The Secondary Amine/Nitric Oxide Complex Ion $R_2N[N(O)NO]^-$ as Nucleophile and Leaving Group in S_NAr Reactions

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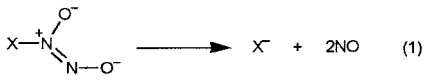
Ions of structure $R_2N[N(O)NO]^-$ and their alkylation products have seen increasing use as nitric oxide (NO)-generating agents for biomedical research applications. Here we show that such diazeniumdiolate anions can readily displace halide from a variety of electrophilic aza- or nitroaromatic substrates to form O^2-arylated derivatives of structure $R_2N-N(O)=N-OAr$. The site of arylation and the cis arrangement of the oxygens were confirmed by X-ray crystallography. Displacement by various nucleophiles showed $R_2N[N(O)NO]^-$ to be a reasonably good leaving group, with rate constants for displacement by hydroxide, methoxide, and isopropylamine that were between those of chloride and fluoride in the S_NAr reactions we surveyed. The Meisenheimer intermediate could be spectrally observed. These O^2-aryl diazeniumdiolates proved capable of reacting with the nucleophilic sulfur of the HIV-1 p7 nucleocapsid protein's zinc finger assembly to eject the zinc, disrupting a structural motif critical to viral replication and suggesting possible utility in the drug discovery realm.

Introduction

Diazeniumdiolate anions (**1**) are of current interest for their ability to generate bioregulatory nitric oxide (NO) spontaneously in physiological fluids, as in eq 1.[1−4] They can be alkylated at the terminal oxygen to produce charge-neutral derivatives[5] that are proving useful as potential prodrugs, molecules that are stable in aqueous media but that are capable of generating nitric oxide beneficially when the protecting group is hydrolytically or enzymatically removed in the target cell.[6−11]



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We are interested in exploring the potential utility of the corresponding aryl derivatives. To this end, we introduce a series of such compounds below, compare the reactivity of **1** with that of chloride as a leaving group in S_NAr reactions, and report on the ability of the O^2-aryl diazeniumdiolates[12] to degrade a protein's zinc finger assembly by electrophilically attacking the constituent thiolate ligands.

Results and Discussion

Diazeniumdiolate Ions as Nucleophiles. We began by studying the reactivity of various electron-deficient aryl halides with the DiEthylAmine/NO adduct, DEA/NO (**2**; see Scheme 1). In general, aryl rings with two or more activating moieties reacted smoothly to give the anticipated products. Thus, not only could 2,4-dinitrophenyl derivative **3a** be prepared in good yield, but so too could the two analogues in which one or the other of the nitro groups is replaced by trifluoromethyl (**3b** and **3c**). Analogous nitropyridyl compounds (**3d** and **3e**)

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(12) We refer to the compounds of structure $R_2NN(O)=NOAr$ studied herein as "O^2-arylated 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. Rationale for maintaining the "diazeniumdiolate" root for all such ions having potential biomedical relevance is discussed in detail in 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.

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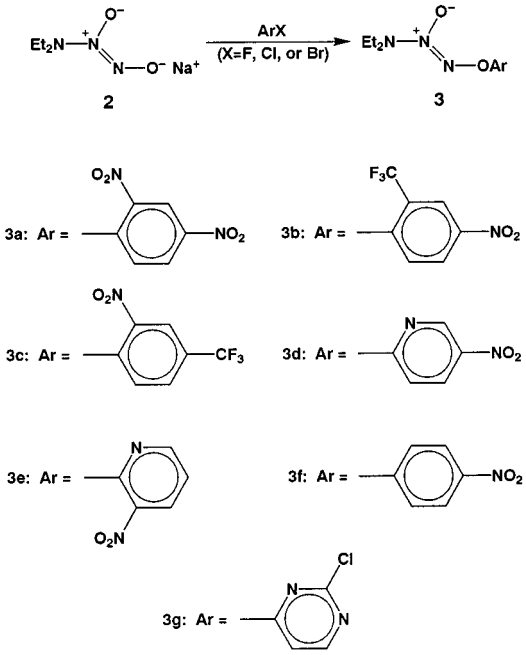
Scheme 1. Scope of Electrophilic Diazeniumdiolation Reaction. Reactions of 2 with Various Aromatic Electrophiles

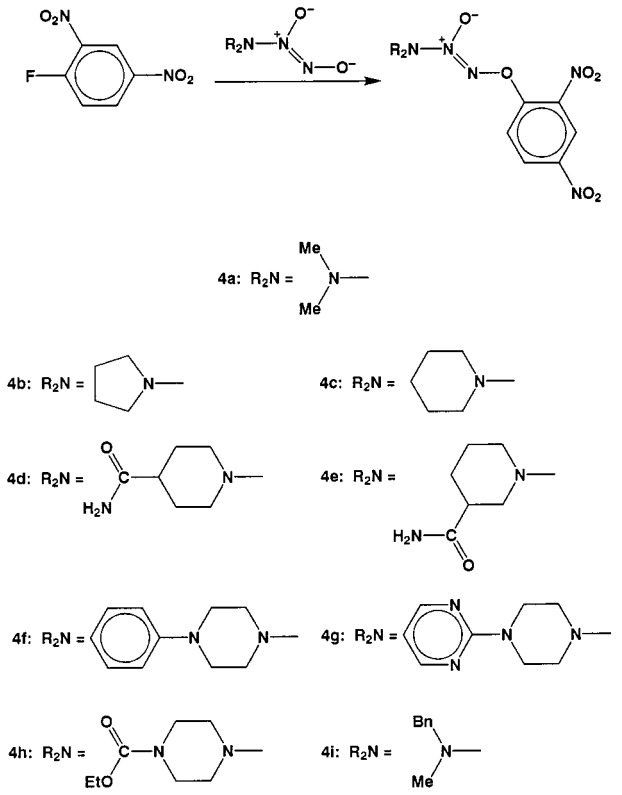


readily formed, but the otherwise unsubstituted halopyridines have thus far not been successfully linked to diazeniumdiolates. However, a single nitro group in the *para* position was sufficiently activating that DEA/NO could displace halide to produce **3f**, consistent with the view that the nitro group is a more potent inducer of S_NAr reactivity than a heteroaromatic nitrogen.[13,14] Two nitrogens in the ring, on the other hand, could under appropriate circumstances allow for facile displacement of halide; an interesting case in point is the reaction of DEA/NO with 2,4-dichloropyrimidine, in which the 4-position was readily substituted by displacing the halide to produce **3g** but the 2-position could not be substituted by the anion of **2** in dimethyl sulfoxide solution even when silver ion was added to the medium. This result is consistent with the known reactivities of the 2 and 4 positions in 2,4-dichloropyrimidine toward other nucleophiles.[15]

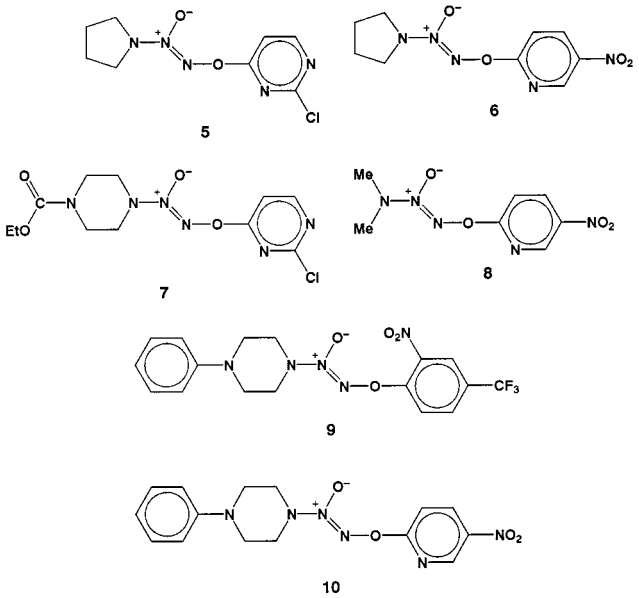
Similar results were seen when the diazeniumdiolate ions formed by reacting nitric oxide with a variety of other secondary amines were in turn reacted with 1-fluoro-2,4-dinitrobenzene, as expected. These reactions are summarized in Scheme 2, with the structures of six additional compounds synthesized for this investigation shown in Scheme 3.

