

SUPPLEMENTARY INFORMATION

An Activatable NIR Fluorescent Rosol for Selectively Imaging Nitroreductase Activity

Jessica L. Klockow^{1†}, Kenneth S. Hettie^{1†*}, Edward L. LaGory², Eui Jung Moon², Amato J. Giaccia², Edward E. Graves^{1,2}, Frederick T. Chin^{1*}

¹Department of Radiology, ²Department of Radiation Oncology, Stanford University, Stanford, CA, 94305, USA

*Co-corresponding authors:

Kenneth S. Hettie, Ph.D.
3165 Porter Drive
Palo Alto, CA 94304
Email: khettie@stanford.edu

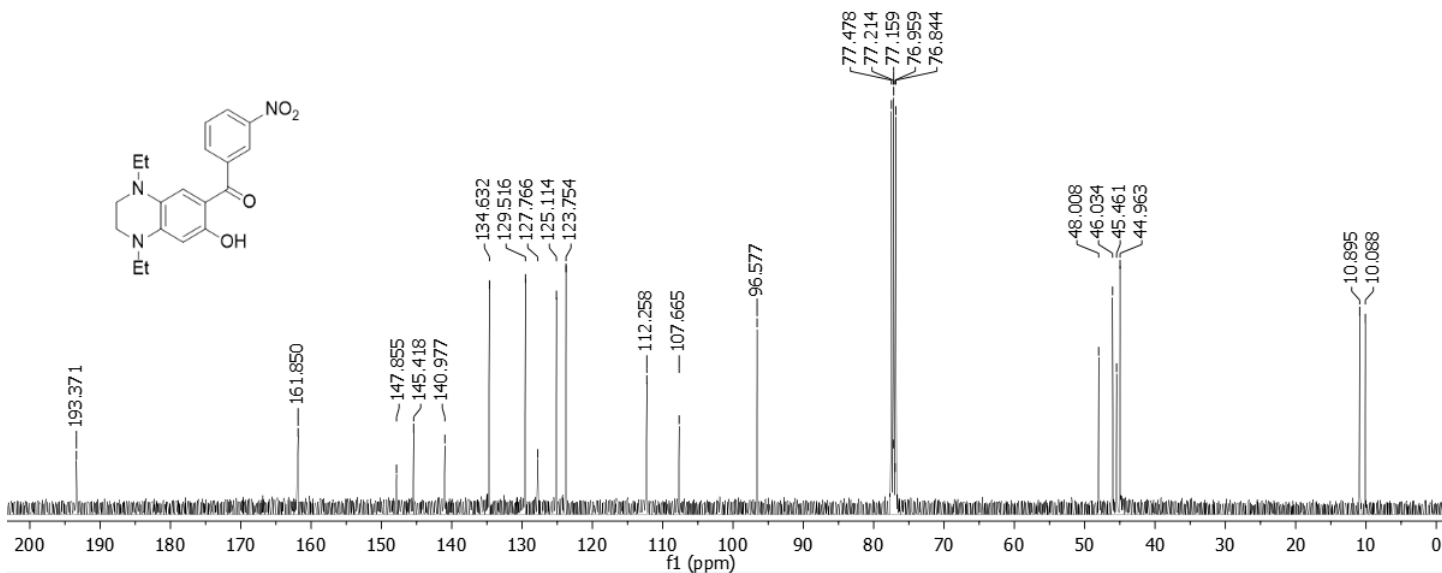
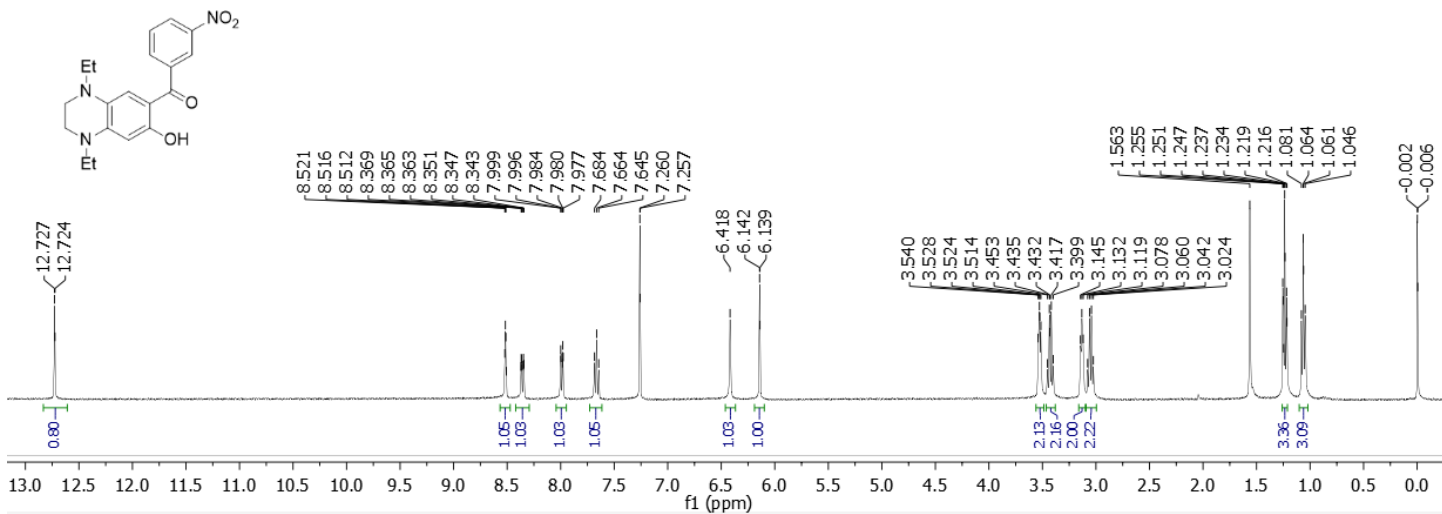
Frederick T. Chin, Ph.D.
3165 Porter Drive, Room 2129
Palo Alto, CA 94304
Email: chinf@stanford.edu

