O-Alkylation of Cupferron: Aiming at the Design and Synthesis of Controlled Nitric Oxide Releasing Agents

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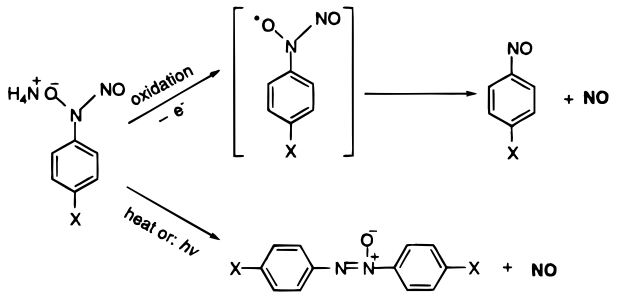
O-Alkylation of *N*-nitroso-*N*-phenylhydroxylamine ammonium salt (cupferron) was studied for the synthesis of novel nitric oxide (NO) releasing agents. The alkylation occurred regioselectively at the terminal oxygen, leading to a single product *N*-(alkyloxy)-*N*′-phenyldiimide *N*′-oxide as indicated by NMR and X-ray analysis. The *O*-alkyl derivatives exhibited significantly improved stability compared to their parent compound, cupferron. It was demonstrated that the cupferron *O*-alkyl derivatives could function as photoreleasing NO donor compounds. *N*-(*N*′′-acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide), which linked the cupferron portion with an amino acid via an acetal moiety, was synthesized as an model NO prodrug where controlled NO release would occur either by increasing pH or by a protease-catalyzed hydrolysis.

Introduction

Nitric oxide (NO) has been identified as a major mediator in physiological processes.[1−4] Besides the three "classical" NO-mediated functions, which are vasodilatory and antiplatelet effects,[5,6] macrophage-induced cytotoxicity,[7,8] and neurotransmission,[9,10] new and more detailed biological roles of NO are being uncovered.[11,12] NO is synthesized in vivo from arginine mediated by nitric oxide synthase (NOS).[13,14] Administration of NO-donating compounds, such as glycerin trinitrate (GTN), has been practiced in cardiovascular medicine for over a century.[15] Most NO donors release NO either by spontaneous decomposition or by metabolic transformation. One of the major problems associated with the current NO donors is the indiscriminate release of NO. Therefore, the development of donors that can deliver NO at specific time and space to evoke the desired biological function is of great current interest.[16−18]

Scheme 1. Cupferronand Its Substituted Derivatives Can Release NO via Oxidation, Thermal Activation, and Photoactivation



Cupferron, an analogue of diazeniumdiolate, is commonly used in industry as a metal chelator[19] and a polymerization inhibitor.[20] It is also a donor that can release NO upon enzymatic,[21] electrochemical, [22,23] as well as chemical oxidation.[24] Hwu et al. also discovered that cupferron can thermally and photochemically decompose to azoxy compounds and release NO (Scheme 1).[25]

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Table 1. O-Alkylation Derivatives of Cupferron[a]

RX	PhN(O)NOR		yield (%)
MeI	Me	**1**	55
EtI	Et	**2**	49
n-PrI	*n*-Pr	**3**	40
CH_2=CH_2CH_2Br	CH_2=CH_2CH_2	**4**	31
$HOCH_2CH_2CH_2Br$	$HOCH_2CH_2CH_2$	**5**	23
CH_3OCH_2Br	CH_3OCH_2	**6**	35
CH_3SCH_2Cl	CH_3SCH_2	**7**	16
$PhSCH_2Cl$	$PhSCH_2$	**8**	17
$C_6H_5CH_2Br$	$C_6H_5CH_2$	**9**	41
p-CH_3O-$C_6H_5CH_2Cl$	*p*-CH_3O-$C_6H_5CH_2$	**10**	36
p-NO_2-$C_6H_5CH_2Br$	*p*-NO_2-$C_6H_5CH_2$	**11**	41
o-NO_2-$C_6H_5CH_2Br$	*o*-NO_2-$C_6H_5CH_2$	**12**	44

[a] Ratio of RX to Cupferron: 3:1; isolated yield %.

