Chemistry of the Diazeniumdiolates. 3. Photoreactivity

Aloka Srinivasan,[‡] Naod Kebede,[§] Joseph E. Saavedra,[⊥] Alexander V. Nikolaitchik,[§] Daniel A. Brady,[§] Emily Yourd,[§] Keith M. Davies,[†] Larry K. Keefer,[‡] and John P. Toscano*[,§]

Contribution from the Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218, Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, Maryland 21702, Intramural Research Support Program, SAIC Frederick, National Cancer Institute at Frederick, Frederick, Maryland 21702, and Department of Chemistry, George Mason University, Fairfax, Virginia 22030

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Abstract: We have found O^2-substituted diazeniumdiolates, compounds of structure $R_2N-N(O)=NOR'$ that are under development for various possible pharmaceutical uses, to be rather photosensitive. With R = ethyl and R′ = methyl, benzyl, or 2-nitrobenzyl, the observed product distributions suggest that two primary pathways are operative. A minor pathway involves the extrusion of nitrous oxide (N_2O) with simultaneous generation of $R_2N^•$ and $R'O^•$, which may then form amines, aldehydes, and alcohols. The major reaction pathway is an interesting photochemical cleavage of the N=N bond to form a nitrosamine ($R_2NN=O$) and an oxygen-substituted nitrene (R′ON). The intermediacy of the O-nitrene was inferred from the production of abundant oxime, via rearrangement of the O-nitrene to a C-nitroso compound (R′ON → O=NR′), and subsequent tautomerization to the more stable oxime. Involvement of the O-nitrene was confirmed by trapping with 2,3-dimethyl-2-butene to form the aziridine and with oxygen to generate the nitrate ester. 2-Nitro substitution on the benzyl derivative had surprisingly little effect on the reaction course. For each compound examined, minor amounts of nitric oxide (NO), presumably produced by secondary photolysis of the nitrosamine, were observed. Time-resolved infrared experiments provided additional support for the above reaction pathways and confirmed that the nitrosamine is a primary photoproduct. We have also found that the relative contributions of the reaction pathways can be altered in certain derivatives. For example, when R′ = 2,4-dinitrophenyl, the contribution of the nitrosamine/O-nitrene-forming pathway was diminished. Pharmacological implications of these results are discussed.

Introduction

Diazeniumdiolates have proven useful as research tools in a variety of applications requiring spontaneous release of the critical bioregulatory molecule nitric oxide (NO).[1] Recent efforts to develop pharmaceuticals based on this chemistry have concentrated on use of derivatives of such compounds to deliver NO specifically to a targeted site. Given the large number of biological phenomena now known to be mediated by NO, such targeting will be important to the ultimate success of most medical applications. One proposed strategy is to anchor diazeniumdiolates in polymeric matrices, restricting the release of NO to only those cells with which the polymer is in physical contact.[2] Another strategy is the use of prodrug derivatives that cannot release NO until they have been metabolically reconverted to the diazeniumdiolate by enzymes specific to the target cell type.[3] For example, a diazeniumdiolate masked by vinylation at the terminal oxygen of the $[N(O)NO]^-$ function has been shown to protect the liver from cell death without dangerously lowering blood pressure via its selective dealkylation by oxidative enzymes concentrated in the liver.[4]

An approach distinct from those described above that is often used to release a biological agent from a prodrug involves photochemistry. Photosensitive precursors, usually called "caged compounds" or "phototriggers", have become important biomedical research tools.[5] 2-Nitrobenzyl is a photosensitive protecting group commonly used to mask a variety of functionalities including alcohols, ketones, carboxylic acids, amines, and phosphates.[5]

[‡] National Cancer Institute.
[§] Johns Hopkins University.
[⊥] SAIC Frederick.
[†] George Mason University.

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(5) (a) McCray, J. A.; Trentham, D. R. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18*, 239−270. (b) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755−784. (c) Givens, R. S.; Kueper, L. W., III *Chem. Rev.* **1993**, *93*, 55−66. (d) Corrie, J. E. T.; Trentham, D. R. *Biological Applications of Photochemical Switches*; Morrison, H., Ed.; John Wiley and Sons: New York, 1994; p 243. (e) *Methods in Enzymology*; Marriot, G., Ed.; Caged Compounds, Vol. 291; Academic Press: New York, 1998.

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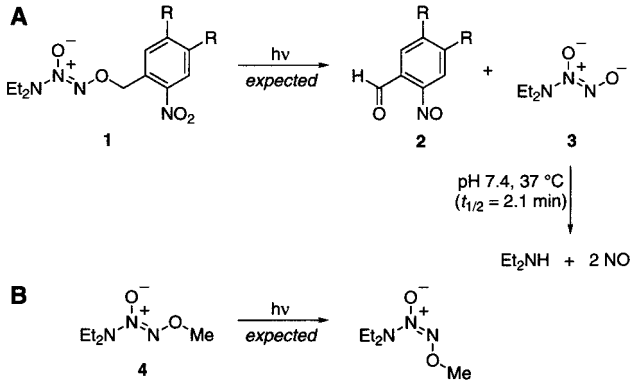
Scheme 1. Pathways of O^2-Substituted Diazeniumdiolate Phototransformations Initially *Expected* in the Study of Makings and Tsien[6] (A) and in the Present Work (B)[a]

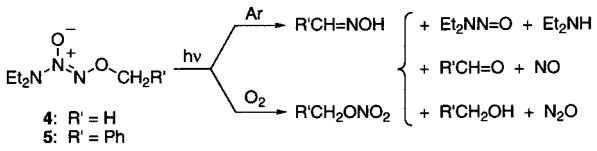


[a] Subsequent investigation has shown the photolysis of these compounds to follow the alternate mechanisms summarized herein.

In an attempt to apply this strategy to the release of NO through photochemically generated diazeniumdiolates, Makings and Tsien synthesized diazeniumdiolate derivatives **1** (Scheme 1, R = H, OCH_3, OCH_2CO_2Et) and studied the kinetics of NO release.[6,7] On the basis of known 2-nitrobenzyl chemistry,[8] these potential prodrugs were *expected* to photodecompose rapidly to nitrosoaldehydes **2** and diazeniumdiolate **3**, which has been shown[1b] to release NO spontaneously with a half-life of 2.1 min at pH 7.4 and 37 °C (Scheme 1A). Instead, however, NO was produced much faster, within the 5-ms time-resolution of Makings and Tsien's flash photolysis experiments. In addition, quantum yields for NO formation were disappointingly low, ranging from 0.02 to 0.05. Nonetheless, these phototriggered NO donors were used to inhibit thrombin-stimulated platelet aggregation,[6] to examine the induction of long-term depression in the cerebellum,[9] and to study long-term potentiation in cultured hippocampal neurons.[10]

We were studying the photolysis of O^2-substituted diazeniumdiolates for a different reason when the work of Makings and Tsien was published. We had noted that the two oxygen atoms of all diazeniumdiolates whose structures had been determined were cis to each other, with no spectral evidence of a trans configuration even in solution.[11] We *expected* that *E*/*Z* isomerization might occur in the excited state, as has been documented for the partial double bond in nitrosamines[12] and the N=N bond in azoxyalkanes;[13] however, when we irradiated O^2-substituted diazeniumdiolates[14] we observed extensive degradation, with carcinogenic nitrosamines as principal products (vide infra).

