Diazonium Salts Hot Paper

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Water-Soluble Triazabutadienes that Release Diazonium Species upon Protonation under Physiologically Relevant Conditions**

*Flora W. Kimani and John C. Jewett**

Abstract: *Triazabutadienes are an understudied structural motif that have remarkable reactivity once rendered water-soluble. It is shown that these molecules readily release diazonium species in a pH-dependent manner in a series of buffer solutions with pH ranges similar to those found in cells. Upon further development, we expect that this process will be well suited to cargo-release strategies and organelle-specific bioconjugation reactions. These compounds offer one of the mildest ways of generating diazonium species in aqueous solutions.*

Whereas many biochemical events are prompted by subtle changes in the pH value, there are relatively few small-molecule reactions that are equally responsive. Our work on the pH-responsive dengue virus[1] has prompted our entry into this area. We are looking for processes that are responsive within the relevant pH and temporal ranges of infection[2] and ideally generate species that go on to perform other reactions. Herein, we report a chemical system that brings us closer to meeting this challenge, as a stable species is transformed into a reactive species in a physiologically relevant pH range.

There are two general types of reactions that probe changes in the pH value: Some dyes undergo a reversible bathochromic shift upon protonation,[3] and some acid-labile protecting groups can irreversibly uncage to reveal a reactive, therapeutic, or diagnostic functionality. Whereas the former are rapid indicators, it is challenging to parlay their protonation into further chemical reactivity. The most prominent uncaging reactions, namely those of Schiff bases,[4] acetals,[5] or imidazole-based cross-linkers,[6] do not have unmasking rates with the dramatic pH dependence that we desire. Our search for reactivity led us to the work of Fanghänel and co-workers and their two-decade-long study of 1,2,3-triazabutadienes **1** (Figure 1b).[7] Inspired by their work, the processes reported herein represent a clear departure from existing pH-triggered transformations and, to the best of our knowledge, feature unique reactivity.

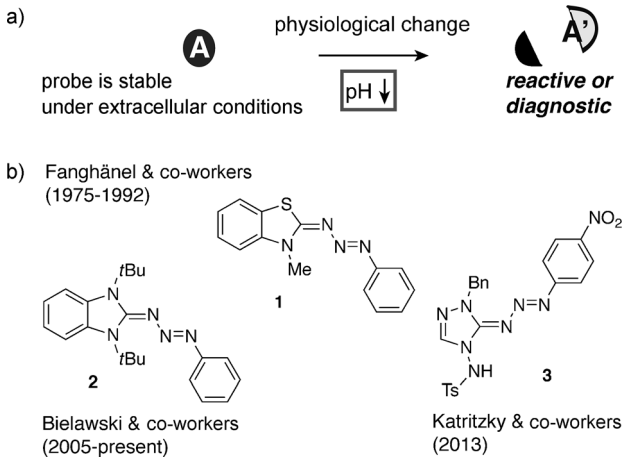

Figure 1. a) The transformation of a probe triggered by physiological changes. b) Several classes of triazabutadienes that have been reported previously. Bn = benzyl, Ts = *para*-toluenesulfonyl.

Triazabutadienes have undergone a small renaissance in recent years mainly because of the work of Bielawski and Khramov (compound **2**; Figure 1b).[8] While these molecules are now commonly called triazenes, for the purpose of our discussion, we will continue to call this functional group a triazabutadiene, in part to distinguish it from the more conventional *N*,*N*-dialkyltriazenes,[9] but also in recognition of the role that the left half of the molecule plays in determining its reactivity. Much of the more recent work on these compounds has focused on their "push–pull" properties (see compound **3**, Figure 1b)[10] and Staudinger-like rearrangements to expel nitrogen upon heating of the compounds.[8,11] What fascinated us about triazabutadienes was their early reported instability in the presence of strong acids (Figure 2a). The mechanism for their degradation, which is summarized in Figure 2b, involves the protonation of the N3 nitrogen atom followed by elimination of an aryl diazonium species. This simple mechanism is complicated by *E*/*Z* isomerization about the N1–N2 bond[7c] and the fact that protonation and alkylation of the N1 nitrogen atom is highly preferred.[7d,12] The vast majority of the work on such structures has been carried out with molecules featuring a benzothiazole or benzimidazole moiety in organic solvents, and upon making a departure from both, we were excited by what we found.

