JOC The Journal of Organic Chemistry

Article

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Development of N-Substituted Hydroxamic Acids with Pyrazolone Leaving Groups as Nitrosocarbonyl Precursors

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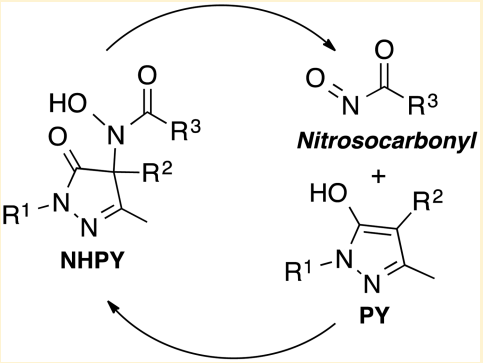
Supporting Information

ABSTRACT: A novel class of nitrosocarbonyl precursors, N-substituted hydroxamic acids with pyrazolone leaving groups (NHPY), has been synthesized. Under physiological conditions, these compounds generate nitrosocarbonyl intermediates, which upon hydrolysis release nitroxyl (azanone, HNO) in excellent yields. The amount and rate of nitrosocarbonyl generation are dependent on the nature of the pyrazolone leaving groups and significantly on the structural properties of the NHPY donors. Pyrazolones have been found to be efficient nitrosocarbonyl traps, undergoing an N-selective nitrosocarbonyl aldol reaction. This trapping reaction has been used to confirm the involvement of nitrosocarbonyl intermediates in NHPY aqueous decomposition. In addition, NHPY compounds are shown to generate nitrosocarbonyls efficiently under mild basic conditions in organic solvent and may therefore also enjoy synthetic utility.



INTRODUCTION

Nitroxyl (azanone, HNO) is the one-electron reduced and protonated relative of nitric oxide (NO) that has recently been proposed as a potential alternative to current treatments for cardiac failure.[1−9] HNO possesses unique physiological properties that affect vasorelaxation and enhancement of cardiac contractility and may also find use in the treatment of alcoholism and cancer.[8,9]

HNO is a very reactive molecule that spontaneously dimerizes to yield hyponitrous acid (HON═NOH), which subsequently dehydrates to nitrous oxide (N_2O).[10] Because of this inherent chemical reactivity, donors are needed for the generation and study of HNO. Angeli's salt (AS, Figure 1) is a well-known HNO donor with a relatively short half-life of 2−3 min under physiological conditions.[11] Accessing other HNO donors with different release rates under physiological conditions is important to assess the impact that HNO may potentially have on the treatment of diseases. Piloty's acid (PA) derivatives,[12] acyloxy nitroso (AcON) compounds,[13] (hydroxylamino)pyrazolone (HAPY),[14,15a] and (hydroxylamino)barbituric acid (HABA) derivatives[16] (Figure 1) are among a limited number of physiologically compatible HNO donors with tunable half-lives that have been reported.[17−19] Continued efforts are required to develop other potential HNO donors that can be used under physiological conditions.

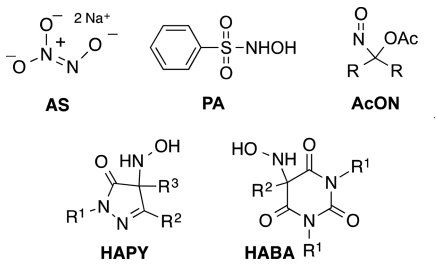


Figure 1. Representative examples of HNO donors.

One approach that has been used to generate HNO is based on the hydrolysis of nitrosocarbonyl intermediates.[20] Nitrosocarbonyls are transient electrophiles that can react with nucleophiles including water to release HNO. These intermediates can be generated by a variety of chemical processes including oxidation of hydroxamic acids, thermal fragmentation of Diels−Alder adducts,[21−23] photocleavage of 1,2,4-oxadiazole-4-oxides,[24] and the rearrangement of nitrocarbenes.[25−28] In general, these methods are not suitable for HNO generation under physiological conditions. Notable among compounds reported to generate HNO through nitrosocarbonyl hydrolysis are *N,O*-bis-acylated derivatives such as *N,O*-dibenzoyl-*N*-hydroxycyanamide that release HNO under enzymatic or basic conditions.[29] Based on this initial work, we reported modified *N,O*-bis-acylated hydroxylamine derivatives with arenesulfonyl leaving groups that produce nitrosocarbonyls nonenzymatically under physiological conditions.[17] Mechanistic studies revealed that the chemistry of these donors is complicated and that non-HNO producing pathways (acyl migration and amide hydrolysis) can be dominant.

Nitrosocarbonyls have been used as important components in a variety of synthetic approaches.[30−50] Aerobic oxidation of

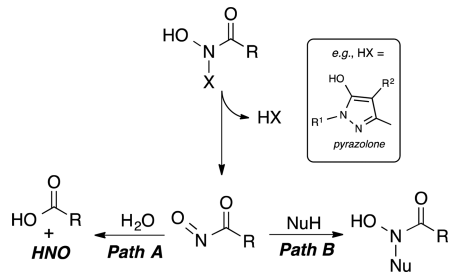
Received: July 15, 2016
Published: September 12, 2016

DOI: 10.1021/acs.joc.6b01705
J. Org. Chem. 2016, 81, 9138−9146

hydroxamic acids under mild conditions that avoid over-oxidation of products has been used to generate nitrosocarbonyl intermediates.[33−50] These reactive species can also be generated via metal catalysis and have been extensively utilized in ene reactions, Diels−Alder reactions, or as electrophiles in nitrosocarbonyl aldol reactions.

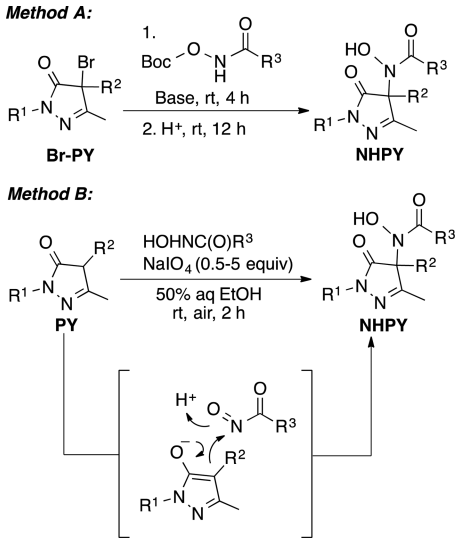
Herein, we report a new class of nitrosocarbonyl donors that decompose based on the general strategy shown in Scheme 1.