Scheme 2. Products Observed upon Irradiation of **4** or **5** in the Absence and Presence of Oxygen



Herein we report on several representative examples of this interesting transformation, which takes a course that neither we nor Makings and Tsien anticipated.

Results and Discussion

Products of Photolysis. Our initial study was undertaken with simple diazeniumdiolates whose aliphatic O^2-substitutents (e.g., methyl, ethyl) were ultraviolet inactive and thus would not interfere with irradiation of the diazeniumdiolate chromophore (λ_{max} = 230−240 nm).[3] Analysis of the organic products observed following photolysis (Rayonet, 254 nm) of methyl derivative **4** in argon-saturated acetonitrile-d_3 is summarized in Scheme 2 and Table 1. The quantum yield for photodegradation of **4** at 254 nm was 0.10. As indicated in Table 1, low photochemical conversion of **4** resulted in large yields of *N*-nitrosodiethylamine and formaldoxime. The yields of these products were diminished by further photolysis with a concomitant increase in the formation of diethylamine and methanol. Material balance was poorer at higher conversion. For example, at 35% conversion 93% of the diethylamine group and 85% of the O^2-methyl portion of the starting material were accounted for in the products, but at 73% conversion these numbers decreased to 67% and 64%, respectively. Nitric oxide was formed in only very low yield (4%). Small amounts of nitrous oxide (N_2O) were also produced.

To facilitate characterization of the product mixture, we next examined the photolysis (Rayonet, 254 nm)[15] of O^2-benzyl analogue **5** in argon-saturated acetonitrile-d_3. The results were very similar to those of methyl derivative **4**, but with a much higher quantum yield (0.67) for photoreaction at 254 nm. As noted above, yields (Table 1) indicated that the initially formed products were quite sensitive to secondary photolysis. Again, material balance was better at lower conversions; at 22% conversion 99% of the diethylamine group and 80% of the O^2-benzyl group were accounted for in the products, but at 76% conversion these numbers decreased to 70% and 57%, respectively.

Results analogous to those obtained with **5** were also found upon photolysis (Rayonet, 254 or 350 nm) of the 2-nitrobenzyl compound **1** (R = OMe), studied previously by Makings and Tsien,[6] indicating that the 2-nitro substituent had very little

(6) Makings, L. R.; Tsien, R. Y. *J. Biol. Chem.* **1994**, *269*, 6282−6285.

(7) Other photochemical precursors to NO have been examined: (a) Sexton, D. J.; Muruganandam, A.; McKenney, D. J.; Mutus, B. *Photochem. Photobiol.* **1994**, *59*, 463−467. (b) Pou, S.; Anderson, D. E.; Surichamorn, W.; Keaton, L. L.; Tod, M. L. *Mol. Pharmacol.* **1994**, *46*, 709−715. (c) Murphy, K. P. S. J.; Williams, J. H.; Bettache, N.; Bliss, T. V. P. *Neuropharmacology* **1994**, *33*, 1375−1385. (d) Namiki, S.; Arai, T.; Fujimori, K. *J. Am. Chem. Soc.* **1997**, *119*, 3840−3841. (e) Zavarine, I. S.; Kini, A. D.; Morimoto, B. H.; Kubiak, C. P. *J. Phys. Chem. B* **1998**, *102*, 7287−7292. (f) De Leo, M.; Ford, P. C. *J. Am. Chem. Soc.* **1999**, *121*, 1980−1981 and references therein. (g) Etchenique, R.; Furman, M.; Olabe, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 3967−3968. (h) For an overview of recent advances in NO donor chemistry, including a review of photochemical NO release, see: Hou, Y. C.; Janczuk, A.; Wang, P. G. *Curr. Pharm. Des.* **1999**, *5*, 417−441.

(8) (a) Schupp, H.; Wong, W. K.; Schnabel, W. *J. Photochem.* **1987**, *36*, 85−97. (b) Zhu, Q. Q.; Schnabel, W.; Schupp, H. *J. Photochem.* **1987**, *39*, 317−332. (c) Yip, R. W.; Wen, Y. X.; Gravel, D.; Giasson, R.; Sharma, D. K. *J. Phys. Chem.* **1991**, *95*, 6078−6081.

(9) Lev-Ram, V.; Makings, L. R.; Keitz, P. F.; Kao, J. P. Y.; Tsien, R. Y. *Neuron* **1995**, *15*, 407−415.

(10) Arancio, O.; Kiebler, M.; Lee, C. J.; Lev-Ram, V.; Tsien, R. Y.; Kandel, E. R.; Hawkins, R. D. *Cell* **1996**, *87*, 1025−1035.

(11) Keefer, L. K.; Flippen-Anderson, J. L.; George, C.; Shanklin, A. P.; Dunams, T. M.; Christodoulou, D.; Saavedra, J. E.; Sagan, E. S.; Bohle, D. S. Nitric Oxide: *Biol. Chem.*, in press.

(12) Michejda, C. J.; Davidson, N. E.; Keefer, L. K. *J. Chem. Soc., Chem. Commun.* **1976**, 633−634.

(13) Taylor, K. G.; Riehl, T. *J. Am. Chem. Soc.* **1972**, *94*, 250−255.

(14) We refer to the compounds of structure $R_2NN(O)=NOR'$ studied herein as "O^2-substituted diazeniumdiolates", rather than using strict *Chemical Abstracts* or International Union of Pure and Applied Chemistry nomenclature systems, to emphasize their status as derivatives of the 1-(*N*,*N*-dialkylamino)diazen-1-ium-1,2-diolate ion. The rationale for maintaining the "diazeniumdiolate" root for all such ions having potential biomedical relevance is discussed in detail in ref 11.

(15) Representative experiments with 350-nm photolysis were also conducted. These gave analogous results to the more extensive 254-nm photolysis experiments described in the text.

