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Chemoselective Amide Formation Using *O*-(4-Nitrophenyl)hydroxylamines and Pyruvic Acid Derivatives

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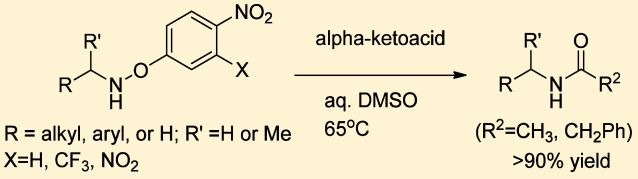
Supporting Information

ABSTRACT: A series of *O*-(4-nitrophenyl)hydroxylamines were synthesized from their respective oximes using a pulsed addition of excess $NaBH_3CN$ at pH 3 in 65−75% yield. Steric hindrance near the oxime functional group played a key role in both the ease by which the oxime could be reduced and the subsequent reactivity of the respective hydroxylamine. Reaction of the respective hydroxylamines with pyruvic acid derivatives generated the desired amides in good yields. A comparison of phenethylamine systems bearing different leaving groups revealed significant differences in the rates of these systems and suggested that the leaving group ability of the N−OR substituent plays an important role in determining their reactivity with pyruvic acid. Competition experiments (in 68% DMSO/phosphate buffered saline) using 1 equiv of *N*-phenethyl-*O*-(4-nitrophenyl)hydroxylamine and 2 equiv of pyruvic acid in the presence of other nucleophiles such as glycine, cysteine, phenol, hexanoic acid, and lysine demonstrated that significant chemoselectivity is present in this reaction. The results suggest that this chemoselective reaction can occur in the presence of excess α-amino acids, phenols, acids, thiols, and amines.



INTRODUCTION

Working at the interface between chemistry and biology, scientists rely heavily on labeling technologies, which tag and identify specific biological targets in aqueous media.[1] The development of water-based chemical-coupling technologies is important, as they can provide improved tools for probing biological processes via efficient tagging reactions. Our continued exploration of the novel amide-forming chemistry between α-keto acids and hydroxylamine derivatives in water led to the discovery of other reactive nitrogen sources for this chemistry, specifically substituted *O*-phenylhydroxylamines. To the best of our knowledge, this is the first comprehensive report of the preparation, purification, relative rate comparisons, solvent sensitivities, and chemoselectivity profile of this class of reactive nitrogen reagents.

Our interest in this reaction was inspired by the findings of Bode et al., who described a high-yield amide-forming reaction in water via the condensation of N-substituted hydroxylamines (**1a**; Scheme 1) and α-keto acids (e.g., **2**).[2−4]

We were interested in extending this reaction beyond N-substituted hydroxylamines (e.g., **1a**) to include O-substituted hydroxylamine starting materials. The rationale for this effort is that unlike N-substituted hydroxylamines, which form nitrone intermediates,[2] the O-substituted systems may instead form the more reactive oximinium ions and lead to improved rates of reaction. Indeed, fast rates are necessary in bioorthogonal tagging reactions, as they typically involve second-order kinetics.[1] Moreover, *N*-alkylhydroxylamines are not stable as their free bases, which complicates their purification by traditional methods. In contrast, O-substituted hydroxylamines are more robust to chromatography.[5]

In an earlier report, we demonstrated that α-keto acids react cleanly with the related *N*-benzoyloxyamines (e.g., **1b**) and found improved yields and reactivity compared to **1a** (Scheme 1).[5] The scope of these reactions was also extended to include the related α-keto phosphonic acids (e.g., **3a**,**b**), which provided high yields of amides and exquisite chemoselectivity in their reactions with *N*-(benzoyloxy)amines. The improved reactivity was thought to be derived from the superior leaving group ability of the benzoyloxy group.

There are two types of hydroxylamines which undergo this amide-forming chemistry. Type I involves the O-*un*substituted hydroxylamines such as **1a**, whereas type II involves O-substituted hydroxylamines such as **1b**. Mechanistic studies by Sanki et al. using ^{18}O labeling revealed that type I hydroxylamines (RNHOH) react via nitrone intermediates (**5**; Scheme 2) and suggested that these were converted to nitrilium (**6**) or hydrated intermediates before generating amides.[2,6] Later experiments by Bode et al. ruled out the nitrilium intermediate.[4b] In 2012, using selectively ^{18}O-labeled substrates, Bode et al. provided support for the involvement of α-lactones (**4a**) and oxaziridine (**4b**) intermediates in the reactions of these O-*un*substituted hydroxylamine systems (type I).[4b] In contrast, no intermediates have been observed in the related *O*-(benzoyloxy)hydroxylamine systems, presumably due to the

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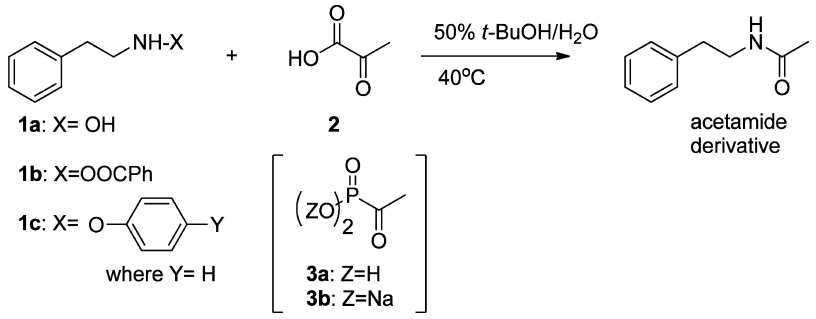
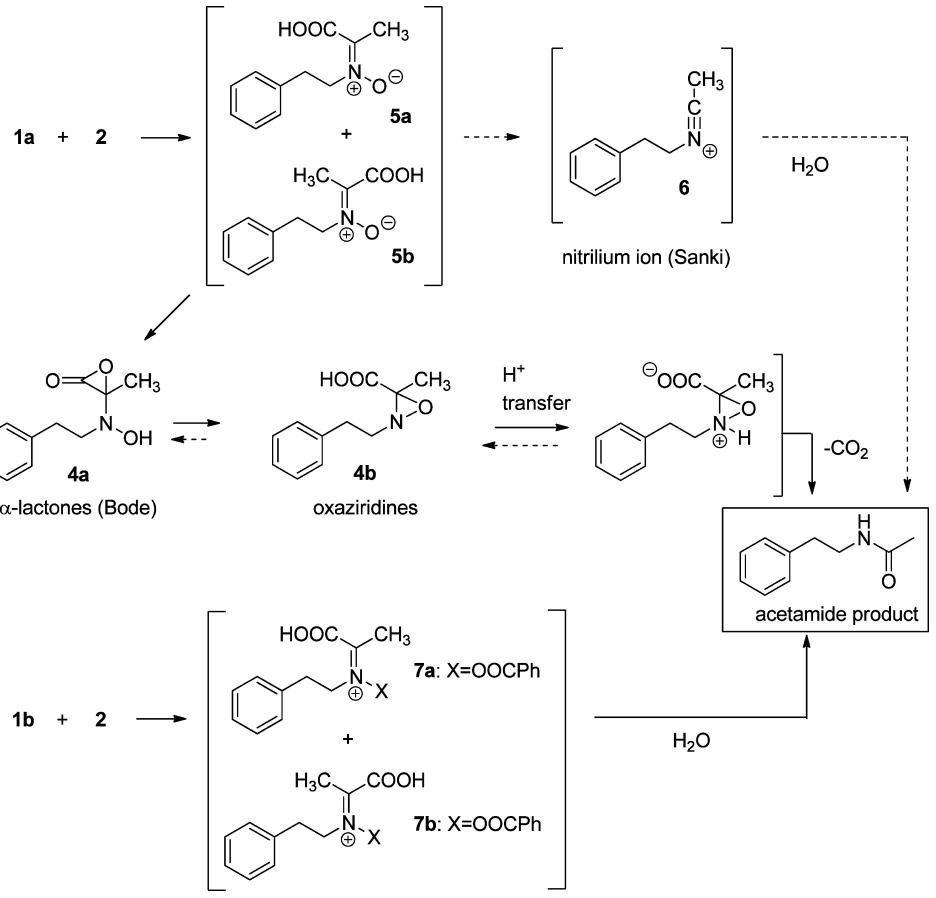
Scheme 1. Ligation of Hydroxylamine Derivatives and α-Keto Acid Motifs To Form Amides



Scheme 2. Proposed Mechanisms for These Reaction Types[2,4b,6]



high reactivity of their putative oximinium ion intermediates in water (e.g., **7** in Scheme 2).[5]

Even though we developed a direct entry to *N*-benzoyloxyamines from amines,[7,8] these systems have caveats associated with their use. While they show excellent utility in both siderophore and *N*-hydroxy-containing peptides synthesis,[9−12] they typically have high chemical reactivity.[5,13] Even though they can be stored for months in the freezer they slowly degrade at room temperature,[7] which could limit their use as chemoselective reagents. Moreover, the ester motif can in principle react with biological amines to give benzamides and hydroxylamines. In addition, a potentially problematic O-to-N acyl transfer reaction has been observed with adjacent amines.[12,14,15] In our efforts to develop more robust reagents, we investigated the *O*-phenylhydroxylamines as an alternative motif.

