J|A|C|S
A R T I C L E S

Controlled Photochemical Release of Nitric Oxide from *O*²-Benzyl-Substituted Diazeniumdiolates

Patrick H. Ruane, K. Mani Bushan, Christopher M. Pavlos, Raechelle A. D'Sa, and John P. Toscano*

Contribution from the Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218

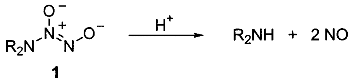
Received May 14, 2002

Abstract: An investigation of potential photosensitive protecting groups for diazeniumdiolates ($R_2N{-}N(O){=}NO^-$) has been initiated, and here the effect of meta electron-donating groups on the photochemistry of *O*²-benzyl-substituted diazeniumdiolates ($R_2N{-}N(O){=}NOCH_2Ar$) is reported. Photolysis of the parent benzyl derivative (Ar = Ph) results almost exclusively in undesired photochemistry—the formation of nitrosamine and an oxynitrene intermediate with very little, if any, photorelease of the diazeniumdiolate. We have been able to use meta substitution to tune the photochemistry of these benzylic systems. The desired diazeniumdiolate photorelease has been shown to become more substantial with stronger π-donating meta substituents. This effect has been verified by direct observation of the photoreleased diazeniumdiolate with ^{1}H NMR spectroscopy and by NO quantification measurements conducted in high- and low-pH solutions. In addition, the observed rates of NO release are consistent with that expected for normal thermal decomposition of the diazeniumdiolate in aqueous solutions and also show the same pH dependence.

Introduction

Compounds containing the diazeniumdiolate $[N(O){=}NO]^-$ functional group have proven useful as research tools in a variety of applications requiring spontaneous release of the critical bioregulatory molecule nitric oxide (NO).[1] Anions such as 1-(*N*,*N*-dialkylamino)diazen-1-ium-1,2-diolates **1** are stable as solid salts, but release up to 2 mol of NO when dissolved in aqueous solution at physiologically relevant conditions (Scheme 1). Keefer and co-workers have developed anions **1** with half-lives in aqueous buffer at pH 7.4 and 37 °C ranging from 2 s to 20 h.[2] These compounds have shown great potential in a variety of medical applications[3] requiring the rapid production (e.g., to produce a fast but transient drop in blood pressure[4]) or gradual release (e.g., to study the effects of prolonged cytostasis on vascular smooth muscle cells[5]) of NO.

Scheme 1



Recent efforts to make diazeniumdiolates more effective pharmaceuticals have concentrated on using derivatives of such compounds to deliver NO specifically to a targeted site.[6] 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 way to achieve targeted release of NO is through the use of photochemistry. Photosensitive precursors, usually called "caged compounds" or "phototriggers", have become important biomedical research tools.[7] Several direct photochemical precursors to NO (e.g., metal nitrosyl compounds,[8] nitrosothiols,[9] and bis-*N*-nitroso

---

* To whom correspondence should be addressed. Fax: (410) 516-8420. E-mail: jtoscano@jhu.edu.

(1) For a recent review of the chemistry of diazeniumdiolate derivatives, see: Hrabie, J. A.; Keefer, L. K. *Chem. Rev.* **2002**, *102*, 1135−1154.

(2) (a) Maragos, C. M.; Morley, D.; Wink, D. A.; Dunams, T. M.; Saavedra, J. E.; Hoffman, A.; Bove, A. A.; Isaac, L.; Hrabie, J. A.; Keefer, L. K. *J. Med. Chem.* **1991**, *34*, 3242−3247. (b) Keefer, L. K.; Nims, R. W.; Davies, K. M.; Wink, D. A. *Methods Enzymol.* **1996**, *268*, 281−293.

(3) (a) Morley, D.; Keefer, L. K. *J. Cardiovasc. Pharmacol.* **1993**, *22*, S3−S7. (b) Hanson, S. R.; Hutsell, T. C.; Keefer, L. K.; Mooradian, D. L.; Smith, D. J. *Adv. Pharmacol. (San Diego)* **1995**, *6*, 383−398. (c) Saavedra, J. E.; Southan, G. J.; Davies, K. M.; Lundell, A.; Markou, C.; Hanson, S. R.; Adrie, C.; Hurford, W. E.; Zapol, W. M.; Keefer, L. K. *J. Med. Chem.* **1996**, *39*, 4361−4365. (d) Pulfer, S. K.; Ott, D.; Smith, D. J. *J. Biomed. Mater. Res.* **1997**, *37*, 182−189. (e) Raulli, R. *J. Pharm. Pharmacol.* **1998**, *50*, 75−82. (f) Smith, D. J.; Simmons, M. L. *J. Controlled Release* **1998**, *51*, 153−159. (g) Jacobs, B. R.; Brilli, R. J.; Ballard, E. T.; Passerini, D. J.; Smith, D. J. *Am. J. Respir. Crit. Care Med.* **1998**, *158*, 1536−1542. (h) Fernandez-Tome, P.; Lizasoain, I.; Leza, J. C.; Lorenzo, P.; Mora, M. A. *Neuropharmacology* **1999**, *38*, 1309−1315. (i) Saavedra, J. E.; Shami, P. J.; Wang, L. Y.; Davies, K. M.; Booth, M. N.; Citro, M. L.; Keefer, L. K. *J. Med. Chem.* **2000**, *43*, 261−269. (j) Fitzhugh, A. L.; Keefer, L. K. *Free Radical Biol. Med.* **2000**, *28*, 1463−1469.

(4) Diodati, J. G.; Quyyumi, A. A.; Keefer, L. K. *J. Cardiovasc. Pharmacol.* **1993**, *22*, 287−292.

