Synthesis of Caged Rhodamine and Resorufin and the Production of Caged Rhodamine- and Caged Resorufin-Antibody Conjugates

Dr. Olga Vasalatiy, Shannon Cofiell, Colin Wilson

Imaging Probe Development Center, National Heart, Lung, and Blood Institute, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, ipdc@nhlbi.nih.gov

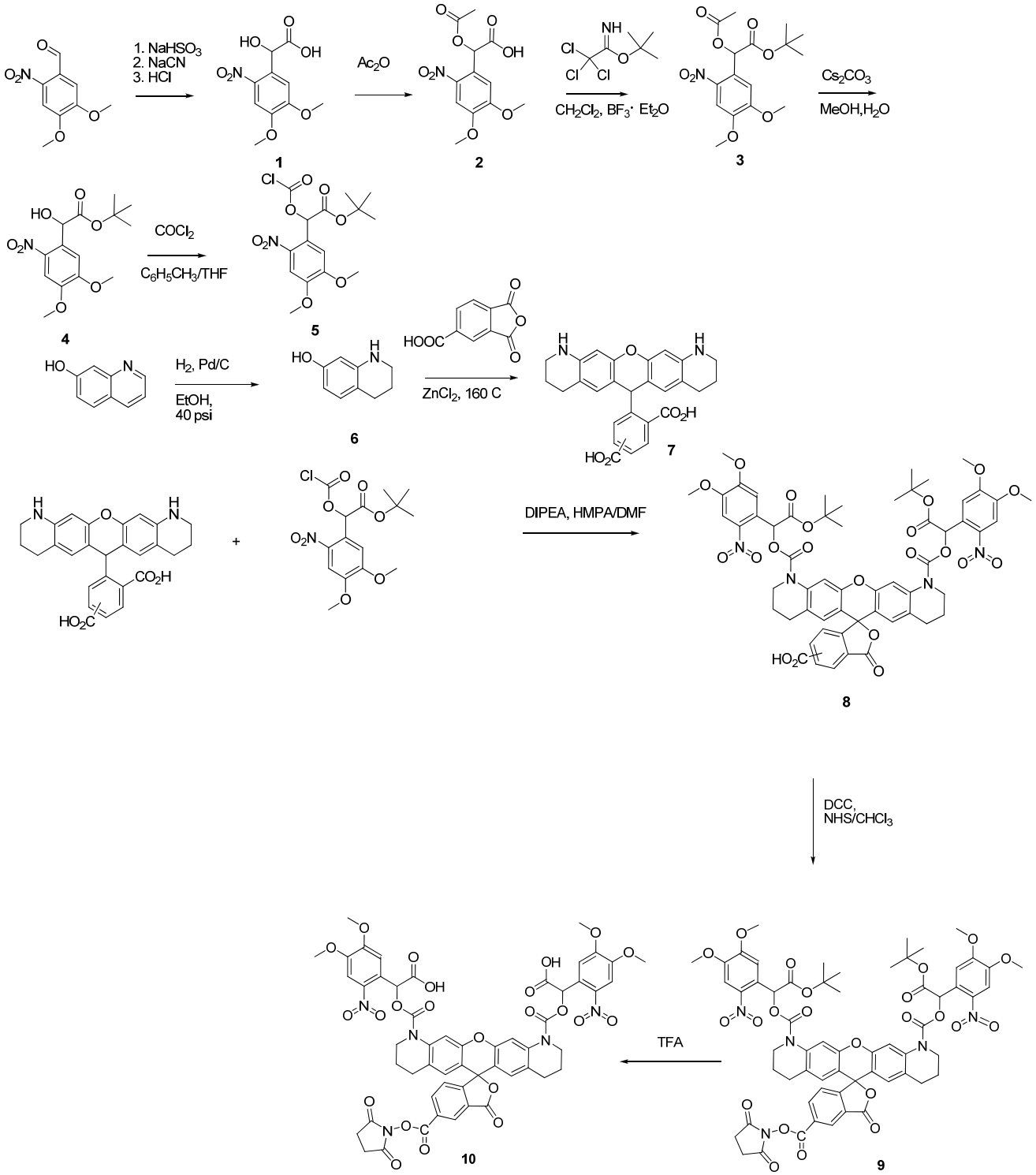
Background

Recently, a new technique capable of providing spatial resolutions 10-100-fold better than conventional optics has been introduced[1]. In this diffraction-breaking technique, called PhotoActivation Localization Microscopy (PALM), small populations of photoactivatable fluorescent molecules are photoactivated and imaged until photodestruction. Photoactivation of small populations of molecules allow maintenance of the density of imaged molecules to be less than one molecule per resolution limit and while photodestruction of activated molecules allow a new subset of molecules to be imaged. The probes for PALM have been primarily photoactivatable fluorescent proteins from marine organisms[2]. However, photoactivatable fluorescent proteins have several characteristics that limit their sole use in PALM, such as the emission of <1000 photons before photodestruction and difficulties performing double labeling experiments. The use of photoactivatable small molecule caged dye-protein conjugates has the potential to overcome these problems.

