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Supporting Information

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Hydrolytic Stability of Hydrazones and Oximes**

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Experimental Procedures

Materials. Anhydrous DMF and CH_2Cl_2 were withdrawn from a CYCLE-TAINER® solvent delivery system (J.T. Baker, Phillipsburg, NJ). Other solvents and chemicals were from Sigma–Aldrich (St. Louis, MO). Synthetic reactions were monitored by thin-layer chromatography with visualization by UV-light, or staining with phosphomolybdic acid. Flash chromatography was performed with columns of silica gel 60, 230–400 mesh (Silicycle, Québec City, Québec, Canada).

Instrumentation. NMR spectra for compound characterization were acquired with a Bruker DMX-400 Avance spectrometer (^{1}H: 400 MHz, ^{13}C: 100 MHz) at the National Magnetic Resonance Facility at Madison (NMRFAM). Samples for compound characterization were prepared in DMSO-d_6 unless stated otherwise. NMR spectra for kinetic analysis were acquired with a Bruker AC+ 300 spectrometer (^{1}H: 300 MHz) at the Magnetic Resonance Facility in the Department of Chemistry. Mass spectra were obtained with a Micromass LCT (electrospray ionization, ESI) in the Mass Spectrometry Facility in the Department of Chemistry. Elemental analyses were performed by Midwest Microlab LLC (Indianapolis, IN).

General procedure for the synthesis of *t*BuCH=NNHCH$_3$ (1), *t*BuCH=NN(CH$_3$)$_2$ (2), and *t*BuCH=NNHCOCH$_3$ (4). *t*BuCHO (13.61 mL, 123.07 mmol) was stirred with the alkylhydrazine or acetylhydrazine (123.07 mmol) for 25 min at 0 °C. The mixture was allowed to warm to room temperature, and stirred for 1.5 h. Anhydrous $MgSO_4$(s) was added, and the mixture was stirred for 15 min. The solid was removed by filtration to yield the hydrazone in >90% yield. Compounds **1** and **2** were obtained as light-yellow liquids, and compound **4** was a white solid.

*t*BuCH=NNHCH$_3$ **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.83 (s, 1H), 4.93 (bs, 1H), 2.78 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 148.8, 35.3, 34.3, 28.2; anal. calcd. for $C_6H_{14}N_2$: C 63.11, H 12.36, N 24.53; found: C 62.05, H 12.00, N 23.20.

*t*BuCH=NN(CH$_3$)$_2$ **2**: HRMS (ESI) $[M+H]^+$ calcd. for $C_7H_{17}N_2$, 129.1392, found 129.1398; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.58 (s, 1H), 2.69 (s, 6H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 147.4, 43.5, 34.4, 28.4.

*t*BuCH=NNHCOCH$_3$ **3**: HRMS (ESI) $[M+Na]^+$ calcd. for $C_7H_{14}N_2ONa$, 165.1004; found 165.0999; ^{1}H NMR (400 MHz, 2 rotamers) δ = 10.83 and 10.74 (s, 1H), 7.36 and 7.21 (s, 1H), 2.04 and 1.83 (s, 3H), 1.03 (s, 9H); ^{13}C NMR (100 MHz, 2 rotamers) δ = 171.5 and 165.1, 156.7 and 153.4, 34.4 and 34.2, 27.2, 21.5 and 20.1.

Synthesis of *t*BuCH=NOCH$_3$ (3). CH_3ONH_2·HCl (4.85 g, 58.08 mmol) was dissolved in DMF (15 mL), and *N,N*-diisopropylethylamine (10.11 mL, 58.08 mmol) and *t*BuCHO (6.42 mL, 58.08 mmol) were added to the resulting solution. The mixture was cooled to 0 °C, stirred for 25 min, and allowed to warm to room temperature. After stirring for 1.5 h, anhydrous $MgSO_4$(s) was added, and the mixture was stirred for 15 min. The solid was removed by filtration, and the filtrate was distilled to yield *t*BuCH=NOCH$_3$ (**3**) as a colorless liquid (1.62 g, 24%, b.p. = 65 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29 (s, 1H), 3.80 (s, 3H), 1.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 158.3, 61.3, 33.6, 27.7; anal. calcd. for $C_6H_{13}NO$: C 62.57, H 11.38, N 12.16, O 13.89; found: C 62.71, H 11.70, N 11.83, O 13.77.