Whereas Fanghänel and co-workers had previously shown that strong mineral acids such as hydrochloric acid could facilitate the decomposition of **1**, they did not perform the

[*] F. W. Kimani, Dr. J. C. Jewett
Department of Chemistry and Biochemistry
University of Arizona
1306 E University Blvd, Tucson, AZ 85721 (USA)
E-mail: jjewett@email.arizona.edu

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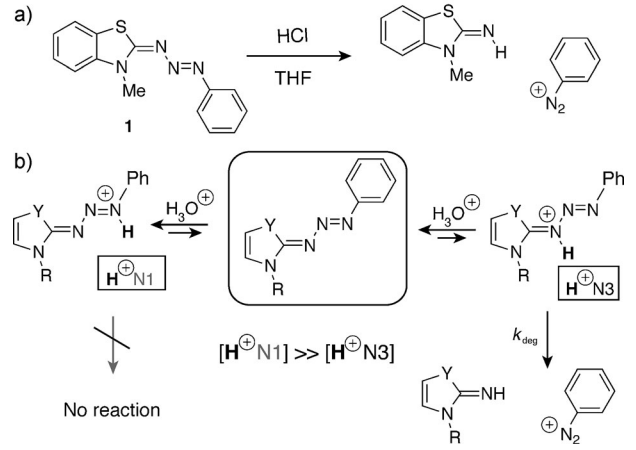
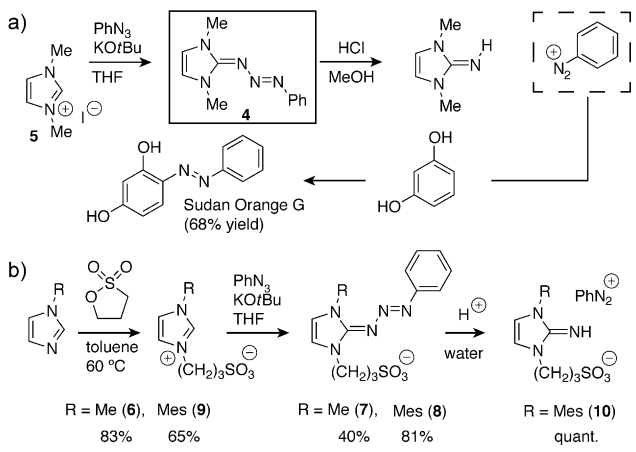


Figure 2. a) Fanghänel and co-workers reported that **1** decomposed in the presence of a strong acid. b) Reaction of **1** by protonation of the N3 nitrogen atom.

reaction in water or assess its susceptibility to degradation in physiologically more relevant pH ranges.[7e] Based on the harsh conditions used to degrade triazabutadienes with benzothiazole or benzimidazole moieties, we hypothesized that these derivatives would be too stable for our purposes. Instead, we selected an imidazole core, in no small part because of the wealth of N-heterocyclic carbene (NHC) literature and synthetic precedent.[13,14] To assess the reactivity of the core, we synthesized triazabutadiene **4** from dimethylimidazolium iodide (**5**) and phenyl azide (Scheme 1a). We chose this azide-based synthetic approach, which was reported by Bielawski, over Fanghänel's original coupling of an appropriately deprotonated cyclic guanidine with an aryl diazonium salt for reasons of synthetic ease and versatility. No changes were observed for a methanolic mixture of equimolar amounts of **4** and resorcinol (to trap any diazonium species formed) until we added HCl. Upon acidification, the solution underwent a rapid color change and provided the known aryl azo dye Sudan Orange G (Scheme 1a). When we increased the pK_a value of the acid by employing acetic acid instead of HCl, the same reaction occurred, albeit at a slower rate.

