

Supplementary Data for:

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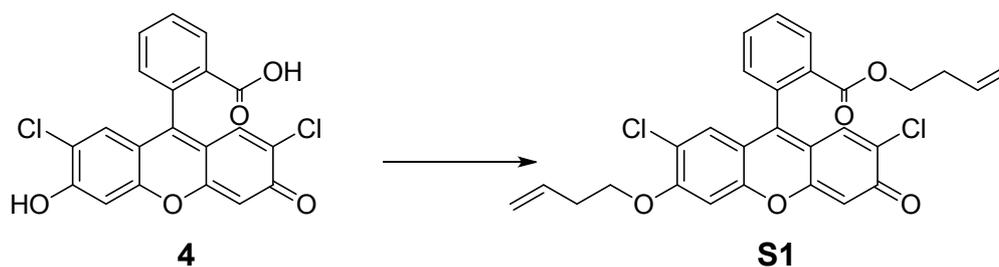
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General Experimental Details

All infrared spectra were obtained using a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer; thin film spectra were acquired using sodium chloride plates. All ^1H and ^{13}C NMR spectra were measured at 400.13 and 100.62 MHz using a Bruker Avance 400 MHz spectrometer. The solvent used for NMR spectroscopy was CDCl_3 (unless stated otherwise) using TMS (tetramethylsilane) as the internal reference. Chemical shifts are given in parts per million (ppm) and J values are given in Hertz (Hz). Mass spectra were recorded on a Thermo Fisher Exactive with an ion max source and ESI probe fitted with an Advion traversa nanomate. Mass range 20-2000 with a resolution of better than 100000. Melting points were recorded using Stuart Scientific melting point apparatus SMP3 and are uncorrected. Microanalyses were performed on an Exeter Analytical Inc. CE-440 Elemental Analyser. All chromatographic manipulations used silica gel as the adsorbent. Reactions were monitored using thin layer chromatography (TLC) on glass backed plates with Merck Kiesel 60 F254 silica gel. TLC visualised by UV radiation at a wavelength of 254 nm, or stained by exposure to an ethanolic solution of phosphomolybdic acid (acidified with concentrated sulphuric acid), followed by charring where appropriate. Purification by column chromatography used Merck Kiesel 60 H silica adsorbent. Reaction solvents were obtained commercially dry. Ozone was generated by feeding high purity oxygen into a Fischer Ozone Generator 500MM or into an AMBIO₃X, Ozone Generator. Absorption spectra were acquired in Quartz Cells with a 10mm path length UV/VIS on a Shimadzu UV-Visible Spectrophotometer UV-1601PC. Fluorescence spectra were recorded in a Quartz Fluorimeter Cell with a 10mm path length on a Perkin-Elmer Luminiscence Spectrometer LS SOB. The samples were excited at 497 nm and the emission intensities were collected from 500 to 650 nm.

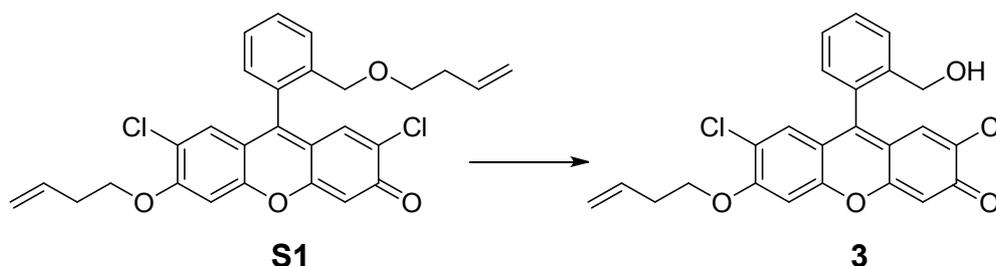
Synthesis of the Ozone Probe (3):

But-3-en-1-yl-2-(6-(but-3-en-1-yloxy)-2,7-dichloro-3-oxo-3H-xanthen-9-yl)benzoate S1:¹



Potassium carbonate (2.88 g, 20.8 mmol) and 4-bromobutene (2.1 mL, 20.7 mmol) were added to a stirred solution of 2',7'-dichlorofluorescein **4** (2.76 g, 6.88 mmol) in DMF (25 mL) in a 100-mL round bottom flask at rt and the reaction mixture was stirred at 70 °C overnight. The mixture was added to a beaker containing distilled water (750 mL) and the resulting precipitate filtered using a Büchner funnel and further washed with distilled water (250 mL). The solid was dissolved in CH₂Cl₂, dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure and the solid dried under vacuum to afford the title compound **S1** as a red solid (3.19 g, 91% yield); Mp 182-183 °C [lit. 184-185 °C]; IR (KBr): 1719, 1591, 1520, 1278, 1000 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 293K): δ 8.31 (dd, J= 7.6, 1.2 Hz, 1H), 7.73 (td, J=7.6, 1.2 Hz, 1H), 7.28 (dd, J=7.6, 1.2 Hz, 1H), 7.03 (s, 1H), 7.02 (s, 1H), 6.94 (s, 1H), 6.59 (s 1H), 5.94 (ddt, J= 17.2, 10.4, 6.8 Hz, 1H), 5.59 (ddt, 17.2, 9.6, 6.8 Hz 1H), 5.23 (dd, J= 17.2, 1.2 Hz, 1H), 5.17 (dd, J= 10.4, 1.2 Hz, 1H), 5.00-5.01 (m, 1H), 4.97 (m, 1H), 4.21 (t, J= 6.8 Hz, 2H), 4.07-4.17 (m, 2H), 2.66 (q, J=6.8 Hz, 2H), 2.22 (q, J=6.8 Hz, 2H). ¹³C NMR (400MHz, CDCl₃, 293k): δ 177.7, 164.9, 158.6, 157.8, 152.5, 149.7, 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, 69.3, 64.5, 33.1, 32.6.

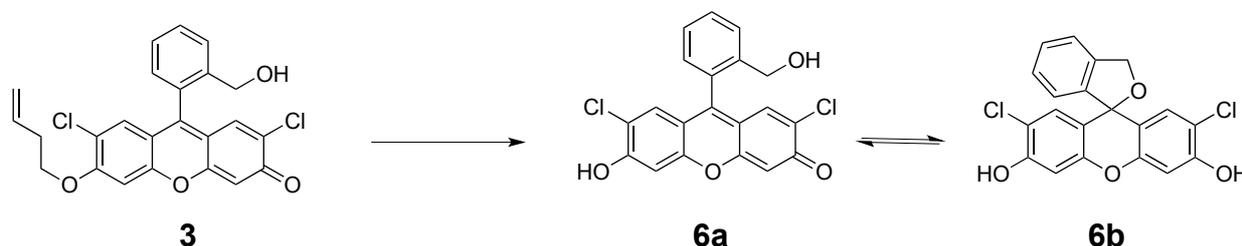
6-(But-3-en-1-yloxy)-2,7-dichloro-9-(2-(hydroxymethyl) phenyl) -3H-xanthen-3-one (3):¹



But-3-en-1-yl-2-(6-(but-3-en-1-yloxy)-2,7-dichloro-3-oxo-3H-xanthen-9-yl)benzoate **S1** (2.05 g, 4.0 mmol) was dissolved in dried CH₂Cl₂ (15 mL) in a 100 mL round bottom flask and purged with N₂ over 10 min with stirring. DIBAL (14.0 mL, 1M in hexanes,

14.0 mmol) was added dropwise over 15 min at $-78\text{ }^{\circ}\text{C}$ under N_2 . After 2h the mixture was warmed to $0\text{ }^{\circ}\text{C}$ and Et_2O (20 mL) and sat. NH_4Cl aq. (6 mL) were added. After 30 min DDQ (1.00 g, 4.4 mmol) was added to the mixture and the colour changed from orange to dark green. The mixture was stirred for a further 30 min at rt before being filtered through Celite using EtOAc as eluent. Silica gel chromatography of the crude material (5-10% gradient elution with EtOAc in hexanes) afforded the product as light orange solid (1.41 g, 79% yield). The product was recrystallised from hexane to obtain the title compound **1** as light orange crystals (1.03 g, 58% yield); Mp $169\text{-}170\text{ }^{\circ}\text{C}$ [lit. $167\text{-}168\text{ }^{\circ}\text{C}$]. IR (KBr): $3231, 2921, 2869, 1608\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3 , 293K): δ 7.38-7.43 (m, 2H), 7.26-7.31 (m, 1H), 6.90 (s, 1H), 6.88 (s, 1H), 6.86 (s 1H), 6.81-6.83 (m, 1H), 6.74 (s, 1H), 5.92 (ddt, $J = 17.2, 10.4, 6.4\text{ Hz}$, 1H), 5.32 (s, 2H), 5.19 (dd, $J = 17.2, 1.6\text{ Hz}$ 1H), 5.13 (dd, $J = 10.4, 1.6\text{ Hz}$, 1H), 4.08 (t, $J = 6.8\text{ Hz}$, 2H), 2.61 (td, $J = 6.8, 6.4\text{ Hz}$, 2H); ^{13}C NMR (400MHz, CDCl_3 , 293k): δ 154.9, 151.9, 150.1, 149.6, 143.9, 138.6, 133.8, 129.5, 128.8, 128.6, 123.7, 121.0, 118.2, 118.1, 117.5, 117.1, 115.5, 103.6, 101.1, 83.1, 77.3, 72.3, 68.6, 33.3.