Table 1. Yields of Products Detected Following Photolysis of O^2-Substituted Diazeniumdiolates

		Yields of products (%)[a]							
reactant	% conversion	Et_2NNO	R′CHNOH	R′CHO	Et_2NH	$R'CH_2OH$	$R'CH_2ONO_2$	N_2O	NO
4 under Ar	7	97	77	*b*	1	0	0	7	*b*
	35	72	80	*b*	21	5	0	*b*	*b*
	73	49	42	*b*	18	22	0	*b*	*b*
	99	48	25	*b*	20	39	0	*b*	4
5 under Ar	11	96	66	7	0	4	0	5	*b*
	22	92	59	7	7	14	0	*b*	*b*
	76	49	38	10	21	9	0	*b*	*b*
	98	16	27	21	53	11	0	6	6
5 under O_2	16	59	0	24	0	20	28	6	*b*
	34	58	0	34	8	18	21	*b*	*b*
	99	11	0	48	63	34	10	*b*	5
1, R = OMe, under Ar	59	90	36	27	0	14	0	*b*	3

[a] Average of at least three measurements (estimated error = ±5%) based on percent reactant converted. [b] Not determined.

influence on the photochemistry of **1**. We were able to detect only trace amounts of nitrosoaldehyde **2** (R = OMe). Product distributions under argon in pH 7.4 phosphate buffer did not differ greatly from those seen in acetonitrile for any of the above compounds (**1**, **4**, and **5**).

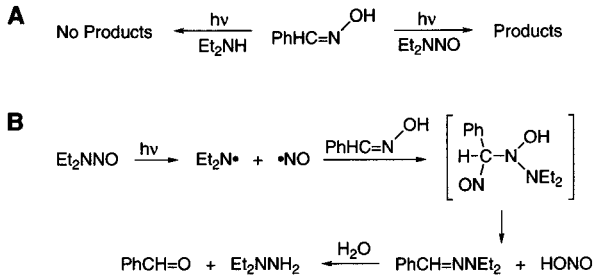
Given the high yields of *N*-nitrosodiethylamine observed for **1** (and also for **4** and **5**), NO was likely produced not by the expected photodeprotection of the diazeniumdiolate anion **3** (Scheme 1A), but rather by secondary photolysis of the nitrosamine[16] (eq 1). Consistent with this hypothesis, the yield of *N*-nitrosodiethylamine decreased at higher conversions (Table 1). Thus, we believe that the NO yields reported in Table 1 for nearly complete consumption of starting material represent an upper limit to the amount of NO produced upon photolysis of these O^2-substituted diazeniumdiolates.

$$R_2NN{=}O \xrightarrow{h\nu} R_2N\bullet + \bullet NO \qquad (1)$$

In addition to the expected photosensitivity of the initially produced nitrosamine, we have found that initially produced oximes are also consumed under the conditions of our experiment (Table 1). To gain insight into this process, we examined the photochemistry of benzaldoxime.[17] Photolysis (Rayonet, 254 nm) of benzaldoxime or benzaldoxime/diethylamine solutions resulted only in *E*/*Z* isomerization. However, photolysis of acetonitrile solutions containing equimolar amounts of benzaldoxime and *N*-nitrosodiethylamine produced benzaldehyde (61% yield based on consumption of the oxime) and 1,1-diethylhydrazine (47% yield based on consumption of the nitrosamine). A comparison with previously observed oxime photoreactivity[17] and known nitrosamine photochemistry[16] led us to hypothesize that these products arise via the reaction pathway shown in Scheme 3B. Consistent with this hypothesis, we observed formation of 1,1-diethylhydrazine in O^2-substituted diazeniumdiolate solutions photolyzed to high conversion.

The identities and yields of major products from photolysis of benzyl derivative **5** were dramatically affected by the presence of oxygen (Scheme 2, Table 1). Irradiation (Rayonet, 254 nm) of **5** in oxygen-saturated acetonitrile-d_3 afforded significant amounts of *N*-nitrosodiethylamine, as previously observed under argon; however, no benzaldoxime was detected. In its place, enhanced yields of benzaldehyde and newly formed benzyl nitrate were observed. Nitrates were never detected for any of the compounds examined when photolyses were conducted under argon.

Scheme 3. Observations (A) and Proposed Mechanism of Photolysis (B) for the Irradiation of Benzaldehyde Oxime in the Presence of Either Diethylamine or *N*-Nitrosodiethylamine



Time-Resolved Infrared Studies. To elucidate further the photochemistry of O^2-substituted diazeniumdiolates, we undertook a time-resolved infrared (TRIR) investigation. TRIR spectroscopy is a mechanistically powerful tool that can provide *both* structural and kinetic information concerning intermediates involved in photochemical reactions. In addition, flash photolysis experiments generally avoid secondary photochemical reactions. Typical TRIR data observed following 266-nm laser excitation of **5** in argon- and oxygen-saturated acetonitrile-d_3 are shown in Figure 1. Data were obtained in the form of difference spectra. Band assignments given in Figure 1 are based on comparison with literature data and when possible with the IR spectra of authentic samples. The negative bands in Figure 1 are due to depletion of **5**. In agreement with the product studies described above, TRIR spectroscopy demonstrates that the photochemistry of O^2-substituted diazeniumdiolates is dramatically affected by the presence of oxygen. Kinetic traces for major positive bands are displayed in Figure 2.

Photolysis of an Anionic Diazeniumdiolate. Photosensitivity in the diazeniumdiolate series was not limited to the O^2-substituted derivatives. A striking illustration of this reactivity was encountered in a recent study of **6** (Scheme 4) as a drug for promoting healing after vascular graft placement in baboons.[18] Anionic diazeniumdiolates normally dissociate to bioactive NO in the simple, proton-catalyzed hydrolysis reaction shown in Scheme 4A.[18,19] As a prelude to picking the optimum pH for the infusate to be used in these experiments, we

(16) Chow, Y. L. *Acc. Chem. Res.* **1973**, *6*, 354−360.

(17) The photochemistry of oximes has been investigated: (a) Just, G.; Ng, L. S. *Can. J. Chem.* **1968**, *46*, 3381−3389. (b) Haley, M. F.; Yates, K. *J. Org. Chem.* **1987**, *52*, 1817−1824.

(18) Lundell, A.; Marijianowski, M.; Keefer, L. K.; Davies, K. M.; Saavedra, J. E.; Hanson, S. *J. Vasc. Surg.* Submitted; the dissociation rate for **6** was strongly dependent on sodium hydroxide concentration even in the range [NaOH] = 50 mM to 1 M.

(19) Davies, K. M.; Wink, D. A.; Saavedra, J. E.; Keefer, L. K. *J. Am. Chem. Soc.* **2001**, *123*, 5473−5481.

