

# Supporting information

## New Organocatalyst Scaffolds with High Activity in Promoting Hydrazone and Oxime Formation at Neutral pH

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## Experimental

*Chemicals:* All chemicals and spectroscopic grade solvents were purchased from commercial suppliers and used without further purification. Phosphate buffered saline (PBS) contained 137 mM NaCl, 2.7 mM KCl and 11.9 mM sodium and potassium phosphates (pH 7.4) and was freshly prepared daily from a commercial 10X stock solution. Stock solutions of reagents and catalysts were freshly made daily, with exception of phenylhydrazine stock solutions, which were discarded after 2-3 hours and made anew.

*Instrumentation:* All UV/Vis experiments were performed on a Cary 100 Bio UV-Visible Spectrophotometer. LC-HRMS was performed at Department of Chemistry, University of Copenhagen on a Dionex Ultimate 3000 UPLC connected to a Bruker MicroTOF QII mass spectrometer.

## Kinetic Experiments

*Preparing reactions:* Samples were mixed in 600  $\mu\text{L}$  1 cm path length cuvettes. Samples were prepared by adding 3.0  $\mu\text{L}$  of a 200 mM stock solution of the aldehyde in DMF and 3.0  $\mu\text{L}$  of a 200 mM stock solution of the catalyst in DMF into 591  $\mu\text{L}$  of a 10:1 PBS/DMF.\* Mixing was performed by rapidly pipetting up and down 70  $\mu\text{L}$  at least 25 times and immediately a background spectrum was recorded. The experiment was started by adding 3.0  $\mu\text{L}$  of a 4.00 mM phenylhydrazine stock solution in 10:1 PBS/DMF and repeating the mixing procedure just described. This results in a 9:1 PBS/DMF mixture with 1.0 mM aldehyde, 1.0 mM catalyst and 20  $\mu\text{M}$  phenylhydrazine. Data collection was started after a fixed amount of time (usually 2 minutes) to allow time for the operator to perform the pipetting and mixing procedures.

*Performing measurements:* Experiments were done by scanning a specified region of the UV/vis spectrum (e.g. from 300 nm to 380 nm) at specific time intervals (e.g. one measurement every 2.5 minutes). Representative examples of these time-dependent UV/Vis spectral changes are found in Figure S1 and Figure S2. After measurement, absorption at the most desirable wavelength (e.g. the one with the highest change in absorption) for every measurement was extracted and plotted against time. Representative examples of these time-dependent single-wavelength absorbance changes are found (black squares) in Figure S3 and Figure S4.

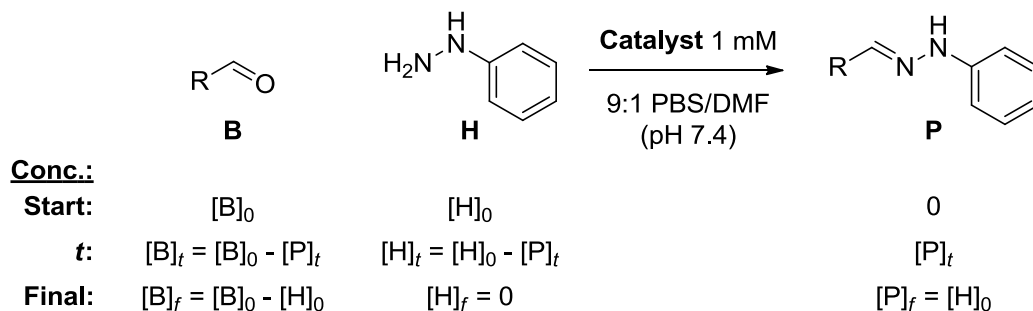
*Equations used for fitting:* The equation derived in the following allow for extraction of time-dependent data at any wavelength (assuming a meaningful change can be detected at the given wavelength), as the concentration

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\* Some substrates/catalysts were not soluble at 200 mM in DMF. This was generally solved by diluting these stock solutions (e.g. to 20 mM) with 10:1 PBS/DMF and then adjusting volumes accordingly when preparing samples.

of product during the reaction is not calculated based on the extinction coefficient of neither product nor starting materials.

- For  $[B]_0 \gg [H]_0$ , the following concentration relationships can be deduced:



- According to Lambert-Beer's law:

$$[P]_t = \frac{A}{A_{max}} \cdot [P]_f = \frac{A}{A_{max}} \cdot [H]_0$$

where  $A$  is the measured absorbance at time  $t$  and  $A_{max}$  is the absorbance when the reaction has proceeded to use all the hydrazine.<sup>†</sup>

- It follows that:

$$[H]_t = [H]_0 - \frac{A}{A_{max}} \cdot [H]_0 = [H]_0 \cdot \left(1 - \frac{A}{A_{max}}\right)$$

and:

$$[B]_t = [B]_0 - \frac{A}{A_{max}} \cdot [H]_0 = [H]_0 \cdot \left(\frac{[B]_0}{[H]_0} - \frac{A}{A_{max}}\right)$$

- Combining these two will give the following relation:

$$\frac{[H]_t}{[B]_t} = \frac{1 - \frac{A}{A_{max}}}{\frac{[B]_0}{[H]_0} - \frac{A}{A_{max}}} = \frac{A_{max} - A}{A_{max} \cdot \frac{[B]_0}{[H]_0} - A} = \frac{A_{max} - A}{c \cdot A_{max} - A}$$

with  $c = \frac{[B]_0}{[H]_0}$ .

<sup>†</sup> It is assumed that the hydrazine is converted fully to the hydrazone, so that at the endpoint, where the maximum absorbance,  $A_{max}$ , is reached, a hydrazone concentration equivalent to the starting concentration of the hydrazine has been reached. Based on the high excess (50 eq.) of aldehyde, this assumption is deemed to be reasonable.

- For  $[H]_0 \neq [B]_0$ , the integrated second order rate law (assuming 1<sup>st</sup> order in both H and B) can be written as:

$$\frac{[H]_t}{[B]_t} = \frac{[H]_0}{[B]_0} \cdot e^{([H]_0 - [B]_0)kt} = c^{-1} \cdot e^{([H]_0 - [B]_0)kt}$$

with  $k$  being the second order rate constant and still using the above-mentioned definition of  $c = \frac{[B]_0}{[H]_0}$ .

- When using the relation derived above in the integrated rate law:

$$\frac{A_{max} - A}{c \cdot A_{max} - A} = c^{-1} \cdot e^{([H]_0 - [B]_0)kt}$$

- This is rewritten to give (parenthesis added for clarity):

$$A_{max} - A = A_{max} \cdot e^{([H]_0 - [B]_0)kt} - A \cdot (c^{-1} \cdot e^{([H]_0 - [B]_0)kt}) = A_{max} \cdot e^{([H]_0 - [B]_0)kt} - p \cdot A$$

if one defines  $p = (c^{-1} \cdot e^{([H]_0 - [B]_0)kt})$ .

- Moving all terms incorporating  $A$  to one side of the equation, you arrive at:

$$p \cdot A - A = A_{max} \cdot e^{([H]_0 - [B]_0)kt} - A_{max}$$

which can be rewritten to:

$$A(p - 1) = A_{max} \cdot e^{([H]_0 - [B]_0)kt} - A_{max}$$

and finally:

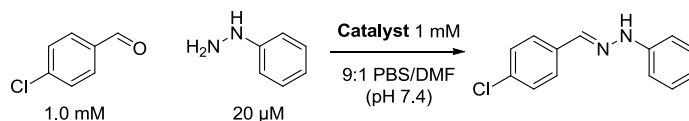
$$A = \frac{A_{max} \cdot e^{([H]_0 - [B]_0)kt} - A_{max}}{p - 1}$$

- Substituting  $p$  and  $c$  gives the final equation:

$$A = \frac{A_{max} \cdot e^{([H]_0 - [B]_0)kt} - A_{max}}{\frac{[H]_0}{[B]_0} \cdot e^{([H]_0 - [B]_0)kt} - 1}$$

Fitting this equation using non-linear regression (Origin 8.6) to the measured  $A$  vs.  $t$  data will give  $k$  and  $A_{max}$ . Representative examples of best fits are illustrated by the red lines in Figure S3 and Figure S4.

