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Hydrolytic Stability of Hydrazones and Oximes[**]
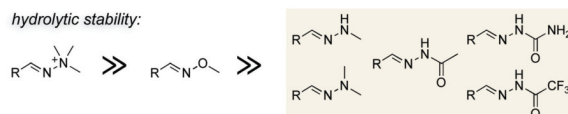
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Abstract

Hydrazones and oximes are common conjugates, but are labile to hydrolysis. The hydrolytic stability of isostructural hydrazones and an oxime have been determined at pD 5.0–9.0. The hydrolysis of each adduct was catalyzed by acid. Rate constants for oxime hydrolysis were nearly 10^3-fold lower than those for simple hydrazones; a trialkylhydrazonium ion (formed after condensation) was even more stable than the oxime. The data suggest a general mechanism for conjugate hydrolysis.

Keywords

conjugates; hydrazones; hydrolysis; oximes; reaction mechanisms

Molecules containing carbon–nitrogen double bonds are prevalent in both chemical and biological contexts. The foundations for our current understanding of carbon–nitrogen double-bond formation and hydrolysis were laid by seminal early work on hydrazone hydrolysis and formation,[1] and by contributions from mechanistic studies on enzymes that utilize pyridoxal phosphate.[2] In particular, the meticulous kinetic analyses of Jencks resulted in the delineation of a carbinolamine intermediate in carbon–nitrogen double-bond formation and hydrolysis, and elucidation of the general mechanism of carbonyl-group addition reactions.[3,4] These principles were summarized in a landmark review.[5]

Hydrazones and oximes ($C^1=N^1-X^2$) possess greater intrinsic hydrolytic stability than do imines. The textbook explanation for this greater stability invokes the participation of X^2 in electron delocalization (Figure 1).[6] The contribution of resonance form **II** in alkylhydrazones and oximes, and resonance form **IV** in acylhydrazones increases the negative-charge density

on C^1 and hence reduces its electrophilicity, thereby imparting greater hydrolytic stability to hydrazones and oximes. An alternative explanation is based on the repulsion of the lone pairs of N^1 and X^2 being relieved in the conjugates.[7]

Although the greater stability of hydrazones and oximes than imines is well-appreciated, a consensus on the comparative stability of hydrazones and oximes is lacking. To the best of our knowledge, the only report of a direct comparison of the rates of hydrolysis of hydrazones and oximes was from Stieglitz and coworkers in 1934.[8] These workers assayed the hydrolysis of benzophenonehydrazone and benzophenoneoxime in extremely acidic solutions by titrating the respective hydrazine and hydroxylamine products. This rudimentary study provided little insight. More recently, other workers have discussed the stability of the hydrazones and oximes used in particular applications,[9,10] but without direct comparisons.

Here, we report the first detailed investigation of the hydrolysis of isostructural alkylhydrazones, acylhydrazones, and an oxime. Half-lives for the hydrolysis of these conjugates were measured with ^1H NMR spectroscopy in deuterated buffers (pD 5.0–9.0) to obtain pD–rate profiles. In addition, pD-titrations of the conjugates were performed with ^1H NMR spectroscopy to determine relevant pK_a values and thereby provide mechanistic insight. Our findings establish oximes as the linkage of choice for the stable conjugation of molecules via a carbon–nitrogen double bond.

Conjugates **1**–**6** were synthesized by condensation of the respective nitrogen bases with pivalaldehyde (*t*BuCHO), and removing the water byproduct with anhydrous $MgSO_4(s)$ (Scheme 1). Pivalaldehyde was chosen because it lacks enolizable protons, thus preventing obfuscating side reactions such as aldol condensations. Methoxyamine and all the alkylhydrazines and acylhydrazines were available commercially except for trifluoroacetylhydrazine, which was generated in situ by the deprotection of Boctrifluoroacetylhydrazine (compound **8**, see Supporting Information). Trimethylhydrazonium ion **7** was synthesized by reacting dimethylhydrazone **2** with methyl iodide (Scheme 1). The synthesis of **7** by the condensation of trimethylhydrazinium ion and pivalaldehyde was unsuccessful, consistent with reports by others;[11] nor was this condensation reaction facilitated to a detectable extent by aniline[3d,10] at pD 5.0–9.0. (As trimethylhydrazinium ion did not even condense with the unhindered carbonyl group of formaldehyde, the likely problem is that nucleophilic attack by trimethylhydrazinium ion, $H_2N^1-N^2(CH_3)_3{}^+$, generates a positive charge on N^1 when N^2 already bears a positive charge.) ^1H NMR spectroscopy in deuterated phosphate buffers (pD 5.0–9.0) was used of the aldehydic proton of pivalaldehyde ($\delta = 9.4$ ppm), a signal for conjugate hydrolysis.