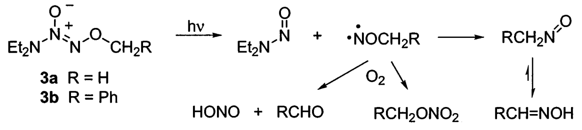
(5) Mooradian, D. L.; Hutsell, T. C.; Keefer, L. K. *J. Cardiovasc. Pharmacol.* **1995**, *25*, 674−678.

(6) (a) Saavedra, J. E.; Dunams, T. M.; Flippen-Anderson, J. L.; Keefer, L. K. *J. Org. Chem.* **1992**, *57*, 6134−6138. (b) Smith, D. J.; Chakravarthy, D.; Pulfer, S.; Simmons, M. L.; Hrabie, J. A.; Citro, M. L.; Saavedra, J. E.; Davies, K. M.; Hutsell, T. C.; Mooradian, D. L.; Hanson, S. L.; Keefer, L. K. *J. Med. Chem.* **1996**, *39*, 1148−1156. (c) Saavedra, J. E.; Billiar, T. R.; Williams, D. L.; Kim, Y.-M.; Watkins, S. C.; Keefer, L. K. *J. Med. Chem.* **1997**, *40*, 1947−1954. (d) Tang, X.; Xian, M.; Trikha, M.; Honn, K. V.; Wang, P. G. *Tetrahedron Lett.* **2001**, *42*, 2625−2629.

(7) For reviews, see: (a) McCray, J. A.; Trentham, D. R. *Annu. Rev. 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. In *Biological Applications of Photochemical Switches*; Morrison, H., Ed.; Wiley: New York, 1994; pp 243−305. (e) *Methods in Enzymology*; Marriott, G., Ed.; Academic Press: New York; Vol. 291, 1998. (f) Zacharias, D. A.; Baird, G. S.; Tsien, R. Y. *Curr. Opin. Neurobiol.* **2000**, *10*, 416−421. (g) Pellicciolі, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441−458.

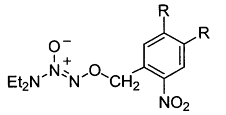
10.1021/ja026900s CCC: $22.00

Scheme 2



compounds[10]) have been developed and used in a variety of applications.[11] Photochemical precursors to diazeniumdiolate anions **1** have the added advantage of ultimately releasing NO at controlled, well-defined rates that can be varied by the substituents R, pH, or temperature.

The commonly employed 2-nitrobenzyl photosensitive protecting group[7] has been used to develop potential photochemical precursors **2** to diazeniumdiolates.[12] These derivatives have been used to inhibit thrombin-stimulated platelet aggregation,[12] to examine the induction of long-term depression in the cerebellum,[13] and to study long-term potentiation in cultured hippocampal neurons.[14]

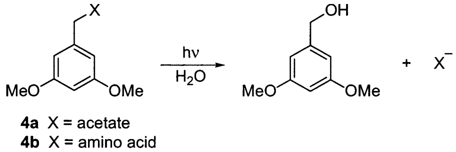


2a R = H
2b R = OMe
2c R = OCH$_2$CO$_2$Et

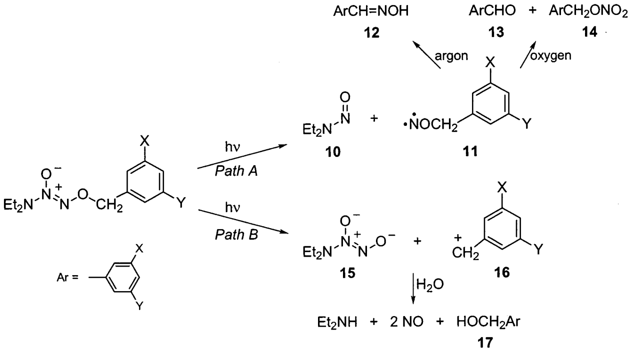
We have found, however, that the photochemistry of **2** is severely complicated by an efficient photochemical reaction that produces potentially carcinogenic nitrosamines and oxygen-substituted nitrenes.[15] Indeed, the photochemistry of **2** is exactly analogous to that of simple O^2-alkylated derivatives **3** (Scheme 2).[16]

We have begun an investigation of alternate photosensitive protecting groups for diazeniumdiolates. This study examines the use of the well-established meta effect of electron-donating groups in benzylic systems.[17] Such substitution has been shown to favor the formation of ionic products in photochemical reactions. Zimmerman first demonstrated the efficient photosolvolysis of 3,5-dimethoxybenzyl acetate (**4a**, Scheme 3) in 50% aqueous dioxane.[18] Benzyl acetates lacking π-electron-donating groups in the 3- or 5-position, on the other hand, undergo a photohomolysis reaction leading to radical-derived products. Barltrop[19] and Chamberlin[20] later described the use of the 3,5-dimethoxybenzyl group as an efficient phototrigger for the release of amino acids in aqueous ethanolic or dioxane solutions (**4b**, Scheme 3).

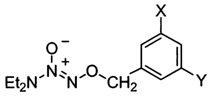
Scheme 3



Scheme 4



On the basis of this previous work, we have prepared a series of O^2-benzyl-substituted diazeniumdiolates, **2a,b**, **3b**, and **5**−**9**, and have examined the effect of electron-donating meta substituents on the observed photochemistry and on the efficiency of NO release.



5 X = OMe, Y = H
6 X = Y = OMe
7 X = NMe$_2$, Y = H
8 X = Y = NMe$_2$
9 X = Y = OH

Results and Discussion

Products of Photolysis. Analysis of the organic products observed following photolysis (Rayonet, 300 nm) of compounds **3b** and **5**−**8** (1 mM) in both argon- and oxygen-saturated 90% aqueous acetonitrile is summarized in Scheme 4 and Table 1. (Results observed with compound **9** will be discussed in more detail below.)