The N-substituted *O*-phenylhydroxylamines (e.g., **1c** in Scheme 1) were attractive for numerous reasons. First, they represented new type II hydroxylamine systems for this amide-forming chemistry. Second, the *O*-phenyl motif was in principle more stable to pH changes and hydrolysis than the benzoate motif present in the *N*-(benzoyloxy)amines. Third, methods exist to access the *O*-phenylhydroxylamines[16−25] and a recent patent detailed a new high-yield entry to *O*-phenylhydroxylamine derivatives.[16] Fourth, the N−OR bond is cleaved during these reactions and alteration of the leaving group ability of the −OR group via substituted phenoxy systems (e.g., RNH−OC_6H_4−Y) could provide a way to modulate the chemical reactivity and stability of these systems. Indeed, the pK_a values of phenol (pK_a 9.95) and most substituted phenols lie between those of water (pK_a 15.74) and benzoic acid (pK_a 4.2).[26] In this regard, the *O*-phenylhydroxylamines could in principle be

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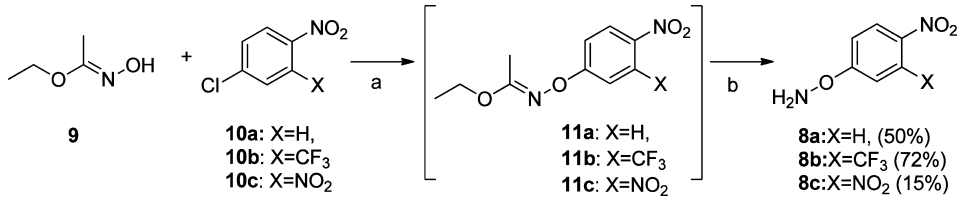
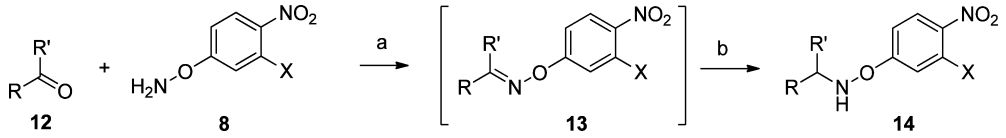
Scheme 3. [a]



[a]Reagents: (a) DMF, solid NaOH at 42 °C; (b) 37% HCl at room temperature.

Table 1. Synthesis of *O*-Substituted Hydroxylamines[a]



12	8	yield of oxime **13**, %	yield of **14**, %
12a (phenacetaldehyde)	**8a** (X = H)	82 (**13a**; R = Bn, R′ = X = H)	65 (**14a**)
12b (acetaldehyde)	**8a** (X = H)	94 (**13b**; R = Me, R′ = X = H)	75 (**14b**)
12c (heptaldehyde)	**8a** (X = H)	98 (**13c**; R = *n*-C_6H_{13}, R′ = X = H)	73 (**14c**)
12d (acetone)	**8a** (X = H)	100 (**13d**; R = R′ = Me, X = H)	65 (**14d**)
12a (phenacetaldehyde)	**8b** (X = CF_3)	100[b] (**13e**; R = Bn, R′ = H, X = CF_3)	15 (**14e**)
12c (heptaldehyde)	**8c** (X = NO_2)	60% (**13f**; R = *n*-C_6H_{13}, R′ = H, X = NO_2	5 (**14f**)
12e (benzaldehyde)	**8a** (X = H)	90 (**13g**; R = Ph, R′ = X = H	NA
12f (acetophenone)	**8a** (X = H)	100 (**13h**; R = Ph, R′ = Me, X = H)	NA
12g (1*H*-indole-3-carbaldehyde)	**8a** (X = H)	40[c] (**13i**; R = 1*H*-indol-3-yl, R′ = X = H)	NA

[a]Reagents: (a) EtOH; (b) pulsed additions of $NaBH_3CN$, pH 3 maintained via aliquots of CH_3SO_3H. NA = not applicable, as no reduction was observed from the corresponding oxime. [b]Used as crude oxime directly in the next step. [c]Recrystallized yield.

tailored to have improved reactivity, chemoselectivity, and stability depending upon their O-substituent.

A previous report by Sheradsky et al. demonstrated that the *N*-alkyl-*O*-phenylhydroxylamines such as **1c** were unstable liquids, which could not be purified by distillation or chromatography.[27] We hypothesized that this was due to the nucleophilic properties of the appended phenol ring.[27] Since the related *N*-benzoyloxyamine systems did not show the same degree of instability, we reasoned that an electron-deficient phenol could impart additional stability to the system. Indeed, the 4-nitrophenol motif (e.g., **1c**, where Y = NO_2) solved this issue and provided stable reagents for developing new methods. Since the hydroxylamine N−O bond is cleaved as these systems react with α-keto acids to form amides, they also have the added advantage of releasing 4-nitrophenol, which provides a convenient indicator of the reaction. This discovery was then evaluated in other *O*-(nitrophenyl)hydroxylamine derivatives and was found to be a general transformation for this compound class. In addition, remarkable chemoselectivity was observed for this amide-forming step in comparison to the more classic approach using an amine and an acid chloride.

RESULTS AND DISCUSSION

Synthesis. While there are several methods to generate the *O*-phenylhydroxylamines, there are only limited synthetic entries to the *N*-alkyl-*O*-phenylhydroxylamines, some of which rely on organometallic reagents.[22,27−31] After an extensive screening of possible chemistries, we elected to pursue the reductive amination approach using *O*-(4-nitrophenyl)hydroxylamine (**8a**) and the desired ketone or aldehyde. While the commercial cost of **8a** is significant, a recent patent described a high-yield entry to these systems using ethyl acetimidate (**9**) and 4-nitrochlorobenzene (**10a**).[16] Using a modified method described in the Experimental Section, we generated multigram quantities of *O*-(4-nitrophenyl)hydroxylamine **8a** (Scheme 3).

Coupling of this reagent **8a** with a series of aldehydes and ketones (**12**) provided the respective intermediate *O*-(4-nitrophenyl) oximes (**13**), which could be isolated by column chromatography or used directly in subsequent steps (see the Supporting Information). Attempts to reduce these intermediate oximes to their related N-substituted *O*-(4-nitrophenyl)-hydroxylamines **14**, however, proved problematic. Sodium borohydride, $LiAlH_4$, $NaBH(OAc)_3$,[32] and H_2 gas over Pd−C all provided the over-reduced amine product, complex mixtures, or no reaction. An extensive review of the literature found limited reports associated with the reduction of N-alkyl O-substituted oximes to their corresponding O-substituted hydroxylamines.[27−31] The consensus from these references indicated that moderate yields could be obtained using $NaBH_3CN$ at pH 3, when the pH is maintained by the addition of inorganic acids dissolved in alcoholic solvents.[27−31]

$NaBH_3CN$ is a unique hydride source due to its stability to hydrolysis at low pH.[34] This feature is critical, as a competition likely exists between hydrolysis of the reactive hydride reagent at low pH[34] and the desired hydride addition to the oxime (which is presumably activated via protonation at low pH).

Our own investigation of this challenging selective oxime reduction revealed that a two-stage pulsed approach worked best at pH 3. Specifically, addition of $NaBH_3CN$ (3 equiv) to the oxime **13a** (R = Bn, R′ = X = H, Table 1) at pH 3 (monitored via a methyl orange indicator) gave approximately 50% conversion after 2 h. The pH was carefully maintained over this 2 h period by addition of an organic acid,

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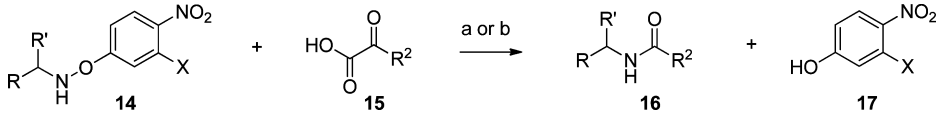
Table 2. Amide Synthesis Reactions Using *O*-Substituted Hydroxylamines[a]



O-arylhydroxylamine **14**	reagent a or b	yield of amide **16**, %	phenol **17**
14a (R = Bn, R′ = H, X = H)	a	99 (**16a**; R = Bn, R′ = H, R^2 = Me)	**17a** (X = H)
14b: (R = Me, R′ = H, X = H)	a	95 (**16b**; R = R^2 = Me, R′ = H)	**17a** (X = H)
14c (R = *n*-C_6H_{13}, R′ = H, X = H)	a	71 (**16c**; R = *n*-C_6H_{13}, R′ = H, R^2 = Me)	**17a** (X = H)
14d (R = R′ = Me, X = H)	a	59 (**16d**; R = R′ = R^2 = Me)	**17a** (X = H)
14e (R = Bn, R′ = H, X = CF_3)	a	83 (**16b**; R = Bn, R′ = H, R^2 = Me)	**17b** (X = CF_3)
14f (R = *n*-C_6H_{13}, R′ = H, X = NO_2)	a	75 (**16c**; R = *n*-C_6H_{13}, R′ = H, R^2 = Me)	**17c** (X = NO_2)
14a (R = Bn, R′ = H, X = H)	b	100 (**16e**; R = R^2 = Bn, R′ = H)	**17a** (X = H)

[a]Reagents: (a) 75% *t*-BuOH in water, 65 °C, 18 h; (b) 68% DMSO in water, 65 °C, 15 h. Note: yields were determined by ^{1}H NMR using an internal standard.

methanesulfonic acid, in small increments (0.1 equiv). Typically 3 equiv of methanesulfonic acid was required to maintain the pH over this 2 h period. However, the amount of CH_3SO_3H needed was system dependent. Importantly, no further reaction was observed upon continued stirring beyond 2 h, indicating that the hydride reagent was consumed during this time period. Additional $NaBH_3CN$ (3 equiv) and subsequent methanesulfonic acid additions were used to push the reaction closer to completion and provided good conversions to the desired N-substituted *O*-(4-nitrophenyl)hydroxylamine **14a**.