The hydrolytic cleavage of carbon–nitrogen double bonds is reversible. An excess of a deuterated aldehyde or ketone can be used to trap the liberated nitrogen base and thereby push the hydrolysis reaction to completion, allowing the forward (hydrolysis) reaction to be monitored without interference from the reverse (condensation) reaction. Various aldehydes and ketones were tested as potential chemical traps. Deuterated acetone was an inefficient trap —a 100-fold excess drove the hydrolysis of a methylhydrazone to only 62% completion at pD 7.0 (data not shown). Another dialkyl ketone, levulinic acid, has been used for a similar purpose,[12] but would have added a muddling carboxyl group to the reaction mixture. Hexachloroacetone, tribromoacetaldehyde, and calcium mesoxylate could not be used due to their low aqueous solubility. Alloxan, an electrophilic ketone, was unstable in water. Finally, a 10-fold excess of deuterated formaldehyde (CD_2O) was identified as an effective trap, driving the hydrolysis reactions of all the conjugates (except that of trimethylhydrazonium ion **7**) to completion at pD 5.0–9.0. A typical kinetic trace obtained is shown in Figure 2.

At pD 5.0–9.0, the half-life of oxime **3** was much larger that those of each hydrazone, except for trimethylhydrazonium ion **7** (Table S1 in Supporting Information). At pD 7.0, the first-order rate constant for the hydrolysis of oxime **3** was approximately 600-fold lower than methylhydrazone **1**, 300-fold lower than acetylhydrazone **4**, and 160-fold lower than semicarbazone **5**. Although the linkage in a trialkylhydrazonium ion (such as conjugate **7**) is highly stable, it is not suitable for bioconjugation because its synthesis involves treatment with methyl iodide—a reagent that is not chemoselective in a biological system—subsequent to condensation. Thus, oximes are the most preferable linkages for carbon–nitrogen double bondmediated bioconjugation.

The hydrolysis of the conjugates is catalyzed by acid (Figure 3). This finding is consistent with conjugate hydrolysis being accelerated by protonation. The hydrolysis of oxime **3** at pD > 7.0 and that of trimethylhydrazonium ion **7** at pD >5.0 were too slow to yield a complete kinetic trace within a reasonable time-frame.

pD-Titration experiments monitored with ^{1}H NMR spectroscopy revealed that some (but not all) of the conjugates experience a substantial change in protonation state between pD 0.7 and 13.4 (Figure 4). The δ value of $C^1\underline{H}$ for methylhydrazone **1** (pK_a = 5.5), dimethylhydrazone **2** (5.8), and trifluoroacetylhydrazone **6** (7.9) exhibited a sigmoidal dependence on pD. The δ value of $C^1\underline{H}$ in conjugates **3**–**5** and **7** was not a function of pD, indicating that an insignificant fraction of these conjugates is protonated at pD 0.7– 13.4.

What is the site of protonation in the conjugates? The titration curves for methylhydrazone **1** and dimethylhydrazone **2** are presumably due to the protonation of either N^1 or N^2. The similarity of δ values for the protonated forms of **1** and **2** to the δ value for the trimethylhydrazonium ion **7** (Figure 4), in which N^2 bears a positive charge, suggests that the site of protonation of methylhydrazone **1** and dimethylhydrazone **2** is N^2 (**VI**). This interpretation is also supported by N^2 of dimethylhydrazone **2** being more nucleophilic than N^1 toward methyl iodide (Scheme 1). The only other literature report of attempts to determine the site of hydrazone protonation reached the same conclusion.[13] The observed titration of trifluoroacetylhydrazone **6** is due to the loss of its N^2 proton, which is made acidic by the proximal trifluoromethyl group.

The value of δ does not correlate with conjugate stability. A high δ value of $C^1\underline{H}$ is indicative of low electron density on C^1, which portends a high susceptibility to attack by nucleophiles. Surprisingly, despite having the largest δ value (Figure 4), trimethylhydrazonium ion **7** is the most stable conjugate (Figure 3 and Table S1 in Supporting Information). Moreover, oxime **3** and acetylhydrazone **4** have similar δ values, but at pD 7.0 the half-life of oxime **3** is 25 d whereas that of acetylhydrazone **4** is 2 h (Table S1 in Supporting Information).

