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Triazine-Substituted and Acyl Hydrazones: Experiment and Computation Reveal a Stability Inversion at Low pH

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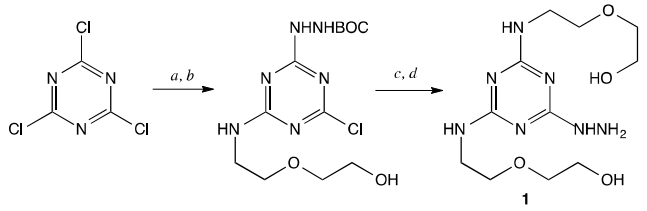
ABSTRACT: Condensation of a hydrazine-substituted [s]-triazine with an aldehyde or ketone yields an equivalent to the widely-used, acid-labile acyl hydrazone. Hydrolysis of these hydrazones using a formaldehyde trap as monitored using HPLC reveal that triazine-substituted hydrazones are more labile than acetyl hydrazones at pH >5. The reactivity trends mirror the corresponding acetylhydrazones, with hydrolysis rates increasing along the series (aromatic aldehyde < aromatic ketone < aliphatic ketone). Computational and experimental studies indicate a reversal in stability around the triazine pKa, pH~5. Protonation of the triazine moiety retards acid-catalyzed hydrolysis of triazinylhydrazones in comparison to acetyl hydrazone analogues. This behavior supports mechanistic interpretations suggesting that resistance to protonation of the hydrazone-N_1 is the critical factor in affecting reaction rate.

Labile bonds in general, and hydrazones in particular, find diverse roles in chemistry ranging from materials to medical sciences. Hydrazones have been employed in the creation of dynamic combinatorial libraries (1) and as reagents and auxiliaries in organic synthesis (2). Fragrant aldehydes and ketones have been incorporated into materials as pro-perfumes using hydrazone chemistry (3). Hydrazones can be bioactive in their own right (4) including uses as anti-trypanasomals (5) and tools for molecular biology (6). The lability of hydrazones in acid inspires the application of these groups as linkers in drug conjugates. Calicheamicin is conjugated to an oxidized antibody through an acylhydrazone in Mylotarg (7). Conjugates of other drugs have been reported including doxorubicin (8). Hydrazones have been used as the basis for switches, sensors, and other materials (9). While the literature is replete with examples of aliphatic, acyl and aromatic hydrazine derivatives of triazines and resulting hydrazones, the mechanism of hydrazone hydrolysis is unreported (10).

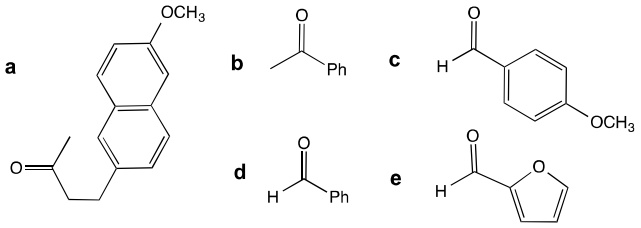
Our longstanding interest in triazine chemistry(11) led us to examine whether hydrazine derivatives of triazines—so-called triazinylhydrazines—might mirror acylhydrazines in hydrazone formation and hydrolysis. *A priori*, it was unclear whether such hydrazones would be more or less stable than the acyl analogues. To start, a four-step synthesis of triazinylhydrazine **1** (Scheme 1) was adopted. The aminoethoxyethanol substituents were included to enhance water solubility. The moderate reactivity of BOC-$NHNH_2$ (BOC is *t*-butoxycarbonyl) led to its installation on a dichlorotriazine intermediate that could be subsequently elaborated to **1** as shown**.** In contrast, reaction of cyanuric chloride with BOC-$NHNH_2$ led to a mixture of products including the desired mono-addition product, the di-addition product and other impurities that were not identified.(12) Similarly, the elevated temperatures required for installation of BOC-$NHNH_2$ (or hydrazine) on a suitably derivatized monochlorotriazine led to the desired compound, but also additional impurities identified in HPLC chromatograms. Our interest in applying poly(dichlorotriazines) as intermediates in dendrimer synthesis or on other scaffolds makes this seemingly laborious synthesis generally applicable, if not desirable (13).