2,7-Dichloro-9-(2-(hydroxymethyl)phenyl)-3-oxo-3H-xanthen-6-olate 6a,b:¹

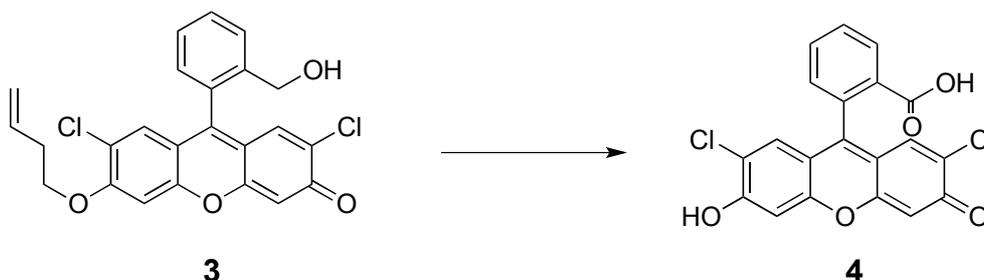


A solution of 6-(But-3-en-1-yloxy)-2,7-dichloro-9-(2-(hydroxymethyl)phenyl)-3H-xanthen-3-one **1** (100 mg, 0.227 mmol) in 30 mL of acetone: water (95:5) was cooled to $0\text{ }^{\circ}\text{C}$. Ozone generated in an AMBIOX ozone generator was bubbled through the solution for 15 min. KI (15 mg, 0.090 mmol) was added. The mixture was stirred for one hour and solvent was removed under reduced pressure to afford the product as an orange solid that was isolated using column chromatography (23 mg, 25% yield).

6a: IR (KBr): 3413, 1571, 1486, 1358; ^1H NMR (400 MHz, CDCl_3 , 293K): δ 7.43-7.49 (m, 2H), 7.33 (t, $J = 7.5\text{ Hz}$, 1H), 6.91 (s, 1H), 6.84-6.86 (m, 2H), 6.81 (s 1H), 6.78 (s, 1H), 5.31 (s, 1H); ^{13}C NMR (400MHz, CDCl_3 , 293k): δ 156.4, 155.5, 151.2, 151.1, 145.4, 140.0, 130.6, 130.5, 129.9, 124.6, 122.3, 118.6, 118.6, 117.9, 117.7, 104.5, 102.2, 96.9, 84.6, 73.2; HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{13}\text{Cl}_2\text{O}_4$ (M+H) 387.0185, found 387.0177.

6b: ^1H NMR (400 MHz, CDCl_3 , 293K): δ 7.67 (d, J = 7.6 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.93 (s, 2H), 6.50 (s 2H), 4.24 (s, 2H); ^{13}C NMR (400MHz, CDCl_3 , 293k): δ 176.6, 158.8, 154.3, 140.9, 132.6, 131.1, 130.3, 129.8, 129.2, 129.0, 128.7, 112.5, 105.1, 62.6.

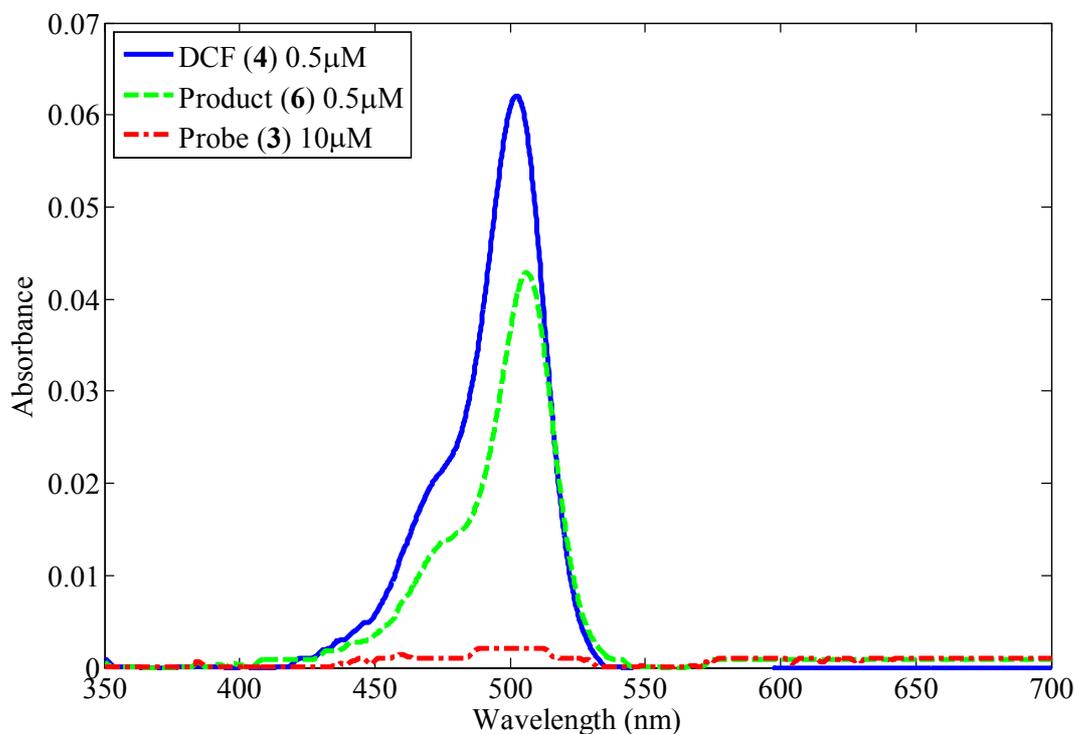
2',7'-dichlorofluorescein 4:¹



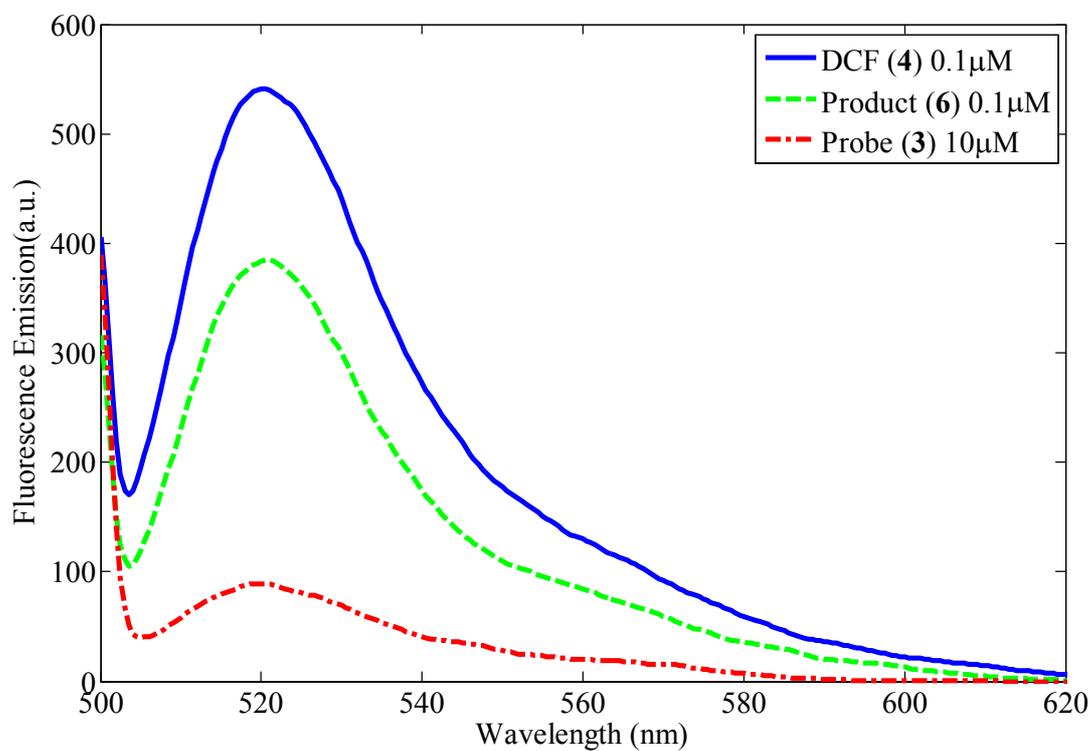
A solution of 6-(But-3-en-1-yloxy)-2,7-dichloro-9-(2-(hydroxymethyl)phenyl)-3H-xanthen-3-one **1** (100 mg, 0.227 mmol) in 30 mL of acetone: water (95:5) was cooled to 0 °C. Ozone generated in an AMBIOX ozone generator was bubbled through the solution for 15 min. KI (15 mg, 0.090 mmol) was added. The mixture was stirred for one hour and solvent was removed under reduced pressure to afford the product as an orange solid that was isolated using column chromatography (34 mg, 37% yield). ^1H NMR (400 MHz, d_4 -MeOD, 293K): δ 8.08 (d, J = 7.6 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.77 (t, J = 7.6, 1H), 7.28 (d, J =7.6, 1H), 6.86 (s, 1H), 6.66 (s, 1H); ^{13}C NMR (400MHz, CDCl_3 , 293k): δ 169.4, 155.3, 152.2, 150.8, 135.6, 130.2, 128.1, 126.4, 124.7, 123.7, 116.8, 110.8, 103.4, 82.6. HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{10}\text{Cl}_2\text{NaO}_5$ ($\text{M}+\text{Na}$) 422.9798, found 422.9792.