The data are consistent with a mechanism of C^1=N^1−X^2 hydrolysis that entails protonation of N^1 (Figure 5). The resultant protonated species (**VII**) would be highly susceptible to hydrolysis due to the enhanced electrophilicity of C^1. None of the conjugates is protonated to a significant extent on N^1 at pD 0.7–13.4 (Figure 4), indicating that the pK_a value of species **VII** is <0.7 in each conjugate, consistent with estimates of pK_a values for protonated oximes.[14] The protonation of N^1 of trimethylhydrazonium ion **7** is discouraged by the adjacent quaternary ammonium group. Consequently, trimethylhydrazonium ion **7** is highly stable (Figure 3), even without the ability to access resonance form **II** or the presence of a repulsive lone pair on X^2. This finding belies the textbook[6] and alternative[7] explanation for the stability of hydrazones and oximes being greater than that of imines. Rather, these conjugates are more stable than imines because of the inductive effect of X^2 = N or O. This explanation is analogous to one for the origin of the α-effect.[15]

The protonation of N^1 of oxime **3** is more favorable than that of trimethylhydrazonium ion **7**, accounting for the lower stability of oxime **3**. Still, the protonation of the oxime is less favorable than is the protonation of alkylhydrazones **1**–**2** and acylhydrazones **4**–**6**, due to the higher electronegativity of X^2 in the oxime ($\chi_O = 3.5$[16]) *versus* the hydrazones ($\chi_N = 3.0$). Hence, oxime **3** is more resistant to hydrolysis than are alkylhydrazones **1**–**2** and acylhydrazones **4**–**6**.

Finally, we note that the NMR spectra revealed no evidence of a carbinolamine intermediate (**VIII**). This observation, along with the high acidity of species **VII** ($pK_a < 0.7$), indicates that the rate-limiting transition state is that for the attack of water on species **VII**. The decomposition of a carbinolamine intermediate limits the rate of hydrolysis only under extremely acidic conditions.[4,14]

In summary, we have evaluated the hydrolytic stability of a series of isostructural hydrazones and an oxime. We found the oxime to be *much* more stable than the simple hydrazones. pD-Rate profiles and pD-titrations suggest that the anomalous stabilities of the oxime (as well as a trialkylhydrazonium ion) is due to its resistance to protonation. These data can inform the proper use of compounds containing carbon–nitrogen double bonds.[9,10]

Experimental Section

See the Supporting Information for experimental details.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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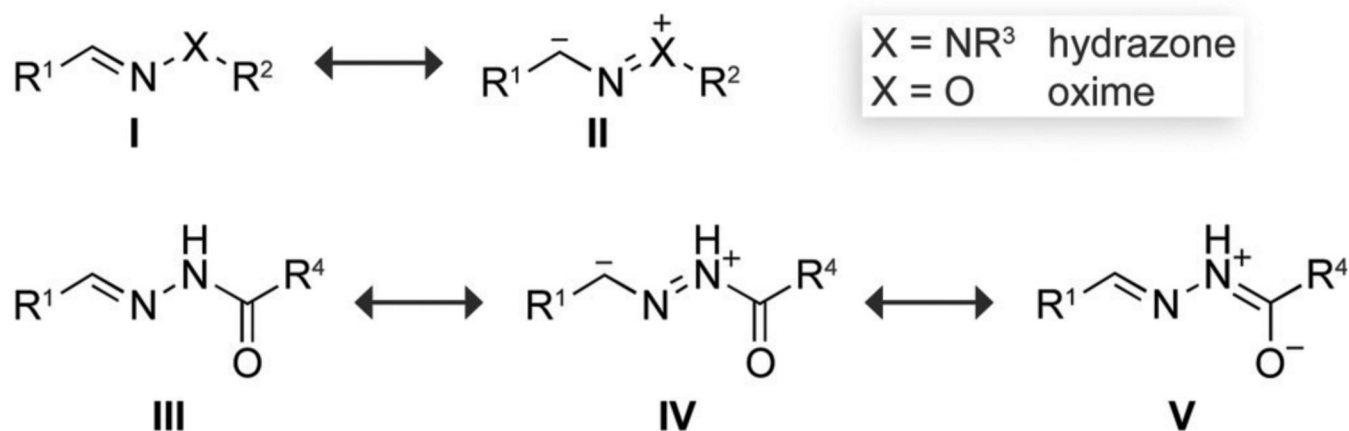16. Pauling, L. The Nature of the Chemical Bond. Vol. 1st ed. Ithaca, NY: Cornell University Press; 1939. p. 60