Cupferron has a NONO moiety attached directly to a carbon instead of a nitrogen atom. The advantage of this type of donor is that, after the release of NO, the byproducts are noncarcinogenic.[24] Balaban and coworkers[24a] synthesized a series of ortho-substituted derivatives of cupferron. They found that these derivatives were good donors both in vitro and in vivo. These derivatives showed a faster decomposition rate than cupferron due to the ortho substitution, which prevents the NONO moiety from becoming planar.[24b] Recently, our group has developed a series of para-substituted cupferron derivatives.[23] These compounds evolve NO and a nitrosobenzene derivative by a spontaneous dissociation mechanism after undergoing a one-electron oxidation. These para-substituted cupferron derivatives constitute a series of redox-sensitive NO donors. However, the instability of cupferron or its ortho- and para-substituted derivatives can be a liability in the pharmaceutical realm where targeted delivery is crucial to the success of NO donor drug efforts. To provide better donor compounds for the controlled release of NO, we designed and synthesized a series of *O*-alkyl derivatives of cupferron. We found that the spontaneous NO-release rate of these *O*-alkyl derivatives is significantly slower than that of cupferron. In addition, these derivatives could be used as a new type of photoreleasing NO donor, as well as NO prodrugs that release NO chemically and enzymatically.

Results and Discussion

The Alkylation Reaction. *O*-Alkyl derivatives of cupferron can be prepared by adding an excess amount of an alkyl halide to a solution of cupferron in DMF at 0 °C (Scheme 2). Upon completion of the reaction, as monitored by TLC, the mixture is diluted with water, extracted with methylene chloride, and dried with Na_2SO_4. The product is then further purified by silica gel chromatography using ethyl acetate−hexane as an eluent.

Scheme 2. Synthesis of *O*-Alkyl Derivatives of Cupferron

A series of *O*-alkyl derivatives of cupferron were synthesized (Table 1). Similar to diazenumdiolate,[17] an analogue of cupferron, only one type of alkylation product, *N*-(alkyloxy)-*N*′-phenyldiimide *N*′-oxide, was isolated from the reaction. Thus, the alkylation was highly regioselective. For example, in compound **10**, the only product isolated from the reaction of *p*-methoxybenzyl chloride, the benzyl group is attached to the terminal oxygen rather than the interior oxygen. The products were characterized by ^{1}H NMR, ^{13}C NMR, and MS and confirmed by X-ray analysis in certain cases.

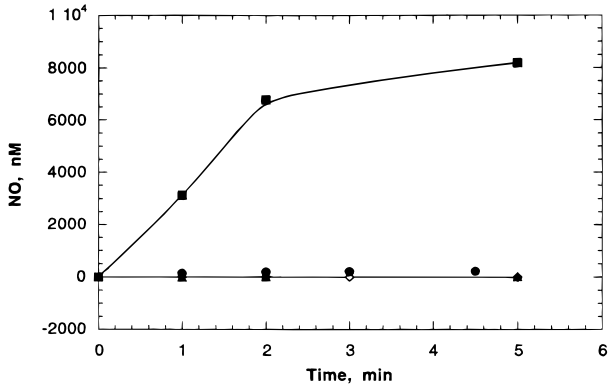

Figure 1. Stability of cupferron and its *O*-alkyl derivatives at 25 °C: (■) cupferron, (◇) *N*-(*o*-nitrobenzyl)oxy)-*N*′-phenyldiimide *N*′-oxide (**12**), (▲) *N*-methoxy-*N*′-phenyldiimide *N*′-oxide (**1**), and (●) *N*-(*N*″-acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide (**14**). The NO donors were dissolved at 6.0 mM in a mixture of CH_3CN and phosphate buffer (10 mM, pH 7.0, with 1.0 mM EDTA) (4/1 (v/v)). The solutions were deoxygenated with argon.

The O-alkylation reactions of cupferron gave reasonable yields in most cases. The highest yields were obtained with active alkyl halides (e.g., methyl iodide). The reaction yield was slightly higher than that of the O-alkylation of diazeniumdiolate.[17a] We have explored different reaction conditions to increase the reaction yield. Raising the reaction temperature and increasing the reaction time only resulted in the formation of more byproduct azoybenzene,[25] which was the decomposition product from cupferron. An increase in yield, however, was observed by increasing the ratio of alkyl halide to cupferron.