Scheme 1. Reactivity of N-Substituted Hydroxamic Acids with Good Leaving Groups X



N-Substituted hydroxamic acids with pyrazolone leaving groups (NHPY, shown in Scheme 2) are efficient nitrosocarbonyl

Scheme 2. Strategies for the Synthesis of NHPY Derivatives



donors that upon *O*-deprotonation and loss of HX (HX = pyrazolone) generate nitrosocarbonyl intermediates either under physiological conditions or in basic organic solvent. In aqueous solutions, subsequent hydrolysis of the nitrosocarbonyl (path A) generates a carboxylic acid and HNO in excellent yield. In organic solution, as has been elegantly demonstrated in recent reports,[37,39,40,44−46,49] nitrosocarbonyl intermediates can react with nucleophiles at the nitrogen of the nitroso group through an N-selective nitrosocarbonyl aldol reaction (path B) to produce N-substituted hydroxamic acid adducts. We have observed this same reactivity using pyrazolone traps that have also been used to confirm nitrosocarbonyl generation upon NHPY decomposition.

On the basis of previously reported HAPY donors,[14,15a] pyrazolones have been shown to be efficient leaving groups with the rate of HAPY decomposition dependent on the nature of the pyrazolone. This observation was utilized to design HAPY donors with varying half-lives. In this report, we demonstrate that the rate of nitrosocarbonyl generation from NHPY compounds depends both on the nature of the leaving groups and also unexpectedly on the structural properties of the substituted hydroxylamino group as discussed in detail below.

RESULT AND DISCUSSION

Synthesis. We have developed two general methodologies for the synthesis of NHPY derivatives: (1) the reaction of the corresponding brominated pyrazolone (Br-PY) with an *O*-*tert*-butoxycarbonyl protected hydroxamic acid, followed by subsequent acid deprotection (Scheme 2, method A), and (2) an N-selective nitrosocarbonyl aldol reaction initiated by oxidation of hydroxamic acids in the presence of pyrazolones (Scheme 2, method B). A 50% v/v aqueous ethanol solution enhances the solubility of reactants, and potassium carbonate is used to adjust the pH of solution in the range of 7−8 to favor nitrosocarbonyl reaction with the deprotonated pyrazolone.

We first synthesized NHPY donors **1a,b** using the two described methods; yields are reported in Table 1. Method A

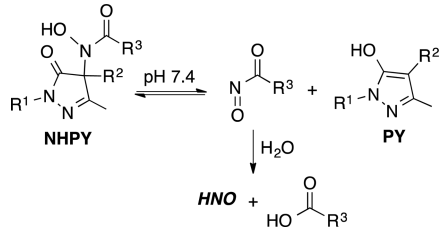
Table 1. Synthetic Yields for NHPY Donors

				% yield	
NHPY	R^1	R^2	R^3	method A	method B
1a	Ph	C(=NOMe)Me	Me	<10	73
1b	Me	C(=NOMe)Me	Me	<10	75
1c	Ph	C(=NOMe)Me	OMe	48	71
1d	Me	C(=NOMe)Me	OMe	50	69
1e	Ph	C(=NOMe)Me	NMe_2	35	48
1f	Me	C(=NOMe)Me	NMe_2	38	47
1g	Ph	Me	NMe_2	11	48

provides **1a,b** in very poor yields, whereas the nitrosocarbonyl aldol reaction affords these precursors in much higher yields (73−75%). The synthesis of donors **1c,d** via either of two methods resulted in moderate to good yields (48−71%). Methods A and B gave donors **1e−g** in low to moderate yields (11−48%). The nitrosocarbonyl aldol reaction (method B) is straightforward, single pot, and scalable; donor **1a** can be prepared on a gram scale under conditions identical to a 100 mg scale without any significant loss in isolated yield. For future studies, it may be possible to expand this synthetic route to other active enolates that may undergo further synthetic manipulation.

Decomposition under Physiological Conditions. HNO generation following decomposition of the synthesized NHPY donors under physiological conditions was explored. Presumably, upon deprotonation of oxygen, a nitrosocarbonyl intermediate and pyrazolone byproduct are formed (Scheme 3). As described above, the reactions of nitrosocarbonyls and

Scheme 3. HNO Release Pathway from NHPY Donors



DOI: 10.1021/acs.joc.6b01705
J. Org. Chem. 2016, 81, 9138−9146

pyrazolones can be efficient, and therefore, there is a competition between the reverse reaction to produce the initial NHPY compound and hydrolysis of the nitrosocarbonyl intermediate to generate HNO.

We have recently used ^{1}H NMR spectroscopy to measure half-lives of donor decomposition by quantifying donor and pyrazolone byproduct as a function of time.[15,16] On the basis of the distinctive chemical shifts of the methyl groups of the NHPY donors and the pyrazolone byproducts, the decomposition of the donors and release of byproducts is easily monitored. Utilizing this assay, the half-lives of NHPY donors were determined under physiologically relevant conditions (Table 2).

Table 2. Half-lives and HNO Yields for NHPY Donors

NHPY	R^1	R^2	R^3	$t_{1/2}$[a]	% HNO[c]
1a	Ph	C(=NOMe)Me	Me	5 days	82[d]
1b	Me	C(=NOMe)Me	Me	stable[b]	
1c	Ph	C(=NOMe)Me	OMe	2 days	104[d]
1d	Me	C(=NOMe)Me	OMe	4 days	94[d]
1e	Ph	C(=NOMe)Me	NMe_2	25 min	>95[e]
1f	Me	C(=NOMe)Me	NMe_2	46 min	>95[e]
1g	Ph	Me	NMe_2	stable[b]	

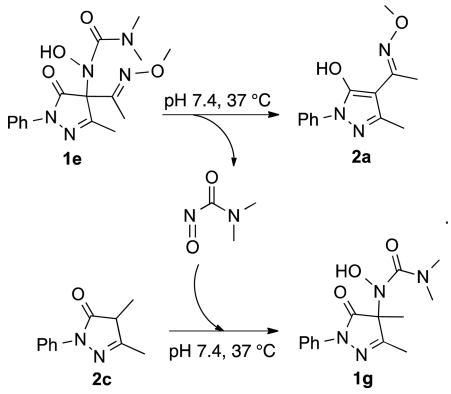
[a]Determined from ^{1}H NMR analysis of the decomposition of 5 mM of the donor in 10% DMSO-d_6, 10% D_2O, and 80% H_2O, phosphate buffer (0.25 M) with DTPA (0.2 mM), pH 7.4 at 37 °C under argon. [b]Less than 5% decomposition after 2 days. [c]Donors (0.1 mM) were incubated in phosphate buffer (0.25 M) with DTPA (0.2 mM), pH 7.4 at 37 °C under argon. [d]HNO yields are measured at the half-life of the donor. [e]HNO yields are measured after complete decomposition of the donor and are reported relative to the standard HNO donor, Angeli's salt, as determined by N_2O headspace analysis (SEM ± 5%; n = 3).