Synthesis of *t*BuCH=NNHCONH$_2$ (5). $NH_2CONHNH_2$·HCl (2.00 g, 17.93 mmol) was dissolved in DMF (20 mL), and Et_3N (2.75 mL, 19.73 mmol) and *t*BuCHO (2.38 mL, 21.52 mmol) were added to the resulting solution. The mixture was cooled to 0 °C, stirred for 25 min, and allowed to warm to room temperature. After stirring for 1.5 h, anhydrous $MgSO_4$(s) was added, and the mixture was stirred for 15 min. After filtration, the organic layer was concentrated under reduced pressure, and the residue was purified by flash chromatography (silica gel, 10% (*v/v*) methanol in methylene chloride) to give *t*BuCH=NNHCONH$_2$ (**5**) as a white solid (1.49 g, 58%). HRMS (ESI) $[M+Na]^+$ calcd. for $C_6H_{13}N_3ONa$:

166.0956, found 166.0964; ^{1}H NMR (400 MHz) δ = 9.76 (s, 1H), 7.08 (s, 1H), 6.11 (bs, 2H), 1.02 (s, 9H); ^{13}C NMR (100 MHz) δ = 157.0, 150.5, 34.0, 27.4.

Synthesis of BocNHNHCOCF$_3$ (8). BocNHNH$_2$ (5.00 g, 37.83 mmol) was dissolved in CH$_3$CN (100 mL). The mixture was cooled to 0 °C, and Et$_3$N (5.8 mL, 41.61 mmol) and (CF$_3$CO)$_2$O (5.25 mL, 37.77 mmol) were added. The reaction mixture was stirred for 1 h. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, ethyl acetate). BocNHNHCOCF$_3$ was obtained as a white solid (7.15 g, 83%). HRMS (ESI) [M+Na]$^+$ calcd. for C$_7$H$_{11}$F$_3$N$_2$O$_3$Na: 251.0619, found 251.0623; ^{1}H NMR (400 MHz) δ = 11.27 (bs, 1H), 9.30 (s, 1H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) δ = 156.3 (q, J(C,F) = 36.1 Hz), 154.5, 115.9 (q, J(C,F) = 288.0 Hz), 80.2, 28.0.

Synthesis of *t*BuCH=NHNHCOCF$_3$ (6). HCl.H$_2$NNHCOCF$_3$ was synthesized by dissolving BocNHNHCOCF$_3$ (**8**) (5.00 g, 21.92 mmol) in HCl (4N) in dioxane (140 mL). The mixture was then stirred for 1h. The solvent was removed under reduced pressure to give an off-white powder. This powder (3.0 g) was transferred to another flask, and dissolved in DMF (20 mL). The resulting solution was cooled to 0 °C, and *t*BuCHO (2.42 mL, 21.88 mmol) and Et$_3$N (2.78 mL, 20.00 mmol) were added. After stirring for 30 min, anhydrous MgSO$_4$(s) was added, and the reaction mixture was allowed to warm to room temperature. After stirring for 1.5 h, the solid was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, methylene chloride). *t*BuCH=NHNHCOCF$_3$ was obtained as a white solid (2.58 g, 95%). HRMS (ESI) [M+Na]$^+$ calcd. for C$_7$H$_{11}$F$_3$N$_2$ONa: 219.0721, found 219.0715; ^{1}H NMR (400 MHz) δ = 7.75 (s, 1H), 1.08 (s, 9H), 1.04 (s, 1H); ^{13}C NMR (100 MHz) δ = 164.7, 152.5 (q, *J*(C,F) = 36.7 Hz), 115.9 (q, *J*(C,F) = 289.4 Hz), 35.0, 26.8.

Synthesis of *t*BuCH=NN(CH$_3$)$_3$I (7). CH$_3$I (0.73 mL, 11.69 mmol) was added to compound **2** (0.50 g, 3.89 mmol), and the mixture was stirred for 15 min at rt. Unreacted CH$_3$I was removed under reduced pressure to yield *t*BuCH=NN(CH$_3$)$_3$I as a yellow solid (1.00 g, 95%). HRMS (ESI) [M]$^+$ calcd. for C$_8$H$_{19}$N$_2$: 143.1548, found 143.1543; ^{1}H NMR (400 MHz) δ = 8.43 (s, 1H), 3.37 (s, 9H), 1.13 (s, 9H); ^{13}C NMR (100 MHz) δ = 174.3, 54.4, 36.0, 26.1.

