

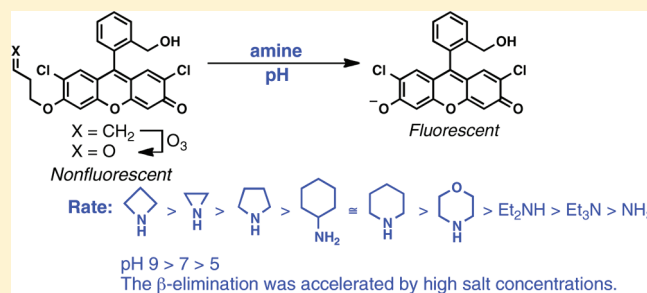
Amine-Promoted β -Elimination of a β -Aryloxy Aldehyde for Fluorogenic Chemodosimeters

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Supporting Information

ABSTRACT: We previously reported a fluorescent chemodosimeter for ozone. The β -elimination step after the ozonolysis of the chemodosimeter was too slow to be practical for real-time monitoring of ozone. We examined primary, secondary, and tertiary amines at various pHs. It was found that pyrrolidine in pH 9 buffer could accelerate the elimination to generate a fluorescence signal. The elimination step is now sufficiently rapid to monitor ozone exposure in real time. We also discovered that azetidine was distinctly effective for the same elimination reaction in a pH 6 buffer.



Instrument-generated ozone is frequently used for the sanitization of swimming pools, drinking water,¹ food contact surfaces,² houses, and surgical rooms.³ In contrast to these beneficial applications of ozone, natural atmospheric ozone negatively impacts human health. It has been argued that⁴ ozone at ground level may induce acute respiratory symptoms among asthmatics⁵ and might be a cause of asthma.⁶ Therefore, measurement of personal exposure to ozone would prove highly beneficial for workers in high-ozone areas and those who already suffer from respiratory illness. Ozone badges are commercially available, but more sensitive, selective, and robust colorimetric or fluorometric methods need to be developed.

We reported a fluorescence-based method that was selective for ozone (Scheme 1).⁷ In this method, chemodosimeter **1** reacted with ozone by a 1,3-dipolar cycloaddition mechanism to form molozonide **2**.⁸ This species underwent retro-[3 + 2] cyclization to form aldehyde **3** and compound **4**. In water, these compounds presumably did not react with each other to form the corresponding ozonide.⁹ In the final step, a β -elimination occurred to convert the nonfluorescent aldehyde **3** to the fluorescent compound **5**. In tissue culture media, the β -elimination was found to be facile,⁷ presumably due to the presence of albumin.¹⁰ However, for environmental samples, the β -elimination required more than 1.5 h at 37 °C⁷ and even more hours at room temperature (see below). This slow step would be detrimental to real-time monitoring of ozone exposure.

In order to enable real-time monitoring of ozone, it was necessary to develop a method to accelerate the β -elimination step (**3** to **5**). Such a method would be beneficial not only for this ozone detection project but also for the development of chemodosimeters that exploit a similar β -elimination.¹¹ Compound **5** displays maximal fluorescence between pH 5 and pH 9.¹² Therefore, we set out to develop a method to accelerate the β -elimination step within this pH range.

At the outset of this study, it was unclear whether the β -elimination proceeds by an E2 mechanism or an E1cb mechanism¹³ or both. Considering the ease of fluorescence measurement in a high-throughput manner and the multiple parameters that can influence the rate of β -elimination near neutral pH,¹⁴ we chose to screen for optimal pH and reagents.

Unlike our previous work,⁷ in this work we isolated and characterized aldehyde **3** after exposure of compound **1** to ozone, although this aldehyde could not be fully purified because of its instability.¹⁵ Using the moderately pure aldehyde **3** as the starting material, we examined ammonia, cyclohexylamine, pyrrolidine, and triethylamine as primary, secondary, and tertiary amines at pH 5, 6, 7, 8, and 9 (Figure 1). Anilines, although potentially catalytically active in this case,¹⁶ were excluded from the screening efforts because of their air sensitivity. Generally, the β -elimination, as determined by the fluorescence signal from compound **5**, was faster at higher pHs (Figure 1, “no amine”). Ammonia ($pK_a = 9.2$ for NH_4^+) and Et_3N ($pK_a = 10.75$ for Et_3NH^+) did not appear to accelerate the β -elimination significantly. Cyclohexylamine ($pK_a = 10.66$ for $c\text{-HexNH}_3^+$) accelerated the β -elimination, particularly in pH 8 and 9 buffers. Pyrrolidine ($pK_a = 11.3$ for pyrrolidinium ion) was found to be even more effective, especially in the pH 9 buffer. These results indicate that it is critical to form an iminium ion with a secondary amine (e.g., **6**, Scheme 2) or an imine with a primary amine during the elimination reaction.