Based on our previous studies of HAPY donors,[15a] we investigated the effect of the leaving group on NHPY donor half-life. As expected, we observe a substantial impact on half-life that is related to the pK_a values of the respective pyrazolone byproducts. For example, pyrazolone **2a** (R^1 = Ph, R^2 = C(=NOMe)Me) has a pK_a of 6, making it anionic under physiological conditions and consequently a better leaving group compared with pyrazolone **2c** (R^1 = Ph, R^2 = Me), which has a pK_a of 7.6.[15a] Accordingly, when R^2 is changed from an *O*-methyloxime group (donors **1e**,**f**) to a methyl group (donor **1g**), the observed half-life increases dramatically from tens of minutes to days (Table 2). Exchanging the R^1 group from phenyl to methyl also has a small effect on half-life, consistent with that previously reported for HAPY donors.[15a] Further exploration of NHPY derivatives revealed that the R^3 group has a surprisingly large effect on half-life (Table 2). The origin of this unexpected effect is examined in more detail below.

Dimerization of HNO and subsequent dehydration provides N_2O, which is a common benchmark for HNO production.[10] Using Angeli's salt as a standard, the relative amounts of N_2O released from the NHPY donors were measured via gas chromatography headspace analysis following incubation in pH 7.4 phosphate buffer solutions containing the metal chelator, diethylenetriaminepentaacetic acid (DTPA), at 37 °C under argon. In all examples (Table 2), we find that the NHPY compounds are excellent HNO donors, even for the very long-lived donors (**1a** and **1c**,**d**). HNO was confirmed as the source of N_2O from donor **1e** by membrane inlet mass spectrometry (MIMS), which is a useful technique to detect dissolved gases, including HNO, directly in aqueous solutions (Supporting Information).[51,52]

Mechanistic Studies. Pyrazolone **2c** has recently been shown to be an effective trap for HNO and nitrosocarbonyl intermediates under physiological conditions.[15] Our proposed mechanism for the decomposition of the NHPY class of HNO donors involves initial oxygen deprotonation followed by formation of a nitrosocarbonyl intermediate. Further hydrolysis of this reactive species generates HNO. Based on analogy with HNO and recent reports of nitroso aldol and nitrosocarbonyl aldol reactions,[15,16,37,39,40,44,46,48−50,53] we examined pyrazolone **2c** as an efficient trap for nitrosocarbonyls (Scheme 4).

Scheme 4. Nitrosocarbonyl Trapping by Pyrazolone 2c

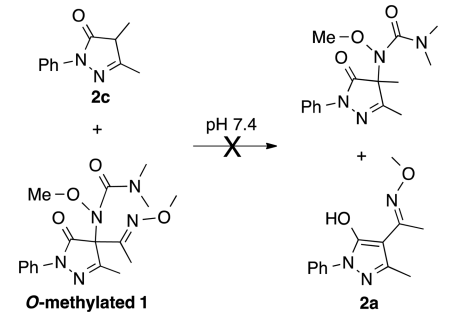


Donor **1e** was incubated in the presence of pyrazolone **2c** and the reaction was followed by ^{1}H NMR spectroscopy in pH 7.4 phosphate buffer at 37 °C (Supporting Information). Upon decomposition of donor **1e**, we observe efficient trapping of the nitrosocarbonyl intermediate by pyrazolone **2c** through an N-selective nitrosocarbonyl aldol reaction to generate NHPY **1g**, confirming that pyrazolones are efficient traps of these reactive intermediates.

Although the above findings strongly suggest the formation of a nitrosocarbonyl intermediate, based on Glover's extensive reactivity studies of bis-heteroatom-substituted amides,[54] attack of pyrazolone **2c** at the amide nitrogen of the hydroxamic acid moiety of donor **1e** to form NHPY **1g** directly may be possible. To eliminate this possibility, *O*-methylated NHPY **1** was synthesized and its stability in the presence of pyrazolone **2c** was confirmed by ^{1}H NMR spectroscopy (Scheme 5).

Stability Studies. As described previously, decomposition analysis of NHPY compounds revealed a surprisingly strong

Scheme 5. Possible Reaction of *O*-Methylated NHPY 1 with Pyrazolone 2c



DOI: 10.1021/acs.joc.6b01705
J. Org. Chem. 2016, 81, 9138−9146

dependence of the R^3 group on donor stability. Donors **1a** (R^3 = Me), **1c** (R^3 = OMe), and **1e** (R^3 = NMe_2) all have the same pyrazolone leaving group but decompose with dramatically different half-lives of 5 days, 2 days, and 25 min, respectively.

Our previous studies determined that HAPY decomposition rates are correlated with donor pK_a, confirming a very low barrier for decomposition once the oxyanion is formed.[15a,55] To determine if the R^3 group has an unexpected impact on the pK_a values of NHPY compounds, we evaluated the pK_a of donors **1a**, **1c**, and **1e**. Decomposition of donor **1e** was monitored by examining the growth of the anionic pyrazolone byproduct **2a** (λ = 265 nm) at pH 7.4 and 37 °C in phosphate buffer as a function of pH (Figure 2).[14,15a] The decomposition rate is pH

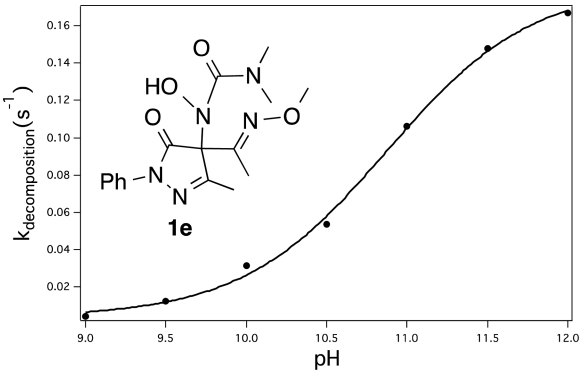


Figure 2. Plot of the observed decomposition rates of donor **1e** (20 μM) as a function of pH in 0.25 M phosphate buffer containing 0.2 mM DTPA at 25 °C determined by UV–vis analysis of the growth of the anionic pyrazolone byproduct **2a** (λ_{max} = 265 nm). The solid curve is the calculated best fit to a sigmoid function.

dependent, and on the basis of the experimental kinetic data, the pK_a of donor **1e** is estimated to be 10.7. Under these conditions, donor **1c** decomposes slowly, and donor **1a** is observed to be stable for 1 h. Thus, we measured the pK_a values of donor **1a** (10.0) and **1c** (9.1) in 50% (v/v) aqueous ethanol by titration with NaOH solution. These measurements reveal that donors **1a**, **1c**, and **1e** have similar pK_a values. Interestingly, HAPY compounds with pK_a values near 10.5 have half-lives of approximately 30 min,[15a,55] consistent with our results for donor **1e**. In contrast to the HAPY compounds (and **1e** (R^3 = NMe_2)), the stability of **1a** (R^3 = Me) and **1c** (R^3 = OMe) suggests a barrier for the decomposition of these donors even after oxyanion formation.