Surprisingly, the related oximes generated from benzaldehyde, acetophenone, and 1*H*-indole-3-carbaldehyde did not react under these optimized reduction conditions. Efforts to reduce these bulky oximes with Pyr-BH_3 gave no reaction, even though the related *O*-methyloximes were converted in high yield to *O*-methylhydroxylamines with this reagent by Kawase et al.[33]

Using our pulsed-reduction approach, the desired *O*-(4-nitrophenyl)hydroxylamines (**14a−d**) were synthesized in good yields (65−75%, Table 1). These new materials **14a−d** were stable to column chromatography on silica gel and were characterized by ^{1}H and ^{13}C NMR and mass spectrometry and elemental analysis (see the Supporting Information). These N-substituted hydroxylamines (**14a−d**) were then evaluated by ^{1}H NMR for their ability to react with pyruvic acid in 75% *t*-BuOH−water, and the results are shown in Table 2.[2] The *N*-(2-phenylethyl) system **14a** was prepared in order to compare to the prior system **1b**.

Yields of the acetamide products in Table 2 were determined by ^{1}H NMR using a dibenzyl ether internal standard (after an aqueous workup to remove unreacted pyruvic acid). These initial ^{1}H NMR results (Table 2) suggested high conversion of **14a** and high yield of the product acetamide **16a**.

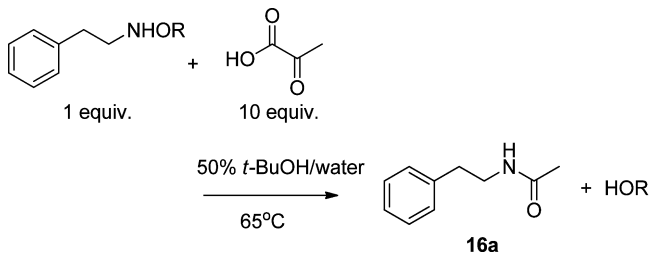
Other substituted *O*-(aryl)hydroxylamines were also synthesized to investigate the relative reactivities of substituted *O*-phenyl systems in this reaction (Scheme 3). It was expected that electron-deficient systems would enhance the leaving group ability of the *N*-phenoxy substituent and provide systems with increased reactivity. Thus, the related *O*-(4-nitro-3-(trifluoromethyl)phenyl)hydroxylamine (**8b**; 72%) and *O*-(3,4-dinitrophenyl)hydroxylamine (**8c**; 15%) were synthesized using modifications of the methods used to synthesize the 4-nitrophenyl system **8a**. Although the trifluoromethyl system **8b** was well-behaved and stable to the workup, the related dinitro system **8c** was highly reactive and decomposed when exposed to aqueous base (aqueous Na_2CO_3) during its isolation. This was circumvented by using acidified water during the workup of **8c** (see the Experimental Section) but still resulted in low yields for **8c.**

The substituted phenyl systems **8b,c** were reacted with phenacetaldehyde and heptaldehyde, respectively, to form their crude respective oximes **13e,f**, which were reduced with $NaBH_3CN$ at pH 3 to the respective *N*-alkylhydroxylamines **14e** (15%) and *n*-heptylhydroxylamine **14f** (5%; see Table 1). Again, the dinitro system was problematic in general and decomposed during isolation using column chromatography at both the oxime and the hydroxylamine stages. The 4-nitro-(3-trifluoromethyl)phenyl system derived from **8b** (**14e**) was better behaved throughout its synthesis and was stable to silica gel based chromatography.

Compounds **14e,f** were also shown to react with pyruvic acid (2 equiv) to give the desired acetamides **16a,c** in 83% and 75% yields, respectively (Table 2). In an effort to demonstrate the general reactivity of α-keto acids to these systems, hydroxylamine **14a** was reacted with phenylpyruvic acid (2 equiv) and gave a 100% yield of the desired 2-phenyl-acetamide **16e** at 86% conversion in 68% DMSO−water after 15 h at 65 °C (Table 2).

Next, we compared the relative reactivities of **1b**, **14a**, and **14e** with pyruvic acid using pseudo-first-order reaction conditions (Table 3) by HPLC. The rate constant for the

Table 3. Comparative Rates for 1b, 14e, and 14a with Pyruvic Acid at 65 °C in 50% *t*-BuOH/Water As Determined by HPLC[a]



compd	rate (min^{-1})	pK_a
1b (R = benzoyl)	0.0184	4.20
14e (R = 3-(trifluoromethyl)-4-nitrophenyl)	0.0122	6.07
14a (R = 4-nitrophenyl)	0.0047	7.08

[a]Pseudo-first-order conditions (10 equiv of pyruvic acid) were used. A plot of pK_a (*x* axis) vs rate (min^{-1}, *y* axis) gave a line defined by $y = -0.0048x + 0.0395$; $r^2 = 0.96$.

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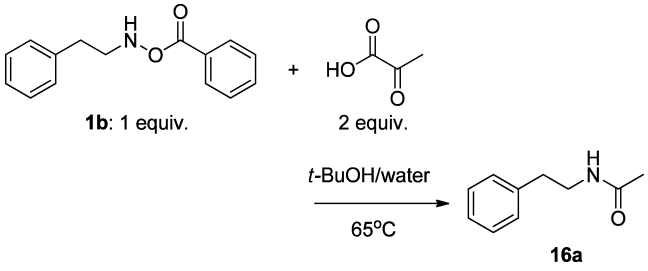
respective reactions between pyruvic acid (100 mM) and **1b**, **14a**, or **14e** (10 mM) were calculated using diphenylmethane (DPM, 10 mM) as an internal standard to determine the amount of starting material consumed per unit time (min). As shown in Table 3, the rate for the benzoyloxy derivative **1b** was approximately 1.5 times faster than that for **14e** and nearly 4 times faster than that for **14a**. This is consistent with the relative leaving group ability of these systems: e.g., the pK_a value of benzoic acid (4.2), 3-trifluoromethyl-4-nitrophenol (6.07), and 4-nitrophenol (7.08). This relationship was approximated via linear regression (r^2 = 0.96) to give a line defined as

$$\text{rate (in min}^{-1}) = -0.0048\ (\text{p}K_a \text{ value of the substituted phenol}) + 0.0395 \quad (1)$$

This equation describes the relationship between rate and pK_a and can be used to estimate the rates of future *O*-phenyl systems via their respective phenol's pK_a value.

Using the HPLC analysis method, these reactions were further optimized for solvent composition. As shown in Table 4, a 70% solution (by volume) of *t*-BuOH in water at pH 7.4 using 2 equiv of pyruvic acid and 1 equiv of the *N*-benzoyloxy system **1b** gave the highest yield of **16a** (85%). In contrast, the *N*-(4-nitrophenoxy)amine **14a** (Table 5) derivative gave the highest yield of **16a** (57%) in 90% *t*-BuOH in water at the same pH and molarity of reagents.

Table 4. Solvent Study in *t*-BuOH−Water Mixtures for *N*-Benzoyloxy Derivative 1b at 65 °C



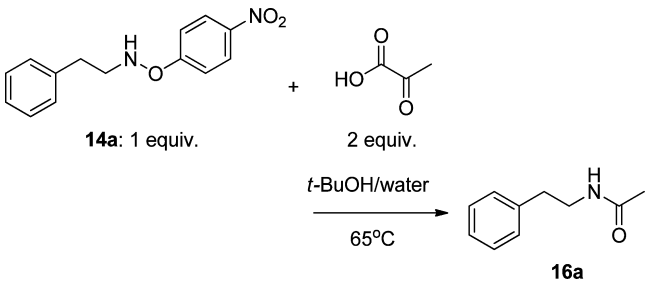
amt of *t*-BuOH, %	conversn,[a] %	yield of amide **16a**, %	est yield of **16a** based upon conversn,[b] %
50	83	43	52
60	93	79	85
70	94	80	85
80	94	78	83
90	98	74	76
100	89	26	29

[a]Based on starting material consumed. [b]Taking into account the percent conversion: e.g., for the 70% reaction 80/0.94 = 85.