In our previous study of the photoreactivity of O^2-substituted diazeniumdiolates **3**, we reported that in acetonitrile the formation of oxime was completely quenched by molecular oxygen concomitant with increased yields of aldehyde and newly formed nitrate (Scheme 2).[15] In the present study, we find that although the yield of oxime is suppressed by oxygen, it is not completely quenched and attribute this difference to the lower solubility of oxygen in water vs acetonitrile.[21]

Quantification of NO released upon photolysis was performed electrochemically with an inNO Nitric Oxide Measuring System

---

(8) (a) Bettache, N.; Carter, T.; Corrie, J. E. T.; Ogden, D.; Trentham, D. R. In *Methods in Enzymology*; Packer, L., Ed.; Academic Press: New York, 1996; Vol. 268, pp 266−281. (b) Works, C. F.; Ford, P. C. *J. Am. Chem. Soc.* **2000**, *122*, 7592−7593 and references therein.

(9) (a) Sexton, D. J.; Muruganandam, A.; McKenney, D. J.; Mutus, B. *Photochem. Photobiol.* **1994**, *59*, 463−467. (b) Etchenique, R.; Furman, M.; Olabe, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 3967−3968.

(10) (a) Namiki, S.; Arai, T.; Fujimori, K. *J. Am. Chem. Soc.* **1997**, *119*, 3840−3841. (b) Namiki, S.; Kaneda, F.; Ikegami, M.; Arai, T.; Fujimori, K.; Asada, S.; Hama, H.; Kasuya, Y.; Goto, K. *Bioorg. Med. Chem.* **1999**, *7*, 1695−1702. (c) Yoshida, M.; Ikegami, M.; Namiki, S.; Arai, T.; Fujimori, K. *Chem. Lett.* **2000** 730−731.

(11) For reviews of NO-donor chemistry, see: (a) Gasco, A.; Fruttero, R.; Sorba, G. *Il Farmaco* **1996**, *51*, 617−635. (b) Hou, T.-C.; Janszuk, A.; Wang, P. G. *Curr. Pharm. Des.* **1999**, *5*, 417−441. (c) Feelisch, M. *Arch. Pharmacol.* **1998**, *358*, 113−122. (d) Yamamoto, T.; Bing, R. J. *Proc. Soc. Exp. Biol. Med.* **2000**, *225*, 200−206. (e) Janero, D. R. *Free Radical Biol. Med.* **2000**, *28*, 1495−1506. (f) Lehmann, J. *Expert Opin. Ther. Pat.* **2000**, *10*, 559−574. (g) Megson, I. L. *Drugs Future* **2000**, *25*, 701−715. (g) Wang, P. G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk, A. J. *Chem. Rev.* **2002**, *102*, 1091−1134.

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

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

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

(15) Srinivasan, A.; Kebede, N.; Saavedra, J. E.; Nikolaitchik, A. V.; Brady, D. A.; Yourd, E.; Davies, K. M.; Keefer, L. K.; Toscano, J. P. *J. Am. Chem. Soc.* **2001** *123*, 5465−5472.

(16) A very minor reaction pathway involving the extrusion of nitrous oxide (N$_2$O) is also observed upon photolysis of **3**.[15]

(17) (a) Zimmerman, H. E. *J. Am. Chem. Soc.* **1995**, *117*, 8988−8991. (b) Pincock, J. A. *Acc. Chem. Res.* **1997**, *30*, 43−49. (c) Zimmerman, H. E. *J. Phys. Chem. A* **1998**, *102*, 5616−5621.

(18) Zimmerman, H. E.; Sandel, V. R. *J. Am. Chem. Soc.* **1963**, *85*, 915−922.

(19) Barltrop, J. A.; Schofield, P. *J. Chem. Soc.* **1965**, 4758−4765.

(20) Chamberlin, J. W. *J. Org. Chem.* **1966**, *31*, 1658−1659.

(21) The concentration of oxygen in saturated water solutions is 1.3 mM, while that in saturated acetonitrile solutions is 9.1 mM (Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993; pp 289 and 293).

Table 1. Yields of Products Detected by HPLC Analysis Following Photolysis of *O*²-Substitued Diazeniumdiolates[a]

reactant	purge	nitrosamine **10**	oxime **12**	aldehyde **13**	nitrate **14**	alcohol **17**
3b	Ar	90	78	7	0	0
3b	O_2	85	53	21	27	0
5	Ar	95	*b*	*b*	0	6
5	O_2	95	*b*	*b*	*b*	6
6	Ar	74	37	5	0	9
6	O_2	90	31	14	*b*	13
7	Ar	67	44	13	0	40
7	O_2	71	37	12	*b*	36
8	Ar	53	35	30	0	27
8	O_2	53	16	16	*b*	27

[a] Based on percent reactant converted (approximately 10%). Estimated error ±2%. [b] Not determined.

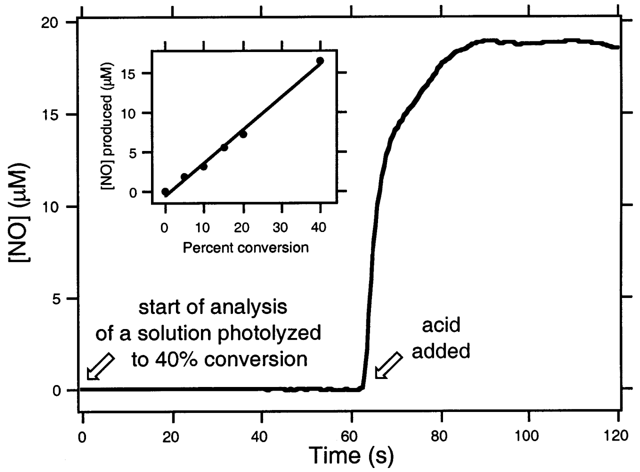


Figure 1. Quantification of NO yields observed after 300 nm photolysis of **7** (0.1 mM in 99/1 H_2O/CH_3CN) from 0 to 40% conversion (determined by HPLC analysis). See the text for details.