Structural Studies. To explore the origin of the R^3 group's impact on donor stability, we examined NHPY structural properties. Resonance structures **A**–**C** (Figure 3) can be used as a guide to describe the structures of NHPY compounds. We assume that nitrosocarbonyl formation should be favored from structures **A** and **C** but not from structure **B**.

X-ray Crystallographic Studies. For NHPY compounds **1a**, **1b**, **1d**, and **1g**, we were able to obtain suitable crystals for

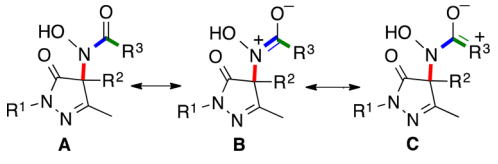
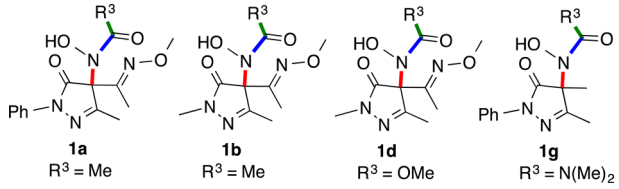


Figure 3. Important resonance structures for NHPY compounds.

X-ray diffraction experiments. Distances for the C–N bond (red) between the pyrazolone ring and the hydroxamic acid nitrogen are similar in all cases; however, the N–C(O) bond (blue) distance depends on the electron-donating ability of the R^3 group (Table 3). This bond is shortest for R^3 = Me and

Table 3. Selected X-ray Crystallographic Geometry Parameters for NHPY 1a, 1b, 1d, and 1g

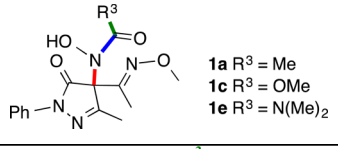


NHPY	d_{C-N} (Å)	$d_{N-C(O)}$ (Å)	$d_{C(O)-R^3}$ (Å)	$d_{C=O}$ (Å)	< HO-N-C=O (°)
1a	1.459	1.355	1.500	1.229	157.7
1b	1.454	1.349	1.492	1.240	164.0
1d	1.455	1.385	1.335	1.209	157.2
1g	1.471	1.409	1.341	1.238	139.4

longest for R^3 = NMe_2. The C(O)–R^3 bond (green) lengths are similarly impacted by R^3. These bond lengths suggest that resonance structure **C** is most important for R^3 = NMe_2. The HO–N–C=O dihedral angle of NHPY **1g** (R^3 = NMe_2) (139.4°) is also consistent with resonance structure **C**. On the other hand, the bond length and angles observed for NHPY **1a** (R^3 = Me) and **1b** (R^3 = Me) suggest that resonance structure **B** is most important for these precursors. This resonance structure should not favor nitrosocarbonyl generation, and as a result, these donors decompose very slowly (**1a**) or are stable (**1b**) even after deprotonation.

Computational Studies. To gain additional insight into the structural properties and further compare these with half-life measurements, we calculated the geometries of NHPY donors **1a** (R^3 = Me), **1c** (R^3 = OMe), and **1e** (R^3 = NMe_2). All calculations were performed with Spartan '14 at the B3LYP/6-31G(d) level;[56] optimized geometries and vibrational frequencies were calculated for each compound. Based on the calculated N–C(O) and C(O)–R^3 bond distances and the HO–N–C=O dihedral angles, the degree of pyramidalization at the amide nitrogen is markedly increased in donor **1e** compared with donors **1a** and **1c** (Table 4). This result, along with the observed half-lives, suggests that an analysis of the contributions of resonance structures **A**–**C** is a good indicator of the stability of the donors. Donor **1e** ($t_{1/2}$ = 25 min), with dominant resonance structure **C**, possesses a pyramidal amide nitrogen and is a much shorter-lived donor compared with donors **1a** ($t_{1/2}$ = 5 days) and **1c** ($t_{1/2}$ = 2 days). Resonance

Table 4. Selected B3LYP/6-31G(d) Calculated Geometry Parameters for NHPY 1a, 1c, and 1e



NHPY	d_{C-N} (Å)	$d_{N-C(O)}$ (Å)	$d_{C(O)-R^3}$ (Å)	$d_{C=O}$ (Å)	< HO-N-C=O (°)
1a	1.470	1.385	1.512	1.222	169.5
1c	1.466	1.394	1.343	1.215	154.7
1e	1.468	1.413	1.369	1.232	136.5

DOI: 10.1021/acs.joc.6b01705
J. Org. Chem. 2016, 81, 9138–9146

structure **B**, the major contributor for NHPY **1a**, does not favor nitrosocarbonyl generation, consistent with the long half-life of this donor.

Hydroxyl group deprotonation is expected to be the first step for decomposition of NHPY donors. As mentioned above, donors **1a** and **1c** are still relatively stable to dissociation even after deprotonation. This observation suggests a barrier to decomposition for the oxyanionic species. To gain insight into the stability of anionic NHPY compounds, we calculated barriers for dissociation upon deprotonation. Consistent with the results discussed earlier, these calculations indicate the lowest barrier of dissociation for deprotonated **1e** ($R^3 = NMe_2$) and highest for deprotonated **1a** (R^3 = Me) (Table 5), again consistent with the resonance structure discussion above.

Table 5. B3LYP/6-31G(d) Calculated Decomposition Barriers for Anionic Donors

NHPY	$\Delta G^{\ddagger}$ (kcal/mol)
1a	11.8
1c	7.9
1e	5.2

The relationship between resonance structures and donor decomposition was further studied by examining the barrier for rotation around HON−C(O) bonds in three model hydroxamic acid derivatives **3a−c** (Figure 4). The lowest

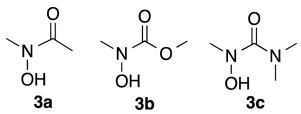


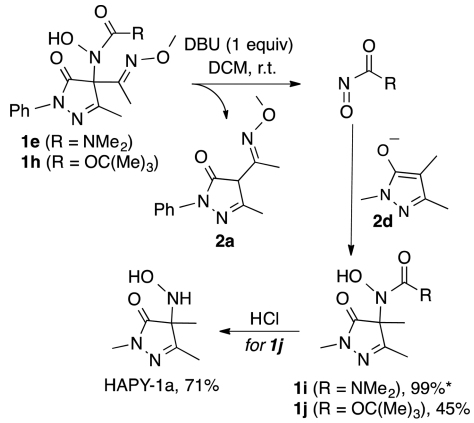
Figure 4. Model hydroxamic acid derivatives.

barriers to rotation around the HON−C(O) bonds for these three model compounds were calculated to be 15, 11.2, and 5.1 kcal/mol, respectively (Supporting Information). This observed trend indicates a correlation between the nature of substituted carbonyl and the rotational barrier.[57] The highest barrier to rotation for compound **3a** with R = Me suggests a strong contribution from resonance structure **B** in Figure 3. On the other hand, the low barrier to rotation calculated for compound **3c** with R = NMe_2 suggests a large contribution from resonance structure **C**.