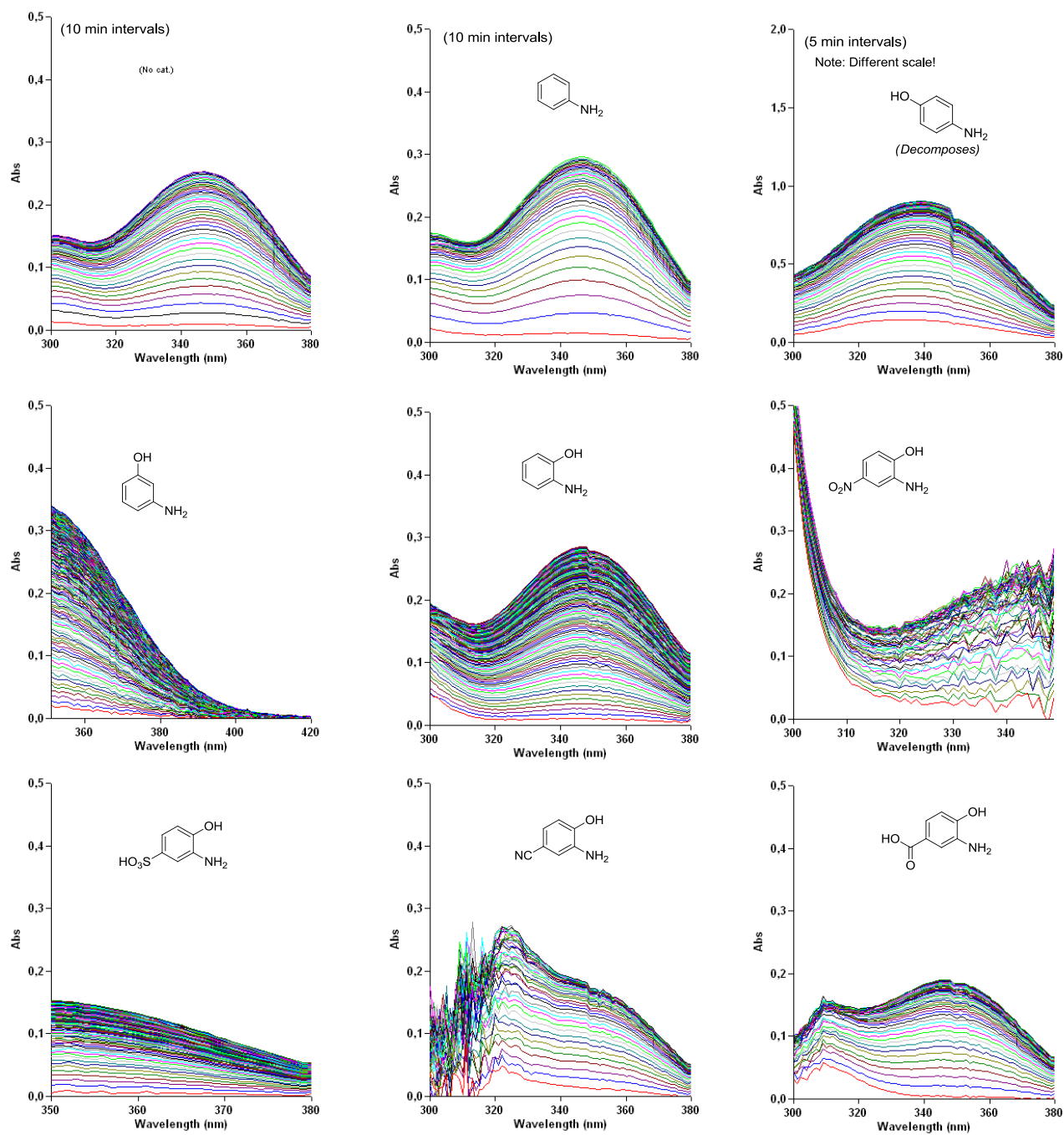
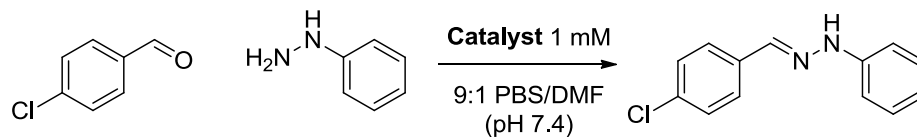
**Table S1: Full Table of Aminophenol (and Similar) Catalysts Investigated**

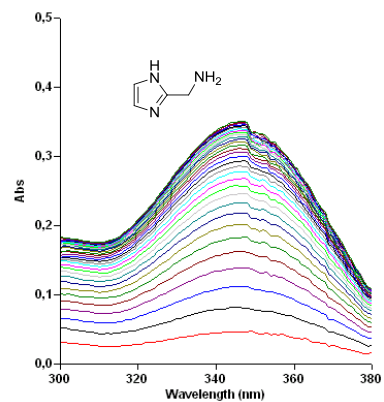
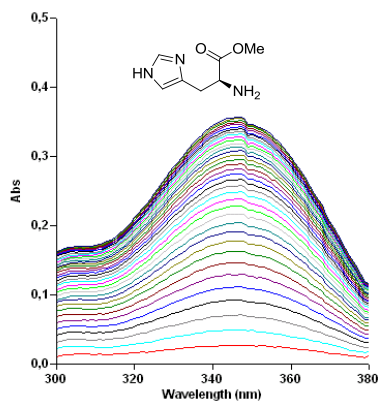
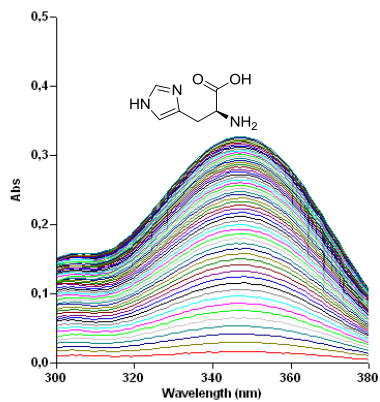
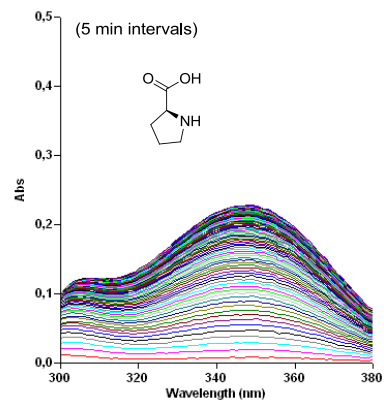
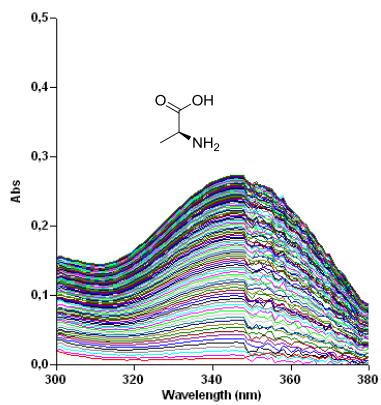
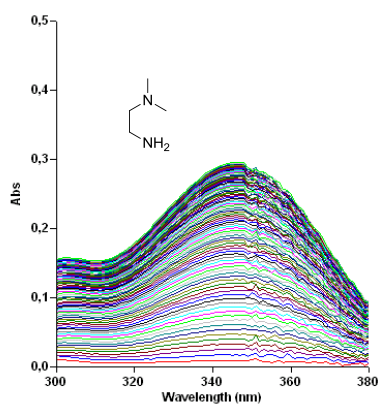
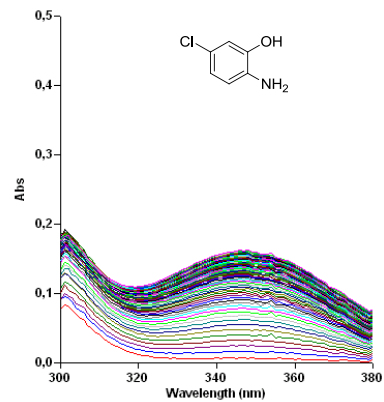
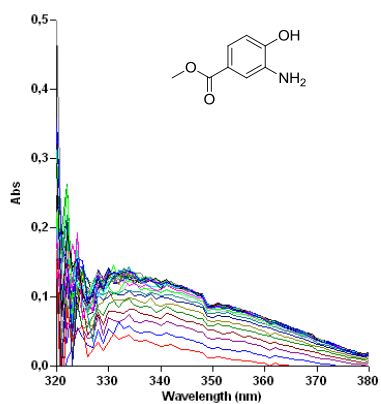
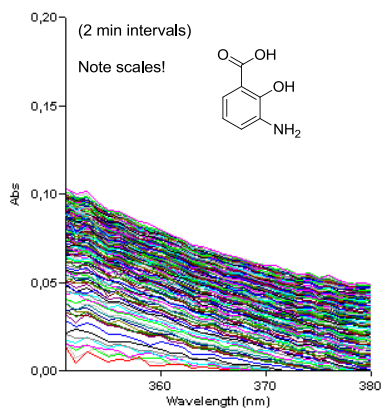


