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Specific fluorogenic probes for ozone in biological and atmospheric samples

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Ozone exposure is a growing global health problem, especially in urban areas. While ozone in the stratosphere protects the earth from harmful ultraviolet light, tropospheric or ground-level ozone is toxic and can damage the respiratory tract. It has recently been shown that ozone may be produced endogenously in inflammation and antibacterial responses of the immune system; however, these results have sparked controversy owing to the use of a non-specific colorimetric probe. Here we report the synthesis of fluorescent molecular probes able to unambiguously detect ozone in both biological and atmospheric samples. Unlike other ozone-detection methods, in which interference from different reactive oxygen species is often a problem, these probes are ozone specific. Such probes will prove useful for the study of ozone in environmental science and biology, and so possibly provide some insight into the role of ozone in cells.

The investigation of reactive oxygen species (ROS) has had a rather controversial history[1]. The existence of singlet oxygen and superoxide was first proposed in the late 1960s; however, because of the lack of sensitive and specific probes these results were met with scepticism, and these species were only confirmed in the late 1980s. Recently, ozone has been placed in the spotlight. It has been proposed that ozone is produced endogenously from singlet oxygen in both neutrophils and atherosclerotic plaque, which implicates ozone in inflammatory responses[2–4]. This conclusion was drawn from the premise that indigo carmine (1, Fig. 1)[5] differentially reacted with ozone as opposed to other ROS. However, 1 has since been shown not only to react with ozone, but also with superoxide in a similar manner[6], and thus challenges the proposal for endogenous ozone[7–9]. Despite the scepticism, a more recent report independently indicated the generation of ozone-like molecules in human neutrophils[10].

Chemiluminescent methods, which include the use of 1, are also used to measure ozone in ambient air[11,12], but other atmospheric compounds also absorb in this region of the electromagnetic spectrum and false-positive ozone readings are often reported[13,14]. In addition, many of these methods are sensitive to humidity. We reasoned that if a specific probe was available then ozone detection in air and biological systems would be achieved more reliably.

Several problems are associated with the use of 1 as a probe for ozone. First, the dye readily undergoes oxidative cleavage[15], which is most likely responsible for the lack of specificity (Supplementary Fig. S2b). Additionally, the reaction of blue 1 with ROS yields 2 (Fig. 1), which is colourless and cannot be visualized directly without the use of a spectrometer, particularly in biological samples. Although 1 emits a characteristic chemiluminescence signal[5] and absorbance maximum[16,17] (430 nm and 610 nm, respectively), which have been used to measure ozone in atmospheric samples, biological samples are analysed using high-performance liquid chromatography (HPLC) or mass spectrometry in combination with these spectroscopic techniques to confirm the products because the signal for 2 (ultraviolet, $\lambda_{max} = 245$ and 298 nm) overlaps with the signals for many other bioorganic compounds[2,4,6]. A similar approach has recently been reported for the simultaneous detection of ozone and carbonyls in air[18]. Although this system was shown to be resistant to humidity, it requires extensive extraction and derivatization prior to HPLC analysis, and its stability in the presence of other oxidizing agents was not reported.

Small molecule-based turn-on probes are particularly useful because their spectral properties are easily discernable and because they are compatible with aqueous media. Also, fluorescent samples can be visualized easily with the aid of a handheld fluorescence spectrometer, fluorescence microscope or even a simple laser pen. Although selective fluorescent probes exist for singlet oxygen[19], superoxide[19] and hydrogen peroxide[20], previously no such probe has been reported for ozone (ozone probes adsorbed on solid surfaces used at present typically suffer from variability in temperature, pressure and humidity[19]). As such, we set out to design a fluorescent turn-on probe selective for ozone.

While compound 7 (Fig. 2) is as fluorescent as 2′,7′-dichlorofluorescein (DCF)[21], when the phenolic hydroxy group is alkylated such compounds are nearly non-fluorescent[22–27]. Our hypothesis was that compound 4, which was also predicted to be nearly non-fluorescent, would react with ozone, and the resulting aldehyde 6 would undergo β-elimination to yield the fluorescent compound 7 and acrolein (Fig. 2). Hydrogen peroxide is also a by-product of this aqueous ozonolysis reaction, which could oxidatively stress cells[28]. Since non-toxic, physiological concentrations of hydrogen peroxide are between 10 and 100 μM (ref. 29,30) and the amount of hydrogen peroxide produced is stoichiometric with respect to ozone, the level of this ROS should not reach the toxic, millimolar range concentrations that would inhibit catalase activity[31]. This terminal alkene probe should be specific for ozone because it contains an isolated (non-conjugated) olefin and should not yield a fluorescence signal on exposure to other ROS[32,33] (Supplementary Fig. S2c). Based on this hypothesis, we designed and synthesized the homoallyl ether 4 in two steps from commercially available DCF in 64% yield (Fig. 2)[23]. We then bubbled ozone through a solution of 4 in 95:5 acetone:water and the fluorescent compound 7 was obtained in quantitative yield, presumably through intermediates 5 and 6, and thus provided a positive indication for our probe design.

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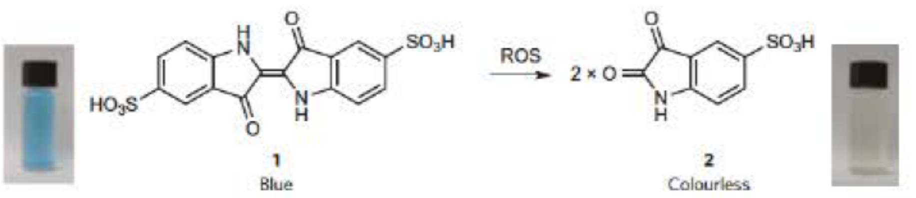

Figure 1 | Indigo carmine (1) is a non-selective, colorimetric probe for ROS. In this case, a blue solution of **1** reacts with various ROS to form colourless isatin sulfonic acid (**2**).

To develop a fluorescent method amenable to both biological and atmospheric detection, we chose a pH 7 buffer ($PO_4^{3-} = 5$ mM) to perform the conversion of 4 into 7. A solution of 4 in MeOH and pH 7 buffer (5:95) at 24 °C was exposed to ozone and, after incubation at 37 °C for one hour to facilitate the conversion of 4 into 7 (see Supplementary Information for time-dependence data), 7 was observed (signal-to-background (S/B) = 80) (Fig. 3a). We envisaged that this detection method would be performed in the presence of cells, so we also carried out the ozonolysis reaction in a mixture of 10% fetile bovine serum in RPMI-1640 cell-culture media and pH 7 buffer (1:3). Indeed, the method is compatible with components of the cell-culture media (S/B = 104; not shown), which indicates that this method should be amenable to *in vitro* and possibly *in vivo* studies. It is important to note that the conversion of 4 into 7 was found to be much faster in the presence of the components of cell-culture media and that the reaction was complete in 30 minutes (Supplementary Fig. S7).

Given our successful results with ozone, we next wanted to determine the specificity of our probe to ROS. No reaction or fluorescence signal was observed with the ROS examined (Fig. 3a), although fluorescence bleaching of 4 was observed when a large excess of these species was present. Hydrogen peroxide and the hydroxyl radical were also examined because the former is a by-product of the aqueous ozonolysis reaction[34], the latter is a product of ozone decomposition in aqueous solution[11] and both are endogenous ROS. For each, no fluorescence-signal enhancement was observed (Fig. 3a). Thus, our probe generates fluorescence specifically with ozone and can be used to detect ozone even in the presence of other ROS. A similar set of experiments was also performed using 1 to demonstrate the lack of specificity and the turn-off nature of this colorimetric probe (see Fig. 1 and Supplementary Information).

We next examined the sensitivity of our fluorescent method for ozone detection. The fluorescence intensity correlated with the concentration of ozone in the 50 nM−12.5 μM (2.4−600 ppb) range with S/B = 2.6−310 after tenfold dilution of the original samples (0−500 nM, Fig. 3b).

Ozone's role in damaging human tissues is unclear, so there is a need for a biomedical probe to better understand the localization of this highly reactive species. Cellular effects of ozone exposure have been observed; however, it is speculated that ozone is much too reactive to reach and permeate airway epithelial cells[35–37]. Thus, to examine the utility of our detection method in such biological samples, we first attempted ozone detection in the presence of various antioxidants found in the airway liquid lining. Ascorbic acid, glutathione and uric acid are the most predominant antioxidants present[35–37] and, as Fig. 3c shows, our fluorescent method works in the presence of physiologically relevant antioxidants. We then proceeded to test our method with human pleural fluid to mimic the epithelial lining fluid of the lung. Indeed, a strong fluorescence signal was produced (S/B = 92; Fig. 3d), which indicates that probe 4 should provide a tool to

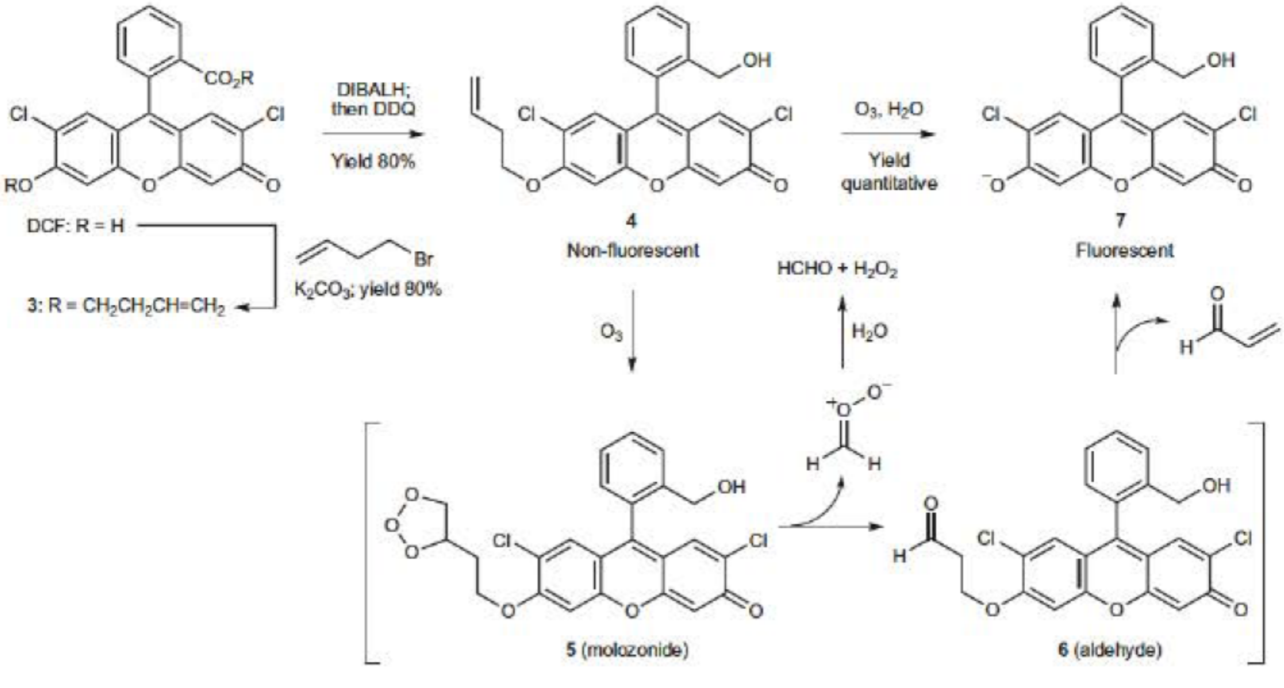

Figure 2 | Synthesis and mechanistic design of ozone probe 4. Probe **4** was prepared in two steps from DCF. The terminal olefin of **4** reacts with ozone to form molozonide **5** via a (3 + 2) cycloaddition, which undergoes retro (3 + 2) cycloaddition to form aldeyde **6**, HCHO and H_2O_2. Aldehyde **6** then undergoes β-elimination to form the fluorescent compound **7** and $CH_2{=}CHCHO$. DIBALH = diisobutylaluminium hydride, DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone.