Potential Synthetic Utility of NHPY Compounds. Nitrosocarbonyl compounds have been utilized as versatile electrophiles in a range of synthetic procedures. Recently, mild oxidative pathways to generate nitrosocarbonyls have significantly expanded the scope of their applications. Diels−Alder reactions, ene reactions, and nitrosocarbonyl aldol reactions are examples of useful methodologies that involve nitrosocarbonyl intermediates.[33−46,48,50]

To demonstrate the potential synthetic utility of NHPY compounds, the reaction of donor **1e** with anionic pyrazolone **2d** was examined in organic solvent (dichloromethane) in the presence of 1 equiv of the non-nucleophilc base diazabicycloundecene (DBU) (Scheme 6). After complete decomposition of NHPY **1e**, ^{1}H NMR spectroscopy confirmed the formation of pyrazolone byproduct **2a** and a new N-substituted hydroxamic acid adduct **1i** in excellent yield (99%), which was independently synthesized and characterized. The formation of pyrazolone **1i** is the result of nitrosocarbonyl formation and

Scheme 6. Nitrosocarbonyl Generation from NHPY Compounds in Organic Solvent



subsequent trapping by pyrazolone byproduct **2d** and can be thought of as a nitrosocarbonyl-transfer reaction.

We also examined a hydroxamic acid derivative that can be further manipulated after completion of the reaction. Donor **1h** generates a nitrosocarbonyl intermediate under mild basic conditions, and in the presence of anionic pyrazolone **2d**, N-substituted hydroxamic acid adduct **1j** is formed in good yield (45%) (Scheme 6). Acid-catalyzed deprotection of the Boc group leads to the formation of HAPY-**1a**, which has previously been reported.[16] These results demonstrate that NHPY compounds are efficient nitrosocarbonyl precursors that can be used under mild basic conditions in organic solvent without the need of metal-based catalysts. This nonoxidative protocol importantly avoids any problems associated with product overoxidation.

CONCLUSION

The NHPY class of nitrosocarbonyl precursors quantitatively generates nitrosocarbonyl intermediates (and ultimately HNO) under physiological conditions with tunable half-lives. The nitrosocarbonyl release rate is dependent on the nature of pyrazolone leaving groups and also significantly on the structural properties of the donors. Under the conditions of our experiments, pyrazolones are demonstrated to be efficient nitrosocarbonyl traps through an N-selective nitrosocarbonyl aldol reaction. This trapping reaction has been used to provide evidence for the involvement of nitrosocarbonyl intermediates in the aqueous decomposition of NHPY compounds. Additionally, NHPY compounds are shown to release nitrosocarbonyls under mild basic conditions in organic solvent and may enjoy further synthetic applications.

EXPERIMENTAL SECTION

Method and Materials. All starting materials were of reagent grade and used without further purification. 4-(Acetyl-*O*-methoxyoxime)-*N*-phenyl-3-methyl-pyrazolone (**2a**), 4-(acetyl-*O*-methoxyoxime)-1,3-dimethylpyrazolone (**2b**), 3,4-methyl-*N*-phenylpyrazolone (**2c**), 1,3,4-trimethylpyrazolone (**2d**),[15a] brominated pyrazolones (Br-PY),[14] *C*-methoxycarbohydroxamic acid, and *N*-hydroxy-*N*′,*N*′-dimethylurea[58] were prepared according to literature procedures. Acetohydroxamic acid was purchased and used without further purification. NMR spectra were obtained on a 300 or 400 MHz FT-NMR spectrometer. All chemical shifts are reported in parts per million (ppm) relative to residual $CHCl_3$ (7.26 ppm for ^{1}H, 77.23 ppm for ^{13}C). High-resolution mass spectra were collected on a magnetic

DOI: 10.1021/acs.joc.6b01705
J. Org. Chem. 2016, 81, 9138−9146

sector mass spectrometer working in fast atom bombardment (FAB) mode. Gas chromatography (GC) headspace analysis was performed on an instrument equipped with ECD detection and a packed carbon molecular sieve column. UV−vis absorption spectra were collected using a diode array spectrophotometer.

Method A for the Synthesis of NHPY Compounds. Base (1 equiv) was added to a solution of Boc-protected hydroxamic acids (1 equiv) in an appropriate solvent at room temperature and stirred for 1 h. The solution was added dropwise to a solution of brominated pyrazolone and stirred for 3 h. The reaction was followed to completion by TLC. The organic solvent was then removed by rotary evaporation, and the NHPY product was purified either by column chromatography (ethyl acetate/hexane) on silica gel or by recrystallization from dichloromethane and hexane.

Method B for the Synthesis of NHPY Compounds. Hydroxamic acid (1−5 equiv) was added to a solution of pyrazolone (1 equiv) in 50% aqueous ethanol. The pH of the solution was adjusted to 7−8 using 0.2 equiv of potassium carbonate. Sodium periodate (0.5−5 equiv) was added to the solution, and the reaction mixture was sonicated for 10 min and stirred at room temperature for 3 h until the reaction was complete as determined by TLC. The white solid was removed by filtration and the resulting filtrate concentrated under reduced pressure. Recrystallization from dichloromethane and hexane gave the desired NHPY compound.

*N-Hydroxy-N-(4-(acetyl-O-methoxyoxime)-3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-N-acetamide (**1a**).* According to method A, triethylamine (0.05 mL, 0.39 mmol) was added to a solution of *N*-(*tert*-butoxycarbonyloxy)acetamide (0.068 g, 0.39 mmol) in acetonitrile (4 mL) at room temperature, and the reaction was stirred for 1 h. This solution was added dropwise to 4-(acetyl-*O*-methoxyoxime)-4-bromo-*N*-phenyl-5-methylpyrazolone (0.126 g, 0.39 mmol), and the reaction proceeded at room temperature for 3 h. The reaction was concentrated by rotary evaporation, and the resulting solid was redissolved in dichloromethane and washed with water. The organic phase was collected and concentrated via rotary evaporation. Without further purification, the compound was dissolved in methanol (3 mL) and cooled to 0 °C, and acetyl chloride (0.2 mL) was added. The reaction was allowed warm to room temperature and stirred overnight. The solution was concentrated via rotary evaporation, redissolved in dichloromethane, and filtered, and the filtrate was concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (9 mg, 7%).

