Supporting Information for

A Boronate-Caged $[^{18}\text{F}]$FLT Probe for Hydrogen Peroxide Detection Using Positron Emission Tomography

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General Methods

All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry N₂. When dry solvent was used the solvent was passed over activated alumina. Other reagents were used without further purification. Silica gel P60 (SiliCycle) was used for column chromatography and SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick) was used for analytical thin layer chromatography and visualized by fluorescence quenching under UV light. 3’-Deoxy-3’-fluorothymidine was purchased from Carbosynth LLC (San Diego, CA). 3-N-Boc-5’-O-dimethoxytrityl-3’-O-nosyl-thmidine was purchased from ABX advanced biochemical compounds (Radeberg, Germany). All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO). ¹H and ¹³C NMR spectra for characterization of new compounds were collected in CDCl₃ (Cambridge Isotope Laboratories) at 25 °C at the reported frequency at the College of Chemistry NMR Facility at the University of California, Berkeley. All chemical shifts are reported in parts per million and referenced to the residual solvent peak from CHCl₃ at 7.27 ppm. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets. Low-resolution mass spectral analyses were carried out using an LC-MS (Agelent Technology 6130, Quadrupole LC/MS). HPLC kinetic studies were performed using a Hitachi Elite LaChrome system with L-2455 diode array detector (Hitachi, Tokyo, Japan) using a Phenomenex Luna C18(2) column, 100 Å 250 x 4.6 mm 5 micron (Phenomex, Torrance, CA). HPLC purification of radiolabeled compounds was performed using a Waters 600 system (Waters, Milford, MA) with a Shimadzu-10A UV-vis detector (Shimadzu, Kyoto, Japan) and an in line CsI(Tl) radiation detector (Carroll & Ramsey, Berkeley, CA) also using a Phenomenex Luna C18(2) column. A Perkin Elmer 1480 wizard 3” automatic gamma counter (Perkin Elmer, Waltham, MA) was used to count activity for in vitro studies.
Synthetic Procedures

Synthesis of 1

Compound 1 was prepared according to a slightly modified procedure previously reported.\(^1\) To a 250 mL round-bottomed flask with a stir bar was weighed carbonyldiimidazole (1.82 g, 11.2 mmol, 1.1 equiv.). Nitrogen atmosphere was established and dry acetonitrile (75 mL) was charged to the flask. Next, the boronic ester (2.36 g, 10.1 mmol, 1.0 equiv.) was charged to the flask under positive pressure of nitrogen and the resulting mixture was heated to 50 °C for 1 h, at which point TLC analysis indicated complete consumption of the alcohol. The reaction mixture was cooled to room temperature, concentrated and directly purified by flash chromatography eluting with a solvent gradient of 40 – 60% EtOAc in hexanes. Pure fractions were combined to provide the product 1 (2.58 g, 7.86 mmol) as a white solid in 78% yield. Analytical data matched that of the reported compound.

Synthesis of PC-[\(^{19}\)F]FLT-1

To a 50 mL two-necked round-bottomed flask was weighed the imidazole carbamate 1 (101.3 mg, 0.309 mmol, 2.0 equiv.), 3'-deoxy-3'-fluorothymidine ([\(^{19}\)F]FLT) (37.7 mg, 0.154 mmol, 1.0 equiv.), and 4-dimethylaminopyridine (37.7 mg, 0.309 mmol, 2.0 equiv.). A water-chilled condenser was affixed to the flask and nitrogen atmosphere was established, then dry THF (3.5 mL) was charged to the flask and the reaction mixture was heated to reflux for 3.5 h. TLC analysis indicated complete consumption of FLT. The reaction mixture was directly purified by flash chromatography eluting with a solvent gradient of 40 – 70% EtOAc in hexanes. Pure fractions were combined to provide the product PC-[\(^{19}\)F]FLT-1 (58.6 mg, 0.116 mmol) as a white solid in 75% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 9.10 (s, 1H), 7.81 (d, \(J = 7.8\) Hz, 2H), 7.37 (d, \(J = 7.9\) Hz, 2H), 7.33 (s, 1H), 6.45 (dd, \(J = 9.2, 5.5\) Hz, 1H), 5.23 (dd, \(J = 53.3, 5.1\) Hz, 1H), 5.20 (s, 2H), 4.33-4.50 (m, 3H), 2.58 (ddd, \(J = 20.6, 14.6, 5.6\) Hz, 1H), 2.14 (m, 1H), 1.80 (s, 3H), 1.34 (s, 12H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 163.5, 154.4, 150.3, 137.2, 135.1, 134.9, 127.6, 111.8, 93.6 (d, \(J_{C,F} = 179.5\) Hz), 84.7, 84.0, 82.1 (d, \(J_{C,F} = 27\) Hz), 75.1,
70.3, 67.0 (d, $J_{C,F} = 10.9$ Hz), 38.0 (d, $J_{C,F} = 20.9$ Hz), 24.8, 12.5. HRMS calculated for $C_{24}H_{31}BFN_2O_8$ (M+H⁺) 505.5152; found 505.2154