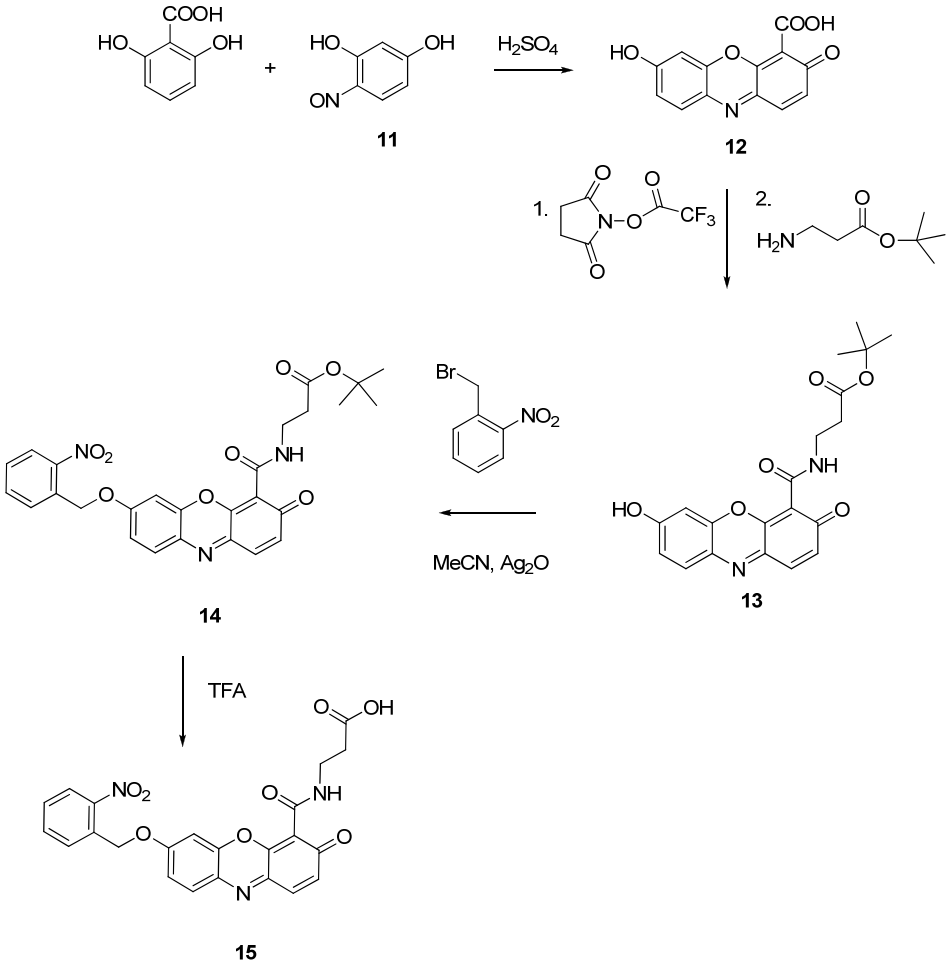
Chemistry

Double-caged rhodamine dye (**8**), activated for protein conjugation as its succinimide ester (**10**) was synthesized according to Scheme 1. Mono-caged resorufin dye with carboxylic acid functionality (**15**) was prepared as outlined in Scheme 2. The conjugations of double-caged rhodamine dye and mono-caged resorufin dye to two different IgG antibodies are shown in Schemes 3 and 4, respectively.