According to method B, acetohydroxamic acid (0.161 g, 2.14 mmol) was added to a solution of pyrazolone byproduct **2a** (0.105 g, 0.43 mmol) in 50% aqueous ethanol (7 mL), and potassium carbonate (0.012 g, 0.09 mmol) was added to adjust the pH to 7−8. Sodium periodate (0.458 g, 2.14 mmol) was added to the reaction mixture, sonciated for 10 min, and then stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (10 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, and the resulting solid was redissolved in ethyl acetate (50 mL) and washed three times with a saturated solution of ammonium chloride (30 mL). The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.1 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (m, 2H), 7.51 (s, 1H), 7.42 (m, 2H), 7.22 (m, 1H), 3.95 (s, 3H), 2.25 (s, 3H), 2.19 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 159.0, 157.0, 151.4, 137.8, 128.8, 125.8, 119.5, 76.3, 62.5, 21.0, 15.4, 11.4. HR-MS (FAB): found m/z = 319.14094 (MH^+), calcd for $C_{15}H_{18}N_4O_4$ 319.14063. Mp: 168−170 °C.

*N-Hydroxy-N-(4-(acetyl-O-methoxyoxime)-1,3-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-N-acetamide (**1b**).* Following the methods described above for the synthesis of NHPY **1a** and using 4-(acetyl-*O*-methoxyoxime)-4-bromo-1,3-dimethyl-pyrazolone in method A and pyrazolone **2b** in method B, the title compound was obtained in 8% and 75% yields, respectively, as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (s, 1H), 3.92 (s, 3H), 3.32 (s, 3H), 2.25 (s, 3H), 2.10 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.9, 161.4, 157.0, 151.4, 75.0, 62.5, 31.9, 21.3, 15.5, 11.6. HR-MS (FAB): found m/z = 257.12526 (MH^+), $C_{10}H_{16}N_4O_4$ 257.12498. Mp: 171−173 °C.

*N-Hydroxy-N-(4-(acetyl-O-methoxyoxime)-3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-N-methylcarbamate (**1c**).* According to method A, sodium hydride 60% (0.061 g, 1.52 mmol) was added to a solution of *N*-(*tert*-butoxycarbonyloxy)methylcarbamate (0.265 g, 1.38 mmol) in dimethylformamide (6 mL) at room temperature, and the reaction was stirred for 1 h. This solution was added dropwise to 4-(acetyl-*O*-methoxyoxime)-4-bromo-*N*-phenyl-5-methylpyrazolone (0.447 g, 1.38 mmol), and the reaction proceeded at room temperature for 3 h. The reaction was diluted with ether (10 mL) and washed with ammonium chloride, water, and brine. The organic phase was collected and concentrated via rotary evaporation. Without further purification, the compound was dissolved in methanol (10 mL) and cooled to 0 °C, and acetyl chloride (0.6 mL) was added. The reaction was allowed to warm to room temperature and stirred overnight. The solution was concentrated via rotary evaporation, redissolved in dichloromethane, and filtered, and the filtrate was concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.221 g, 48%).

According to method B, to a solution of pyrazolone **2a** (0.245 g, 1 mmol) and *C*-methoxycarbohydroxamic acid (0.109 g, 1.2 mmol) in 50% aqueous ethanol (5 mL) was added potassium carbonate (0.028 g, 0.2 mmol) to adjust the pH to 7−8. Sodium periodate (0.257 g, 1.2 mmol) was added to the reaction mixture, which was sonicated for 10 min and then stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (10 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, redissolved in ethyl acetate (50 mL), and washed three times with saturated solution of ammonium chloride (30 mL). The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.237 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.59 (s, 1H), 7.88 (m, 2H), 7.41 (m, 2H), 7.23 (m, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 2.26 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.6, 158.3, 158.1, 152.2, 138.0, 128.9, 125.6, 119.6, 76.5, 62.6, 54.5, 16.0, 10.9. HR-MS (FAB): found m/z = 335.13550 (MH^+), calcd for $C_{15}H_{18}N_4O_5$ 335.13554. Mp: 160−162 °C.

*N-Hydroxy-N-(4-(acetyl-O-methoxyoxime)-1,3-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-N-methylcarbamate (**1d**).* Following the methods described above for the synthesis of NHPY **1c** and using brominated pyrazolone 4-(acetyl-*O*-methoxyoxime)-4-bromo-1,3-dimethylpyrazolone in method A and pyrazolone **2b** in method B, the title compound was obtained in 50% and 69% yields, respectively, as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.05 (s, 1H), 3.89 (s, 3H), 3.82 (s, 3H), 3.30 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.4, 157.9, 157.5, 152.0, 75.4, 62.5, 54.3, 31.9, 15.7, 11.0. HR-MS (FAB): found m/z = 273.11964 (MH^+), calcd for $C_{10}H_{16}O_5N_4$ 273.11989. Mp: 162−164 °C.

*N′,N′-Dimethyl-N-hydroxy-N-(4-(acetyl-O-methoxyoxime)-3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)urea (**1e**).* According to method A, triethylamine (0.07 mL, 0.5 mmol) was added to a solution of *N*-(*tert*-butoxycarbonyloxy)-*N′*,*N′*-dimethylurea (0.102 g, 0.5 mmol) in acetonitrile (6 mL) at room temperature and the reaction stirred for 1 h. This solution was added dropwise to 4-(acetyl-*O*-methoxyoxime)-4-bromo-*N*-phenyl-5-methylpyrazolone (0.161 g, 0.5 mmol), and the reaction proceeded at room temperature for 3 h. The reaction was concentrated by rotary evaporation, redissolved in dichloromethane, and washed with water. The organic phase was collected and concentrated via rotary evaporation. Without further purification, the compound was dissolved in methanol (7 mL) and cooled to 0 °C, and acetyl chloride (0.3 mL) was added. The reaction was allowed warm to room temperature and stirred overnight. The solution was concentrated via rotary evaporation, redissolved in dichloromethane, and filtered, and the filtrate was concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (61 mg, 35%).

According to method B, to a solution of pyrazolone byproduct **2a** (0.711 g, 2.9 mmol) and *N*-hydroxy-*N′*,*N′*-dimethylurea (0.302 g, 2.9 mmol) in 50% aqueous ethanol (15 mL) was added potassium

DOI: 10.1021/acs.joc.6b01705
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carbonate (0.08 g, 0.58 mmol) to adjust the pH to 7−8. Sodium periodate (0.299 g, 1.4 mmol) was added to the reaction mixture, which was sonicated for 10 min and stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (5 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, redissolved in ethyl acetate (40 mL), and washed three times with saturated solution of ammonium chloride (20 mL). The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.483 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (m, 2H), 7.44 (m, 2H), 7.40 (s, 1H), 7.22 (m, 1H), 3.89 (s, 3H), 3.00 (s, 6H), 2.22 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 161.1, 157.0, 152.6, 138.0, 128.8, 125.3, 119.3, 76.2, 62.5, 37.9, 15.56, 11.4. HR-MS (FAB): found m/z = 348.16732 (MH^+), calcd for $C_{16}H_{22}N_5O_4$ 348.16718. Mp: 140−142 °C.