The reaction between **14a** and pyruvic acid was also performed in other solvents (i.e., EtOAc, DMF, acetonitrile, and DMSO) in an effort to increase the yield of **16a**. Of these solvents, DMSO had the fewest byproducts formed and the highest yields and was chosen for further optimization experiments by the addition of water at pH 7.4.

As shown in Table 6, a 60% solution of DMSO in water (by volume at pH 7.4) gave high conversion (86%) and 78% yield. This yield corresponds to a high yield of **16a** (91% yield) when one corrects for the unreacted starting material. Note: experiments performed at higher water loadings (e.g., 10−50% DMSO in water) provided lower percent conversions and turbid mixtures and introduced significant solubility constraints of the internal standard due to the higher water content. Nevertheless, this survey of reaction conditions allowed us to identify optimal conditions for this transformation (60% DMSO/water mixture).

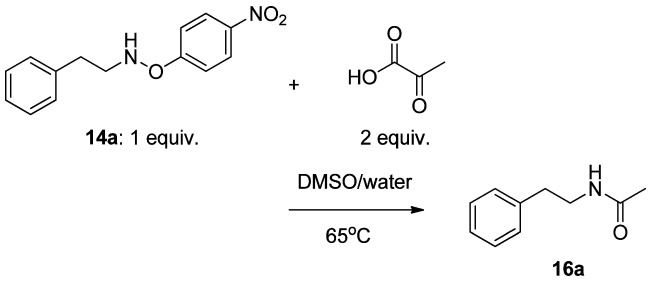
Table 5. Solvent Study in *t*-BuOH−Water Mixtures for *N*-4-Nitrophenoxy Derivative 14a at 65 °C



t-BuOH, %	conversn,[a] %	yield of amide **16a**, %	est yield of **16a** based upon conversn,[b] %
50	34	15	44
60	43	20	47
70	42	22	52
80	49	26	53
90	56	32	57
100	62	22	36

[a]Based on starting material consumed as measured by HPLC. [b]Taking into account the percent conversion: e.g., for the 90% reaction 32/0.56 = 57.

Table 6. Solvent System Study for 14a in DMSO−Water Mixtures at 65 °C and pH 7.4



entry	amt of DMSO, %	conversn,[a] %	yield of amide **16a**, %	est yield of **16a** based upon conversn,[b] %
1	10[c]	32	8	23
2	20[c]	32	11	34
3	30[c]	64	20	31
4	40[c]	76	43	56
5	50[c]	76	73	96
6	60	86	78	91
7	70	80	67	84
8	80	73	58	79
9	90	67	38	57
10	100	63	30	48

[a]Based on starting material consumed as measured by HPLC. [b]Taking into account the percent conversion: e.g., for the 60% reaction 78/0.86 = 91. [c]Reaction mixtures appeared turbid.

The dramatic solvent effect on this reaction, where a 60% DMSO/water mixture resulted in significantly higher reactivities, yields, and conversions for the *N*-(4-nitrophenoxy)-amine system **14a**, can be explained by two factors. First, water plays a functional role in the mechanism of this reaction and is

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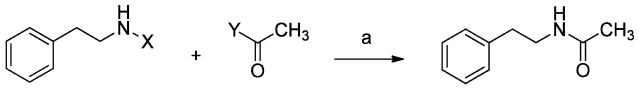
needed for hydration of the putative oximinium ion intermediate **7** to form the product amide. We have shown that mixed solvent systems containing water further facilitate conversion to the product (Table 6: entry 6 vs entry 10). Second, solvents with high dielectric constants such as water (ε = 78) and DMSO (ε = 47) greatly facilitate this reaction. Although dielectric constants are a bulk property of the solvent, they are a good indicator of the solvent's ability to accommodate separation of charge.[35a] Indeed, mixtures containing solvents with high dielectric constants (DMSO and water) were more optimal than the related *t*-BuOH (ε = 12.5)/water mixtures. We speculate that these mixed aqueous solvents containing polar aprotic solvents such as DMSO may help lower the energy of the transition state, leading to ionic intermediates such as **7**.[35a] In general, solvents with lower dielectric constants resulted in lower yields (e.g., solvent/dielectric constant ε: DMF/38; CH_3CN/37, EtOAc/6).

The fact that the chemical reactivity of these systems can be modulated via changes in the steric demands of the N-alkyl substituent, the electron richness of the phenoxy substituent, and the reaction solvent composition provides additional flexibility in developing new biological applications for these self-reporting systems. Indeed, a preliminary assessment in Chinese hamster ovary (CHO) cells demonstrated that both **14a** and **14c** were nontoxic at 100 μM after a 48 h incubation at 37 °C.

An assessment of the chemoselectivity of this reaction was conducted, and the results are shown in Table 7. Compound

Table 7. Chemoselectivity Experiments[a]



14a: X=O-(4-nitrophenyl)
18: X=H

16a

entry	starting compd	Y	additive	yield of **16a**, %
1	**14a**	COOH	lysine and phenol	98
2	**14a**	COOH	glycine	93
3	**14a**	COOH	cysteine	36
4	**14a**	COOH	cysteine and hexanoic acid	39
5	**18**	Cl	cysteine and hexanoic acid	0
6	**18**	Cl	lysine and phenol	1

[a]Reagents: 68% by volume DMSO in phosphate buffered saline at 65 °C for 18 h in the presence of a diphenylmethane internal standard (see the Experimental Section for more details).

14a (1 equiv) and pyruvic acid (2 equiv) were challenged to react in the presence of competing nucleophiles (2 equiv each) such as cysteine, hexanoic acid, lysine, glycine, and phenol in 68% DMSO/water at 65 °C for 18 h. Competition experiments involving glycine, lysine, and phenol each provided high yields of the desired acetamide (≥93% yield), indicating that the presence of these functional groups was not detrimental to the coupling chemistry to form **16a**.

In contrast, cysteine (Table 7, entry 3) was shown to compete with this reaction at pH 7.4, whereas glycine did not (entry 2). This suggested that thiol groups can compete with **14a** for pyruvic acid. It is well-known that, during aerobic cellular respiration, thiols can react with pyruvic acid to form thioesters. For example, acetyl-coA is formed by this chemistry in the presence of NAD^+ and pyruvate dehydrogenase.[35b] Even so, in the presence of *2 equiv* of cysteine hydrochloride monohydrate, *1 equiv* of **14a** still provided a 36% yield of **16a**.

In sharp contrast, control experiments using the more classic reagents phenethylamine and acetyl chloride gave trace yields of **16a** (≤1% yield) after identical challenges and workup. These experiments demonstrated that significant chemoselectivity is present in the α-keto acid reaction with **14a**.

Conclusion. Using a pulsed addition of $NaBH_3CN$ at pH 3, a series of *O*-(4-nitrophenyl)hydroxylamines were synthesized from their respective oximes. Careful pH control was necessary and was maintained by a methyl orange indicator and incremental additions of methanesulfonic acid. Yields of the desired O-substituted hydroxylamines ranged from 5 to 75%. Steric hindrance near the oxime functional group was shown to play a key role in both the ease by which the oxime could be reduced and the subsequent reactivity of the respective hydroxylamine. As expected, the less hindered systems were more reactive.

Reaction of the respective hydroxylamines with pyruvic acid generated the desired amide derivatives in good yields. A solvent composition study was conducted and revealed that high yields could be obtained with **14a** in 60% DMSO/water at pH 7.4. A rate comparison of phenethylamine systems bearing *N*-OBz, *N*-3-trifluoro(4-nitrophenoxy), and *N*-(4-nitrophenoxy) groups revealed significant differences in the rates of these systems and suggested that the leaving group ability of the N substituent plays a defining role in determining the reactivity of these systems. This insight allows one to further refine the selectivity and reactivity of these systems by modulating the electron richness of the appended *O*-phenyl substituent. Alterations in the size of the N-alkyl substituent and the solvent system also were shown to influence the rate of these reactions.

Many preparation methods exist to provide amides from amine starting materials using reactive electrophiles such as acid chlorides. These reactive electrophiles can react with numerous competing nucleophiles, including thiols, phenols, and amines (e.g., Table 7, entry 6). In contrast, our results suggest that high chemoselectivity is present in the coupling of N,O-substituted hydroxylamines and α-keto acids in aqueous solvent mixtures (e.g., Table 7, entry 1).[5] Other type II hydroxylamine systems have recently been shown to have high chemoselectivity in their reactions with α-keto acids. For example, unprotected peptides containing a terminal cyclic hydroxylamine (5-oxaproline) were shown to couple with unprotected α-keto acid containing peptides in 46% yield (in 90% DMSO/water at 60 °C for 20 h) in the presence of 0.1 M oxalic acid).[4c] In this regard, it may be possible to further increase reaction efficiencies by the addition of specific additives such as oxalic acid. We also note that a related chemoselective reaction between α-keto acids and oximes has been described by Bode et al.[36a] Since these reactions work particularly well at pH 7.4, future work will explore the biological applications of N,O-substituted hydroxylamine–α-keto acid couplings.[36b] Indeed, the high chemoselectivity observed here can be used to develop future reagents, which perform selective acylation reactions in the presence of biological nucleophiles.

