Far-red BODIPY-based Oxime Esters: Photo-uncaging and Drug Delivery

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Experimental section

General

All reagents and solvents were purchased from commercial suppliers and used as received without further purification. Compound 1 was synthesized according to their previous reported procedures. 1H and 13C NMR spectra were carried out in CDCl3 solution on a Bruker AVANCE spectrometer (500 MHz). Orbitrap mass spectrometry analyses were performed at the Prof. Hao Chen lab, Department of Chemistry and Environmental Science, New Jersey Institute of Technology (NJIT). Flash column chromatography was performed on a CombiFlash® Rf+ automated flash chromatography using RediSep Rf Gold® normal-phase HP silica columns. UV-vis absorption spectra were recorded on a Tecan Infinite M200 PRO plate reader spectrometer in 1 cm path length cuvettes. Fluorescence emission spectra were measured using an Edinburgh FLS980 fluorescence spectrometer. Cell images were recorded using a Zeiss LSM 780 confocal microscope. Fiji, a freely available image processing software, was used to process all the cell images. Details of the optically computed phase microscopy imaging were described in previous publications (ref. 2). The light source (low coherence light) illuminates a Michelson interferometer that has a reference arm and a sample arm. Exiting the interferometer, the interferometric light is process by an optical computation module with a grating disperses the light, a spatial light modulator imposes a 2D sinusoidal function for modulation, and a camera for light detection.
Reagents: a) DMF/acetic acid/piperidine; b) DMF/POCl$_3$; c) NH$_2$OH-HCl/ethanol; d) 3a: triethylamine/DCM/2-propylentaoyl chloride, 3b: triethylamine/DCM/acetyl chloride.

Compound 2 and precursors were synthesized using previously reported methods by our group. General procedure for the synthesis of styryl-BODIPY oxime esters (3a and 3b): A solution of compound 1 in dry dichloromethane was cooled to 0 °C under nitrogen gas, triethylamine (0.1 mL) was added followed by acid chloride (2 eq) in dry dichloromethane and the mixture was stirred for 2 h with the temperature slowly rising to 25 °C. The organic phases were then washed with saturated sodium chloride/water solution and the residue was purified by column chromatography (hexane/ethyl acetate, 4/1, v/v). The styryl-BODIPY oxime ester was obtained as a red solid in good yield.

(3a): $^1$H NMR (500 MHz, CDCl$_3$) δ 8.43 (s, 1H), 7.71-7.66 (q, 2H), 7.56-7.53 (t, 4H), 7.46-7.27 (m, 15H), 7.02 (d, 1H), 6.74 (s, 1H), 6.55 (s, 2H), 3.92 (s, 3H). 13C NMR (126 MHz, CDCl$_3$) δ 173.9, 156.5, 154.5, 152.7, 151.5, 150.5, 145.3, 140.4, 139.6, 139.2, 138.7, 138.6, 137.9, 135.4, 135.1, 134.8, 134.1, 133.9, 132.6, 132.3, 132.0, 130.4, 129.6, 128.5, 128.1, 127.7, 119.8, 118.5, 105.5, 105.4, 61.5, 61.4, 61.2, 56.5, 56.4, 44.0, 34.7, 20.7, 15.0, 14.0, 13.5 ppm.

C$_{57}$H$_{56}$BF$_2$N$_3$O$_5$S$_2$ + H$^+$ Calculated Mass: 976.3800, Found: 976.3815.

(3b): $^1$H NMR (500 MHz, CDCl$_3$) δ 8.43 (s, 1H), 7.70-7.65 (q, 2H), 7.56-7.53 (t, 4H), 7.46-7.27 (m, 15H), 7.02 (d, 1H), 6.74 (s, 1H), 6.55 (s, 2H), 3.93 (s, 3H), 2.19 (s, 3H), 1.84 (s, 3H), 1.65 (s, 3H) ppm.

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.9, 156.6, 154.4, 151.0, 150.5, 145.4, 140.3, 140.2, 139.7, 139.2, 138.8, 137.9, 135.4, 135.0, 134.8, 134.3, 134.1, 133.8, 132.7, 132.2, 132.0, 130.4, 129.6, 129.5, 129.4, 128.5, 128.2, 128.1, 127.7, 119.7, 119.5, 118.5, 105.4,
61.4, 56.5, 19.7, 15.0, 14.1, 13.5 ppm.


Cell culture: HeLa cell line was cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO2 incubator. Cellular Uptake Studies: To investigate the cellular of 3a, HeLa cells were employed. All cells were seeded on the confocal dish (MatTek) at the density of 4 x 10^4 cells per dish and incubated for 24 h at 37 °C. Stock solutions of 3a dissolved in DMSO were prepared at a nominal concentration of 10^4 μM. The stock solution was diluted to 1 and 10 μM with the DMEM cell medium respectively and freshly placed over cells for 1 h and 4 h incubation period separately. Cells were washed three times with PBS and the live cell imaging solution (Molecular Probes) was added to confocal dishes. Fluorescent images were obtained using Zeiss LSM 780 confocal microscope.

Cell viability before Photolysis: To assess the cytotoxicity of 3a, HeLa cells were cultured in the Dulbecco’s modified Eagle’s medium (DMEM) cell medium supplemented with 10% fetal bovine serum, 1% penicillin, and streptomycin at 37 °C and 5% CO2. Cells were plated in 96 well plates and incubated until there were no fewer than 5 x 10^3 cells per well for the experiments. Next, cells were incubated with different concentrations of 3a (0.1, 0.5, 1, 2.5, 5, 10, 20, 40 μM) for 4 h, where the values within parentheses refer to the nominal concentrations. Then, 3a was removed and regular DMEM was added for 4 h washed + 24 h group. additional 22 h incubation was performed for both 4 h washed + 24 h and 4 h +24 h groups. After 22 h incubation, 20 μL of the Cell Titer 96 Aqueous One solution reagent (for MTS assay) was added to each well, followed by further incubation for 2 h at 37 °C.