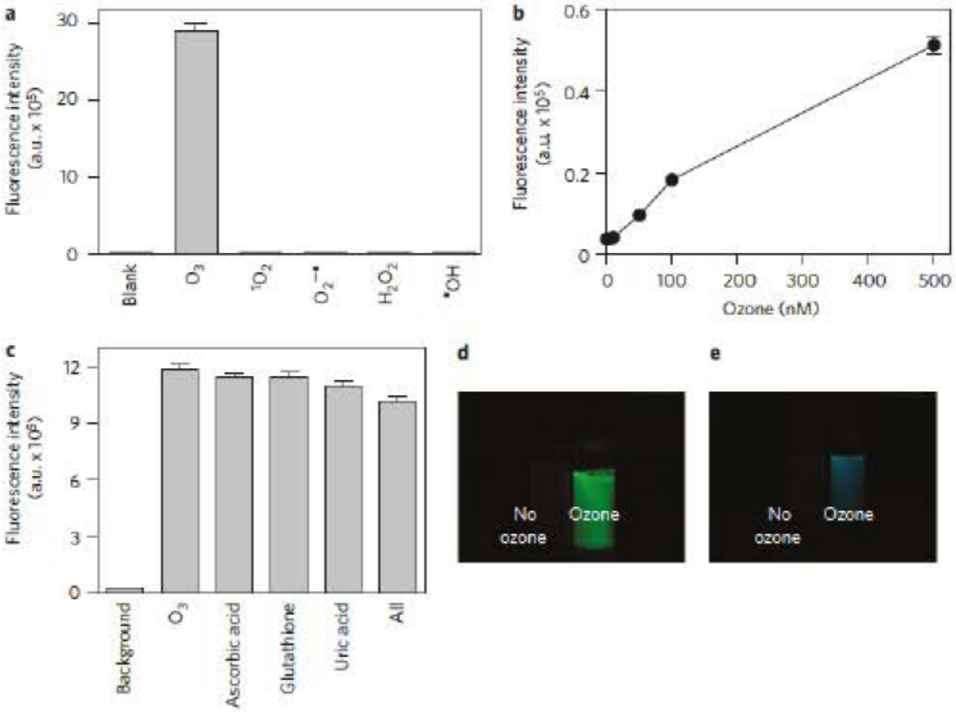

Figure 3 | Ozone detection in aqueous and biological media. Experimental details are given in the Methods section. Each experiment was performed in triplicate and error bars were determined from the mean and standard deviation (Prism 5.0a, GraphPad Software). **a**, ROS specificity. **b**, Correlation between fluorescence intensity and ozone concentration in MeOH and pH 7 buffer (5:95) after tenfold dilution of the original samples. The intensity was linear up to 12.5 μM (see Supplementary Information). **c**, Ozone detection in the presence of antioxidants. In all cases, the concentration of ascorbic acid was 50 μM and concentration of glutathione and uric acid were 100 μM. **d**, Ozone detection in human pleural fluid and pH 7 buffer (1:3) visualized under a long-range ultraviolet lamp. **e**, Ozone detection in human serum and pH 7 buffer (1:3) visualized under a long-range ultraviolet lamp.

visualize indirectly this reactive gas in the study of ozone and the respiratory system.

As a further demonstration of the robustness of probe **4**, we next examined the detection of ozone in human serum. As Fig. 3e shows, probe **4** is able to detect ozone even in the presence of other potentially reactive species in serum ($S/B = 92$). It is interesting that, although the maximum emission wavelength shifted to 531 nm, the observed colour was blue. This observation proved to be general for this fluorophore scaffold, as both DCF and **7** behaved in a similar manner in serum. Although the exact reason for the red-shift is unclear at present, it is known that the emission spectra of fluoresceins can be influenced by various factors in serum[38].

To determine whether our probe can be used in studies related to ozone's role in cell biology we incubated human bronchial epithelial cells with compound **4**. Although compound **4** was cell permeable, it was found that it had been converted to a fluorescent compound inside the cells in the absence of external ozone within ten minutes. Based on our ongoing studies as to why compound **4** and structurally related compounds are not fluorescent, it was thought that the hydroxymethyl group of **4** may have been oxidized to give a more electron-withdrawing group, possibly a carboxy or aldehyde group, and so emit a strong fluorescence signal. To circumvent such an oxidation event, we prepared compound **8** and confirmed that this compound was also specific for ozone (see Supplementary Information for the details). As expected, this compound was nearly non-fluorescent and thus was used for the live-cell experiment. Unlike compound **4**, compound **8** remained weakly fluorescent within the cells. Once a steady baseline signal had been established and maintained for five minutes, ozone gas was generated within the airtight environmental chamber that housed the cell-culture dishes, and fluorescence signals were monitored using live-cell microscopy for ~15 minutes. As Fig. 4 shows (see Supplementary Information for a film), a time-dependent increase in fluorescence emissions occurred inside the cells after ozone generation, which indicates that ozone can penetrate through cell membranes. These results show that further studies on the intracellular biological activity of ozone are warranted.

Detection in ambient indoor and outdoor air is also important because of the toxic effects of this gas[39,40]. Exposure to environmental ozone is particularly a problem for those who suffer from respiratory ailments, such as asthma, bronchitis and cystic fibrosis. These patients rely solely on community-based monitoring to adjust their behaviour so as to minimize their exposure. As such, one of our goals was to assess whether compound **4** could be used to develop a simple method by which ordinary citizens could monitor their total ozone exposure, and thus make more informed decisions about their outdoor activity. As a first step towards this goal, we carried out a proof-of-principle experiment. A solution of compound **4** (500 nmol) in dimethylsulfoxide (DMSO) was absorbed on a piece of adsorbent paper and allowed to dry; **1** was used for comparison because this is more amenable to continuous measurement than is a method based on ultraviolet absorbance. The paper strips were stored in an office room that had two photocopiers and two laser printers. After eight hours, the paper strips were removed and placed in a vial that contained pH 7 buffer to elute the dyes. Figure 5a and the fluorescence emission of compound **7** (see Supplementary Information) show that 0.95 nmol of **7** was generated, which indicates that at least 0.95 nmol of ozone was exposed to the strip over eight hours. The paper strip that contained **1** displayed a 77% decrease in absorbance at 610 nm (Fig. 5b), which correlates to the consumption of 387 nmol of **1**. The deviation in these values is presumably because of photobleaching and the lack of specificity of **1** towards various ROS, including singlet oxygen. Although the use of paper strips is not an optimal format, we envisage that a badge that contains probe **4** may be developed for patients to wear and so continuously measure their own total ozone exposure through the day.

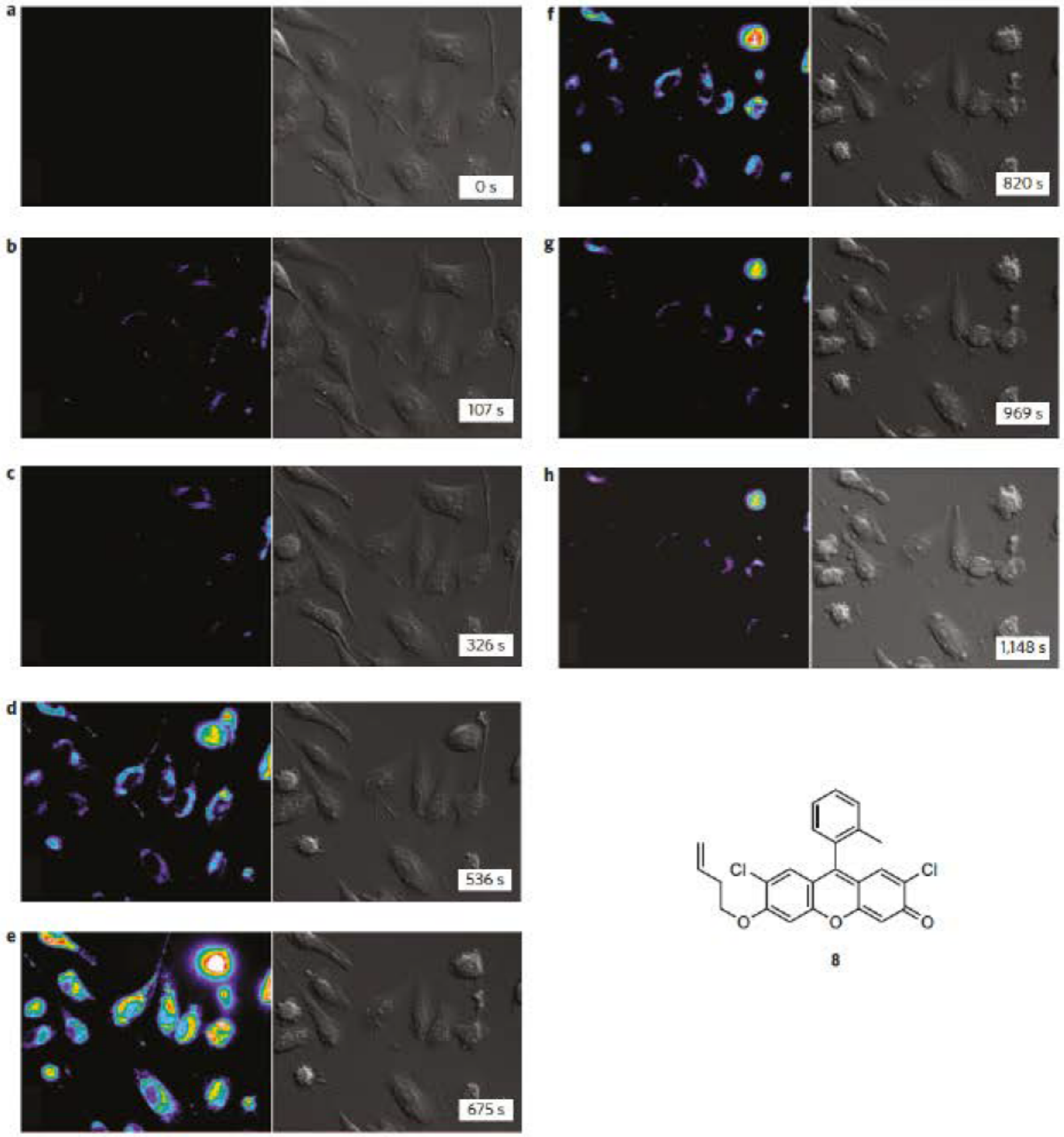

Figure 4 | Live-cell imaging of human bronchial epithelial cells in the presence of ozone using compound 8. a–h, The left-hand panels are pseudocolour images that indicate the changes in emission intensity over time, and the right-hand panels show differential interference contrast images of the bronchial epithelial cells. **a**, Before the addition of **8** (0 s). **b**, At 107 s a steady-state intensity was reached with compound **8** at 250 nM. **c**, At 326 s an ozone flow started. **d**, At 536 s the emission intensity increased. **e**, At 675 s the emission maximum was reached. **f**, At 820 s cells started to die. **g**, At 969 s the emission continued to fall. **h**, At 1,148 s the ozone flow stopped.