Interestingly, the ortho and para nitro groups of 1,3-difluoro-4,6-dinitrobenzene (**11**; see Scheme 4) activated both fluorines for displacement by diazeniumdiolate ions. Thus exposure to excess DEA/NO converted the difluoride to symmetrically substituted **12** (Scheme 4), but reaction of the difluoride first with *N*-methylaniline to produce **13** followed by exposure to the dimethylamino analogue of **2** (DMA/NO) produced **14**. This order of addition was

Scheme 2. Scope of Electrophilic Diazeniumdiolation Reaction (part 2). Reactions of 1-Fluoro-2,4-dinitrobenzene with Various $R_2N[N(O)NO]^-$ Ions



Scheme 3. Additional *O*^{2}-Aryl Diazeniumdiolates Prepared for This Investigation



necessary because reaction of monodiazeniumdiolate **15** with *N*-methylaniline led to extensive displacement of the diazeniumdiolate ion as well as of fluoride. Similarly, *N*-methylaniline displaced the diazeniumdiolate ion from **14** as well. These reactions are shown in Scheme 4.

The Diazeniumdiolate Ion as a Leaving Group. These latter reactions demonstrated that the diazeniumdiolate ion can serve as a leaving group as well as a nucleophile in S_NAr reactions. To probe this behavior further, we studied the reaction of **3a** with hydroxide,

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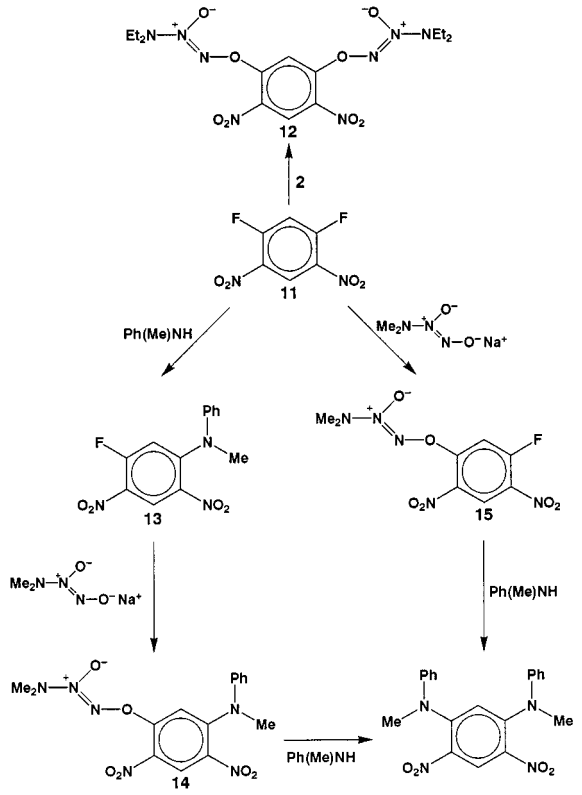
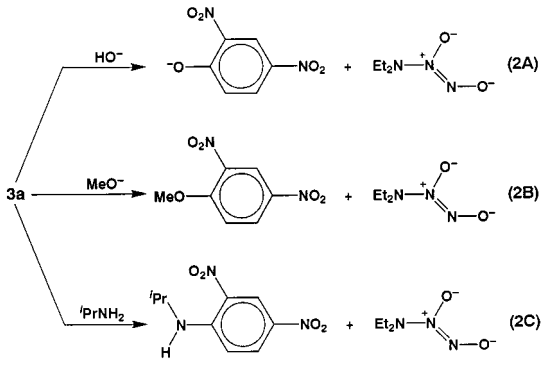
Scheme 4. Amination versus Diazeniumdiolation of 1,3-Difluoro-4,6-dinitrobenzene (11)



methoxide, and isopropylamine (Table 1). While no displacement of the diazeniumdiolate ion was detectable when **3a** was placed in water at around pH 7, raising the pH led to release of 2,4-dinitrophenoxide and diazeniumdiolate ions (eq 2A). When the reaction of **3a** with hydroxide was studied kinetically by monitoring loss of substrate by HPLC while maintaining constant ionic strength with sodium perchlorate, it was found to be first-order in substrate and hydroxide ion, with a second-order rate constant, 7×10^{-3} M^{-1} s^{-1} at 25 °C, that compares with values of 1.2×10^{-1} and 1.2×10^{-4} M^{-1} s^{-1} reported for the reaction of hydroxide with 1-fluoro- and 1-chloro-2,4-dinitrobenzene, respectively.[14]



The reaction of **3a** with methoxide (eq 2B) was examined both in methanol and in dimethyl sulfoxide. Ultraviolet spectral changes observed for the reaction of 0.2 mM substrate with excess sodium methoxide in methanol (Figure 1) showed the characteristic **3a** absorption maximum ($\lambda_{max} = 302$ nm, $\epsilon = 16$ mM^{-1} cm^{-1}) shifting to 292 nm ($\epsilon = 12$ mM^{-1} cm^{-1}) and a second maximum appearing at 250 nm ($\epsilon = 16$ mM^{-1} cm^{-1}), leading to a final

Table 1. Rate and Activation Parameters for Reaction of 3a with OH^-, CH_3O^-, and *i*-$PrNH_2$ [a]

nucleophile	solvent	k_2 at 25 °C (M^{-1} s^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})
OH^-	H_2O	7.4×10^{-3}	45.2	−134
MeO^-	MeOH	110×10^{-3}	57.5	−70.2
i-$PrNH_2$	Me_2SO	80×10^{-3}	59.9	−65.2

[a] Rate constants k at 25 °C were calculated from the corresponding activation parameters.

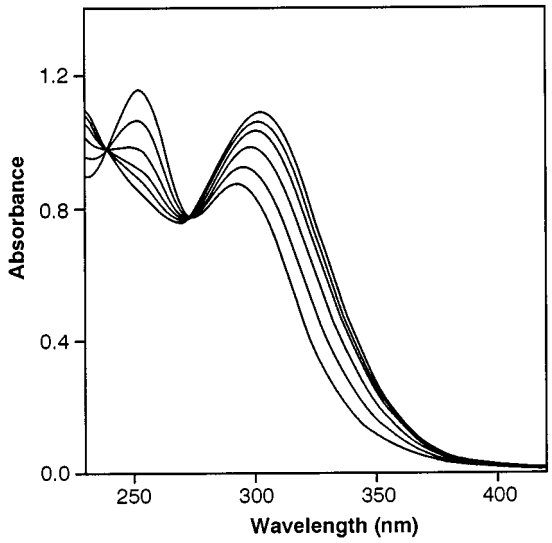


Figure 1. Ultraviolet spectral changes observed after adding sodium methoxide at a concentration of 1.75 mM to a methanolic solution of 96 μM **3a** at 37 °C. Spectra were recorded 10, 60, 120, 240, 480, and 3000 s after mixing.

product spectrum that was consistent with the presence of 2,4-dinitroanisole ($\lambda_{max} = 292$ nm, $\epsilon = 10.5$ mM^{-1} cm^{-1}) and DEA/NO ($\lambda_{max} = 250$ nm, $\epsilon = 8.0$ mM^{-1} cm^{-1}) in the solution. When the rate of disappearance of **3a** was followed by HPLC, the measured second-order rate constant (0.11 M^{-1} s^{-1}; see Table 1) was found to be ca. four times as large as that (0.03 M^{-1} s^{-1}) reported[16] for 1-chloro-2,4-dinitrobenzene.

Reactions of dinitrohalobenzenes with anionic nucleophiles in hydroxylic solvents are generally assumed to follow an S_NAr reaction mechanism involving formation of a σ, or Meisenheimer, complex resulting from attack of the nucleophile on the ipso carbon. In polar aprotic solvents, such as dimethyl sulfoxide, enhanced stability has been found for such complexes, which are characterized by strong absorption bands in the visible region. In some cases, stable Meisenheimer complexes have been identified.[14,17] Direct spectral evidence for an intermediate Meisenheimer complex was obtained during the reaction of sodium methoxide with **3a** in dimethyl sulfoxide, a transformation illustrated in Scheme 5. Strong visible absorbance [λ_{max} 504 and 346 nm (ϵ 26 and 15 mM^{-1} cm^{-1}, respectively)] appeared within seconds of mixing **3a** with excess methoxide and slowly decayed, ultimately yielding a solution showing UV absorbance consistent with the expected reaction products (2,4-dinitroanisole and the free diazeniumdiolate, DEA/NO), with the latter species reacting slowly to generate amine and nitric oxide. The spectral changes documenting this

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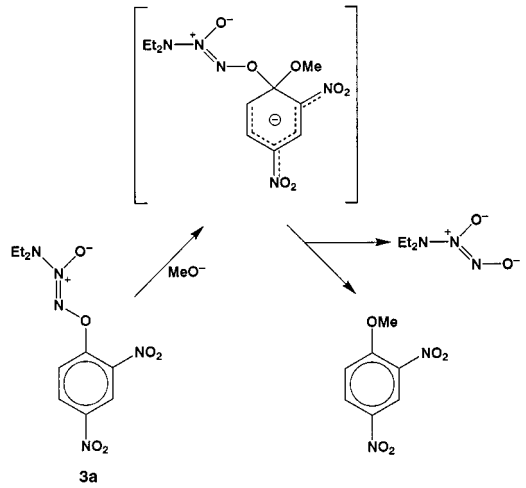
Scheme 5. Intermediacy of a Meisenheimer Complex in the Reaction of 3a with Methoxide Ion in Dimethyl Sulfoxide



mechanism of S_NAr reactivity are shown in Figure 2. A transient visible coloration was also observed on mixing **3a** with sodium methoxide in methanol; while this was not evident among the spectral changes of Figure 1, it nevertheless suggests that loss of the diazeniumdiolate anion from the Meisenheimer complex was also rate limiting in that solvent.

