SUPPORTING INFORMATION

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

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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 °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 \(\tau\) 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

\[
t_{(1/2)} = \frac{0.693}{k_{ps}}
\]

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.**

\[ \text{Acetone oxime.}^4 \quad \text{Acetone (3.6 mL, 50 mmol), hydroxylamine hydrochloride (5.2 g, 75 mmol) and sodium carbonate (8 g) were stirred together in 10 mL H}_2\text{O for 16 h. The reaction solution was extracted multiple times with diethyl ether, the organic extract dried with anhydrous magnesium sulfate and evaporated to give a white solid as the product (75% yield).} \]

$^1\text{H NMR (CDCl}_3, 400 \text{ MHz}) \quad \delta (\text{ppm}) = 1.890 (s, 3\text{H}), 1.878 (s, 3\text{H}). \quad ^1\text{C NMR (CDCl}_3, 500 \text{ MHz}) \quad \delta (\text{ppm}) = 155.79, 21.921, 15.134. \]

\[ \text{Acetone O-(2-(dimethylamino)ethyl) oxime.}^5 \quad \text{Acetone oxime (2 g, 27 mmol) was stirred overnight with 54 mL of sodium methoxide solution (5 M in MeOH, 54 mmol) at 50 °C. The colorless reaction solution turned orange in an hour after addition of sodium methoxide. 2-chloro-N,N-dimethylethan-1-amine hydrochloride (3.88 g, 27 mmol) was added to the sealed tube and the reaction mixture stirred at 100 °C for 1 h. The crude product mixture was distilled under vacuum to retrieve 1.8 g of the crude product, an almost colorless yellow-tinted liquid.} \]

$^1\text{H NMR (D}_2\text{O, 400MHz}) \quad \delta (\text{ppm}) = 4.032 (t, 2\text{H}), 2.503 (t, 2\text{H}), 2.187 (s, 6\text{H}), 1.764 (s, 3\text{H}), 1.753 (s, 3\text{H}). \]
2-(Aminooxy)-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 (elucent 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 ($\Delta t$ 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 | $\lambda_{\text{max}}$ (nm) | Hydrazone | $\lambda_{\text{max}}$ (nm) |
|-----------|-------------------|-----------|-------------------|
| ![Structure 1](image1) | 355 | ![Structure 2](image2) | 290 |
| ![Structure 3](image3) | 340 | ![Structure 4](image4) | 338 |
| ![Structure 5](image5) | 327 | ![Structure 6](image6) | 268 |
| ![Structure 7](image7) | 292 | ![Structure 8](image8) | 385 |
| ![Structure 9](image9) | 366 | ![Structure 10](image10) | 288 |
| ![Structure 11](image11) | 356 | ![Structure 12](image12) | 272 |
| ![Structure 13](image13) | 272 | ![Structure 14](image14) | 282 |
| ![Structure 15](image15) | 401 | ![Structure 16](image16) | 272 |
| ![Structure 17](image17) | 286 | ![Structure 18](image18) | 253 |
| ![Structure 19](image19) | 338 | ![Structure 20](image20) | 260 |
| ![Structure 21](image21) | 324, 366sh | ![Structure 22](image22) | 286 |
| ![Structure 23](image23) | 262 | ![Structure 24](image24) | 257 |
| ![Structure 25](image25) | 300 | ![Structure 26](image26) | 274 |
| ![Structure 27](image27) | 268 | ![Structure 28](image28) | 387 |
| ![Structure 29](image29) | 350 | ![Structure 30](image30) | 273 |

*aConditions: pH 7.4, 25°C, phosphate-buffered saline buffer with 10% DMF*
| Hydrazone | $\lambda_{\text{max}}$ (nm) | Hydrazone | $\lambda_{\text{max}}$ (nm) |
|-----------|-----------------|-----------|-----------------|
| ![Hydrazone 1](image1.png) | 270 | ![Hydrazone 2](image2.png) | 284 |
| ![Hydrazone 3](image3.png) | 274 | ![Hydrazone 4](image4.png) | 384 |
| ![Hydrazone 5](image5.png) | 272 | ![Hydrazone 6](image6.png) | 283 |
| ![Hydrazone 7](image7.png) | 274 | ![Hydrazone 8](image8.png) | 285 |
| ![Hydrazone 9](image9.png) | 274 | ![Hydrazone 10](image10.png) | 285 |
| ![Hydrazone 11](image11.png) | 274 | ![Hydrazone 12](image12.png) | 285 |
| ![Hydrazone 13](image13.png) | 274 | ![Hydrazone 14](image14.png) | 270 |
| ![Hydrazone 15](image15.png) | 344 | ![Hydrazone 16](image16.png) | 278 |
| ![Hydrazone 17](image17.png) | 340 | ![Hydrazone 18](image18.png) | obscured |

*aConditions: pH 7.4, 25°C, phosphate-buffered saline buffer with 10% DMF*
| reactants                      | buffer   | $k_1$(obs) (min$^{-1}$) | $k_2$(app) (M$^{-1}$sec$^{-1}$) | $k_{rel}$ |
|-------------------------------|----------|-------------------------|---------------------------------|-----------|
| ![Chemical Structure 1](image1) | 10% DMF  | 0.037 (0.005)           | 1.2 (0.2)                       | 1         |
|                               | aqueous  | 0.073 (0.004)           | 2.4 (0.12)                      | 2.0       |
| ![Chemical Structure 2](image2) | 10% DMF  | 0.079 (0.002)           | 2.6 (0.05)                      | 1         |
|                               | aqueous  | 0.046 (0.005)           | 1.5 (0.18)                      | 0.6       |
| ![Chemical Structure 3](image3) | 10% DMF$^b$ | 0.027 (0.002)       | 0.88 (0.05)                      | 1         |
|                               | aqueous$^b$ | 0.023 (0.005)       | 0.77 (0.18)                      | 0.9       |

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

$^b$Data 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. sigma(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) | $k_{1\text{(app)}}$ (M⁻¹sec⁻¹) | $k_{\text{rel}}$ | Substrate | $k_{1\text{(obs)}}$ (min⁻¹) | $k_{1\text{(app)}}$ (M⁻¹sec⁻¹) | $k_{\text{rel}}$ |
|-----------|---------------------------|-------------------------------|-----------------|-----------|---------------------------|-------------------------------|-----------------|
| 1         | 0.003 (0.001)              | 0.10 (0.003)                  | 0.8             | 7         | 0.026 (0.002)              | 0.87 (0.007)                  | 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             |           |                           |                               |                 |

*Conditions: [2-butanone] = 500 μM, [hydrazine] = 10 μM, 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, 10% DMF, 25 °C. Values measured 3 times and averaged (std. dev. in parentheses). Pseudo-first-order $k_{1\text{obs}}$ 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

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Ethanamine, 2-(aminoxy)-M,N-dimethyl-, hydrochloride

$^1$H NMR (DMSO, 500MHz)

$\text{NH}_2 \cdot 2\text{HCl}$

Ethanamine, 2-(aminoxy)-M,N-dimethyl-, hydrochloride (1:2)

$^{13}$C NMR (DMSO, 500MHz)

$\text{NH}_2 \cdot 2\text{HCl}$