Figure 1.
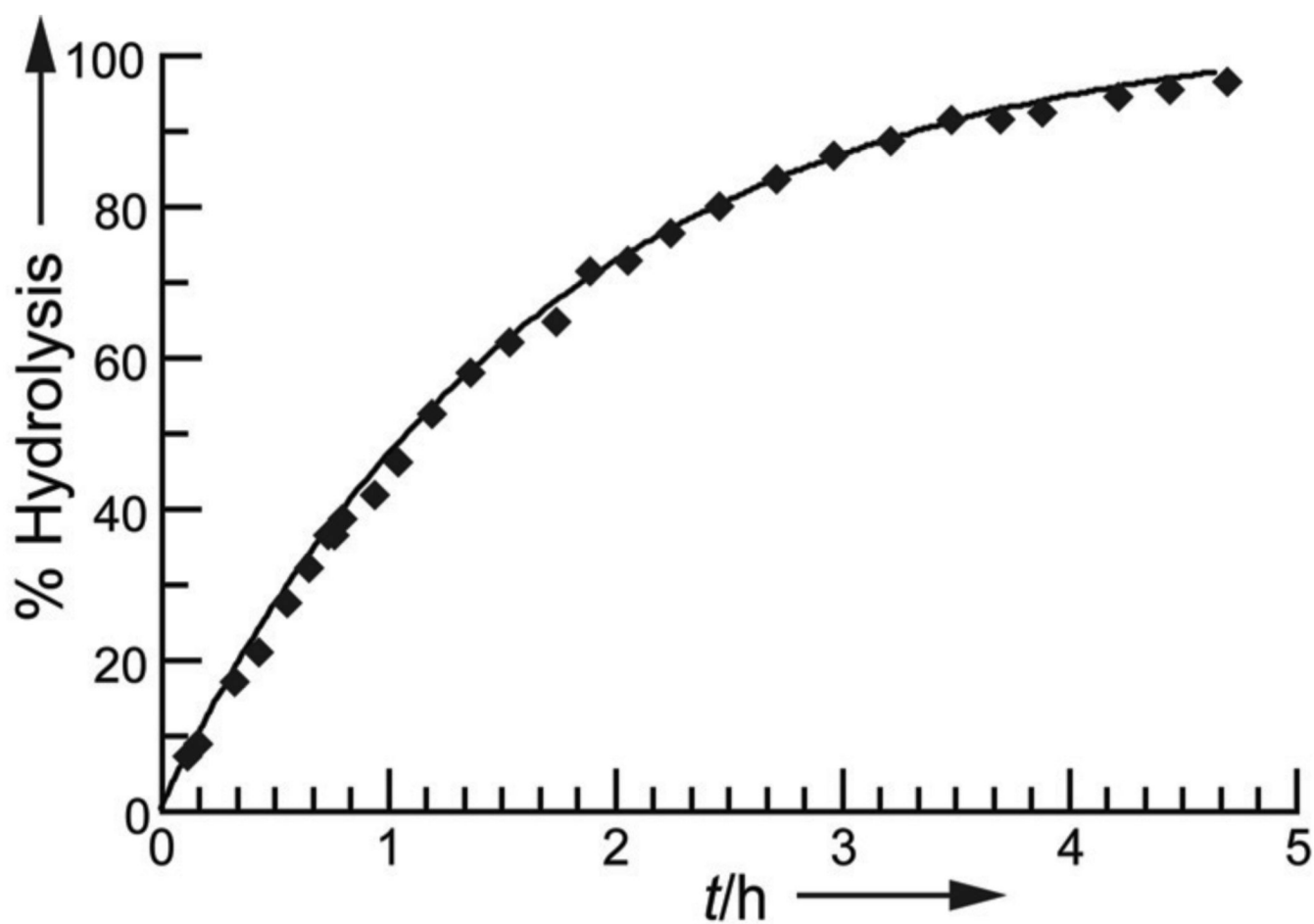Major resonance forms of conjugates.

