Absorption Spectra of Radical Ions*; Elsevier: New York, 1988, p 288.

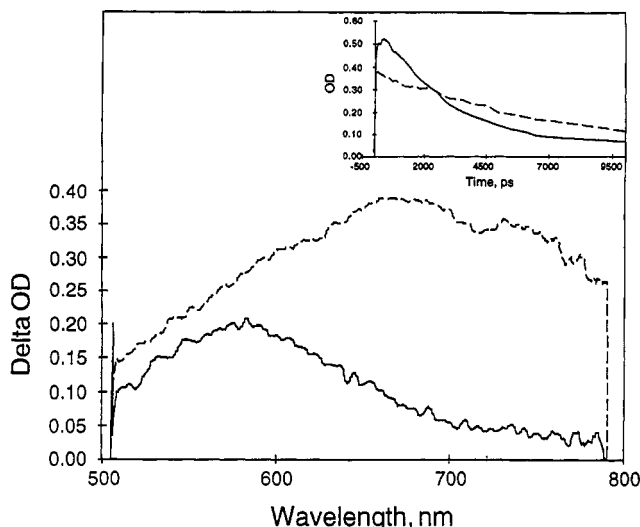
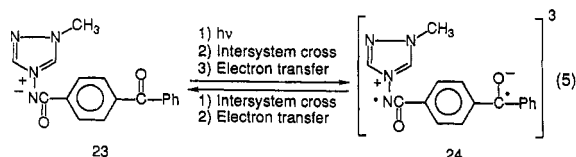


Figure 5. Transient absorption spectra of ylide **23** recorded 90 ps after pulsed excitation (355 nm, 20 ps) in air-saturated acetonitrile solution (broken line). The solid line is the transient spectrum recorded 10 ns after irradiation of a similar solution containing 10% acetic acid. The inset shows the decay of the initially formed transient monitored at 700 nm for solutions without (broken line) and with (solid line) acetic acid, respectively.

of **23** to the aryl-substituted ketone triplet excited state as shown in eq 5. Since, according to this proposal, **24** is



formed in its triplet state, back electron transfer to regenerate **23** can occur only after triplet **24** intersystem crosses to the singlet. We attribute the observed 8.0-ns lifetime of triplet **24** to the rate of its intersystem crossing. In principle, triplet **24** could undergo back electron transfer to form the triplet of **23** directly. We consider this process to be unlikely since the efficiency of nitrene formation from photolysis of **23** is low. The absence of back electron transfer from the triplet is probably due to the fact that the energy of **24** is below that of triplet **23**.

These spectroscopic results show that intervention of the unanticipated electron transfer route for the deactivation of excited **22** and **23** slows the formation of nitrene so much that secondary photochemical reactions come to dominate the products. Consequently, these aryl-substituted carbonyl group-containing ylides are not likely to be useful photolabeling reagents.

Conclusions

We sought to modify the structure of the substituted nitrogen ylides so that their irradiation with long wavelength UV light would lead to formation of an aroylnitrene. Three classes of ylides were investigated that extend the absorption to longer wavelength by (i) incorporation of an aromatic chromophore, or (ii) modification of the nitrogen-containing heterocycle by benzannulation, or (iii) incorporation of a carbonyl group. Only the first strategy was successful and only for the case of biphenyl-containing triazolium ylides. Those compounds with a naphthyl group as the absorbing chromophore are unreactive—possibly because their triplet energy is too low to give the nitrene from the ylide. Unfortunately, benzannulation of the nitrogen heterocycle inhibits the formation of the nitrene. And, finally, incorporation of a carbonyl chromophore provides an energy-wasting electron transfer pathway that inhibits nitrene formation.

Experimental Section

General. NMR spectral data were recorded on a General Electric QE-300 (300 MHz) instrument. UV absorption spectra were measured with a Cary-1E UV-vis spectrophotometer. Low-resolution mass spectra were recorded on a Varian-MAT CH-5 mass spectrometer (70 eV). A Hewlett-Packard 5890 GC and HP-5970 mass selective detector were used for GC-MS analyses which were performed at 70 eV with 0.25 mm × 12 m HP-1 capillary column programmed at 150 °C for 3 min and then 250 °C at the rate of 15 °C min⁻¹. A Hewlett-Packard 5890 GC equipped with a flame ionization detector and an HP 3390A electronic integrating recorder was used for analytical separations. GC analyses were performed with a 0.53 mm × 10 m HP-1 column. Preparative scale irradiations were performed in a Rayonet Photochemical Reactor equipped with either 300- or 350-nm lamps. The time-resolved absorption apparatus has been described previously.¹⁰ Elemental analyses were performed by the Microanalytical Laboratory of the University of Illinois.