We also tested other secondary amines, piperidine, morpholine, and Et_2NH (Figure 1). The most sterically hindered Et_2NH was found to be the least effective promoter for the conversion of **3** to **5**. Piperidine ($pK_a = 11.2$ for piperidinium ion) accelerated the reaction better than less basic but sterically similar morpholine

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Scheme 1. Fluorescence-Based Detection of Ozone Using Compound 1 as an Indicator

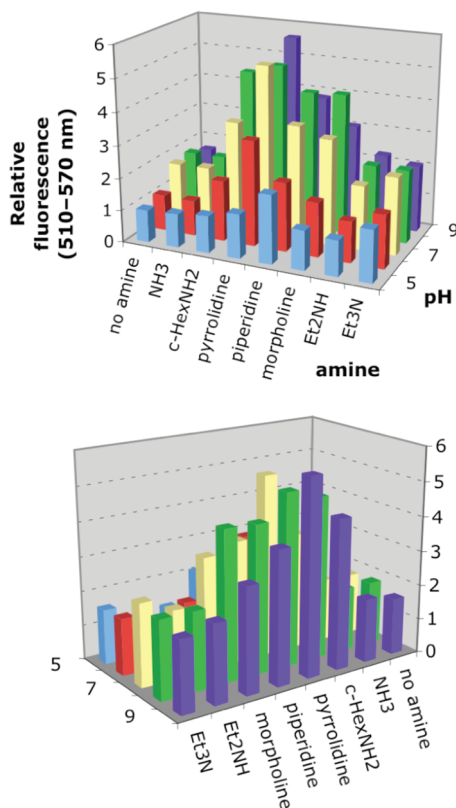
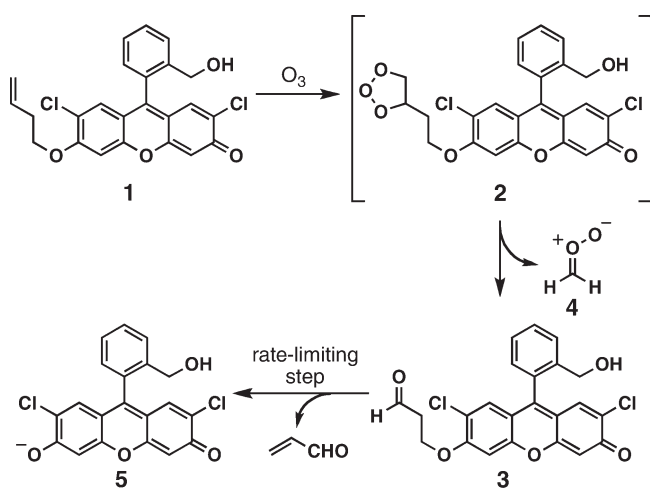
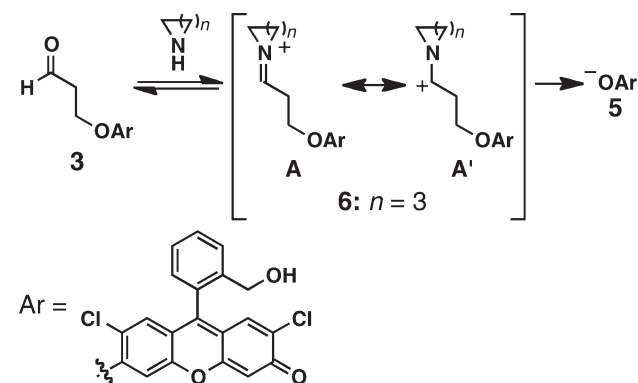


Figure 1. Conversion of the nonfluorescent aldehyde 3 to the fluorescent compound 5 in the presence of various amines in pH 5–9 buffers. $[3] = 1 \mu M$, $[amine] = 1 mM$, $25^\circ C$, 20 min, 1:9 (v/v) MeOH/buffer. The fluorescence intensity was normalized. The same graph is shown from two different angles.