Figure 2.
Kinetic trace for the hydrolysis of methylhydrazone **1** at pD 7.0 in the presence of a 10-fold molar excess of D_2CO. Each data point was obtained by integration of a ^1H NMR spectrum. Similar kinetic traces were obtained for other hydrolysis reactions.
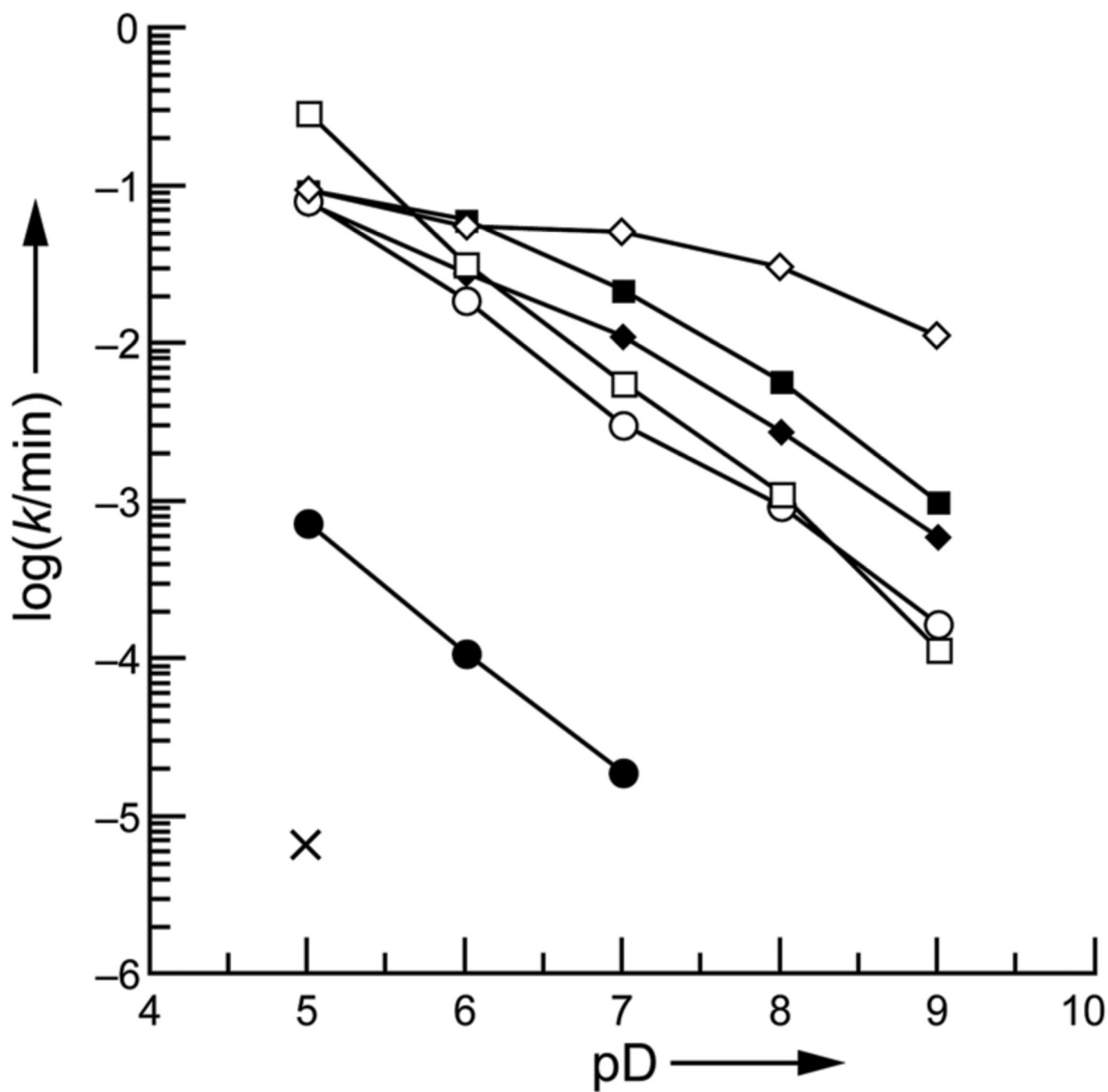
Figure 3.