■ EXPERIMENTAL SECTION

General Considerations. All solvents and most reagents were used as received from the vendor, and column chromatography was run on silica gel (40–63 μm) with the indicated solvent systems. High-

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resolution mass spectra (HRMS) were acquired via electrospray ionization and an Agilent 6224 time-of-flight mass spectrometer.

***O*-(4-Nitrophenyl)hydroxylamine (8a).**[16] A solution of ethyl acetimidate **9** (7.48 g: 72.5 mmol) and 4-chloronitrobenzene **10a** (9.13 g: 58 mmol) in DMF (15 mL) was stirred mechanically at room temperature. The yellow solution was warmed to 30 °C, and solid NaOH (2.83 g: 70.7 mmol) was added in six portions. The reaction mixture was sonicated to facilitate reaction. The reaction mixture was then warmed to 42 °C and stirred for 1.5 h. The solid NaOH pellets slowly dissolved and formed a yellow suspension over time (presumably containing a NaCl precipitate). The reaction mixture was stirred overnight. ^{1}H NMR ($CDCl_3$) provided a convenient way to monitor the reaction using the following signals: δ 7.53 (d, from chloronitrobenzene **10a**), 7.23 (d, product **11a**), 4.21 (q, **11a**), 3.99 (q, starting ester **9**). The aromatic signal integration ratio of δ 7.53 (**10a**) vs δ 7.23 (**11a**) or TLC (33% hexane in $CHCl_3$) with R_f = 0.62 for **10a** and R_f = 0.41 for **11a** was convenient to monitor percent conversion over time. Water (24 mL) was then added at 40 °C, the slurry that formed was cooled to 15 °C and filtered, and the solid was washed with water. The yellow solid was air-dried and gave the intermediate **11a** (14.24 g, 88% yield). The subsequent hydrolysis step involved adding **11a** (14.24 g) to 37% HCl (11.9 g solution, 10 mL, 4.4 g of HCl: 0.12 mol) in portions over 20 min at room temperature. The white suspension was vigorously sonicated in order to ensure complete conversion to **8a**. After 2 h of sonication, the white suspension was neutralized with 33% NaOH solution (55 mL), cooled to room temperature, and filtered and the yellow solid washed with water and air-dried (11.82 g). ^{1}H NMR of the crude solid showed complete conversion (e.g., loss of ethyl pattern). Efforts to further purify this material by column chromatography (100% $CHCl_3$) gave significant reductions in yield and provided the pure *O*-(4-nitrophenyl)hydroxylamine product **8a** (4.5 g, 50% yield, R_f(100% $CHCl_3$) = 0.38) as a solid. *Later experiments demonstrated that the air-dried crude solid could be used without further purification for oxime **13** formation in the next step*. We noted that the main impurity was 4-nitrophenol (**17a**).

Caution! A series of related *O*-phenylhydroxylamines have been screened by differential scanning calorimetry (DSC) and accelerating rate calorimetry (ARC), and the *O*-(4-nitrophenyl)hydroxylamine **8a** had an onset temperature for its decomposition of 120 °C (vs 90 °C for the related *O*-(2,4-dinitrophenyl)hydroxylamine).[37] Although no violent decompositions were observed during our investigations, caution should be taken not to heat these samples near their decomposition temperature, as a violent explosion could result.[37]

Compound **8a**: ^{1}H NMR ($CDCl_3$) δ 8.20 (d, 2H), 7.24 (d, 2H), 6.10 (br s, 2H, O-NH_2); ^{13}C NMR ($CDCl_3$) δ 166.4, 141.8, 125.7, 113.3.

***O*-(4-Nitrobenzotrifluoride)hydroxylamine (8b).** A solution of ethyl acetimidate (**9**; 0.5 g, 4.8 mmol) was stirred with 5-chloro-2-nitrobenzotrifluoride (**10b**; 0.85 g, 3.8 mmol) in DMF (2 mL) at room temperature. The yellow solution was warmed to 30 °C (via an oil bath), and solid NaOH (0.192 g; 4.8 mmol) was added in four to five portions. A mechanical stirrer was used to maintain good stirring. The reaction mixture was then sonicated for 1.5 h to ensure that all the solid dissolved into the solution. The reaction was completed after 1.5 h of sonication (color changed from yellow to yellowish orange). The reaction was monitored by TLC (40% CH_2Cl_2/hexane; R_f = 0.6 for **10b** and R_f = 0.4 for **11b**) and ^{1}H NMR ($CDCl_3$) by following the appearance of δ 4.21 (q from **11b**), δ 2.16 (s from **11b**), and δ 1.38 (t from **11b**). After all the starting material was consumed as indicated by TLC, water (2 mL) was added at 40 °C and the reaction mixture was cooled to 15 °C and stirred for 15 min. A yellow solid appeared, which was filtered, washed with water, and air-dried to give the intermediate **11b** (900 mg, 86% yield). The subsequent hydrolysis step involved adding **11b** (100 mg, 0.3 mmol) to 37% HCl (0.225 mL, 2.7 mmol, 12 M) in portions over a period of 20 min. A mechanical stirrer was used for proper stirring and to facilitate conversion to **8b**. The reaction was then sonicated for 2.5 h at room temperature and monitored by TLC (40% hexane/CH_2Cl_2; R_f = 0.5 for **11b** and R_f = 0.3 for **8b**). The ^{1}H NMR ($CDCl_3$) spectra showed the disappearance of peaks δ 4.21 (q from **11b**), δ 2.16 (s from **11b**), and δ 1.38 (t from **11b**). The reaction mixture was cooled to room temperature, 5 mL of acidified water (at pH 2) was added to the reaction flask, and the solution was extracted three times with CH_2Cl_2. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give **8b** (530 mg, 72%) as a solid.[38] Significantly higher yields were obtained when these compounds were isolated under acidic conditions.

Compound **8b**: ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 1H), 7.61 (d, 1H), 7.41 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 163.4, 140.1, 126.8, 124.8 (q, $^2J_{C-F}$ = 34 Hz), 120.9 (q, $^1J_{C-F}$ = 272 Hz), 115.2, 112.1 (q, $^3J_{C-F}$ = 6 Hz).

***O*-(3,4-Dinitrophenyl)hydroxylamine (8c).** A solution of ethyl acetimidate (**9**; 5.0 g, 48 mmol) was stirred with 3,4-dinitrochlorobenzene (**10c**; 7.85 g, 38 mmol) in DMF (10 mL) at room temperature. The yellow solution was warmed to 30 °C (via an oil bath), and solid NaOH (1.92 g, 48 mmol) was added in four to five portions. A mechanical stirrer was used for good stirring. The reaction mixture was then sonicated for 1−1.5 h to ensure that all the solid dissolved into the solution and then warmed to 40 °C for 2 h. The solid NaOH pellets began to dissolve and formed a yellow suspension. The reaction was completed in 3 h and was monitored by TLC and ^{1}H NMR ($CDCl_3$). ^{1}H NMR provided a better way of monitoring using the disappearance of the singlet at δ 7.89 (from **10c**) and appearance of signals at δ 8.00 (d, from **11c**), δ 7.94 (d, from **11c**), δ 7.71 (s, from **11c**), and δ 4.2 (q, from **11c**). On TLC two spots were observed, whose intensities corresponded to the integration ratio in ^{1}H NMR for the *E* and *Z* isomers. TLC (33% hexane/CH_2Cl_2) gave R_f = 0.37 for **10c** and R_f = 0.53 and 0.28 for the two isomers of **11c**. Water (20 mL) was added at 40 °C, and the reaction mixture was cooled to 15 °C and stirred for 15 min. A yellow solid appeared, which was filtered and washed with fresh water to give the intermediate **11c** (9.0 g, 68% yield). The subsequent hydrolysis step involves adding **11c** (9.0 g) into 37% HCl solution (23.3 mL, 280 mmol, 12 M) in portions over a period of 20 min at room temperature. A mechanical stirrer was used for complete conversion to **8c**. The reaction was then sonicated for 2.5 h and monitored by TLC (40% hexane/CH_2Cl_2): R_f = 0.55 and 0.34 (*E* and *Z* isomers of **11c**) and R_f = 0.25 for **8c**. The reaction was cooled to room temperature, 50 mL of acidified water (at pH 2, see note below) was added to the reaction flask, and the solution was extracted three times with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to give **8c**[21a] as a crude unstable solid (1.0 g, yield 15%). (Note: this yield was later improved to 52% by not adding acidified water and simply extracting the material with CH_2Cl_2 from the concentrated HCl layer as its HCl salt. Excess CH_2Cl_2 was needed, as the HCl salt is only partially soluble in CH_2Cl_2.)