†Equal contribution

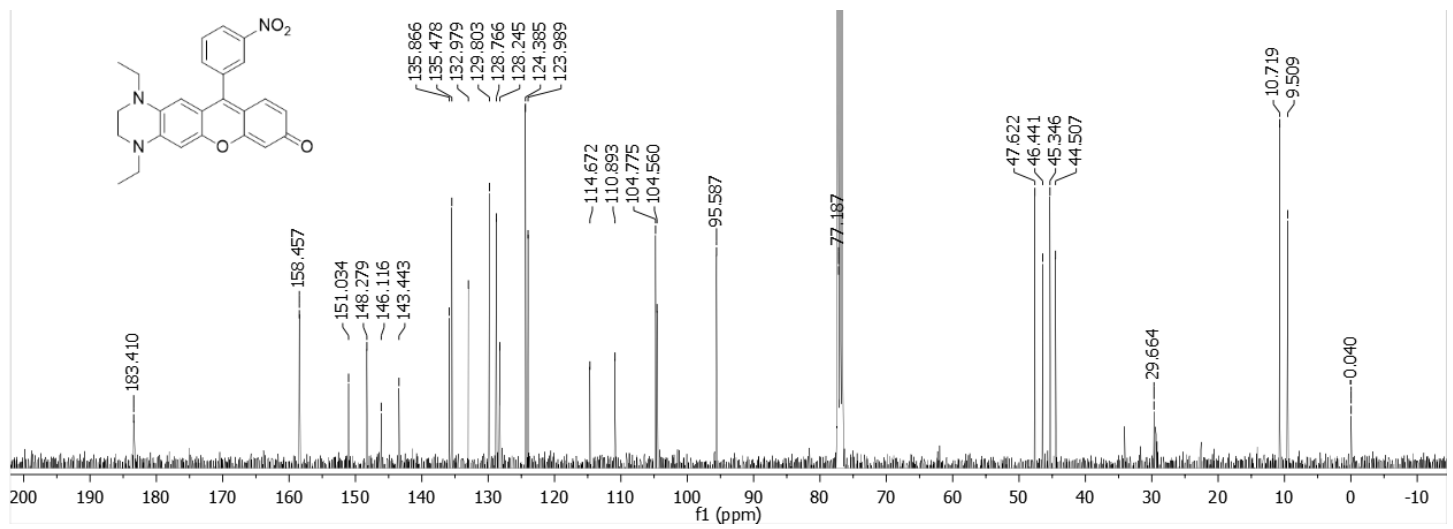
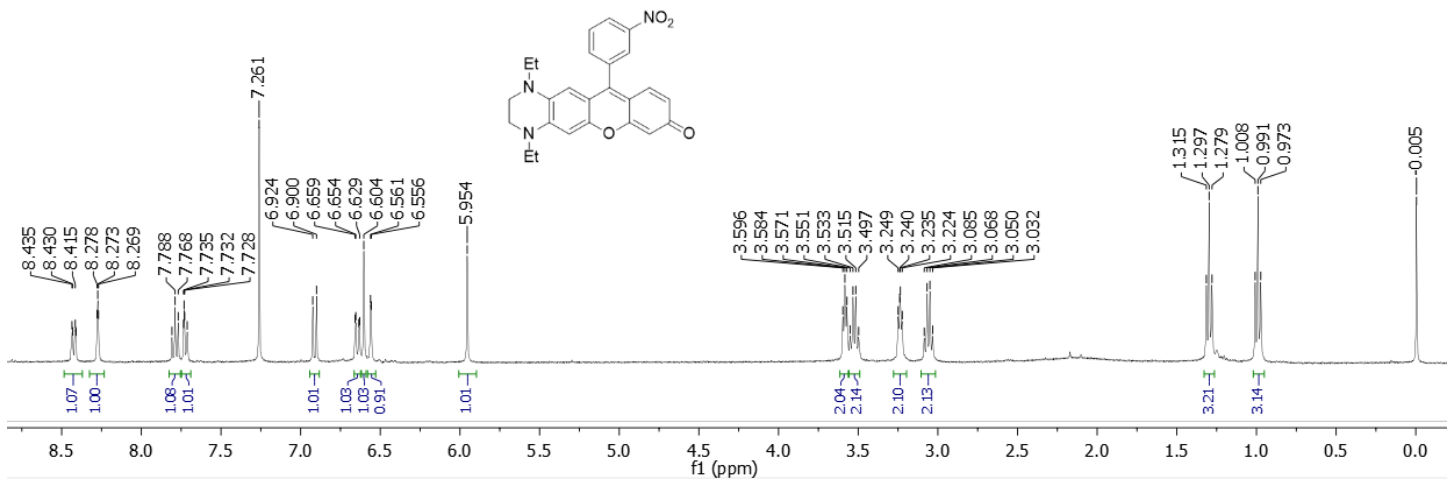