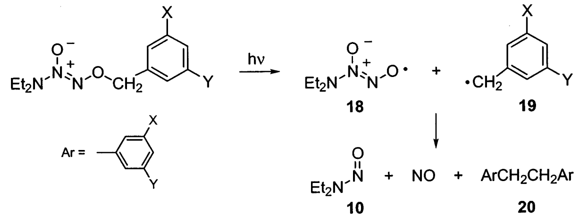
using an amiNO-2000 probe (Innovative Instruments Inc.). The amiNO-2000 probe was calibrated using ascorbic acid/sodium nitrite before and after NO measurements were conducted. To differentiate NO production arising from diazeniumdiolate **15** from that of non-diazeniumdiolate-derived NO, we utilized the pH dependence of diazeniumdiolate stability. At room temperature diazeniumdiolate **15** has a lifetime of several hours at pH 11, but only several seconds at pH 3. Aqueous solutions at pH 11 of each reactant were irradiated and then analyzed for NO. Any NO detected under these conditions must arise from a non-diazeniumdiolate pathway. For all reactants examined, the amount of NO formed from such a pathway was negligible. To liberate the NO associated with photoreleased diazeniumdiolate **15**, solutions were acidified with 1.0 M H_2SO_4 to pH 2−3 and NO analysis was continued. Substantial amounts of NO were then observed (e.g., Figure 1). (Control experiments indicate that there is no electrochemical response associated with this large pH change.) In each case, the yield of NO was determined from a series of measurements following photolysis to a range of percent conversions. NO yields were found to be linear for up to 20−40% conversion (e.g., Figure 1, inset). The NO yields determined from these experiments, along with the observation that diazeniumdiolate **15** dissociates to give 1.5 equiv of NO,[22,23] were used to derive yields of photoreleased diazeniumdiolate from the systems studied. These yields along with quantum yields of photodecomposition are reported in Table 2. (Again, results observed with compound **9** will be discussed in more detail below.)

Table 2. Quantum Yields for Photodecomposition and Yields of Diazeniumdiolate **15** Following Photolysis of *O*²-Substitued Diazeniumdiolates

reactant	solvent % H_2O/CH_3CN	photolysis λ (nm)	quantum yield[a]	% diazeniumdiolate **15**[b]
2a	99/1	300	*c*	5
2b	99/1	300	*c*	3
3b	99/1	300	*c*	trace
5	99/1	300	*c*	6
6	99/1	300	*c*	10
	99/1	254	0.17	*c*
	0/100	254	0.35	*c*
7	99/1	300	0.07	35
	0/100	300	0.27	*c*
	99/1	254	0.66	*c*
	0/100	254	0.81	*c*
8	99/1	300	0.08	47
	0/100	300	0.28	*c*
	99/1	254	0.51	*c*
	0/100	254	0.74	*c*

[a] Of reactant photodecomposition, determined with the potassium ferrioxalate actinometer.[24] [b] Based on percent reactant converted (determined by HPLC analysis), the yield of NO measured, and the observation that diazeniumdiolate **15** gives 1.5 equiv of NO.[22,23] Estimated error ±5%. [c] Not determined.

Scheme 5



Mechanistic Analysis. We have interpreted our results concerning the photochemistry of *O*²-substituted diazeniumdiolates **5**−**8** in terms of path A (undesired) and path B (desired) shown in Scheme 4. Other minor reaction pathways may contribute slightly to the overall photochemistry, so each individual product yield of Table 1 is not a quantitative measure of the relative contributions of paths A and B, but the results taken as a whole, along with the diazeniumdiolate yields of Table 2, give a very good indication of the partitioning between these two major reaction pathways.

Path A involves the production of carcinogenic[25] nitrosamine **10** and oxynitrene **11** and has been discussed in detail for **3** previously.[15] The oxynitrene either rearranges to an intermediate *C*-nitroso compound that forms oxime **12** or is trapped by oxygen to give aldehyde **13** (and HONO) and nitrate **14**. Path B involves the formation of ionic products, diazeniumdiolate **15** and benzyl cation **16**. In water, cation **16** is trapped to provide benzyl alcohol **17**; diazeniumdiolate **15** dissociates to diethylamine and NO.

In addition to production via path A, nitrosamine **10** could potentially be formed by way of the reaction pathway shown in Scheme 5. Here, photoinduced homolytic cleavage gives radicals **18** and **19**, which would be expected to provide

---

(22) Quantification of NO yields by chemiluminescence measurements[2] or ultrafiltration experiments[23] has indicated that diazeniumdiolate **15** dissociates to 1.5 equiv of NO, in contrast to most other derivatives, which provide 2 equiv of NO. We have independently confirmed these observations by electrochemical detection of NO. (See the Supporting Information.)

(23) Ramamurthi, A.; Lewis, R. S. *Chem. Res. Toxicol.* **1997**, *10*, 408−413.

(24) Calvert, N.; Pitts, N. J. *Photochemistry*; Wiley: New York, 1966; p 783.