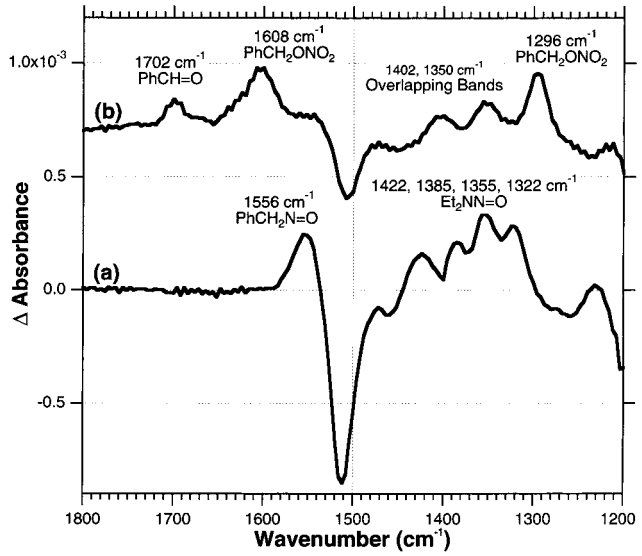


Figure 1. TRIR difference spectra observed from 7 to 9 μs following 266-nm laser photolysis (10 ns, 0.4 mJ) of benzyl derivative **5** (30 mM) in argon- (a) and oxygen-saturated (b) acetonitrile-d_3. See text for a discussion of band assignments.

determined the rates of dissociation in aqueous solutions of different alkalinities. In following these reactions by ultraviolet spectrophotometry, we found that absorbance at this compound's characteristic maximum of 250 nm decreased much more rapidly when continuously monitored in the light path of the spectrophotometer than when aliquots taken from a shielded flask were assayed intermittently. Moreover, although spontaneous hydrolysis of anionic diazeniumdiolates in the dark is first order in hydrogen ion[19] even in strongly basic media,[18] solutions of **6** placed in the spectrophotometer beam exhibited similar decay rates in 10 and 100 mM sodium hydroxide.

To investigate this reaction further, a 0.3 mM solution of **6** in 10 mM sodium hydroxide was prepared and divided into two portions. One portion was placed in the light path of a diode array spectrophotometer for 3 h until photolysis was complete, while the other was allowed to stand for a week to hydrolyze in the dark (half-life $\approx$ 20 h). The predominant inorganic product in both reactions was nitrite (54% versus 59%, respectively), but the nitrate yield differed significantly (12% versus 2.5%, respectively).

Mechanisms of Diazeniumdiolate Photolysis. An important clue to the mechanism by which anionic diazeniumdiolate **6** is photolyzed is the 5-fold increase in nitrate yield when the reaction was carried out in the light path of the spectrophotometer rather than in the dark. This result is reminiscent of that seen with Angeli's salt [NaON(O)═NONa], an anionic diazeniumdiolate whose N═N bond Donald et al. have reported to cleave photolytically; these authors concluded that their primary photoproducts were nitrite ion and triplet NO^-, with the latter species autoxidizing to nitrate via rearrangement of an intermediate peroxynitrite ($ONOO^-$) ion.[20] By analogy with the mechanism of Donald et al., we postulate that **6** photolyzes according to the pathway shown in Scheme 4B.

For the O^2-substituted diazeniumdiolates, we interpret our results in terms of the two competing reaction pathways (Paths A and B) shown in Scheme 5. Path A produces N_2O along with aminyl radical **7** and benzyloxy radical **8**. These radicals then proceed to observed products (diethylamine, benzaldehyde, benzyl alcohol). Path B involves initial formation of *N*-

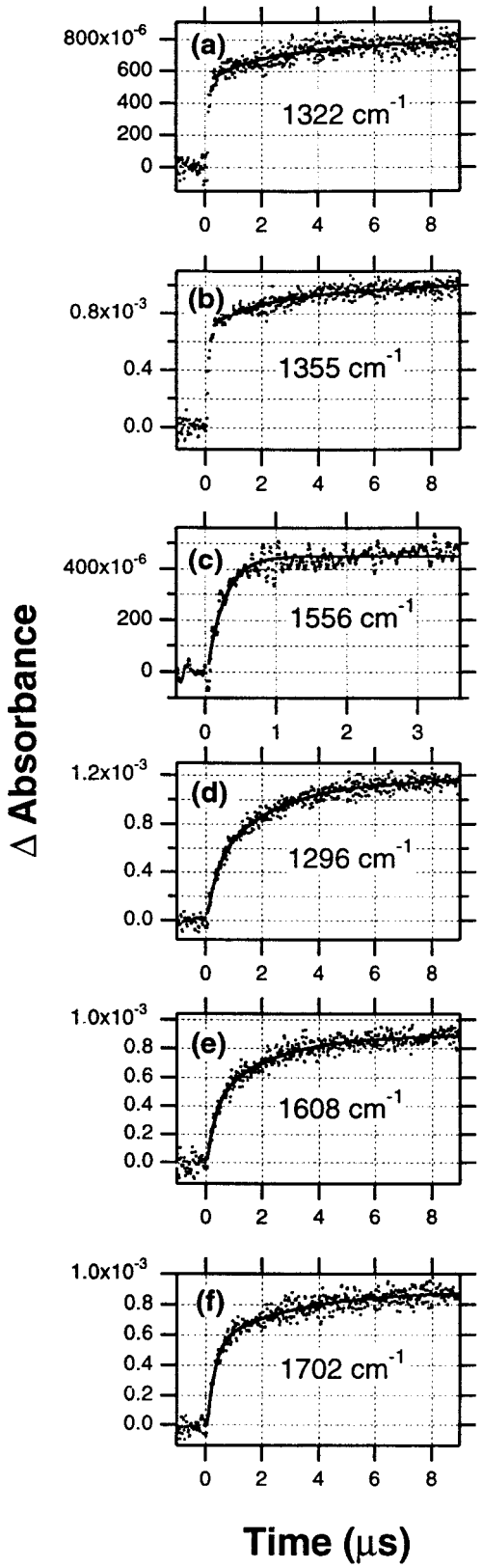
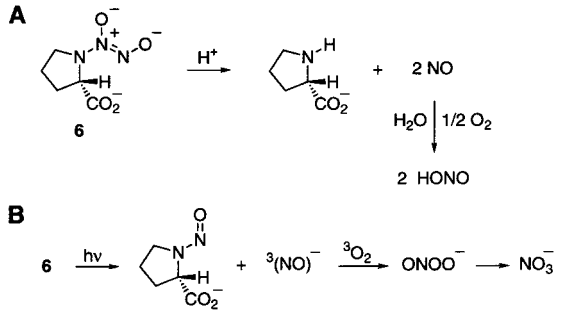


Figure 2. Kinetic traces observed following 266-nm laser photolysis (5 ns, 2.6 mJ) of benzyl derivative **5** (20 mM) in argon- (a−c) and oxygen-saturated (d−f) acetonitrile-d_3. The dotted curves are experimental data; the solid curves are calculated best fits to second-order kinetics (a, b, and d−f) or a single-exponential function (c). See text for details.