($pK_a = 8.46$ for morpholinium ion). Pyrrolidine and piperidine are similarly basic ($pK_a = 11.3$ for pyrrolidinium ion), but pyrrolidine was reproducibly¹⁷ superior as a promoter in this system. Proline was also tested but was less effective than pyrrolidine (data not shown). It is noteworthy that a series of Spencer's seminal studies showed that pyrrolidine was more effective than other amines¹⁴

Scheme 2. Plausible Pathway Leading to Compound 5



and that the pH values of the reaction solutions and the pK_a values of amines were related to the rates of the β -eliminations of β -hydroxy and β -acetoxy ketones.^{14,18}

In order to gain insight into the mechanism of the pyrrolidine-promoted β -elimination, preliminary kinetic studies were performed in a pH 7 phosphate buffer.¹⁹ The reaction rate, as determined by fluorescence, appeared to show a saturation curve with regard to the concentrations of pyrrolidine in the 0–2 mM range (Figure 2a). A likely scenario is that the rate-determining step (RDS) in this range is the reversible formation of iminium ion 6 (Scheme 2). The RDS presumably starts shifting to the elimination step when the concentration of pyrrolidine is above 2 mM, as the rate becomes linearly proportional to the concentration of pyrrolidine. The rate could not be measured accurately at higher pyrrolidine concentrations due to the altered pH of the reaction media. The reaction was too fast to be measured accurately when the concentrations of phosphate salts were high (see below).

The reaction rate increased nearly linearly when the concentrations of phosphate ions were high (Figure 2b). We used a low concentration of pyrrolidine (0.25 mM) in this experiment to slow down the reaction so that we could measure the rate accurately. Under these conditions, phosphate ions accelerated the reaction possibly by either enhancing the formation of iminium ion 6 with high ionic strength or deprotonating the α -proton of 3 and 6 to facilitate the elimination reaction. This is the second system from our laboratory that benefited from a phosphate-accelerated fluorogenic reaction.²⁰

At this point, our findings are that the rate of the β -elimination (3 to 5) increases as the concentrations of pyrrolidine and phosphate ions increase and that pH 9 was optimal within the aforementioned allowed pH range. Therefore, it became apparent that higher concentrations of pyrrolidine and borate²¹ at pH 9 would further facilitate the β -elimination. In the next experiment, the concentration of pyrrolidine was raised to 47.5 mM, and a commercially available concentrated pH 9 buffer (0.5 M borate) was used. As Figure 2c shows, the initial rate of the β -elimination was 30 times greater in the presence of pyrrolidine (47.5 mM) under these conditions. The β -elimination reaction was essentially complete in 3 min at room temperature unlike our previous work where the β -elimination required >1.5 h.

We proceeded to determine whether chemodosimeter 1 could be used to detect ozone in a pyrrolidine solution at pH 9 (Figure 2d). In the absence of pyrrolidine, the chemodosimeter apparently reacted with ozone as indicated by stronger fluorescence