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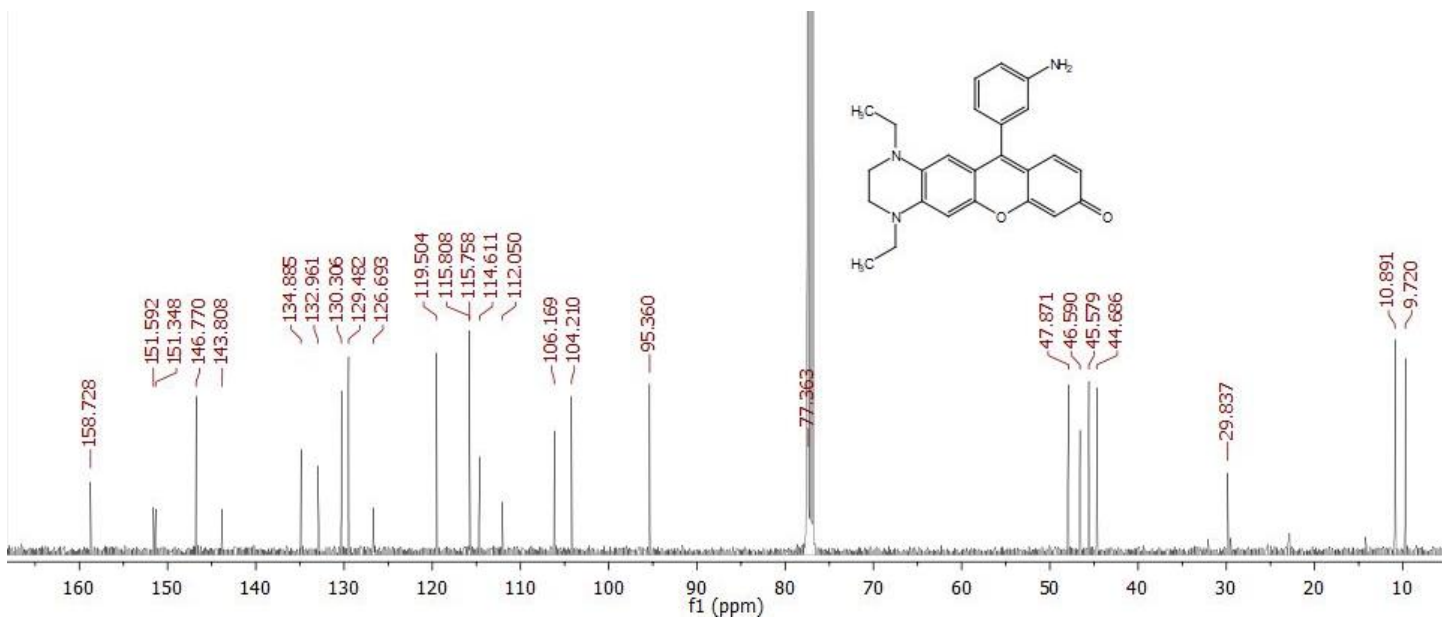
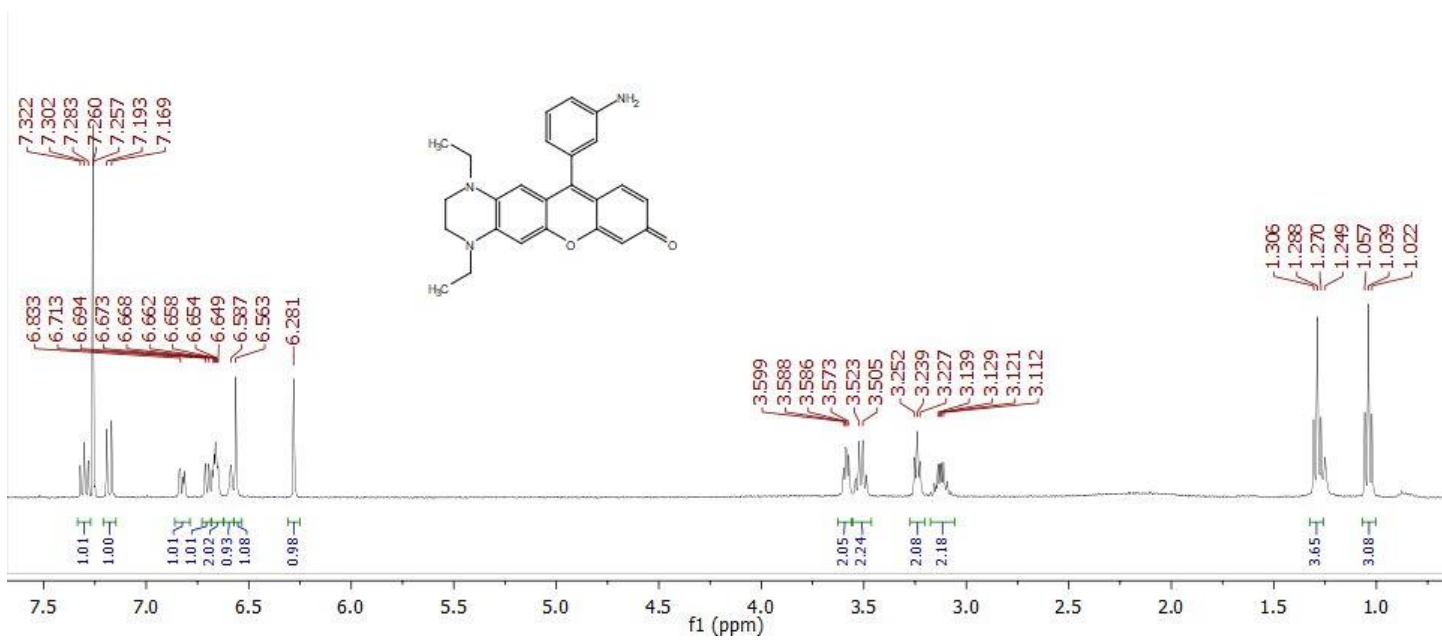
¹H- & ¹³C-NMR Spectra