pD-rate profiles for the hydrolysis of conjugates **1** (◆), **2** (■), **3** (●), **4** (□), **5** (○), **6** (◇), and
7 (×). First-order rate constants (*k*) were calculated from kinetic traces (*e.g.*, Figure 2).
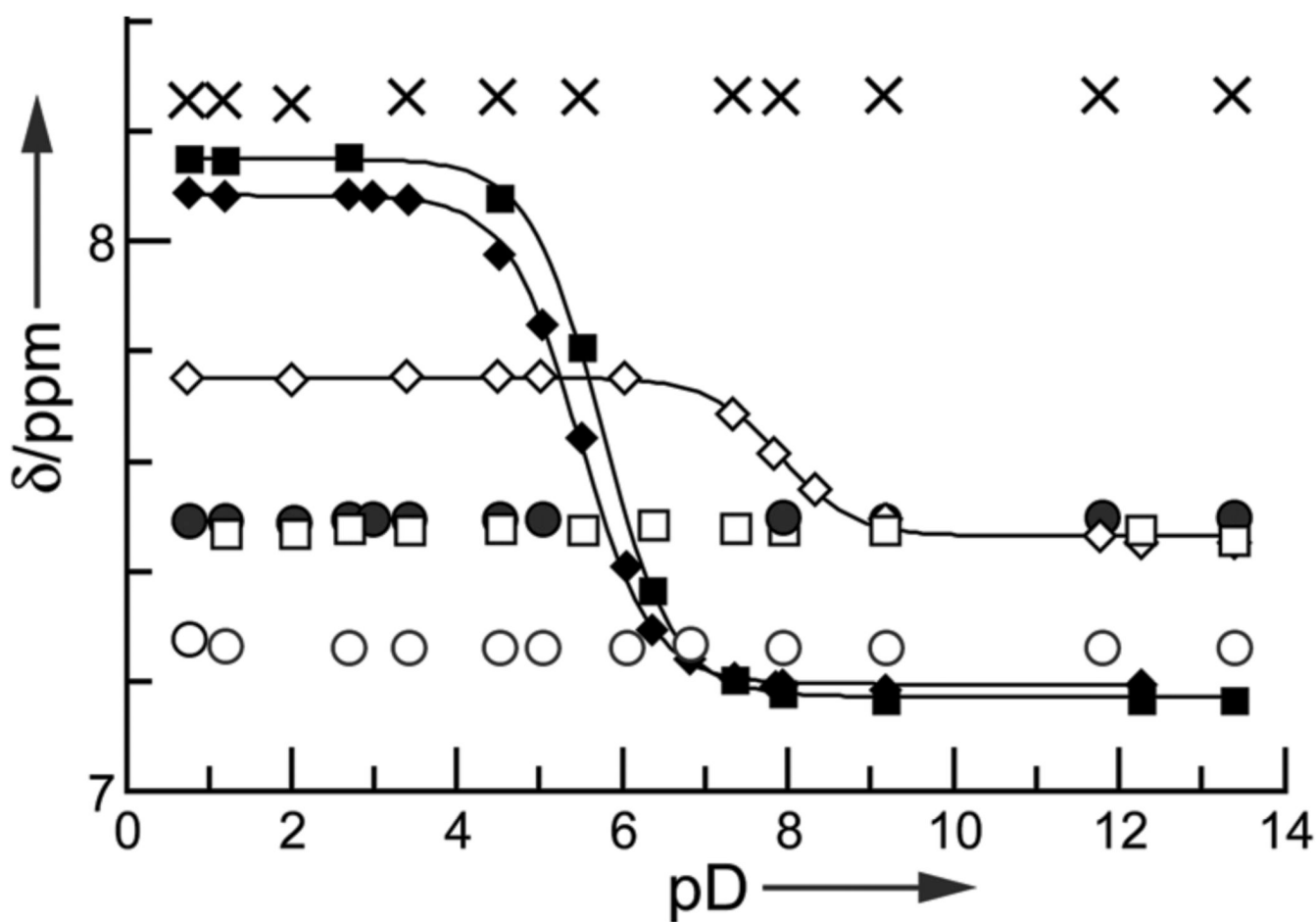
Figure 4.
pD-Titration of the chemical shift of C$^1\underline{H}$ of conjugates **1** (◆), **2** (■), **3** (●), **4** (□), **5** (○), **6** (◇), and **7** (×).