Synthesis of Reagents. Typical Procedures. **1:** Terephthaloyl chloride (510 mg, 2.5 mmol) and 4-amino-1-methyl-1,2,4-triazolium tosylate (1.35 g, 5.0 mmol) were heated at reflux in dry CH₃CN (30 mL) solution under a N₂ atmosphere for 2 h. Removal of the solvent gave a white solid which was dissolved in aqueous NaHCO₃ solution (1.68 g, 20 mmol in 30 mL of water) and stirred for 30 min to afford a white precipitate. The precipitate was filtered and then recrystallized from water to yield **1** (520 mg, 64%) as colorless prisms: mp 268 °C dec; ¹H NMR (DMSO-*d*₆) δ 4.02 (s, 6 H), 7.95 (s, 4 H), 9.19 (s, 2 H), 10.62 (s, 2 H); MS (EI, low res) (70 eV, 265 °C) *m/e* 326 (M⁺, 50%), 56 (100%). Anal. Calcd for C₁₄H₁₄N₈O₂: C, 51.53; H, 4.32; N, 34.34. Found: C, 51.38; H, 4.36; N, 34.07.

2: Terephthaloyl chloride (1.0 g, 5.0 mmol) and 4-amino-1-methyl-1,2,4-triazolium tosylate (1.35 g, 5.0 mmol) were heated at reflux in dry CH₃CN (40 mL) solution under a N₂ atmosphere. After 1 h, hydroxysuccinimide (590 mg, 5.0 mmol) was added to the solution and heated at reflux for 5 h. Removal of the solvent gave a white solid, which was dissolved in aqueous NaHCO₃ solution (1.26 g, 15.0 mmol in 40 mL of H₂O) and stirred for 30 min. The solution was extracted with CH₂Cl₂ (4 × 100 mL) and the combined organic phases were washed with H₂O (2 × 50 mL) and dried (Na₂SO₄), and the solvent was evaporated. Flash chromatography (silica gel; 3% CH₃OH/CH₂Cl₂) and recrystallization from CH₂Cl₂/*n*-hexane gave **2** (390 mg, 24%) as colorless needles: mp 203 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.89 (s, 4 H), 4.03 (s, 3 H), 8.10 (d, *J* = 9 Hz, 2 H), 8.20 (d, *J* = 9 Hz, 2 H), 9.25 (s, 1 H), 10.66 (s, 1 H). MS (EI, low res) *m/e* 146 (M⁺ - 185, 100%), no M⁺ peak. Anal. Calcd for C₁₅H₁₃N₅O₅: C, 52.48; H, 3.82; N, 20.40. Found: C, 52.47; H, 3.87; N, 20.27.

13: Terephthaloyl chloride (1.0 g, 5.0 mmol) and 4-amino-1-methyl-1,2,4-triazolium tosylate (1.35 g, 5.0 mmol) were heated at reflux in a dry CH₃CN (40 mL) solution under a N₂ atmosphere. After 1 h, *n*-butylamine (440 mg, 6.0 mmol) and K₂CO₃ (1.38 g, 10.0 mmol) were added to the solution and heated at reflux for 3 h. The resulting mixture was cooled to room temperature and poured into H₂O (50 mL). The aqueous solution was extracted with EtOAc (3 × 150 mL), and the combined organic layers were washed with H₂O, 2 × 50 mL, dried (Na₂SO₄), and evaporated. Flash chromatography (silica gel; 8% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave **13** (420 mg, 45%) as colorless needles: mp 207 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.89 (t, *J* = 8 Hz, 3 H), 1.32 (m, 2 H), 1.49 (m, 2 H), 3.25 (m, 2 H), 4.02 (s, 3 H), 7.81 (d, *J* = 9 Hz, 2 H), 8.01 (d, *J* = 9 Hz, 2 H), 8.45 (t, *J* = 6 Hz, 1 H), 9.20 (s, 1 H), 10.63 (s, 1 H); MS (EI, low res) *m/e* 301

(10) Zhu, Y.; Koefed, R. S.; Devadoss, C.; Shapley, J. R.; Schuster, G. B. *Inorg. Chem.* 1992, 31, 3505.

(M⁺, 38%), 146 (M⁺ - 155, 100%). Anal. Calcd for C₁₅H₁₉N₅O₂: C, 59.78; H, 6.36; N, 23.24. Found: C, 59.65; H, 6.39; N, 23.18.