When isopropylamine was employed as a nucleophile in dimethyl sulfoxide, its reaction with **3a** (eq 2C) proceeded cleanly to products with no evidence of base catalysis. Spectral changes between 200 and 450 nm showed a decrease in the **3a** absorbance ($\lambda_{max} = 310$ nm, $\epsilon = 16$ mM^{-1} cm^{-1}) accompanied by formation of a product with a strong visible maximum at 376 nm and a shoulder at 432 nm. An isosbestic point, observed at 345 nm, was maintained for the duration of the reaction (Figure A of Supporting Information). The values of the second-order rate constant, measured under pseudo first-order conditions with the isopropylamine concentration (0.059−0.118) in large excess over that of **3a** (94 μM), were found to be independent of the excess amine concentration and the wavelengths used (310, 370, and 432 nm) to monitor the reaction. The value obtained spectrally was also in good agreement with that measured by HPLC in experiments having [isopropylamine] = 12−29 mM. The reaction of **3a** with isopropylamine occurred five times more rapidly than the reaction of isopropylamine with 1-chloro-2,4-dinitrobenzene measured in this laboratory.

X-ray Crystallographic Studies. The regio- and stereochemistry of these ArO2N2═N1(O1)N3R_2 systems were in keeping with those of the alkyl derivatives. The structure of **3a** (see Supporting Information for full structural details) shows the electrophilic moiety to be attached to the O2-oxygen, with the two diazeniumdiolate oxygens being in the typical cis arrangement. This was true even with the sterically more demanding substitution pattern of **14**. The O1−N1−N2−O2 torsion angles were −2.5° and −3.9° for the two molecules in **3a** and −0.6° in **14**. The substitution in **14** also caused the para nitro group to be rotated out of the plane of the six-membered ring. In **3a** only one ortho nitro group was rotated out of the plane of the six-membered ring, due in this case to intermolecular packing forces rather than intramolecular interactions as in **14**. In both **3a** and **14**, the R_2N nitrogen (N3) was slightly pyramidal, with N3 being from 0.27 to 0.37 Å out of the plane formed by N1 and the two R groups. Crystal data and structure refinement parameters are summarized in Table 2.

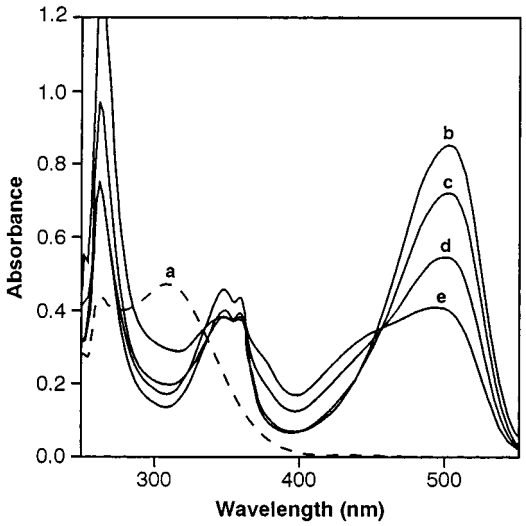


Figure 2. Electronic spectra of: (a) **3a** at a concentration of 35 μM in dimethyl sulfoxide; (b−e) same as a but 0.3, 60, 145, and 267 min, respectively, after adding excess (44 mM) sodium methoxide at 37 °C to generate the transient Meisenheimer intermediate, as in Scheme 5.

Antiviral Activity of the O^2-Aryl Diazeniumdiolates. The results for the N- and O-nucleophiles presented above suggested that the O^2-aryl diazeniumdiolates might be selectively activated for nitric oxide release by reaction with cellular thiolate groups. Known to be potent nucleophiles, these RS^- species retain their high reactivity even when coordinated to metal centers, such as those in the zinc finger motifs of many proteins.[18] The human immunodeficiency virus (HIV-1) synthesizes a nucleocapsid protein (NCp7) containing two copies of a specific zinc finger motif without which packaging of viral genomic RNA and infectivity are blocked.[19−23] Several classes of electrophilic agent have been shown to bind these sulfurs covalently, ejecting the zinc and inhibiting viral replication.[24] In fact, 1-chloro-2,4-dinitrobenzene has itself shown promise as a treatment for early HIV disease in human patients.[25]

To determine whether the O^2-arylated diazeniumdiolates are sufficiently electrophilic to react with the NCp7 thiolate moiety, the protein was dissolved in 10

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Table 2. Crystal Data and Structure Refinement Parameters

	3a	14
empirical formula	$C_{10}H_{13}N_5O_6$	$C_{15}H_{16}N_6O_6$
formula weight	299.25	376.34
temperature (K)	293(2)	293(2)
wavelength (Å)	1.54178	1.54178
crystal system	triclinic	monoclinic
space group	$P\bar{1}$	$P2_1/n$
unit cell dimensions	a = 7.3410(10) Å, α = 92.52(1)°	a = 13.152(3) Å, α = 90°
	b = 7.748(2) Å, β = 93.54(1)°	b = 7.3661(15) Å, β = 98.33(2)°
	c = 24.434(4) Å, γ = 99.98(2)°	c = 17.821(4) Å, γ = 90°
volume (Å^3)	1364.0(5)	1708.3(6)
Z	4	4
density (calculated) (Mg/m^3)	1.457	1.463
absorption coefficient (mm^{-1})	1.055	0.99
F(000)	624	784
crystal size (mm^3)	0.66 × 0.60 × 0.40	0.28 × 0.24 × 0.04
θ range for data collection	5.81 to 56.28%	1.81 to 22.88%
index ranges	$-7 \le h \le 7$, $-8 \le k \le 8$, $-26 \le l \le 0$	$-1 \le h \le 14$, $-2 \le k \le 8$, $-19 \le l \le 19$
reflections collected	3802	3300
independent/observed reflections	3580/2964 [R(int) = 0.0172]	2339/1538 [R(int) = 0.0212]
completeness to theta = 22.88°	99.7%	100.0%
absorption correction	face corrected	face corrected
max. and min. transmission	0.960 and 0.925	0.962 and 0.802
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/parameters	3580/0/380	2339/0/244
goodness-of-fit on F^2	1.102	1.035
final R indices [I >20 (I)]	R1 = 0.0592, wR2 = 0.1408	R1 = 0.0560, wR2 = 0.1282
R indices (all data)	R1 = 0.0739, wR2 = 0.1596	R1 = 0.0943, wR2 = 0.1474
largest diff. peak and hole	0.327 and −0.241 eÅ^3	0.264 and −0.253 e Å^3

mM phosphate buffer at 22 °C and pH 7.0 and mixed with a selection of these novel compounds; evidence of zinc loss was followed by fluorescence,[24] as shown in Figure C of Supporting Information. Time-dependent loss of fluorescence due to the Trp^{37} residue of the protein's C-terminal zinc finger was seen for all four O^2-aryl diazeniumdiolates studied, with the observed decreases of 27−45% reflecting significant loss of bound zinc, as predicted. Upon the basis of this reactivity, we next assessed the compounds' ability to inhibit HIV-1$_{Rf}$ replication in the XTT cytoprotection assay. In this screen, T-cells of the lymphoblastoid line CEM-SS are infected with the laboratory-adapted cytopathic HIV strain Rf and antiviral activity is measured as cytoprotection. While none of the 19 compounds tested provided consistent inhibition of HIV-1 replication, three of them did show measurable activity in at least one antiviral determination (IC_{50} values of: 2.7 μM for **4b** in two of four determinations; 47 μM for **6** in one of four experiments; and 16 μM for **8** in one of three determinations). The data suggest that these compounds do interact with NCp7, but that there are other factors affecting their ability to inhibit viral replication. One such factor is cytotoxicity; **3b**, **4f**, and **12** were all toxic to the lymphoblastic CEM-SS cells at submicromolar concentrations, suggesting reactivity against a cell-based target. As a reference, the positive control 3′-azido-3′-deoxythymidine (AZT), a nucleoside reverse transcriptase inhibitor, inhibited virus replication with an IC_{50} between 1 and 9 nM and was nontoxic at 1 μM. Cytotoxicity values for the aryl diazeniumdiolates evaluated in this assay are summarized in Table B of Supporting Information. Despite their lack of consistent activity in the anti-HIV screen, the ability of these compounds to disrupt the NCp7 protein's zinc finger as predicted from the physicochemical data suggests that this family of compounds may be useful starting points for drug design activities. Efforts to exploit their reactivity with other nucleophilic biomolecules for therapeutic benefit are currently underway.