The respective absorbance values were read on a Tecan Infinite M200 PRO plate reader spectrometer at 490 nm. Cell viabilities were calculated on the basis of the following equation

\[
\text{Cell Viability (\%) = \frac{Abs_{490nm}^D - Abs_{490nm}^C}{Abs_{490nm}^D - Abs_{490nm}^D2} \times 100\%}
\]

where \(Abs_{490nm}^D\) is the absorbance of the cells incubated with different concentrations of experimental probe solutions, \(Abs_{490nm}^C\) is the absorbance of cell-free well containing only oxime probe at the concentration that was studied, \(Abs_{490nm}^D2\) is the absorbance of cells alone incubated in the medium, and \(Abs_{490nm}^D2\) is the absorbance of the cell-free well. The data is shown in Figure S19.

Time-resolved fluorescence measurement The fluorescence lifetime was measured with a home-built time-resolved photoluminescence (TRPL) system, which had been described in detail in ref. 2. Briefly, the system was powered by a pulse regenerated amplifier (Regen, Phidia-C, Uptek), from which 800 nm laser pulses with a pulse-width of 150 fs pulses were generated at a repetition rate of 1 kHz. 2mJ, 800 nm pulses were directed to an optical parametric amplifier (OPA-C, Light-conversion). The 1140 nm signal was picked up after OPA and sent to a type I BBO crystal for the second harmonic generation of 570 nm pump pulses, which were employed to excite the sample. A 590
nm long pass filter was used to remove the pump scattering. The emission of the sample was measured with a photodiode, and the kinetic traces were recorded by an oscilloscope. The instrument response of the setup was 0.57 ns.

**Transient absorption spectroscopy** Sub-picosecond-to-nanosecond transient absorption (TA) experiments were carried out with a home-built system. Details about the system can be found in ref. 3. Briefly, TA measurement used the same 570 nm pump as the TRPL system to excite the sample. The pump beam was chopped to 500 Hz, attenuated to 100 nJ per pulse, and softly focused on the sample contained in a 2 mm pathlength quartz cuvette. The TA system employed a CaF$_2$ crystal (2 mm thick) as the medium to generate a white light continuum (WL) to produce the probe beam. An optical delay setup using a retroflector and an automated delay stage provided a time window up to 4200 ps. The WL was divided into two beams: one was focused on the sample and overlapped with the pump to take the TA measurements, and the other served as the reference to compensate for the pulse-to-pulse discrepancy. The diverged sample and reference beams were dispersed by a spectrometer (SpectralPro-300i, Acton) and recorded separately by two vertically aligned photodiode arrays (1024 pixels, EB Stresing). TA signals were calculated pixel-by-pixel using the formula $\Delta OD = -\log \left( \frac{I_S}{I_R} \right)_{\text{pump-on}} / \left( \frac{I_R}{I_S} \right)_{\text{pump-off}}$, where $I_S$ and $I_R$ were the intensities of the sample and reference, respectively. The TA system was automated with a homemade program coded with LabView. A set of metMb transient absorption spectra at a different time delay for excitation at 360 nm was compared and showed that 3a has enough time to utilize absorbed light energy to initiate N-O bond cleavage.
Figure S1. $^1$H NMR of compound 3a.

Figure S2. $^{13}$C NMR of compound 3a.
Figure S3. Mass spectrum of compound 3a.

Figure S4. $^1$H NMR of compound 3b.
Figure S5. $^{13}$C NMR of compound 3b.

Figure S6. Mass spectrum of compound 3b.
**Figure S7.** Absorption spectra of compound 3a irradiated with 660 nm LED light.

**Figure S8.** $^1$H NMR of styryl-BODIPY-CN (BD-CN).
Figure S9. $^{13}$C NMR of styryl-BODIPY-CN (BD-CN).

Figure S10. Mass spectrum of styryl-BODIPY-CN (BD-CN).

[M+H]$^+$
Calculated: 832.2645
Found: 832.2636
Figure S11. Mass spectrum of photolysis of compound 3a.

Figure S12. Absorption and emission spectra of compound 1 (styryl-BODIPY)
Figure S13. Fluorescent quantum yield measurement of compound 3a using cresyl violet as reference.

Figure S14. Molar absorptivity measurement of compound 3a.

Figure S15. Fluorescent quantum yield measurement of styryl-BODIPY-CN (BD-CN) using cresyl violet as reference.
Figure S16. Molar absorptivity measurement of compound 3b.

Figure S17. Fluorescent quantum yield measurement of compound 3b using cresyl violet as reference.

Figure S18. Molar absorptivity measurement of styryl-BODIPY-CN (BD-CN).
Figure S19. Transient absorption spectra and lifetime measurement of BD-CN (styryl-BODIPY-CN) and 3a.
Figure S20. Stability of 3a in cell culture medium (DMEM) in dark up to 4 hours, maximum absorbance were recorded using UV-vis and normalized.

Figure S21. Dark cell viability of compound 3a incubated in HeLa cells.
Figure S22. a) optically computed phase images (ref. 4) of untreated healthy cell, and c) cell treated with 3a and 30 min LED light. b) 3D unwrapped phase image of the same untreated healthy cell, and d) the same cell treated with 3a and 30 min LED light. Untreated cells have regular morphology and large area, while after treatment the cells showed altered morphology and increase height.