The Stability of *O*-Alkyl Cupferron Derivatives. Cupferron and its substituted derivatives are unstable and decompose to release NO (Scheme 1).[25] Using a commercial NO detector, the stability of the *O*-alkyl derivatives was directly monitored and compared to the parent compound, cupferron. It was found that, in general, *O*-alkyl derivatives exhibited remarkable stability compared to cupferron (Figure 1). On the other hand, the stability of each *O*-alkyl derivative differs slightly in a very small range. As shown in Figure 2, when the *O*-alkyl group was *O*-nitrobenzyl, no detectable decomposition was observed at all.

Photochemical Release of NO from *O*-Alkyl Derivatives of Cupferron. With the purpose of developing NO donors for controlled release, we carried out some preliminary studies on photochemical behaviors of the *O*-alkyl cupferron derivatives. It was found that while the alkyl derivatives were remarkably stable at room temperature without light they readily released NO upon

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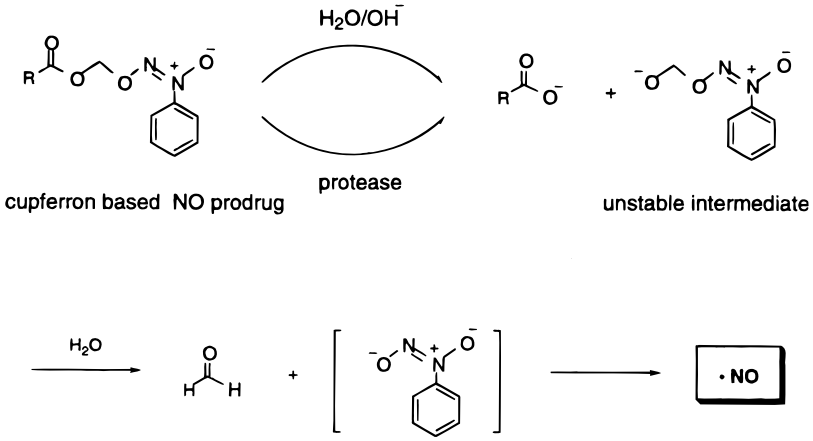
Scheme 3. Acetal-Linked *O*-Alkyl Derivatives as NO Prodrugs



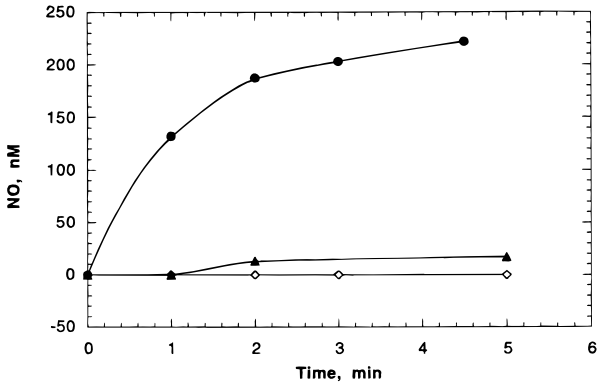


Figure 2. Stability of different *O*-alkyl derivatives: (◇) *N*-(*o*-nitrobenzyl)oxy)-*N*′-phenyldiimide *N*′-oxide (**12**), (▲) *N*-methoxy-*N*′-phenyldiimide *N*′-oxide (**1**), and (●) *N*-(*N*″-acetylphenylalanyl-methylenyloxy)-*N*′-phenyldiimide *N*′-oxide (**14**). The NO donors were dissolved at 6.0 mM in a mixture of CH_3CN and phosphate buffer (10 mM, pH 7.0, with 1.0 mM EDTA) (4/1 (v/v)). The solutions were deoxygenated with argon.