**Synthesis of 2**

![Synthesis of 2](image)

To a 250 mL two-necked round-bottomed flask was weighed bis(pinacolato)diboron (6.09 g, 24 mmol, 1.2 equiv.), KOAc (5.89 g, 60 mmol, 3.0 equiv.) and Pd(dppf)Cl$_2$•CH$_2$Cl$_2$ (817 mg, 1.0 mmol, 0.05 equiv.). The flask was affixed with a water cooled condenser and a nitrogen atmosphere was established. Dioxane (50 mL) was charged to the flask and the mixture was sparged with N$_2$ gas for 15 min. Under a positive pressure of nitrogen the aryl bromide (4.02 g, 20 mmol, 1.0 equiv.) was charged to the flask and the mixture was heated in a 100 °C oil bath for 24 h. The mixture was cooled to room temperature and filtered over a pad of celite and washed with EtOAc. The resulting eluent was concentrated and purified by flash chromatography eluting with a solvent gradient of 30 – 70% EtOAc in hexanes. Pure fractions were combined to provide the product 2 (4.72 g, 19 mmol) as a white solid in 95% yield. $^1$H NMR (400 MHz, CDCl$_3$): δ 7.77 (d, $J = 7.7$ Hz, 2H), 7.25 (d, $J = 7.7$ Hz, 2H), 3.85 (t, $J = 6.6$ Hz, 2H), 2.88 (t, $J = 6.6$ Hz, 2H), 1.35 (s, 12H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 142.2, 135.000, 134.9, 128.5, 83.7, 63.3, 39.4, 24.8. HRMS calculated for $C_{14}H_{21}BO_3Na$ (M+Na⁺) 271.1476; found 271.1479

**Synthesis of Imidazole Carbamate**

![Synthesis of Imidazole Carbamate](image)

To a 250 mL round-bottomed flask with a stir bar was weighed carbonyldiimidazole (713 mg, 4.4 mmol, 1.1 equiv.). Nitrogen atmosphere was established and dry acetonitrile (40 mL) was charged to the flask. Next, the boronic ester 2 (992 mg, 4.0 mmol, 1.0 equiv.) was charged to the flask under positive pressure of nitrogen and the resulting mixture was heated to 50 °C for 3 h, at which point TLC analysis indicated complete consumption of the alcohol. The reaction mixture was concentrated, dissolved in EtOAc and washed with H$_2$O (5×) and brine. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated. The crude mixture was purified by flash chromatography eluting with a solvent gradient of 33 – 66% EtOAc in hexanes. Pure fractions were combined to provide the product (1.03 g, 3.02 mmol) as a white solid in 76% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (s, 1H), 7.77 (d, $J$
= 7.6 Hz, 2H), 7.36 (s, 1H), 7.25 (d, \( J = 7.6 \) Hz, 2H), 7.04 (s, 1H), 4.60 (t, \( J = 6.8 \) Hz, 2H), 3.09 (t, \( J = 6.8 \) Hz, 2H), 1.33 (s, 12H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 148.6, 139.9, 137.3, 135.3, 130.7, 128.3, 117.2, 83.9, 68.4, 35.2, 25.0. HRMS calculated for C\(_{18}\)H\(_{24}\)BN\(_2\)O\(_4\) (M+H\(^{+}\)) 343.1824; found 343.1825