Absorbance and fluorescence data

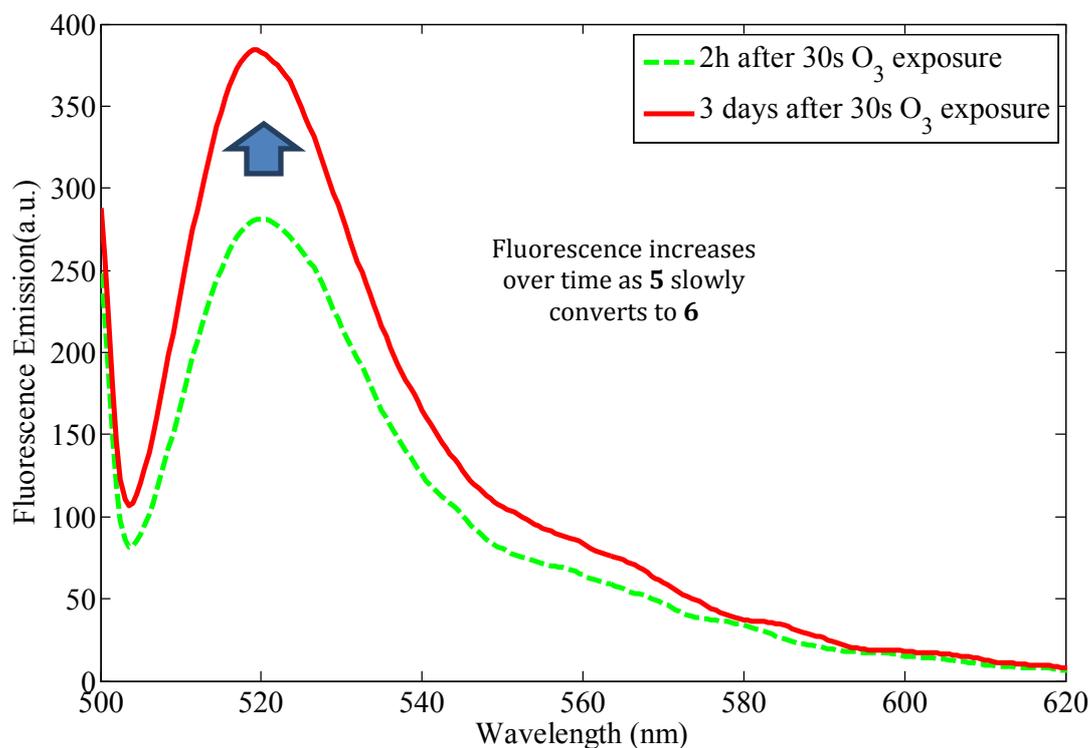
Absorbance spectra of commercial DCF 4 (blue solid line), ozone probe 3 (red dash-dotted line) and the reaction mixture obtained after ozone exposure (green dashed line).



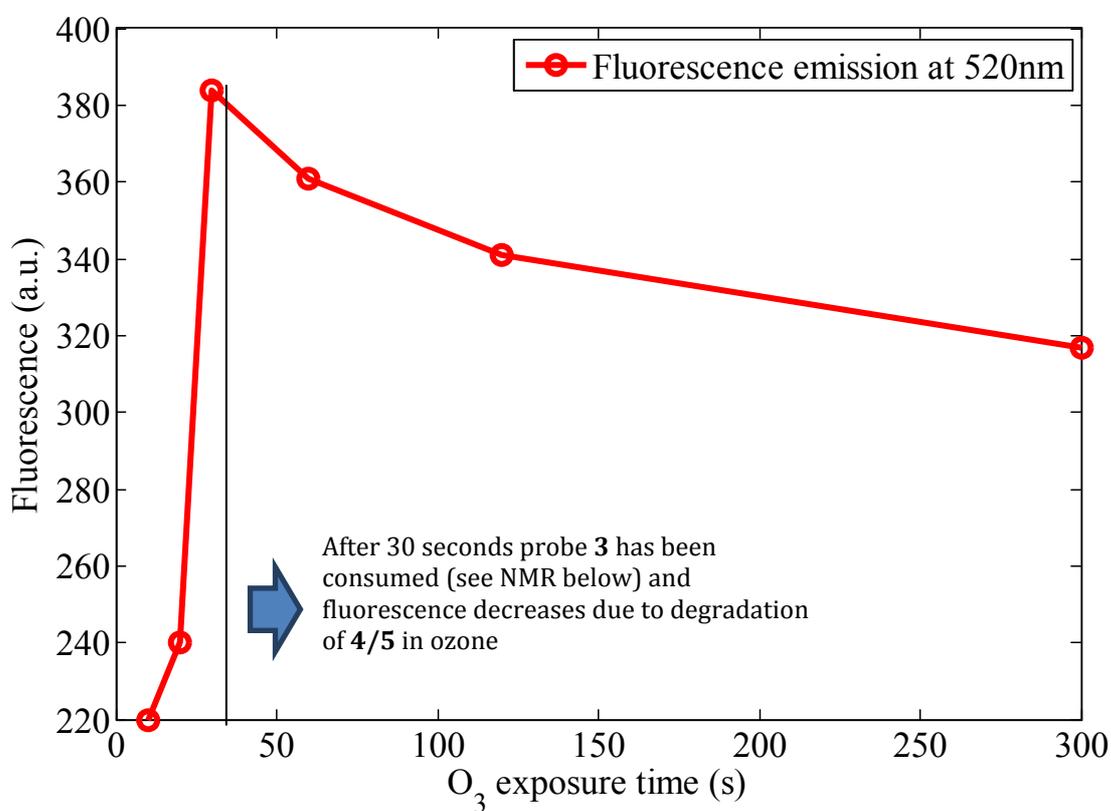
Fluorescence spectra of commercial DCF 4 (blue), ozone probe 4 (red dash-dotted line) and the reaction mixture obtained after ozone exposure (green dashed line).



Time evolution of the fluorescence of reaction mixture obtained after ozone exposure of probe 3 and subsequent addition of KI:

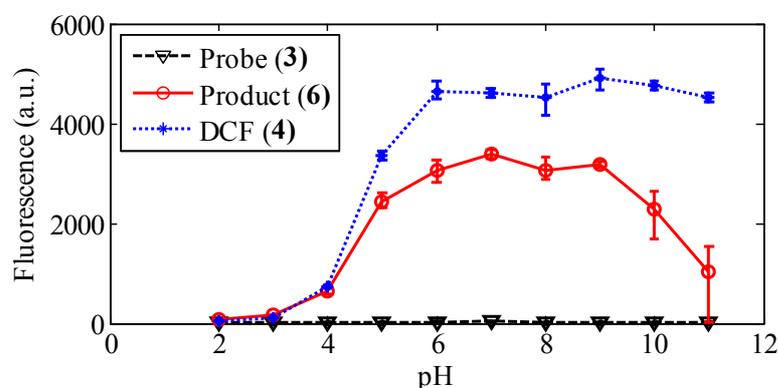


Observed fluorescence over increased O₃ exposure time:



pH stability of DCF (4) and fluorescent product (6)

Fluorescence of DCF (4) and probe product (6) at 1 μ M. Excitation - 502nm, Emission - 522nm.



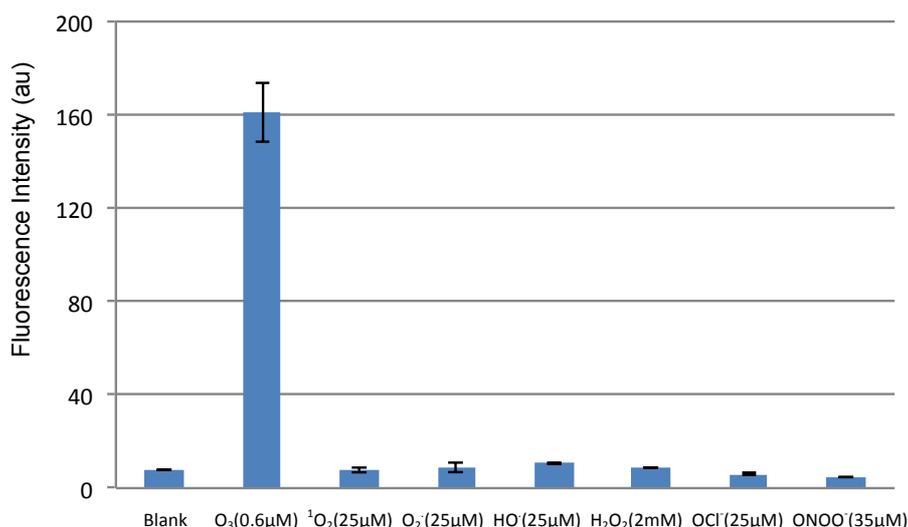
Selectivity of Probe 3

Preparation of stock solutions for selectivity study:

Ozone solution was prepared by bubbling ozone through H₂O at 0 °C for 10 min. Absorbance of the final solution was measured at 260 nm using H₂O as blank.

The peroxynitrite solution was prepared by adding 4 mL of H₂O₂ 0.6 M, 4 mL HCl 0.7M and 4 mL NaNO₂ (0.6 M). Immediately after the sodium nitrite, 4 mL NaOH 3M were added to the solution. Absorbance of the final solution was measured at 302 nm using H₂O as blank.

Entry	Reagent	MW (mol/L)	Quantity	Solvent	V (mL)	Conc.
A	Compound 3	455.29	0.9001mg	DMSO	1.980	1 mM
B	NaMoO ₄ ·2H ₂ O	241.95	6.27mg	pH 10 BS	2.590	10 mM
C	KO ₂	71.10	1.24 mg	DMSO	1.745	10 mM
D	FeSO ₄ ·7H ₂ O	278.02	6.99 mg	H ₂ O	2.515	10 mM



Preparation of solutions for selectivity study:

- Blank: **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Ozone: O₃ aq. 30 µM (0.2 mL) was added to **A** (5 µL). H₂O (0.2 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Singlet Oxygen: **B** (15 µL) and H₂O₂ 1.16 M (5 µL) were added to **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Superoxide: **C** (15 µL) was added to **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Hydrogen peroxide: H₂O₂ 1.16 M (10 µL) was added to **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Hydroxyl radical: **D** (15 µL) and H₂O₂ 11.6 mM (15 µL) were added to **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Hypochlorite: NaOCl 10 mM (15 µL) was added to **A** (5 µL). H₂O (0.4 mL) and pH 7 PBS (4.6 ml) were added to the solution.
- Peroxynitrite: ONOO⁻ 700 µM (0.25 mL) was added to **A** (5 µL). H₂O (0.15 mL) and pH 7 PBS (4.6 ml) were added to the solution.