We also wanted to examine ozone detection in ambient air. Solutions of **4** in MeOH and pH 7 buffer (5:95) were prepared and exposed to ambient air for eight hours on a sunny day (~32 °C) in various locations throughout Pittsburgh, Pennsylvania (data collected on 6 June 2008; for detailed locations, see Supplementary Information). Ozone detection was successful with S/B values of 10–17 (Fig. 5c), which correlates to 13–22 ppb in the probe solution exposed to the air. To ensure that we had selectively detected ozone, we screened a number of other pollutants (Fig. 5d). Nitrogen dioxide and sulfur dioxide are converted into their corresponding acids in aqueous solution, so nitric acid and sulfuric acid were screened. We also screened lead, palladium and platinum (known pollutants emitted from motor vehicles) at environmentally relevant concentrations[41]. None of these species produced a fluorescence signal. Aside from the specificity, it is important to note that because our detection method is performed in aqueous media it will not be affected by humidity. Thus, probe **4** may be used as a sensitive probe for ozone in the atmosphere.

In summary, we have developed specific and robust fluorescent probes for ozone. Our probes are able to fluorescently detect ozone in biological and environmental samples. Additional features include water compatibility and ease of measurement. Our probes should provide valuable tools with which to better understand ozone's role in human health and the atmosphere. Additionally, the simplicity of their use suggests that, after further optimizations (for example, acceleration of the conversion from **6** into **7** in buffer),

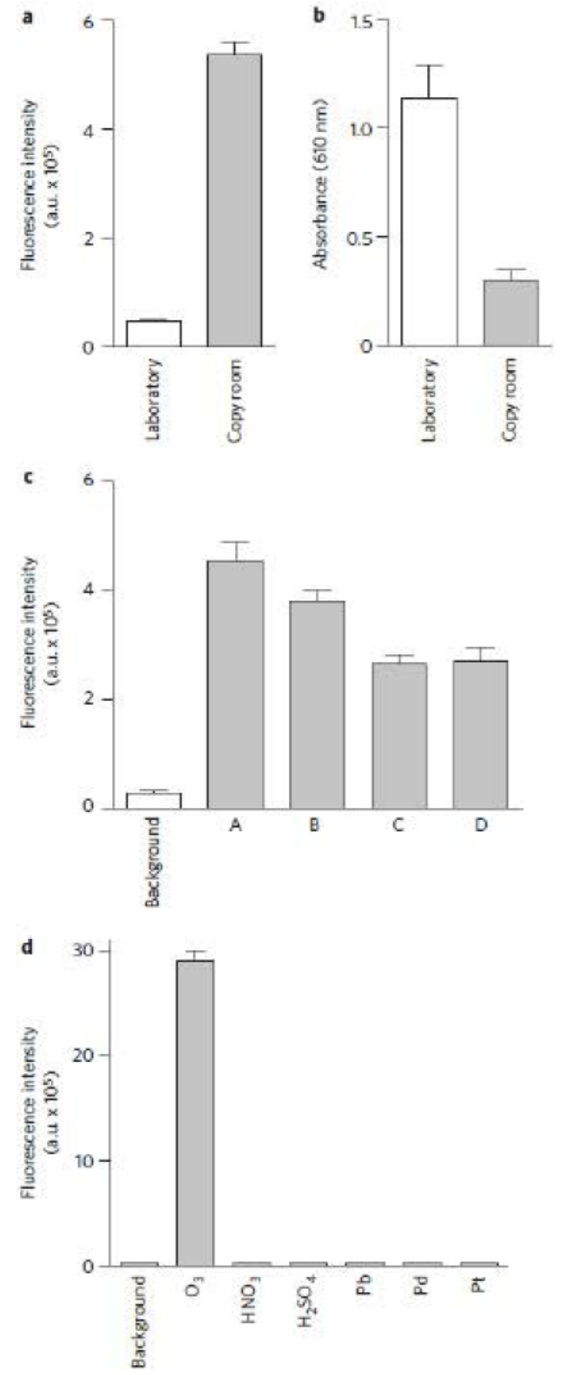

Figure 5 | Conversion of 4 into 7 enables the detection of ozone in air samples. **a**,**b**, Dyes (500 nmol) **4** (**a**) and **1** (**b**) were absorbed onto pieces (11 × 9 cm) of adsorbent paper and used to detect ozone in indoor air for eight hours. **c**,**d**, Solutions of **4** (12.5 μM) in MeOH and pH 7 buffer (5:95) were exposed to ambient air in various locations (A–D) throughout a city and ozone assays performed. Ozone was measured in outdoor air for eight hours (**c**), as was its specificity against other air pollutants (**d**) (acid concentration, 250 μM; metal concentration, 100 nM). Experiments were performed in triplicate and error bars were determined from the mean and standard deviation (Prism 5.0a, GraphPad Software). For experimental details, see Supplementary Information.

it may be possible for individuals to monitor their exposure to ozone at home. This would be of enormous benefit to those who suffer from respiratory diseases, as they will be able to change their behaviour accordingly.

Methods

Ozone detection. Solutions of **4** (final concentration 12.5 μM) in a mixture of MeOH and pH 7 buffer ([PO_4^{3-}] = 5 mM; 5:95; 4.0 ml) were prepared, and the samples were exposed to various amounts of ozone at 24 °C followed by incubation for one hour at 37 °C before fluorescence measurements (except in the ambient air experiments). The concentration of ozone was 5 μM for these studies. For experimental details with other ROS, see Supplementary Information. Fluorescence spectra were recorded in a disposable cuvette (1 × 1 cm) on a Jobin Yvon FluoroMax-3 spectrometer under the control of a Windows-based PC using FluorEssence software. The samples were excited at 497 nm and the emission intensities were collected at 523 nm. All spectra were corrected for emission intensity using the manufacturer-supplied photomultiplier curves.

Ozone detection in indoor air. One adsorbent paper (Kimwipe) was cut into two 11 × 9 cm pieces. Compound **4** (DMSO solution, 500 nmol total) was added to one portion of the Kimwipe and allowed to dry overnight in a laboratory drawer. The paper strip was then moved to a photocopy room that contained two photocopiers and two laser printers, and kept there for eight hours. The control remained in the laboratory drawer. After eight hours, the samples were placed into a scintillation vial and the dye was eluted with pH 7 buffer (6.0 ml; about five minutes) before fluorescence measurement.

Ozone detection in ambient air. Solutions of **4** (final concentration 12.5 μM) in a mixture of MeOH and pH 7 buffer ([PO_4^{3-}] = 5 mM; 5:95; 4.0 ml) were prepared, and the samples were placed for eight hours in outdoor areas with heavy traffic. Care was taken to ensure the samples were not hit by direct sunlight. A negative control was prepared in the same manner, but incubated at 37 °C in a closed vial for eight hours. After incubation for eight hours, the fluorescence of each sample was measured.

Ozone detection in live human epithelial cells. Human bronchial epithelial cells (BEAS-2B) (American Type Culture Collection) were grown in LHC-9 medium (Invitrogen) on glass-bottomed Petri dishes (MatTek) precoated with 0.01 mg ml^{-1} of human fibronectin (Invitrogen), 0.029 mg ml^{-1} Vitrogen 100 (Cohesion) and 0.01 mg ml^{-1} bovine serum albumin (Invitrogen) in LHC-9 medium[42]. Immediately prior to imaging, cells were rinsed with Hank's balanced salt solution, which contained calcium and magnesium, and placed inside a temperature-controlled, airtight environmental chamber and maintained at 37 °C and 5% CO_2 (Tokai Hit). Images were obtained using a Nikon TE2000E-PFS (Perfect Focus System) microscope equipped with a 40× oil-immersion objective (Nikon, CFI PlanFluor, NA 1.3) and, a fluorescein isothiocyanate longpass filter set (Chroma), a Q-Imaging SRV CCD camera (Q-Imaging) and MetaMorph software (Molecular Devices). The motorized XYZ stage used was made by ASI (Eugene). Baseline images were collected before the addition of **8** and time-lapse images obtained at 30-second intervals after the addition of **8** (250 nM). Once a stable baseline had been achieved, multiple washes were carried out and the media replaced. The cells were then exposed to ozone (~2.1 ppm) and monitored every 30 seconds for 15 minutes. Ozone was generated from medical grade oxygen (Valley Gas) via a model V1-0 ultraviolet ozonator (OREC) and analysed with a Dasibi (Glendale) direct-reading instrument. Ozone concentrations were maintained by adjusting both the intensity of the ultraviolet light and the flow rate of oxygen. For each experiment, images were collected from six stage positions, with a minimum of five cells per position (a minimum of 30 cells per experiment).

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Author contributions

A.L.G., C.M.S., B.R.P., G.D.L. and K.K. designed the experiments. A.L.G. generated the data shown in Figs 2, 3 and 5. C.M.S. generated the data shown in Fig. 4. S.A. synthesized and characterized compound 8. A.L.G., C.M.S. and K.K. wrote the manuscript.

Additional information

Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/. Correspondence and requests for materials should be addressed to K.K.

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SUPPLEMENTARY INFORMATION

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GENERAL INFORMATION

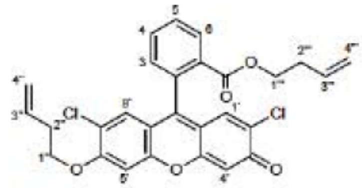
Ozone was generated by feeding high purity oxygen into a Welsbach T-series ozone generator. RPMI-1640 medium (without L-glutamine and phenol red) is a product of HyClone® and was used as received. Human pleural fluid was purchased from Lee Biosolutions and used as received. Human serum (off-the-clot, type AB) was purchased from PAA Laboratories, Inc. and used as received. Kimwipes® is a product of Kimberly-Clark. Buffers were purchased from J. T. Baker (pH 7, catalog number 5608-01) and used after dilution with ARISTAR® ULTRA Water.

All reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium-benzophenone, and methylene chloride (CH_2Cl_2) was distilled from calcium hydride. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous materials, unless otherwise stated.

All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm EMD silica gel plates (60F-254) using UV-light (254 nm). TSI silica gel (230–400 mesh) was used for flash column chromatography.

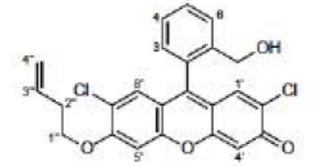
NMR spectra were recorded on AM300 (Bruker) instruments and calibrated using a solvent peak as an internal reference. The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were obtained from a Micromass Autospec double focusing instrument.

EXPERIMENTAL SECTION



Preparation of compound 3. A100-mL round bottom flask was charged with 2',7'-dichlorofluorescein (2.4 g, 5.89 mmol), DMF (20 mL), K_2CO_3 (2.5 g, 17.7 mmol) and 4-bromobutene (1.8 mL, 17.7 mmol) at 24 °C. The reaction mixture was stirred at 70 °C for 8 h and then poured into 750 mL water. The resulting precipitate was washed with water and dried *in vacuo* to yield **3** as a red-orange solid (2.4 g; 80%).[1]

Data for **3**: mp = 184−185 °C; R_f = 0.34 (70% EtOAc in hexanes); IR (KBr pellet): 1725 (C=O), 1593, 1521, 1278, 997 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 293K): δ 8.32 (dd, *J* = 7.5, 1.2 Hz, 6-H), 7.82−7.68 (m, 2H, 4-H, 5-H), 7.26−7.30 (br d, *J* = 7.5 Hz, 1H, 3-H), 7.04 (s, 1H, Ar), 7.03 (s, 1H, Ar), 6.95 (m, 1H, Ar), 6.60 (s, 1H, Ar), 5.93 (ddt, *J* = 16.9, 10.3, 6.6 Hz, 1H, $3'''$-H), 5.60 (ddt, *J* = 16.5, 9.9, 6.6 Hz, 1H, $3''$-H), 5.24 (ddt, *J* = 17.1 , 3.3, 1.5 Hz, 1H, $4'''$-H_{trans}), 5.18 (ddt, *J* = 10.2 , 3.3, 1.5 Hz, 1H, $4'''$-H_{cis}), 5.02 (ddt, *J* = 17.1, 3.3, 1.5 Hz, 1H, $4''$-H_{trans}), 4.97 (ddt, *J* = 10.2, 3.3, 1.5 Hz, 1H, $4''$-H_{cis}), 4.20 (t, *J* = 6.7 Hz, 2H, $1'''$-H), 4.13 (t, *J* = 6.7 Hz, 2H, $1''$-H), 2.68 (q, *J* = 6.6 Hz, 2H, $2'''$-H), 2.23 (q, *J* = 6.6 Hz, 2H, $2''$-H); ^{13}C NMR (75 MHz, $CDCl_3$, 293K): δ 177.7, 169.4, 158.6, 157.8, 152.5, 149.8, 135.2, 133.5, 133.3, 133.2, 133.1, 131.6, 130.4, 130.3, 130.2, 128.0, 127.4, 120.5, 118.1, 117.6, 117.4, 114.9, 105.7, 100.7, 69.2, 64.5, 33.0, 32.6; HRMS (ESI) *m/z* calcd for $C_{28}H_{23}Cl_2O_5$ $[M+H]^+$ 509.0923, found 509.0876.



Preparation of compound 4. A solution of compound **3** (200 mg, 0.392 mmol) in CH_2Cl_2 (1.32 mL) was treated with DIBALH (1.4 mL, 1.0 M in hexanes, 1.4 mmol) dropwise over 15 min at -78 °C under a nitrogen atmosphere. The mixture was stirred for 5 min at the same temperature and then was warmed to 24 °C. After 2 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (700 μL) and diluted with Et_2O (2.9 mL) at 0 °C. DDQ (2.50 g, 11.0 mmol) was then added to the mixture at 0 °C. After stirring 15 min, the mixture was filtered through Celite® and washed with Et_2O. The filtrate was dried over Na_2SO_4 and the solvents were evaporated under reduced pressure. Silica gel flash chromatography of the residue (5 to 10 % EtOAc in hexanes) afforded compound **4** as a light orange solid (134 mg, 80%).

Data for **4**: mp = 167−168 °C; R_f = 0.69 (50% EtOAc in hexanes); IR (KBr pellet): 3387 (br O-H), 3080, 2925, 2857, 1608, 1480, 1416, 1035 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 293K): δ 7.39−7.45 (m, 2H, Ar), 7.28−7.30 (m, 1H, Ar), 6.91 (s, 1H, Ar), 6.90 (s, 1H, Ar), 6.88 (s, 1H, Ar), 6.85 (s, 1H, Ar), 6.76 (s, 1H, Ar), 5.94 (ddt, *J* = 17.1, 10.5, 6.6 Hz, 1H, $3''$-H), 5.26 (ddt, *J* = 17.1, 3.3, 1.5 Hz, 1H, $4''$-H_{trans}), 5.17 (ddt, *J* = 10.2, 3.3, 1.5 Hz, 1H, $4''$-H_{cis}), 5.34 (s, 2H, CH_2OH), 4.11 (t, *J* = 6.7 Hz, 2H, $1''$-H), 2.63 (ddd, *J* = 6.6, 6.6, 1.5 Hz, 2H, $2''$-H); ^{13}C NMR (75 MHz, $CDCl_3$, 293K): δ 154.9, 151.9, 150.1, 149.6, 143.9, 138.6, 133.8, 129.5, 128.7, 128.6, 128.3, 123.7, 121.0, 118.2, 118.1, 117.5, 117.1, 115.5, 103.6, 101.1, 83.1, 72.3, 68.6, 33.2; HRMS (ESI) *m/z* calcd for $C_{24}H_{19}Cl_2O_4$ $[M+H]^+$ 441.0660, found 441.0683.

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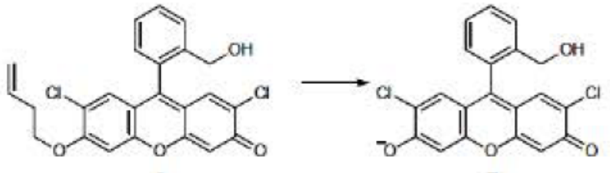
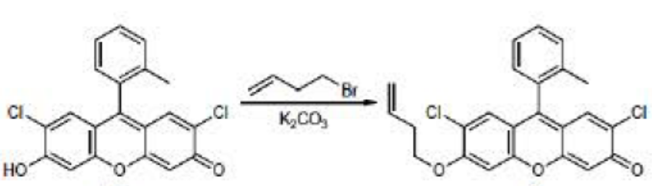
[1] Sparano, B. A.; Shahi, S. P.; Koide, K. *Org. Lett.* **2004**, *6*, 1947.



4 nonfluorescent

7 fluorescent

Conversion of compound 4 to compound 7. A solution of **4** (34.2 mg, 0.0775 mmol) was prepared in 95:5 acetone/water (585 μL total, 0.15 mM). The solution was cooled to 0 °C and ozone was bubbled through the sample for 2 min.[2] Following ozonolysis, a small amount of KI (<5 mg) was added and the solution was stirred at 24 °C for 1 min. TLC analysis (50% EtOAc in hexanes) indicated complete conversion to **7**. Details of the spectroscopic, absorbance and fluorescence properties of **7** can be found in the references shown below.[3,4]



S1

8

Preparation of compound 8. 4-Bromo-1-butene (168 μL, 1.7 mmol) and K_2CO_3 (263 mg, 1.9 mmol) were added to a solution of compound **S1** (176 mg, 0.47 mmol) in DMF (2 mL), and the resulting mixture was heated at 80 °C under N_2 for 36 h. The reaction mixture was then poured to H_2O (30 mL) and extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (10 to 40% EtOAc in hexanes) on silica gel (60 mL) to afford compound **8** (46 mg, 23% yield) as an orange solid.

Data for **8**: $R_f = 0.34$ (50% EtOAc in hexanes); mp = 142–143 °C; IR (KBr): 2923, 2853, 1637 (C=O), 1608, 1514, 1435, 1336, 1280, 1000, 916, 834 cm^{-1}; ^{1}H NMR (300 MHz, 293K, $CDCl_3$) δ 7.56–7.40 (m, 3H), 7.16 (d, J = 9.6 Hz, 1H), 7.14 (s, 1H), 7.06 (s, 1H), 7.04 (s, 1H), 6.60 (s, 1H), 5.93 (ddt, J = 17.1, 10.5, 6.6 Hz, 1H), 5.25 (ddt, J = 17.1, 3.3, 1.5 Hz, 1H), 5.19 (ddt, J = 10.5, 3.3, 1.5 Hz, 1H), 4.23 (t, J = 6.6 Hz, 2H), 2.69 (tdd, J = 6.6, 6.6, 1.5 Hz, 2H), 2.09 (s, 3H); ^{13}C NMR (75 MHz, 293K, $CDCl_3$) δ 177.8, 158.9, 157.8, 152.8, 148.8, 136.0, 135.6, 133.1, 131.5, 131.0, 130.1, 129.0, 128.4, 127.6, 126.5, 120.7, 118.3, 118.1, 114.3, 105.8, 100.7, 69.3, 33.0, 19.7; HRMS (EI+) calcd. for $C_{24}H_{18}Cl_2O_3$ [M$^+$] 424.0633, found 424.0644.

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[2] Procedure based on a recent report: Schiaffo, C. E.; Dussault, P. H. *J. Org. Chem.* **2008**, *73*, 4688.

[3] Song, F.; Garner, A. L.; Koide, K. *J. Am. Chem. Soc.* **2007**, *129*, 12354.

[4] Koide, K.; Song, F.; de Groh, E. D.; Garner, A. L.; Mitchell, V. D.; Davidson, L. A.; Hukriede, N. A. *ChemBioChem* **2008**, *9*, 214.

Preparation of parent stock solutions used for this study.