**Synthesis of 3**

The imidazole carbamate (400 mg, 1.17 mmol, 1.0 equiv.) was weighed into a 500 mL round-bottomed flask containing a stir bar. The flask was put under a nitrogen atmosphere and dry Et\(_2\)O (30 mL) was charged to the vessel. The mixture was cooled in an ice bath and then MeOTf (154 \( \mu \)L) was added dropwise and the solution was stirred at 0 °C for 1 h. A white precipitate formed and no starting material was observable by TLC. The precipitate was filtered off from the still cold ether and washed (1×) with cold ether. The collected solid was dried overnight under vacuum to provide the product 3 (496 mg, 0.98 mmol) in 84% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 9.52 (s, 1H), 7.75 (d, \( J = 7.5 \) Hz, 2H), 7.66 (s, 1H), 7.57 (s, 1H), 7.26 (d, \( J = 7.5 \) Hz, 2H), 4.69 (t, \( J = 7.0 \) Hz, 2H), 4.07 (s, 3H), 3.15 (t, \( J = 7.0 \) Hz, 2H), 1.32 (s, 12H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 145.4, 139.2, 138.3, 135.5, 128.5, 125.2, 119.9, 84.0, 71.5, 37.7, 34.7, 25.1. HRMS calculated for C\(_{19}\)H\(_{26}\)BN\(_2\)O\(_4\) (M\(^+\)) 357.1980; found 357.1982

**Synthesis of CC-[\(^{19}\)F]FLT-1**

To a 50 mL 2-necked round-bottomed flask was weighed 3 (152 mg, 0.30 mmol, 2.0 equiv.), 3’-deoxy-3’-fluorothymidine (FLT) (36.6 mg, 0.15 mmol, 1.0 equiv.), and 4-dimethylaminopyridine (36.7 mg, 0.30 mmol, 2 equiv.). A water-chilled condenser was affixed to the flask and nitrogen atmosphere was established, then dry THF (3.5 mL) was charged to the flask and the reaction mixture was heated to reflux for 16 h. TLC analysis indicated complete consumption of FLT. The reaction mixture was directly purified by flash chromatography eluting with a solvent gradient of 40 – 60% EtOAc in hexanes. Pure fractions were combined to provide the product CC-[\(^{19}\)F]FLT-1 (77 mg, 0.15 mmol) as a white solid in 99% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 9.46 (s, 1H), 7.74 (d, \( J = 7.7 \) Hz, 2H), 7.32 (s, 1H), 7.21 (d, \( J = 7.7 \) Hz, 2H), 4.69 (t, \( J = 7.0 \) Hz, 2H), 4.07 (s, 3H), 3.15 (t, \( J = 7.0 \) Hz, 2H), 1.32 (s, 12H).
= 7.6 Hz, 2H), 6.42 (dd, J = 9.1, 5.5 Hz, 1H), 5.19 (dd, J = 53.3, 4.7 Hz, 1H), 4.48 – 4.28 (m, 5H), 3.00 (t, J = 7.0 Hz, 2H), 2.58 (m, 1H), 2.20 – 2.13 (m, 1H), 1.88 (s, 3H), 1.33 (s, 12H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 163.7, 154.4, 150.4, 139.9, 135.1, 134.9, 128.2, 111.7, 93.6 ($J_{C,F} = 179.6$ Hz), 84.8, 83.8, 82.2 ($J_{C,F} = 27.1$ Hz), 68.7, 66.8 ($J_{C,F} = 10.9$ Hz), 38.0 ($J_{C,F} = 21$ Hz), 35.2, 24.8. HRMS calculated for C$_{25}$H$_{33}$BFN$_2$O$_8$ (M+H$^+$) 519.2309; found 519.2311.

**Evaluation of CC-[¹⁹F]FLT-1 Reactivity**

![Reaction Scheme](image)

To a 1 mM solution of Control-Caged-FLT-1 in a 2:1 solvent mixture of H$_2$O/MeOH and 2% DMSO was charged H$_2$O$_2$ to reach a concentration of 10 mM. The reaction was followed by HPLC-MS. In the absence of H$_2$O$_2$ only the ion corresponding to Control-Caged-FLT-1 was observed. In the presence of 10 mM H$_2$O$_2$, complete cleavage to the phenol was observed within 30 min. No Hydrolysis of the carbonate was observed.
Kinetic Measurements

The rate constant for peroxide reactivity was determined experimentally via pseudo first-order kinetic measurements where \([\text{peroxy-FLT}] \gg [\text{H}_2\text{O}_2]\). Therefore the \([\text{H}_2\text{O}_2]\) is considered to be insignificant and rate = \(k\) [peroxy-FLT]. Briefly, 10 μL of 0.1 mM \(\text{H}_2\text{O}_2\) was added to 990 μL of 1 mM FLT leading to a final concentration of 0.001 mM \(\text{H}_2\text{O}_2\). The rate of generation [FLT] over time was monitored by HPLC with UV-vis detection at 254 nm. Using a 33% isocratic gradient at 1 mL/min FLT was observed at \(t_r = 3.8\) min. Triplicate samples were observed for 5 h.