Compound **8c**: ^{1}H NMR ($CDCl_3$) δ 7.901 (d, 1H, *J* = 8.8 Hz), 7.900 (d, 1H, *J* = 2.2 Hz), 7.02 (dd, 1H, *J* = 8.8 and 2.2 Hz), 6.40 (s, 2H, O-NH_2) (note that the signal at δ 7.90 appears as dd but is actually two separate doublets, as is evident by their respective coupling constants at 7.02 ppm); ^{13}C NMR ($CDCl_3$) δ 156.0, 141.7, 135.7, 126.7, 121.2, 116.2.

General Method for Preparation of *O*-(4-Nitrophenyl) *N*-Substituted Oximes 13. The *O*-4-nitrophenylhydroxylamine **8a** (1 mmol) was dissolved in absolute EtOH (8 mL) and the respective aldehyde or ketone **12** (1 mmol) was added dropwise. Note: a drop of trifluoroacetic acid was added to facilitate some of the reactions (i.e., with acetaldehyde, acetone, and acetophenone). The reaction mixture was stirred at room temperature for 24 h and monitored by TLC (e.g., 50% hexane in $CHCl_3$: R_f = 0.35 for **14a**). Note: the reaction rates varied and the phenacetaldehyde reaction was complete within 2 h. After TLC indicated high conversion, the solution was concentrated to give the crude oxime, which could either be purified by column chromatography or used directly in the next step. The ^{1}H NMR data and isolated yields for the oxime intermediates are listed to show that the first step (oxime formation) proceeded generally in high yield.

*2-Phenylacetaldehyde O-(4-Nitrophenyl) Oxime (**13a**):* 210 mg, 82% yield as a mixture of *E*/*Z* oximes in ∼2:1 ratio was observed, which complicated the spectra; ^{1}H NMR ($CDCl_3$) δ 8.35−8.20 (two doublets, 2H), 7.90 (t, 0.66 H), 7.47−7.20 (m, 7.3 H), 3.93 and 3.70 (two doublets, 2H).

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*Acetaldehyde O-(4-Nitrophenyl) Oxime (**13b**):* 169 mg, 94% yield as a mixture of *E*/*Z* oximes in a 3/2 ratio was observed, which complicated the spectra; ^{1}H NMR ($CDCl_3$) δ 8.25−8.10 (two doublets, 2H), 7.85 (q, 0.6H), 7.30−7.15 (m, 2.4H), 2.10−2.00 (two doublets, 3H).

*Heptanal O-(4-Nitrophenyl) Oxime (**13c**):* 245 mg, 98% yield as a mixture of *E*/*Z* oximes in a ∼2.3:1 ratio was observed, which complicated the spectra; ^{1}H NMR ($CDCl_3$) δ 8.25−8.20 (merged doublets, 2H), 7.81 and 7.07 (two triplets in a 2.3:1 ratio, total 1H), 7.23 (d, 2H), 2.56 and 2.38 (two multiplets, total 2H), 1.60 (m, 2H), 1.45−1.28 (m, 6H), 0.91 (m, 3H).

*Propan-2-one O-(4-Nitrophenyl) Oxime (**13d**):* 194 mg, 100% yield; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, 2H), 7.25 (d, 2H), 2.08 (s, 3H), 2.07 (s, 3H).

Note: oximes **13e**,**f** are described separately in the syntheses of **14e**,**f**.

*Benzaldehyde O-(4-Nitrophenyl) Oxime (**13g**):* 217 mg, 90% yield, presumably the all-*E* isomer; ^{1}H NMR ($CDCl_3$) δ 8.48 (s, 1H), 8.26 (d, 2H), 7.75 (d, 2H), 7.49 (m, 3H), 7.37 (d, 2H).

*Acetophenone O-(4-Nitrophenyl) Oxime (**13h**):* 256 mg, 100% yield; TLC (50% hexane/50% $CHCl_3$) R_f = 0.35; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 2H, J = 8.7 Hz), 7.79 (d, 2H), 7.47 (br s, 3H), 7.40 (d, 2H, *J* = 8.7 Hz), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.2, 160.2, 142.4, 135.1, 130.5, 128.7, 126.7, 125.8, 114.5, 13.8.

*1H-Indole-3-Carbaldehyde O-(4-Nitrophenyl) Oxime (**13i**).* The *O*-(4-nitrophenyl)hydroxylamine **8a** (150 mg, 0.97 mmol) was dissolved in absolute EtOH (7 mL) and TFA (7.22 μL). The indole-3-carboxaldehyde **12g** (141 mg, 0.97 mmol) was added dropwise over 1 min. The mixture was heated to 80 °C and stirred overnight. The solution was concentrated under vacuum to give oxime **13i** as a bright yellow solid. The solid was recrystallized with ethyl acetate/hexane to yield 95 mg (40%) of the product. ^{1}H NMR (acetone-d_6): δ 8.88 (s, 1H) 8.32 (d, 2H) 8.28 (d, 1H) 7.95 (d, 1H) 7.55 (m, 3H), 7.30 (m, 2H).

General Method for Reduction of *O*-(4-Nitrophenyl) *N*-Substituted Oximes to Hydroxylamines. The oxime (1 mmol) was dissolved in MeOH (3 mL) and treated with sodium cyanoborohydride $NaBH_3CN$ (3 mmol). Using a trace of methyl orange as an in situ indicator, pH 3 was attained by adding methanesulfonic acid (1 mmol) to maintain a consistent red color. The pH was controlled by adding incremental aliquots of methanesulfonic acid (0.1 mmol) to the reaction mixture every time it turned from red to orange, which indicated pH >3. At the end of 2 h, no further color changes were observed (and no additional conversion was noted if stirred beyond 2 h). At this point, $NaBH_3CN$ (3 mmol) was again added to drive the reaction to completion and the pH was again controlled via incremental methanesulfonic acid addition. The reaction was stirred for 2 h at pH 3 after the second pulse of $NaBH_3CN$ was added. The reaction was monitored by TLC (e.g., 50% hexane in $CHCl_3$ for **14a**) for high conversion. The solution was concentrated, dissolved in dichloromethane, and washed three times with aqueous Na_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude hydroxylamine derivative, which was purified by column chromatography on silica gel. Isolated yields ranged from 5 to 75%, depending upon the substrate. It was noted that continued pulsed additions of reducing agent (i.e., beyond two 3 equiv $NaBH_3CN$ pulses) resulted in the generation of increasing amounts of the corresponding amine (e.g., phenethylamine), presumably via reduction of the desired hydroxylamine product. In general, two pulses seemed best at balancing over-reduction vs product yield, but this trend was also substrate dependent.

*O-(4-Nitrophenyl)-N-phenethylhydroxylamine (**14a**):* oil, 167 mg, 65% yield; ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 2H; J = 9.4 Hz), 7.33 (t, 2H), 7.25−7.18 (m, 5H), 6.16 (t, 1H), 3.39 (m, 2H), 2.92 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 165.8, 138.6, 128.9, 128.7, 126.6, 125.8, 113.6, 53.2, 33.2; MS calcd for $C_{14}H_{15}N_2O_3$ (M + H) 259.1077, found 259.1073. Anal. Calcd for $C_{14}H_{14}N_2O_3{\cdot}0.1H_2O$: C, 64.65; H, 5.50, N, 10.77. Found: C, 64.88; H, 5.45; N, 10.42.

*N-Ethyl-O-(4-nitrophenyl)hydroxylamine (**14b**):* oil, 136 mg, 75% yield; TLC 50% $CHCl_3$/hexane, R_f = 0.4; ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 2H, $^3J_{H-H}$ = 9.34 Hz), 7.21 (d, 2H, $^3J_{H-H}$ = 9.34 Hz), 6.10 (t, 1H), 3.19 (m, 2H), 1.20 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 166.1, 141.6, 125.7, 113.6, 46.8, 12.2, HRMS calcd for $C_8H_{11}N_2O_3$ (M + H) 183.0764, found 183.0765. Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.86; H, 5.59; N, 15.22.

*N-Heptyl-O-(4-nitrophenyl)hydroxylamine (**14c**):* oil, 184 mg, 73% yield; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, 2H), 7.20 (d, 2H), 6.20 (t, 1H), 3.15 (m, 2H), 1.53 (m, 2H), 1.30 (m, 8H), 0.88 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 166.0, 141.6, 125.8, 113.6, 52.4, 31.7, 29.1, 27.1, 27.0, 22.6, 14.1. Anal. Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10. Found: C, 62.15; H, 8.02; N, 11.07.