Entry	Reagent	Quantity	Solvent (10 mL)	Conc. of stock solution
A	Compound **4**	44.1 mg (0.10 mmol)	DMSO	10.0 mM
B	Indigo carmine	46.6 mg (0.10 mmol)	DMSO	10.0 mM
C	$NaMoO_4{\cdot}2H_2O$	206.0 mg (1.00 mmol)	pH 10 buffer	100 mM
D	KO_2	71.1 mg (1.00 mmol)	DMSO	100 mM
E	$FeSO_4{\cdot}7H_2O$	278.0 mg (1.00 mmol)	pH 7 buffer	100 mM
F	L-Ascorbic acid sodium salt	19.8 mg (0.10 mmol)	pH 7 buffer	10.0 mM
G	Glutathione, reduced	30.7 mg (0.10 mmol)	pH 7 buffer	10.0 mM
H	Uric acid	16.8 mg (0.10 mmol)	pH 7 buffer	10.0 mM
I	$Pb(NO_3)_2$	16.6 mg (50 µmol)	MeOH	5.0 mM
J	$PdCl_2$	9.0 mg (50 µmol)	3:1 Brine/MeOH	5.0 mM
K	$PtCl_2$	13.3 mg (50 µmol)	DMSO	5.0 mM

Notes:

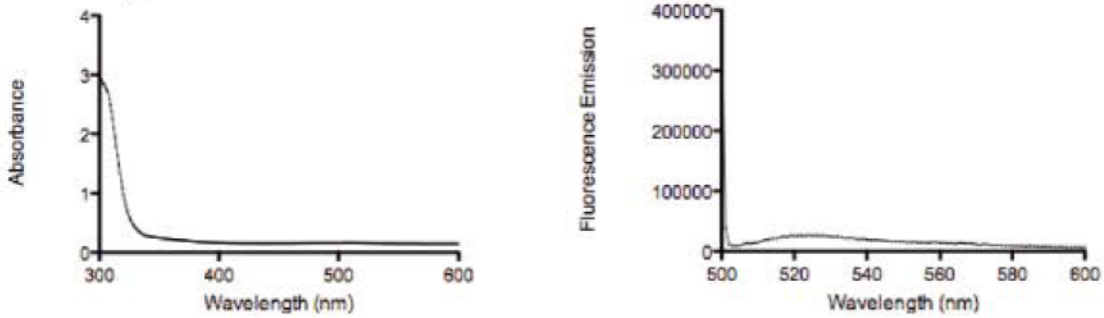
(1) All the solutions were stored at 24 °C.
(2) Solution **A** was stored in the dark as a precautionary measure.
(3) All solutions for testing the ROS specificity were prepared and used immediately.
(4) All solutions for testing detection in the presence of antioxidants were prepared and used immediately.

UV-visible spectroscopy. Absorption spectra were acquired in a 1 × 1-cm quartz cuvette (Spectrocell Inc.; product number RF-2010) on a Perkin Elmer Lambda 19 UV-Visible spectrometer under the control of a Windows-based PC running the manufacturer's supplied software.

Fluorescence spectroscopy. Fluorescence spectra were recorded in a 1 × 1-cm disposable cuvette (VWR; catalog number 58017-880) on a Jobin Yvon FluoroMax-3 spectrometer under the control of a Windows-based PC running FluorEssence software. The samples were excited at 497 nm and the emission intensities were collected at 523 nm. All spectra were corrected for emission intensity using the manufacturer supplied photomultiplier curves.

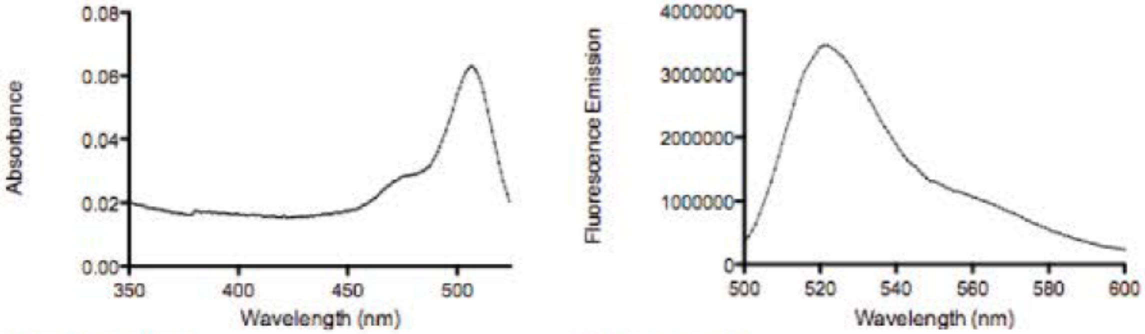
Absorbance and Fluorescence Emission of 4, 7, 8, and **S1.** Fluorescence and emission spectra were measured in 0.1% DMSO in pH 7 buffer (Figure S1).

Synthesis and structural characterization of compound S1: F. Song, M. P. Cook, K. Koide, manuscript in preparation.



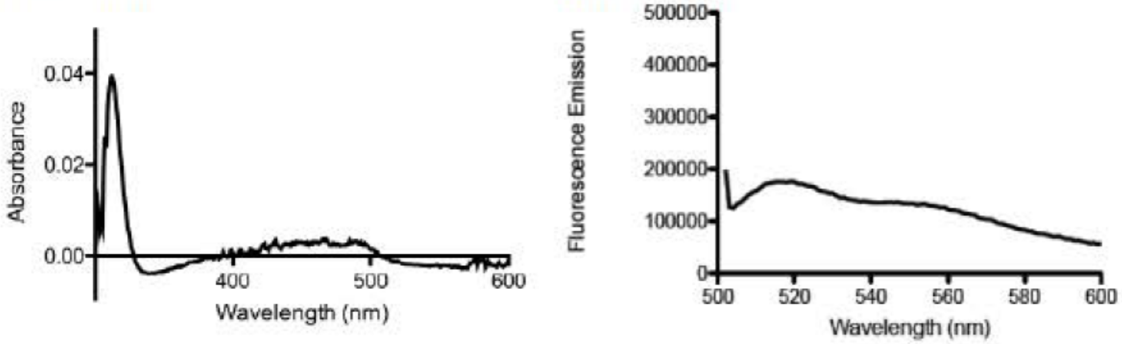
(a) [**4**] = 10 μM

(b) [**4**] = 10 μM



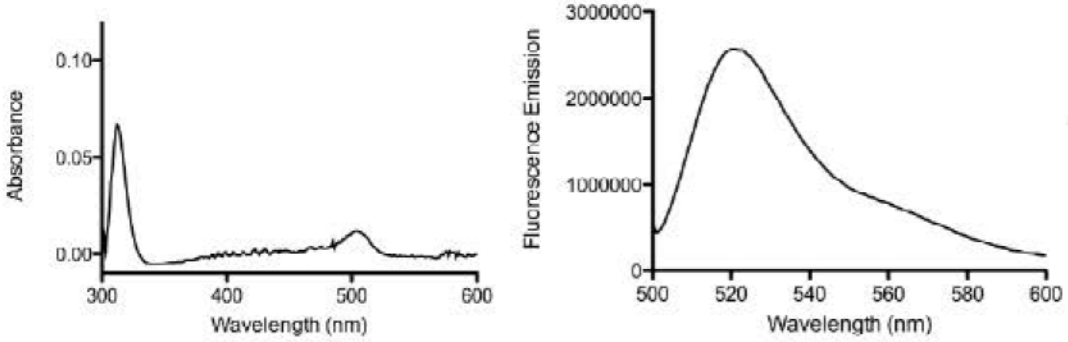
(c) [**7**] = 1.0 μM

(d) [**7**] = 1.0 μM



(e) [**8**] = 1.0 μM

(f) [**8**] = 10 μM



(g) [**S1**] = 1.0 μM

(h) [**S1**] = 1.0 μM

Figure S1. Absorbance and emission spectra of **4**, **7**, **8**, and **S1**. (a) Absorbance spectrum of **4**. (b) Emission spectrum of **4**. (c) Absorbance spectrum of **7**. (d) Emission spectrum of **7**. (e) Absorbance spectrum of **8**. (f) Emission spectrum of **8**. (g) Absorbance spectrum of **S1**. (h) Emission spectrum of **S1**.

Relative Quantum Yields. To determine quantum yields relative to fluorescein, a stock solution of compound **4** was prepared in DMSO (1 mM) and diluted in phosphate buffer (pH = 7.0) to $OD_{490} = 0.09$. The samples were excited at 490 nm and the integrated emission spectra were compared. The quantum yields of all compounds were referenced to fluorescein in 0.1 N NaOH ($\Phi = 0.95$).[5] The quantum yield for compound **4** was calculated as 0.00132.

Mechanistic rationale of ozone specificity.

(a)

$$2\,O_2^- + 2\,H^+ \xrightleftharpoons{K_{pH\,7} = 4 \times 10^{20}} H_2O_2 + O_2$$

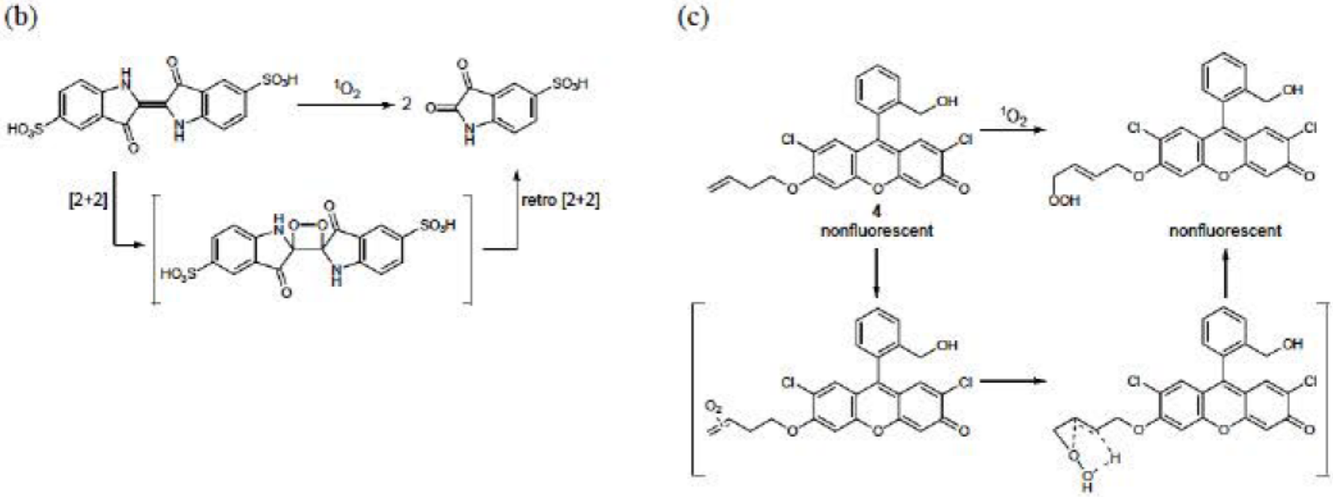


Figure S2. Mechanistic rationale of fluorescence turn-on specificity for ozone. (a) Superoxide disproportionation in pH 7 buffer. (b) The reaction of indigo carmine with singlet oxygen. (c) The reaction of compound **4** with singlet oxygen.