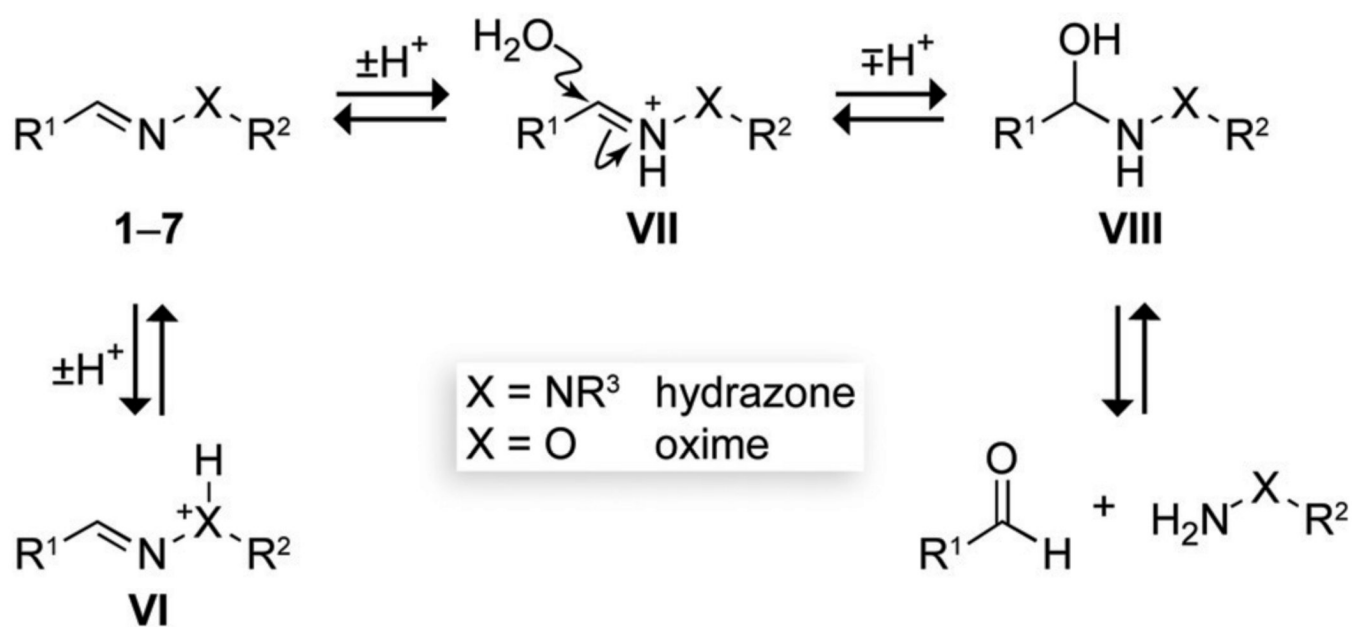
Figure 5.
Putative mechanism for the hydrolysis of hydrazones and oximes.
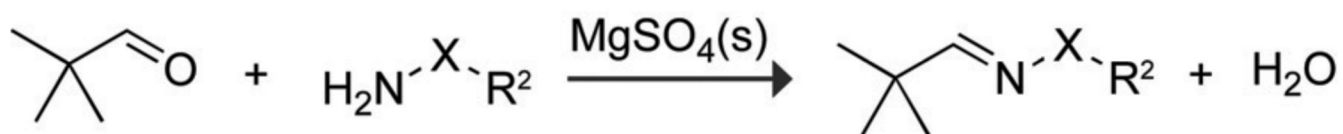
1	X = NH	R^2 = CH_3
2	NCH_3	CH_3
3	O	CH_3
4	NH	$C(O)CH_3$
5	NH	$C(O)NH_2$
6	NH	$C(O)CF_3$
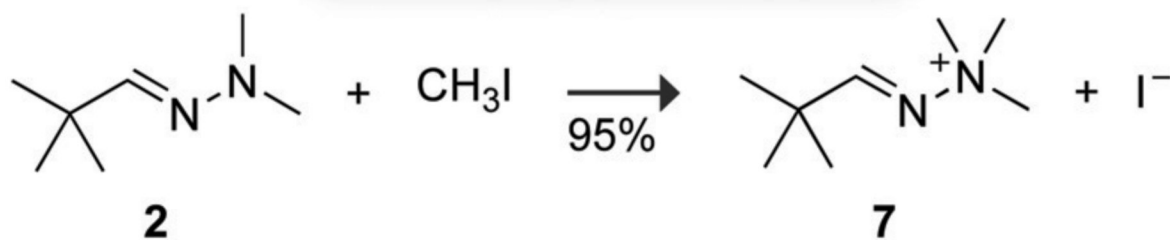
Scheme 1.
Synthesis of conjugates.