Scheme 1. Synthesis of triazinylhydrazine **1**. *a)* Aminoethoxyethanol, THF, DIPEA, -15 to 0 °C, 1h. *b)* BOC-$NHNH_2$, THF, DIPEA, RT 12h. *c)* Aminoethoxyethanol, dioxane, DIPEA, 80 °C, 12h. *d)* 4M HCl:MeOH, 12h.



We examined a suite of five aldehydes and ketones, **a-e** (Chart 1). We denote the hydrazone formed by the condensation of a **1** and **a** as **1-a**, and the corresponding acetylhydrazone as **Ac-a**. The alphabetical ordering of these compounds reflects the measured stabilities to hydrolysis (*vide infra*).

Chart 1. Aldehydes and ketones used in this study.



Condensation of **1** or acetyl hydrazine, $AcNHNH_2$, to form the corresponding hydrazone was accomplished readily by mixing with a 3-fold excess of **a-e** and 0.5 equivalents of acetic acid at room temperature in ethanol overnight followed by chromatographic purification. Structures were validated with ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry. All compounds showed a single peak in HPLC chromatograms with a unique retention time. Hydrolysis rates were measured in a mixed solvent system to account for the varying hydrophobicity of these molecules. Briefly, 50mM stock solutions of **1-a** through **1-e** were prepared in methanol (**Ac-a** through **Ac-e** were prepared in THF) and diluted to 10mM with disodium phosphate/citric acid buffers to reach pH of 5.2, 6.8, and 8.0. Hydrolysis was monitored in the presence of 10x excess formaldehyde as a trap (14) using HPLC (Supporting Information). Hydrolysis was pronounced at pH 5.2. Figure 1a shows the data derived from HPLC traces. Comparatively, acyl hydrazones are more stable than the triazinyl hydrazone counterpart. The stability increases with hydrazones **a**<**b**<**c**<**d**<**e**.

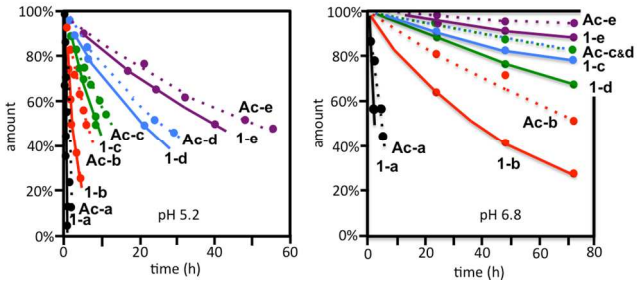


Figure 1. Hydrolysis profiles at pH 5.2 (left) and 6.8 (right).

Table 1. First-order reaction constants and half lives, and DFT computed N1 proton affinities ΔE_{prot} relative to **Ac-b**. Changes in ΔE_{prot} upon triazine protonation are in parenthesis. The values of k are reported as ($x10^3$ min^{-1}) and for $t^{1/2}$ as (min^{-1}) and for ΔE_{prot} as (kcal/mol)

	pH 4.0		pH 5.2		pH 6.8		ΔE_{prot}
Cmpd			k	$t_{1/2}$	k	$t_{1/2}$	
1-a	-	-	39	18	4.7	150	4.4 (-5.0)
Ac-a	-	-	20	34	2.6	270	1.7
1-b	8.0	87	5.2	130	0.3	2310	2.3 (-4.3)
Ac-b	30	23	1.9	370	0.1	6900	0.0
1-c	1.9	360	1.3	530	0.09	-	1.0 (-4.1)
Ac-c	8.6	81	0.5	1400	0.04	-	-0.7
1-d	1.8	390	0.9	770	0.059	-	-1.3 (-4.2)
Ac-d	2.9	240	0.4	1700	0.046	-	-3.0
1-e	0.7	990	0.3	2300	0.014	-	-0.1 (-4.4)
Ac-e	3.1	224	0.2	3500	0.027	-	-1.9

At pH 6.8, similar trends are observed over 72 h as shown in Figure 1b with most showing less than 20% hydrolysis over this period. At pH 8.0, neglible hydrolysis was observed over 72h: only **1-a** and **Ac-a** show measurable hydrolysis rates (0.0006 min^{-1} and 0.0001 min^{-1}, respectively). First-order reaction rate constants and half lives are shown in Table 1.