Experimental Section

Compound **2** was prepared and characterized as previously described,[26] as were the analogous piperidine,[27] pyrrolidine,[6] and substituted piperazine[7] salts. Unless otherwise indicated, nuclear magnetic resonance (NMR) spectra were recorded in chloroform-*d* with a Varian Unity Plus or Varian XL-200 NMR spectrometer and ultraviolet (UV) data were collected on a Hewlett-Packard Model 8451A diode array spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Inc. NO was purchased from Matheson Gas Products (Montgomeryville, PA). Other reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected.

Sodium 1-(*N*,*N*-Dimethylamino)diazen-1-ium-1,2-diolate (DMA/NO). A 40% aqueous solution (25 mL, 0.198 mol) of dimethylamine was cooled to 0 °C. To this cold solution was added 8 g (0.2 mol) of sodium hydroxide. After stirring under nitrogen until the pellets had dissolved, the ice-bath was removed, 250 mL of dioxane was added, and the two-phase mixture was charged with 40 psi of nitric oxide. After 48 h of stirring, the pressure was released, and the mixture containing a white precipitate was poured into 2 L of ether. The product was collected by filtration, washed with copious amounts of ether, and dried under vacuum to give 25 g of product: ^{1}H NMR (D_2O) δ 2.75 (s); ^{13}C NMR (0.1 M NaOD) δ 45.93; UV (0.1 M NaOH) λ_{max} (ϵ) 250 nm (7.6 mM^{-1} cm^{-1}). All DMA/NO preparations contain significant amounts of inorganic salts, and thus a useful elemental analysis was not obtained.

To prove the authenticity of the compound, 1.95 g (0.015 mol) was dissolved in 20 mL of methanol, cooled to 0 °C, treated with 1.5 mL (0.016 mol) of dimethyl sulfate, and kept overnight at 25 °C. The product was concentrated on a rotary evaporator, extracted with dichloromethane, washed with water, dried over sodium sulfate, filtered, and evaporated. A vacuum distillation at 50−51 °C at approximately 1 mmHg gave O^2-methyl 1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate as a colorless liquid: ^{1}H NMR δ 3.0 (s, 6 H), 4.01 (s, 3

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H); ^{13}C NMR δ 42.89, 60.85; UV (water) λ_{max} (ϵ) 234 nm (6.8 mM^{-1} cm^{-1}). Anal. Calcd for $C_3H_9N_3O_2$: C, 30.25; H, 7.62; N, 35.27. Found: C, 30.32, H, 7.63, N, 35.18.

Sodium 1-(*N*-Benzyl-*N*-methylamino)diazen-1-ium-1,2-diolate. A solution of 50 mL (0.39 mol) of *N*-benzyl-*N*-methylamine in 30 mL of methanol was placed in a Parr bottle. The solution was treated with 83 mL (0.38 mol) of 25% sodium methoxide in methanol. The system was evacuated, charged with 40 psi of nitric oxide, and kept at 25 °C for 48 h. The white crystalline product was collected by filtration and washed with ether. The product was dried under vacuum to give 14.7 g of product: mp 150−152 °C; UV (0.01 M NaOH) λ_{max} (ϵ) 252 nm (10.0 mM^{-1} cm^{-1}); ^{1}H NMR (0.01 M NaOD in D_2O) δ 2.82 (s, 3 H); 4.06 (s, 2 H); 7.37−7.38 (m, 5 H); ^{13}C NMR (0.01 M NaOD in D_2O) δ 138.1, 132.5, 131.3, 130.9, 61.88, 44.61. Anal. Calcd for $C_8H_{10}N_3O_2Na\cdot H_2O$: C, 43.44; H, 5.47; N, 19.00; Na, 10.39. Found: C, 43.45; H, 5.53; N, 18.84; Na, 10.67.

Sodium 1-[4-Carboxamido)piperidin-1-yl]diazen-1-ium-1,2-diolate. This compound was prepared from 12.8 g (0.1 mol) of isonipecotamide and nitric oxide as described above. A yield of 13 g of product was obtained: ^{1}H NMR (D_2O) δ 1.87 (m, 2 H), 2.03 (m, 2 H), 2.44 (m, 1 H), 3.08 (m, 2 H), 3.23 (m, 2 H); ^{13}C NMR (0.1 M NaOD) δ 30.29, 43.46, 54.24, 183.44; UV (0.1 M NaOH) λ_{max} (ϵ) 250 nm (8.2 mM^{-1} cm^{-1}). The sample contained approximately 7% starting isonipecotamide and was used without further purification in arylation reactions, all of which gave acceptable elemental analyses.

A portion of this material was methylated with dimethyl sulfate as described above for the dimethylamine analogue to give O^2-methyl 1-[(4-carboxamido)piperidin-1-yl]diazen-1-ium-1,2-diolate: mp 155−157 °C; UV (ethanol) λ_{max} (ϵ) 241 nm (7.1 mM^{-1} cm^{-1}); ^{1}H NMR δ 2.06 (m, 4 H); 2.44 (m, 1 H); 3.08 (m, 4 H); 3.68 (s, 3 H), 6.21 (b, 2 H); ^{13}C NMR δ 27.53, 41.29, 51.01, 61.08, 175.69. Anal. Calcd for $C_7H_{14}N_4O_3\cdot 2/3\ H_2O$: C, 39.25; H, 7.21; N, 26.15. Found: C, 39.41; H, 6.93; N, 25.95.

Sodium 1-[(3-Carboxamido)piperidin-1-yl]diazen-1-ium-1,2-diolate. A solution of 10 g (0.078 mol) of nipecotamide in 25% methanolic sodium methoxide was diluted and charged with nitric oxide as described for its isomer above to give 9.04 g (55%) of a white powder: ^{1}H NMR (0.1 M NaOD) δ 1.52 (m, 1 H), 1.76 (m, 1 H), 1.94 (m, 2 H), 2.77 (m, 1 H), 3.10 (m, 3 H), 3.29 (m, 1 H), 3.34 (s, 1.6 H); ^{13}C NMR (0.1 M NaOD) δ 25.97, 44.88, 55.12, 56.71, 181.73; UV (10 mM NaOH) λ_{max} (ϵ) 250 nm (7.2 mM^{-1} cm^{-1}).

A portion of the sample was methylated in methanol as described for the dimethylamine analogue to give O^2-methyl 1-[(3-carboxamido)piperidin-1-yl]diazen-1-ium-1,2-diolate for characterization: mp 140−142 °C; UV (ethanol) λ_{max} (ϵ) 240 nm (7.1 mM^{-1} cm^{-1}); ^{1}H NMR δ 2.01 (m, 4 H); 2.29 (m, 2 H); 3.02 (sextet, 2 H); 3.89 (m, 2 H), 4.02 (s, 3 H); 5.52 (b, 2 H); ^{13}C NMR δ 22.84, 26.74, 41.68, 51.90, 61.09, 174.77. Anal. Calcd for $C_7H_{14}N_4O_3$: C, 41.58; H, 6.98; N, 27.71. Found: C, 41.69; H, 6.92; N, 27.57.