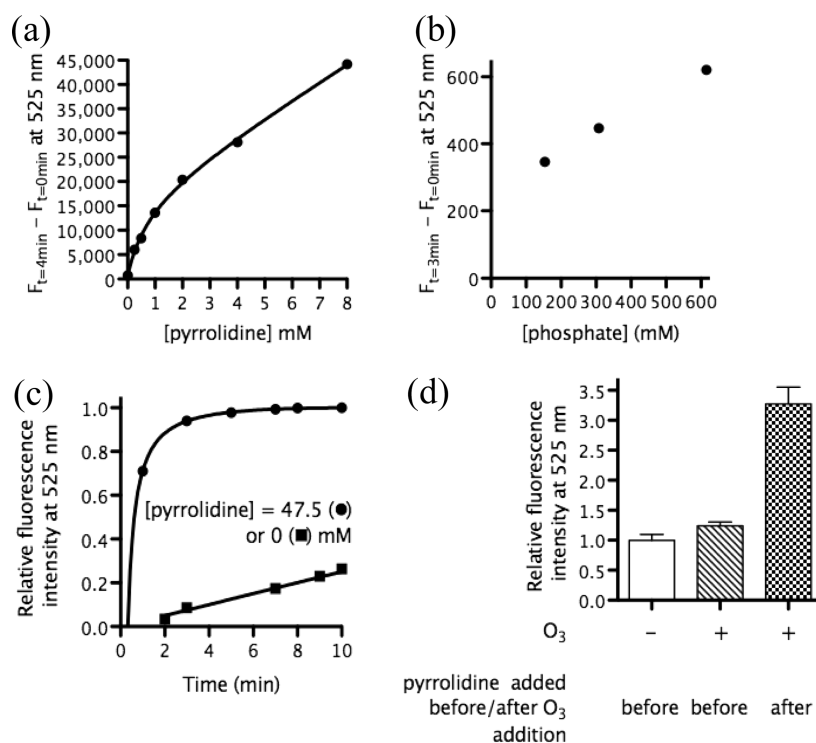


Figure 2. (a) $[3] = 1 \mu\text{M}$, $[\text{pyrrolidine}] = 0, 0.25, 0.5, 1, 2, 4,$ and 8 mM , 25°C , 4 min , $5:95 \text{ (v/v) MeOH/pH } 7 \text{ phosphate (} 50 \text{ mM) buffer}$. (b) $[3] = 1 \mu\text{M}$, $[\text{pyrrolidine}] = 0.25 \text{ mM}$, 25°C , 3 min , $5:95 \text{ (v/v) MeOH/pH } 7 \text{ phosphate (} 154, 308, \text{ and } 615 \text{ mM) buffer}$. (c) $[3] = 10 \text{ nM}$, $[\text{pyrrolidine}] = 0$ or 47.5 mM , 25°C , $5:95 \text{ (v/v) MeOH/pH } 9 \text{ borate (} 0.50 \text{ M) buffer}$. (d) $[1] = 1 \mu\text{M}$, $[\text{pyrrolidine}] = 0$ or 47.5 mM , 25°C , $5:95 \text{ (v/v) MeOH/pH } 9 \text{ borate (} 0.50 \text{ M) buffer}$.

after pyrrolidine was added. On the other hand, the chemodosimeter did not react with ozone in the presence of pyrrolidine. This is consistent with the reactivity of amines with ozone at higher pHs.²² Therefore, in order to develop an ozone detection kit based on compound **1**, it is recommended that compound **1** be exposed to ozone in the absence of pyrrolidine and that pyrrolidine be added before fluorescence measurement (for a representative example, see Figure 3 and “Ozone detection with compound **1**” in the Experimental Section).

To justify the pyrrolidine-promoted β -elimination of **3**, we hypothesized that ring strain of the pyrrolidine-derived iminium ions might play a crucial role. More specifically, the iminium ion **A** (Scheme 2) with a smaller n value might suffer from ring strain to a greater extent, thus increasing a degree of contribution from resonance structure **A'**. The more carbocation-like character of the iminium ion would increase the acidity of the adjacent C–H, which would then facilitate the deprotonation step.

To test this hypothesis, additional cyclic secondary amines were examined in pH 6 and 7 buffers (Figure 4). In both buffers, azetidines promoted the β -elimination of **3** most efficiently. Specifically, the initial rate of the azetidines-promoted elimination in a pH 6 buffer was 33 times and 6 times greater than those of the background and pyrrolidine-promoted reactions, respectively. Although aziridine was an effective catalyst in a different iminium ion system,²³ azetidines were more effective in our system. These data are consistent with our hypothesis, but it would be premature to attribute the rate enhancement exclusively to strain. More rigorous mechanistic studies, as represented by a series of studies by the Spencer group for a related system,^{14,18} are needed to understand the structure–reactivity relationship in a quantitative manner.

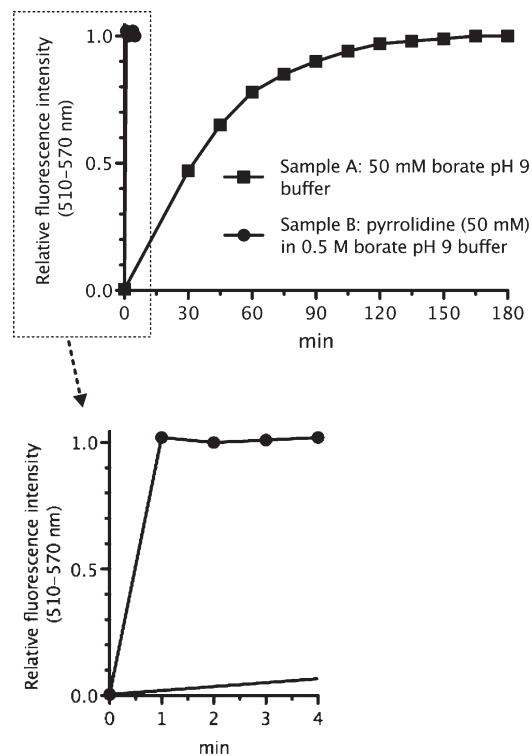


Figure 3. Fluorescence increase after exposure of **1** to ozone.