(25) Lijinsky, W. *Chemistry and Biology of N-Nitroso Compound*; Cambridge University Press: Cambridge, U.K., 1992; p 399.

nitrosamine **10**, NO, and bibenzyl **20**. We observe no evidence for this reaction pathway. We have not detected bibenzyl **20** or any other products that might be expected to arise from benzyl radical **19** for any of the O^2-substituted diazeniumdiolates studied. In addition, NO is not detected at high pH where diazeniumdiolate **15** is stable, but radical **18** would still be expected to dissociate to nitrosamine **10** and NO. Indeed, as discussed above, we have not observed any NO that is produced via a pathway other than that shown in Scheme 4, path B.

Triplet sensitization experiments indicate that path B is a singlet-state reaction, consistent with expectations based on previous studies of analogous benzyl acetates.[17] Triplet-sensitized photolysis of **7** or **8** results in significantly diminished yields of the products of path B. For example, using 4,4′-dihydroxybenzophenone as the triplet sensitizer in argon-saturated 90% aqueous acetonitrile, only trace amounts of NO and the corresponding benzyl alcohol **17** are detected, while the yield of nitrosamine **10** increases dramatically.

The data of Tables 1 and 2 demonstrate that the relative contribution of paths A and B is strongly dependent on the aromatic ring substitution pattern. The yield of nitrosamine **10** decreases and that of NO and alcohol **17** increases with stronger π-donating meta substitutents. (We have found in the case of **8** that the corresponding benzyl alcohol **17** ($X = Y = NMe_2$) is photosensitive and under the conditions of our experiments undergoes secondary photolysis to aldehyde **13** ($X = Y = NMe_2$). In addition, and as observed previously,[15] oxime **12** is photosensitive and can also be converted to aldehyde **13** under the conditions of our experiments.) We estimate that the photodecomposition of O^2-substituted diazeniumdiolates **5**−**8** proceeds approximately 5%, 10%, 35%, and 50% through path B (Scheme 4), respectively.

***O^2*-3,5-Dihydroxybenzyl-Substituted Diazeniumdiolate 9.** Given our observation that the desired path B is favored with strong π-donating meta substituents, we examined dihydroxybenzyl derivative **9**. We hoped to take advantage of the enhanced acidity of phenolic protons in the excited state.[26] Photoinduced deprotonation to yield an oxyanionic meta-substituted derivative was expected to result in significant enhancement of the relative contribution of path B to the observed photochemistry.

Consistent with the expected large effect of oxyanionic meta substitution, when **9** is irradiated (Rayonet, 300 nm) in a pH 11.2 aqueous solution (containing 1% acetonitrile) to 5−35% conversion, the amount of NO detected corresponds to a yield of photoreleased diazeniumdiolate **15** of approximately 92%. A comparison of results observed with O^2-substituted diazeniumdiolate derivatives **5**−**9** is shown in Figure 2.

The photoproduction of **15** from dihydroxybenzyl derivative **9** was further verified by ^{1}H NMR spectroscopy (Figure 3). A 7 mM solution of **9** in D_2O/NaOD (pD 11) was photolyzed (Rayonet, 300 nm) to approximately 20% conversion. New signals for benzyl alcohol **17** ($X = Y = OH$), diazeniumdiolate **15**, nitrosamine **10**, and diethylamine were observed and characterized by spiking with authentic samples of each. Since dissociation of diazeniumdiolate **15** to NO and diethylamine is not expected under such basic conditions, we examined the photolysis of **15** itself. We have found that, under identical conditions, diazeniumdiolate **15** undergoes photolysis to diethylamine (and NO) and nitrosamine **10** (and NO^-).[27] A full characterization of the photochemistry of **15** and related diazeniumdiolates will soon be reported.[28]

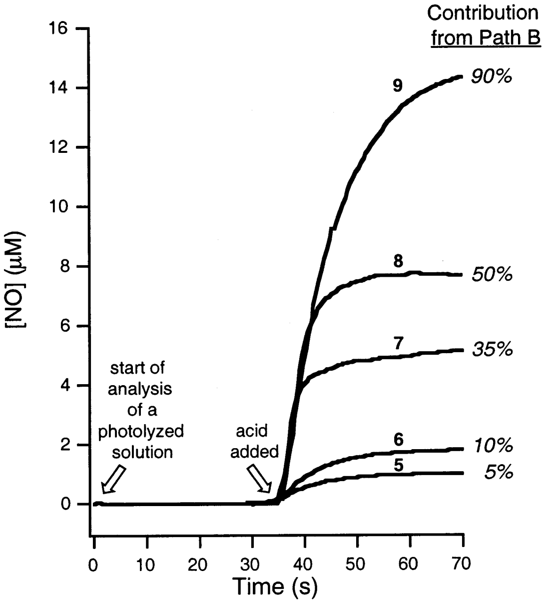


Figure 2. Comparison of NO yields and relative contributions to the observed photochemistry from path B (Scheme 4) following 300 nm photolysis of **5**−**9** (0.1 mM in 99/1 H_2O/CH_3CN). The photolyses were carried out at pH 11.2 to approximately 10% conversion. See the text for details.