![Graph](image)

**Figure S1.** Representative data for rate determination of Peroxy-Caged FLT1 conversion under pseudo first-order conditions.

FLT generation was also studied (+)/(-) 0.1 mM peroxide (See Figure 2 a,b). A solution of 1 mM of Peroxy-Caged-FLT-1 in 20 mM pH 7.4 phosphate buffer was prepared and [FLT] was observed over time by HPLC as described above. Next, 1.3 mg of Peroxy-Caged-FLT-1 was added to 2.97 mL of pH 7 phosphate buffer and 30 μL of 10 mM \(\text{H}_2\text{O}_2\) resulting in a final concentration of 1 mM FLT and 0.1 mM \(\text{H}_2\text{O}_2\).

ROS Reactivity Assays

Figure S2 displays reactivity assays of PC-FLT-1 with a panel of ROS.\(^2\) Of these ROS, only \(\text{H}_2\text{O}_2\) and \(\text{ONOO}^-\) produce FLT at appreciable levels. We note that peroxynitrite is reactive with PC-FLT1 at high concentrations (5 mM), but not at lower ones (50 μM and below) that are within proposed physiological ranges. All ROS species were tested at a concentration of 5 mM with 1 mM peroxy-FL, except for \(\text{O}_2^-\) which was generated enzymatically at a rate of 24 μmol/min. For these assays, 400 μL samples were quenched with 15 μL of 500 mM ascorbic acid at the given time points and [FLT] was determined by
HPLC at a wavelength of 254 nm using a 33% acetonitrile isocratic gradient with a flow rate of 1 mL/min. ROS were generated as follows:

**H₂O₂** – 11.4 μL of 3% wt (0.88 M) H₂O₂ was added to 2 mL of 1 mM peroxy-FLT in 20 mM pH 7.4 HEPES buffer.

**ONOØ** – 354 μL of a 33.4 mM solution of peroxynitrite was added to a solution of 1.0 mg peroxy-FLT dissolved in 2.0 mL of 50 mM pH7 phosphate buffer yielding a final concentration of 5 mM ONOO⁻ and 1 mM peroxy-FLT.

**O₂⁻** – Superoxide was generated enzymatically according to the method of Lippert et al.¹ in the presence of 1 mM peroxy-FLT.

**tBuOOH** - 70% tBuOOH was diluted 1/10x and 12.9 uL of 1/10x dil tBuOOH was added to 1.99 μL of 1 mM peroxy-FLT in HEPES (20 mM pH 7.4).

**tBuO⁻** - 3 mL of 1 mM peroxy-FLT in HEPES (20 mM pH 7.4) were degassed with bubbling N₂ for ~30 min. 10.6 mg FeSO₄ 7H₂O were added to the mixture. Then degassed an additional 30 min with bubbling N₂. 70% tBuOOH was diluted 1/10x and 19.4 uL of the dilute tBuOOH was added to degassed mixture.

**NO⁻** – A 10mM solution of NaOH was degassed with bubbling N₂ for ~30 min. A 25 mM stock solution of PROLInonate was prepared by dissolving 4.77 mg in 870 μL of the 10 mM NaOH and stored on ice. Meanwhile, 0.84 mg peroxy-FLT was dissolved in 1.8 mL HEPES (20 mM pH 7.4) and degassed for ~20 min. 200 μL of the 25 mM PROLInonate solution was added to the solution containing peroxy-FLT resulting in a final concentration of 5 mM NO and 1 mM peroxy-FLT.

**·OCl⁻** – 0.9 mg peroxy-FLT were dissolved in 1.988 mL HEPES (20 mM pH 7.4). Added 12 uL 6.15% NaOCl.

**·OH** – 7.96 mg FeSO₄ 7H₂O was dissolved in 2 mL of 1 mM peroxy-FLT in HEPES (20 mM pH 7.4) and degassed with bubbling N₂ for ~30 min. Then 11.4 μL of 3% wt (0.88 M) H₂O₂ was added to the solution for a final concentration of 5 mM.
Figure S2: Response of 1mM peroxy-FLT to various ROS. All ROS were treated at 5 mM except for O$_2^-$ which was generated enzymatically at a rate of 24 μmol/min. Peroxynitrite was tested at high (5 mM) and low (50 μM) concentrations.