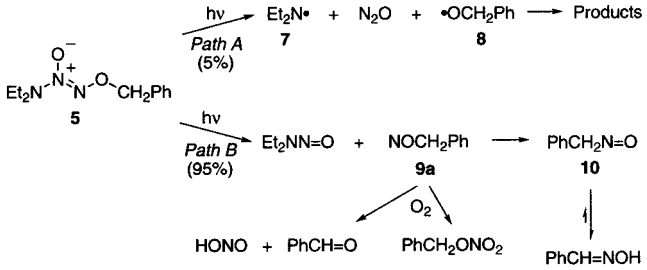
nitrosodiethylamine and the interesting oxygen-substituted nitrene intermediate **9a**. Only very limited reports of oxygen-substituted nitrenes exist in the literature,[21] in contrast to the

(20) Donald, C. E.; Hughes, M. N.; Thompson, J. M.; Bonner, F. T. *Inorg. Chem.* **1986**, *25*, 2676−2677.

Scheme 4. Solvolytic (A) versus Photochemical (B) Decomposition of **6** in Aerobic Sodium Hydroxide Solution



Scheme 5. Observed Photochemical Reaction Pathways for O^2-Substituted Diazeniumdiolate **5**



more thoroughly studied nitrogen-[22] or carbon-substituted[23] nitrenes. In fact, the direct observation of an O-nitrene (i.e., HON) has only very recently been reported.[24] Based on the yields of *N*-nitrosodiethylamine at low conversion and of N_2O, we estimate that photolysis of **5** proceeds approximately 5% through Path A and 95% through Path B.

Very high yields (>90%) of *N*-nitrosodiethylamine at low photochemical conversion suggest that this species is a primary photoproduct of O^2-substituted diazeniumdiolates. More direct evidence for the initial formation of *N*-nitrosodiethylamine upon photolysis of **5** was provided by TRIR spectroscopy. The rates of growth of signals observed at 1422, 1385, 1355, and 1322 cm^{-1} in argon-saturated acetonitrile-d_3 (e.g., Figure 2a−b) all consisted of a fast and unresolvable component (our current instrument response is approximately 50 ns) and a slower resolvable component. Comparison with the IR spectrum of an authentic sample confirmed that all of these bands belong to *N*-nitrosodiethylamine. The observed kinetics indicated that this species was formed via two routes. The fast component is attributable to direct production of the nitrosamine via Path B of Scheme 5. We believe the slower component represents recombination of aminyl radical **7** and NO, which are formed by photolysis in the original laser pulse of some initially produced *N*-nitrosodiethylamine.

$$2\mathrm{R} \xrightarrow{k_2} \mathrm{P} \tag{2}$$

$$[\mathrm{P}] = \frac{1}{2}\left[[\mathrm{R}]_0 - \frac{[\mathrm{R}]_0}{1 + [\mathrm{R}]_0 k_2 t}\right] \tag{3a}$$

or

$$A_\mathrm{P} = \frac{1}{2}\left[A_0 - \frac{A_0}{1 + A_0(k_2/\epsilon_\mathrm{P} l)t}\right] \tag{3b}$$

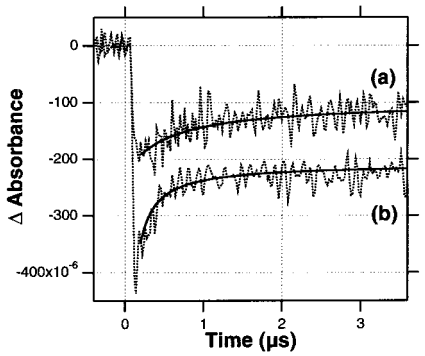


Figure 3. Kinetic traces observed following 266-nm laser photolysis [5 ns, (a) 0.17 and (b) 0.49 mJ] of 10 mM *N*-nitrosodiethylamine in acetonitrile-d_3. The dotted curves are experimental data observed at 1355 cm^{-1}; the solid curves are calculated best fits to second-order kinetics using eq 3. See text for details.

In support of this hypothesis, when *N*-nitrosodiethylamine itself was examined by TRIR spectroscopy, we observed at 1355 cm^{-1} (Figure 3) partial recovery of the depleted signal. This recovery can be fit to the second-order, equal concentration kinetic model[25] of eqs 2 and 3, where [P] is the concentration of P at time t, $[R]_0$ is the initial concentration of R, k_2 is the second-order rate constant, A_P is the absorbance of P at time t, A_0 is the initial absorbance of R, ϵ_P is the IR extinction coefficient of P at the frequency being monitored, and l is the optical path length. Analysis of the observed recovery kinetics with a 0.02-cm path length at 1355 cm^{-1} ($\epsilon = 12$ M^{-1} cm^{-1}) both at low (Figure 3a: 0.17 mJ) and higher (Figure 3b: 0.49 mJ) laser power (leading to low and higher initial radical concentration, respectively) provides the same, within experimental error (estimated as ±10%), second-order rate constant $k_2 = 2.0 \times 10^9$ M^{-1} s^{-1}. The slow kinetic growth observed following photolysis of **5** at 1322 cm^{-1} ($\epsilon = 11$ M^{-1} cm^{-1}) and 1355 cm^{-1} can be fit by means of eq 3 (Figure 2a,b). Such an analysis provides the second-order rate constants $k_2 = 1.3 \times 10^9$ M^{-1} s^{-1} and $k_2 = 1.4 \times 10^9$ M^{-1} s^{-1} at 1322 and 1355 cm^{-1}, respectively, in good agreement with each other and also with that derived for the partial recovery of *N*-nitrosodiethylamine.

To explain the significant amount of benzaldoxime formed upon photolysis of **5** under argon, we propose that the initially formed O-nitrene **9** rearranges to C-nitroso compound **10**. Such primary C-nitroso compounds are known to be unstable, tautomerizing to the corresponding oximes under the conditions of our experiments.[26] Presumably, this tautomerization occurs over time scales longer than were monitored in our TRIR experiments, since we have not detected signals that may be attributed to benzaldoxime over the first 180 μs following flash photolysis. We do, however, observe a band at 1556 cm^{-1} that is consistent with C-nitroso compound **10**.[27] The rate of growth of this signal can be fit to a single-exponential function with $k_{obs} = 4.0 \times 10^6$ s^{-1} (Figure 2c).