General Procedures for the Arylation of Diazeniumdiolates

Method A: solution of 11 mmol of a diazeniumdiolate anion in 20 mL of 5% aqueous sodium bicarbonate is cooled to 0 °C under nitrogen. A solution containing 10 mmol of the activated fluoro- or chloroarene in 10 mL of *tert*-butyl alcohol is added slowly. A precipitate normally forms upon addition. The mixture is allowed to warm gradually to room temperature and then stirred overnight. The product is extracted with dichloromethane and washed subsequently first with cold dilute hydrochloric acid and then with sodium bicarbonate solution. The organic layer is dried over sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated under vacuum to give the crude product. Purification is carried out by recrystallization, flash chromatography, or preparative HPLC.

Method B: To a solution of 7.5 mmol of the diazeniumdiolate in 10 mL of dimethyl sulfoxide at 0 °C under a steady stream of nitrogen is added dropwise 6 mmol of the activated aryl compound. The reaction is allowed to warm to room temperature and stirred for 24 to 72 h. Reaction is quenched with water, extracted with ether, dried over sodium sulfate, and evaporated. The product is either recrystallized or purified by flash chromatography.

Method C: A solution of 1.76 mmol of the activated bromo-, fluoro-, or chloroarene reagent in 3 mL of dimethyl sulfoxide is added to a slurry (1.76 mmol) of the diazeniumdiolate in 3 mL of THF at room temperature under nitrogen. The mixture is stirred for 72 h. The resulting homogeneous solution is treated with 100 mL of water. The precipitate is extracted with ether. The organic layer is dried and evaporated, whereupon the product is purified as described in method A.

***O*^{2}-(2,4-Dinitrophenyl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3a).** This compound was prepared by method A to give 1.3 g of a red oil which crystallized on standing. Recrystallization from ethanol gave yellow-orange needles: mp 76−77 °C; ^{1}H NMR δ 1.25 (t, 6 H), 3.58 (q, 4 H), 7.68 (d, 1 H), 8.44 (m, 2 H), 8.89 (m, 1 H); ^{13}C NMR δ 11.51, 46.92, 117.53, 122.17, 129.13, 142.05, 154.22; UV (ethanol) λ_{max} (ϵ) 218 nm (17.4 mM^{-1} cm^{-1}) and 302 nm (15.6 mM^{-1} cm^{-1}). Anal. Calcd for $C_{10}H_{13}N_5O_6$: C, 40.13; H, 4.35; N, 23.41. Found: C, 40.21; H, 4.43, N, 23.37.

Reaction of 3a with Nucleophiles. A solution of 85 mg (0.28 mmol) of **3a** in 1 mL of ether was cooled to −4 °C and treated with 1 mL of diethylamine. The solution was kept at −4 °C for 1 h, giving a precipitate. The solid was collected by filtration. The filtrate was concentrated and analyzed by NMR; the residue was identical to an authentic sample of 2,4-dinitro-*N*,*N*-diethylaniline. The precipitate was washed with petroleum ether and dried under nitrogen to give 5.4 mg of product having λ_{max} 250 nm (6.5 mM^{-1} cm^{-1}); ^{1}H NMR (D_2O) δ 0.96 (t, 6 H), 1.28 (t, 6 H), 2.94 (q, 4 H), 3.08 (q, 4 H). This product proved to be identical to an authentic sample of the diethylammonium salt of anion **2** prepared as previously described.

A solution of 16 mg (0.064 mmol) of **3a** in 1 mL of ether was treated with 29 mL of 25% sodium methoxide in methanol (0.14 mmol) and allowed to stand at −4 °C for 2 h. The solid precipitate was collected by filtration, washed with ether, and dried under vacuum to give 4 mg of a solid identical to an authentic sample of **2** (sodium salt).

Kinetic Measurements of the Reaction of 3a with Nucleophiles. Kinetic data on reactions of **3a** with hydroxide, methoxide, and isopropylamine were obtained by HPLC under pseudo first-order conditions with the nucleophile concentration in ≥10-fold excess of the diazeniumdiolate substrate. In a typical experiment involving methoxide, the reaction was initiated by treating 10 mL of a 23 mM solution of sodium methoxide in methanol, in a thermostated water bath, with 10 μL of a 0.95 M solution of **3a** in the same solvent. Aliquots of 100 μL each were withdrawn at timed intervals and quenched by addition to 0.10 M hydrogen chloride in methanol. The concentration of **3a** in the resulting solution was determined by HPLC using a Phenomenex Luna column with 60% aqueous acetonitrile as mobile phase. Pseudo first-order rate constants, k_{obs}, were obtained from the linear plots of log[substrate] versus time for runs having methoxide concentrations of 13−23 mM. Second-order rate constants ($k_2 = k_{obs}/[MeO^-]$) were independent of the excess methoxide concentrations employed.

Similar conditions were used to follow the reaction of 1 mM **3a** with 12−29 mM isopropylamine in dimethyl sulfoxide except that the mobile phase was 65% aqueous methanol. The reaction of 64−69 μM **3a** with 6.2−6.5 mM hydroxide in 1% aqueous dioxane was followed by HPLC using similar conditions except that the mobile phase was 60:40 acetonitrile:0.5% aqueous trifluoroacetic acid. Values for the activation parameters were calculated from linear fit of the data [ln(k/T versus 1/T] to the Eyring absolute rate equation.

Spectral changes observed during the reactions of **3a** were obtained using a Hewlett-Packard 8452A Diode Array UV−visible spectrophotometer with the nucleophile (methoxide or isopropylamine) in large excess. In a typical experiment, the reaction (in methanol or dimethyl sulfoxide) was initiated by adding 10- to 50-μL aliquots of nucleophile stock solutions to 1.0 mL of **3a** contained in the same solvent in a thermostated

cuvette. First-order rate constants were calculated from the spectral changes using the Hewlett-Packard kinetics software.

***O*²-(2-Trifluoromethyl-4-nitrophenyl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3b).** A solution of 859 mg (5.54 mmol) of **2** in dimethyl sulfoxide was treated with 1.05 g (5.02 mmol) of 2-fluoro-5-nitrobenzotrifluoride according to method B to give 1.45 g of a pale yellow crystallized product. This was recrystallized from ether−petroleum ether: mp 67−68 °C; ^{1}H NMR δ 1.24 (t, 6 H), 3.52 (q, 4 H), 7.58 (d, 1 H), 8.44 (m, 1 H), 8.57 (d, 1 H); ^{13}C NMR δ 11.53, 47.16, 115.88, 118.25, 119.95, 123.45, 128.90, 142.45, 158.44; UV (ethanol) λ_{max} (ϵ) 302 nm (14.4 mM^{-1} cm^{-1}). Anal. Calcd for $C_{11}H_{13}N_4O_4F_3$: C, 41.00; H, 4.07; N, 17.39. Found: C, 40.93; H, 4.11; N, 17.26.

***O*²-[2-Nitro-4-(trifluoromethyl)phenyl] 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3c).** Method A was followed in the preparation of this compound. Purification of the product was carried out on a KP−Sil column fitted to a Flash 40 apparatus, eluted with 2:1 cyclohexane:ethyl acetate, to give 183 mg (38%) of an oil: ^{1}H NMR δ 1.23 (t, 6 H), 3.50 (q, 4 H), 7.66 (d, 1 H), 7.84 (d, 1 H), 8.28 (s, 1 H); ^{13}C NMR δ 11.48, 47.20, 118.29, 123.64, 131.13, 137.89, 152.16; UV (ethanol) λ_{max} (ϵ) 244 nm (8.26 mM^{-1} cm^{-1}). Anal. Calcd for $C_{11}H_{13}N_4O_4F_3$: C, 41.00; H, 4.07; N, 17.39. Found: C, 41.28; H, 4.16; N, 17.26.

***O*²-(5-Nitropyrid-2-yl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3d).** This reaction was carried out as described in method A. The yellow solid was recrystallized from ether−petroleum ether to give a 73% yield of pure material: mp 77−78 °C; ^{1}H NMR δ 1.24 (t, 6 H), 3.53 (q, 4 H), 7.21 (dd, 1 H), 8.52 (dd, 1 H), 9.17 (dd, 1 H); ^{13}C NMR δ 11.42, 47.21, 109.25, 134.99, 141.15, 145.10, 165.21; UV (ethanol) λ_{max} (ϵ) 300 nm (12.9 mM^{-1} cm^{-1}). Anal. Calcd for $C_9H_{13}N_5O_4$: C, 42.35; H, 5.13; N, 27.44. Found: C, 42.46; H, 5.14; N, 27.53.