The accepted mechanism for hydrazone hydrolysis starts with protonation of nitrogen N1 to yield I (Scheme 2).(15) Addition of a molecule of water yields carbinolamine intermediate, II. Proton migration (III) leads to C-N bond cleavage to complete the hydrolysis. Subject to historical and contemporary inquiry (14-17), this mechanism is consistent with catalysis observed in acid. Three different interpretations have been invoked to rationalize differences in rates of hydrolyses of different hydrazones (and oximes and imines). These include a thermodynamic argument on ground state stabilities (16), resonance stabilization arguments

focusing on the reduction of electrophilicity of C1 (17), and stabilities derived from a resistance to protonation of N1 (Figure 3) recently invoked by Kalia and Raines to explain relative stabilities of hydrazones and oximes (14).

Scheme 2. Mechanism of hydrolysis. R = acetyl or triazinyl.

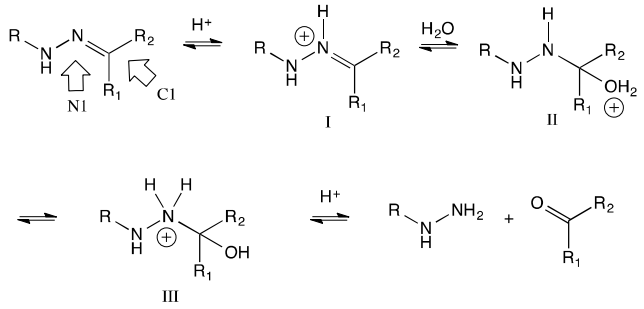
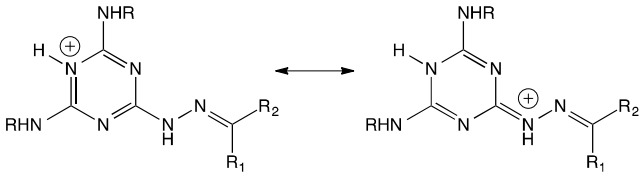


Table 1 includes density functional theory (DFT) calculations (18-20) of proton affinities of N1 of the hydrazone. These values, ΔE_{prot}, are assigned relative to **Ac-b**. Positive values correspond to stable proton binding. Calculations use the B3LYP exchange-correlation functional(18), the 6-31+G(d,p) basis set (19), the SMD continuum model for aqueous solvent (20), and the *Gaussian 09* suite of programs (21). Other computational details, and energies & geometries of all species, are reported as Supporting Information. The measured rates of hydrolysis for **Ac-a - Ac-e** correlate with the computed N1 proton affinities, consistent with the work of Kalia and Raines.(14) For example, ketone-derived **Ac-a** has a shorter half-life and larger proton affinity than **Ac-b**, while aldehyde-derived **Ac-c**, **Ac-d**, and **Ac-e** have longer half-lives and smaller (more negative) proton affinities than **Ac-b**. **Ac-c** has a larger proton affinity and shorter half-life than **Ac-d**, consistent with resonance electron donation from the para-methoxy group to N1 increasing proton affinity.

Table 1 also shows that the triazinyl hydrazones **1-a - 1-e** all have shorter half-lives at pH 5, and larger N1 proton affinities, than the corresponding acetyl hydrazones. Computation clearly supports an interpretation that resistance of N1 to protonation is the key predictor of the stability of hydrazones to hydrolysis. Consistent with this analysis, computational models of simple imines (H_2NMe) and oximes (H_2NOMe) derived from **b** (PhMeC=NMe and PhMeC=NOMe) give a large computed N1 proton affinity 14.9 kcal/mol (relative to **Ac-b**) for the rapidly hydrolyzed imine, and a small proton affinitiy -1.5 kcal/mol for the stable oxime (14).