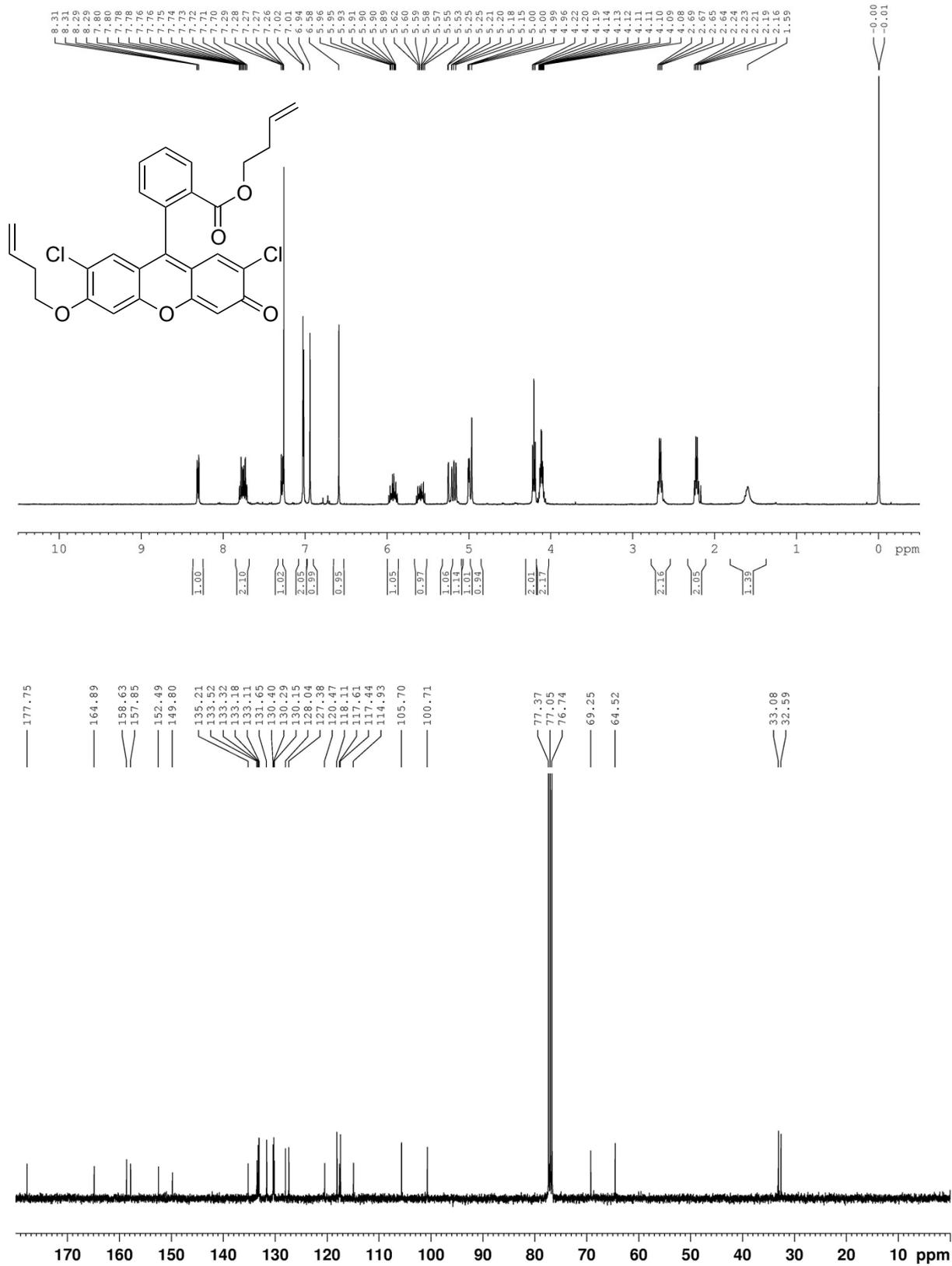
Fluorescence of the solutions were measured a day after the preparation in order to wait for the second step of the ozonolysis to take place. The samples were excited at 502 nm and the emission intensities were collected at 522 nm.

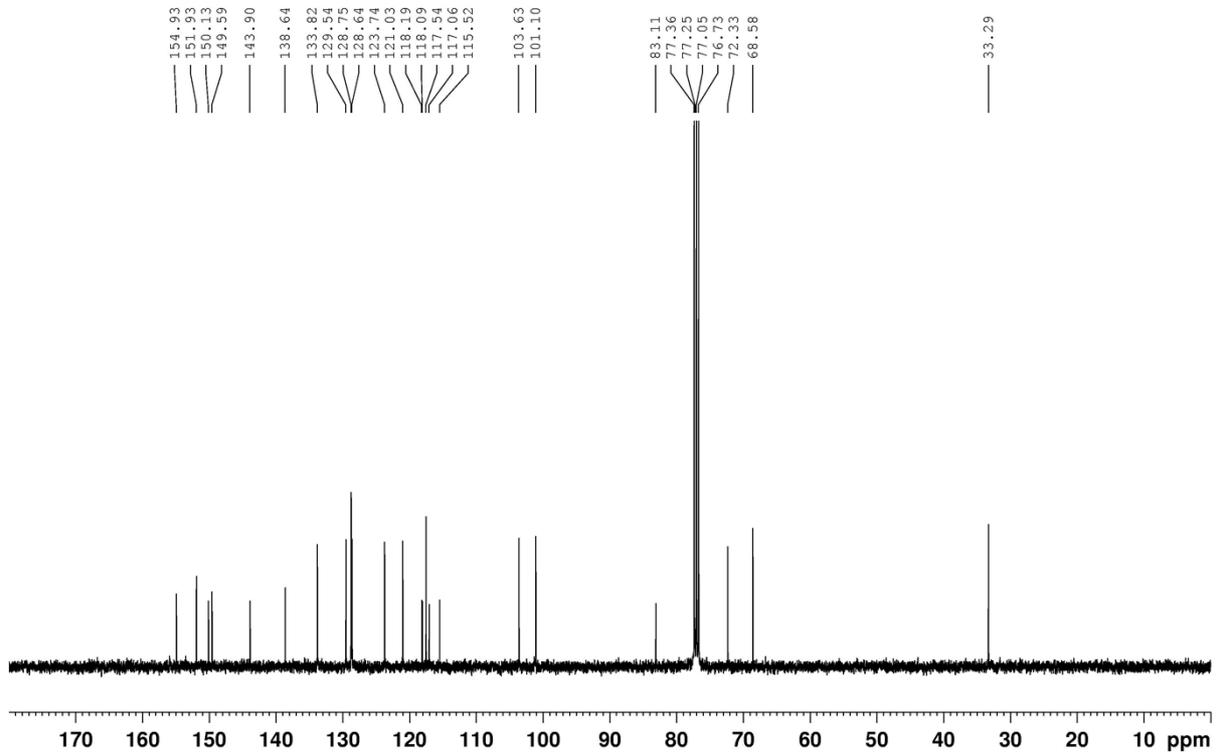
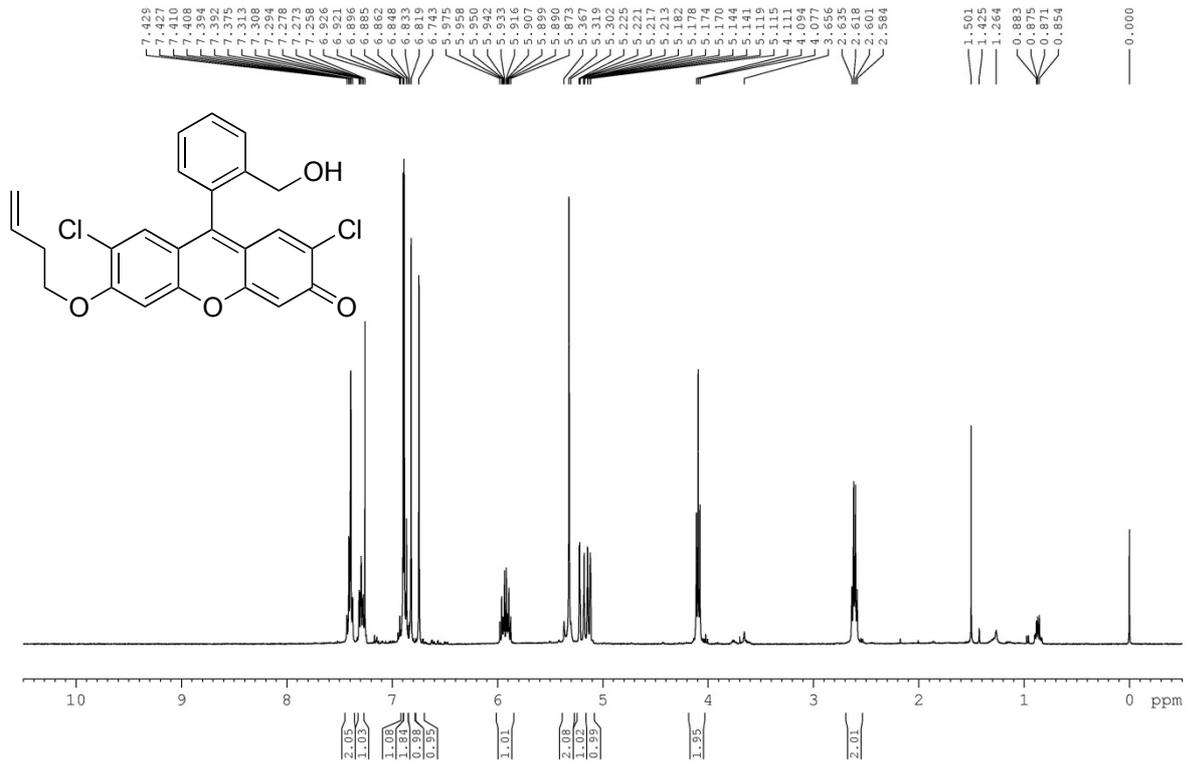
Caution:

As the ozonated water is manipulated to create the solution for the above study ozone escapes in an uncontrolled manner and decays fairly rapidly, causing a variability in the observed fluorescence measurements. Ozone detection using UV absorption in similarly prepared ozonated solutions also resulted in similar variation in ozone content.