Recall (Table 1, Scheme 2) that when the photolysis was conducted under oxygen benzaldoxime was no longer detected as a product and the yields of benzaldehyde and benzyl nitrate increased dramatically. We propose that these products arose by efficient oxygen trapping of O-nitrene **9a**. The observed rate

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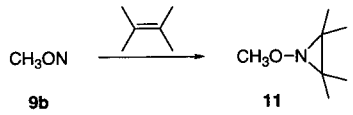
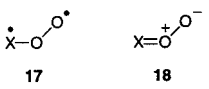
Scheme 6. Trapping of O-Nitrene Intermediate **9b** by 2,3-Dimethyl-2-butene



of growth in Figure 2c corresponded to production of C-nitroso **10** from O-nitrene **9a**, and this nitrene had a lifetime of approximately 250 ns. Based on the observation that the O-nitrene was completely quenched in the presence of millimolar concentrations of oxygen,[28] we estimate that the rate constant for O-nitrene reaction with oxygen is on the order of 10^9-10^{10} M^{-1} s^{-1}. This rate is consistent with that for a triplet carbene reaction with oxygen,[29] but is in stark contrast to reaction rates of aryl nitrenes with oxygen, which are known to be very sluggish,[30] typically near 10^5 M^{-1} s^{-1}.

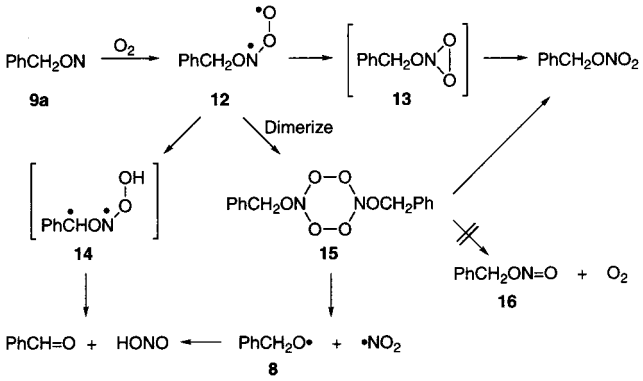
Additional evidence for the O-nitrene intermediate came from trapping **9b** in the presence of 2,3-dimethyl-2-butene to produce the known aziridine **11** (Scheme 6). The identity of the known aziridine produced was confirmed by comparison with an authentic sample. We have also characterized the fundamental chemistry of O-nitrene **9b**, which we show to have a triplet ground state, and will soon report on our findings.[31]

Kinetic studies of oxygen-saturated solutions of **5** by TRIR provide information on the formation of benzyl nitrate (Figure 2d−e) and benzaldehyde (Figure 2f) from the reaction of O-nitrene **9a** and oxygen. On the basis of these experiments and on previous studies of the reaction of aryl nitrenes with oxygen,[30] we have formulated the reaction pathways shown in Scheme 7. Sawaki and co-workers[30c−e] have shown that nitroso oxides are best described by radical structure **17** (X = RN) rather than the zwitterionic form **18**, which is more characteristic of carbonyl oxides (X = R_2C).



By analogy to the studies of Sawaki et al., we propose that the initial product of reacting O-nitrene **9a** with oxygen is biradical **12**, which may then proceed directly to benzyl nitrate via ring-closed intermediate **13**. Alternatively, aryl nitrene analogues of **12** have been shown to dimerize rapidly;[30a,b] thus, benzyl nitrate may also be formed from dimer **15**. Formation of benzaldehyde (and nitrous acid) can also occur directly from

Scheme 7. Proposed Mechanism for the Reaction of O-Nitrene **9a** with Oxygen



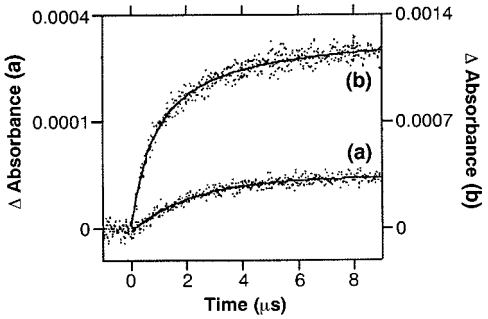


Figure 4. Kinetic traces observed for benzyl nitrate at 1296 cm^{-1} following 266-nm laser photolysis [5 ns, (a) 0.2 and (b) 2.6 mJ] of (a) 7 and (b) 20 mM benzyl derivative **5** in oxygen-saturated acetonitrile-d_3. The dotted curves are experimental data; the solid curves are calculated best fits to a single-exponential function (a) or second-order kinetics (b). See text for details.

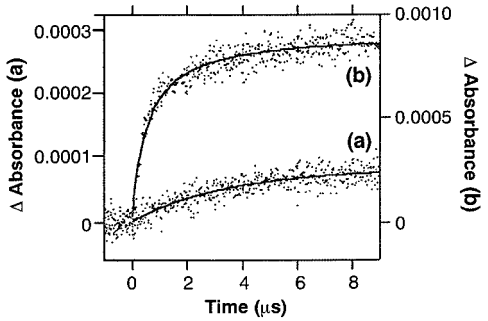


Figure 5. Kinetic traces observed for benzaldehyde at 1702 cm^{-1} following 266-nm laser photolysis [5 ns, (a) 0.2 and (b) 2.6 mJ] of (a) 7 and (b) 20 mM benzyl derivative **5** in oxygen-saturated acetonitrile-d_3. The dotted curves are experimental data; the solid curves are calculated best fits to a single-exponential function (a) or second-order kinetics (b). See text for details.

biradical **12** via **14** or it can be produced from dimer **15** and subsequent hydrogen atom abstraction from benzyloxy radical **8** by nitrogen dioxide.[32]

Consistent with Scheme 7, we have found that the growth kinetics for benzyl nitrate (Figure 4) and benzaldehyde (Figure 5) are affected significantly by the initial concentration of biradical **12**. Consider the case of benzyl nitrate. Once formed, we propose that biradical **12** has available two routes to benzyl nitrate: a direct first-order pathway through intermediate **13** and a second-order pathway involving rate-limiting formation of dimer **15** and its subsequent fast decay to products. The

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(32) The reaction of hydrogen atoms with nitrogen dioxide to form HONO has been studied by low-temperature IR spectroscopy: Guillory, W. A.; Hunter, C. E. *J. Chem. Phys.* **1971**, *54*, 598−603.

relative contributions of these two pathways will depend on the initial concentration of **12**, with the second-order pathway of course being favored at higher initial concentrations.