In conclusion, we have developed a more rapid method to detect ozone by fluorescence than our previous one. Pyrrolidine

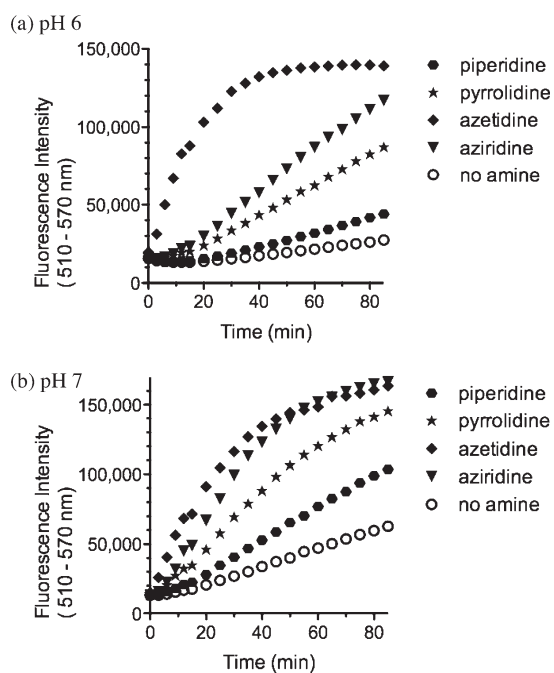


Figure 4. Conversion of the nonfluorescent aldehyde **3** to the fluorescent compound **5** in the presence of secondary cyclic amines in (a) pH 6 or (b) pH 7 buffer. Conditions: $[3] = 1 \mu\text{M}$, $[\text{amine}] = 200 \mu\text{M}$, 25°C , 1:9 (v/v) MeOH/buffer. The experiments were performed in duplicate, and the average values are shown in the graphs. Error bars were omitted for clarity. See Figure S5 in the Supporting Information for error bars.

presumably reacts with aldehyde **3** to form the putative iminium ion **6**, rendering the α -protons more acidic. One of the α -protons is abstracted by a base to trigger the elimination process. The azetidione-promoted elimination reaction suggests that azetidione derivatives may serve as organocatalysts.

EXPERIMENTAL SECTION

General Techniques. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride. The conversion of **1** to **3** was monitored by thin-layer chromatography (TLC) carried out on 0.25-mm silica gel plates (60F-254) using heat or UV light (254 or 365 nm) for visualization. Silica gel (230–400 mesh) was used for flash chromatography. Ultrapure water was prepared from a Barnstead Nanopure Diamond Ultrapure Water System.

NMR spectra were recorded on an AM300 instrument and calibrated using a solvent peak as an internal reference. The following abbreviations are used to indicate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent. High-resolution mass spectra were obtained using either a VG Autospec having EBE geometry and electron impact ionization (EI) or a Q-TOF API-US with electrospray ionization (ESI) in the positive ion modes.

Buffer solutions used in this study are as follows: pH 5.00 buffer solution (50 mM potassium hydrogen phthalate, <50 mM sodium hydroxide), pH 6.00 buffer solution (50 mM potassium hydrogen phthalate, <50 mM sodium hydroxide), pH 7.00 buffer concentrate (potassium phosphate monobasic/sodium hydroxide, 1.23 M), pH 8.00 buffer (50 mM sodium phosphate, dibasic/potassium phosphate), pH 9.00 buffer (50 mM borate), pH 9.00 buffer concentrate (0.5 M borate). The pH 7.00 buffer concentrate was diluted with ultrapure water to prepare the corresponding diluted pH 7 buffer solutions.

Fluorescence spectra for Figure 2c were recorded in a 1-mL cuvette (340–800 nm) on a Jobin Yvon FluoroMax-3 spectrometer under the

Chart 1

	no amine	NH_4Cl	cyclohexylamine	pyrrolidine	piperidine	morpholine	Et_2NH	Et_3N	
A	○	○	○	○	○	○	○	○	pH 5
B	○	○	○	○	○	○	○	○	pH 6
C	○	○	○	○	○	○	○	○	pH 7
D	○	○	○	○	○	○	○	○	pH 8
E	○	○	○	○	○	○	○	○	pH 9
	1	2	3	4	5	6	7	8	

control of a Windows-based PC running FluorEssence software. The samples were excited at 496 nm and the emission intensities were collected between 500 and 600 nm wavelengths. All spectra were corrected for emission intensity using the manufacturer supplied photo-multiplier curves.

Other fluorescence spectra were recorded in black 96-well polypropylene assay plates on a Modulus II Microplate Multimode Reader with a blue optical kit (maximum excitation wavelength 490 nm, emission wavelength 510–570 nm).

The fluorescence signals of compound **5** in 1% DMSO/50 mM phosphate pH 7 buffer were measured at various concentrations as shown in Figure S1 (Supporting Information).