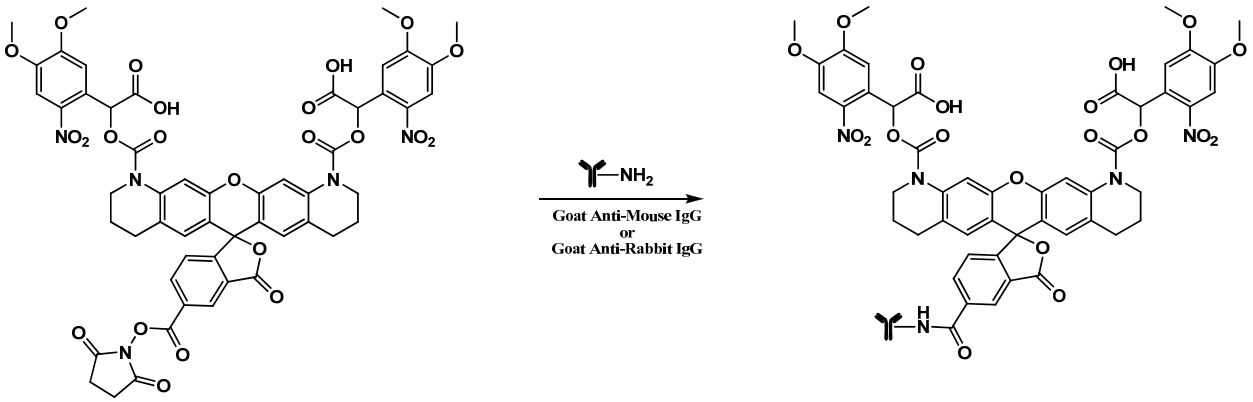
Scheme 1. Synthesis of an *N*-hydroxysuccinimide ester of the double-caged rhodamine dye.



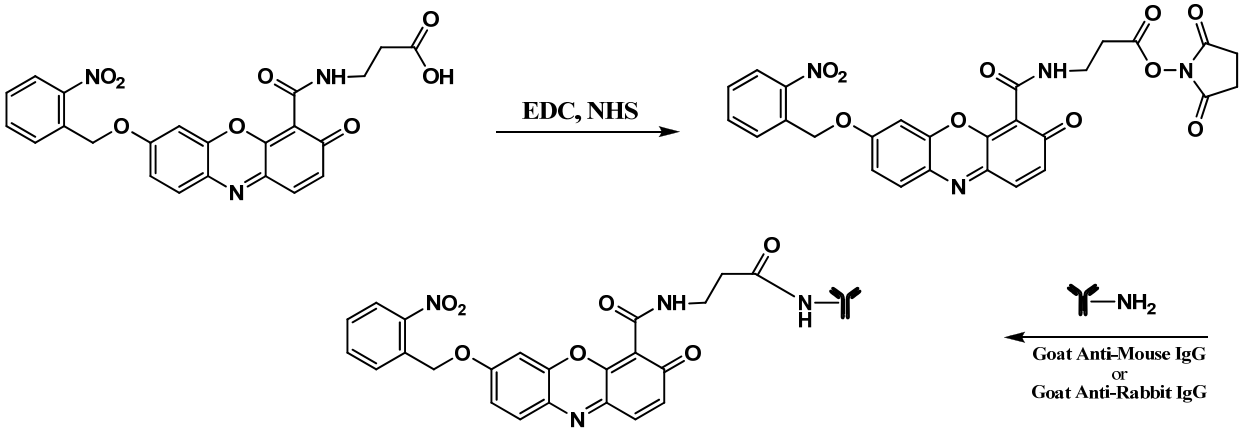
Scheme 2. Synthesis of a mono-caged resorufin dye containing a free carboxyl group.



Scheme 3. Conjugation of double-caged rhodamine dye to IgG molecules.



Scheme 4. Conjugation of mono-caged resorufin dye to IgG molecules.



Experimental

All organic precursors and solvents were obtained from commercial sources and used as received unless otherwise noted. 7-Hydroxyquinoline was purchase from Synbias, Ltd (Donetsk, Ukraine). Flash chromatography was performed on an Analogix IntelliFlash 280 instrument with UV detection at 254 nm. Semi-preparative HPLC purification was performed on an Agilent 1200 Series instrument equipped with a multi-wavelength detector using an Agilent Eclipse XDB C18 column (9.4 x 250 mm, 5µm) with a flow rate of 4 mL/min. The solvent system consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in ACN). A linear gradient of 30% B to 95% B over 15 minutes was used. Atmospheric pressure chemical ionization mass spectrometry (APCI MS) was performed on an LC/MSD TrapXCl Agilent Technologies instrument. Gel filtration purification of the antibody conjugates was performed on an Agilent 1200 Series HPLC equipped with a multi-wavelength detector using a Zorbax GF-250

column (9.4 x 250 mm, 4 μm) with a flow rate of 1 mL/min. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian spectrometer operating at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS). UV-VIS spectrophotometry was carried out on a Perkin Elmer Lambda 25 Spectrophotometer. MALDI-TOF mass spectra were recorded on a Shimadzu Biotech Axima-CFP Plus spectrometer. A supersaturated solution of dihydroxybenzoic acid (DHB) (50% CH_3CN, 50% water with 0.1% trifluoroacetic acid) was used as a matrix for MALDI-TOF analysis of the naked and conjugated antibodies.