23: 4-Benzoylbenzoyl chloride (1.22 g, 5.0 mmol) and 4-amino-1-methyl-1,2,4-triazolium tosylate (1.35 g, 5.0 mmol) were heated at reflux in dry CH₃CN (40 mL) solution under a N₂ atmosphere for 1 h. The resulting solution was cooled to room temperature and then poured into aqueous NaHCO₃ solution (1.26 g, 15.0 mmol). After 30 min of stirring, the solution was extracted with CH₂Cl₂ (3 × 50 mL) and the combined organic layers were washed with H₂O (2 × 50 mL) and dried (Na₂SO₄), and the solvent was evaporated. Flash chromatography (silica gel; 3% CH₃OH/CH₂-Cl₂) and recrystallization from CH₃CN gave **23** (1.35 g, 89%) as colorless needles: mp 184–185 °C dec; ¹H NMR (DMSO-*d*₆) δ 4.03 (s, 3 H), 7.56 (m, 2 H), 7.65–7.75 (m, 5 H), 8.12 (d, *J* = 9 Hz, 2 H), 9.23 (s, 1 H), 10.66 (s, 1 H); MS (EI, low res) *m/e* 306 (M⁺, 66%) 125 (100%). Anal. Calcd for C₁₇H₁₄N₄O₂: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.69; H, 4.63; N, 18.25.

3: Recrystallization from DMF gave a 54% yield as colorless prisms, mp 254 °C dec; ¹H NMR (DMSO-*d*₆) δ 4.02 (s, 6 H), 7.70 (d, *J* = 9 Hz, 4 H), 8.06 (d, *J* = 9 Hz, 4 H), 9.21 (s, 2 H), 10.65 (s, 2 H); MS (EI, low res) *m/e* 402 (M⁺, 19%), 236 (100%). Anal. Calcd for C₂₀H₁₈N₆O₂: C, 59.69; H, 4.51; N, 27.85. Found: C, 59.71; H, 4.58; N, 27.56.

4: Recrystallization from CH₃CN gave a 29% yield as colorless needles, mp 234 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.89 (s, 4 H), 4.03 (s, 3 H), 7.81 (d, *J* = 9 Hz, 2 H), 8.00 (d, *J* = 9 Hz, 2 H), 8.11 (d, *J* = 9 Hz, 2 H), 8.16 (d, *J* = 9 Hz, 2 H), 9.22 (s, 1 H), 10.66 (s, 1 H); MS (EI, low res) *m/e* 419 (M⁺, 1%), 222 (100%). Anal. Calcd for C₂₁H₁₇N₅O₂: C, 60.14; H, 4.09; N, 16.70. Found: C, 60.04; H, 4.09; N, 16.66.

5: Recrystallization from DMF gave a 36% yield as colorless prisms, mp 255 °C dec; ¹H NMR (DMSO-*d*₆) δ 4.04 (s, 6 H), 7.94 (d, *J* = 9 Hz, 2 H), 8.11 (d, *J* = 9 Hz, 2 H), 8.50 (s, 2 H), 9.24 (s, 2 H), 10.67 (s, 2 H); MS (EI, low res) *m/e* 376 (M⁺, 14%), 210 (100%). Anal. Calcd for C₁₈H₁₆N₆O₂: C, 57.44; H, 4.28; N, 29.77. Found: C, 57.19; H, 4.37; N, 29.37.

6: Recrystallization from CH₃CN gave a 38% yield as colorless needles, mp 251 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.92 (s, 4 H), 4.05 (s, 3 H), 8.03 (d, *J* = 9 Hz, 1 H), 8.19–8.29 (m, 3 H), 8.65 (s, 1 H), 8.84 (s, 1 H), 9.27 (s, 1 H), 10.69 (s, 1 H). MS (EI, low res) *m/e* 393 (M⁺, 2%), 196 (100%). Anal. Calcd for C₁₉H₁₅N₅O₅: C, 58.01; H, 3.84; N, 17.80. Found: C, 57.98; H, 3.85; N, 17.75.

7: Recrystallization from water gave a 40% yield as colorless prisms, mp 254 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.37 (s, 8 H), 3.46 (s, 4 H), 4.01 (s, 6 H), 7.25 (d, *J* = 9 Hz, 4 H), 7.90 (d, *J* = 9 Hz, 4 H), 9.16 (s, 2 H), 10.60 (s, 2 H); MS (EI, low res) *m/e* 514 (M⁺, 1%), 132 (100%). Anal. Calcd for C₂₆H₃₀N₁₀O₂: C, 60.68; H, 5.88; N, 27.22. Found: C, 60.36; H, 5.95; N, 26.94.