Preparation of 3. Ozone was bubbled into a stirred solution of alkene **1** (49.0 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at -78°C under an air atmosphere for 5 min, upon which the reaction solution turned from yellow to orange. The solution was then purged with nitrogen at -78°C and then treated with dimethyl sulfide (17 μL , 0.22 mmol). The resulting solution was gradually warmed to 25°C . After 12 h at the same temperature, the solvents were removed in vacuo. The resulting residue was purified by preparative TLC (50% EtOAc in hexanes) to afford aldehyde **3** (27.0 mg, 55% yield) as a pale yellow solid. This material could not be purified further due to its instability.

Data for **3** (because of the equilibrium between **3** and its cyclic ether form, NMR spectra showed more peaks than the structure of **3** indicates): $R_f = 0.29$ (50% EtOAc in hexanes); IR (KBr pellet) 3401 (br), 2925, 1715, 1631, 1591, 1488, 1433, 1412, 1268, 1177 cm^{-1} ; ^1H NMR (300 MHz, 295K, 1% $\text{CD}_3\text{OD}/\text{CDCl}_3$) δ 9.90 (t, 1H, $J = 1.8$ Hz), 7.42–7.29 (m, 3H), 6.91–6.78 (m, 5H), 5.32 (s, 2H), 4.40 (dt, 2H, $J = 6.0, 2.4$ Hz), 3.00 (t, 2H, $J = 6.0$ Hz); ^{13}C NMR (75 MHz, 293K, $\text{DMSO}-d_6$) δ 201.3, 154.0, 153.8, 153.7, 149.0, 148.8, 148.7, 144.6, 138.3, 138.2, 129.0, 128.8, 128.6, 128.44, 128.36, 123.0, 121.5, 118.0, 116.8, 116.7, 115.7, 115.5, 103.2, 101.7, 82.0, 72.2, 72.0, 63.3, 48.6, 42.3 (more than 23 peaks due to the tautomerization); HRMS (EI+) m/z calcd. for $\text{C}_{23}\text{H}_{16}\text{Cl}_2\text{O}_5$ $[\text{M}]^+$ 442.0375, found 442.0370.

Conversion of the Nonfluorescent Aldehyde 3 to the Fluorescent Compound 5 in the Presence of Various Amines in pH 5–9 Buffers (Figure 1). (a). *Preparation of Stock Solutions.* The concentrated pH 7 phosphate buffer was diluted with ultrapure water to a 50 mM phosphate solution. 100 mM solutions of NH_4Cl , cyclohexylamine, pyrrolidine, piperidine, morpholine, Et_2NH , and Et_3N were prepared in the 50 mM phosphate pH 7 buffer. These solutions (0.40 mL of each) were diluted with ultrapure water (3.6 mL) to prepare the 10 mM amine solutions with 5 mM phosphate. Aldehyde **3** was dissolved in MeOH to prepare a 10 μM solution.

(b). *Conversion of 3 to 5.* A black 96-well plate was used (Chart 1). All of the following steps were performed at 25°C .

Step 1: The 50 mM pH 5, 6, 7, 8, or 9 buffer solution (160 μL) was transferred to each well in rows A, B, C, D, or E, columns 1–8, respectively.

Step 2: A 5 mM pH 7 phosphate buffer (20 μL) was added to each well in column 1, rows A–E. The 10 mM NH_4Cl solution (20 μL) was added to each well in column 2, rows A–E. The 10 mM cyclohexylamine solution (20 μL) was added to each well in column 3, rows A–E. The 10 mM pyrrolidine solution (20 μL) was added to each well in column 4, rows A–E. The 10 mM piperidine solution (20 μL) was added to each well in column 5, rows A–E. The 10 mM morpholine solution (20 μL) was added to each well in column 6, rows A–E. The 10 mM Et_2NH solution (20 μL) was added to each well in column 7, rows A–E. The 10 mM Et_3N solution (20 μL) was added to each well in column 8, rows A–E.

Step 3: The MeOH solution of aldehyde **3** (10 μM , 20 μL) was added to each of the 40 wells (columns 1–8, rows A–E). Final conditions: [**3**] = 1 μM , [amine] = 0 or 1 mM, 1:9 (v/v) MeOH:buffer, [buffer salt] = 45 mM.

Step 4: Green fluorescence emission was monitored every minute for 20 min. The raw data at 20 min are shown in Table S1 (Supporting Information).