#	Catalyst	$k_2$ ( $M^{-1}s^{-1}$ )	$k_{rel}$ vs. no cat. (Table 1)	#	Catalyst	$k_2$ ( $M^{-1}s^{-1}$ )	$k_{rel}$ vs. no cat. (Table 1)	#	Catalyst	$k_2$ ( $M^{-1}s^{-1}$ )	$k_{rel}$ vs. no cat. (Table 1)	#	Catalyst	$k_2$ ( $M^{-1}s^{-1}$ )	$k_{rel}$ vs. no cat. (Table 1)
3		Decomposition		6		1.2 $\pm 0.4$	11	10		0.67 $\pm 0.04$	6.1	12		0.35 $\pm 0.02$	3.2
4		0.193 $\pm 0.004$	1.8	7		0.425 $\pm 0.006$	3.9	11		1.4 $\pm 0.4$	13	S10		Decomposition	
5		0.226 $\pm 0.019$	2.0	S04		0.172 $\pm 0.005$	1.6	S06		Decomposition		S11		Decomposition	
S01		Decomposition		S05		0.387 $\pm 0.015$	3.5	S07		Decomposition		S12		Decomposition	
S02		Decomposition		8		0.578 $\pm 0.010$	5.2	S08		Decomposition		S13		Decomposition	
S03		0.48 $\pm 0.07$	4.4	9		0.52 $\pm 0.05$	4.7	S09		Decomposition		S14		Decomposition	

**Table S1: Second-order rate constants ( $k_2$ ) for catalysis of hydrazone formation of phenylhydrazine and *p*-chlorobenzaldehyde by aminophenol (and similar) catalysts investigated in this study. Conditions and concentrations as per Scheme 1 and Table 1 in the manuscript. The relative rate constant ( $k_{rel}$ ) is relative to the uncatalyzed reaction (entry 1, Table 1 in the manuscript). Numbering scheme as in manuscript, except for additional results, which are denoted Sxx.**

**Figure S1: Representative Time-Dependent UV/Vis Spectral Changes from Catalyst Discovery**





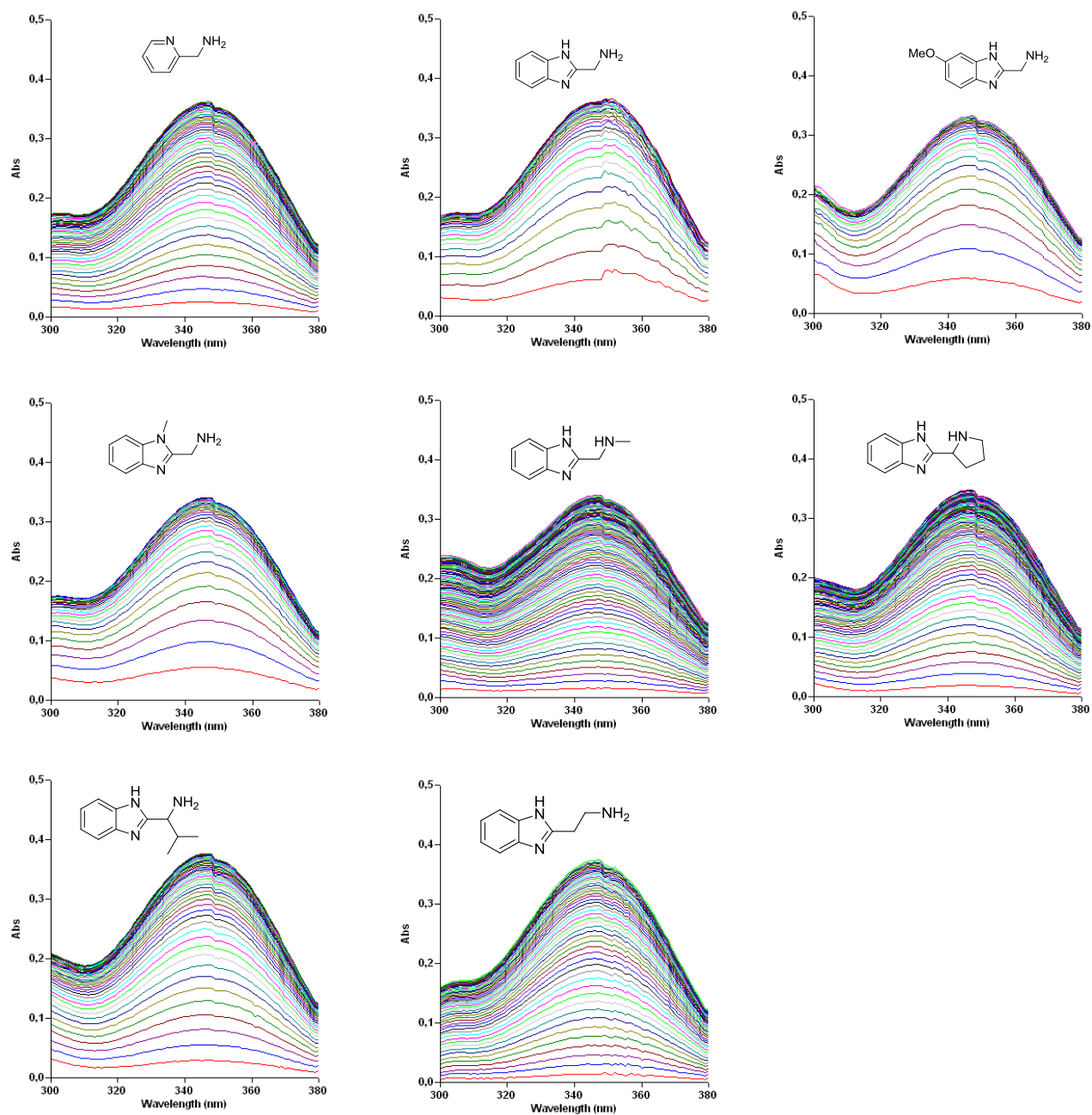
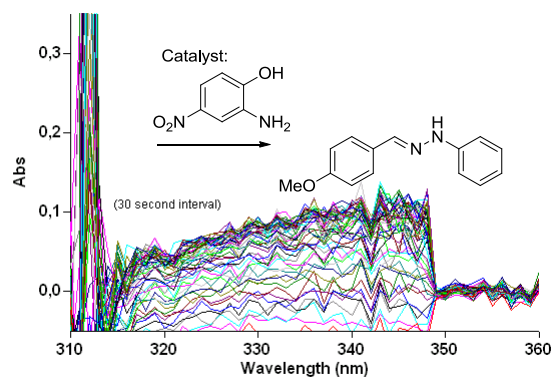
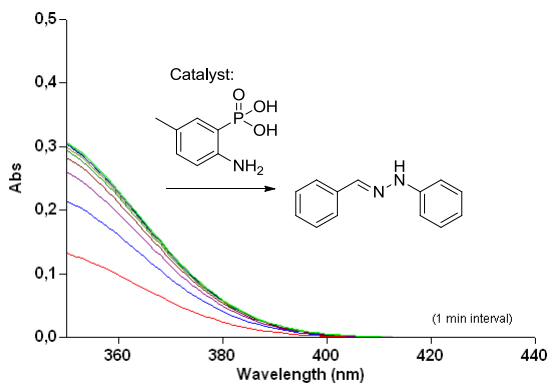
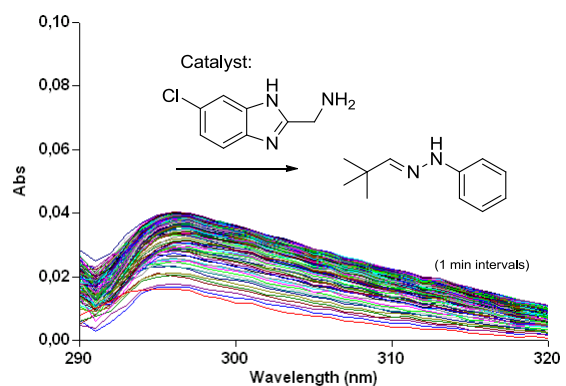
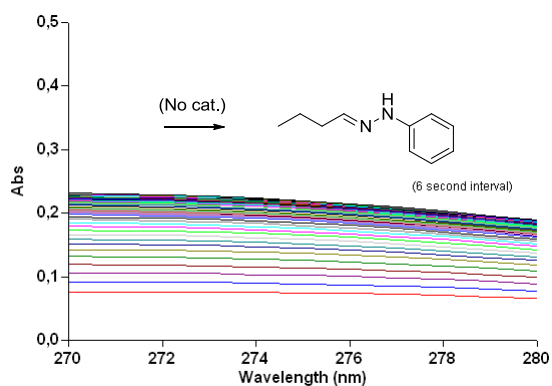
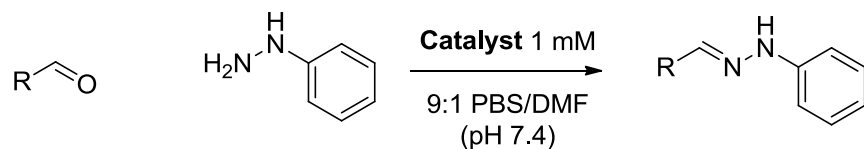
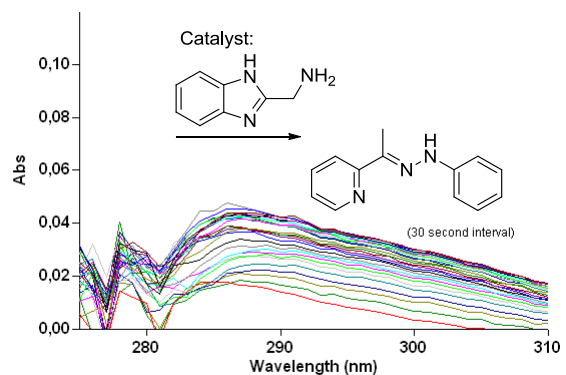
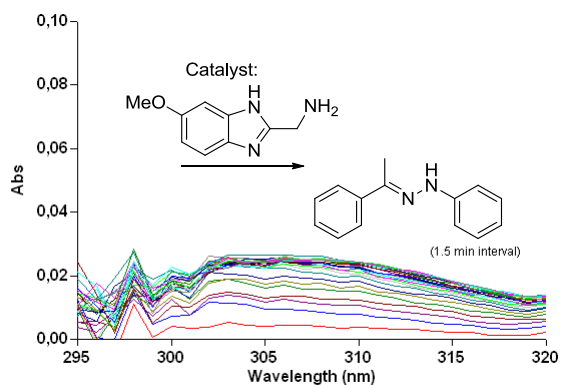
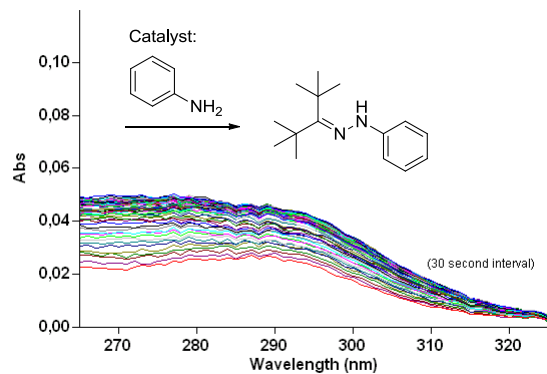
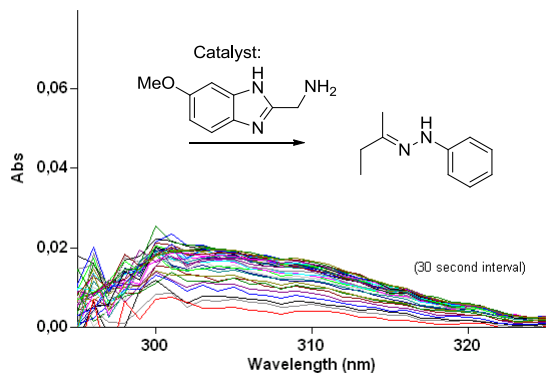
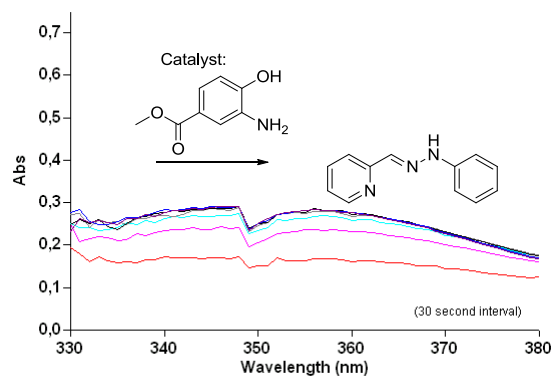
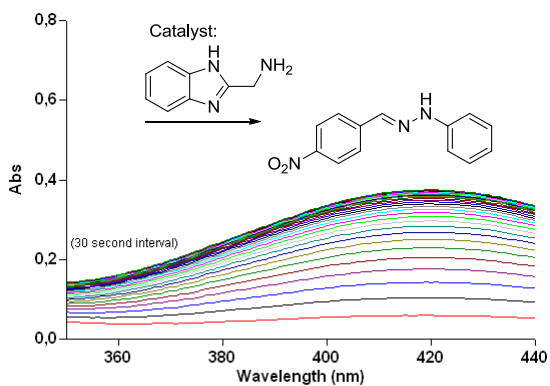