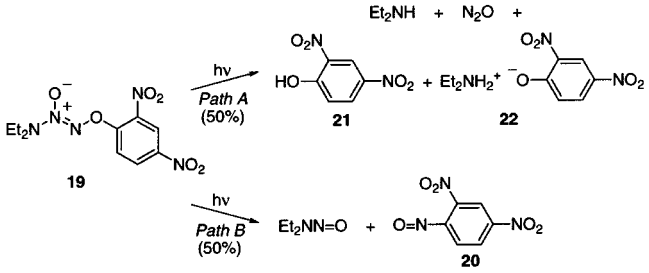
Accordingly, we found that at low initial [**12**] (resulting from a low starting concentration of **5** and low laser power) the formation of benzyl nitrate was best described by a single-exponential function with $k_{obs} = 4.4 \times 10^5\ s^{-1}$. At higher initial [**12**] (resulting from a higher starting concentration of **5** and higher laser power), however, the formation of benzyl nitrate did not fit a single-exponential function, but rather a second-order kinetic model with the rate constant for dimerization $k_2 = 1.2 \times 10^9\ M^{-1}\ s^{-1}$. (The kinetic data at low initial [**12**] could not be fit adequately to a second-order kinetic model.) An analogous treatment of the kinetic data for the formation of benzaldehyde leads to an observed first-order rate constant $k_{obs} = 2.9 \times 10^5\ s^{-1}$ at low initial [**12**] and a second-order rate constant for dimerization $k_2 = 1.7 \times 10^9\ M^{-1}\ s^{-1}$ at higher initial [**12**], both in reasonable agreement with the derived rate constants for benzyl nitrate.[33]

Previous work on products derived from aryl nitrenes plus oxygen[30] suggests that dimer **15** could decompose via a third pathway to nitrite ester **16** and oxygen. Nonetheless, we have found no evidence for the production of **16** in our product studies or in the TRIR experiments. Nitrite esters are known to photolyze efficiently to NO and alkoxy radicals.[34] Thus, if **16** were formed in an appreciable amount one would expect the yield of NO to increase in oxygen-saturated solutions of **5** photolyzed to high conversion. NO yields, however, are equivalent in argon- and oxygen-saturated solutions (Table 1).

Pharmacological Implications. Our findings presented thus far provide little encouragement for the deliberate photolysis of simple O^2-alkylated diazeniumdiolates as a strategy for generating pharmacologically useful NO. Not only are the ultimate yields of NO very low, but the reaction produces large amounts of potentially toxic *N*-nitroso compounds. For example, *N*-nitrosodiethylamine (produced in the cleavage of **1**, **4**, and **5**) has been shown to be carcinogenic to a great diversity of species, ranging from frogs and snakes to monkeys.[35]

Fortunately, nontoxic nitrosamines have been identified. Accordingly, one aspect of our drug discovery efforts has concentrated on O^2-substituted diazeniumdiolates whose photolyses would generate such species. A case in point is diazeniumdiolate **6**. We are presently studying its conversion to O^2-alkylated prodrug forms because the corresponding *N*-nitroso compound has been reported to be noncarcinogenic in numerous previous tests involving its long-term administration to rodents,[36] apparently because it is not metabolized. Other nitrosamines with ionizable functional groups may similarly be too polar to enter the cell for metabolic conversion to a DNA-damaging form, and thus should be quantitatively excreted unchanged.

Scheme 8. Observed Photochemical Reaction Pathways for O^2-(2,4-Dinitrophenyl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate **19**



An alternative to the production of noncarcinogenic nitrosamines is to avoid the production of nitrosamines entirely. Our initial investigations have revealed that the relative contributions of Path A and Path B of Scheme 5 can depend on the O^2-substituent. Thus, when dinitrophenyl derivative **19** was photolyzed to 14% conversion (Scheme 8), we isolated lower yields of *N*-nitrosodiethylamine (51%) and O-nitrene-derived product **20** (49%) and much higher yields of N_2O (45%) than were the cases with methyl and benzyl derivatives **4** and **5**. In addition, phenol **21** plus phenolate salt **22** were formed in high yield (41% total).

Conclusions

We have shown that the photoreactivity of O^2-substituted diazeniumdiolates involves two competing reaction pathways (Scheme 5), one of which produces potentially carcinogenic nitrosamines and an interesting O-nitrene intermediate. Product analysis and TRIR investigations have demonstrated that in the absence of a chemical trap, the O-nitrene rearranges to a C-nitroso compound that subsequently tautomerizes to isolable oxime. The O-nitrene can be trapped with alkene to form 1-alkoxyaziridines and also reacts very efficiently with oxygen to produce ultimately nitrate ester (or aldehyde and nitrous acid).

If drugs based on O^2-substituted diazeniumdiolates are to enjoy routine medical use, formation of carcinogenic nitrosamines must of course be avoided. Fortunately, initial experiments have suggested that electron-withdrawing O^2-aryl substituents inhibit the nitrosamine-forming pathway. Further experiments are in progress that aim to clarify the generality and magnitude of this effect.

Inadvertent photolysis of any diazeniumdiolate-based drug during storage or on formulation with a vehicle for administration is easily envisioned. Thus, the caveats regarding nitrosamine formation emanating from this investigation should be carefully borne in mind upon any attempt at medical application of the diazeniumdiolates.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from Aldrich Chemical Co. and used without further purification. Acetonitrile was distilled from P_2O_5 before use. Acetonitrile-d_3 (Cambridge Isotope Laboratories) was used as received. 2,3-Dimethyl-2-butene was passed through a small neutral alumina column immediately before use.

Melting points were measured with a Thomas-Hoover apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 300 (300 MHz) or a Varian XL200 (200 MHz) Fourier transform NMR spectrometer. Resonances are reported in δ units downfield from tetramethylsilane. Gas chromatography (GC) was

(33) Note that our kinetic model for the formation of benzaldehyde at high initial concentration of biradical **12** also requires that the hydrogen atom transfer reaction between benzyloxy radical **8** and nitrogen dioxide is rapid relative to the rate-limiting formation of dimer **15**.