Reactivity of indigo carmine and 4. Indigo carmine would be expected to be more reactive towards ozone from a frontier molecular orbital view[6] due to the presence of its α,β-unsaturated ketone moiety (higher HOMO, lower LUMO); however, this moiety also makes it more reactive toward other ROS. For example, Kettle and co-workers proposed a superoxide-mediated one-electron reduction of indigo carmine through its α,β-unsaturated ketone moiety.[7] With respect to **4**, because the dominant characteristic of superoxide is to act as a strong Brönsted base,[8] it would likely deprotonate the primary hydroxy group, which would not produce a fluorescence signal. In addition, the disproportionation of superoxide in pH 7 buffer is extremely fast precluding reaction with **4** (Figure S2a). Finally, in the presence of singlet oxygen, because indigo carmine does not contain allylic hydrogens,[9] it will undergo [2+2] cycloaddition followed by a retro-[2+2] cycloaddition to yield isatin sulfonic

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[5] Brannon, J. H.; Magde, D. *J. Phys. Chem.* **1978**, *82*, 705.

[6] Huisgen, R. *Angew. Chem. Int. Ed.* **1963**, *2*, 633. Houk, K. N.; Sims, J.; Duke, Jr., R. E.; Strozier, R. W.; George, J. K. *J. Am. Chem. Soc.* **1973**, *95*, 7287. Sustmann, R. *Tetrahedron Lett.* **1971**, *29*, 2717.

[7] Kettle, A. J.; Clark, B. M.; Winterbourn, C. C. *J. Biol. Chem.* **2004**, *279*, 18521.

[8] Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393.

[9] Clennan, E. L.; Pace, A. *Tetrahedron* **2005**, *61*, 6665.

acid (Figure S2b). Compound **4**, however, contains allylic hydrogens and will undergo an ene-type reaction,[10] which again will not produce a fluorescence signal (Figure S2c). In conclusion, other ROS may react with **4**, but in each case a fluorescence signal will not be produced.

ROS specificity:

Ozone: Solution **A** (5.0 μL, [**4** or **8**]$_{\text{final}}$ = 12.5 μM) was added to a mixture of MeOH/pH 7 buffer ([PO_4^{3-}] = 5 mM) (5:95) (4.0 mL), and the samples were exposed to ozone (~5 μM) at 24 °C followed by 1 h incubation at 37 °C before fluorescence measurement.

Singlet oxygen: Solution **A** (5.0 μL, [**4** or **8**]$_{\text{final}}$ = 12.5 μM), solution **C** (40.0 μL, [$NaMoO_4 \bullet 2H_2O$]$_{\text{final}}$ = 1.0 mM) and 30% H_2O_2 (10 μL, [H_2O_2]$_{\text{final}}$ = 25.0 mM)[11] were added to a mixture of MeOH/pH 10 buffer (5:95) (4.0 mL), and the samples were incubated for 30 min at 24 °C.

Superoxide: Solution **A** (5.0 μL, [**4** or **8**]$_{\text{final}}$ = 12.5 μM) and solution **D** (10.0 μL, [KO_2]$_{\text{final}}$ = 250 μM)[12] were added to a mixture of MeOH/pH 7 buffer solution (5:95) (4.0 mL), and the samples were incubated for 30 min at 37 °C.

Hydrogen peroxide: Solution **A** (5.0 μL, [**4** or **8**]$_{\text{final}}$ = 12.5 μM) and 30% H_2O_2 (10 μL, [H_2O_2]$_{\text{final}}$ = 25.0 mM) were added to a mixture of MeOH/pH 7 buffer solution (5:95) (4.0 mL), and the samples were incubated for 30 min at 37 °C.

Hydroxyl radical: Solution **A** (5.0 μL, [**4** or **8**]$_{\text{final}}$ = 12.5 μM), solution **E** (10.0 μL, [$FeSO_4 \bullet 7H_2O$]$_{\text{final}}$ = 250 μM) and 30% H_2O_2 ([H_2O_2]$_{\text{final}}$ = 250 μM)[13] were added to a mixture of MeOH/pH 7 buffer solution (5:95) (4.0 mL), and the samples were incubated for 30 min at 37 °C.

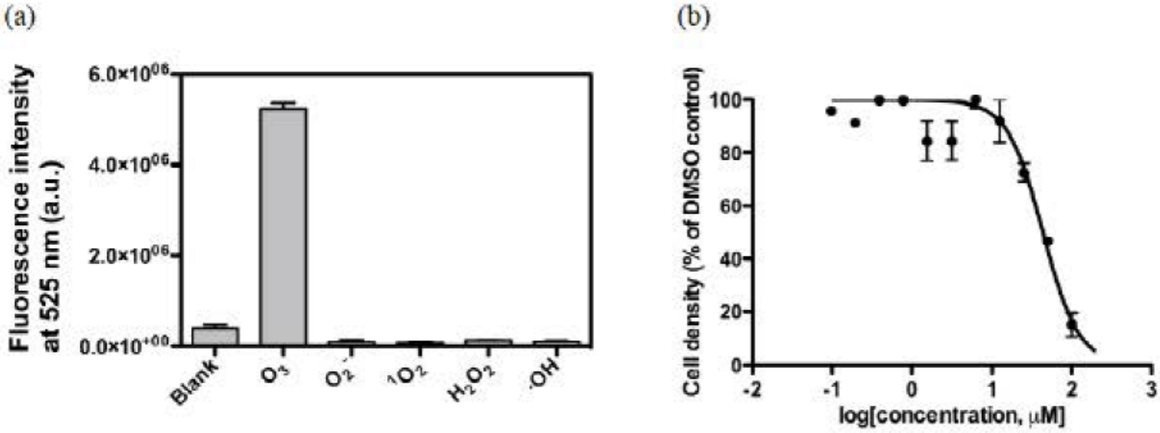

Figure S4. (a) The reaction of ROS with compound **8**. The experiment was performed in duplicate. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.). (b) Cytotoxicity of compound **8** against HeLa cells seeded at a density of 2000 cells/well in a 96-well plate. Concentrations of compound **8** ranged from 10 nM to 200 μM. The assay was performed for 3 days, and

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[10] Singleton, D. A.; Hang, C.; Szymanski, M. J.; Meyer, M. P.; Leach, A. G.; Kuwata, K. T.; Chen, J. S.; Greer, A.; Foote, C. S.; Houk, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 1319.

[11] Procedure from: Aubry, J. M.; Cazin, B.; Duprat, F. *J. Org. Chem.* **1989**, *54*, 726.

[12] Procedure from: Setsukinai, K.; Urano, Y.; Kakinuma, K.; Majima, H. J.; Nagano, T. *J. Biol. Chem.* **2003**, *278*, 3170.

[13] Procedure from: Yan, E. B.; Unthank, J. K.; Castillo-Melendez, M.; Miller, S. L.; Langford, S. J.; Walker, D. W. *J. Appl. Physiol.* **2005**, *98*, 2304.

the relative cell density was determined by an MTS assay (Promega CellTiter 96® AQ$_{ueous}$ Non-Radioactive Cell Proliferation Assay). The IC_{50} of **8** was found to be 43 μM.

Indigo carmine with various ROS. The ROS experiments described above were repeated using indigo carmine (Solution **B**, [Indigo Carmine]$_{final}$ = 12.5 μM in all cases) in triplicate. The data are shown below in Figure S5b. **Conclusion:** Indigo carmine reacts with nearly all ROS with a 11–70% decrease in absorbance at 610 nm.

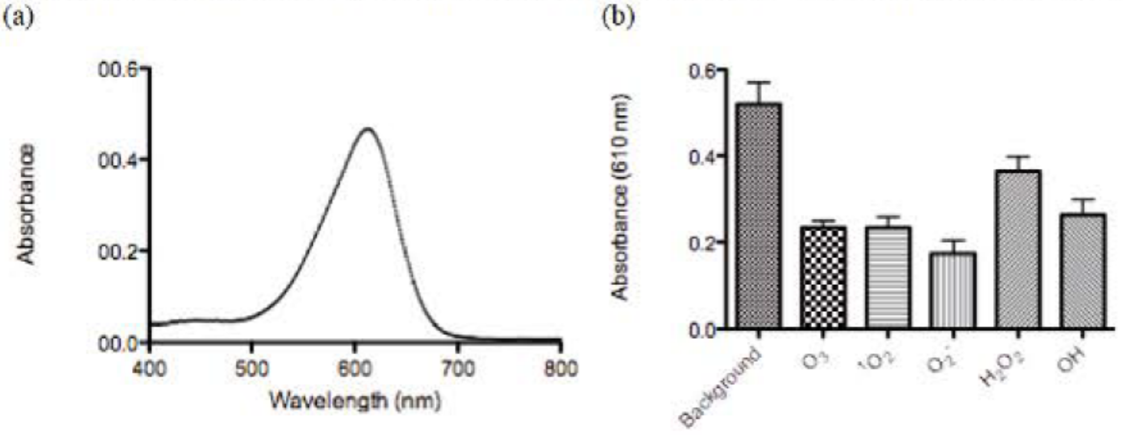

Figure S5. (a) Absorbance spectrum of indigo carmine. (b) ROS with indigo carmine. Each experiment was performed in triplicate and error bars were determined from the mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

Time-dependence of the conversion of 4 to 7 in buffer. Solution **A** ([**4**]$_{final}$ = 12.5 μM) was added to a mixture of MeOH/pH 7 buffer ([PO_4^{3-}] = 5 mM) (5:95) (4.0 mL), and the sample was exposed to ozone (~5 μM) at 24 °C followed by incubation at 37 °C. Aliquots of the reaction mixture were taken out at various times for fluorescence measurement (Figure S6). The conversion of **4** to **7** requires 60 min for completion (presumably the β-elimination is the rate determining step because the formation and fragmentation of molozonides are extremely rapid[14]), but 10–15 min is sufficient to observe fluorescence signal.

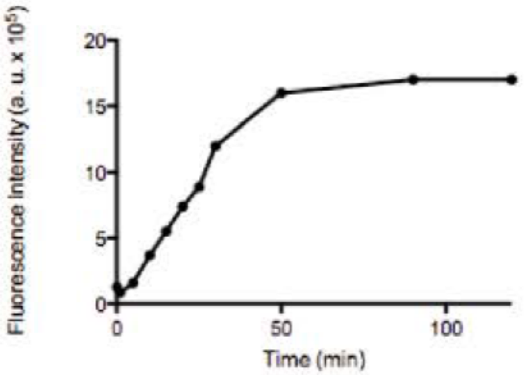

Figure S6. Time-dependence of the conversion of **4** to **7** at 37 °C MeOH/pH 7 buffer. See above for conditions.