Compound 2



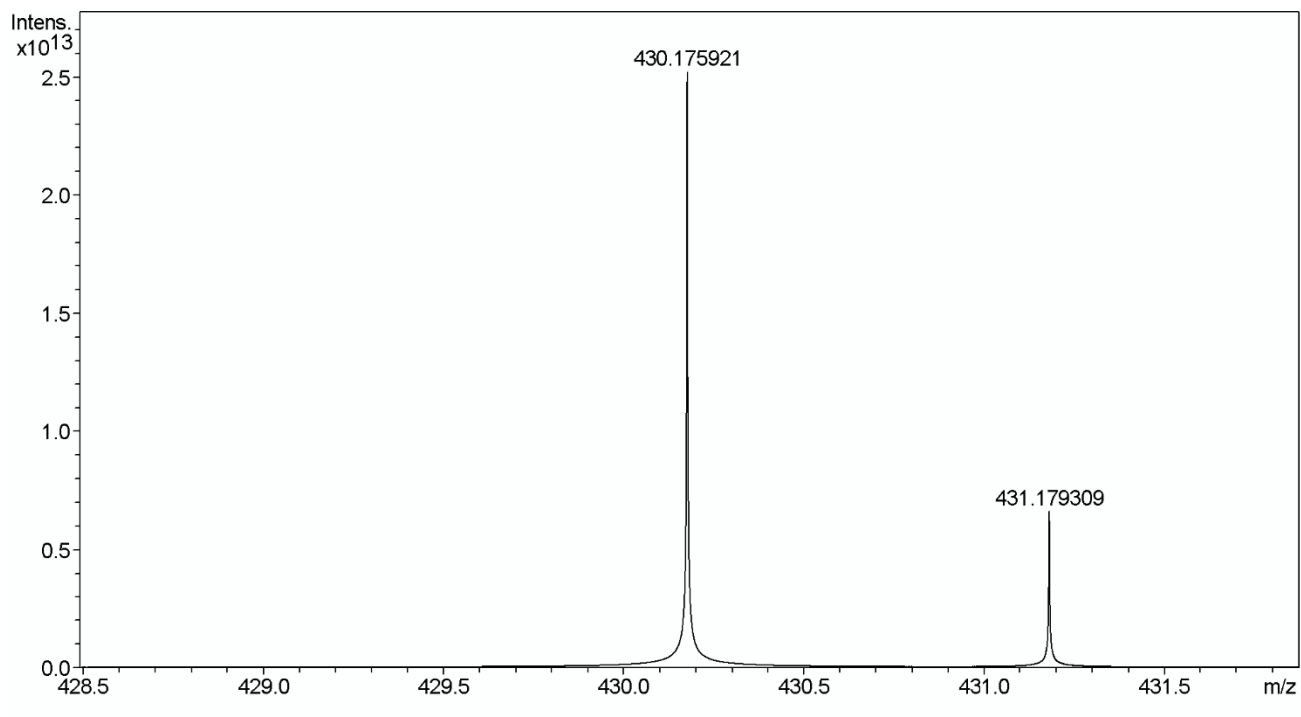
NO₂-Rosol



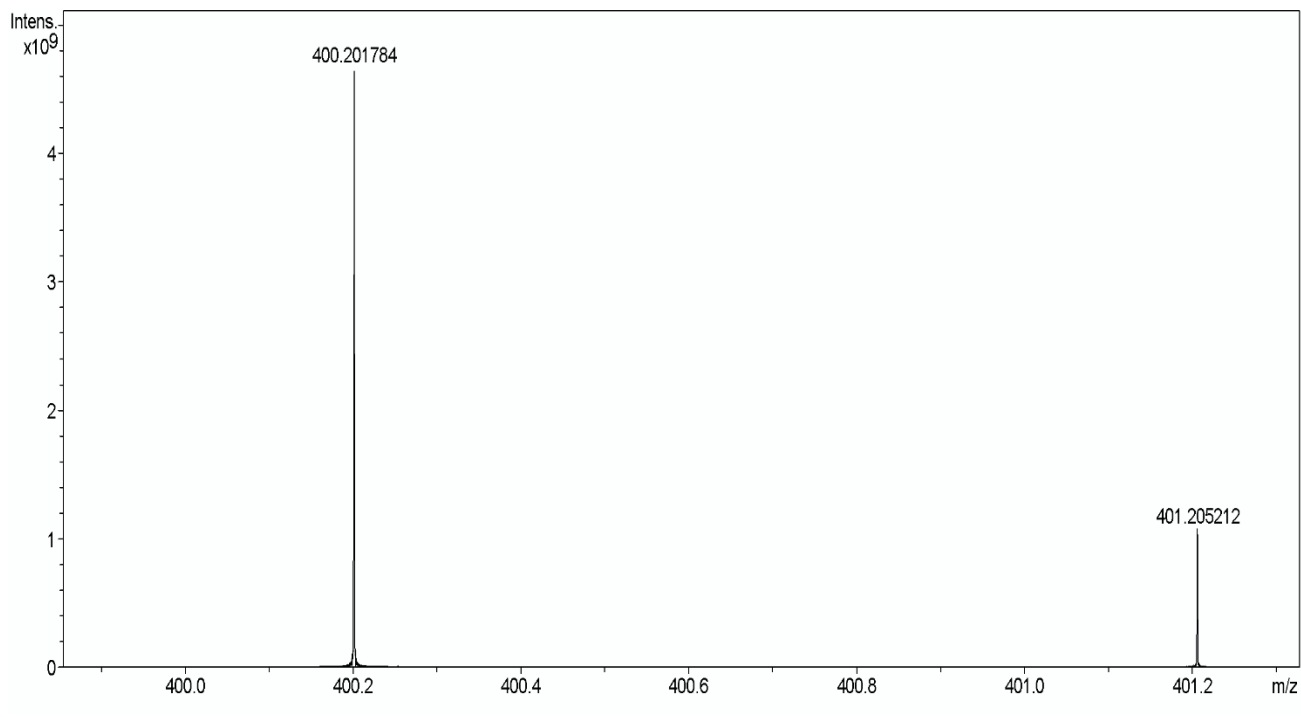


HRMS Spectra of NO₂- and NH₂-Rosol

NO₂-Rosol



NH₂-Rosol



Spectroscopy

Solubility

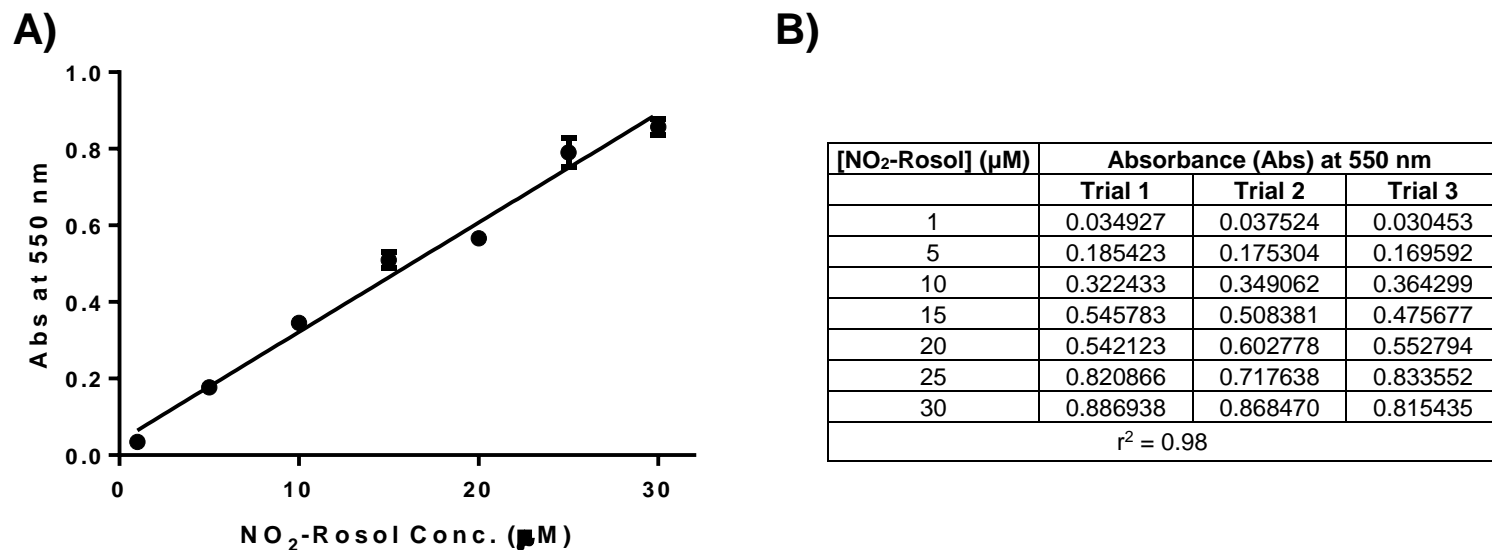


Figure S1. Linear regression analysis and statistical measure between a select set of parameters of **NO₂-Rosol**. A) Correlation plot interrelating the resultant absorbance value of **NO₂-Rosol** measured at 550 nm to the various concentrations of **NO₂-Rosol** that were utilized, which ranged between 1-30 μM. B) The accompanying coefficient of determination (r^2) of the aforementioned analyzed select set of parameters of **NO₂-Rosol**. An r^2 value ≥ 0.70 corresponds to a strong relationship. The calculated r^2 value (0.98) signifies a very strong relationship exists between the resultant absorbance value measured at 550 nm and the corresponding concentration of **NO₂-Rosol**, which suggests that **NO₂-Rosol** demonstrates excellent water solubility throughout the range of **NO₂-Rosol** concentrations that were utilized.

Photostability

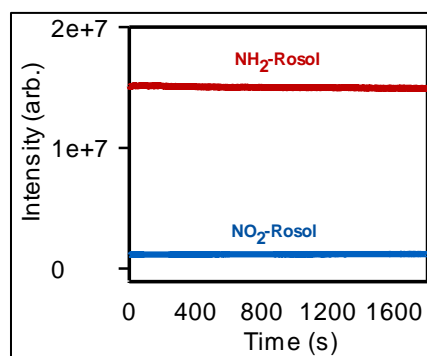


Figure S2. Photostability of the unactivated (**NO₂-Rosol**, blue) and activated (**NH₂-Rosol**, red) form of the NTR-selective activatable molecular probe over 30 min before and after complete reduction of the nitro group of the pendant nitroaromatic moiety of **NO₂-Rosol**, respectively.

