

SUPPORTING INFORMATION

Fast Alpha Nucleophiles: Structures that Undergo Rapid Hydrazone/Oxime Formation at Neutral pH

Eric T. Kool*, Pete Crisalli, Ke Min Chan

Department of Chemistry, Stanford University, Stanford, CA 94305-5080 (USA)

*e-mail: kool@stanford.edu

Table of Contents

General experimental methods.....	S2
Kinetics methods and notes.....	S2
Synthetic methods.....	S3
Fig. S1. Representative time-dependent UV-Vis spectra during reactions.....	S5
Fig. S2. Representative time course plots of spectral changes.....	S9
Fig. S3. Representative pseudo-first-order kinetics fits.....	S12
Table S1. Long-wavelength absorption maxima of hydrazones in this study.....	S15
Table S2. Comparisons of Selected Reaction Rates in 10% DMF buffer vs. Fully Aqueous Buffer.....	S17
Fig. S4. Testing kinetic order in aldehyde (two selected reactions).....	S18
Fig. S5. Hammett plot of reactivity of substituted arylhydrazines.....	S18
Table S3. Reactivity of varied hydrazines with 2-butanone.....	S19
Fig. S6. Plot showing correlation of reactivity of various hydrazines with 2-formylpyridine and 2-butanone.....	S19
References.....	S20
NMR Spectra of dimethylaminoethoxyamine dihydrochloride.....	S21

General. Compounds were obtained from Sigma-Aldrich except the following: 2,4,6-trimethylphenylhydrazine (Matrix Scientific), terephthalaldehydic acid (Fluka), and 8-quinoline carboxaldehyde (Alfa Aesar). Reaction buffer was prepared by mixing 10x phosphate-buffered saline solution pH 7.4 (Fisher Scientific) with deionized water and HPLC grade dimethylformamide (DMF, Fisher Scientific) in 1:9:1 ratio (vol:vol:vol) except where DMF was replaced by water as noted below (Table S2). Reactants were dissolved in spectrophotometric grade dimethylformamide (Fisher Scientific) immediately prior to reaction. Reactions were monitored on a Cary 100 Bio UV-Visible Spectrophotometer.

Kinetics methods. Reactions were performed under pseudo-first-order conditions in quartz cuvettes (10 mm path length; total volume ~700 μL) with solutions pre-equilibrated at room temperature (23 $^{\circ}\text{C}$). 594 μL reaction buffer was pipetted into the cuvette, followed by 3.0 μL of a 100 mM hydrazine stock solution (where hydrazine was used in excess) in spectrophotometric grade DMF. The solution was mixed by rapid pipetting up and down (70 μL volume) 25 times. A background spectrum was measured and stored. The reaction was then initiated by addition of 3.0 μL of 20 mM aldehyde stock solution with mixing by rapid pipetting up and down 25 times. The cuvettes were capped and spectra were measured repeatedly at 2-10 min intervals over 2-4 hours. Representative spectra (with background subtracted) as a function of time are shown in Fig. S1. For each pair of reactants we chose a wavelength near the wavelength of maximal change to record time vs. absorbance data. Representative plots of these curves are shown in Fig. S2. Note that for 2-butanone reactions the ketone was used in excess.

Fitting of the pseudo-first-order kinetics data was done by linear least-squares fits, following the Guggenheim (time lag) method.^{1,2} In this method (see method II in ref. 2), the data obtained at regular intervals τ are divided into halves, and early absorbance data are subtracted from the second-half data as shown in the following equation:

$$\ln(A_{t+\tau} - A_t) = \ln((A_{\text{inf}} - A_0)(1 - e^{-k\tau})) - kt$$

This method removes the need for measuring a zero-time absorbance value and a final (infinity) absorbance value. The natural log of absorbance differences are plotted vs. time, and the slope corresponds to the negative of the pseudo-first-order rate constant. Representative linear fits are shown in Fig. S3. The first-order rate constants were obtained from the slopes of the linear fits, and were adjusted for units of seconds. Experiments were repeated 3-6 times and averaged, and standard deviations were obtained.

To serve as a check on rate constants obtained above, we also used the half-life method to estimate rate constants for the majority of reactions. In this method, we measured the time to reach 50% of the absorbance change ($t_{(1/2)}$) and then obtained the pseudo-first-order rate constant (k_{ps}) from the equation

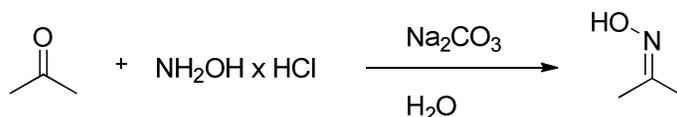
$$t_{(1/2)} = 0.693/k_{\text{ps}}$$

The second-order rate constants were obtained from k_{ps} and the reactant concentration. Very good agreement was seen with our reported rate constants (Tables 1,2 in main text).

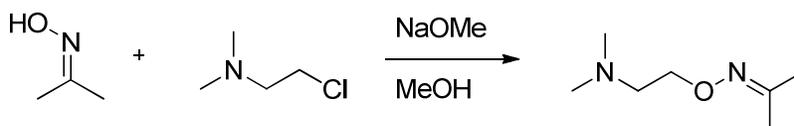
Additional notes on kinetics methods:

- (a) *On the possibility of non-second-order behavior.* The mechanism and kinetic behavior for imine formation between hydrazines and carbonyl compounds depends on multiple factors, including carbonyl structure, hydrazine structure, reactant concentrations and pH. We have obtained direct evidence for second-order behavior in two example cases (see ref. 3 and below). It is possible that not all cases tested here would reflect true second-order behavior if the kinetic mechanism were investigated in detail; the reader is cautioned that the second-order rate constants listed here are apparent values, and were simply obtained by calculation from the first-order rates. The observed pseudo-first-order rates are more reliable since they were measured directly.
- (b) *On the possibility of reaction reversal (hydrolysis of hydrazones).* The reverse reaction, if it occurred on fast timescales that are competitive with hydrazone formation, might affect measured rate constants. The main concern here would be cases in which reversal occurs rapidly, on a timescale of minutes. For the majority of substrates, this appears unlikely, since “standard” hydrazones (2-hydrazinylpyridine with 4-formylbenzamides as calculated by Dawson, *Bioconj. Chem.* 2008, 19, 2543–2548) at pH 7 in the absence of catalyst give a hydrolytic rate (k_{-1}) of approximately 10^{-6} per second, for a half-life of 8 days. However, we cannot rule out this equilibration in select cases without measuring it directly. Such back reaction would lead to an underestimate of forward reaction rates.

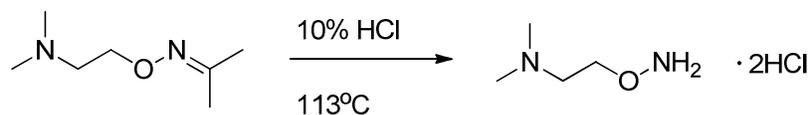
Synthetic methods.



Acetone oxime.⁴ Acetone (3.6 mL, 50 mmol), hydroxylamine hydrochloride (5.2 g, 75 mmol) and sodium carbonate (8 g) were stirred together in 10 mL H₂O for 16 h. The reaction solution was extracted multiple times with diethyl ether, the organic extract dried with anhydrous magnesium sulfate and evaporated to give a white solid as the product (75% yield). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) = 1.890 (s, 3H), 1.878 (s, 3H). ¹³C NMR (CDCl₃, 500 MHz) δ (ppm) = 155.79, 21.921, 15.134.