*N′,N′-Dimethyl-N-hydroxy-N-(4-(acetyl-O-methoxyoxime)-1,3-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)urea (**1f**).* Following the methods described above for the synthesis of NHPY **1e** and using brominated 4-(acetyl-*O*-methoxyoxime)-4-bromo-1,3-dimethylpyrazolone in method A and pyrazolone **2b** in method B, the title compound was obtained in 38% and 47% yields, respectively, as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35 (s, 1H), 3.92 (s, 3H), 3.35 (s, 3H), 3.02 (s, 6H), 2.09 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.7, 161.5, 157.8, 152.8, 75.2, 62.8, 38.2, 31.8, 16.1, 10.7. HR-MS (FAB): found m/z = 286.15192 (MH^+), calcd for $C_{11}H_{19}N_5O_4$ 286.15153. Mp: 144−146 °C.

*N′,N′-Dimethyl-N-hydroxy-N-(3,4-dimethyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)urea (**1g**).* Following the methods described above for the synthesis of NHPY **1e** and using 4-bromo-4,5-dimethyl-*N*-phenyl-pyrazolone in method A and pyrazolone **2c** in method B, the title compound was obtained in 11% and 48% yields, respectively, as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (m, 2H), 7.38 (m, 2H), 7.25 (s, 1H), 7.17 (m, 1H), 3.00 (s, 6H), 2.10 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.7, 162.8, 161.6, 138.1, 128.8, 124.9, 119.0, 69.6, 38.1, 20.1, 12.7. HR-MS (FAB): found m/z = 291.14536 (MH^+), calcd for $C_{14}H_{19}O_3N_4$ 291.14572. Mp: 162−164 °C.

*N-Hydroxy-N-(4-(acetyl-O-methoxyoxime)-3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-tert-butylcarbamate (**1h**).* According to method B, to a solution of pyrazolone **2a** (0.135 g, 0.55 mmol) and *tert*-butyl hydroxycarbamate (0.088 g, 0.66 mmol) in 50% aqueous ethanol (3 mL) was added potassium carbonate (0.018 g, 0.13 mmol) to adjust the pH to 7−8. Sodium periodate (0.141 g, 0.66 mmol) was added to the reaction mixture, which was sonicated for 10 min and then stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (6 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, redissolved in ethyl acetate (50 mL), and washed three times with saturated solution of ammonium chloride (30 mL). The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.142 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.92 (m, 2H), 7.40 (m, 2H), 7.19 (m, 1H), 3.87 (s, 3H), 2.26 (s, 3H), 2.13 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.86, 158.6, 156.9, 152.5, 138.7, 129.0, 124.6, 118.3, 85.7, 76.0, 62.4, 28.2, 15.8, 10.9. HR-MS (FAB): found m/z = 377.18193 (MH^+), calcd for $C_{18}H_{24}O_5N_4$ 377.18250.

*N′,N′-Dimethyl-N-hydroxy-N-(1,3,4-trimethyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)urea (**1i**).* A solution of pyrazolone **2d** (0.023 g, 0.18 mmol) and 1,8-diazabicycloundec-7-ene (0.027 mL, 0.18 mmol) in 1 mL of dichloromethane was stirred for 30 min. The mixture was added to a solution of NHPY **1e** (0.062 g, 0.18 mmol) in dichloromethane (1 mL). To this solution was added dropwise diazabicycloundec-7-ene (0.027 mL, 0.18 mmol) and the resulting mixture stirred for 4 h at room temperature. The reaction mixture was diluted with 5 mL of dichloromethane and washed with HCl 1 N solution. The organic phase was collected and dried over $MgSO_4$, and the solvent was removed by rotary evaporation (99% conversion).

According to method B, to a solution of pyrazolone **2d** (0.063 g, 0.5 mmol) and *N*-hydroxy-*N′*,*N′*-dimethylurea (0.052 g, 0.5 mmol) in 50% aqueous ethanol (5 mL) was added potassium carbonate (0.014 g, 0.1 mmol) to adjust the pH to 7−8. Sodium periodate (0.053 g, 0.25 mmol) was added to the reaction mixture, which was sonicated for 10 min and then stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (5 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, redissolved in ethyl acetate (40 mL), and washed three times with saturated solution of ammonium chloride (20 mL). The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.055 g, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 3.29 (s, 3H), 2.97 (s, 6H), 2.00 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.0, 162.1, 161.4, 68.4, 38.0, 31.4, 19.5, 13.4. HR-MS (FAB): found m/z = 229.13012 (MH^+), calcd for $C_9H_{16}O_3N_4$ 229.13007. Mp: 125−127 °C.

*N-Hydroxy-N-(1,3,4-trimethyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-tert-butylcarbamate (**1j**).* A solution of pyrazolone **2d** (0.035 g, 0.28 mmol) and 1,8-diazabicycloundec-7-ene (0.042 mL, 0.28 mmol) in 2 mL of dichloromethane was stirred for 30 min. The mixture was added to a solution of NHPY **1h** (0.105 g, 0.28 mmol) in dicholoromethane (2 mL). To this solution was added diazabicycloundec-7-ene (0.042 mL, 0.28 mmol) was added dropwise and the resulting mixture stirred for 4 h at room temperature. The reaction mixture was diluted with 10 mL of dichloromethane and washed with HCl 1 N solution. The organic phase was collected, dried over $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation. The residue was purified by column chromatography (20% ethyl acetate/hexane) on silica gel to obtain the title compound as a white solid (0.032 g, 45%).

According to method B, to a solution of pyrazolone **2d** (0.052 g, 0.41 mmol) and *N*-Boc-hydroxylamine (0.065 g, 0.49 mmol) in 50% aqueous ethanol (2 mL) was added potassium carbonate (0.011 g, 0.08 mmol) to adjust the pH to 7−8. Sodium periodate (0.105 g, 0.49 mmol) was added to the reaction mixture, which sonicated for 10 min and then stirred for 3 h at room temperature. The reaction mixture was diluted with ethanol (6 mL), and the solid was filtered. The filtrate was concentrated via rotary evaporation, redissolved in ethyl acetate (30 mL), and washed three times with saturated solution of ammonium chloride (20 mL). The organic phase was collected, dried over $MgSO_4$, and concentrated in vacuo. Recrystallization from dichloromethane and hexane gave the title compound as white solid (0.068 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.81 (s, 1H), 3.32 (s, 3H), 2.07 (s, 3H), 1.60 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.4, 161.5, 155.1, 77.4, 69.0, 32.0, 28.3, 19.6, 13.1. HR-MS (FAB): found m/z = 258.14549 (MH^+), calcd for $C_{11}H_{19}N_3O_4$ 258.14538. Mp: 130−132 °C.