Time-dependence of the conversion of 4 to 7 in cell culture media. Solution **A** ([**4**]$_{final}$ = 12.5 μM) was added to a mixture of 10% FBS in RPMI-1640 cell culture media/pH 7 buffer ([PO_4^{3-}] = 5 mM) (1:3) (4.0 mL), and the

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[14] Greenwood, F. L.; Durham, L. J. *J. Org. Chem.* **1969**, *34*, 3363.

sample was exposed to ozone (~5 μM) at 24 °C followed by incubation at 37 °C. Aliquots of the reaction mixture were taken out at various times for fluorescence measurement (Figure S7). The conversion of **4** to **7** requires 15–20 min for completion, but <5 min is sufficient to observe fluorescence signal. Although the exact mechanism of this enhancement is not known, catalysis of a β-elimination by action of bovine serum albumin has been previously reported.[15]

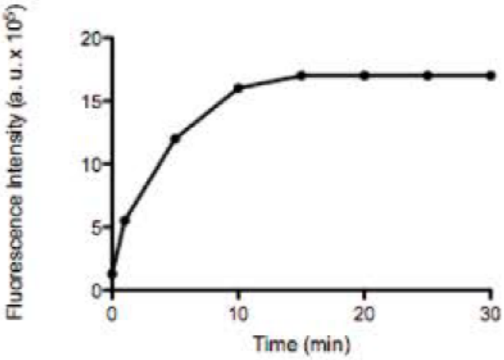

Figure S7. Time-dependence of the conversion of **4** to **7** at 37 °C in 10% FBS in RPMI-1640 cell culture media/pH 7 buffer. See above for conditions.

Concentration dependence. Ozone solutions were prepared in pH 6 buffer and the concentrations were determined by UV absorption (λ_{max} = 258 nm; ε = 2900 l mol^{-1} cm^{-1}.[16] Solution **A** (5.0 μL, $[\mathbf{4}]_{final}$ = 12.5 μM) was added to a mixture of MeOH/pH 7 buffer ($[PO_4^{3-}]$ = 5 mM) (5:95) (4.0 mL), and the samples were exposed to varying amounts of ozone at 24 °C followed by 1 h incubation at 37 °C. The samples were diluted 10-fold with pH 7.0 buffer ($[PO_4^{3-}]$ = 5 mM) before fluorescence measurement to maintain linearity with respect to fluorescence signal (see Figure S8 below for the linearity between 0 and 12.5 μM). For each experiment, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

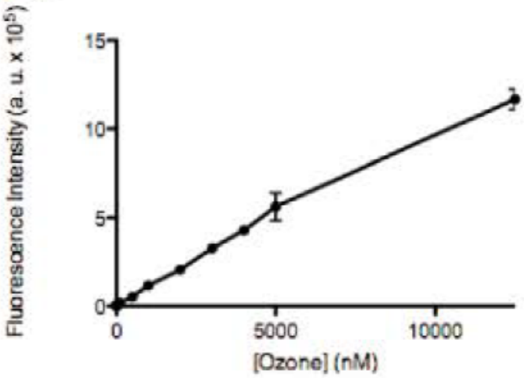

Figure S8. Correlation between fluorescence intensity (λ_{em} = 523 nm) and [ozone] in MeOH/pH 7 buffer (5:95). The background signal is 0.0377 × 10^5. See above for conditions.

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[15] Klein, G.; Reymond, J-L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1113.

[16] Panich, N. M.; Ershov, B. G., Seliverstov, A. F., Basiev, A. G. *Russ. J. Appl. Chem.* **2007**, *80*, 1812. Ershov, B. G.; Panich, N. M.; Seliverstov, A. F.; Belyaeva, M. P. *Russ. J. Appl. Chem.* **2008**, *81*, 723.

Ozone detection in the presence of antioxidants. Solution **A** (5.0 μL, [**4**]$_{\text{final}}$ = 12.5 μM) and solutions **E–G** (**E**: 20.0 μL, [Ascorbic acid]$_{\text{final}}$ = 50 μM; **F**, **G**: 40.0 μL, [Glutathione/Uric acid]$_{\text{final}}$ = 100 μM) were added to a mixture of MeOH/pH 7 buffer ([PO_4^{3-}] = 5 mM) (5:95) (4.0 mL), and the samples were exposed to ozone (~ 5 μM) at 24 °C followed by 1 h incubation at 37 °C before fluorescence measurement. For each experiment, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

Ozone detection in human pleural fluid. Human pleural fluid (1.0 mL) was diluted in pH 7 buffer ([PO_4^{3-}] = 5 mM) (3.0 mL). Solution **A** (5.0 μL, [**4**]$_{\text{final}}$ = 12.5 μM) was added to the resulting human pleural fluid mixture, and the samples were exposed to ozone (~ 5 μM) at 24 °C followed by 1 h incubation at 37 °C before fluorescence measurement. The experiment was performed in triplicate.

Ozone detection in human serum. Human serum (1.0 mL) was diluted in pH 7 buffer ([PO_4^{3-}] = 5 mM) (3.0 mL). Solution **A** (5.0 μL, [**4**]$_{\text{final}}$ = 12.5 μM) was added to the resulting human serum mixture, and the samples were exposed to ozone (~ 5 μM) at 24 °C followed by 1 h incubation at 37 °C before fluorescence measurement. The experiment was performed in triplicate.

Ozone detection in indoor air. One adsorbent paper (Kimwipe®) was cut into two 11 × 9 cm pieces. Solution A (50 μL, 500 nmol of **4**) was added to one portion of the Kimwipe® and allowed to dry (Figure S9, top right). Solution B (50 μL, 500 nmol of indigo carmine) was added to the other portion of the Kimwipe® and allowed to dry (Figure S9, top left). After overnight drying in a laboratory drawer, the paper strips were moved to a poorly ventilated photocopy room (2 photocopiers and 2 laser printers) for 8 h. The controls remained in the laboratory drawer. After 8 h, the samples were placed into a scintillation vial, and the dyes were eluted with pH 7 buffer (6.0 mL) (~5 min) before fluorescence and absorbance measurement. For each compound, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

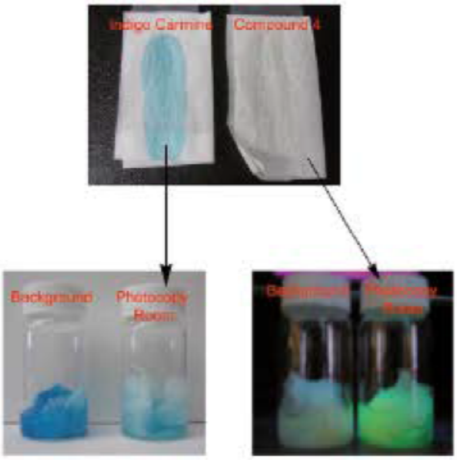

Figure S9. Ozone detection in indoor air. Top photo = Adsorbent paper before placing into photocopy room for 8 h. Bottom photos = Adsorbent paper in buffer for absorbance and fluorescence measurement. Left = indigo carmine (photo taken in room light), right = **4** (photo taken under long-range UV lamp).

Ozone detection in ambient air. Solution **A** (5.0 µL, [**4**]$_{final}$ = 12.5 µM) was added to a mixture of MeOH/pH 7 buffer ([PO_4^{3-}] = 5 mM) (5:95) (4.0 mL), and the samples were placed in 4 outdoor areas throughout University of Pittsburgh campus for 8 h (high temperature = 32.2 °C; AQI = 43; PM2.5 = 64)[17] on June 6, 2008. Care was taken to ensure the samples were not exposed to direct sunlight. The locations are described below. For each location, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

Sample	**Location** (See the map: http://maps.google.com/?ie=UTF8&ll=40.444612,-79.955739&spn=0.00783,0.009527&t=h&z=17&layer=t)
Background	A negative control was prepared in the same manner but incubated at 37 °C in a closed vial for 8 h.
A	Corner of Forbes Avenue* (3 lane road) and South Bouquet Street (2 lane road) next to a lane of traffic frequently traveled by cars and buses
B	Corner of Fifth Avenue* (4 lane road) and Bigelow Boulevard (4 lane road) next to a lane of traffic frequently traveled by cars and buses
C	In a tree ~200 m from Fifth Avenue (University Drive/Parkman Avenue)
D	Outside a 12th floor window of Chevron Science Center, which is ~200 m from Fifth Avenue (the corner between University Drive and Parkman Avenue)

* Forbes and Fifth Avenues are two of the most heavily traveled streets in Pittsburgh, Pennsylvania

Air pollutant specificity:

Acids: HNO_3 or H_2SO_4 (10 µL of 0.1 M solution; [acid]$_{final}$ = 250 µM) and solution **A** (5.0 µL, [**4**]$_{final}$ = 12.5 µM) were added to a mixture of MeOH/pure water solution (5:95) (4.0 mL), and the samples were incubated at 37 °C for 30 min before fluorescence measurement. For each experiment, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

Metals: Pb, Pd or Pt solution (40 µL of 10 µM solution; [Metal]$_{final}$ = 100 nM) and solution **A** (5.0 µL, [**4**]$_{final}$ = 12.5 µM) were added to a mixture of MeOH/pH 7 buffer ([PO_4^{3-}] = 5 mM) (5:95) (4.0 mL), and the samples were incubated at 37 °C for 30 min before fluorescence measurement. For each experiment, triplicate replicas were performed. Error bars were determined from the calculated mean and standard deviation (Prism 5.0a, GraphPad Software, Inc.).