Kinetics of conjugate hydrolysis. Deuterated sodium phosphate buffers were prepared by dissolving Na$_3$PO$_4$ in D$_2$O to a concentration of 0.15 M. Acidity was adjusted by adding D$_3$PO$_4$, to pDs 5.0, 6.0, 7.0, 8.0, and 9.0 (pD = pH meter reading + 0.41).[1] The conjugates were dissolved to a concentration of 25.00 mM in buffer solutions containing D$_2$CO (0.25 M, added from a 20% (*v*/*v*) D$_2$CO solution in D$_2$O). ^{1}H NMR spectra were obtained at the desired time-points, and the extent of hydrolysis was quantitated by peak integration. Hydrolysis resulted in the appearance of the aldehyde, characterized by the formyl proton at 9.4 ppm, a *t*Bu singlet at 1.0 ppm, and a *t*Bu peak of the hydrated aldehyde at 0.8 ppm. As expected, there was a concurrent decrease in the intensities of peaks due to the conjugate, namely, the *t*Bu group at ~1 ppm, and the proton attached to the double-bonded carbon atom at ~7–8 ppm. The area under the three peaks corresponding to the *t*Bu groups were assigned a cumulative value of 9, serving as an internal standard for integration. Hydrolysis was quantitated according to the eq S1:

$$\%\ \text{hydrolysis} = 100\,\frac{A+\frac{B}{9}}{A+\frac{B}{9}+C} \qquad \text{(S1)}$$

where A is the area under the peak at 9.4 ppm, B is the area under peak at 0.8 ppm, and C is the area under the peak at ~7–8 ppm. Hydrolysis was allowed to proceed to >95% completion. % Hydrolysis was plotted versus time, and the data were fitted to eq S2:

[1] A. K. Covington, M. Paabo, R. A. Robinson, R. G. Bates, *Anal. Chem.* **1968**, *40*, 700–706.

$$Y = Y_{max}(1 - e^{-kt}) \quad \text{(S2)}$$

where Y is the % hydrolysis, t is time, k is the first-order rate constant, and Y_{max} is the % hydrolysis at $t = \infty$. Kinetic traces were obtained in duplicate, and half-lives were calculated with eq S3:

$$t_{1/2} = \frac{0.693}{k} \quad \text{(S3)}$$

NMR titration of conjugates. Deuterated buffers were prepared in the pD range of 0.73–13.36. Trichloroacetic acid (0.40 M)–NaOD was used as a buffer in the pD range of 0.73–2.01, chloroacetic acid (0.40 M)–NaOD was used in the pD range of 2.67–3.39, acetic acid (0.40 M)–NaOD was used in the pD range of 4.50–6.02, and sodium phosphate (0.17 M) was used in the pD range of 6.34–13.36. The ionic strength of the buffers was maintained at I = 0.45 M by the addition of KCl. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal standard for referencing the chemical shift. The conjugates were dissolved in the buffers to a concentration of 25.00 mM, and the chemical shift of the proton attached to the double-bonded carbon was obtained at different pDs. The chemical shifts were plotted against the pDs to generate the data-points in Figure 4. Titration curves for methylhydrazone **1**, dimethylhydrazone **2**, and trifluoroacetylhydrazone **6** were obtained by fitting the data-points to eq S4:

$$\delta = \delta_{bottom} + \frac{\delta_{top} - \delta_{bottom}}{1 + \frac{K_a}{[D^+]}} \quad \text{(S4)}$$

where δ is the chemical shift, δ_{bottom} is the chemical shift at high pDs where the conjugate exists as a free base, δ_{top} is the chemical shift at low pDs where the conjugate is completely protonated, and pK_a is the point of inflection of the curve.

Table S1: Values of $t_{1/2}$ for the hydrolysis of conjugates **1**–**7** at pD 5.0–9.0.

Conjugate	pD 5.0	pD 6.0	pD 7.0	pD 8.0	pD 9.0
1	9 ± 1 min	24.5 ± 0.6 min	1.0 ± 0.1 h	4.2 ± 0.6 h	19.5 ± 0.5 h
2	7.4 ± 0.5 min	11.3 ± 0.2 min	32 ± 3 min	2.0 ± 0.1 h	11.7 ± 0.1 h
3	15.7 ± 0.4 h	4.4 ± 0.3 d	~25 d	not determined	not determined
4	2.4 ± 0.4 min	21.4 ± 0.8 min	2.0 ± 0.2 h	10.17 ± 0.02 h	4.2 ± 0.7 d
5	8.5 ± 0.4 min	36 ± 2 min	3.8 ± 0.5 h	12.3 ± 0.8 h	2.9 ± 0.1 d
6	7.5 ± 0.9 min	12.4 ± 0.8 min	14 ± 1 min	23 ± 1 min	1.0 ± 0.1 h
7	10.3% hydrolysis in 17 d	not determined	no hydrolysis detected in 22 d	not determined	not determined