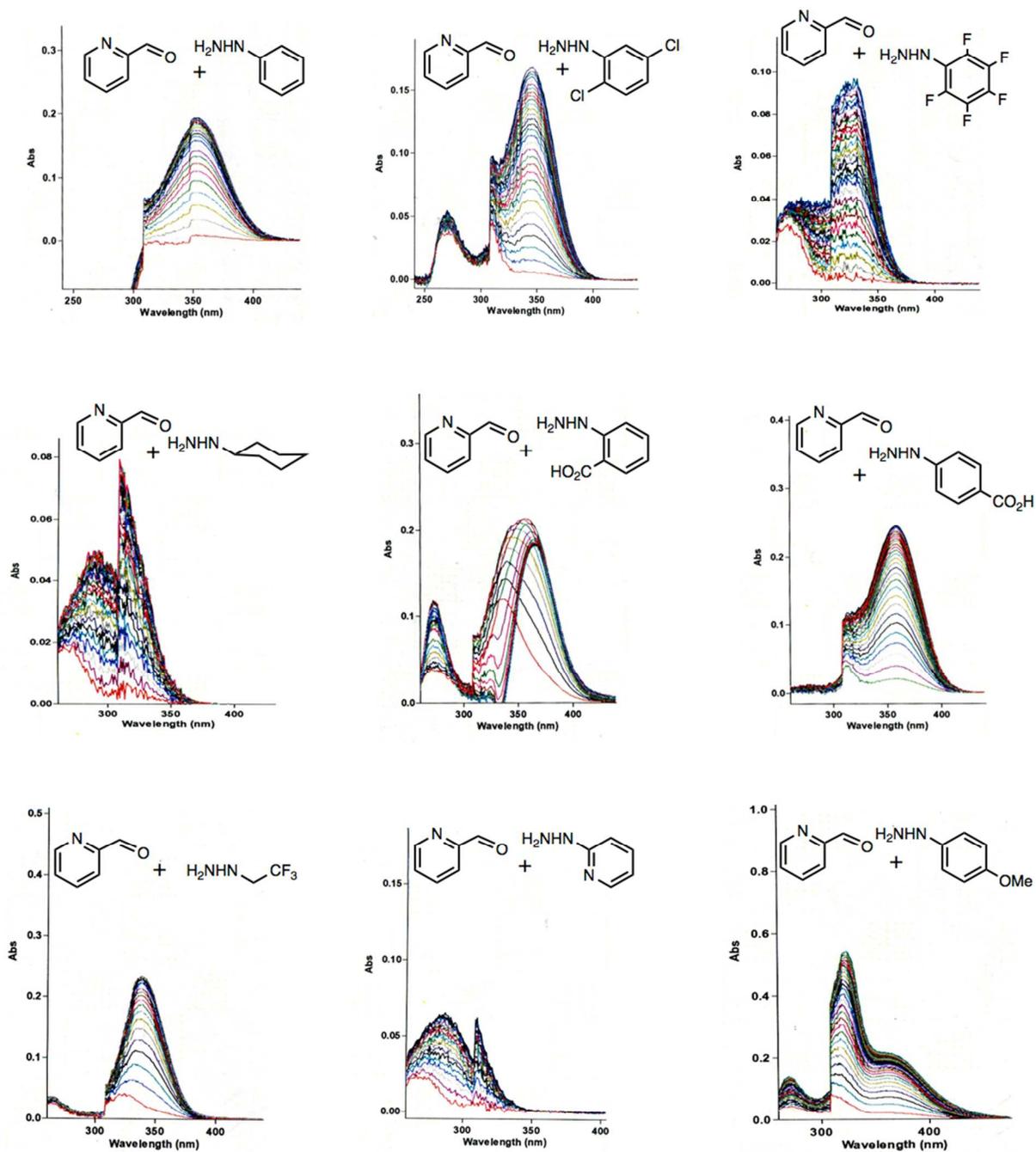


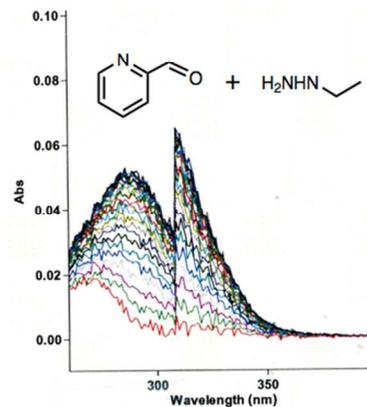
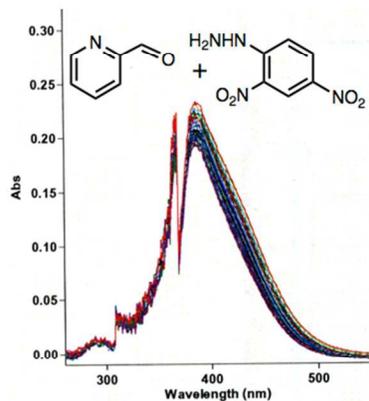
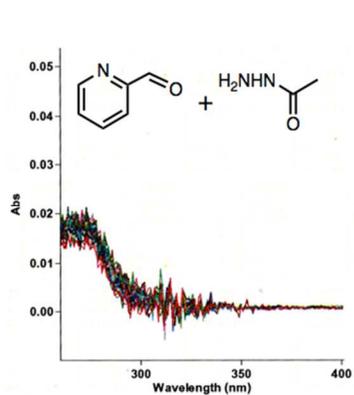
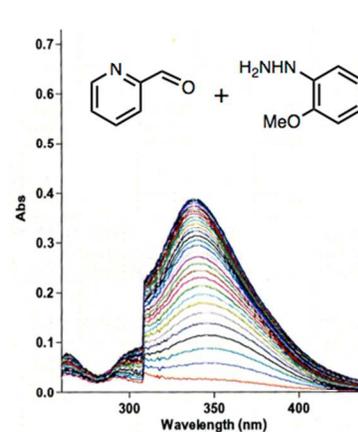
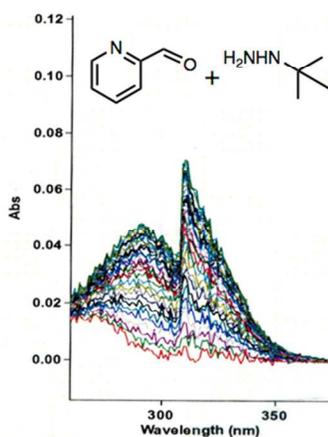
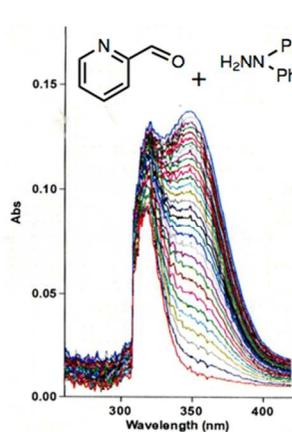
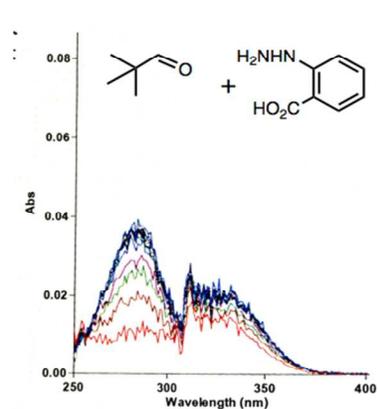
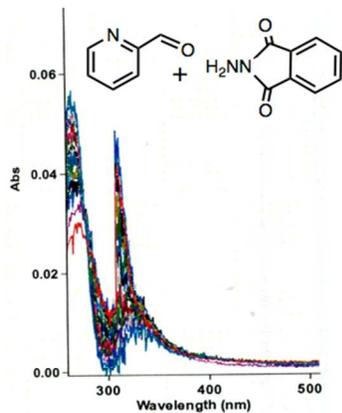
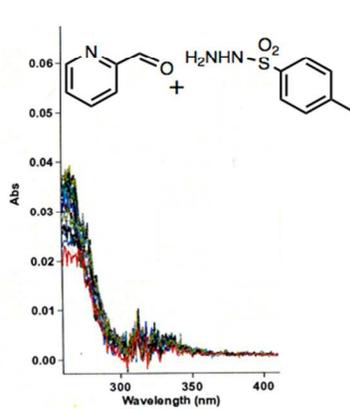
Acetone O-(2-(dimethylamino)ethyl) oxime.⁵ Acetone oxime (2 g, 27 mmol) was stirred overnight with 54 mL of sodium methoxide solution (5 M in MeOH, 54 mmol) at 50 °C. The colorless reaction solution turned orange in an hour after addition of sodium methoxide. 2-chloro-N,N-dimethylethan-1-amine hydrochloride (3.88 g, 27 mmol) was added to the sealed tube and the reaction mixture stirred at 100 °C for 1 h. The crude product mixture was distilled under vacuum to retrieve 1.8 g of the crude product, an almost colorless yellow-tinted liquid. ¹H NMR (D₂O, 400MHz) δ (ppm) = 4.032 (t, 2H), 2.503 (t, 2H), 2.187 (s, 6H), 1.764 (s, 3H), 1.753 (s, 3H).