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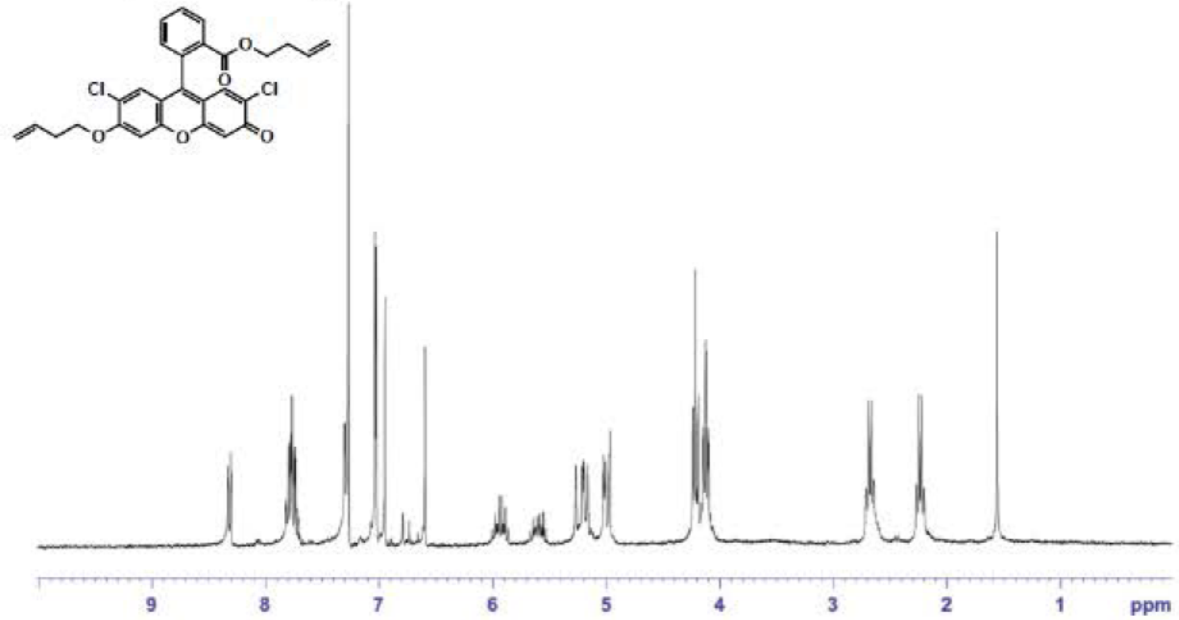
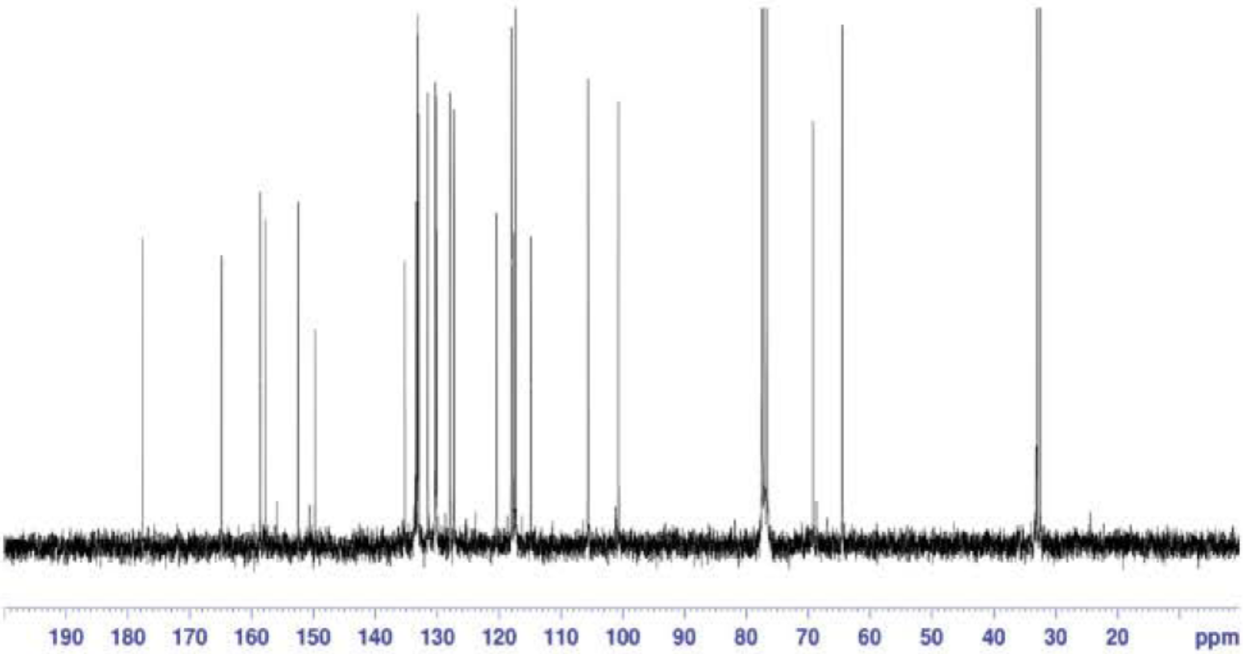
[17] Data taken from http://www.airnow.gov. AQI = air quality index. PM2.5 = particulate matter less than 2.5 µm.

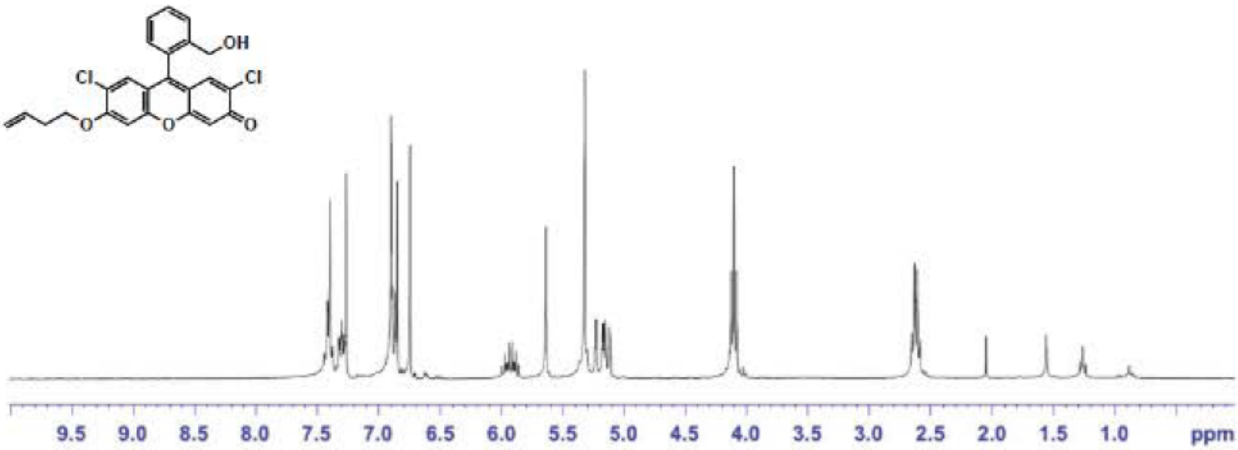
^{1}H NMR spectrum of compound **3**: $CDCl_3$, 293K, 300 MHz



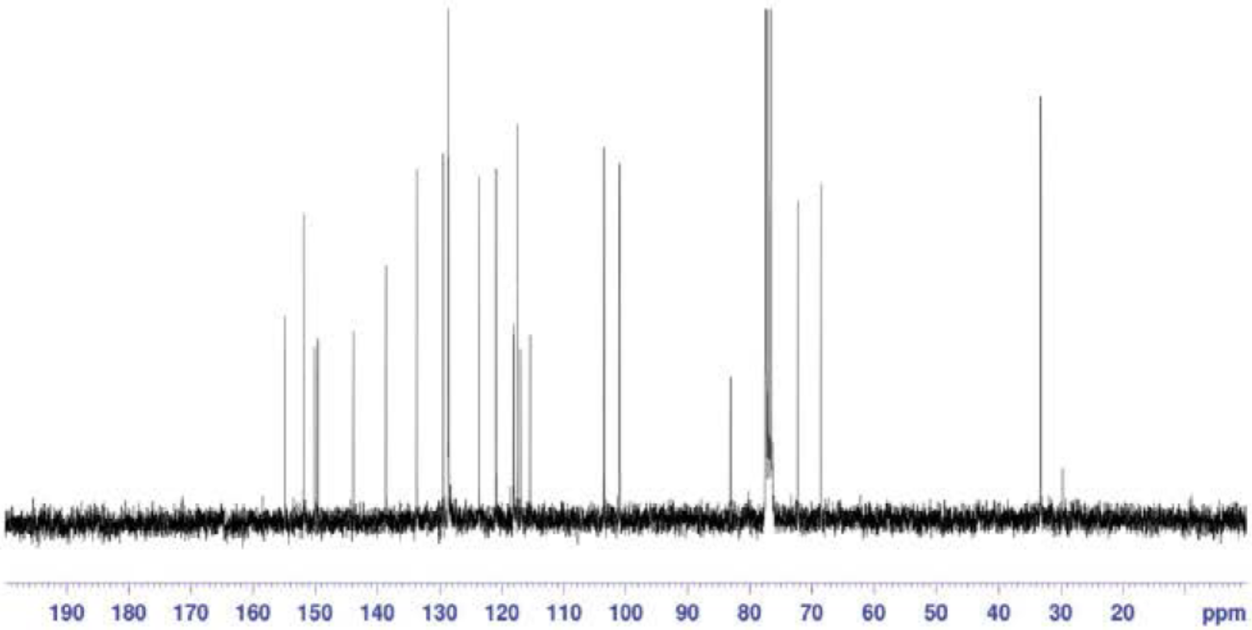
^{13}C NMR spectrum of compound **3**: $CDCl_3$, 293K, 75 MHz



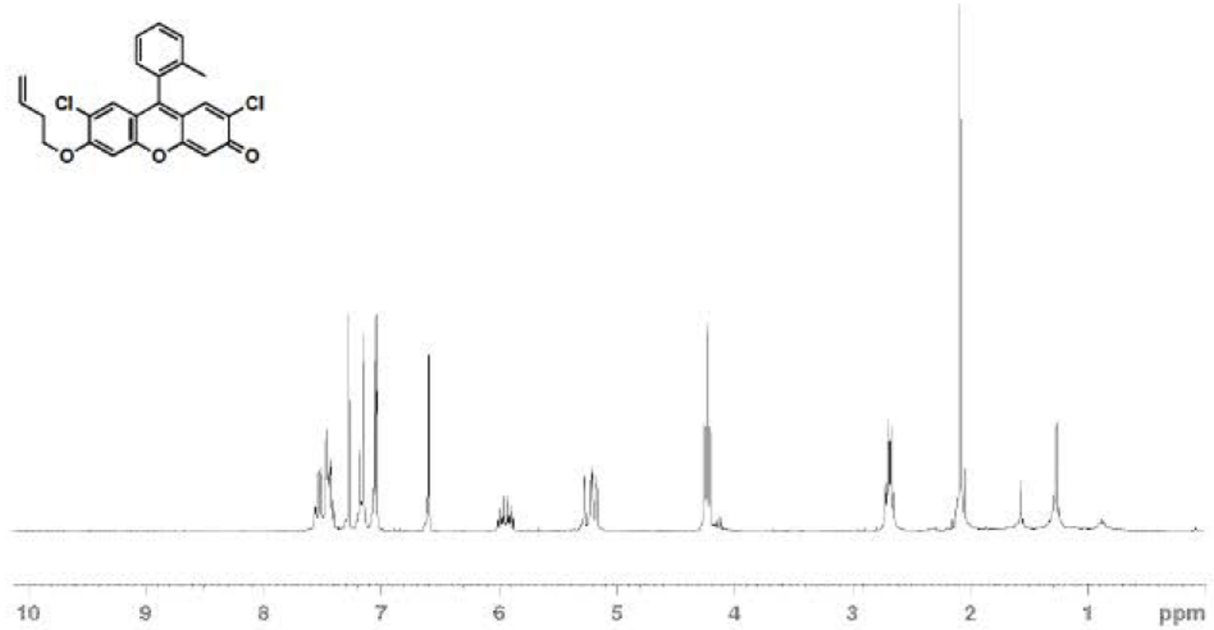
^{1}H NMR spectrum of compound **4**: $CDCl_3$, 293K, 300 MHz



^{13}C NMR spectrum of compound **4**: $CDCl_3$, 293K, 75 MHz



^{1}H NMR spectrum of compound **8**: $CDCl_3$, 293K, 300 MHz



^{13}C NMR spectrum of compound **8**: $CDCl_3$, 293K, 75 MHz