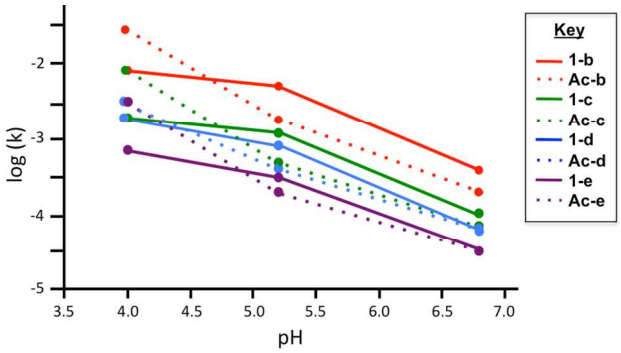
Triazinyl hydrazones, unlike acyl hyrazones, imines and oximes, however, offer additional basic sites on the triazine. Kalia and Raines note that protonation of N1 is disfavored across a range of pH because the pKa of N1 is <0.7. Accordingly, activated species will exist at very low concentrations in the pH range of interest. The pKa of protonated triazines is ~5 (22), thus protonated triazines must be considered at low pH. Triazine protonation should promote hydrolysis by increasing the electrophilicity of C1 through induction, but counterproductively, should increase the resistance of N1 to protonation (Scheme 3), the perceived critical event in hydrolysis.

Scheme 3. A protonated triazine (pKa~5) could increase electrophility of C1 through induction (increasing rate) or increase resistance to N1 protonation (decreasing rate).



Computation bears this prediction out. Table 1 shows that *para*-protonation of the **1-a - 1-e** triazine reduces the predicted N1 proton affinities by 4-5 kcal/mol. *Ortho*-protonation of 1-d reduces the predicted N1 proton affinities by 6-10 kcal/mol, due in part to steric clashes between *ortho*-N-H and N1-H protons. We note that *ortho*-protonation could conceivably accelerate hydrolysis proceeding without N1 protonation, by increasing the electrophilicity of carbon C1. Test calculations suggest that this is a small effect. The reaction of the C1 carbon of molecule **1-d** with OH(-) is made 45 kcal/mol more favorable by N1 protonation, but only 11 kcal/mol more favorable by ortho protonation.

Figure 3. Hydrolysis profiles at pH 4 in the presence of 10-fold molar excess of formaldehyde.



To explore this effect, we measured hydrolysis of **1-b** and **Ac-b** at pH 4. Indeed, an inversion in stability is observed with **Ac-b** hydrolyzing more rapidly than **1-b** (Figure 3). Specifically, at pH 4, the rate constants (k x 10^3 min^{-1}) for **1-b**, **Ac-b**, **1-c**, and **Ac-c** are 8.4, 2.7, 1.9, and 0.9, respectively. The half-lives (min) for **1-b**, **Ac-b**, **1-c**, and **Ac-c** are 83, 25, 364, and 81, respectively.

In summary, the data here support the Kalia/Raines mechanism for hydrolysis. This "resistance to protonation" hypothesis is further supported by the inversion of stability seen in triazinyl and acetyl hydrazones at low pH. This inversion in stability occurs at a pH that is biologically relevant—that associated with the cellular uptake and the endosome. Whether it can be exploited advantageously for the delivery of drugs or other agents remains to be seen.

Supporting Information. Synthetic details, characterization data including spectra and kinetic studies, computational details, and computed structures and energies are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

ACKNOWLEDGMENT

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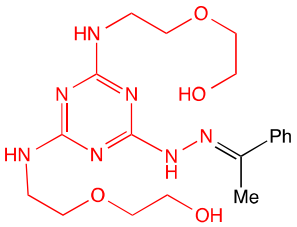
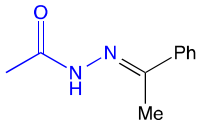


***More stable at* pH<5**



***More stable at* pH>5**