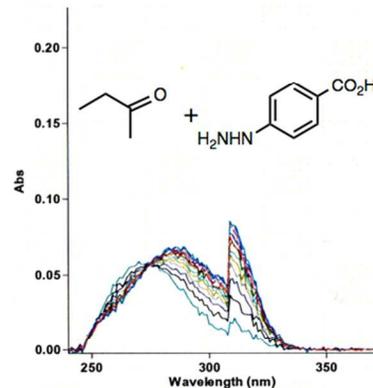
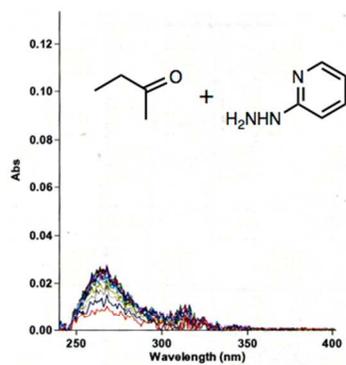
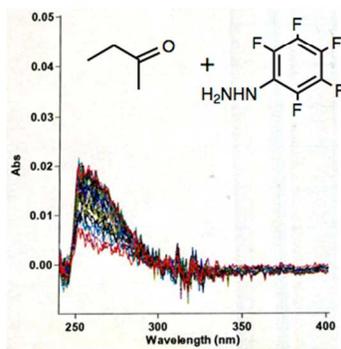
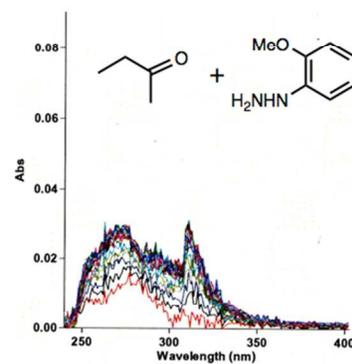
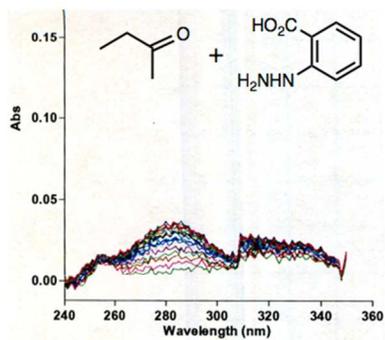
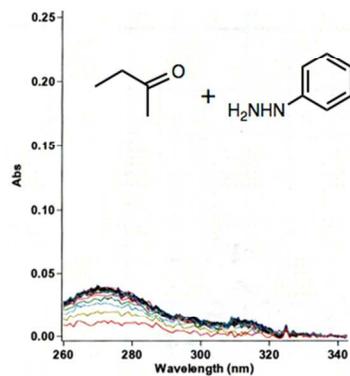
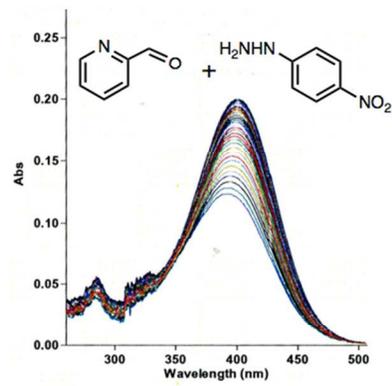
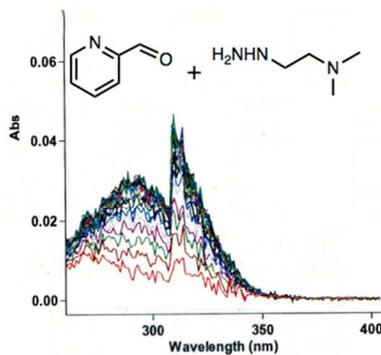
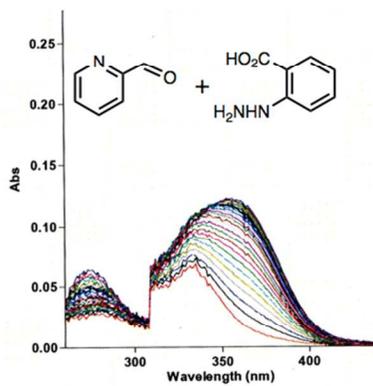


2-(Aminoxy)-*N,N*-dimethyl-ethanamine dihydrochloride.⁵ The crude oxime product (200 mg) from the previous step was placed in an open round bottom flask and stirred with 5 mL 10% HCl solution. The reaction flask was heated in an oil bath at 113 °C for 5 h until TLC (eluent 100% methanol) showed full conversion of the starting material. During heating, the reaction mixture was topped up with 10% HCl solution as it evaporated. After complete conversion of the starting material, the reaction mixture was allowed to boil off completely, leaving a yellow liquid. To remove water from the crude product, 0.5 mL of n-butanol was added and the resultant solution heated at 118 °C to obtain a yellow-white solid. The crude solid was recrystallized from methanol to obtain white crystals as the pure product (70% yield). ¹H NMR (DMSO, 500MHz) δ (ppm) = 4.445 (t, 2H) , 3.427 (t, 2H) , 2.780 (s, 6H). ¹³C NMR (DMSO, 500MHz) δ (ppm) = 68.354, 53.634, 42.305. ESI-MS m/e 105 [MH⁺]

Fig. S1. Representative time-dependent UV-Vis spectral changes during hydrazone formation of various hydrazines at pH 7.4. Spectra were measured at 4-10 min time points as described above. (The discontinuity at ca. 305 nm in some of the spectra is an artifact from a damaged spring in the instrument; it does not impact the kinetic analyses.)