Kinetic Analysis

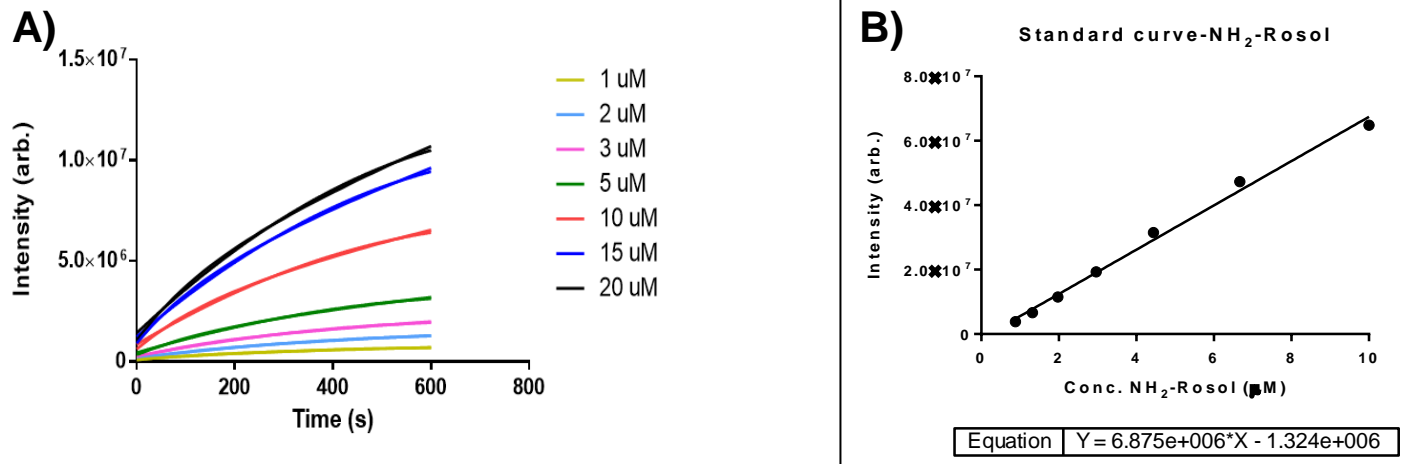


Figure S3. Determination of initial kinetic velocities. (a) Various concentrations of $\text{NO}_2\text{-Rosol}$ were activated by nitroreductase, wherein their fluorescence intensity at the maximum emission wavelength of 705 nm was monitored over time. A) The slope of the curve from the first 90 seconds was converted to velocity using B) a standard curve of the activated form of $\text{NO}_2\text{-Rosol}$ (i.e., $\text{NH}_2\text{-Rosol}$) fluorescence. $r^2 = 0.99$.

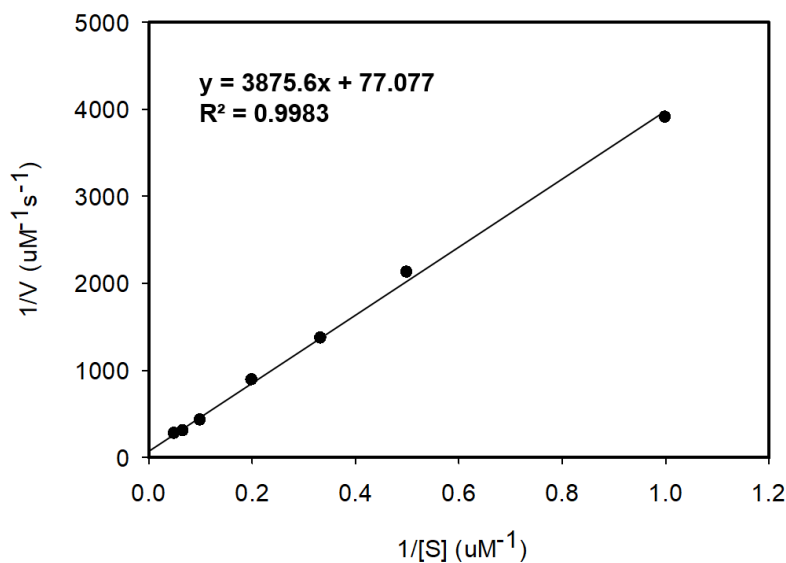


Figure S4. Lineweaver-Burk plot for evaluating the kinetic parameters of nitroreductase activation of $\text{NO}_2\text{-Rosol}$. $[S]$ = concentration of the $\text{NO}_2\text{-Rosol}$. V = velocity as determined from above.