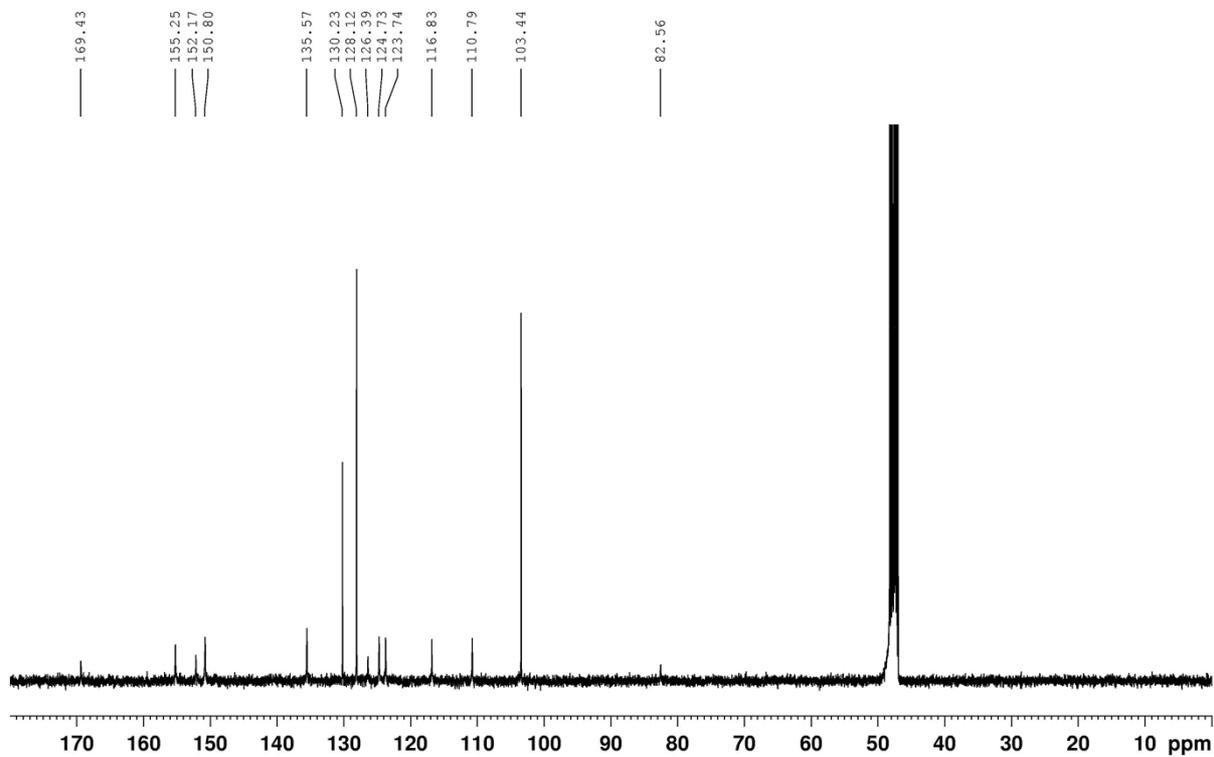
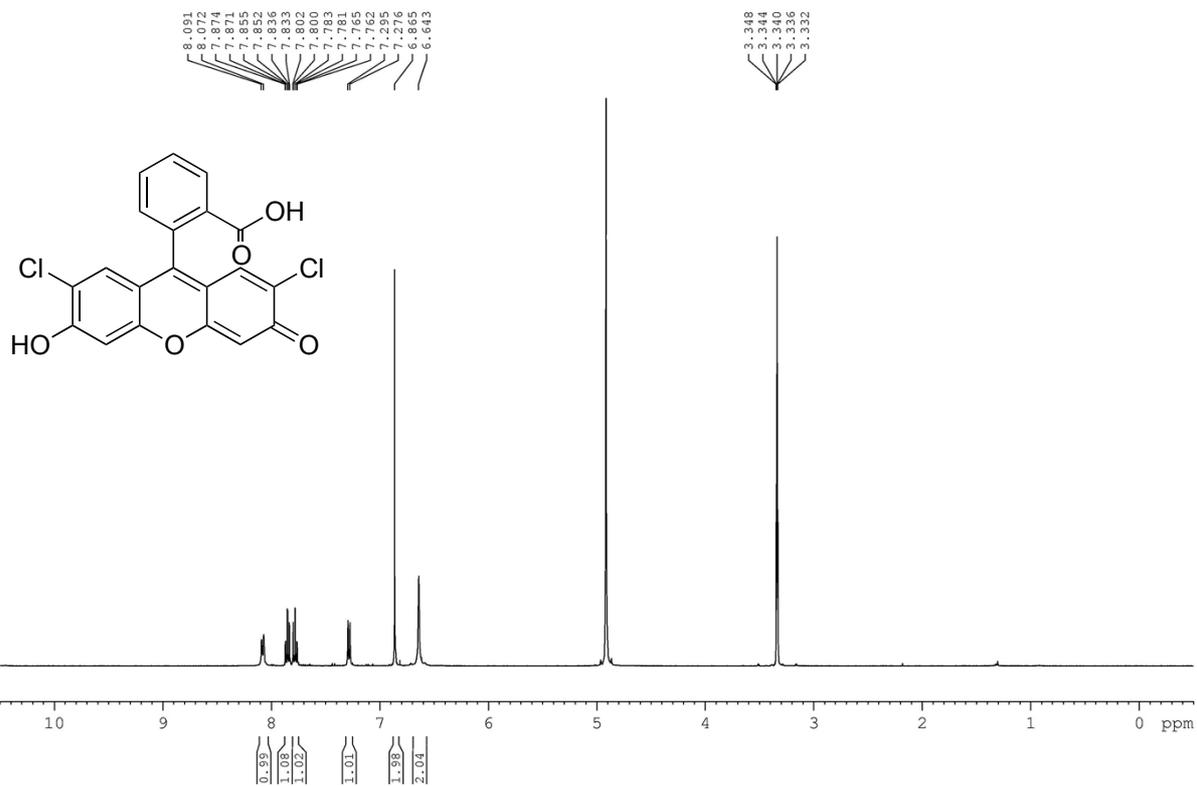
We were able to decrease this error by adding the ozone solution slowly and vertically, directly into the probe solution without touching the walls of the vial.

¹H & ¹³C NMR Spectra:



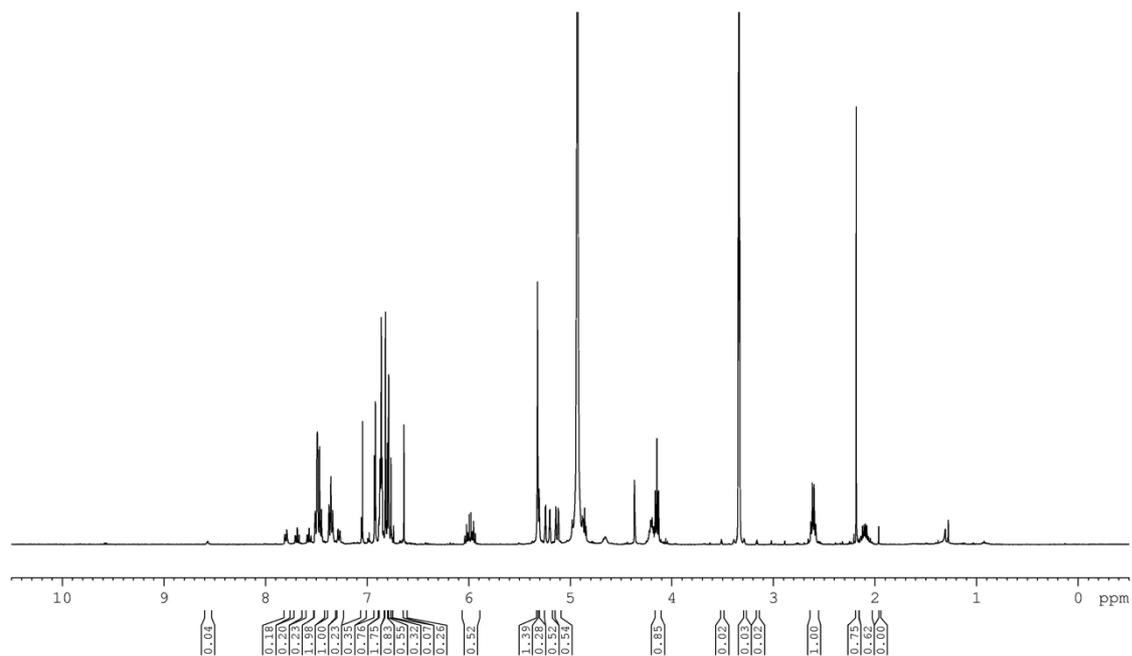


Authentic DCF 4, (from a commercial supplier)