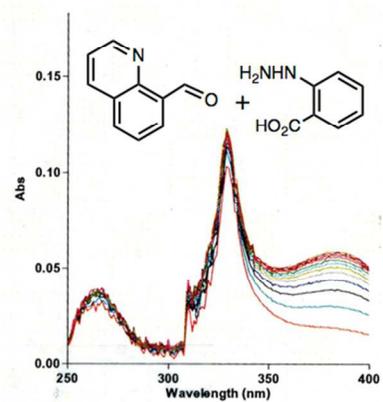
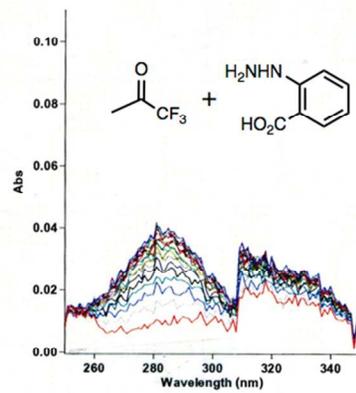
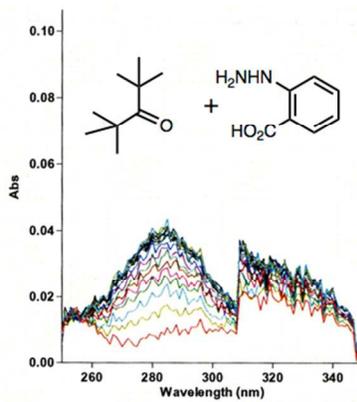
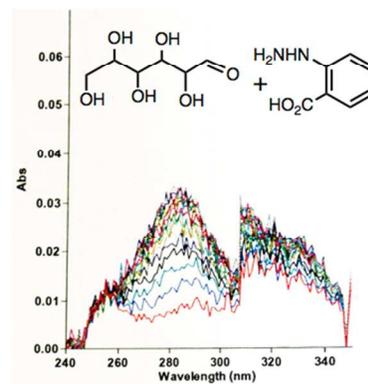
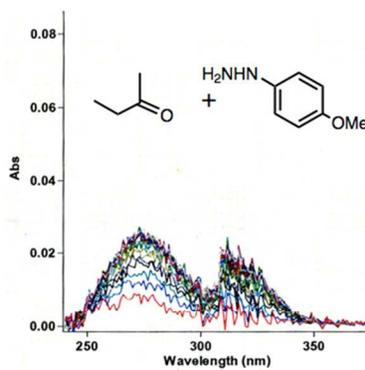
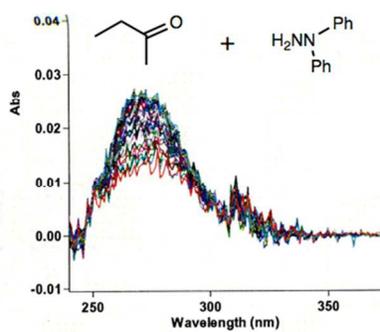
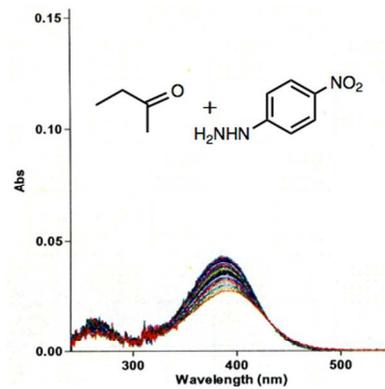
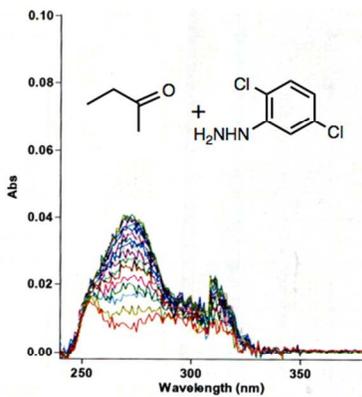
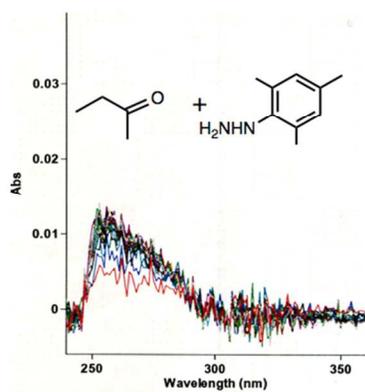
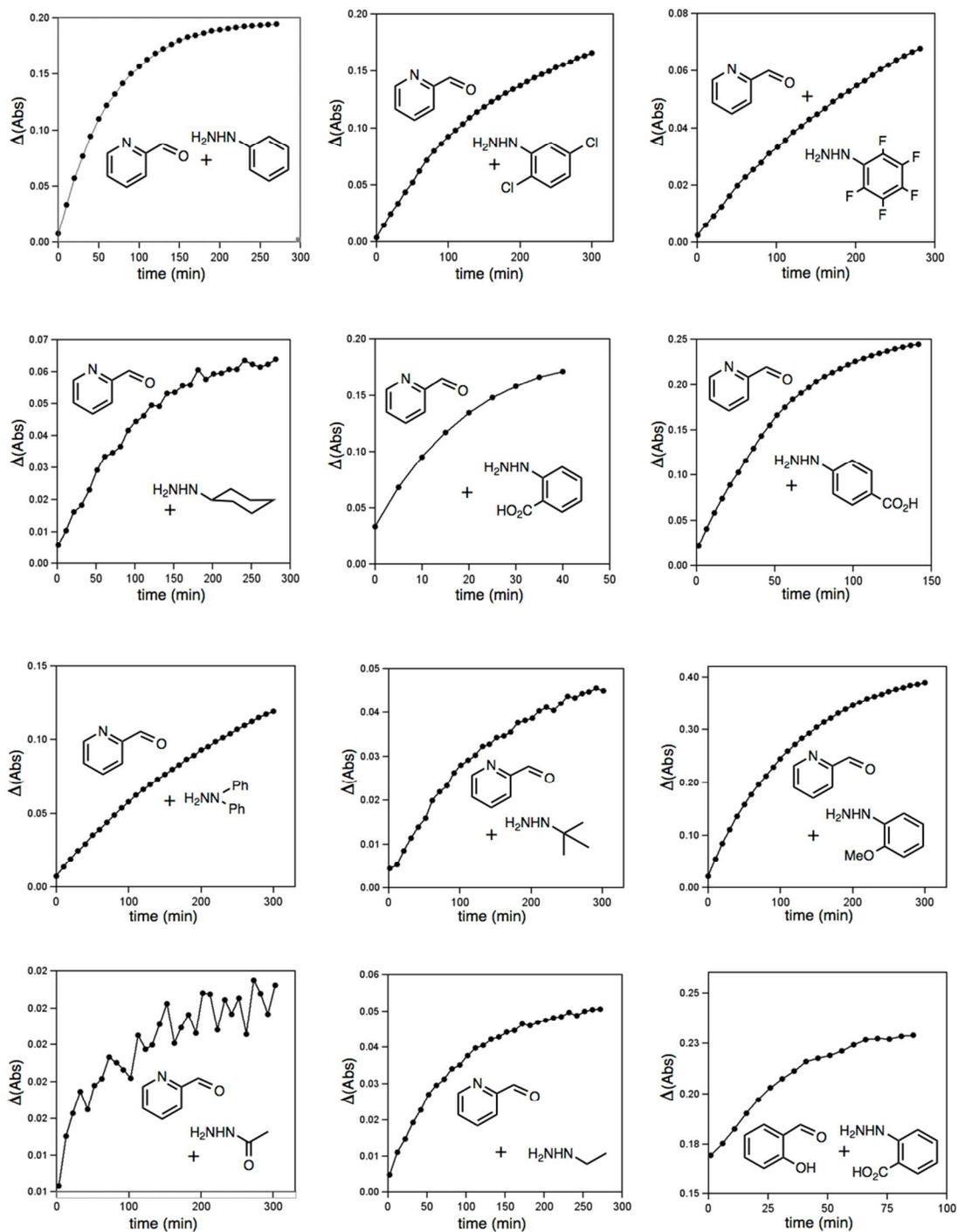
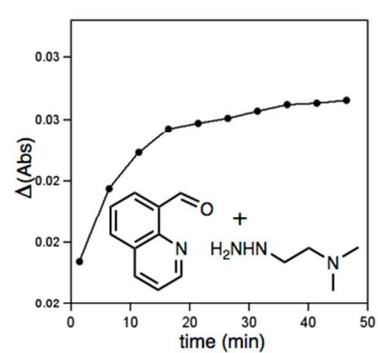