We synthesized a water-soluble analogue by treating methylimidazole with 1,3-propanesultone to obtain zwitterionic NHC precursor **6**. Upon treatment with base in the presence of phenyl azide, triazabutadiene **7** was formed in acceptable yield (Scheme 1b). This compound was highly water-soluble, but upon dissolution, we were met with a surprising level of instability. Whereas this species was stable in methanol or dimethylsulfoxide (DMSO), it degraded in water with a half-life of approximately 24 hours.[15] Upon increasing the steric hindrance by replacing the methyl substituent with a mesityl group, we obtained compound **8** from **9**; only 10% of **9** degraded upon sitting in D_2O for



Scheme 1. a) Synthesis of triazabutadiene **4** and acid-promoted diazonium generation. The diazonium ion is trapped to provide Sudan Orange G. b) Synthesis of triazabutadienes **7** and **8**. Mes = mesityl.

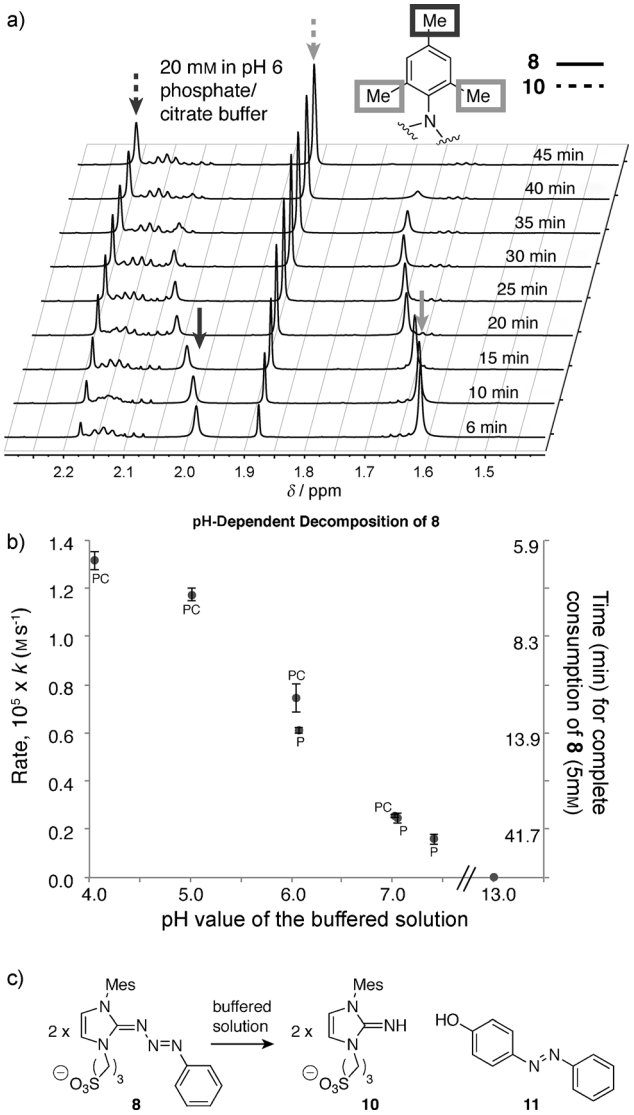


Figure 3. a) A representative NMR experiment showing that **8** is converted into **10** in a pH 6 phosphate/citrate buffer over time. b) The reaction rate of the benzenediazonium salt formation from **8** in buffers is highly pH-dependent. Error bars indicate the standard deviation from three separate reactions. PC = phosphate/citrate buffer, P = phosphate buffer. c) Formation of azobenzene **11** from **8**.

24 hours. Presumably, the bulky mesityl group disfavors protonation of the N3 nitrogen atom.

While establishing the stability of **8** in water, we noted that the degradation reaction slowed down and eventually stopped over the course of the 24 hours. We realized that we were consuming a reagent in the form of hydronium ions and upon monitoring the pH value over time, we found that it rapidly jumped from neutral pH to pH 9.7 and eventually levelled off at pH 9.9 after 24 hours. Consistent with this observation and the proposed mechanism, both compounds were found to be stable in 0.1N NaOH with no detectable degradation after 24 hours.