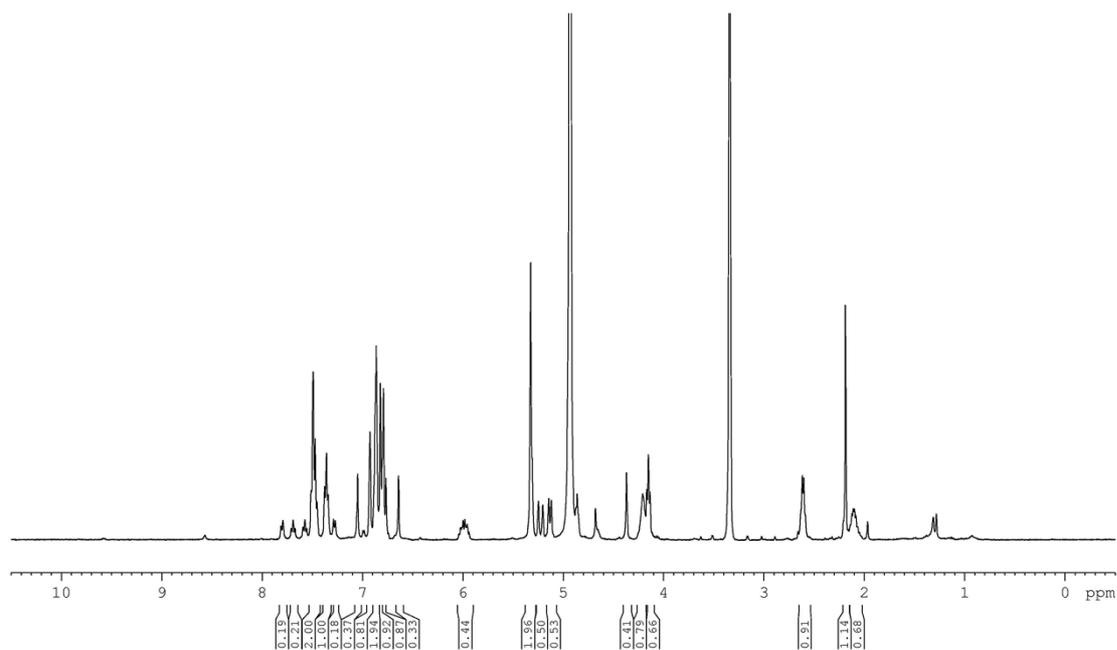


¹H NMR Spectra correlating to the fluorescence spectra taken at various O₃ exposure times:

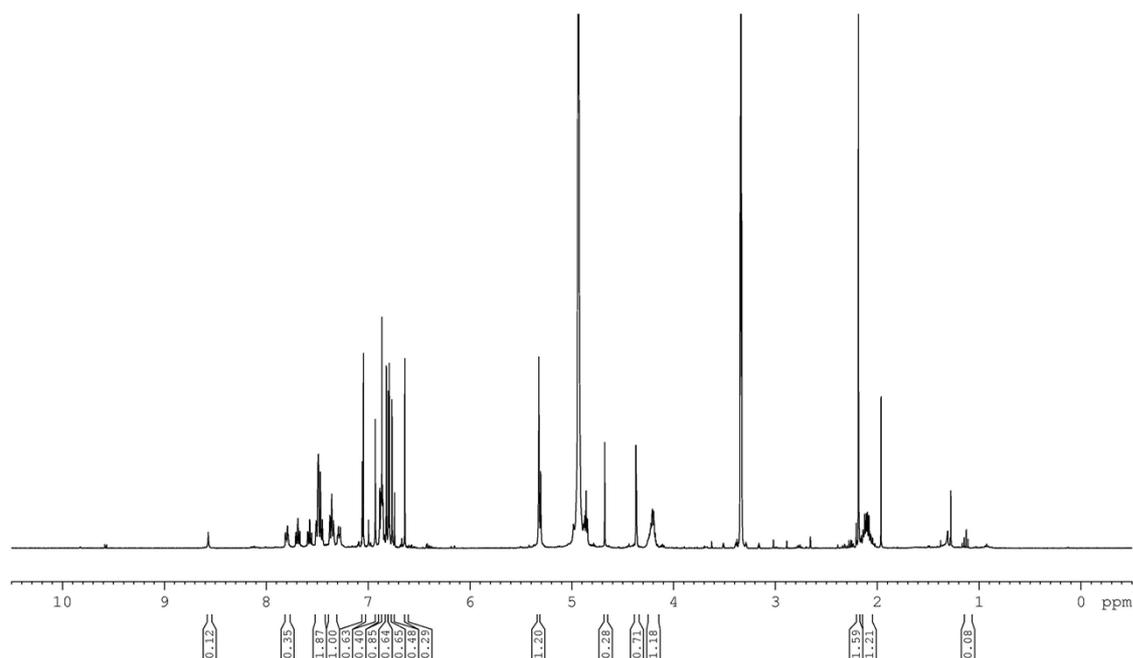
10s Exposure: Alkene CH peaks from probe **3** present



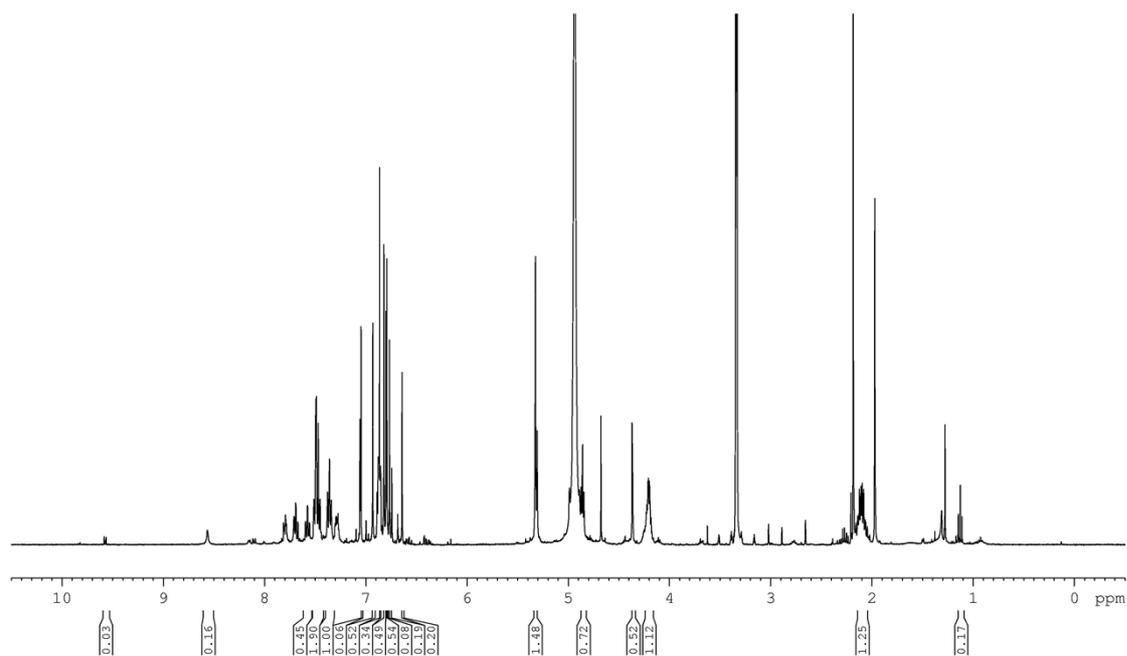
20s Exposure: Alkene CH peaks from probe **3** present



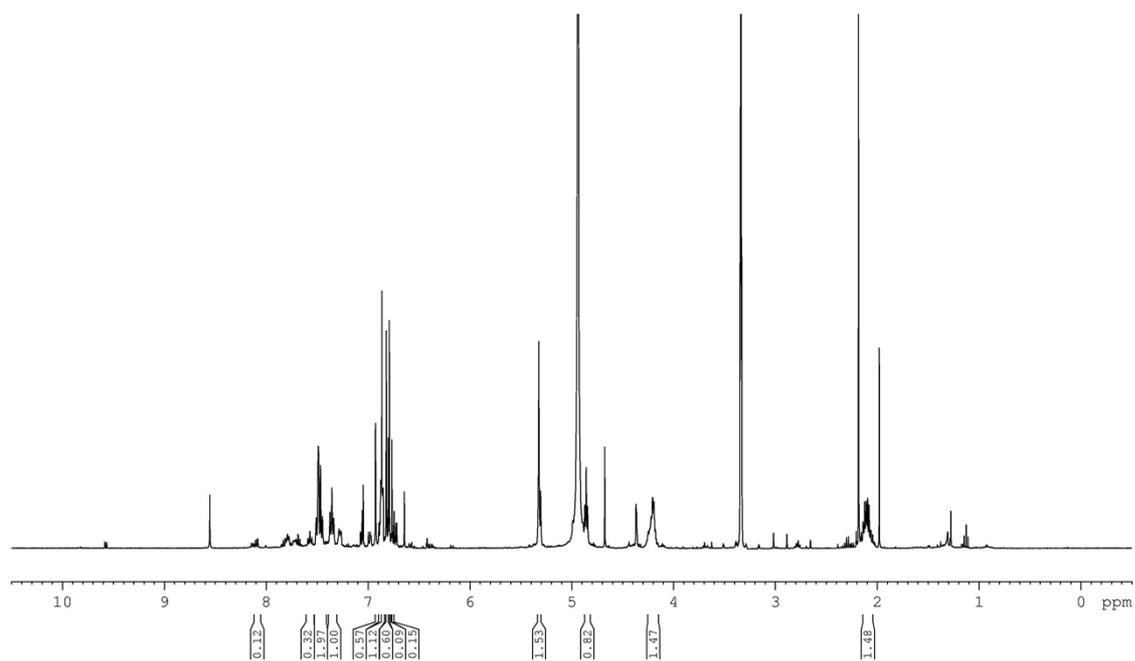
30s Exposure: Alkene peaks from probe **3** completely consumed