Figure S1 (on this and the two preceding pages): UV/vis spectra were recorded at 2.5 min intervals during the reaction unless stated otherwise.



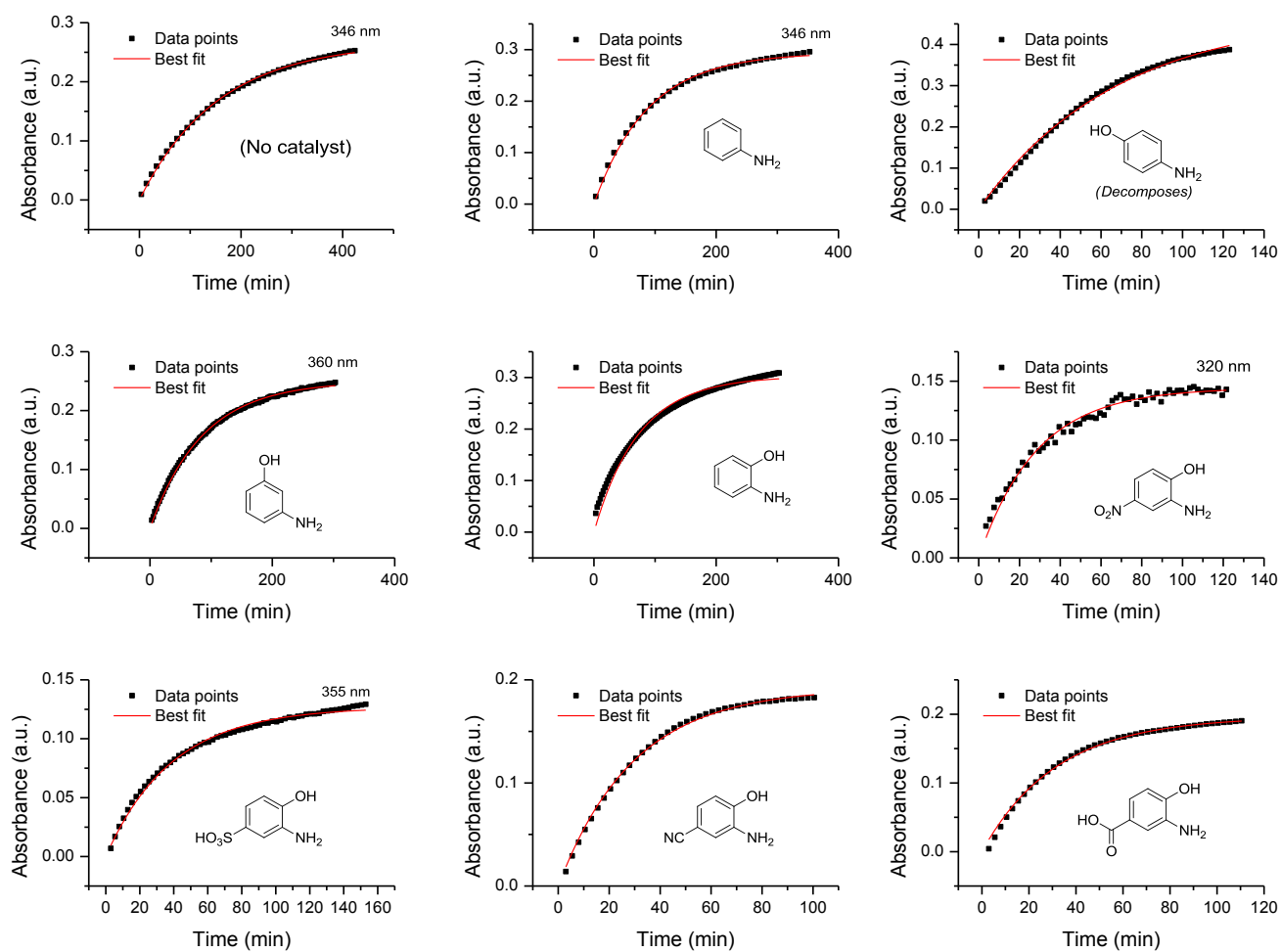
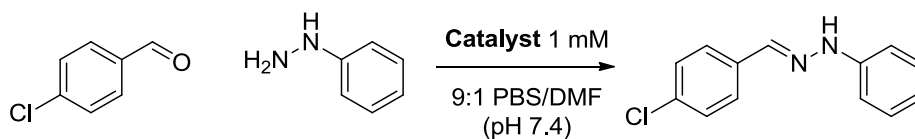
**Figure S2: Representative Time-Dependent UV/Vis Spectral Changes from Scope Investigation**