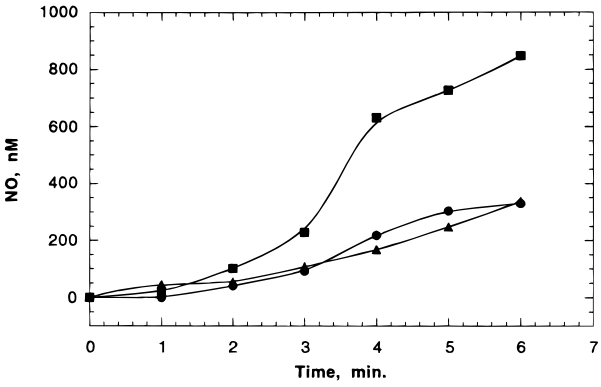
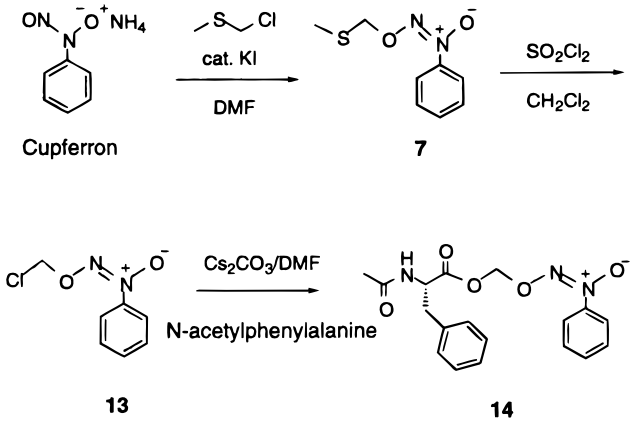


Figure 3. Photochemical NO release from *O*-alkyl derivatives of cupferron: (■) *N*-(*o*-nitrobenzyl)oxy)-*N*′-phenyldiimide *N*′-oxide (**12**), (●) *N*-(*p*-nitrobenzyl)oxy)-*N*′-phenyldiimide *N*′-oxide (**11**), and (▲) *N*-methoxy-*N*′-phenyldiimide *N*′-oxide (**1**). The NO donors were dissolved at a concentration of 6.0 mM in CH_3CN. The solutions were deoxygenated with argon and irradiated with an UV lamp of 254 nm in the dark.

UV irradiation (λ_{max} = 254 nm). As shown in Figure 3, after a 2-min initiation period, NO release occurred rapidly. The NO release was confirmed using an NO detector and as well as by an oxyhemoglobin (oxyHb) assay. In the oxyhemoglobin assay, irradiation at 254 nm released NO, which in turn converted the oxyHb to methemoglobin (metHb). Positive conversion was indicated by a characteristic peak shift from 413 nm (oxyHb) to 406 nm (metHb) in the UV spectra.[27] *N*-(*O*-Nitrobenzyloxy)-*N*′-phenyldiimide *N*′-oxide (entry 12 in Table 1) showed the best NO- releasing ability under UV irradiation. This property of the *O*-alkyl cupferrons provides a unique feature for this class of compounds in realizing photocontrolled NO-release.

***O*-Alkyl Derivatives as NO Prodrugs.** It has been our intention to develop novel NO prodrugs that possess targeting effects and can deliver NO in vivo at the desired time to the desired region. Due to its poor stability, cupferron itself cannot function as a prodrug. We have demonstrated that, by O-alkylation, the stability of cupferron can be remarkably improved. This proved to be crucial for the development of cupferron-based prodrugs. To demonstrate that *O*-alkyl derivatives of cupferron could be designed to act as NO prodrugs, we designed and synthesized a model compound *N*-(*N*″-acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide (**14**). As illustrated in Scheme 3, our strategy was to conjugate a ligand (e.g., a peptide) with an *O*-alkyl derivative of cupferron via an acetal linkage. The binding portion can be then specifically recognized by a protease and hydrolyzed at the ester bond to generate an unstable NO releasing intermediate. By varying the binding ligand, NO can be specifically delivered and released. The synthesis of compound **14** is illustrated in Scheme 4.