*N′,N′-Dimethyl-N-methoxy-N-(4-(acetyl-O-methoxyoxime)-3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)urea (**1**).* According to method A, sodium hydride 60% (0.016 g, 0.4 mmol) was added to a solution of *N*-methoxy-*N′*,*N′*-dimethylurea (0.042 g, 0.36 mmol) in dimethylformamide (5 mL) at room temperature and the reaction stirred for 1 h. This solution was added dropwise to 4-(acetyl-*O*-methoxyoxime)-4-bromo-*N*-phenyl-5-methylpyrazolone (0.116 g, 0.36 mmol) and the reaction proceeded at room temperature for 3 h. The reaction was diluted with ether (8 mL) and washed with ammonium chloride, water, and brine. The organic phase was collected and concentrated by rotary evaporation. The residue was purified by column chromatography (20% ethyl acetate/hexane) on silica gel to obtain the title compound as yellowish oil (0.099 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (m, 2H), 7.38 (m, 2H), 7.17 (m, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.01 (s, 6H), 2.31 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 158.9, 158.4, 152.1, 138.3, 128.8, 125.0, 119.3, 76.7, 64.0, 62.4, 38.1, 17.3, 12.5. HR-MS (FAB): found m/z = 362.18259 (MH^+), calcd for $C_{17}H_{23}N_5O_4$ 362.18283.

*4-(N-Hydroxylamino)-4-methyl-N-methyl-3-methylpyrazolone (HAPY-**1a**).* Compound **1j** (32 mg, 0.13 mmol) was dissolved in methanol (3 mL) at 0 °C, and acetyl chloride (0.2 mL) was added.

DOI: 10.1021/acs.joc.6b01705
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The reaction was allowed to warm to room temperature, and stirring was continued overnight. The reaction was concentrated in vacuo, redissolved in dichloromethane, and filtered, and the filtrate was concentrated via rotary evaporation to give the title compound (71%, 14 mg), whose characterization was consistent with that previously reported.[15] ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.51 (d, 1H), 6.25 (d, 1H), 3.10 (s, 3H), 1.98 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 175.3, 161.9, 69.0, 30.7, 16.0, 12.7.

***N*-(*tert*-Butoxycarbonyloxy)-*N*′,*N*′-dimethylurea.** For this synthesis, we modified a procedure that was previously reported.[59] *N*-Hydroxy-*N*′,*N*′-dimethylurea (2.73 mmol, 0.284 g, 1 equiv) and triethylamine (2.73 mmol, 0.38 mL, 1 equiv) in 15 mL of acetonitrile were stirred for 1 h at room temperature. Di-*tert*-butyl dicarbonate (2.73 mmol, 0.595 g, 1.0 equiv) was added dropwise to the reaction mixture and stirred for 3 h. The reaction was diluted with dichloromethane and washed twice with 1 N HCl solution. The organic phase was dried over $MgSO_4$ and filtered, and the organic solvent was removed by rotary evaporation, which gave the title compound as a white solid (0.407 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (s, 1H), 2.98 (s, 6H), 1.56 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.0, 154.6, 85.3, 36.2, 27.8. HR-MS (FAB): found m/z = 205.11902 (MH^+), calcd for $C_8H_{16}N_2O_4$ 205. 11883.

***N*-(*tert*-Butoxycarbonyloxy)methylcarbamate.** The synthetic procedure is the same as described above using *C*-methoxycarbohydroxamic acid (0.354 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (s, 1H), 3.84 (s, 3H), 9.15 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 157.5, 153.6, 86.4, 53.9, 27.8. HR-MS (FAB): found m/z = 192.08716 (MH^+), calcd for $C_7H_{13}NO_5$ 192.08720.

***N*-(*t*-Butoxycarbonyloxy)acetamide.** For this synthesis, we modified a procedure that was previously reported.[59] Di-*tert*-butyl dicarbonate (9.3 mmol, 2.03 g, 1.0 equiv) was added to a suspension of acetohydroxamic acid (9.3 mmol, 0.7 g, 1.0 equiv) in 50 mL of dichloromethane at room temperature. Sodium *tert*-butoxide (5.6 mmol, 0.54 g, 0.6 equiv) was added dropwise to the reaction mixture, which was then allowed to stir overnight. The reaction was diluted with dichloromethane and washed twice with saturated NH_4Cl solution. The organic phase was dried over $MgSO_4$ and filtered, and the organic solvent was removed by rotary evaporation. The resulting solid, without further purification, was used for the synthesis of NHPY donors using method A. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.87 (s, 1H), 1.52 (s, 9H), 1.45 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.2, 155,7, 86.2, 31.1, 29.8. HR-MS (FAB): found m/z = 176.09325 (MH^+), calcd for $C_7H_{13}NO_4$ 176.09228.

***N*-Methoxy-*N*′,*N*′-dimethylurea.** To a solution of *O*-methylhydroxylamine hydrochloride (46 mmol, 3.84 g, 1 equiv) and potassium carbonate (41.6 mmol, 5.75 g, 0.9 equiv) in 2 mL of water and 100 mL of ethyl acetate at 0 °C was added (37.2 mmol, 4 g, 0.8 equiv) of dimethylcarbamyl chloride. The reaction mixture was stirred overnight. The solid was filtered off and washed with boiling ethyl acetate. The organic solution was dried over $MgSO_4$ and filtered, and the organic solvent was removed by rotary evaporation, which gave the title compound as a colorless oil (3.80 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (s, 1H), 3.66 (s, 3H), 2.92 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.5, 63.3, 35.7. HR-MS (FAB): found m/z = 119.08228 (MH^+), calcd for $C_4H_{10}N_2O_2$ 119.08205.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01705.

Single-crystal X-ray crystallographic data; details concerning N_2O, MIMS, and NMR analysis; computational results including optimized geometries, energies, and vibrational frequencies and intensities (PDF)

X-ray data for NHPY-**1a** (CIF)

X-ray data for NHPY-**1b** (CIF)

X-ray data for NHPY-**1d** (CIF)

X-ray data for NHPY-**1g** (CIF)

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Notes

The authors declare the following competing financial interest(s): J.P.T. is a co-founder and stockholder and served on the Scientific Advisory Board of Cardioxyl Pharmaceuticals.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the National Science Foundation (CHE-1213438 and CHE-1566065) and Cardioxyl Pharmaceuticals for generous support of this research. We also thank the Johns Hopkins University Department of Chemistry Facility Managers, Dr. Maxime A. Siegler (X-ray Crystallography), and Dr. I. Phil Mortimer (Mass Spectrometry) for assistance with compound identification and characterization. We also thank Dr. Tyler A. Chavez for his assistance with MIMS experiments.

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