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performed on a Varian 3700 instrument attached to a Hewlett-Packard 3590E Chemstation. GC-mass spectral (GC-MS) analysis was conducted on a Hewlett-Packard 5890 Series II gas chromatograph attached to a 5971A mass selective detector using HP-G10341B software. Ultraviolet (UV) data were obtained with a Hewlett-Packard 8451A or 8453 diode array spectrophotometer. Infrared (IR) spectra were recorded on a Nicolet-510 FTIR spectrometer or a Bruker IFS-55 Fourier transform IR spectrometer at 4-cm^{-1} resolution. HPLC analysis was performed with one of two systems: (1) a Waters system equipped with a 680 gradient controller, two Waters 510 pumps, a Waters 440 fixed wavelength detector, and a Hewlett-Packard 3590E Chemstation; and (2) a Perkin-Elmer Series 4 chromatograph equipped with PE 85B variable wavelength detector. Capillary electrophoresis was done using a Beckman P/ACE System 2000 equipped with a diode array detector and System Gold data station. Elemental analysis was done by Atlantic Microlab, Inc. (Norcross, GA).

Sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate[37] and compounds **1** (R = H),[6] **4**,[3a] **6**,[38] and **19**[39] were prepared as separately described.

***O*^{2}-Benzyl 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (5).** A slurry of sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (7.0 g, 0.045 mol) and 2 g of anhydrous sodium carbonate in 50 mL of dimethyl sulfoxide was cooled to 0 °C. To this mixture, under nitrogen, was added 18 mg (0.11 mmol) of silver acetate, then 4.76 mL (0.04 mol) of benzyl bromide dropwise. The reaction mixture was allowed to warm gradually to room temperature and was stirred overnight. Water (200 mL) was added, the mixture was filtered, and the filtrate was extracted with ether. The organic layer was dried over sodium sulfate and filtered through a layer of magnesium sulfate; evaporation of the solvent gave an orange oil. Purification was carried out on a 250-mL glass column packed with silica gel and eluted with 5:1 dichloromethane:ethyl acetate to give 3.9 g (51%) of **5** as a pale yellow oil: ^{1}H NMR δ 1.01 (6H, t, J = 7.1 Hz), 3.05 (4H, q, J = 7.1 Hz), 5.28 (2H, s), 7.38 (5H, m); ^{13}C NMR δ 11.31, 48.57, 75.51, 128.35, 128.37, 128.38, 138.58; IR (neat) 3071, 3036, 2875, 1728, 1510, 1461, 1414, 1377, 1243, 1053, 1004, 843, 758, 702 cm^{-1}; UV λ_{max} (CH_3CN) 238 nm (ϵ = 9.9 mM^{-1} cm^{-1}). Anal. Calcd for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 59.16; H, 7.67; N, 18.64.

Continuous Photolysis. Irradiations were performed on solutions of the reactants in acetonitrile or acetonitrile-d_3 in 1.0-cm path length quartz cuvettes, sealed with a rubber septum, and purged prior to irradiation with argon or oxygen for 15−20 min. Solutions were irradiated in a Rayonet reactor equipped with either 254- or 350-nm low-pressure Hg lamps. Quantum yields were determined with respect to the Crystal Violet actinometer.[40]

Analysis of the Reaction Mixtures. The products of photolysis were preliminarily identified by GC-MS and confirmed by GC and HPLC through co-injection of authentic compounds. The nongaseous products were quantified by GC or HPLC and NMR spectroscopy. Gas chromatography was done on a DB5 column (30 m, 0.32 mm ID, Alltech Associates, Deerfield, IL) and a temperature program of 5 min at 40 °C followed by an increase of 8 °C/min to 250 °C. We conducted HPLC employing either a Waters Symmetry column (4.6 × 250 mm), using acetonitrile/water with a gradient of [time (min), % water] (0, 80), (8, 80), (15, 65), (40, 50), (45, 50), (55, 80), or a reversed phase column from Phenomenex (Luna C18(2) 4.6 × 250 mm), using isocratic acetonitrile:water (55:45). In both cases, products were detected at 254 nm with a flow rate of 1.0 mL/min.

Assays for Nitrous Oxide and Nitric Oxide. The presence of nitric oxide and nitrous oxide was confirmed by GC-MS. Nitrous oxide was analyzed by gas chromatography on a Quardrex Mol Sieve 5-Å Plot column (30 m, 0.32 mm i.d.; Alltech, Deerfield, IL) and a mass selective detector. Nitric oxide was quantified indirectly as nitrate and nitrite. Oxygen was injected into the reaction vessel after the irradiation and the reaction mixture was allowed to stand for 1 h, after which it was directly analyzed by capillary electrophoresis with UV detection at 214 nm on a polyacrylamide-coated fused silica column (column dimensions: total length = 57 cm; detection length = 50 cm; i.d. = 75 μm) and 10 mM phosphate buffer of pH 3.2 containing 0.1% of Brij-30 (Calbiochem, La Jolla, CA).

Trapping of the O-Nitrene Intermediate upon Photolysis of 4 by 2,3-Dimethyl-2-butene. In a typical experiment, 49 mg (0.22 mmol) of **4**, 5.3 mg (0.043 mmol) of 2-methoxytoluene as internal standard, and 296 mg (3.35 mmol) of 2,3-dimethyl-2-butene were dissolved in 10 mL of acetonitrile. The mixture was degassed and then irradiated at 254 nm for 1 h. The reaction mixture was analyzed by GC-MS and the presence of 1-methoxy-2,2,3,3-tetramethylaziridine was confirmed from the mass spectra and by peak enhancement with an authentic sample prepared according to Brois.[21a] The amount of aziridine was estimated to be 0.013 mmol (6% yield).

Time-Resolved IR Methods. We conducted TRIR experiments following the method of Hamaguchi and co-workers[41] as described previously.[42] Briefly, the broadband output of a $MoSi_2$ IR source (JASCO) is crossed with excitation pulses from a Nd:YAG laser. Changes in IR intensity are monitored by an MCT photovoltaic IR detector (Kolmar Technologies, KMPV11-1-J1), amplified, and digitized with a Tektronix TDS520A oscilloscope. The experiment is conducted in the dispersive mode with a JASCO TRIR-1000 spectrometer. We collected the TRIR difference spectra using either a Continuum HPO-300 diode pumped Nd:YAG laser (266 nm, 10 ns, 0.4 mJ) or a Quantronix Q-switched Nd:YAG laser (266 nm, 90 ns, 0.4 mJ) operating at 200 Hz. Kinetic traces were collected by means of a Continuum Minilite II Nd:YAG laser (266 nm, 5 ns, 0.2−4 mJ) operating at 20 Hz.

Acknowledgment. J.P.T. gratefully acknowledges the Camille Dreyfus Teacher-Scholar Awards Program, the National Institutes of Health (R01 GM58109), and the American Cancer Society (IRG-58-005-39) for generous support of this research. N.K. gratefully acknowledges support from a UNCF/Parke-Davis Postdoctoral Fellowship. We also thank Professors Gerald J. Meyer and Alex Nickon for helpful comments. This work was supported in part by the National Cancer Institute under contract No. NO1-CO-56000.

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