Scheme 4. Synthesis of *N*-(*N*″-Acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide (14)



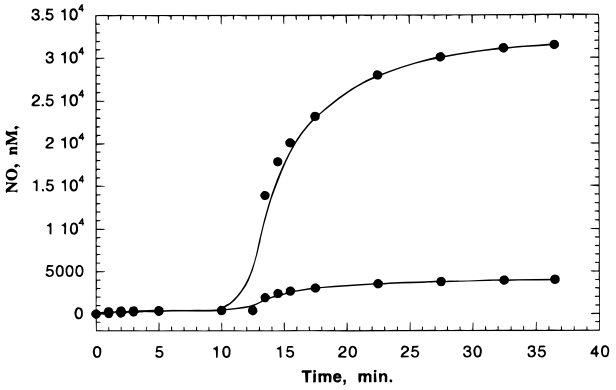


Figure 4. Chemical release of NO from *N*-(*N*′′-acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide (**14**). The compound was dissolved at 6.0 mM in a mixture of CH_3CN and phosphate buffer (10 mM with 1.0 mM EDTA) (1/1 (v/v)). Top line, pH, 11.0; Bottom line, pH 8.0.

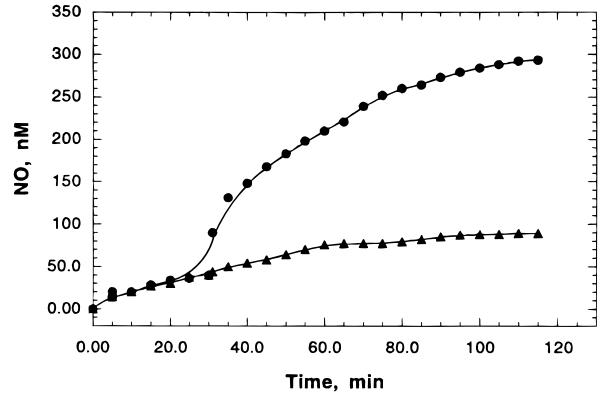


Figure 5. Enzymatic release of NO from *N*-(*N*′′-acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-oxide (**14**, ●). The lower line is the control experiment without the addition of the enzyme (▲). The compound was dissolved at 1.0 mM in a mixture of CH_3CN and phosphate buffer (10 mM, pH 7.0, with 1.0 mM EDTA) (10% (v/v)). α-Chymotryptsin (EC 3.4.21.1) was then added to a final concentration of 0.2 mg/mL.

Cupferron was first allowed to react with chloromethyl methyl sulfide; after treatment with sulfuryl chloride, compound **13** was obtained in situ. *N*-Acetylphenylalanine was then neutralized by Cs_2CO_3, and reacted with compound **13** to afford the model compound **14**.

Compound **14** was essentially stable at neutral pH. Only very slow NO-release was observed. However, as shown in Figure 4, upon increasing the pH to 8 or above, it quickly hydrolyzes and releases NO. To test the initial design, compound **14** was treated with α-chymotrypsin (EC 3.4.21.1), a protease from bovine pancreas. As expected, NO-release was significantly accelerated upon addition of the enzyme (Figure 5). The results from this model study proved the feasibility of our cupferron-based prodrug design. Further studies are in progress focusing on the design of different binding ligands.

In summary, we have demonstrated that O-alkylation of cupferron, which occurred regioselectively at the terminal oxygen, led to a single type of product, *N*-(alkyloxy)-*N*′-phenyldiimide *N*′-oxide. The *O*-alkyl derivatives exhibited remarkably improved stability. It has been shown that *O*-alkyl cupferron derivatives can function as photoreleasing NO donor agents. They can also be used for the design and synthesis of novel NO prodrugs, which expect to find wide applications in biomedical and clinic research.

Experimental Section

All the chemicals were purchased from commercial suppliers and used without further purification. Melting points were measured on a digital apparatus without correction.

Synthesis of *O*-Alkyl Derivatives of Cupferron. All reactions were carried out under argon in dark. In general, cupferron was dissolved in DMF and cooled to 0 °C. Alkyl halides were then injected through a septum and stirred overnight. TLC was used to monitor the reaction progress. The reaction mixture was diluted with water, extracted with methylene chloride and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the product was purified by chromatography using ethyl acetate−hexane as an eluent.