***O*²-(3-Nitropyrid-2-yl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3e).** This compound was prepared as described in method A to give a yellow oil. The crude product was purified on a KP−Sil column fitted to a Flash 40 apparatus, eluted with 5:1 dichloromethane:ethyl acetate, to give a 52% yield of an oil: ^{1}H NMR δ 1.25 (t, 6 H), 3.55 (q, 4 H), 7.26 (m, 1 H), 8.48 (m, 2 H); ^{13}C NMR δ 11.44, 47.06, 119.62, 122.94, 135.63, 152.06, 152.39; UV (ethanol) λ_{max} (ϵ) 262 nm (15.4 mM^{-1} cm^{-1}). Anal. Calcd for $C_9H_{13}N_5O_4$: C, 42.35; H, 5.13; N, 27.44. Found: C, 42.59; H, 5.20; N, 27.20.

***O*²-(4-Nitrophenyl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3f).** Method B was used in the preparation of this compound. It was obtained in 31% yield after recrystallization from ether−petroleum ether: mp 81−82 °C; ^{1}H NMR δ 1.25 (t, 6 H), 3.43 (q, 4 H), 7.36 (d, 2 H), 8.28 (d, 2 H); ^{13}C NMR δ 11.45, 47.58, 115.12, 125.77, 143.62, 160.87; UV (ethanol) λ_{max} (ϵ) 306 nm (13.7 mM^{-1} cm^{-1}). Anal. Calcd for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04. Found: C, 47.16; H, 5.51; N, 21.95.

***O*²-(2-Chloropyrimidin-4-yl) 1-(*N*,*N*-Diethylamino)diazen-1-ium-1,2-diolate (3g).** A solution of 600 mg (4 mmol) of 2,4-dichloropyrimidine in 2 mL of dimethyl sulfoxide and 5 mL of tetrahydrofuran was added via syringe to a slurry of 678 mg (4.37 mmol) of **2** in 15 mL of tetrahydrofuran at room temperature under nitrogen, and the resulting mixture was stirred for 72 h. The mixture was shaken with 100 mL of ether. The organic layer was washed with water, dried over sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated to give 679 mg of an oil which crystallized in the freezer. This material was recrystallized from ether−petroleum ether: mp 37−38 °C; ^{1}H NMR δ 1.25 (t, 6 H), 3.56 (q, 4 H), 7.00 (d, 1 H), 8.50 (d, 1 H); ^{13}C NMR δ 11.35, 46.90, 104.68, 160.61, 160.71, 169.23; UV (ethanol) λ_{max} (ϵ) 268 nm (9.3 mM^{-1} cm^{-1}). Anal. Calcd for $C_{18}H_{12}N_5O_2Cl$: C, 39.11; H, 4.92; N, 28.51; Cl, 14.43. Found: C, 38.96; H, 4.96; N, 28.35; Cl, 14.60.

Attempts to displace the second chlorine by addition of excess **2** resulted in no reaction.

***O*²-(2,4-Dinitrophenyl) 1-(*N*,*N*-Dimethylamino)diazen-1-ium-1,2-diolate (4a).** This compound was prepared according to method B. The crude product was recrystallized from methanol to give a 33% yield of **4a**: mp 146−147 °C; ^{1}H NMR (DMSO-d_6) δ 3.26 (s, 6 H), 7.88 (d, 1 H), 8.54 (dd, 1 H), 8.85 (d, 1 H); ^{13}C NMR (DMSO-d_6) δ 41.17, 117.68, 121.68, 129.66, 136.66, 141.73, 153.14; UV (methanol) λ_{max} (ϵ) 220 nm (23.8 mM^{-1} cm^{-1}) and 304 nm (23.4 mM^{-1} cm^{-1}). Anal. Calcd for $C_8H_9N_5O_6$: C, 35.43; H, 3.34; N, 25.82. Found: C, 35.56; H, 3.37; N, 25.66.

***O*²-(2,4-Dinitrophenyl) 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (4b).** Method A was followed to give a product which was recrystallized from ethanol: mp 94−95 °C; ^{1}H NMR δ 2.04 (m, 4 H), 3.35 (m, 4 H), 6.90 (d, 1 H), 8.20 (dd, 1 H), 8.67 (d, 1 H); ^{13}C NMR δ 23.44, 50.53, 117.17, 122.17, 129.10, 136.88, 141.69, 154.53; UV (ethanol) λ_{max} (ϵ) 300 nm (11.4 mM^{-1} cm^{-1}). Anal. Calcd for $C_{10}H_{11}N_5O_6$: C, 40.41; H, 3.73; N, 23.56. Found: C, 40.36; H, 3.77; N, 23.42.

***O*²-(2,4-Dinitrophenyl) 1-(Piperidin-1-yl)diazen-1-ium-1,2-diolate (4c).** Method A gave a product from sodium 1-(piperidin-1-yl)diazen-1-ium-1,2-diolate[27] that was purified by flash chromatography using a KP−Sil column and a Flash 40 system with dichloromethane as the eluant: mp 111−112 °C; ^{1}H NMR (500 MHz) δ 1.60 (m, 2 H), 1.82 (m, 4 H), 3.63 (m, 4 H), 7.68 (d, J = 9.3 Hz, 1 H), 8.45 (d, J_1 = 2.7 Hz, J_2 = 9.3 Hz, 1 H), 8.87 (d, J = 2.7 Hz, 1 H); ^{13}C NMR δ 23.15, 24.31, 51.62, 117.54, 122.08, 129.10, 137.04, 142.01, 154.10; UV (ethanol) λ_{max} (ϵ) 216 nm (14.6 mM^{-1} cm^{-1}) and 300 nm (14.7 mM^{-1} cm^{-1}). Anal. Calcd for $C_{11}H_{13}N_5O_6$: C, 42.45; H, 4.21; N, 22.50. Found: C, 42.53; H, 4.25; N, 22.50.

***O*²-(2,4-Dinitrophenyl) 1-(4-Carboxamidopiperidin-1-yl)diazen-1-ium-1,2-diolate (4d).** Method A was followed in this preparation. The crude product was recrystallized from 1:1 methanol:dichloromethane to give an 85% yield of pale yellow crystals: mp 165−166 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.71 (m, 2 H), 1.90 (m, 2 H), 2.32 (m, 1 H), 3.20 (m, 2 H), 4.18 (m, 2 H), 6.89 (br s, 1 H), 7.36 (br s, 1 H), 7.92 (d, J = 9.3 Hz, 1 H), 8.54 (d, J_1 = 2.8 Hz, J_2 = 9.3 Hz, 1 H), 8.87 (d, J = 2.8 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 26.63, 39.86, 49.46, 118.01, 121.75, 129.74, 136.81, 142.01, 152.86, 175.33; UV (ethanol) λ_{max} (ϵ) 302 nm (7.5 mM^{-1} cm^{-1}). Anal. Calcd for $C_{12}H_{14}N_6O_7$: C, 40.68; H, 3.98; N, 23.72. Found: C, 40.61; H, 3.99, N, 23.70.

***O*²-(2,4-Dinitrophenyl) 1-(3-Carboxamidopiperid-1-yl)-diazen-1-ium-1,2-diolate (4e).** This compound was prepared as described in method A. A 45% yield of product was obtained after recrystallization from 1:1 methanol:dichloromethane: mp 149−150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56 (m, 2 H), 1.86 (m, 2 H), 2.55 (m, 1 H), 3.15 (m, 2 H), 4.17 (m, 2 H), 6.99 (br s, 1 H), 7.44 (br s, 1 H), 7.90 (d, J = 9.3 Hz, 1 H), 8.56 (d, J_1 = 2.6 Hz, J_2 = 9.3 Hz, 1 H), 8.87 (d, J = 2.8 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 22.60, 26.51, 40.40, 50.10, 51.99, 118.08, 121.83, 129.86, 136.89, 142.07, 152.88, 173.78; UV (ethanol) λ_{max} (ϵ) 222 nm (6.9 mM^{-1} cm^{-1}) and 302 nm (8.2 mM^{-1} cm^{-1}). Anal. Calcd for $C_{12}H_{14}N_6O_7$: C, 40.68; H, 3.98; N, 23.72. Found: C, 40.50; H, 3.98; N, 23.61.