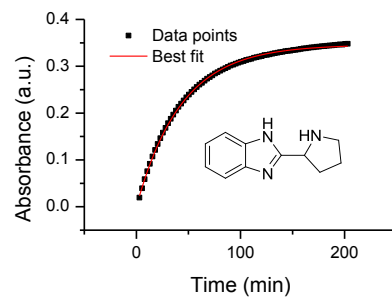
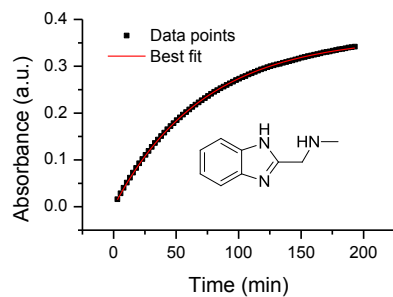
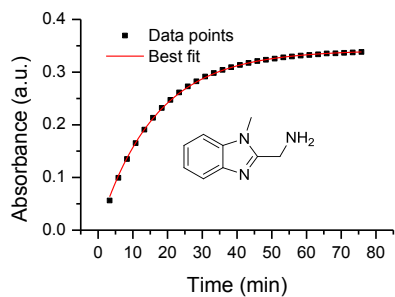
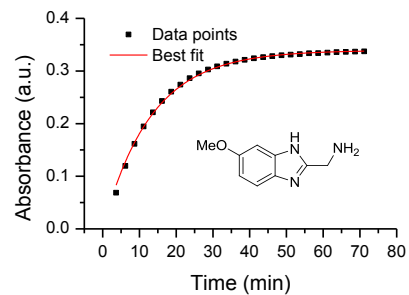
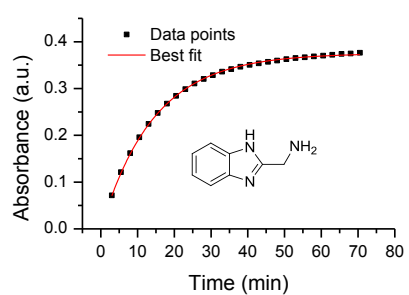
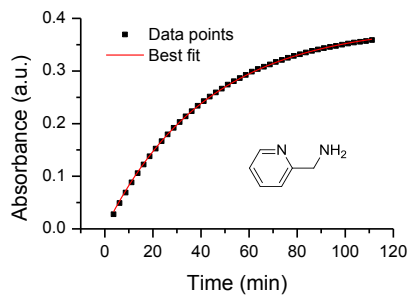
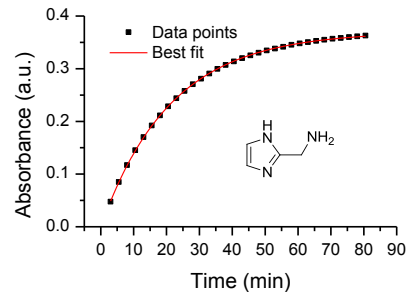
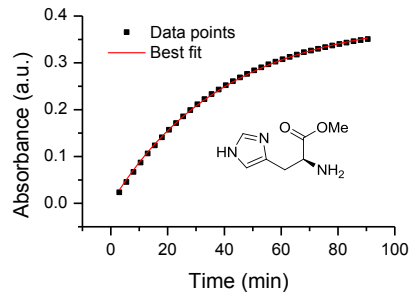
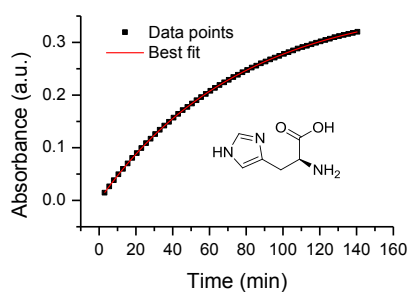
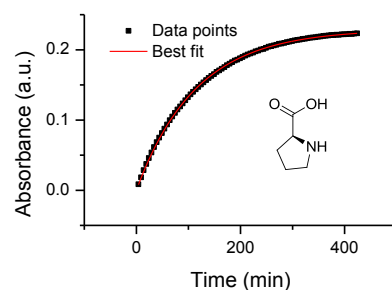
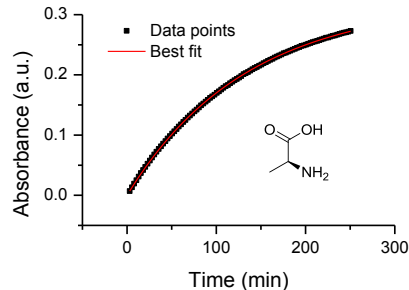
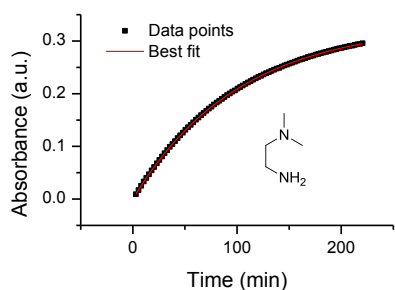
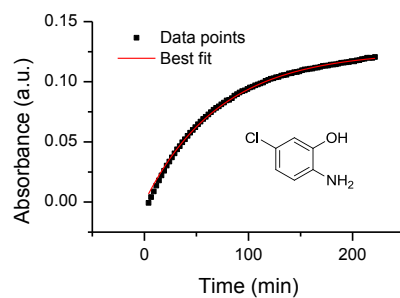
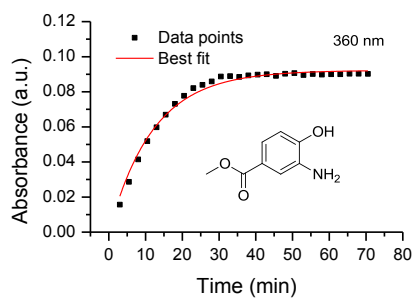
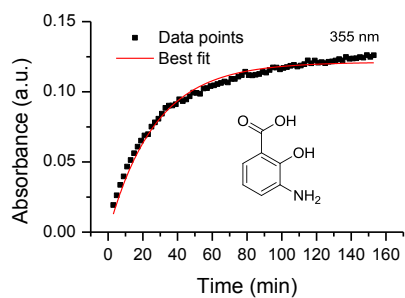


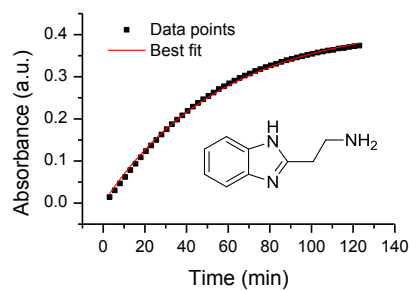
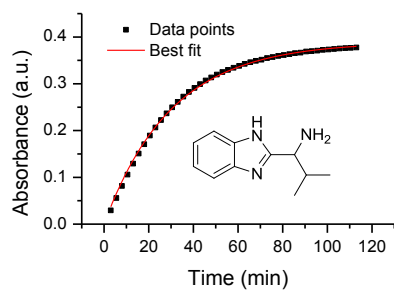