*N-Isopropyl-O-(4-nitrophenyl)hydroxylamine (**14d**):* oil, 127 mg, 65% yield; ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 2H, *J* = 9.2 Hz), 7.23 (d, 2H, *J* = 9.2 Hz), 5.86 (d, 1H, NH), 3.43 (m, 1H), 1.13 (d, 6H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 166.5, 141.0, 125.7, 113.7, 52.3, 19.8; HRMS calcd for $C_9H_{13}N_2O_3$ (M + H) 197.0921, found 197.0916. Anal. Calcd for $C_9H_{12}N_2O_3$: C, 55.04; H, 6.16; N, 14.28. Found: C, 55.36; H, 6.20; N, 14.28.

*O-(4-Nitrobenzotrifluoride)-N-phenethylhydroxylamine (**14e**):* The *O*-(4-nitrobenzotrifluoride)hydroxylamine **8b** (500 mg, 2.25 mmol) was dissolved in absolute EtOH (20 mL), and phenylacetaldehyde (**12a**; 270 mg, 2.25 mmol) was added dropwise at room temperature. The mixture was stirred at room temperature and monitored by TLC (40% CH_2Cl_2/hexane; R_f = 0.45 for oxime **13e**) and ^{1}H NMR ($CDCl_3$). The reaction was completed in 4 h. The solution was concentrated under vacuum. A crude oil (990 mg) was obtained, which was used directly in the next reaction. Crude **13e**: ^{1}H NMR ($CDCl_3$) δ 8.1−8.0 (d, 2H), 7.90 (t, 1H), 7.5−7.3 (m, 5H), 3.9 (d, 2H), 3.4 (d, 1H). The crude oxime **13e** (990 mg) was dissolved in methanol (20 mL), and $NaBH_3CN$ (188 mg, 3.0 mmol) was added. A trace of methyl orange was added as an indicator to monitor the pH needed for the reduction. Methanesulfonic acid (28 mg, 0.3 mmol) was added, and the pH was controlled by adding aliquots (0.3 mmol) of methanesulfonic acid to the reaction mixture every time it turned orange, indicating a higher pH. The reaction was monitored by TLC (40% CH_2Cl_2 in hexane). After each addition of hydride, the reaction mixture was stirred for 2 h and the pH adjusted accordingly. Four additional rounds of $NaBH_3CN$ addition were performed to facilitate conversion (total $NaBH_3CN$ added 412 mg, 6.56 mmol) and the reaction mixture was stirred at room temperature overnight. After 15 h at room temperature, the reaction was approximately 60% complete by TLC. The solution was concentrated, dissolved in dichloromethane, and washed three times with water (pH 2). *Note: exposure to aqueous Na_2CO_3 decomposed the product.* The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product **14e**. The product mixture was purified by flash column chromatography at R_f = 0.45 with 10% CH_2Cl_2/hexane to give product **14e** as a yellow oil (105 mg, 15% yield). Compound **14e**: ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 2H), 7.60 (s, 1H), 7.3−7.45 (m, 5H), 6.25 (t, 1H, NH), 3.40 (t, 2H), 2.9 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 163.7, 138.2, 128.8, 128.7, 127.8, 126.7, 116.5, 113.5, 113.4, 53.2, 33.2; HRMS calcd for $C_{15}H_{14}F_3N_2O_3$ (M + H) 327.0951, found 327.0958.

*N-Heptyl-O-(3,4-dinitrophenyl)hydroxylamine (**14f**):* The *O*-(3,4-dinitrophenyl)hydroxylamine **8c** (100 mg, 0.5 mmol) was dissolved in absolute EtOH (5 mL), and heptaldehyde (**12c**; 57 mg, 0.5 mmol) was added dropwise. The mixture was stirred at room temperature and monitored by TLC (40% CH_2Cl_2/hexane), showing two spots corresponding to the two isomers of oxime **13f** at R_f = 0.70 and 0.65. The reaction was completed in 4 h. The solution was concentrated under vacuum. and the product obtained (oil, 110 mg, 60%) was used directly in the next step. Crude **13f**: ^{1}H NMR ($CDCl_3$; a 3:1 mixture of *E*/*Z* isomers was obtained, which complicated the spectra) δ 7.92 (m, 2H), 7.75 (s, 0.25H), 7.72 (s, 0.75H), 7.13 (t, 0.25H), 7.05 (t, 0.75H), 2.62 (q, 0.5H), 2.39 (q, 1.5H), 1.57 (m, 4H), 1.39 (m, 2H), 1.33 (m, 4H), 0.91 (t, 3H). The oxime **13f** (110 mg, 0.3 mmol) was dissolved in methanol (2 mL), and sodium cyanoborohydride (56 mg, 0.9 mmol) was added. A trace of methyl orange was added as an indicator to monitor the pH during the reduction.

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Methanesulfonic acid (28 mg, 0.3 mmol) was added, and the solution turned red. The pH was carefully controlled by adding methanesulfonic acid aliquots (0.3 mmol) to the reaction mixture every time it turned orange, which indicated a higher pH. The reaction was monitored by TLC (40% CH_2Cl_2/hexane). Four successive aliquots of sodium cyanoborohydride (1.2 eq.; 224 mg) were added to the reaction mixture, and the pH was adjusted after each addition. The reaction mixture was then stirred overnight at room temperature. The reaction was approximately 60% complete by TLC. The solution was concentrated, dissolved in dichloromethane, and washed three times with pH 7 water (note: exposure to an aqueous Na_2CO_3 wash decomposed the compound). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product **14f**. The product mixture was purified by flash column chromatography with 10% CH_2Cl_2/hexane to give **14f** as an unstable yellow oil (5 mg, 5% yield). Three bands eluted in the following order: 3,4-dinitrophenol (a decomposition product), unreduced oxime **13f**, and then **14f**. Compound **14f**: ^{1}H NMR ($CDCl_3$) δ 7.87 (d, 1H, J = 8.7 Hz), 7.81 (d, 1H, J = 2 Hz), 6.97 (dd, 1H, J = 8.7 and 2 Hz), 6.37 (br s, 1H, NH), 3.16 (br t, 2H), 1.58 (m, 2H), 1.31 (m, 6H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 155.6, 140.9, 135.7, 126.8, 120.8, 116.7, 52.5, 31.7, 29.1, 27.0, 26.9, 22.6, 14.1. HRMS for M = $C_{13}H_{19}N_3O_5$ (297.1325) was not observed, likely due to the high temperature used for ionization. Instead, the HRMS provided a signal consistent with facile N−O bond cleavage, loss of the 3,4-dinitrophenoxy fragment ($C_6H_3N_2O_5$), and observation of a $C_7H_{16}N$ fragment. HRMS (M − $C_6H_3N_2O_5$): calcd for $C_7H_{16}N$ 114.1283, found 114.1282.

General Method for the Synthesis of Amides 16. Each of the 4-nitrophenylhydroxylamines **14a−d** (25 mM solution in 75% *t*-BuOH in a 7.4 pH buffer) was combined with an equivalent volume of a pyruvic acid solution (50 mM in the same solvent system) to give a final concentration of the hydroxylamine (12.5 mM) and pyruvic acid (25 mM), respectively. Dibenzyl ether (DBE) was added as an internal standard. The reactions were swirled in an incubator-shaker at 65 °C overnight. The volatiles were removed under reduced pressure, and dichloromethane was added to the residue. The organic layer was washed with aqueous Na_2CO_3 and the organic layer separated, dried over anhydrous Na_2SO_4, filtered, and concentrated. Yields were determined via integration of the DBE standard versus a diagnostic chemical shift for each acetamide derivative. Typically, either the acetamide Me group near 2.0 ppm or the α proton on the carbon attached to the amide nitrogen was used for quantification. NMR spectra are provided for the relevant compounds in the Supporting Information. The results are given in Table 2.

*N-Phenethylacetamide (**16a**):* see percent yield given in Table 2; ^{1}H NMR ($CDCl_3$) δ 7.4−7.1 (m, 5H), 5.40 (s, 1H), 3.51 (q, 2H), 2.76 (t, 2H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.2, 138.9, 128.7, 128.6, 126.5, 40.7, 35.6, 23.3.[39]

The ^{1}H NMR spectra of **16b**[40] and **16d**[40] matched their literature spectra. Compound **16c** has been synthesized previously.[41]

*N-Phenethyl-2-phenylacetamide (**16e**).*[5] The *O*-(4-nitrophenyl)-*N*-phenethylhydroxylamine **14a** (25 mg, 0.096 mmol) and phenylpyruvic acid (**15**, where R^2 = Bn; 31 mg, 0.19 mmol) were dissolved in DMSO (1.8 mL). Diphenylmethane (DPM; 6.76 mg, 0.04 mmol) was added into the vial as an internal standard followed by addition of H_2O (pH 7.4, 1.2 mL) to form a 60% DMSO/H_2O mixture for the reaction. The addition of H_2O in the vial resulted in formation of a white precipitate, which was dissolved by adding some more DMSO (0.8 mL). The reaction (now in a 68% DMSO/H_2O solution) was then swirled in an incubator shaker at 65 °C overnight. The reaction mass was then dissolved in dichloromethane (50 mL) and extracted with water (four times) to remove the DMSO present in the reaction mass. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The product was confirmed by ^{1}H NMR ($CDCl_3$), looking at the distinctive CH_2 (s, δ 3.4 ppm) from the amide **16e**. The DPM standard gives a singlet at 3.9 ppm. The relative integrations were used to calculate the yield of **16e**: 22 mg (86% conversion, 100% yield). The ^{1}H NMR matched the literature spectrum obtained previously for **16e**.[5]