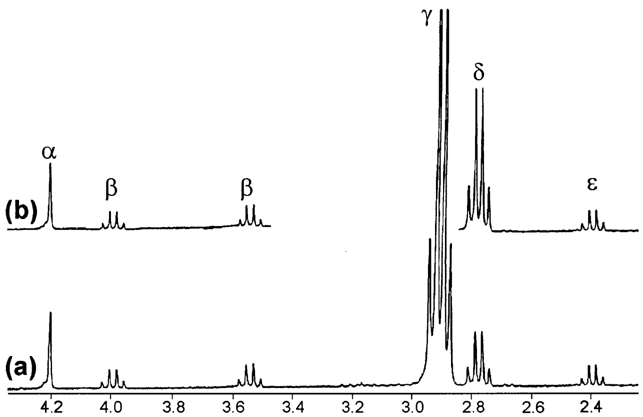


Figure 3. (a) ^{1}H NMR analysis of the products observed following photolysis (Rayonet, 300 nm) of a 7 mM solution of **9** in D_2O/NaOD (pD 11) to approximately 20% conversion. α = benzyl alcohol **17** ($X = Y = OH$), β = nitrosamine **10**, γ = dihydroxybenzyl derivative **9**, δ = diazeniumdiolate **15**, and ϵ = diethylamine. (b) ^{1}H NMR spectrum observed after spiking with an authentic sample of diazeniumdiolate **15**.

When **9** is irradiated (Rayonet, 300 nm) in pH 8.4 or 7.4 solutions, however, the yield of diazeniumdiolate **15** is substantially reduced (to 18% and 16%, respectively), while that of nitrosamine **10** is significantly enhanced. Excited-state deprotonation must not be rapid enough to compete with path A under these conditions. The pH 11.2 results are easily rationalized by simply considering that the pK_a of the phenolic protons of **9** is

---

(26) (a) Bartok, W.; Lucchesi, P. J.; Snider, N. S. *J. Am. Chem. Soc.* **1962**, *84*, 1842−1844. (b) Tolbert, L. M.; Solntsev, K. M. *Acc. Chem. Res.* **2002**, *35*, 19−27.

(27) The UV−vis absorption spectrum of diazeniumdiolate **15** in aqueous solution has $\lambda_{max} = 248$ nm and tails out to $A = 0$ at approximately 320 nm. Thus, to avoid secondary photolysis of photoreleased diazeniumdiolates, O^2-protected precursors should ideally be photolyzed with light of wavelength greater than 320 nm.

(28) D'Sa, R. A.; Ruane, P. H.; Kumar, N. A.; Relyea, H. A.; Toscano, J. P. Manuscript in preparation.

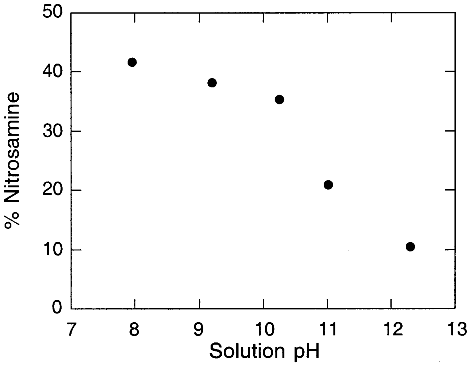


Figure 4. HPLC-derived yield of nitrosamine **10** observed after 300 nm photolysis of **9** (0.1 mM in 99/1 H_2O/CH_3CN) as a function of solution pH.

approximately 10. Thus, at pH 11.2 this compound is already deprotonated when the excited state is formed and path B becomes dominant. Consistent with this interpretation, the yield of nitrosamine is dependent on solution pH and shows a marked decrease at pH values above 10 (Figure 4).[29]

The quantum yield for photodecomposition of **9** in aqueous solution is high. For 254 nm photolysis, the quantum yield (determined with the 1,3-cycloheptadiene actinometer[30]) is 0.87 at both pH 7 and pH 12; for 300 nm photolysis, the quantum yield (determined with the Reinecke's salt actinometer[31]) is 0.14 at pH 7 and 0.36 at pH 12.

NO Release Rate. The rate of NO release from diazeniumdiolates **1** can be controlled by factors such as the substituents R, pH, and temperature.[2] Thus, an advantageous feature of photoprecursors **5**−**9** is that the flux of NO can be controlled (and varied) by these factors. Since 2-nitrobenzyl derivatives **2** have been reported to release NO much faster than expected (within 5 ms, rather than over the course of several minutes) upon photolysis,[12] we wished to confirm that the NO release rate observed with photoreleased diazeniumdiolate **15** is indeed identical to that observed upon normal thermal decomposition of **15**. As demonstrated in Figure 5, the NO release rate observed following photolysis of dimethylamino derivative **7** compares very favorably to that found for thermal dissociation of the sodium salt of **15** and, moreover, shows the same pH dependence. These release rates are consistent with those that have been reported previously.[2,23,32]

Conclusions

The effect of meta electron-donating groups on the photochemistry of O^2-benzyl-substituted diazeniumdiolates has been examined. Photolysis of parent benzyl derivative **3b** results almost exclusively in undesired photochemistry—the formation of nitrosamine **10** and oxynitrene **11** with very little, if any, photorelease of diazeniumdiolate **15**. We have been able to use meta substitution to tune the photochemistry of these benzylic systems. The desired photorelease of diazeniumdiolate **15** has been shown to become more substantial with stronger π-donating meta substituents. This photorelease has been verified by direct observation of diazeniumdiolate **15** with ^1H NMR spectroscopy and by NO quantification measurements conducted in high- and low-pH solutions. In addition, the observed rates of NO release are consistent with that expected for normal thermal decomposition of diazeniumdiolate **15** in aqueous solutions and also show the same pH dependence.