To test the stability at a "constant", non-basic pH value, we performed a series of experiments in buffered solutions. Within the studied buffering range, we observed a sigmoidal dependence of the rate constant on the pH value, centered at pH 6 (Figure 3a,b).[16] Imidazole **10**, which remains in solution, offers a convenient handle for comparing the amounts of starting material and product.[17] To our surprise, we observed pseudo-zeroth-order reaction kinetics as the concentration of **8** decreased linearly with time. We rationalized this observation with deference to the proposed overall mechanism, which states that the reactive N3-protonated form is much less abundant than the non-reactive N1-protonated form. To avoid complications associated with the buffer capacity, resorcinol was not added to these reactions. Over time, we observed the formation of a yellow precipitate, which was confirmed to be 4-phenylazophenol (**11**, Figure 3c). This product was formed by the hydrolysis of one diazonium ion to yield phenol (likely by the pH-independent formation of the aryl cation)[18] followed by a reaction with a second diazonium ion.

The relative instability of **8** at pH 7 prompted us to synthesize several analogues to test the effect of electronic perturbations on the reactivity. For the purposes of this discussion, we have focused on the aryl moiety, but plan to explore other parameters that could influence these reactions. Based on previous work[7e] and first principles,[19] we expected that electron-donating substituents would increase the partial charge on the N3 nitrogen atom, favoring protonation and decreasing the stability. Indeed, triazabutadiene **12**, with an electron-donating *para*-methoxy substituent, was found to be roughly twice as reactive as **8** at pH 4, 5, 6, and 7 ($2.4 \pm 0.1 \times 10^{-5}$ M s^{-1}, $1.9 \pm 0.2 \times 10^{-5}$ M s^{-1}, $1.0 \pm 0.1 \times 10^{-5}$ M s^{-1}, and $0.34 \pm 0.03 \times 10^{-5}$ M s^{-1}, respectively).

To test the corollary and study a derivative with increased stability, we synthesized triazabutadiene **13** with a *para*-nitro substituent. Triazabutadiene **13** was stable in pure water,[20] but rapidly precipitated from buffered solutions (even at pH 7). Fearing that an unexpected degradation pathway might be occurring in buffered solutions, we isolated the solid, dissolved it in deuterated methanol, and confirmed that the starting material had remained unaltered. Upon acidification of the methanolic solution with HCl, a color change occurred, and the solution started bubbling upon prolonged exposure to the acid.[21] The problems with aqueous solubility foiled our attempts to examine **13** in physiologically relevant buffers. To directly compare the reactivity of **13** with those of **8** and **12**, we monitored their reaction rates in a 1:1 mixture of D_2O/MeOD with a ten-fold excess of formic acid (Figure 4a). While completely abiotic, this solvent system was unique in its ability to keep electron-poor **13** in solution in the presence of a high concentration of water and acid. Compounds **8** and **12** behaved as expected with **12** reacting at a faster rate than **8**, but unlike in the buffered systems, the rate took on a decidedly more first-order appearance while not being strictly first order. Comparing the initial slopes to those in water, these reactions were considerably slower than those in buffered water, but this finding can be attributed to the large percentage of organic solvent present in solution, which leads to significant alterations of the solvation and pK_a values. Upon addition of the acid to **13**, we observed a bathochromic shift of the solution and a new species by NMR spectroscopy. After twelve hours, only 38% of **13** had been consumed to yield the diazonium species and the cyclic guanidine. Based on these data, we hypothesized that the new species is