HPLC Method for Rate Constant Determination. A gradient elution using 0.1% TFA in acetonitrile and water was developed which provided baseline separation between the starting materials, product amide **16a**, and the diphenylmethane standard. Using this method, the respective starting materials (1 equiv of **1b** and **14a**) were dosed into respective vials along with 10 equiv of pyruvic acid and heated in an incubator-shaker at 65 °C in 50% *t*-BuOH/pH 7.4 buffer. Vials were taken out at specific time points and cooled to 0 °C to stop the reaction. Then a sample from each vial (10 μL) was injected into the HPLC for analysis. The ratio of the area percent of the starting material vs the internal standard was determined for each time point. A plot of ln([starting material]/[std]) vs time (min) gave a line with negative slope of $-k$ for this pseudo-first-order condition. The respective values of k are given in Table 3. Note: additional HPLC experiments were performed to determine yields in the different solvent systems and can be found in the Supporting Information.

Chemoselectivity Test with 14a. *(a). Glycine Challenge.* Into a glass vial equipped with a phenolic screw-capped lid, glycine (11.6 mg, 155 μmol), *N*-phenethyl-*O*-(4-nitrophenyl)hydroxylamine **14a** (10.6 mg, 41 μmol), and diphenylmethane (DPM; 5.0 mg, 29.7 μmol) were dissolved in DMSO (0.65 mL) and freshly distilled pyruvic acid (10.9 μL, 155 μmol) was added immediately followed by the addition of phosphate-buffered saline (PBS, 0.3 mL). The vial was then capped and shaken, and the 68% DMSO/water solution was swirled overnight in an orbital shaker at 65 °C. The transparent solution turned dark green. Workup involved diluting the sample with CH_2Cl_2 (4 mL), separating the organic layer, and washing it twice with saturated aqueous Na_2CO_3. After base addition, the aqueous phase was bright yellow due to the generation of sodium *p*-nitrophenolate. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated to give a colorless oil. ^{1}H NMR ($CDCl_3$) was used to estimate the yield using the DPM singlet at 3.98 ppm and the acetamide singlet at 1.93 ppm. The yield of acetamide **16a** was 93%.

(b). Cysteine Challenge. Into a vial equipped with a phenolic screw-capped lid, cysteine hydrochloride hydrate (27 mg, 154 μmol), *N*-phenethyl-*O*-(4-nitrophenyl)hydroxylamine **14a** (9.0 mg, 34.8 μmol), and diphenylmethane (12.3 mg, 73.1 μmol) were dissolved in DMSO (0.65 mL) and freshly distilled pyruvic acid (10.9 μL, 13.9 mg, 155 μmol) was added immediately followed by the addition of phosphate-buffered saline (0.3 mL). The vial was then capped and shaken, and the 68% DMSO/water solution was swirled overnight in an orbital shaker at 65 °C. The heterogeneous mixture was dark brown. Workup involved diluting the sample with CH_2Cl_2 (4 mL), separating the organic layer, and washing it twice with saturated aqueous Na_2CO_3. The aqueous phase was dark brown. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated to give a dark yellow oil. ^{1}H NMR ($CDCl_3$) was used to estimate the yield using the DPM singlet at 3.98 ppm and the acetamide singlet at 1.93 ppm. The yield of acetamide **16a** was 36.4%.

(c). Cysteine and Hexanoic Acid Challenge. Into a vial equipped with a phenolic screw-capped lid, cysteine hydrochloride hydrate (27.4 mg, 156 μmol), hexanoic acid (18 mg, 19.4 μL, 156 μmol), *N*-phenethyl-*O*-(4-nitrophenyl)hydroxylamine **14a** (20.9 mg, 80.9 μmol), and diphenylmethane (6.0 mg, 35.6 μmol) were dissolved in DMSO (1.3 mL) and freshly distilled pyruvic acid (13.6 mg, 10.9 μL, 155 μmol) was added immediately followed by the addition of phosphate-buffered saline (0.6 mL). The vial was then capped and shaken, and the 68% DMSO/water solution was swirled overnight in an orbital shaker at 65 °C. Workup involved diluting the sample with CH_2Cl_2 (4 mL), separating the organic layer, and washing it twice with saturated aqueous Na_2CO_3. The aqueous phase was dark brown. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated to give a dark yellow oil. ^{1}H NMR ($CDCl_3$) was used to estimate the yield of **16a** using the DPM singlet at 3.98 ppm and the acetamide singlet at 1.93 ppm. The yield of **16a** was 39%.

(d). Lysine and Phenol Challenge. Into a vial equipped with a phenolic screw-capped lid, lysine hydrochloride (16.7 mg, 91 μmol), phenol (8.6 mg, 91 μmol), *N*-phenethyl-*O*-(4-nitrophenyl)-hydroxylamine **14a** (11.8 mg, 45.7 μmol), and diphenylmethane (12.4 mg, 73.7 μmol) were dissolved in DMSO (0.65 mL) and freshly

distilled pyruvic acid (6.43 μL, 91 μmol) was added immediately followed by the addition of water (0.3 mL). The vial was then capped and shaken, and the 68% DMSO/water solution was swirled overnight in an orbital shaker at 65 °C. The transparent solution turned yellow with no precipitate noted. Workup involved diluting the sample with CH_2Cl_2 (4 mL), separating the organic layer, and washing it twice with saturated aqueous Na_2CO_3. The aqueous phase was bright yellow. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated to give a light yellow oil. ^{1}H NMR ($CDCl_3$) was used to estimate the yield using the DPM singlet at 3.98 ppm (2H) and the quartet at 3.44 ppm (2H). The yield of acetamide was 98.1%.

Control Experiments with Acetyl Chloride and Phenethylamine. *(a). In the Presence of Cysteine and Hexanoic Acid.* In a glass vial equipped with a phenolic screw cap, phenethylamine (17.6 mg, 0.145 mmol), cysteine hydrochloride hydrate (52 mg, 0.29 mmol), hexanoic acid (34 mg, 36 μL, 0.29 mmol), and diphenylmethane (13.4 mg, 0.08 mmol) were combined in DMSO (2.6 mL). Deionized water (1.2 mL) was then added to provide a translucent 68% DMSO/H_2O solution. Acetyl chloride (22.8 mg, 20.7 μL, 0.29 mmol) was added, and the vial was capped and shaken vigorously for 10 s to provide a transparent solution. The reaction vial was attached to an orbital shaker equilibrated at 65 °C and swirled overnight. The solution was cooled to room temperature and then diluted in dichloromethane (50 mL) and washed with water (four times) to remove the DMSO present in the reaction mass. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give 33 mg of the crude product as a colorless oil. Using the relative integrations compared to the DPM standard (δ 3.93, s, 2H), the ^{1}H NMR ($CDCl_3$) of the oil revealed a 66% recovery of hexanoic acid (δ 2.34, t, 2H) and no trace of **16a** (0% yield). No unreacted phenethylamine was detected in the organic layer. Investigation of the water phase revealed the presence of phenethylamine salts.

(b). In the Presence of Lysine and Phenol. Into a vial equipped with a phenolic screw-capped lid, lysine hydrochloride (16.6 mg, 0.091 mmol), phenethylamine (5.5 mg, 5.85 μL, 0.04568 mmol), phenol (8.56 mg, 0.091 mmol), and diphenylmethane (12.7 mg, 0.073 mmol) were dissolved in DMSO (0.65 mL) and acetyl chloride (7.14 mg, 6.49 μL, 0.091 mmol) was added immediately followed by the addition of PBS (0.3 mL). The vial was then shaken and capped and the 68% DMSO/PBS solution swirled overnight in an orbital shaker at 65 °C. The transparent solution was then diluted with CH_2Cl_2 (4 mL) and washed twice with a 10% saturated Na_2CO_3 solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a colorless oil. ^{1}H NMR ($CDCl_3$) revealed unreacted phenethylamine (75% recovered) and a trace of the acetamide product (1% yield).

■ ASSOCIATED CONTENT

Supporting Information

Figures, tables, and text giving ^{1}H NMR spectra for **8a−c**, oxime intermediates **13a−d,g−i**, compounds **14a−f**, and a crude mixture containing **16e**, ^{13}C NMR spectra for compounds **8a−c**, **13h**, **14a−f**, **16a**, details of the kinetic studies of **1b** and **14a** with 10 equiv of pyruvic acid, details of the solvent studies of **1b** and **14a** in *t*-BuOH/pH 7.4 buffer mixtures and of **14a** in DMSO/pH 7.4 buffer mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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