8: Flash chromatography (silica gel; 3% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 40% yield as colorless prisms, mp 221 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.90 (s, 4 H), 4.49 (s, 3 H), 7.72 (m, 1 H), 7.85 (m, 2 H), 8.15 (d, *J* = 9 Hz, 2 H), 8.20 (m, 1 H), 8.30 (d, *J* = 9 Hz, 2 H); MS (EI, low res) *m/e* 393 (M⁺, 0.1%), 105 (100%) no M⁺ peak. Anal. Calcd for C₁₉H₁₅N₅O₅: C, 58.04; H, 3.84; N, 17.77. Found: C, 57.83; H, 3.69; N, 17.77.

9: Flash chromatography (silica gel; 3% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 30% yield colorless needles: mp 235 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.90 (s, 4 H), 4.49 (s, 3 H), 7.71–7.88 (m, 5 H), 8.03 (d, *J* = 9 Hz, 2 H), 8.20 (m, 5 H); MS (EI, low res) *m/e* 222 (M⁺ - 247, 100%). Anal. Calcd for C₂₅H₁₉N₅O₅: C, 63.96; H, 4.08; N, 14.92. Found: C, 63.48; H, 4.14; N, 14.88.

10: Flash chromatography (silica gel; 3% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 35% yield as colorless needles, mp 243 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.93 (s, 4 H), 4.50 (s, 3 H), 7.72 (m, 1 H), 7.86 (m, 2 H), 8.05 (d, *J* = 9 Hz, 1 H), 8.19–8.38 (m, 4 H), 8.78 (s, 1 H), 8.88 (s, 1 H); MS (EI, low res) *m/e* 196 (M⁺ - 247, 100%), no M⁺ peak. Anal. Calcd for C₂₃H₁₇N₅O₅: C, 62.30; H, 3.86; N, 15.79. Found: C, 62.33; H, 3.86; N, 15.79.

11: Flash chromatography (silica gel; 3% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 23% yield as colorless prisms, mp 239 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.90 (s, 4 H), 4.06 (s, 3 H), 7.59 (m, 2 H), 7.83 (d, *J* = 9 Hz, 2 H), 7.89–7.96 (m, 2 H), 8.02 (d, *J* = 9 Hz, 2 H), 8.17 (d, *J* = 9 Hz, 2 H), 8.24 (d, *J* = 9 Hz, 2 H), 10.56 (s, 1 H); MS (EI, low res) *m/e* 468 (M⁺, 0.2%), 132 (100%). Anal. Calcd for C₂₆H₂₀N₄O₅: C, 66.66; H, 4.30; N, 11.96. Found: C, 66.74; H, 4.32; N, 11.98.

14: Flash chromatography (silica gel; 8% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 25% yield as colorless needles, mp 232 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.89 (t, *J* = 9 Hz, 3 H), 1.33 (m, 2 H), 1.51 (m, 2 H), 3.27 (m, 2 H), 4.02 (s, 3 H), 7.72 (d, *J* = 8 Hz, 2 H), 7.78 (d, *J* = 8 Hz, 2 H), 7.92 (d, *J* = 8 Hz, 2 H), 8.07 (d, *J* = 8 Hz, 2 H), 8.50 (t, *J* = 6 Hz, 1 H), 9.21 (s, 1 H), 10.65 (s, 1 H); MS (EI, low res) *m/e* 337 (M⁺, 13), 222 (100%). Anal. Calcd for C₂₁H₂₃N₅O₂: C, 66.82; H, 6.14; N, 18.56. Found: C, 66.75; H, 6.15; N, 18.49.

15: Flash chromatography (silica gel; 5% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 37% yield colorless needles, mp 212 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.91 (t, *J* = 8 Hz, 3 H), 1.36 (m, 2 H), 1.54 (m, 2 H), 3.31 (m, 2 H), 4.04 (s, 3 H), 7.90 (d, *J* = 9 Hz, 1 H), 7.97 (d, *J* = 9 Hz, 1 H), 8.04 (d, *J* = 9 Hz, 1 H), 8.17 (d, *J* = 9 Hz, 1 H), 8.41 (s, 1 H), 8.54 (s, 1 H), 8.61 (t, *J* = 6 Hz, 1 H), 9.24 (s, 1 H), 10.67 (s, 1 H); MS (EI, low res) *m/e* 351 (M⁺, 8%), 196 (100%). Anal. Calcd for C₁₉H₂₁N₅O₂: C, 64.94; H, 6.02; N, 19.93. Found: C, 65.04; H, 6.00; N, 19.95.