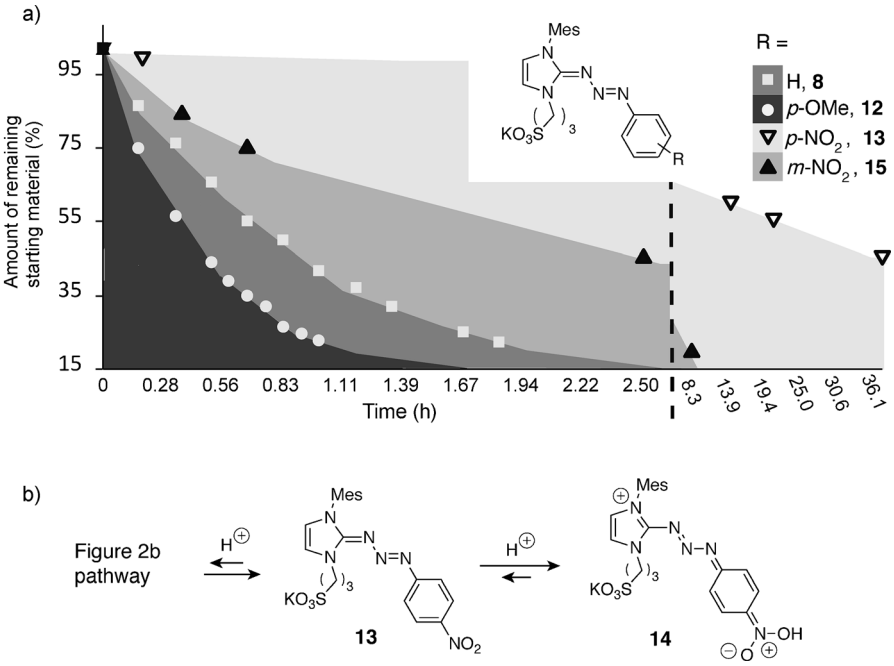


Figure 4. a) Time-dependent diazonium salt release of compounds **8**, **12**, **13**, and **15** (15 μM) in a water/methanol mixture (1:1) containing formic acid (10 equiv). b) Unlike **8** and **12**, upon exposure to formic acid, compound **13** exists in a different form (compound **14**, observable by its unique NMR spectrum), which slowly releases a diazonium species and **10**.

compound **14** (Figure 4b), which adds an extra layer of complication to the reaction analysis.[22]

We thought to circumvent the complication imposed by the formation of protonated species such as **14** by synthesizing *meta*-nitro-substituted derivative **15**, where a similar process is unlikely. As for the other electron-deficient compounds that we had synthesized and evaluated, **15** was soluble and stable in D_2O but insoluble in buffers. The yellow precipitate that formed upon acidification was collected and dissolved in [D_6]DMSO. This compound was identified as a new species and assigned as the stable N1-protonated form. The addition of triethylamine to the NMR tube returned **15** unaltered, thus bolstering our hypothesis (Supporting Information, Figure S1). Again, because of its insufficient solubility in water, we treated **15** with formic acid in a methanol/water mixture (as before), and this species was indeed found to be reactive in the presence of an acid. Moreover, **15** formed the diazonium species at a much faster rate than **13** (Figure 4a). After eight hours, **15** had been nearly completely consumed whereas only approximately 50% of **13** was consumed over 24 hours.

In conclusion, we have reported a water-soluble triazabutadiene system that reacts in a pH-dependent manner in buffered solutions. We expect that its bond-cleaving reactivity in combination with the liberation of a reactive species will make this process suitable for the interrogation of complex systems. For example, diazonium species have previously been shown to be useful in biochemical conjugations to tyrosine.[23] Moreover, exploiting the diazonium ion into phenol conversion (Figure 3c) opens the door to pH-dependent fluorogenic probes and cargo-release strategies. The current pH reactivity profile likely precludes the level of precision that we require for diazonium salt release, but additional design elements, such as internal acids and bases or enzymatically triggered variations, could enable the development of a more broadly applicable reactivity profile.[24] These studies are currently ongoing and will be reported in due course.

Keywords: diazonium salts · kinetics · protonation · triazabutadienes

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[15] Based on integration of the mesityl region.
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[20] No degradation was observed after 24 hours, and the pH value of the solution remained neutral over this time period.
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[22] Initial attempts to avoid this complication with other electron-deficient aryl species (such as *para*-chloro and *para*-trifluoromethyl-substituted systems) were thwarted by a lack of water solubility preventing a comparison of the stabilities at neutral pH values.
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[24] Preliminary experiments to confirm the reactivity of the tyrosine analogue and the lack of thiol background reactivity have been promising; see the Supporting Information.

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