***N*-Methoxy-*N*′-phenyldiimide *N*′-oxide (1)**. Synthesized from the reaction of cupferron with MeI: mp 39.5 °C (lit.[28] mp 38−40 °C); UV λ_{max} = 259 nm, 1.8 × 10^2 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.00 (s, 3H), 7.26 (m, 3H), 7.75 (d, 2H, *J* = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 61.62, 120.86, 128.80, 131.11, 142.86. MS *m*/*z* (EI, relative intensity) 77 (100), 152 (M^+, 54); HRMS (EI) calcd for $C_7H_8N_2O_2$ (M^+) 152.0586, found 152.0590 (M^+).

***N*-Ethoxy-*N*′-phenyldiimide *N*′-Oxide (2).** Synthesized from the reaction of cupferron with EtI: mp 41.3 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (t, 3H, *J* = 6.0 Hz), 4.49 (q, 2H, *J* = 6.0 Hz), 7.40−7.49 (m, 3H), 7.93−7.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.56, 70.66, 121.11, 128.90, 131.11, 143.29; MS *m*/*z* (EI, relative intensity) 77 (100), 166 (M^+, 21); HRMS (EI) calcd for $C_8H_{10}N_2O_2$ (M^+) 166.0742, found 166.0743 (M^+).

***N*-*n*-Propoxy-*N*′-phenyldiimide *N*′-Oxide (3).** Synthesized from the reaction of cupferron with *n*-PrI: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.02 (t, 3H, *J* = 7.5 Hz), 1.83−1.90 (m, 2H), 4.39 (t, 2H, *J* = 7.0 Hz), 7.43−7.51 (m, 3H), 7.95−7.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 10.83, 23.09, 77.19, 121.84, 129.64, 131.83, 143.99; MS *m*/*z* (EI, relative intensity) 108 (100), 180 (M^+, 20); HRMS (EI) calcd for $C_9H_{12}N_2O_2$ (M^+) 180.0899, found 180.0903 (M^+).

***N*-(Allyoxy)-*N*′-phenyldiimide *N*′-Oxide (4).** Synthesized from the reaction of cupferron with allyl bromide:. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.89−4.92 (m, 3H), 5.31−5.46 (m, 2H), 6.00−6.14 (m, 1H), 7.45−7.53 (m, 3H), 7.94−7.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 75.36, 119.98, 121.17, 128.93, 131.23, 132.05, 143.27;. MS *m*/*z* (EI, relative intensity) 41 (100), 178 (M^+, 3); HRMS (EI) calcd for $C_9H_{10}N_2O_2$ (M^+) 178.0742, found 178.0742 (M^+).

***N*-(3-Hydroxypropoxy)-*N*′-phenyldiimide *N*′-Oxide (5).** Synthesized from the reaction of cupferron with 3-bromo-1-propanol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.04−2.08 (m, 2H), 2.84 (s, 1H), 3.79 (t, 2H, *J* = 5.9 Hz), 4.57 (t, 2H, *J* = 6.1 Hz), 7.40−7.48 (m, 3H), 7.92 (d, 2H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.75, 58.96, 72.16, 121.06, 128.96, 131.25, 143.06. MS *m*/*z* (EI, relative intensity) 108 (100), 180 (M − 17, 16); MS *m*/*z* (CI, relative intensity) 197 (M + H^+, 87); HRMS (EI) calcd for $C_9H_{12}N_2O_3$ (M − 17) 180.0898, found 180.0897 (M − 17).

***N*-((Methoxymethyl)oxy)-*N*′-phenyldiimide *N*′-Oxide (6).** Synthesized from the reaction of cupferron with bromomethyl methyl ether: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.58 (s, 3H), 5.45 (s, 2H), 7.47−7.57 (m, 3H), 8.02−8.05 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 58.48, 99.71, 122.43, 130.12, 132.65, 144.33; MS *m*/*z* (EI, relative intensity) 77 (100), 183 (M + H^+, 0.3); MS *m*/*z* (CI, relative intensity) 183 (M + H^+, 100); HRMS (EI) calcd for $C_8H_{10}N_2O_3$ (M − 30) 152.0712, found 152.0713 (M − 30).