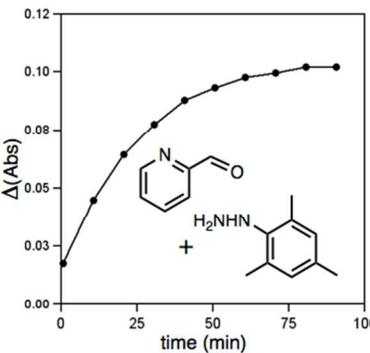
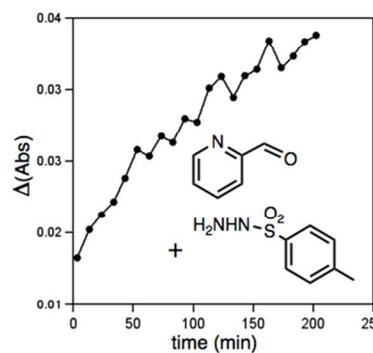
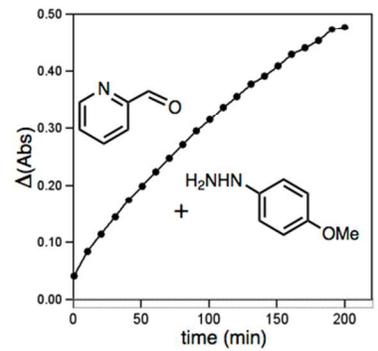
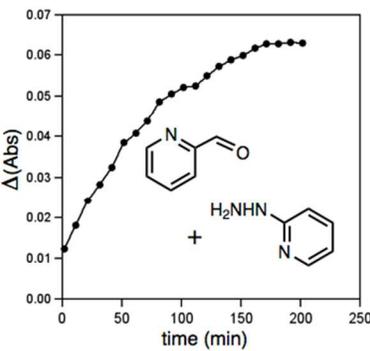
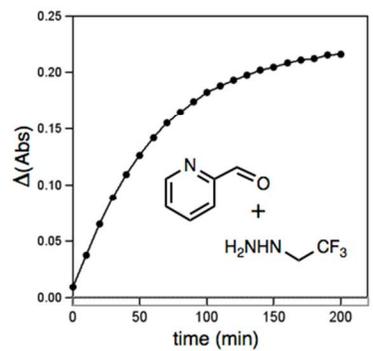
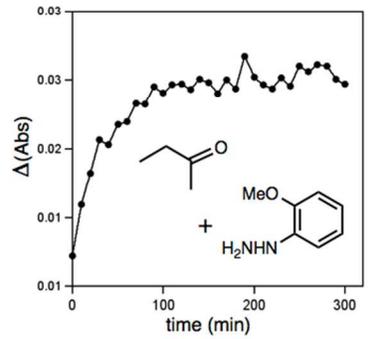
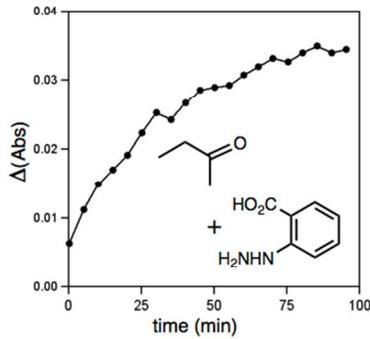
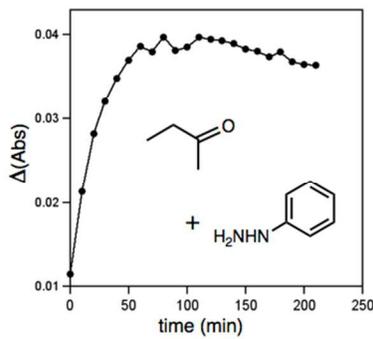
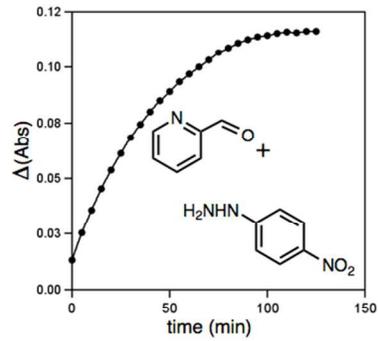
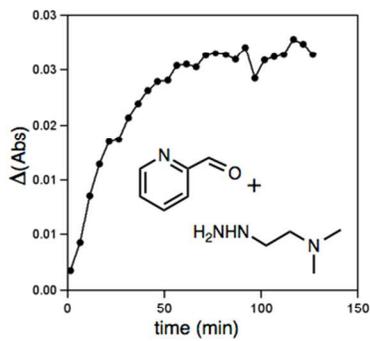
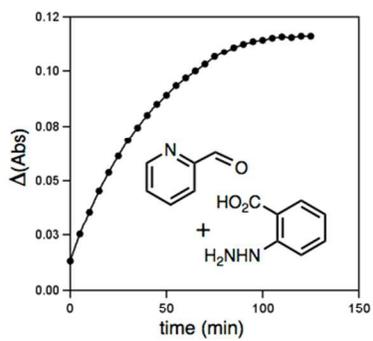