***O*²-(2,4-Dinitrophenyl) 1-(4-Phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate (4f).** Sodium 1-(4-phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate (732 mg; 3 mmol) was allowed to react with 372 mg (3 mmol) of 1-fluoro-2,4-dinitrobenzene as described in method A. Recrystallization from methanol gave a pure product in 90% yield: mp 144−145 °C; ^{1}H NMR (500 MHz) δ 3.42 (m, 4 H), 3.84 (m, 4 H), 6.96 (m, 3 H), 7.31 (m, 2 H), 7.69 (d, J = 9.2 Hz, 1 H), 8.76 (d, J_1 = 2.7 Hz, J_2 = 9.2 Hz, 1 H), 8.88 (d, J = 2.7 Hz, 1 H); ^{13}C NMR δ 48.28, 50.55, 116.89, 117.62, 121.16, 122.15, 129.10, 129.35, 137.21, 142.28, 149.97, 153.82; UV (ethanol) λ_{max} (ϵ) 246 nm (9.3 mM^{-1} cm^{-1}) and 298 nm (6.6 mM^{-1} cm^{-1}). Anal. Calcd for $C_{16}H_{16}N_6O_6 \cdot {}^1/_4H_2O$: C, 48.92; H, 4.23; N, 21.39. Found: C, 48.78; H, 4.04; N, 21.46.

***O*²-(2,4-Dinitrophenyl) 1-[4-(Pyrimidin-2-yl)piperazin-1-yl]diazen-1-ium-1,2-diolate (4g).** Method A was used to prepare **4g** in 90% yield after recrystallization from ethanol: mp 149−150 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.73 (m, 4 H), 4.00 (m, 4 H), 6.71 (t, J = 4.8 Hz, 1 H), 7.95 (d, J = 9.3 Hz, 1 H), 8.41 (d, J = 4.8 Hz, 2 H), 8.54 (d, J_1 = 2.8 Hz, J_2 = 9.3 Hz, 1 H), 8.86 (d, J = 2.8 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 41.74, 49.66, 110.87, 118.04, 121.69, 129.64, 136.73, 142.01, 152.82, 157.98, 160.83; UV (ethanol) λ_{max} (ϵ) 242 nm (21.1 mM^{-1} cm^{-1}) and 300 nm (8.5 mM^{-1} cm^{-1}). Anal. Calcd for $C_{14}H_{14}N_8O_6$: C, 43.08; H, 3.62; N, 28.71. Found: C, 43.04; H, 3.62; N, 28.84.

***O*²-(2,4-Dinitrophenyl) 1-[(4-Ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (4h).** The product, prepared

as in method A, was recrystallized from ethanol:dichloromethane: mp 140−141 °C; ^{1}H NMR δ 1.32 (t, 3 H), 3.63 (m, 4 H), 3.74 (m, 4 H), 4.19 (q, 2 H), 7.66 (d, 1 H), 8.48 (q, 1 H), 8.88 (d, 1 H); ^{13}C NMR δ 14.60, 42.18, 50.55, 62.07, 113.23, 117.72, 122.19, 129.07, 142.48, 153.69, 154.99; UV (ethanol) λ_{max} (ϵ) 300 nm (12 mM^{-1} cm^{-1}). Anal. Calcd for $C_{13}H_{16}N_6O_8$: C, 40.61; H, 4.20; N, 21.87. Found: C, 40.74; H, 4.13; N, 21.98.

***O*^{2}-(2,4-Dinitrophenyl) 1-(*N*-Benzyl-*N*-methylamino)-diazen-1-ium-1,2-diolate (4i).** This material was prepared in 49% yield as described in method A: mp 86−87 °C; ^{1}H NMR (500 MHz) δ 3.25 (s, 3 H), 4.76 (s, 2 H), 7.36 (m, 6 H), 8.35 (dd, $J_1 = 2.7$ Hz, $J_2 = 9.3$ Hz, 1 H), 8.84 (d, $J = 2.8$ Hz, 1 H); ^{13}C NMR δ 39.50, 57.63, 117.41, 122.01, 128.48, 128.57, 128.91, 129.05, 134.03, 136.87, 141.91, 154.06; UV (ethanol) λ_{max} (ϵ) 302 nm (11.0 mM^{-1} cm^{-1}). Anal. Calcd for $C_{14}H_{13}N_5O_6$: C, 48.42; H, 3.77; N, 20.17. Found: C, 48.34; H, 3.80; N, 19.97.

***O*^{2}-(2-Chloropyrimidin-4-yl) 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (5).** Method B gave a pure product that was collected by filtration: mp 150−151 °C; ^{1}H NMR δ 2.00 (t, 4 H), 3.77 (t, 4 H), 6.99 (d, 1 H), 8.49 (d, 1 H); ^{13}C NMR δ 23.36, 50.49, 104.56, 160.43, 160.58, 169.35; UV (ethanol) λ_{max} (ϵ) 274 nm (18.5 mM^{-1} cm^{-1}). Anal. Calcd for $C_8H_{10}ClN_5O_2$: C, 39.44; H, 4.14; N, 28.74. Found: C, 39.51; H, 4.24; N, 28.87.

***O*^{2}-(5-Nitropyrid-2-yl) 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (6).** To a slurry of 459 mg (3 mmol) of sodium 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate in 4 mL of dimethyl sulfoxide at 4 °C was added a solution of 406 mg (2 mmol) of 2-bromo-5-nitropyridine in 6 mL of tetrahydrofuran. The reaction mixture was stirred overnight under nitrogen at room temperature. The mixture was treated with water and extracted with ether. The organic solution was washed with water, dried over sodium sulfate, filtered through a layer of magnesium sulfate, and concentrated on a rotary evaporator. The product was recrystallized from methanol: mp 162−163 °C; ^{1}H NMR (500 MHz) δ 2.10 (m, 4 H), 3.80 (m, 4 H), 7.17 (d, $J = 9.1$ Hz, 1 H), 8.50 (d, $J_1 = 2.7$ Hz, $J_2 = 9.1$ Hz, 1 H), 9.14 (d, $J = 2.7$ Hz, 1 H); ^{13}C NMR δ 23.34, 50.57, 109.02, 134.84, 140.80, 145.03, 165.46; UV (ethanol) λ_{max} (ϵ) 302 nm (11.2 mM^{-1} cm^{-1}). Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.35; N, 27.67. Found: C, 42.72; H, 4.37; N, 27.51.

***O*^{2}-(2-Chloropyrimidin-4-yl) 1-[(4-Ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (7).** Prepared according to method C, this compound had mp 136−137 °C; ^{1}H NMR δ 1.29 (t, 3 H), 3.67 (m, 4 H), 3.71(m, 4 H), 4.18 (q, 2 H), 6.99 (d, 1 H), 8.52 (d, 1 H); ^{13}C NMR δ 14.59, 42.14, 50.55, 61.98, 104.80, 155.04, 160.73, 168.79; UV (ethanol) λ_{max} (ϵ) 268 nm (11.7 mM^{-1} cm^{-1}). This compound undergoes nucleophilic substitution with methoxide displacing the chlorine at C-2 and the diazeniumdiolate at C-4 to give 2,4-dimethoxypyrimidine. Anal. Calcd for $C_{11}H_{15}N_6O_4Cl$: C, 39.95; H, 4.57; N, 25.41; Cl, 10.72. Found: C, 40.07; H, 4.44; N, 25.32; Cl, 10.80.

***O*^{2}-(5-Nitropyrid-2-yl) 1-(*N*,*N*-Dimethylamino)diazen-1-ium-1,2-diolate (8).** This reaction was carried out in tetrahydrofuran:dimethyl sulfoxide as described in Method C. The product was crystallized from methanol to give a 56% yield of **8**: mp 180−181 °C; ^{1}H NMR δ 3.24 (s, 6 H), 7.20 (m, 1 H), 8.51 (m, 1 H), 9.15 (b, 1 H); ^{13}C NMR δ 41.33, 109.02, 136.11, 141.15, 144.84, 164.97; UV (ethanol) λ_{max} (ϵ) 218 nm (11 mM^{-1} cm^{-1}) and 302 nm (13.6 mM^{-1} cm^{-1}). Anal. Calcd for $C_7H_9N_5O_4$: C, 37.00; H, 3.96; N, 30.84. Found: C, 37.10; H, 4.00; N, 30.92.