***N*-(Methylthiomethyloxy)-*N*′-phenyldiimide *N*′-Oxide (7).** Synthesized from the reaction of cupferron with chloro-

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methyl methyl sulfide: mp 40.1 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.32 (s, 3H), 5.46 (s, 2H), 7.44−7.51 (m, 3H), 7.96−7.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.12, 79.99, 121.93, 129.74, 132.20, 143.93; MS *m*/*z* (EI, relative intensity) 61 (100), 168 (M − 30, 5); MS *m*/*z* (CI, relative intensity) 61 (100), 199 (M + H^+, 6); HRMS (EI) calcd for $C_8H_{10}N_2O_3$ (M − 30) 168.0483, found 168.0486 (M − 30).

***N*-(Phenylthiomethyloxy)-*N*′-phenyldiimide *N*′-Oxide (8).** Synthesized from the reaction of cupferron with chloromethyl phenyl sulfide: mp 42−43 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76 (s, 2H), 7.27−7.36 (m, 3H), 7.43−7.47 (m, 2H), 7.50−7.57 (m, 3H), 7.88−7.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 122.94, 128.52, 129.67, 129.92, 132.20, 132.25, 134.79, 143.92; MS *m*/*z* (EI, relative intensity) 77 (100), 230 (M − 30, 6); MS *m*/*z* (CI, relative intensity) 123 (100), 230 (M − 30, 6); HRMS (EI) calcd for $C_8H_{10}N_2O_3$ (M − 30) 230.0640, found 230.0640 (M − 30).

***N*-(Benzyloxy)-*N*′-phenyldiimide *N*′-Oxide (9).** Synthesized from the reaction of cupferron with benzyl bromide: mp 80.2−80.8 °C (lit.[29] mp 81−82 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.44 (s, 2H), 7.35−7.51 (m, 8H), 7.92−7.95 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 76.46, 121.20, 128.62, 128.73, 128.92, 131.23; MS *m*/*z* (EI, relative intensity) 91 (100), 198 (M − 30, 7); MS (FAB, M + H^+, 229); HRMS (EI) calcd for $C_{13}H_{12}N_2O_2$ (EI, M − 30) 198.0919, found 198.0922 (M − 30).

***N*-(*p*-Methoxybenzyloxy)-*N*′-phenyldiimide *N*′-Oxide (10).** Synthesized from the reaction of cupferron with 4-methoxybenzyl chloride: mp 84.4−84.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.80 (s, 3H), 5.37 (s, 2H), 6.89−6.93 (m, 2H), 7.38−7.50 (m, 5H), 7.92−7.95 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.25, 76.31, 113.99, 121.18, 127.50, 128.89, 130.65, 131.14, 144.30, 160.05; MS *m*/*z* (EI, relative intensity) 121 (100), 228 (M-30, 0.4); HRMS (EI) calcd for $C_{14}H_{14}N_2O_3$ (EI, M − 30) 228.1026, found 228.1024 (M − 30).

***N*-(*p*-Nitrobenzyloxy)-*N*′-phenyldiimide *N*′-Oxide (11).** Synthesized from the reaction of cupferron with 4-nitrobenzyl bromide: mp 101.2−101.5 °C; UV λ_{max} 269 nm ($CHCl_3$, ϵ 2.2 × 10^4 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.52 (s, 2H), 7.42−7.55 (m, 3H), 7.59−7.62 (m, 2H), 7.89−7.94 (m, 2H), 8.22−8.25 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 74.60, 121.14, 123.88, 128.78, 129.05, 131.59, 142.67; MS *m*/*z* (EI, relative intensity) 77 (100), 243 (M − 30, 16); HRMS (EI) calcd for $C_{13}H_{11}N_3O_4$ (EI, M^+) 273.0750, found 273.0749 (M^+).

***N*-(*o*-Nitrobenzyloxy)-*N*′-phenyldiimide *N*′-Oxide (12).** Synthesized from the reaction of cupferron with 2-nitrobenzyl bromide: mp 96.3−97.1 °C; UV λ_{max} 261 nm ($CHCl_3$, ϵ 2.8 × 10^4 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92 (s, 2H), 7.44−7.56 (m, 4H), 7.66−7.75 (m, 2H), 7.95−7.98 (m, 2H), 8.17−8.20 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 72.53, 121.17, 125.06, 128.64, 128.83, 129.04, 131.56, 134.18; MS *m*/*z* (EI, relative intensity) 136 (100), 243 (M − 30, 5); MS (FAB) 274 (M + H^+); HRMS (EI) calcd for $C_{13}H_{11}N_3O_4$ (M − 30) 243.0770, found 243.0768 (M − 30).