**Radiosynthesis**

**Preparation of $[^{18}\text{F}]$FLT**

540 mCi of $[^{18}\text{F}]$F was obtained on a ORTG MP1 resin cartridge. Aqueous $[^{18}\text{F}]$F$^-$ was eluted with 500 μL of 15mg/mL kryptofix (K$_{2,2,2}$) and 3 mg/mL K$_2$CO$_3$ in 10% water/acetonitrile. The solution was heated to dryness and resuspended in 400 μL acetonitrile. 460 mCi $[^{18}\text{F}]$F$^-$ in 400 μL was loaded on to a Nanotek LF microfluidics reactor with 500 μL of a 25 mg/mL solution of 3-N-Boc-5’-O-dimethoxytrityl-3’-O-nosyl-thymidine precursor in acetonitrile. The solution two loop volumes were pushed through the reactor loop at a rate of 100 μL/min with temperature maintained at 170°C then the product was hydrolyzed with 1 mL 1 M HCl at 80 °C for 8 min. The solution was neutralized with 1 mL of 1 M NaHCO$_3$. 72.2 mCi $[^{18}\text{F}]$FLT was collected after HPLC purification.

**Preparation of Peroxy-Caged-$[^{18}\text{F}]$FLT**

$[^{18}\text{F}]$FLT was heated to dryness and then redissolved in acetonitrile. Meanwhile, 6 mg of the imidazole precursor 1 and 1 mg DMAP were dissolved in 200 μL acetonitrile and 4 μL TEA. 100 μL (17.8 mCi) was added to the reaction mixture and heated to 80°C for 30 min. The solution was cooled and the
boronic pinacol ester was deprotected with the addition of 3 mL 10% citric acid. The solution was purified by HPLC using a Phenomenex C-18(2) Luna semi-prep column with a gradient of 5% to 95% acetonitrile/water over 30 min at 5 mL/min and UV monitored at 265 nm. PC-[¹⁸F]FLT-1 (t_r = 18 min) was collected and concentrated in 1 mL DMSO using a C-18 light sep-pak. The collected fraction was analyzed using an analytical Phenomenex C-18(2) Luna column with 33% acetonitrile/water at 1 mL/min isocratic with (Figure S4) and without (Figure S3) coinjection of 4 μg cold standard (t_r = 8 min). The specific activity of [¹⁸F]FLT was determined to be 400 ± 150 mCi/μmol.

**Figure S3**: Analytical HPLC of collected PC-[¹⁸F]FLT-1 (pre sep-pak) top UV, bottom radio t_r = 8 min.
Figure S4: PC-[\(^{18}\)F]FLT-1 coinjected with 4 μg non-radioactive standard top UV, bottom radio, \(t_r = 8\) min.

**Preparation of Control-Caged-[\(^{18}\)F]FLT**

\([^{18}\)F]FLT was heated to dryness and then redissolved in acetonitrile. Meanwhile, 7.5 mg of precursor 3 and 4 mg DMAP were dissolved in 250 μL acetonitrile. 100 μL (21 mCi) was added to the reaction mixture and heated to 100 °C for 30 min. The solution was cooled and the boronic pinacol ester was deprotected with the addition of 3 mL 10% citric acid. The solution was HPLC purified using a Phenomenex C-18(2) Luna semi-prep column with a gradient of 5% to 95% acetonitrile/water over 30 min at 5 mL/min, UV monitored at 265 nm. CC-[\(^{18}\)F]FLT-1 (\(t_r = 17.2\) min) was collected and concentrated in 1 mL DMSO using a C-18 light sep-pak.
Cell Culture Studies

Normal (-) H\textsubscript{2}O\textsubscript{2}

0.5 million UOK 262 cells in 0.5 mL serum free RPMI were placed in 1.5 mL microcentrifuge tubes. ~1.5 μCi of either FLT or peroxy-FLT in 3 μL DMSO was added to each vial by bath application. Samples were incubated at 37°C for 30 min, 1 h, and 2 h (n=4). The samples were removed at the prescribed time point then centrifuged and the media was removed. Each sample was washed 3 times with 1 mL cold PBS. The remaining cell pellets were then counted using a Perkin Elmer gamma detector. Results are displayed as % Cell Associated Activity ± standard error (Figure 3a).