**Figure S2 (on this and the preceding page): A representative range of UV/vis spectra measured with different aldehydes reacting with phenylhydrazine in presence of different catalysts. One example of each substrate is shown, and at least one reaction with each of the catalysts investigated is presented.**

**Figure S3: Representative Time-Dependent Single-Wavelength Absorption Changes and Non-Linear Regression Fits from Catalyst Discovery**

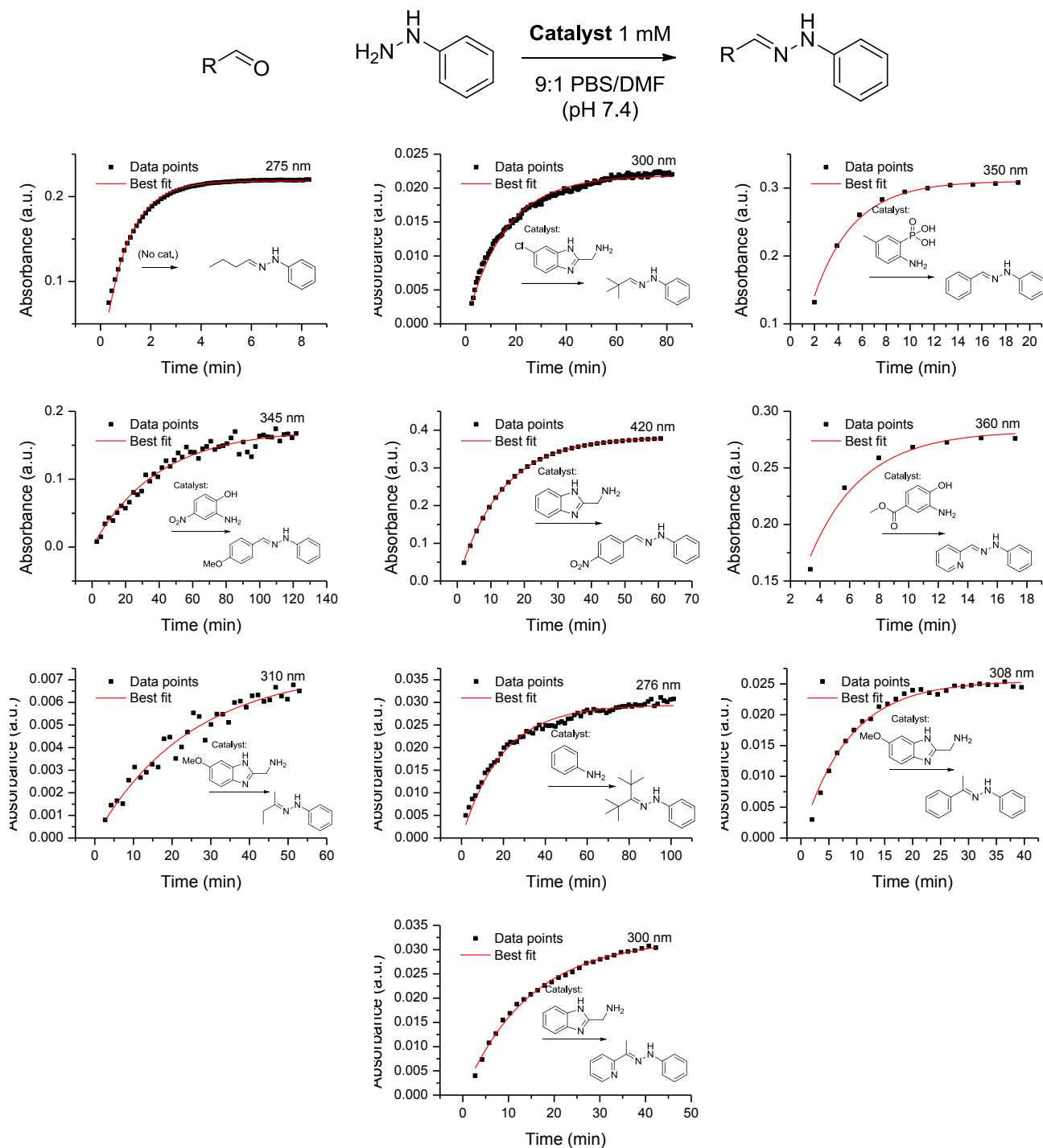






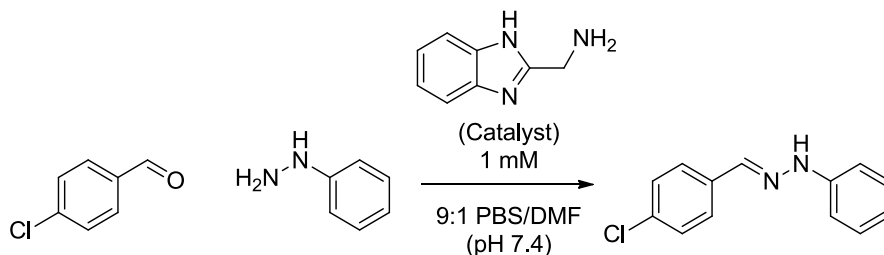
**Figure S3 (on this and the two preceding pages):** Time-dependent single-wavelength absorbance changes during the reaction between phenylhydrazine and *p*-chlorobenzaldehyde. Fits from non-linear regression (Origin 8.6) to the equation derived above are represented in red lines. Unless otherwise noted, the absorbance changes were measured at 347 nm.

**Figure S4: Representative Time-Dependent Single-Wavelength Absorbance Changes and Non-Linear Regression Fits from Scope Investigation**



**Figure S4: Time-dependent single-wavelength absorbance changes during the investigation of scope of catalysis. One example of each substrate is shown, and at least one reaction with each of the catalysts investigated is presented.**

## Kinetic Order of Reactants



Two experiments were carried out, both using phenylhydrazine reacting with *p*-chlorobenzaldehyde under catalysis of 2-aminomethylbenzimidazole (1.0 mM).

In the first experiment, the concentration of phenylhydrazine was altered (10, 20, and 40  $\mu$ M was used, aldehyde was at 1.0 mM), and the relative rate of product formation was plotted as a function of relative concentration of the hydrazine (see Figure S5 left). A slope of 0.9 indicates that the reaction is first order in hydrazine.

In the second experiment, the concentration of *p*-chlorobenzaldehyde was altered (0.50, 1.0 and 2.0 mM used, with phenylhydrazine at 20  $\mu$ M), and the relative rate of product formation was plotted as a function of relative concentration of the aldehyde (see Figure S5 right). A slope of 0.8 indicates that the reaction is first order in aldehyde.

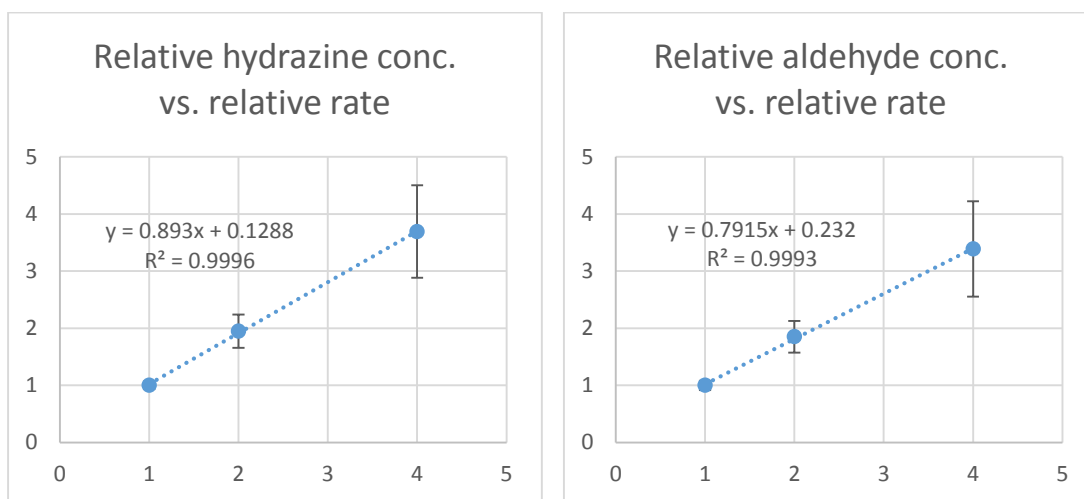


Figure S5: Order of reactants. Plotting the relative concentration of each reactant against the relative rate of product formation gives a straight line with a slope near unity, supporting that the reaction of phenylhydrazine with *p*-chlorobenzaldehyde is first order in both reactants. Error bars based on standard deviation (from triplicate measurements) and assuming a 5 % error on concentration.