***O*^{2}-[2-Nitro-(4-trifluoromethyl)phenyl] 1-(4-Phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate (9).** A solution of 4-fluoro-3-nitrobenzotrifluoride in *tert*-butyl alcohol was added to a cold solution of sodium 1-(4-phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate as described in method A. The product was purified by preparative HPLC using an Altima-C_{18} column with a mobile phase of 20/80 water/acetonitrile: mp 136−137 °C; ^{1}H NMR (500 MHz) δ 3.40 (m, 4 H), 3.78 (m, 4 H), 6.96 (m, 3 H), 7.31 (m, 2 H), 7.64 (d, $J = 8.7$ Hz, 1 H), 7.85 (d, $J_1 = 0.6$ Hz, $J_2 = 2.3$ Hz, $J_3 = 8.8$ Hz, 1 H), 8.26 (d, $J = 1.9$ Hz, 1 H); ^{13}C NMR δ 48.32, 50.69, 116.90, 118.36, 121.11, 123.67, 129.35, 131.09, 131.12, 137.99, 150.07, 151.91; UV (ethanol) λ_{max} (ϵ) 250 nm (22.9 mM^{-1} cm^{-1}). Anal. Calcd for $C_{17}H_{16}N_3O_4F_3$: C, 49.64; H, 3.92; N, 17.03. Found: C, 49.64; H, 3.95; N, 16.95.

***O*^{2}-(5-Nitropyrid-2-yl) 1-(4-Phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate (10).** A tetrahydrofuran solution of 2-bromo-5-nitropyridine was added to sodium 1-(4-phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate as described in method B. The product was recrystallized from acetonitrile to give 248 mg (36%) of light yellow crystals: mp 175−176 °C; ^{1}H NMR (500 MHz) δ 3.42 (m, 4 H), 3.84 (m, 4 H), 6.96 (m, 3 H), 7.21 (d, $J = 9.0$ Hz, 1 H), 7.31 (m, 2 H), 8.53 (d, $J_1 = 2.8$ Hz, $J_2 = 9.0$ Hz, 1 H), 9.17 (d, $J = 2.8$ Hz, 1 H); ^{13}C NMR δ 48.30, 50.72, 109.39, 116.84, 121.04, 129.34, 135.06, 141.27, 145.04, 150.07, 164.89; UV (ethanol) λ_{max} (ϵ) 246 nm (5.2 mM^{-1} cm^{-1}) and 296 nm (4.9 mM^{-1} cm^{-1}). Anal. Calcd for $C_{15}H_{16}N_6O_4$: C, 52.33; H, 4.65; N, 24.42. Found: C, 52.09; H, 4.65; N, 24.28.

1,5-Bis{[1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolato]-*O*^{2}}-2,4-dinitrobenzene (12). This reaction was carried out in tetrahydrofuran:dimethyl sulfoxide according to method C. The product was recrystallized from ethanol: mp 128−129 °C; ^{1}H NMR δ 1.26 (t, 12 H), 3.59 (q, 8 H), 7.60 (s, 1 H), 8.89 (s, 1 H); ^{13}C NMR δ 11.54, 46.76, 104.52, 125.57, 131.22, 154.51; UV (ethanol) λ_{max} (ϵ) 294 nm (11.4 mM^{-1} cm^{-1}). Anal. Calcd for $C_{14}H_{22}N_8O_8$: C, 39.07; H, 5.15; N, 26.04. Found: C, 39.05; H, 5.20; N, 25.95.

***N*-Methyl-*N*-phenyl-2,4-dinitro-5-fluoroaniline (13).** To a solution of 3.15 g (0.0154 mol) of 1,5-difluoro-2,4-dinitrobenzene in 15 mL of tetrahydrofuran was added 2.1 g (0.02 mol) of anhydrous sodium carbonate. To the slurry was added a solution of 1.67 mL (0.02 mol) of *N*-methylaniline in 10 mL of tetrahydrofuran. This was done dropwise over a 20-min period. After 24 h, the tetrahydrofuran was removed on a rotary evaporator, the residue was extracted with dichloromethane, and the organic layer was filtered into a separatory funnel. The solution was washed with water, dried over sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated to give 4.46 g of an orange solid. The product was crystallized from ethanol: mp 158−159 °C; ^{1}H NMR δ 3.45 (s, 3 H), 6.88 (d, 1 H), 7.12 (m, 2 H), 7.28 (m, 1 H), 7.42 (m, 2 H), 8.58 (d, 1 H); ^{13}C NMR δ 42.99, 107.99, 108.33, 123.74, 126.50, 127.07, 130.27, 145.67, 156.03, 159.87; UV (ethanol) λ_{max} (ϵ) 248 nm (12 mM^{-1} cm^{-1}) and 369 nm (13.2 mM^{-1} cm^{-1}). Anal. Calcd for $C_{13}H_{10}N_3O_4F$: C, 53.62; H, 3.46; N, 14.43. Found: C, 53.73; H, 3.57; N, 14.26.

***O*^{2}-[2,4-Dinitro-5-(*N*-methyl-*N*-phenylamino)phenyl] 1-(*N*,*N*-Dimethylamino)diazen-1-ium-1,2-diolate (14).** This compound was prepared using a slight modification of method A. The arylating agent, **13**, was dissolved in dioxane rather than in *tert*-butyl alcohol. After 15 h the dioxane and water were removed on a rotary evaporator, and the residue was extracted with dichloromethane. The solution was washed with water, dried over magnesium sulfate, and evaporated to give 2.7 g of a red-orange solid. The crude product was purified on a 4-cm by 15-cm KP−Sil column of a Flash 40 system and eluted with dichloromethane: mp 163−164 °C; ^{1}H NMR δ 3.18 (s, 6 H), 3.45 (s, 3 H), 7.08 (s, 1 H), 7.15 (m, 3 H), 7.38 (m, 2 H), 8.63 (s, 1 H); ^{13}C NMR δ 41.85, 42.51, 107.79, 113.22, 123.23, 126.18, 126.72, 128.63, 130.04, 134.20, 146.17, 147.82; UV (DMSO) λ_{max} (ϵ) 258 nm (33.2 mM^{-1} cm^{-1}) and 386 nm (15.6 mM^{-1} cm^{-1}). Anal. Calcd for $C_{15}H_{16}N_6O_6$: C, 47.84; H, 4.29; N, 22.33. Found: C, 47.70; H, 4.34; N, 22.04.

X-ray Study. Diffraction data for both **3a** and **14** were collected on a Bruker P4 automated diffractometer with a graphite monochromator on the incident beam. All measurements were performed using the $\theta/2\ \theta$ scan technique with a varying scan speed depending upon the intensity of a reflection. Crystal and experimental data are summarized in Table 2. The data sets were corrected for Lorentz and polarization effects and for absorption using the face-indexed algorithm in program XPREP.[28] The structures were solved by direct methods and refined by full matrix least-squares on F^2 values

(28) XPREP. 1997. Madison, WI, Bruker Analytical Instruments. Ref Type: Computer Program

using all the independent data. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using a riding model in which the C−H distances are fixed at 0.96 Å, and the hydrogen coordinates are reset after each cycle of least-squares. The structure solution, refinement, and preparation of publication plots and tables were accomplished using the SHEXLTL set of computer programs.[29]

Zinc Ejection Assay. Fluorescence measurement of the Trp^{37} residue in the C-terminal zinc finger of recombinant HIV-1 NCp7 protein (kindly provided by L. O. Arthur of the AIDS Vaccine Program of NCI at Frederick, Frederick, MD) was performed as previously described.[19] Briefly, a solution of 20 μg/mL of NCp7 in 10 mM sodium phosphate buffer (pH 7.0) was treated with the indicated compounds for various lengths of time at concentrations of 25 μM each. Aliquots were removed at the indicated times and diluted 10-fold in 10 mM sodium phosphate buffer (pH 7.0). Fluorescence intensity was measured with a Shimadzu RF5000 spectrofluorimeter at excitation and emission wavelengths of 280 and 352 nm, respectively. The disulfide corresonding to 4-[*N*-(2-mercapto)-benzoyl]aminobenzene sulfonamide[19] was used as a positive control for zinc ejection.

Antiviral Assay. Antiviral activity was measured by inhibition of virus cytopathic effects using $HIV\text{-}1_{Rf}$ infection of CEM-SS lymphoblastoid T-cells.[30] Virally induced cytopathogenicity and cell survival (cytoprotection) were measured using a mitochondrion-activated dye to assess cell viability. Absorbance at 450 nM of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-[5-(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide dye reduction following activation with phenazine methosulfate was taken as an index of cell survival at 6 days post-infection. The IC_{50} (concentration resulting in 50% reduction in virus replication) and TC_{50} (concentration resulting in 50% loss of cell viability in cells without virus) values were calculated using linear regression analysis. AZT was included as a positive control for all antiviral determinations.

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Supporting Information Available: Spectral changes accompanying the reaction of **3a** with isopropylamine, rate and activation parameters for reaction of **3a** with nucleophiles, results from the zinc-ejection assay, toxicity data from the anti-HIV screen, and full crystal structure reports for **3a** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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