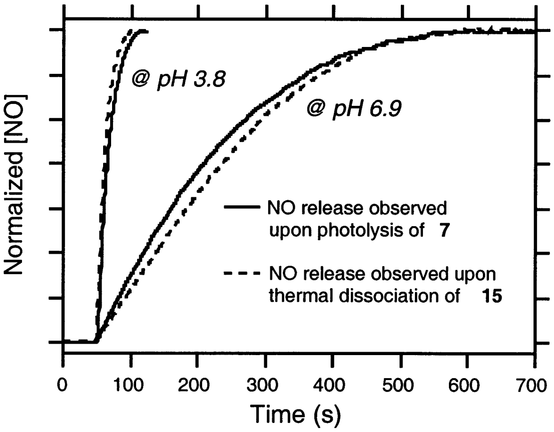


Figure 5. NO release rates observed following photolysis of pH 3.8 and 6.9 solutions of **7** (0.1 mM in 99/1 H_2O/CH_3CN) compared with the corresponding rates observed for the thermal dissociation of the sodium salt of diazeniumdiolate **15**.

We are currently extending the systems studied here to related derivatives that show efficient photochemical release of diazeniumdiolates at neutral pH upon photolysis with longer (≥350 nm) wavelength light.[33] These results will soon be reported.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from Aldrich Chemical Co. and used without further purification. Acetonitrile and dichloromethane were distilled from calcium hydride, and tetrahydrofuran was distilled from sodium/benzophenone before use. *N,N*-Dimethylformamide (DMF) was dried by azeotropic distillation with benzene and then further distilled over neutral alumina under vacuum. Melting points were determined on a Melt Temp II apparatus and are uncorrected. Infrared spectra were recorded on a Bruker IFS 55 Fourier transform IR spectrometer at 4 cm^{-1} resolution. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 300 (300 MHz) or a Varian Unity Plus 400 (400 MHz) Fourier transform NMR spectrometer. Resonances are reported in δ units downfield from that of tetramethylsilane. Mass spectra were collected with a VG 70-S mass spectrometer in the fast atom bombardment mode with sample introduction via direct probe. HPLC analysis was performed on a Waters Delta 600 system equipped with a model 6000A pump, a model 2487 dual-wavelength UV detector, and a model U6K injector with a 20 μL injector loop (Rheodyne). A Waters C-18 symmetry analytical column (3.9 × 150 mm) was used. Absorption spectra were obtained using a Hewlett-Packard 8453 diode array spectrophotometer.

General Procedure for Coupling 1-(*N,N*-Diethylamino)diazen-1-ium-1,2-diolate (15) with Benzyl Bromides. A slurry of 0.39 g (2.52 mmol) of diazeniumdiolate **15**, prepared as described previously,[2,34] in 10 mL of dry tetrahydrofuran was cooled to −78 °C. To this mixture was added 1 equiv of the appropriate benzyl bromide (prepared by literature methods[35]) in 2 mL of DMF via cannula. The reaction mixture was stirred under an inert atmosphere and allowed to warm to room

---

[29] The products observed upon photolysis of derivatives **5**−**8** are not dependent on solution pH.

[30] Numao, N.; Hamada, T.; Yonemitsu, O. *Tetrahedron Lett.* **1977**, *19*, 1661−1664.

[31] Wegner, E. E.; Adamson, A. W. *J. Am. Chem. Soc.* **1966**, *88*, 394−404.

[32] Horstmann, A.; Menzel, L.; Gabler, R.; Jentsch, A.; Urban, W.; Lehmann, J. *Nitric Oxide: Biol. Chem.* **2002**, *6*, 135−141.

[33] Bushan, K. M.; Xu, H.; Ruane, P. H.; D'Sa, R. A.; Pavlos, C. M.; Smith, J. A.; Toscano, J. P. Manuscript in preparation.

[34] Drago, R. S.; Karsetter, B. R. *J. Am. Chem. Soc.* **1961**, *83*, 1819−1822.

[35] (a) 3-Methoxybenzyl bromide: Vejdelek, Z. J.; Zemecek, O.; Musil, V.; Simek, A. *Collect. Czech. Chem. Commun.* **1964**, *29*, 776−794. (b) 3,5-Dimethoxybenzyl bromide: Takatori, K.; Nishihara, M.; Nishiyama, Y.; Kagiwara, M. *Tetrahedron* **1998**, *54*, 1585−1588.

temperature over 24 h. Diethyl ether (20 mL) was then added; the sodium bromide was removed by gravity filtration, diethyl ether and tetrahydrofuran were removed by rotary evaporation, and DMF was removed under vacuum. The resulting yellow oil was dissolved in dichloromethane and washed with water. The organic layer was dried over magnesium sulfate and concentrated by rotary evaporation to yield the desired product, which was purified on a silica column using dichloromethane as the eluent.

Data for *O*^{2}-(3-methoxybenzyl)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate (5): 80% yield as a yellow oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.05 (t, 6H, $J = 8$ Hz), 3.07 (q, 4H, $J = 8$ Hz), 3.82 (s, 3H), 5.25 (s, 2H), 6.86−6.88 (d, 1H, $J = 8.5$ Hz), 6.93−6.99 (m, 2H), 7.23−7.28 (m, 1H); ^{13}C NMR (400 MHz, CDCl$_3$) δ 11.64, 48.89, 55.39, 75.71, 113.62, 114.56, 128.81, 129.71, 137.38, 159.86; MS (FAB) *m*/*z* (rel intens) 276 (M + Na, 100), 254 (M + H, 3); UV−vis (CH$_3$CN) $\lambda_{\max}$ 273 nm ($\epsilon = 3555$ M^{-1} cm^{-1}).

Data for *O*^{2}-(3,5-dimethoxybenzyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (6): 90% yield as a yellow oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.05 (t, 6H, $J = 7$ Hz), 3.05 (q, 4H, $J = 7$ Hz), 3.80 (s, 6H), 5.27 (s, 2H), 6.41 (t, 1H), 6.53 (d, 2H).