Fig. S2. Representative plots of time-dependent absorbance changes during hydrazone formation of varied hydrazine compounds (shown) at pH 7.4. Points were taken at 4-10 min intervals.





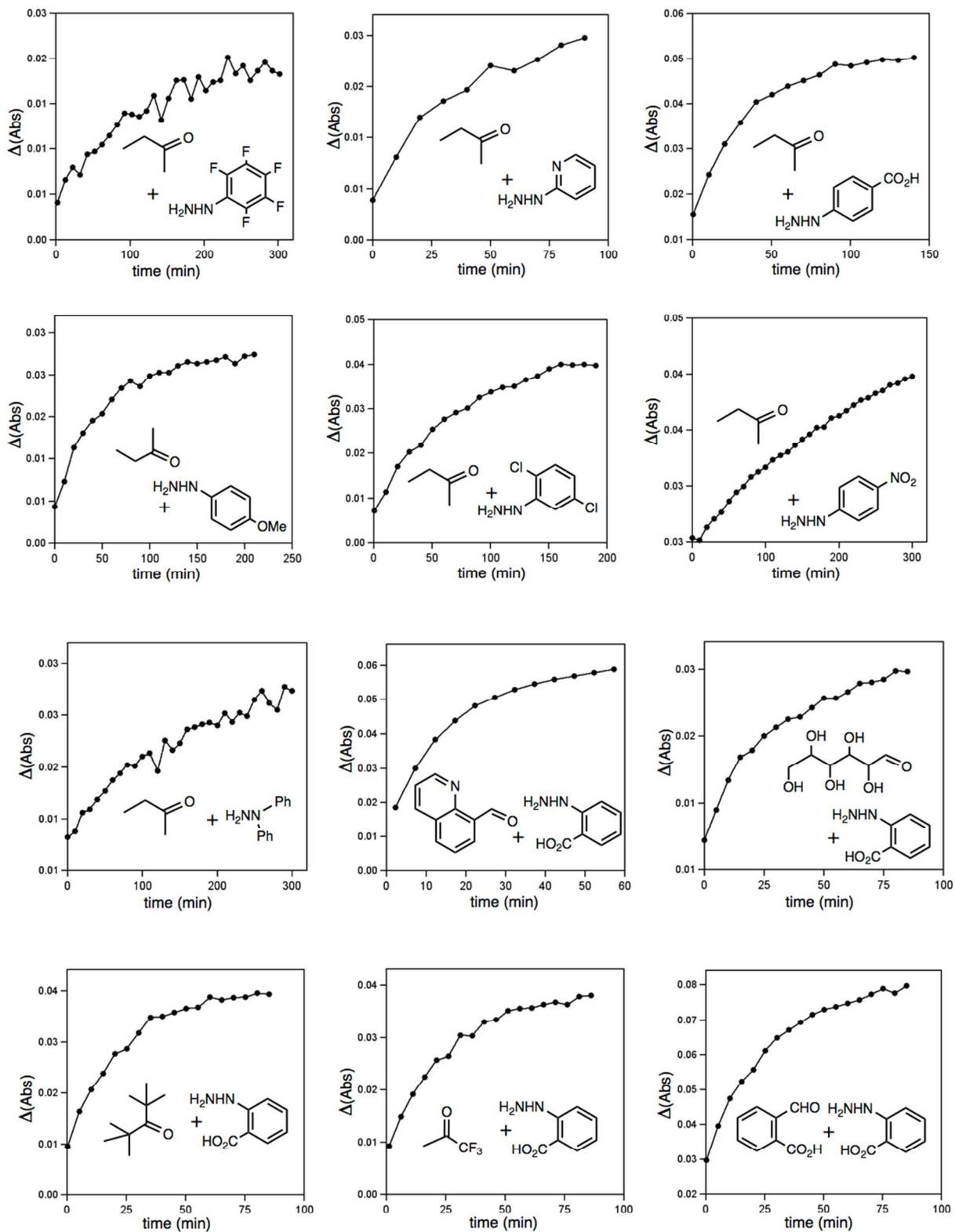
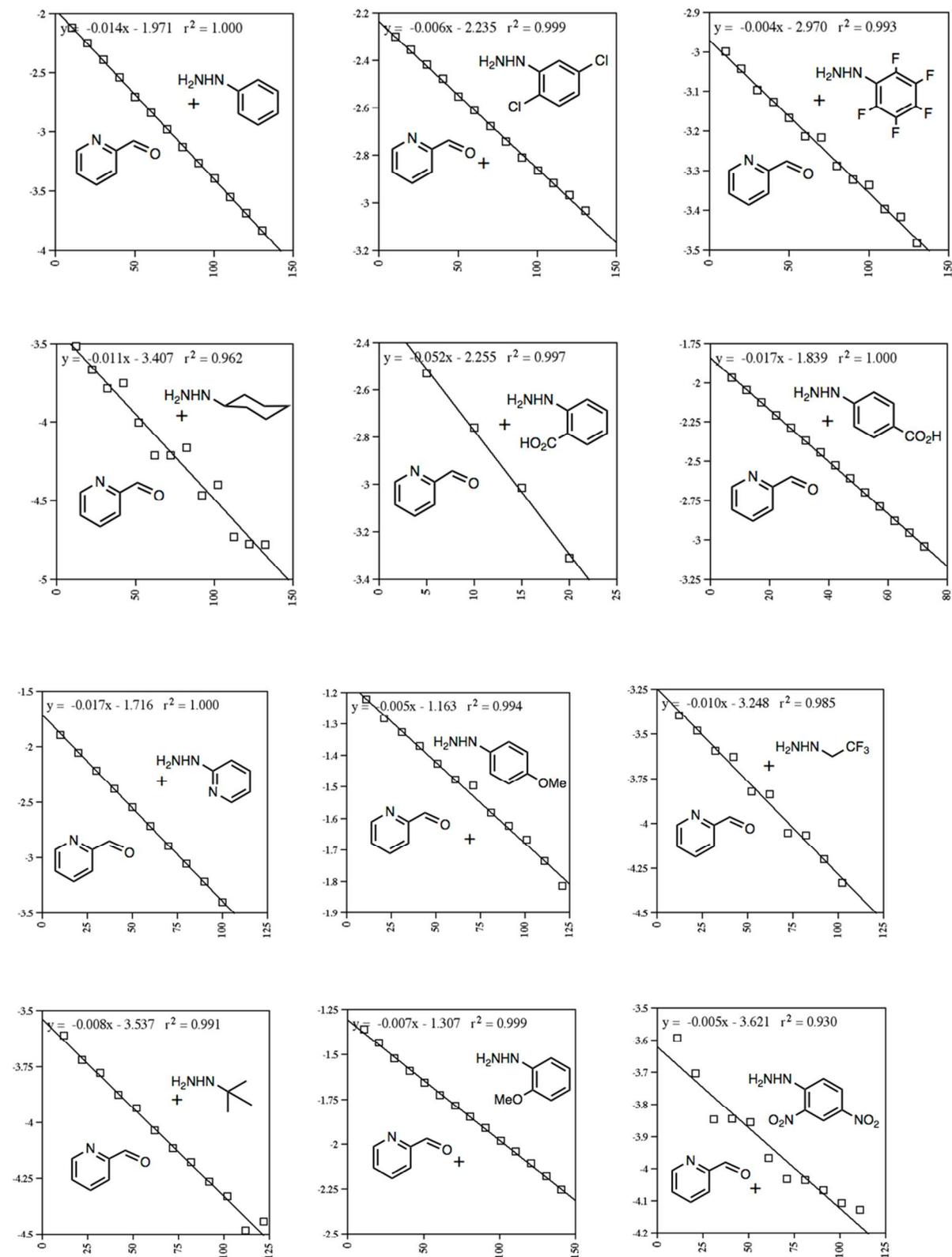
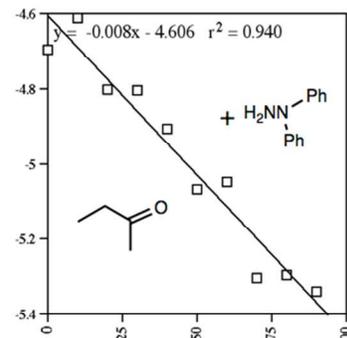
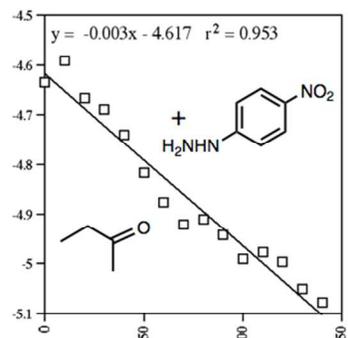
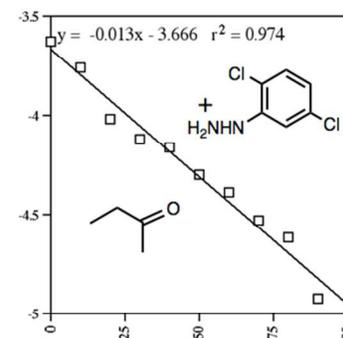
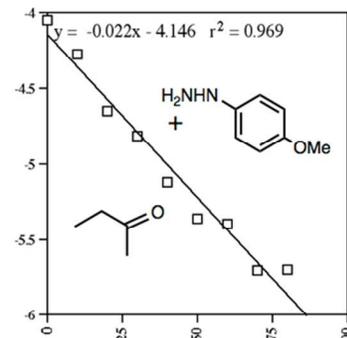
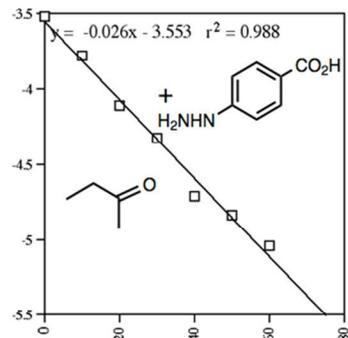
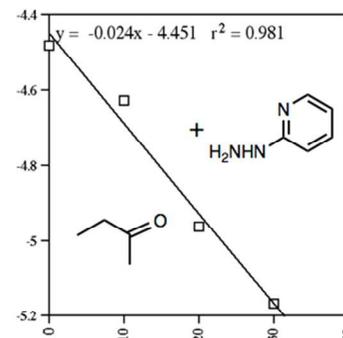
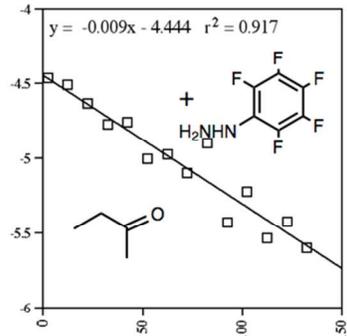
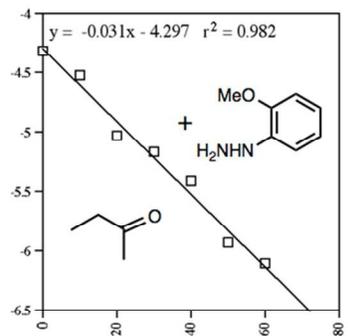
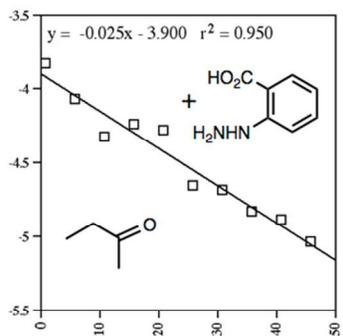
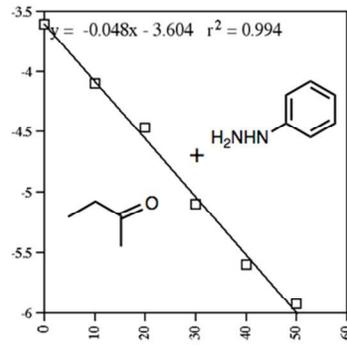
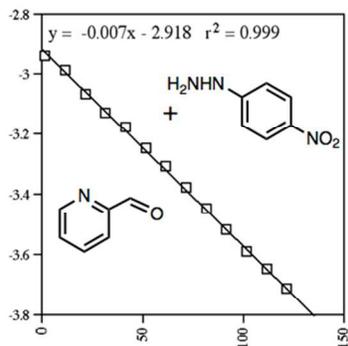
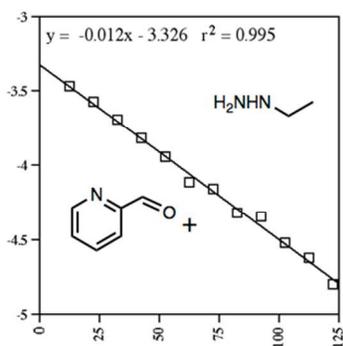