## Evidence for Linear Response to Catalyst Concentration

Due to spectral overlap from the catalysts themselves, it was necessary to perform some reactions at lower catalyst concentration (usually 0.50 mM) to avoid detector saturation. The following data support that there is a linear relationship between catalyst concentration and observed second order rate constant ( $k_2$ ), and that this linear relationship has its onset at the rate of the uncatalyzed reaction (Figure S6):

$$k_2 = k_{\text{uncat}} + k_{\text{cat}}[C]_0$$

with  $[C]_0$  being the starting concentration of the catalyst and  $k_{\text{uncat}}$  and  $k_{\text{cat}}$  being the rate constants for the uncatalyzed (background) reaction and the catalyzed reaction respectively (See the following section for further details).

Thus, one can normalize the second order rate constant to 1.0 mM catalyst concentration by extrapolation from the background reaction rate and the measured (at lower catalyst concentration) reaction rate.

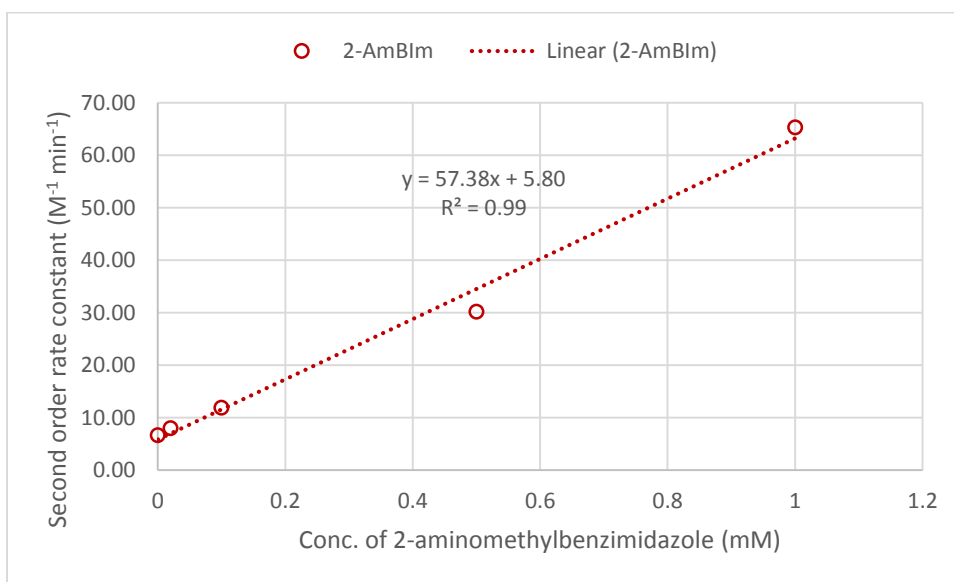


Figure S6: Plotting the rate constant vs concentration of catalyst (in this case 2-(aminomethyl)benzimidazole (21)) results in a straight line that intersects, within error, at the rate constant for the uncatalyzed reaction ( $k_{\text{uncat}} = 6.62 \text{ M}^{-1} \text{ min}^{-1}$ ).



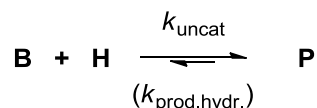
## On the Order of Reactants and Dependence on Catalyst Concentration

Data on the two preceding pages support the following approximate equation for the total reaction rate,  $r_{\text{tot}}$ :

$$\begin{aligned} \text{(S1)} \quad r_{\text{tot}} &= r_{\text{uncat}} + r_{\text{cat}} \\ &= k_{\text{uncat}}[\text{B}][\text{H}] + k_{\text{cat}}[\text{C}]_0[\text{B}][\text{H}] \\ &= (k_{\text{uncat}} + k_{\text{cat}}[\text{C}]_0)[\text{B}][\text{H}] \end{aligned}$$

where  $[\text{B}]$  and  $[\text{H}]$  are the concentrations of aldehyde **B** and hydrazine **H** respectively.  $[\text{C}]_0$  is the starting concentration of catalyst **C**, and the two rate constants,  $k_{\text{uncat}}$  and  $k_{\text{cat}}$ , represent the rate constants for the uncatalyzed (e.g. background) and the catalyzed part of the reaction. This relationship is rationalized by the following discussion.

The uncatalyzed background reaction is expressed as follows:

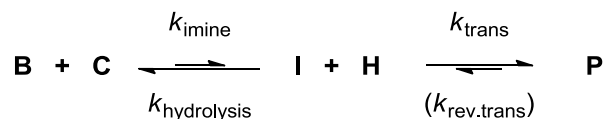


with **H** being the hydrazine, **B** being the aldehyde and **P** being the product (the hydrazone). The rate of hydrolysis of the product,  $k_{\text{prod.hydr.}}$ , is assumed to be of no relevance, since hydrazone hydrolysis is slow at neutral pH.<sup>‡</sup>  $k_{\text{uncat}}$  is measured simply by performing the reaction without catalyst.

The uncatalyzed part of the reaction rate,  $r_{\text{uncat}}$ , is derived from the above reaction scheme:

$$\text{(S2)} \quad r_{\text{uncat}} = k_{\text{uncat}} [\text{B}][\text{H}]$$

The catalyzed reaction goes via the following pathway:



with **I** being the intermediate imine formed between the aldehyde **B** and catalyst **C**.  $k_{\text{hydrolysis}}$  denotes the rate of hydrolysis of the imine **I**, while  $k_{\text{trans}}$  denotes the rate of transimination between the intermediate imine **I** and the hydrazine **H** to form product **P**. The rate of reverse transimination,  $k_{\text{rev.trans}}$ , is considered neglectable.

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<sup>‡</sup> Kalia, J.; Raines, R. T. *Angew. Chem. Int. Ed.* **2008**, *47*, 7523.

The catalyzed part consists of two steps, and thus the correct equation can only be derived after considering the available data. The data shows approx. first order in both **B** and **H**, and thus both  $[B]$  and  $[H]$  must be part of  $r_{\text{cat}}$  as well. This can be explained by applying the quasi-steady-state assumption. In this, the concentration of the intermediate imine **I** is assumed constant, thus  $k_{\text{imine}}[B][C] = k_{\text{hydrolysis}}[I] + k_{\text{trans}}[I][H]$ . Under the assumption that  $K_{m^*} = \frac{k_{\text{hydrolysis}} + k_{\text{trans}}[H]}{k_{\text{imine}}}$  (a modification of the Michaelis constant) is large<sup>§</sup> the concentration of imine **I** can be written as  $[I] = \frac{[C]_0[B]}{K_{m^*} + [B]}$ , \*\* and thus:

$$(S3) \quad r_{\text{cat}} = k_{\text{trans}} [I][H] = k_{\text{trans}} \left( \frac{1}{K_{m^*} + [B]} \right) [C]_0 [B][H]$$

Since  $[B]$  is almost constant during the reaction, one can define  $k_{\text{cat}} = k_{\text{trans}} \left( \frac{1}{K_{m^*} + [B]} \right)$ , and one arrives at:

$$(S4) \quad r_{\text{cat}} = k_{\text{cat}} [C]_0 [B][H]$$

Substitution with equations (S2) and (S4) into equation (S1) gives the final result:

$$(S1) \quad \begin{aligned} r_{\text{tot}} &= r_{\text{uncat}} + r_{\text{cat}} \\ &= k_{\text{uncat}} [B][H] + k_{\text{cat}} [C]_0 [B][H] \\ &= (k_{\text{uncat}} + k_{\text{cat}} [C]_0) [B][H]^{\dagger\dagger} \end{aligned}$$

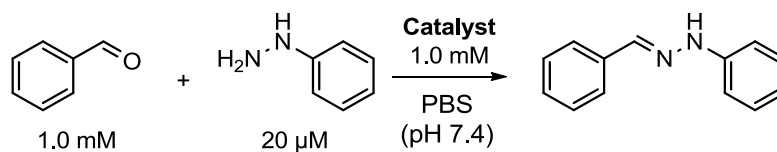
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<sup>§</sup> Since  $[B]_0 = [C]_0$ , it is necessary for  $K_{m^*}$  to be large to fulfill the prerequisite  $\frac{[C]_0}{[B]_0 + K_{m^*}} \ll 1$  (see ref. below).