Preparation of *O*^{2}-(3-(Dimethylamino)benzyl)- and *O*^{2}-(3,5-Bis-(dimethylamino)benzyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (7, 8). The methyl esters of the commercially available 3-amino- and 3,5-diaminobenzoic acids were prepared according to literature procedures.[36] Each amino group was then methylated to the corresponding trimethylammonium salt using a 5-fold excess of methyl iodide.[37] Reduction of the ammonium salts with a large excess of lithium aluminum hydride[38] gave the desired 3-(dimethylamino)- and 3,5-bis-(dimethylamino)benzyl alcohols, respectively, in 80% yield. The bromide precursors were prepared by reacting the corresponding benzyl alcohols with bromide ion.[34] These bromides were too reactive to be isolated and were coupled immediately with diazeniumdiolate **15** to afford **7** and **8** in yields of ca. 20%.

Data for *O*^{2}-(3-(dimethylamino)benzyl)-1-(*N*,*N*-diethylamino)-diazen-1-ium-1,2-diolate (7): 30% yield as a yellow oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.07 (t, 6H, $J = 8$ Hz), 2.97 (s, 6H), 3.06 (q, 4H, $J = 8$ Hz), 5.24 (s, 2H), 6.67−6.75 (m, 3H), 7.18−7.26 (m, 1H); ^{13}C NMR (400 MHz, CDCl$_3$) δ 11.6, 40.69, 48.86, 76.4, 112.46, 112.82, 116.73, 129.28, 136.61, 150.85; MS (FAB) *m*/*z* (rel intens) 289 (M + Na, 100), 265 (M − H, 5); UV−vis (CH$_3$CN) $\lambda_{\max}$ 306 nm ($\epsilon = 3058$ M^{-1} cm^{-1}).

Data for *O*^{2}-(3,5-bis(dimethylamino)benzyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (8): 20% yield as a yellow oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.05 (t, 6H, $J = 8$ Hz), 2.96 (s, 12H), 3.07 (q, 4H, $J = 8$ Hz), 5.20 (s, 2H), 6.02−6.03 (s, 1H), 6.19−6.20 (s, 2H); ^{13}C NMR (400 MHz, CDCl$_3$) δ 11.81, 41.03, 48.95, 77.235, 97.78, 102.53, 137.24, 152.01; MS (FAB) *m*/*z* (rel intens) 332 (M + Na, 82), 309 (M$^+$, 20); UV−vis (CH$_3$CN) $\lambda_{\max}$ 317 nm ($\epsilon = 3704$ M^{-1} cm^{-1}).

***O*^{2}-(3,5-Dihydroxybenzyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (9).** The commercially available 3,5-dihydroxybenzoic acid was esterified in quantitative yield to afford the corresponding methyl ester.[39] The phenolic hydroxy groups were converted to methoxymethyloxy functionalities.[40] Lithium aluminum hydride reduction gave the alcohol,[39] which was brominated under nonacidic conditions.[34] The bromide was coupled with **15** as described above in high yield, and the methoxymethyloxy groups were removed using aqueous acid to afford **9** in quantitative yield: ^{1}H NMR (300 MHz, CDCl$_3$), δ 1.15 (s, 6H, $J = 7$ Hz), 3.09 (q, 4H, $J = 7$ Hz), 5.12 (s, 2H), 6.30 (t, 1H, $J = 2$ Hz), 6.44 (d, 2H, $J = 2$ Hz), 6.80 (s br, 2H).

Preparation of Authentic Samples for Product Analysis. The required benzaldehyde[41] and oxime[42] derivatives were synthesized (from the corresponding benzyl alcohols) according to literature procedures.

Acknowledgment. J.P.T. gratefully acknowledges the National Institutes of Health (Grant R01 GM58109) for generous support of this research. A Camille Dreyfus Teacher-Scholar Award and an Alfred P. Sloan Research Fellowship also support this research.

Supporting Information Available: Quantification of the amount of NO formed following thermal dissociation of varying concentrations of diazeniumdiolate **15** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA026900S

---

(36) (a) Methyl 3-aminobenzoate: Elhadi, F. E., Ollis, W. D., Stoddart, J. F. *J. Chem. Soc., Perkin Trans. 1* **1982**, *8*, 1727−1730. (b) Methyl 3,5-diaminobenzoate: Kraft, A.; Osterod, F. *J. Chem. Soc., Perkin Trans. 1* **1998**, *6*, 1019−1025.

(37) Drewes, S. E.; Magojo, H. E. M.; Sutton, D. A. *J. Chem. Soc., Perkin Trans. 1* **1975**, *13*, 1283−1284.

(38) (a) 3-(Dimethylamino)benzyl alcohol: Sakurai, H.; Izuoka, A.; Sugawara, T. *J. Am. Chem. Soc.* **2000**, *122*, 9723−9734. (b) 3,5-Bis(dimethylamino)-benzyl alcohol: Connolly, C. J. C.; Hamby, J. M.; Schroeder, M. C.; Barvian, M.; Lu G. H.; Paneck, R. L.; Amar, A.; Shen, C.; Kraker, A. J.; Fry, D. W.; Klohs, W. D.; Doherty, A. M. *Biol. Org. Med. Chem. Lett.* **1997**, *7*, 2415−2420.

(39) Stewart, G. M.; Fox, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 4354−4360.

(40) Duffley, R. P.; Handrick, G. R.; Uliss, D. B.; Lambert, G.; Dalzell, H. C.; Razdan, R. K. *Synthesis* **1980**, *9*, 733−736.

(41) Sakurai, H.; Izuoka, A.; Sugawara, T. *J. Am. Chem. Soc.* **2000**, *122*, 9723−9734.

(42) Gale, D. J.; Wilshire, J. F. K. *Aust. J. Chem.* **1975**, *28*, 2447−2458.