Fig. S3. Representative linear least-squares fits (Δt vs. $\ln(\Delta A)$ (Guggenheim method)) for hydrazone formation by reactants shown at pH 7.4. Conditions were as described above. Fitting was done as described as above (details available in ref 2). Slopes correspond to pseudo-first-order rate constants; R^2 values shown.





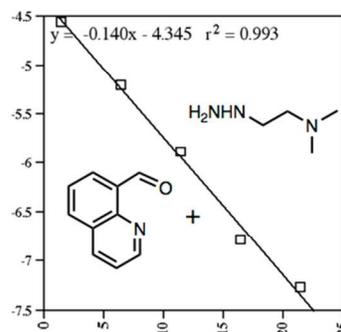
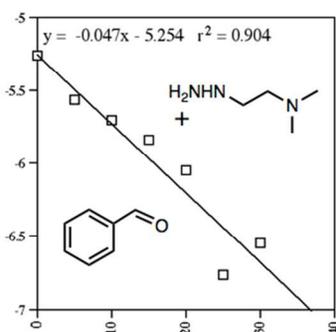
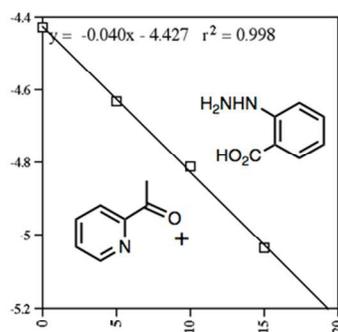
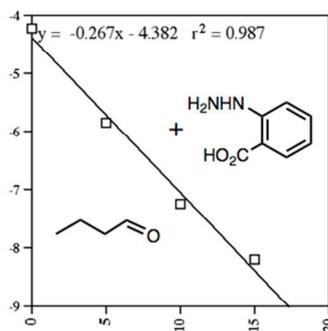
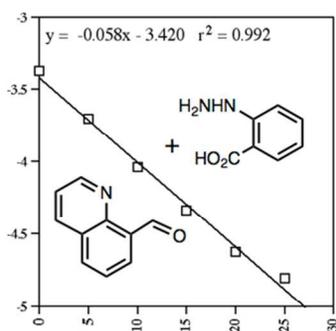
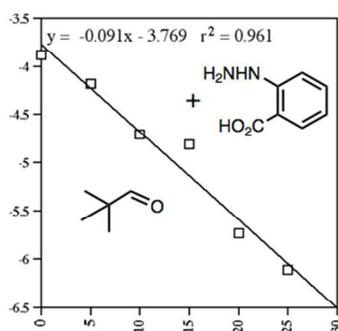
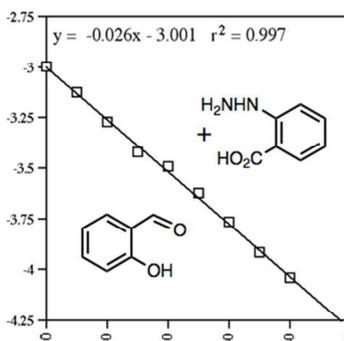
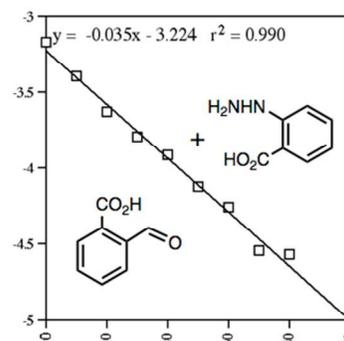
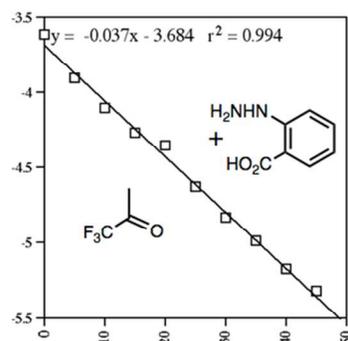
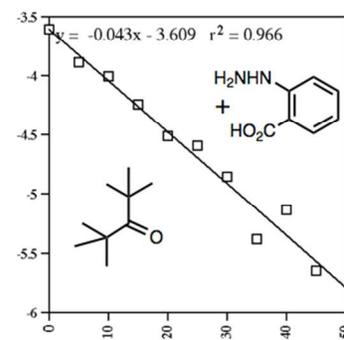
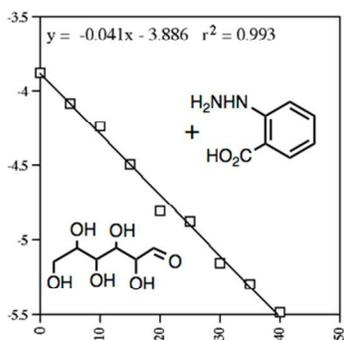
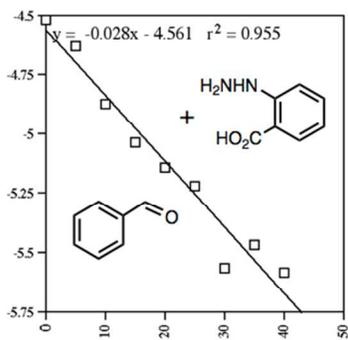
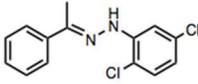
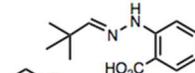
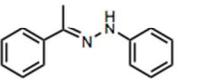
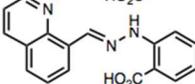
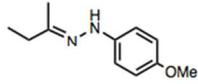
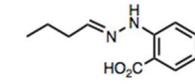
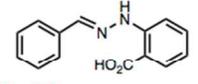
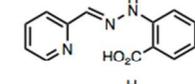
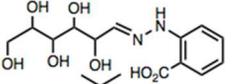
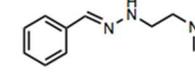
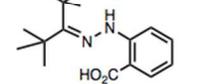
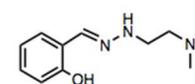
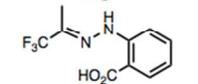
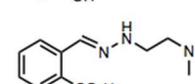
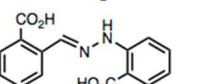
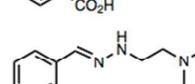
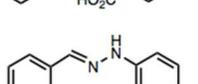


Table S1. Long-wavelength absorption maxima of hydrazones in this study.^a

hydrazone	λ_{max} (nm)	hydrazone	λ_{max} (nm)
	355		290
	340		338
	327		268
	292		385
	366		288
	356		272
	272		282
	401		272
	286		253
	338		265
	324, 366sh		286
	262		257
	300		274
	268		387
	350		273

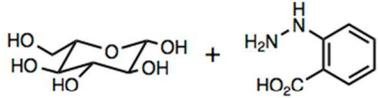
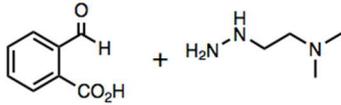
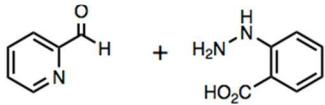
^aConditions: pH 7.4, 25°C, phosphate-buffered saline buffer with 10% DMF

Table S1(contd.). Long-wavelength absorption maxima of hydrazones in this study.^a

hydrazone	λ_{\max} (nm)	hydrazone	λ_{\max} (nm)
	270		284
	274		384
	272		283
	274		285
	284		285
	284		270
	284		278
	344		obscured
	340		

^aConditions: pH 7.4, 25°C, phosphate-buffered saline buffer with 10% DMF

Table S2. Effect of 10% DMF vs. Fully Aqueous Buffer on Selected Reaction Rates.^a

reactants	buffer	$k_{1(\text{obs})}$ (min^{-1})	$k_{2(\text{app})}$ ($\text{M}^{-1}\text{sec}^{-1}$)	k_{rel}
	10% DMF	0.037 (0.005)	1.2 (0.2)	1
	aqueous	0.073 (0.004)	2.4 (0.12)	2.0
	10% DMF	0.079 (0.002)	2.6 (0.05)	1
	aqueous	0.046 (0.005)	1.5 (0.18)	0.6
	10% DMF ^b	0.027 (0.002)	0.88 (0.05)	1
	aqueous ^b	0.023 (0.005)	0.77 (0.18)	0.9

^aConditions: pH 7.4, 25°C, phosphate-buffered saline buffer with or without 10% DMF.
[Carbonyl compound] = 500 μM , [hydrazine] = 10 μM .

^bData from ref. 3 below.

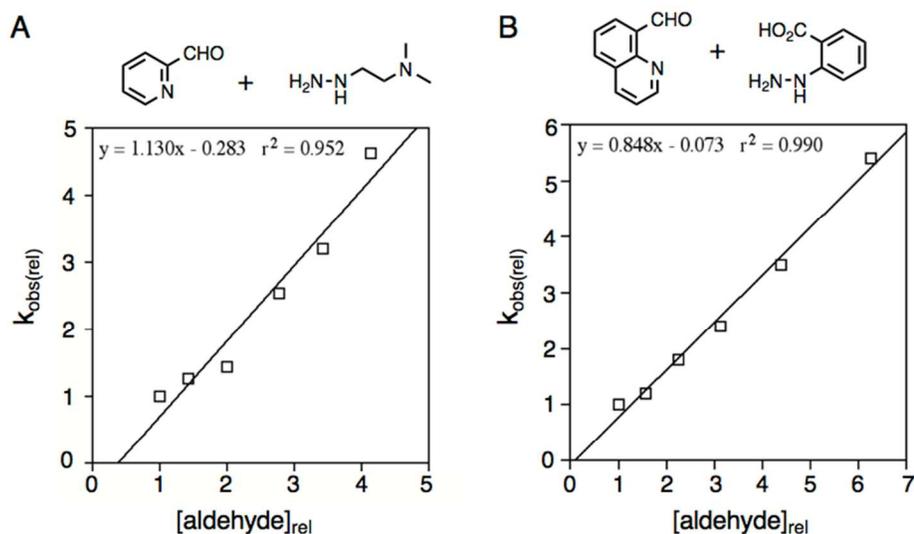


Fig. S4. Testing second-order behavior, as shown by measurements of kinetic order in aldehyde for two representative reactions involving fast alpha nucleophiles. Shown are plots of relative observed pseudo-first-order reaction rates as a function of relative aldehyde concentration. Slopes denote kinetic order in aldehyde, which is ca. one in both cases, consistent with second-order behavior overall. A. [aldehyde] range 150-750 μM ; [hydrazine] = 7.5 μM . B. [aldehyde] range 80-500 μM ; [hydrazine] = 5 μM (data from ref. 3). Buffer is as per Table 1 legend.