ROS Dependent (+) H\textsubscript{2}O\textsubscript{2}

0.5 million UOK 262 cells in 1 mL serum free RPMI with the given amount of H\textsubscript{2}O\textsubscript{2} were placed in 1.5 mL microcentrifuge tubes. ~ 1.5 μCi of peroxy-FLT in 3 μL DMSO was added to each vial by bath application. Samples were incubated at 37°C for 30 min, 1 h, and 2 h (n=4). The samples were removed at the prescribed time point then centrifuged and the media was removed. Each sample was washed 3 times with 1 mL cold PBS. The remaining cell pellets were then counted and decay corrected using a Perkin Elmer gamma detector. Results are displayed as % Cell Associated Activity ± standard error (Figure 3b). Next the cells were washed with 1 mL 0.1 M pH 3 glycine buffer to release surface bound activity. The cells were centrifuged and the supernatant was retained. Then remaining cells were lysed with 1 mL of 1 M NaOH, centrifuged and the supernatant was retained. Both washes and remaining pellets were counted and decay corrected. Figure S5 represents surface bound, internalized, and membrane associated activity for the 1 h incubation at 100 μM H\textsubscript{2}O\textsubscript{2}. Results are displayed as % Cell Associated Activity ± standard error. Both NaOH and glycine washes from each level were collected and a microPET image was generated (Figure 3c).

![Graph](image-url)

**Figure S5:** Surface bound, internalized and membrane associated activity at 1 h incubation with 100 μM H\textsubscript{2}O\textsubscript{2}.
Control-Caged FLT Studies

Next, a control compound which does not exhibit any conversion to FLT in the presence of ROS was evaluated and compared to the Peroxy-Caged-FLT-1 probe. 0.5 million UOK 262 cells in 1 mL serum free RPMI (+)/(-) 100 μM H₂O₂ were placed in 1.5 mL microcentrifuge tubes (n = 4 each). ~ 1.5 μCi of either Control-Caged-FLT-1 (control) or Peroxy-Caged-FLT-1 (Peroxy-FLT) in 3 μL DMSO was added to each vial by bath application. After 1h incubation at 37°C the samples were removed at the prescribed time point then centrifuged and the media was removed. Each sample was washed 3 times with 1 mL cold PBS. The remaining cell pellets were then counted and decay corrected using a Perkin Elmer gamma detector. Results are displayed as % Cell Associated Activity ± standard error.

![Bar chart](image)

**Figure S6:** Peroxide dependent uptake of control compound compared to peroxy-FLT at 1 h incubation (* p value = 0.9).

Paraquat-Treated Cell Studies

Finally, Peroxy-Caged-FLT-1 was evaluated in cells induced to generate endogenous ROS with addition of paraquat (See Figure 4). 0.5 million UOK 262 cells in 0.5 mL serum free RPMI (+)/(-) 1.0 mM paraquat (PQ) were placed in 1.5 mL microcentrifuge tubes (n = 4 each). ~ 1.5 μCi of peroxy-FLT in 3 μL DMSO was added to each vial by bath application. After 2 h, 4 h and 6 h incubation at 37°C the samples were removed, centrifuged and the media was aspirated. Each sample was washed 3 times with 1 mL cold PBS and the remaining cell pellets were then counted, decay corrected and normalized for cell viable cell count. Results are displayed as % Cell Associated Activity ± standard error.

References:

1) Broaders, K. E.; Grandhe, S.; Fréchet, J. M. J. *J. Am. Chem. Soc.* 2011, 133, 756.

2) Lippert, A. R.; Keshari, K. R.; Kurhanewicz, J.; Chang, C. *J. J. Am. Chem. Soc.* 2011, 133, 3776.
Figure S7. $^1$H NMR spectrum of PC-FLT-1.
Figure S8. $^{13}$C NMR spectrum of PC-FLT-1.
Figure S9. $^1$H NMR spectrum of 2.
Figure S10. $^{13}$C NMR spectrum of 2.
Figure S11. $^1$H NMR spectrum of control imidazole carbamate.
Figure S12. $^{13}$C NMR spectrum of control imidazole carbamate.
Figure S13. $^1$H NMR spectrum of 3.
Figure S14. $^{13}$C NMR spectrum of 3.
Figure S15. $^1$H NMR spectrum of CC-FLT-1.
Figure S16. $^{13}$C NMR spectrum of CC-FLT-1.