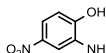
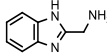
\*\* Segel, L. A.; Slemrod, M. *SIAM Review* **1989**, *31*, 446.

†† Note that this supports the notion of  $k_2 = k_{\text{uncat}} + k_{\text{cat}} [C]_0$ , which is supported by the graph in Figure S6.

## Reactions in Phosphate-buffered Saline (No DMF)

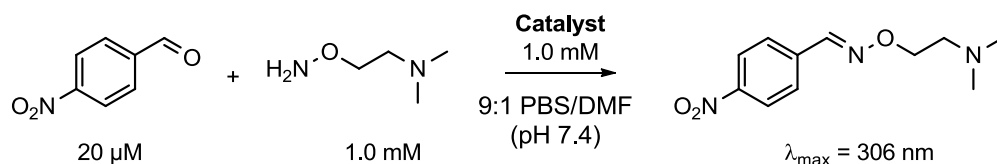


Reactions were carried out and measured as described above, except no DMF was added to the reaction mixture, and all stock solutions were prepared from PBS. Second order rate constants ( $k_2$ ) were determined using the non-linear regression technique described above. The results are presented in the following table.


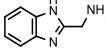
Catalyst	$k_2$ ( $\text{M}^{-1}\text{s}^{-1}$ )	$k_{\text{rel}}$
(none)	$0.21 \pm 0.02$	<u>1.0</u>
<b>2</b> 	$0.45 \pm 0.04$	2.2
<b>7</b> 	$0.98 \pm 0.04$	4.8
<b>21</b> 	$1.86 \pm 0.04$	9.0

Reaction rate in phosphate buffered saline (137 mM NaCl, 2.7 mM KCl and 11.9 mM sodium and potassium phosphates) at pH 7.4. Entry numbers refer to the manuscript.

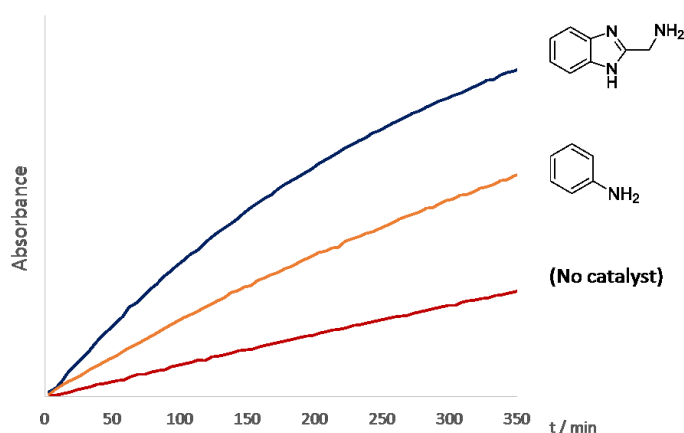
## Catalysis of Oxime Formation



Reactions were carried out and measured as described above. 4-Nitrobenzaldehyde was used for spectral reasons, as conjugation to the aliphatic aminoxy compound produced only a small shift in absorbance (e.g. benzaldehydes that do not absorb above 270 nm (DMF UV cutoff) could not be used with this aminoxy compound).<sup>\*\*</sup> Second order rate constants ( $k_2$ ) were determined as described above, using non-linear regression. The results are presented in the table below and Figure S7 illustrates product formation over time. Note that oxime formation is roughly one order of magnitude slower than hydrazone formation.

	Catalyst	$k_2$ $\text{M}^{-1}\text{s}^{-1} \cdot 10^{-3}$	$k_{\text{rel}}$
	(none)	$10.1 \pm 0.8$	<u>1.0</u>
<b>2</b>		$24.3 \pm 0.3$	2.4
<b>21</b>		$51.9 \pm 0.8$	5.2

Reaction performed in 1:9 DMF/water with 123 mM NaCl, 2.4 mM KCl, and 10.7 mM sodium and potassium phosphates (pH 7.4). Entry numbers refer to the manuscript.



**Figure S7: Oxime formation between (2-dimethylamino)ethoxyamine and 4-nitrobenzaldehyde as a function of time (measured as absorbance at 306 nm). Shown are representative examples of reaction curves using no catalyst and using aniline or 2-aminomethylbenzimidazole as catalysts.**

<sup>\*\*</sup> To minimize spectral overlap, the aminoxy compound was used in excess in these cases, but it has no effect on the fitting procedure, as the equation does not differentiate between nucleophile and electrophile.

## LC-HRMS Analysis of Reaction Mixture

To support the expectation that the product formed in catalyzed reactions between aryl aldehydes and hydrazines is indeed the hydrazone, a reaction mixture consisting of benzaldehyde (1.0 mM) and phenylhydrazine (20  $\mu$ M) was prepared in an ammonium acetate buffer at pH 7.0 with 10 volume % DMF. After 20 hours, the mixture was analyzed by high performance liquid chromatography, and solutions with benzaldehyde only and phenylhydrazine only were run for comparison. The UV chromatograms at 255 nm are shown below, and they illustrate that only one new compound (ret. time 2.4 min) is formed. By direct high resolution mass spectrometry, it was found that the mass of the compound in the new peak corresponded (within 2.0 ppm) to the expected hydrazone.

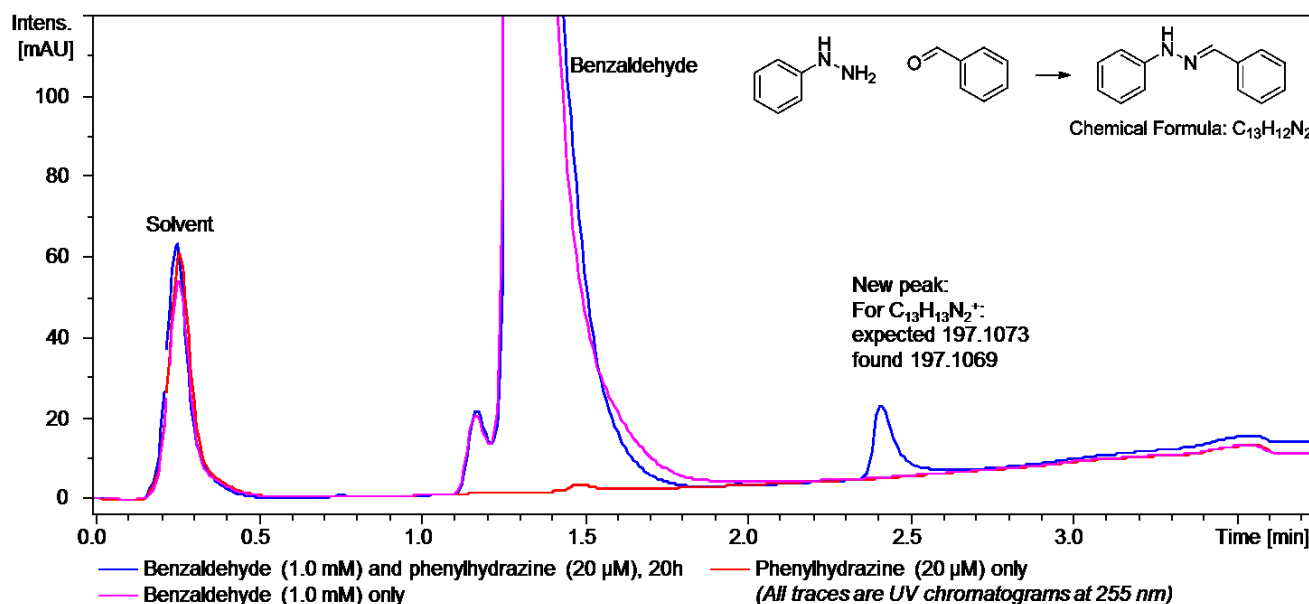


Figure S8: LC-HRMS analysis of reaction between phenylhydrazine and benzaldehyde in ammonium acetate buffer (pH 7.0) with 10 % by volume of DMF. The reaction mixture was analyzed after 20 hours, and chromatograms of benzaldehyde only and phenylhydrazine only are included for reference. Only one new peak had formed, and by direct HRMS this peak was found to correspond to the desired product. Note that a 50-fold excess of aldehyde was used, analogous to pseudo-first-order conditions used in the kinetics experiments.