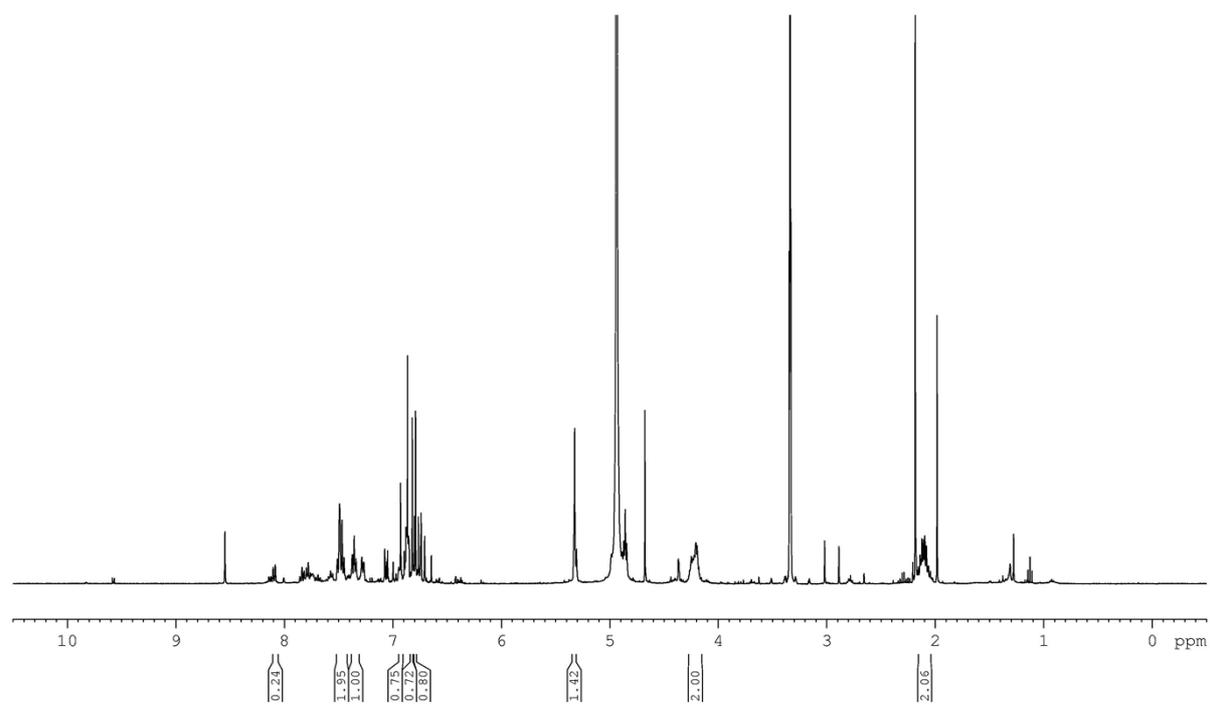
60s Exposure: Formation of DCF **4** beginning to appear at 8.1 ppm



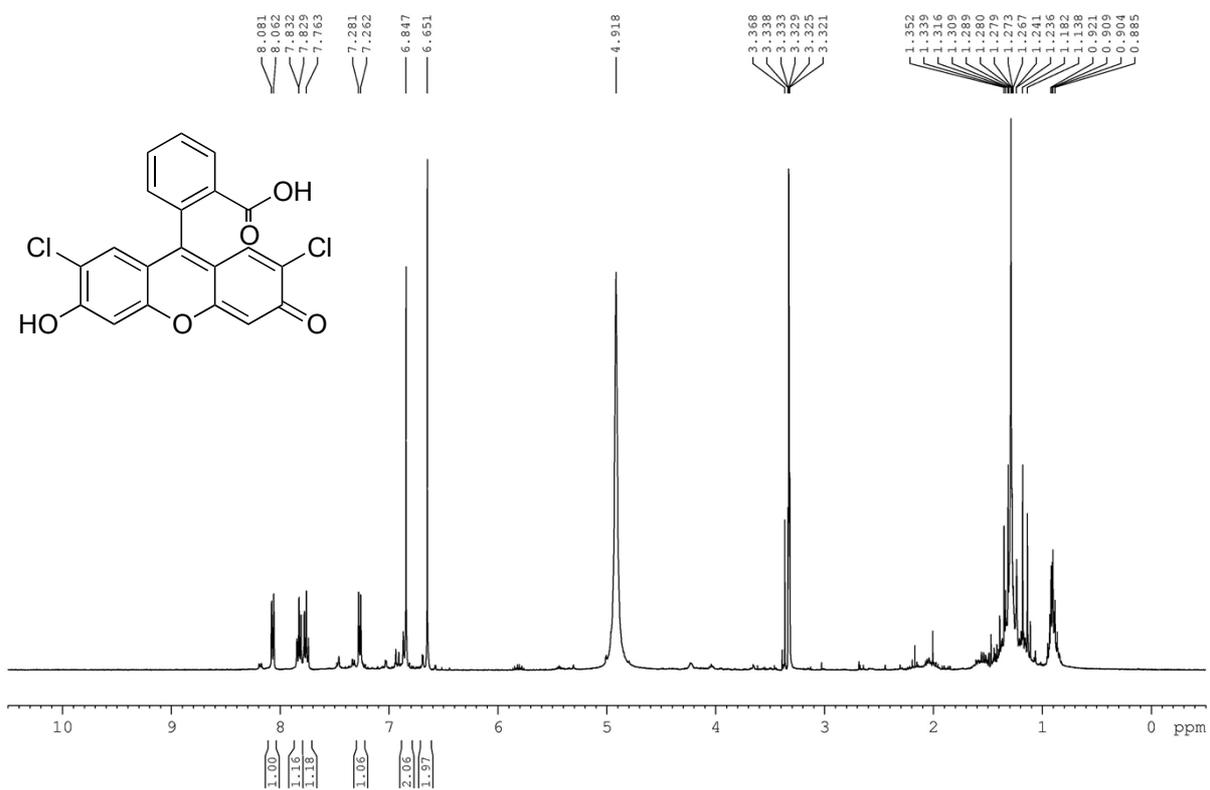
120s Exposure:



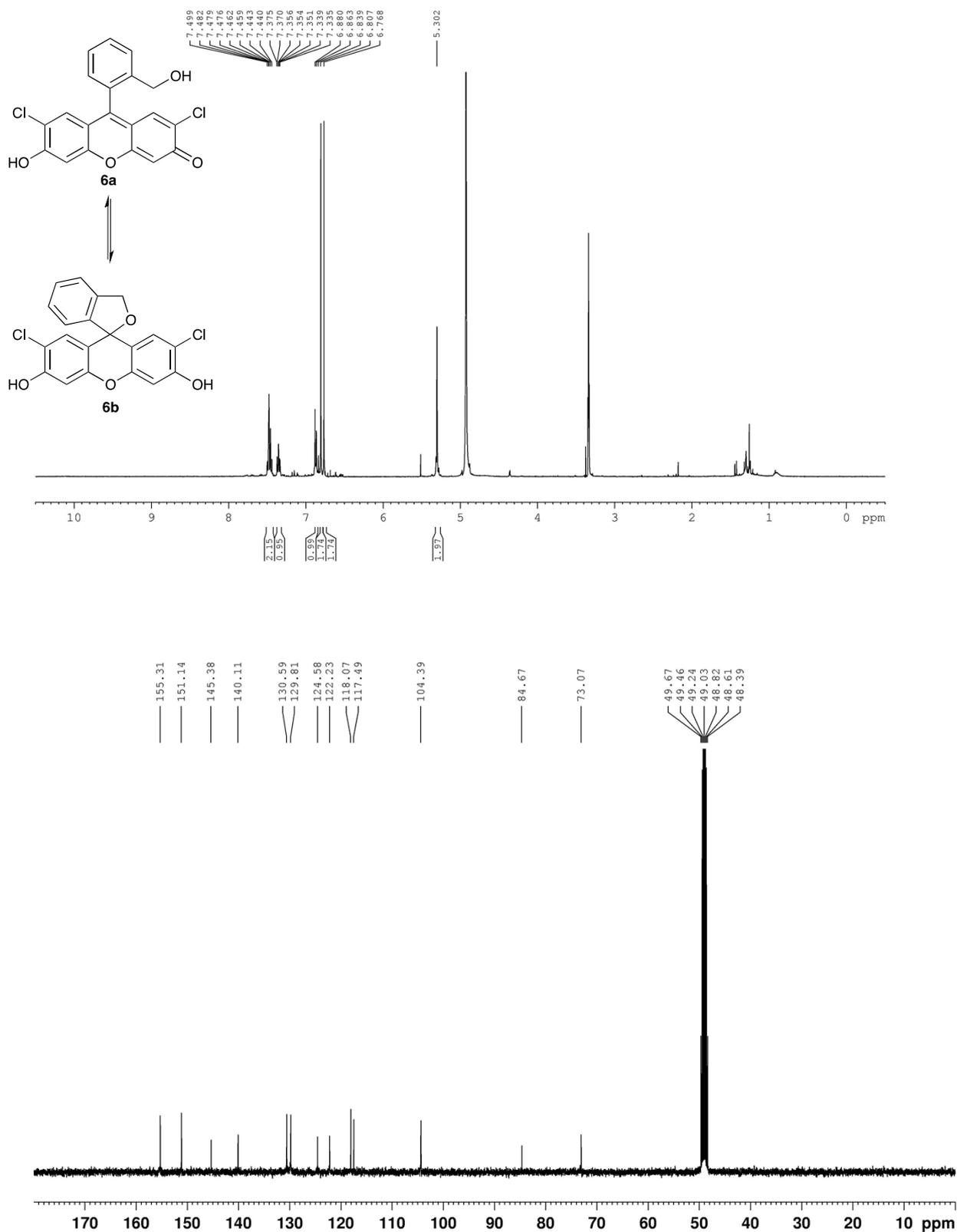
300s Exposure:



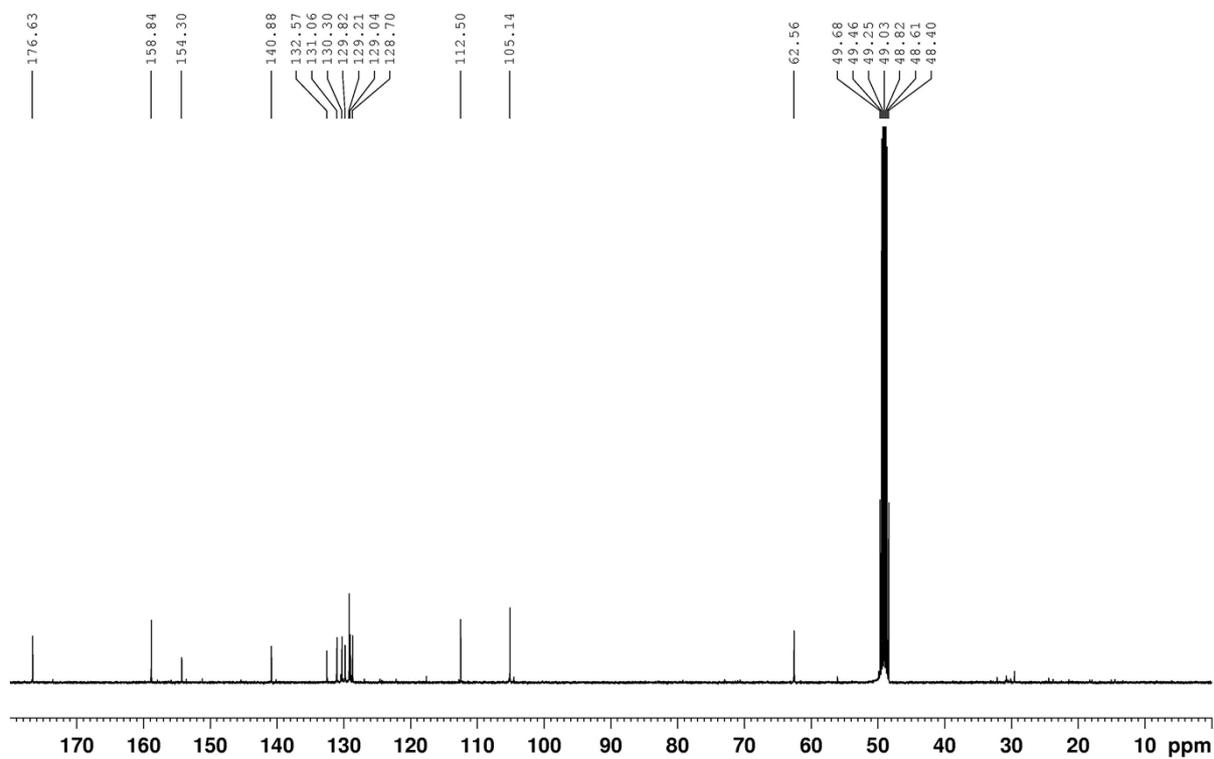
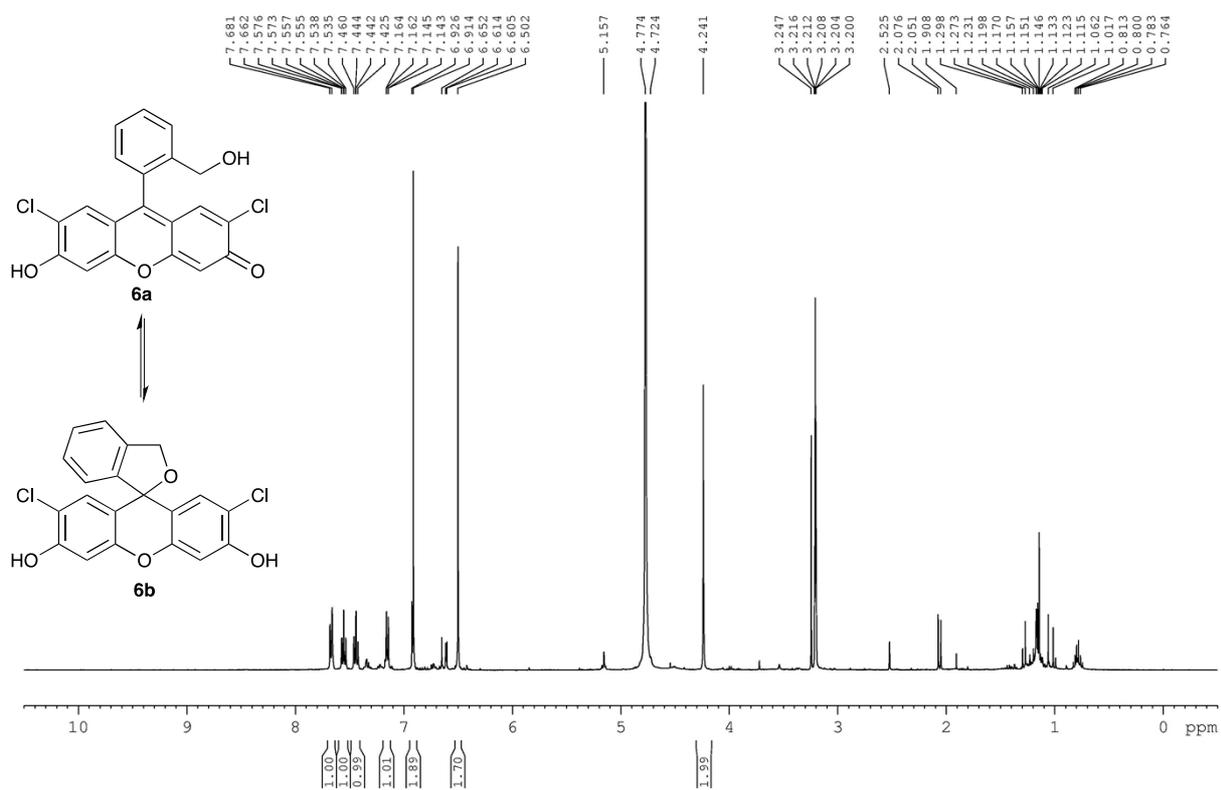
DCF 4 isolated from the reaction of 3 with ozone (15 min exposure time).



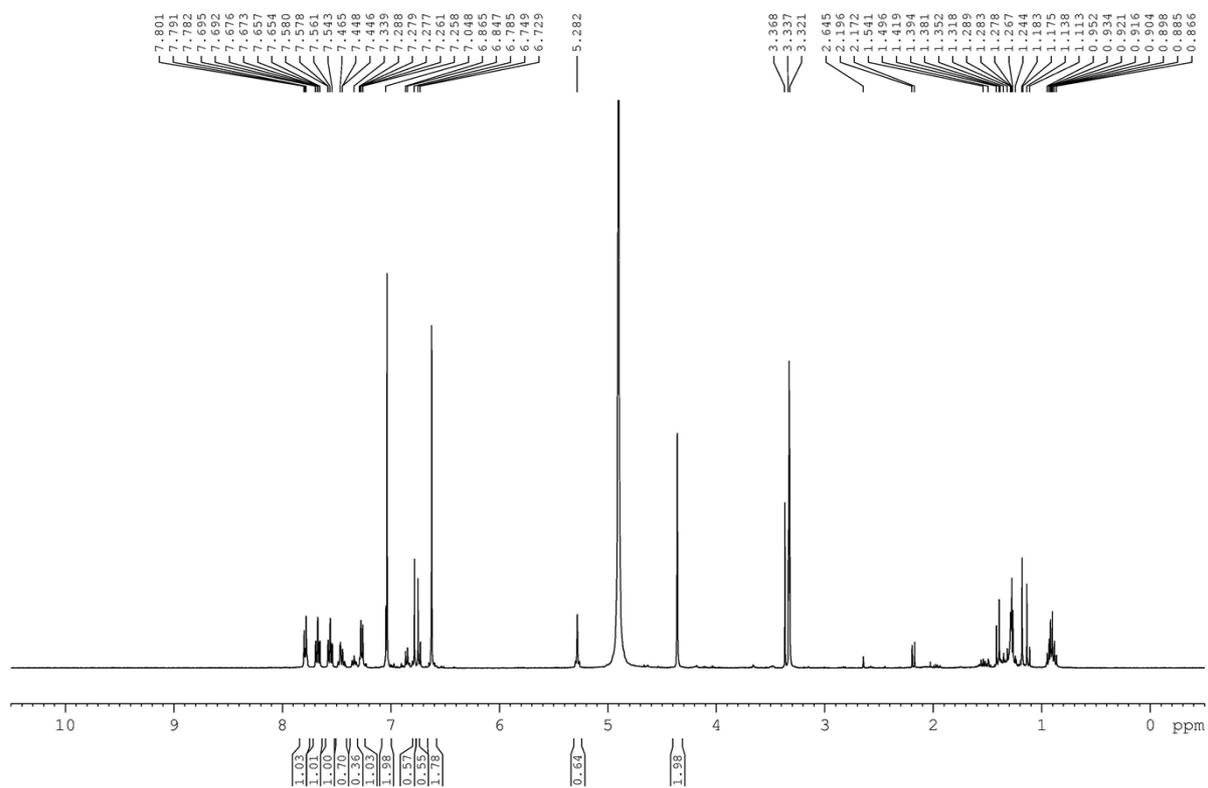
6a or 6b: NMR spectra varied on isolation of **6**, on two occasions we were able to gain almost pure **6a** or **6b** (see both sets of spectra below). In general, however, we often observed a mixture of the two (see the third data set below).



6a or 6b



Typical mixture from chromatography on silica gel.



¹ Song, F., Garner, A. L. & Koide, K. *Nat. Chem.* **1**, 316-321 (2009).