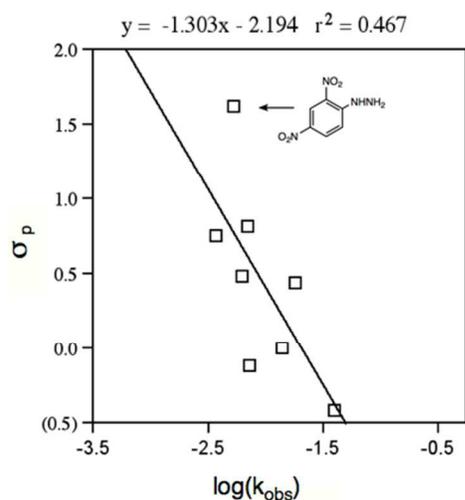
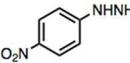
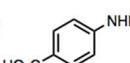
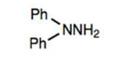
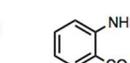
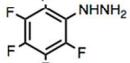
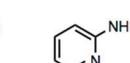
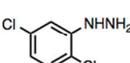
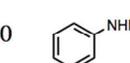
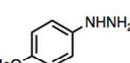
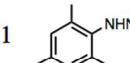
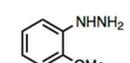


Fig. S5. Hammett plot of observed rate constant vs. σ_p values for arylhydrazines on rates of reaction with 2-formylpyridine, showing generally negative effect of electron-withdrawing substituents and positive effect of electron-donating ones. Dinitrophenylhydrazine is indicated as it falls further off the line than the other cases; twice the sigma value of (nitro) was used in that case.

Table S3. Effects of hydrazine structure on reactivity with 2-butanone

Substrate	$k_{1(\text{obs})}$ (min^{-1})	$k_{2(\text{app})}$ ($\text{M}^{-1}\text{sec}^{-1}$)	k_{rel}	Substrate	$k_{1(\text{obs})}$ (min^{-1})	$k_{2(\text{app})}$ ($\text{M}^{-1}\text{sec}^{-1}$)	k_{rel}
1 	0.003 (0.001)	0.10 (0.003)	0.8	7 	0.026 (0.002)	0.87 (0.07)	7.0
2 	0.007 (0.001)	0.23 (0.03)	1.9	8 	0.026 (0.001)	0.87 (0.03)	7.0
3 	0.007 (0.002)	0.23 (0.07)	1.9	9 	0.026 (0.005)	0.87 (0.17)	7.0
4 	0.011 (0.002)	0.37 (0.07)	3.0	10 	0.044 (0.006)	1.5 (0.2)	12
5 	0.022 (0.004)	0.73 (0.13)	5.9	11 	0.037 (0.010)	1.2 (0.3)	10
6 	0.031 (0.008)	1.0 (0.3)	8.4				

^aConditions: [2-butanone] = 500 μM , [hydrazine] = 10 μM , 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, 10% DMF, 25 $^{\circ}\text{C}$. Values measured 3 times and averaged (std. dev. in parentheses). Pseudo-first-order $k_{(\text{obs})}$ normalized to standard 500 μM [ketone].

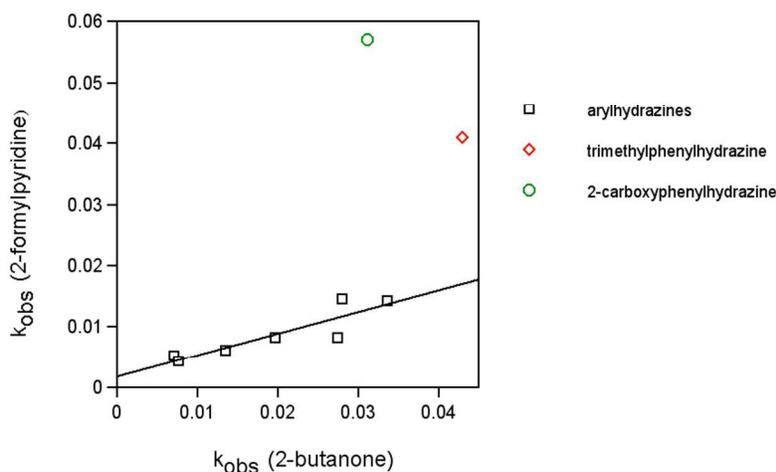
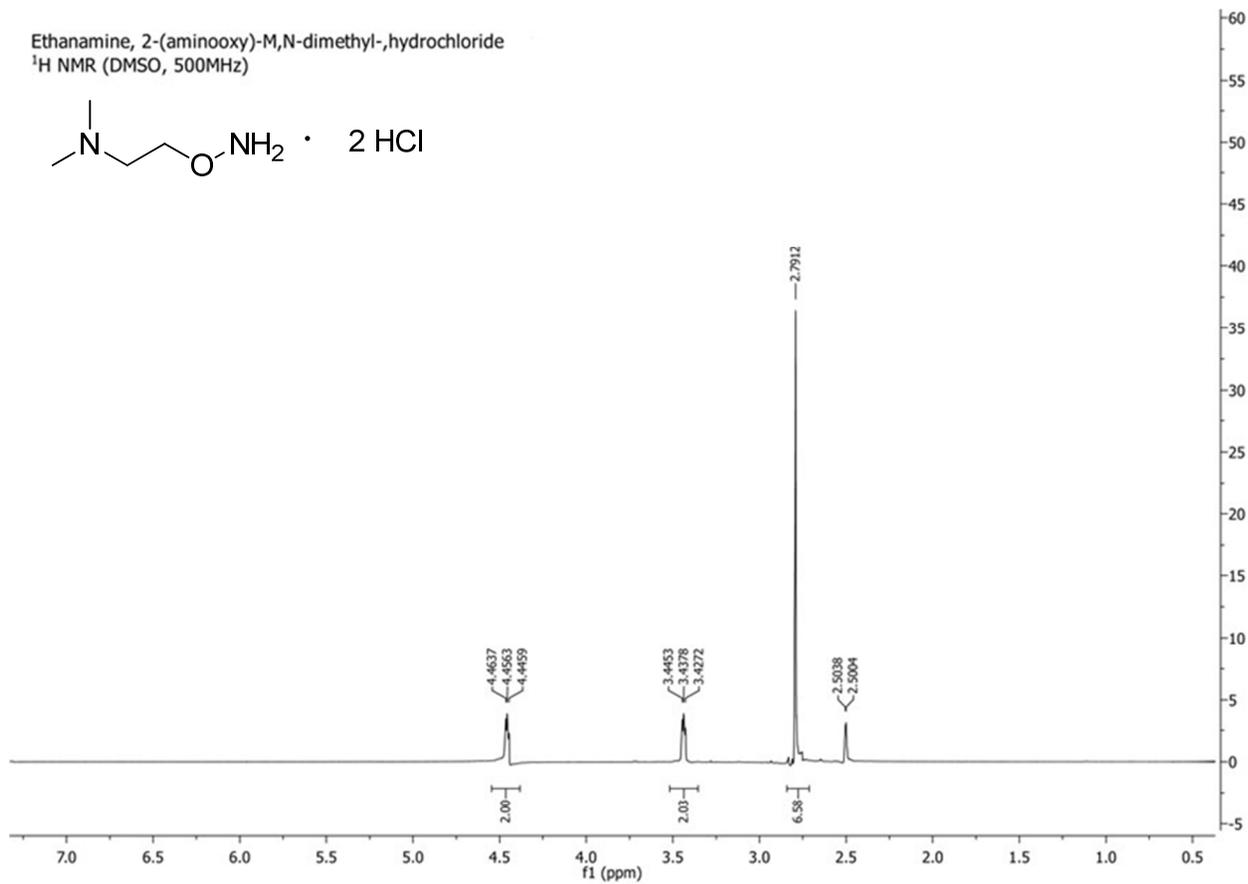
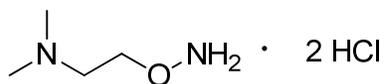


Fig. S6. Plot showing correlation of arylhydrazine reactivity with 2-formylpyridine versus 2-butanone, showing generally consistent effect of electron-withdrawing vs. electron-donating substituents with both an aldehyde and a ketone substrate. Outliers (trimethylphenylhydrazine and 2-carboxyphenylhydrazine) were added separately to the plot, to illustrate their anomalously high reactivity relative to other arylhydrazines.

References

1. Guggenheim, E. A. *Philos. Mag.* **1926**, 2, 538.
2. Hemalatha, M. R. K.; NoorBatcha, I. *J. Chem. Ed.* **1997**, 74, 972.
3. Kool, E. T; Park, D.; Crisalli, P. *J. Am. Chem. Soc.* **2013**, 135, 17663.
4. Pohjakallio, A.; Pihko, P. M. *Chemistry* **2009**, 15, 3960.
5. Villani, F. J.; Tavares, R. F.; Ellis, C. A. *J. Pharm. Sci.* **1969**, 58, 138.

Ethanamine, 2-(aminoxy)-N,N-dimethyl-,hydrochloride
¹H NMR (DMSO, 500MHz)



Ethanamine, 2-(aminoxy)-N,N-dimethyl-,hydrochloride (1:2)
¹³C NMR (DMSO, 500MHz)