Pyrrolidine-Accelerated β -elimination of Aldehyde **3 (Figure 2a).** (a). *Preparation of Stock Solutions.* The 100 mM pyrrolidine solution in 50 mM pH 7 phosphate buffer (192 μL) was diluted with a 50 mM pH 7 phosphate buffer (108 μL) to prepare a 64 mM pyrrolidine solution in a 50 mM phosphate pH 7 buffer. This solution was serially diluted (2 \times) with a 50 mM pH 7 phosphate buffer to prepare 32, 16, 8, 4, 2, and 1 mM pyrrolidine solutions. A 2.5 μM solution of **3** was prepared in 1:4 (v/v) MeOH/water.

(b). *Conversion of **3** to **5**.* A black 96-well plate was used. All of the following steps were performed at 25 $^\circ\text{C}$. This experiment was performed in duplicate. One of the two sets of data is reported in this paper for clarity.

Step 1: The 50 mM pH 7 phosphate buffer (100 μL) was transferred to each of 7 wells.

Step 2: The 0, 1, 2, 4, 8, 16, or 32 mM pyrrolidine solution in 50 mM phosphate pH 7 buffer (50 μL) was added to each of these wells.

Step 3: The 2.5 μM solution of **3** (50 μL) was added to each of these wells.

Step 4: Green fluorescence emission was monitored every 2 min for 10 min. The data at 4 min were analyzed. For each pyrrolidine concentration, $F_{t=4 \text{ min}}$ (fluorescence intensity at 4 min) – $F_{t=0 \text{ min}}$ (fluorescence intensity at 0 min) was calculated. The raw data are shown in Table S2 (Supporting Information).

Phosphate-Accelerated β -Elimination of Aldehyde **3 (Figures 2b and S3 (Supporting Information)).** (a). *Preparation of Stock Solutions.* A 50 mM pyrrolidine solution in 50 mM phosphate pH 7 buffer (100 μL) was diluted with ultrapure water (9.9 mL) to prepare a 1 mM pyrrolidine solution in 0.5 mM phosphate pH 7 buffer. A 1.23 M phosphate pH 7 buffer concentrate was serially diluted to prepare 615, 307.5, 153.8, and 76.9 mM phosphate pH 7 buffer solutions.

(b). *Conversion of **3** to **5**.* A black 96-well plate was used. All of the following steps were performed at 25 $^\circ\text{C}$. This experiment was performed in duplicate. One of the two sets of data is reported in this paper for clarity.

Step 1: The 1230, 615, 307.5, 153.8, or 76.9 mM phosphate pH 7 buffer solution (100 μL) was transferred to each of five wells.

Step 2: The 1 mM pyrrolidine solution in 0.5 mM phosphate pH 7 buffer (50 μL) was added to each of the five wells.

Step 3: The 2.5 μM solution of **3** (50 μL) was added to each of the five wells.

Step 4: Green fluorescence emission was monitored every minute for 4 min. The data at 3 min were analyzed. For each phosphate concentration, $F_{t=3 \text{ min}}$ (fluorescence intensity at 3 min) – $F_{t=0 \text{ min}}$ (fluorescence intensity at 0 min) was calculated. The raw data are shown in Table S3 (Supporting Information).

Kinetics of the β -Elimination of Aldehyde **3 with High [Pyrrolidine] and High [Borate] (Figure 2c).** (a). *Preparation of Stock Solutions.* Pyrrolidine (14.3 mg, 0.20 mmol) was dissolved in 0.5 M borate pH 9 buffer (4.0 mL) to prepare a 50 mM pyrrolidine solution in the borate buffer.

(b). *Conversion of **3** to **5**.* All of the following steps were performed at 25 $^\circ\text{C}$.

Step 1: A 0.5 M borate pH 9 buffer solution (0.95 mL) was transferred to a 1-mL cuvette. The 50 mM pyrrolidine solution in 0.5 M borate pH 9 buffer (0.95 mL) was transferred to another 1-mL cuvette.

Step 2: A 0.2 μM solution of aldehyde **3** in MeOH (50 μL) was added to each of these two cuvettes.

Step 3: Fluorescence spectra were recorded at the indicated times (Figure 2c) in these cuvettes. The raw data are shown in Figure S4 (Supporting Information).

Fluorometric Detection of Ozone Using Compound **1 in the Presence and Absence of Pyrrolidine (Figure 2d; for Flowchart, See Chart S1 in the Supporting Information).** (a).