16: Flash chromatography (silica gel; 5% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 27% yield as colorless needles, mp 183–184 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.90 (t, *J* = 8 Hz, 3 H), 1.34 (m, 2 H), 1.51 (m, 2 H), 3.27 (m, 2 H), 4.48 (s, 3 H), 7.70 (m, 1 H), 7.80 (m, 2 H), 7.86 (d, *J* = 9 Hz, 2 H), 8.11 (d, *J* = 9 Hz, 2 H), 8.18 (d, *J* = 9 Hz, 1 H), 8.50 (t, *J* = 6 Hz, 1 H); ME (EI, low res) *m/e* 351 (M⁺, 0.2%), 146 (100%). Anal. Calcd for C₁₉H₂₁N₅O₂: C, 64.94; H, 6.02; N, 19.93. Found: C, 65.00; H, 6.05; N, 19.95.

17: Flash chromatography (silica gel; 5% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 29% yield as colorless prisms, mp 230 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.90 (m, 3 H), 1.34 (m, 2 H), 1.52 (m, 2 H), 3.28 (m, 2 H), 4.48 (s, 3 H), 7.70–7.96 (m, 9 H), 8.16–8.20 (m, 3 H), 8.51 (t, *J* = 6 Hz, 1 H); MS (EI, low res) *m/e* 222 (M⁺ - 205, 100%), no M⁺ peak. Anal. Calcd for C₂₆H₂₅N₅O₂: C, 70.24; H, 5.89; N, 16.38. Found: C, 70.16; H, 5.86; N, 16.50.

18: Flash chromatography (silica gel; 5% CH₃O/CH₂Cl₂) and recrystallization from CH₃CN gave a 30% yield as colorless prisms, mp 174 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.91 (t, *J* = 8 Hz, 3 H), 1.37 (m, 2 H), 1.55 (m, 2 H), 3.32 (m, 2 H), 4.49 (s, 3 H), 7.73 (m, 1 H), 7.81–7.94 (m, 3 H), 8.03 (d, *J* = 9 Hz, 1 H), 8.10 (d, *J* = 9 Hz, 1 H), 8.19 (d, *J* = 9 Hz, 1 H), 8.27 (d, *J* = 9 Hz, 1 H), 8.45 (s, 1 H), 8.64 (t, *J* = 6 Hz, 1 H), 8.67 (s, 1 H); MS (EI, low res) *m/e* 196 (M⁺ 253, 100%), no M⁺ peak. Anal. Calcd for C₂₃H₂₃N₅O₂: C, 68.80; H, 5.77; N, 17.45. Found: C, 68.69; H, 5.80; N, 17.47.

19: Flash chromatography (silica gel; 5% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 49% yield as colorless needles, mp 213 °C dec; ¹H NMR (DMSO-*d*₆) δ 0.90 (t, *J* = 8 Hz, 3 H), 1.34 (m, 2 H), 1.51 (m, 2 H), 3.27 (m, 2 H), 4.05 (s, 3 H), 7.57 (m, 2 H), 7.84 (d, *J* = 9 Hz, 2 H), 7.89 (d, *J* = 8 Hz, 1 H), 7.95 (d, *J* = 8 Hz, 1 H), 8.14 (d, *J* = 9 Hz, 2 H), 8.46 (t, *J* = 6 Hz, 1 H), 10.52 (s, 1 H); MS (EI low res) *m/e* 350 (M⁺, 74%), 174 (100%). Anal. Calcd for C₂₀H₂₂N₄O₂: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.55; H, 6.32; N, 16.01.

22: Flash chromatography (silica gel; 5% CH₃OH/CH₂Cl₂) and recrystallization from CH₃CN gave a 42% yield as colorless needles, mp 230 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.58 (s, 3 H), 4.02 (2, 3 H), 7.95 (d, *J* = 9 Hz, 2 H), 8.08 (d, *J* = 9 Hz, 2 H), 9.22 (s, 1 H), 10.64 (s, 1 H); MS (EI low res) *m/e* 244 (M⁺, 71%), 125 (100%). Anal. Calcd for C₁₂H₁₂N₄O₂: C, 59.01; H, 4.95; N, 22.94. Found: C, 58.97; H, 4.90; N, 22.92.

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