Synthesis of *N*-(*N*″-Acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-Oxide (14). Sulfuryl chloride (84 μL, 1.04 mmol), dissolved in 3 mL of CH_2Cl_2 (anhydrous), was added dropwise to a solution of *N*-(methylthiomethyloxy)-*N*′-phenyldiimide *N*′-oxide (204 mg, 1.03 mmol) in 3 mL of CH_2Cl_2 (anhydrous) at 0 °C. The stirring mixture was allowed to gradually warm to room temperature. Upon completion and removal of the solvent and the excess sufuryl chloride, *N*-(choloromethyloxy)-*N*′-phenyldiimide *N*′-oxide **13** was obtained as a yellowish liquid without further purification.

N-Acetylphenylalanine (248 mg, 1.20 mmol) and $CsCO_3$ (391 mg, 1.20 mmol) were combined in 5 mL of anhydrous DMF. The resultant suspension was stirred for 30 min at room temperature. Compound **13** was dissolved in 3 mL of DMF and cooled to 0 °C. The compound **13** was then added to the suspension via syringe. The mixture was stirred overnight at room temperature. After completion, the mixture was diluted with 30 mL of water, extracted with CH_2Cl_2, and dried with anhydrous Na_2SO_4. The desired product **14** was isolated as a white powder after column chromatography (hexanes/acetyl acetate, 3:2) (158 mg, 0.43 mmol, yield, 43%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.97 (s, 3H), 3.09−3.17 (m, 2H), 4.93−4.97 (m, 1H), 5.91−5.92 (d, 1H, J = 7.0), 5.96−5.97 (d, 1H, NH, J = 8.0), 6.12−6.14 (d, 1H, J = 7.5), 7.06−7.20 (m, 5H), 7.47−7.58 (m, 3H), 7.96−7.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 23.29 (CH_3), 37.65, 53.17, 88.54, 121.61, 127.46, 128.87, 129.39, 129.47, 130.58, 132.21, 135.46, 143.24, 169.97, 170.60; MS *m*/*z* (EI, relative intensity) 120 (100), 357 (M^+); HRMS (EI) calcd for $C_{18}H_{19}N_3O_5$ (M^+) 357.1325, found 357.1327 (M^+).

Stability Tests. Cupferron was dissolved in a 10 mM phosphate buffer (pH, 7.0; EDTA, 1.0 mM). *O*-Alkyl derivatives were dissolved in CH_3CN and diluted with a 10 mM phosphate buffer (pH, 7.0; EDTA, 1.0 mM). Solutions were deoxygenated with argon. NO generation was monitored and measured using a commercial ISO-NO Mark II isolated nitric oxide probe[30] in the dark.

Photochemical NO Release. *O*-Alkyl derivatives, dissolved in CH_3CN and deoxygenated with argon, were irradiated at a wavelength of 254 nm with a UV lamp (Spectroline, Model ENF-240C) at a fixed position. NO evolution was monitored and measured using the NO probe in the dark.

Chemical NO Release from *N*-(*N*″-Acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-Oxide. Compound **14** (10 mg) was dissolved in 3 mL of CH_3CN and diluted with 1 mL of a 10 mM phosphate buffer (pH 7.0, EDTA 1 mM). The solution was stirred and deoxygenated with argon. The pH was adjusted using a 0.1 M aqueous solution of KOH. NO evolution was monitored and measured using the NO probe in the dark.

Enzymatic NO Release from *N*-(*N*″-Acetylphenylalanylmethylenyloxy)-*N*′-phenyldiimide *N*′-Oxide. The compound was dissolved at 1.0 mM in a 10% (v/v) mixed solvent of CH_3CN and phosphate buffer (10 mM, pH 7.0, with 1.0 mM EDTA). α-Chymotryptsin (EC 3.4.21.1) was then added to a final concentration of 0.2 mg/mL. NO release was monitored and measured using the NO probe in the dark.

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Supporting Information Available: ^{1}H and ^{13}C NMR for compounds **1**−**12** and **14** and complete crystal structure parameters for compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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