Preparation of Stock Solutions. Pyrrolidine (286 mg, 4.0 mmol) was dissolved in a 0.5 M borate pH 9 buffer (40 mL) to prepare a 100 mM pyrrolidine solution. This solution (20 mL) was diluted with a 0.5 M borate pH 9 buffer (20 mL) to prepare a 50 mM pyrrolidine solution. A saturated solution of ozone in MeOH was prepared by continuously bubbling ozone into MeOH at $-78 \text{ }^\circ\text{C}$.

(b). *Conversion of **1** to **5**.* All of the following steps were performed at 25 $^\circ\text{C}$.

Step 1: The 50 mM pyrrolidine solution in a 0.5 M borate pH 9 buffer (1.8 mL) was transferred to each of vials 1–6. The 0.5 M borate pH 9 buffer (1.8 mL) was transferred to each of vials 7–9.

Step 2: A 20 μM solution of chemodosimeter **1** in MeOH (0.20 mL) was added to each of vials 1–9.

Step 3: MeOH (20 μL) was added to each of vials 1–3. The saturated solution of ozone in MeOH (20 μL) was added to each of vials 4–9.

Step 4: Approximately 1 min after step 3, the 50 mM pyrrolidine solution in 0.5 M borate pH 9 buffer (1.8 mL) was added to each of vials 1–6. The 100 mM pyrrolidine solution in 0.5 M borate pH 9 buffer (1.8 mL) was added to each of vials 7–9. These solutions were incubated for 5 min.

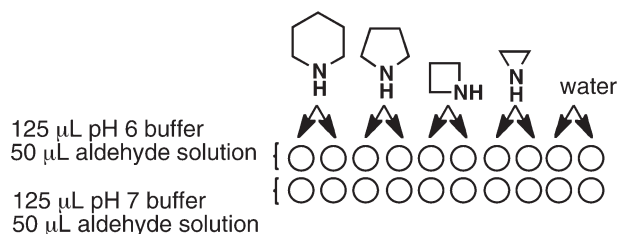
Step 5: Each reaction solution (150 μL) from the nine vials was transferred to a black 96-well plate. The fluorescence was measured using the plate reader. The average fluorescence intensity and standard deviations are reported in Figure 2d after normalization. The raw data are shown in Table S4 (Supporting Information).

Ozone Detection with Compound **1 (Figure 3).** A 3 mM solution of compound **1** in EtOH (50 μL ; 150 nmol) was applied to each of two pieces of 5 \times 5 cm adsorbent paper. The organic solvent was then evaporated in an open atmosphere. These two pieces of paper (samples A and B) were placed for 15 min in a fume hood containing an operating ozone generator. Subsequently, samples A and B were placed in vials. Sample A was treated with 50 mM borate pH 9 buffer (5 mL), and sample B was treated with 500 mM borate pH 9 buffer containing 50 mM pyrrolidine (5 mL). Each solution (200 μL) was transferred to a 96-well plate, and the fluorescence signal was monitored. All of the operations were conducted at 25 $^\circ\text{C}$. As Figure 3 shows, the conversion of **3** to **5** was complete in less than 1 min for sample B and in ~ 2 h for sample A.

Secondary Cyclic Amine-Accelerated β -Elimination of Aldehyde **3 (Figure 4).** Aldehyde **3** was prepared by the ozonolysis of **1** 1 day prior to this experiment and stored at $-80 \text{ }^\circ\text{C}$.

Stock Solutions for This Experiment. Solutions: 100 mM piperidine in 50 mM phosphate pH 7 buffer; 50 mM pyrrolidine in 50 mM phosphate pH 7 buffer; 100 mM azetidine hydrochloride in 50 mM HCl; 10 mM aziridine hydrochloride in 10 mM HCl. Each amine

Chart 2



solution was diluted with ultrapure water to prepare a solution of 1.6 mM amine. A 2.5 μM solution of **3** was prepared in 2:3 v/v MeOH/H₂O.

Conversion of 3 to 5. A black 96-well plate was used (Chart 2). All of the following steps were performed at 25 °C. The experiment was performed in duplicate.

Step 1: The 50 mM phosphate pH 6 or 7 buffer (125 μL) was transferred to a black 96-well plate according to the diagram.

Step 2: The 1.6 mM amine solution or water (25 μL) was transferred to the plate according to the diagram.

Step 3: The 2.5 μM solution of **3** (50 μL) was added to all of the wells. Final reaction conditions: [**3**] = 1 μM , [amine] = 200 μM , [pH 6 or 7 buffer salt] = 31 mM, 1:9 MeOH/buffer.

Step 4: Green fluorescence emission was monitored for 80 min.

ASSOCIATED CONTENT

S Supporting Information. Spectroscopic data and Chart S1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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