LC-HRMS Chromatograms & Spectra of Reaction between NTR with NO₂-Rosol

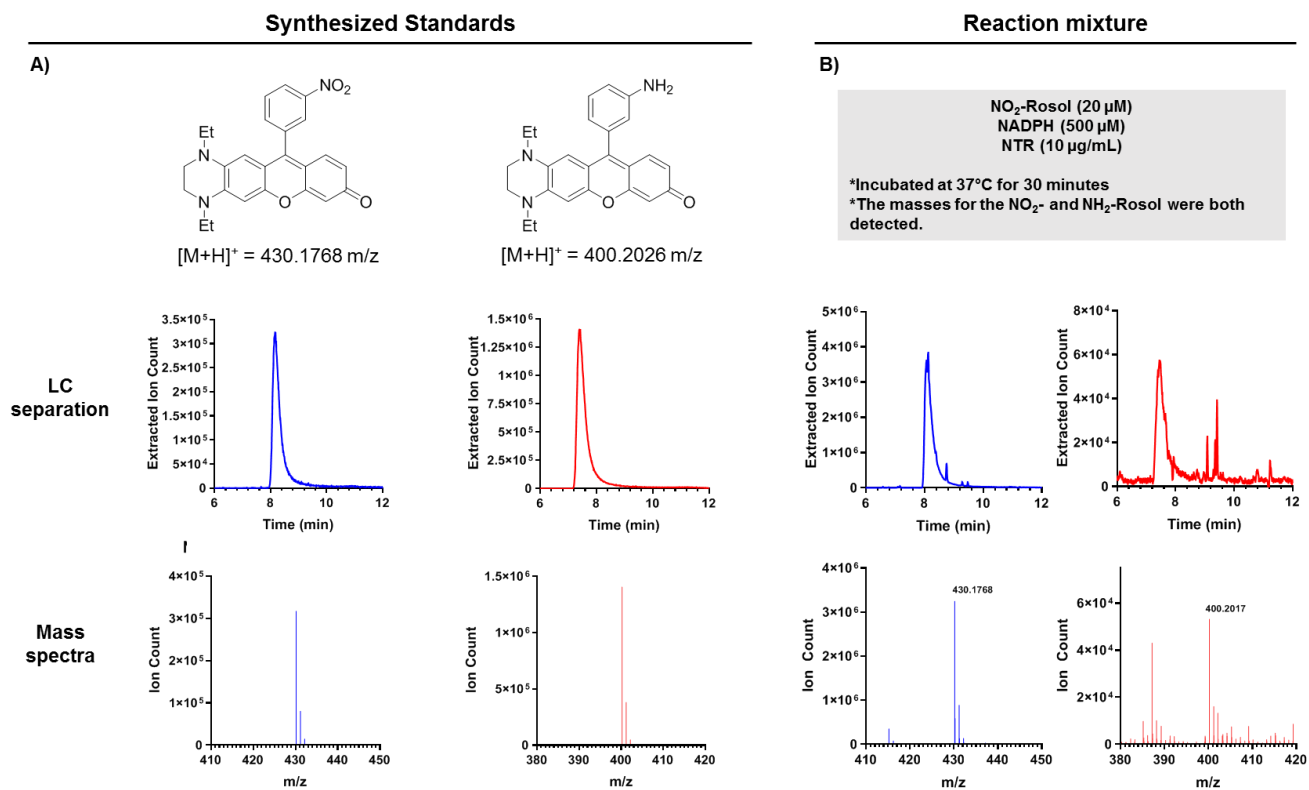


Figure S5. A) LC-HRMS extracted ion count chromatograms and mass spectra of synthesized **NO₂-Rosol** and **NH₂-Rosol** used as standards. B) LC-HRMS extracted ion count chromatograms and mass spectra of the reaction between NTR and **NO₂-Rosol** after 30 minutes.

Cell Viability Assay

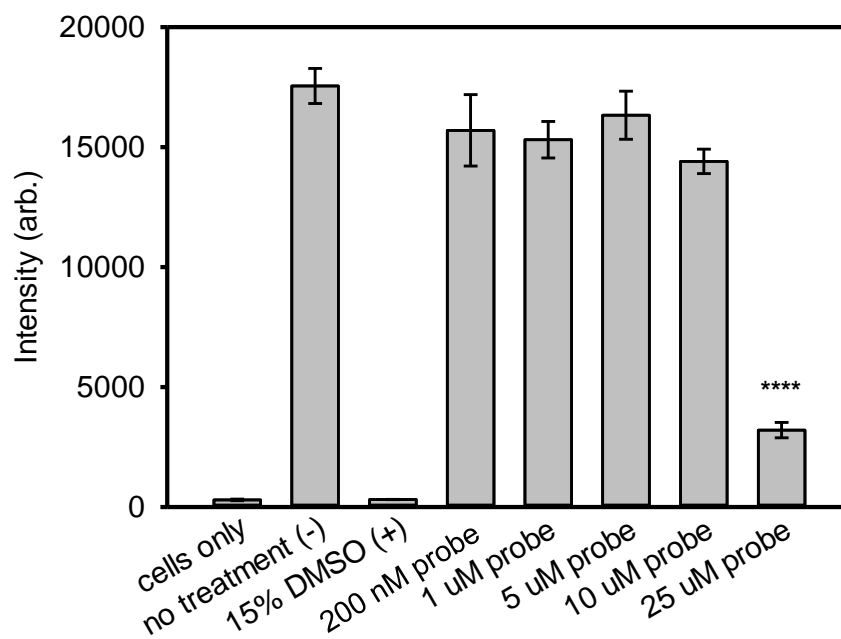


Figure S6. Cell viability assay results from applying **NO₂-Rosol** at relevant concentrations (200 μ M - 10 μ M) to U251 cells. Untreated cells were used as a negative control and cells treated with 15% DMSO as a positive control. Cells were stained with a live cell stain, Calcein-AM, wherein the fluorescence intensity was measured at 516 nm (λ_{ex} = 494 nm). N = 6, **** P < 0.0001