2-(4,5-dimethoxy-2-nitrophenyl)-2-hydroxyacetic acid (1) was synthesized according to literature[3]. 6-Nitrovertraldehyde (85% tech. grade, Acros Organics) (14 g, 66.3 mmol) was suspended in water (130 mL) and stirred for 30 minutes at room temperature. To the yellow suspension was added sodium bisulfite solution (100 mL, 2.11 M) in two portions. A precipitate formed and was removed by filtration. Sodium cyanide (7.9 g, 161.11 mmol) was added to the filtrate and the mixture was stirred at room temperature for 2 hours. The reaction was extracted with ether (5 x 300 mL) and the organic fractions were combined, dried over sodium sulfate, and the solvents were removed under reduced pressure. The intermediate was dried under high vacuum to afford a brown solid (10.5 g). The intermediate was dissolved in concentrated hydrochloric acid (12 M, 35 mL) and the reaction mixture was heated at 90°C for 48 hours. This mixture was extracted with ethyl acetate (5 x 200 mL), the organic fraction was washed with water (1x10 mL) and brine (1x10 mL), then dried over sodium sulfate. The solvent was removed under reduced pressure to afford a brown solid (**1**, 7.25 g, 50% yield). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.66 (1H, s, Ph), 7.35 (1H, s, Ph), 5.88 (1H, s, C<u>H</u>(OH), 3.94 (3H, s, CH_3), 3.90 (3H, s, CH_3). ^{13}C NMR (100.56 MHz, CD_3OD): δ = 173.4, 153.7, 148.6, 140.4, 129.8, 110.3, 108.1, 69.5, 55.7, 55.6.

2-Acetoxy-2-(4,5-dimethoxy-2-nitrophenyl)acetic acid (2). The hydroxyacid (**1**) (5.48 g, 21.3 mmol) was dissolved in acetic anhydride (40 mL) at room temperature. The reaction mixture was refluxed at 140°C for 1 hour and allowed to cool to room temperature. Water (50 mL) was added to the reaction mixture and allowed to stir for an additional 2 hours at room temperature. The solution was extracted with dichloromethane (3 x 250 mL). The organic fractions were combined

and washed with water (5x15 mL) and brine (1x30 mL), then dried over sodium sulfate. The solvent was removed under reduced pressure to obtain a brown semisolid (**2**, 6.33 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67 (1H, s, Ph), 7.03 (1H, s, Ph), 6.93 (1H, s, C<u>H</u>(OH), 3.97 (3H, s, CH_3), 3.95 (3H, s, CH_3), 2.18 (3H, s, CH_3). ^{13}C NMR(100.56 MHz, $CHCl_3$): δ = 172.2, 169.8, 153.6, 149.5, 123.6, 111.3, 108.6, 69.9, 56.8, 56.7, 20.8.

***Tert*-Butyl-2-acetoxy-2-(4,5-dimethoxy-2-nitrophenyl)acetate (3).** Compound (**2**) (0.32 g, 1.07 mmol) was dissolved in anhydrous dichloromethane (3 mL) at room temperature under argon. *Tert*-Butyl-2,2,2-trichloroacetimidate (0.383 mL, 2.14 mmol) was dissolved in anhydrous cyclohexane (6 mL) and added to the reaction mixture. The reaction mixture was purged with argon for 10 minutes followed by addition of boron trifluoride etherate in diethyl ether (0.045 mL), and stirred for 30 minutes at room temperature. To the reaction mixture 5% sodium bicarbonate solution (10 mL) was added and the reaction was extracted with ethyl acetate (4x200 mL). The combined organic layers were washed with water (2x10 mL) and brine (1x10 mL) then dried over sodium sulfate. The residue was purified by flash chromatography (dichloromethane:methanol from 10:0 to 9.5:0.5). The fractions containing the product were combined and solvents were removed under reduced pressure. The title compound was obtained as a light brown oil (**3**, 207 mg, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (1H, s, Ph), 7.01 (1H, s, Ph), 6.79 (1H, s, C<u>H)</u>, 3.97 (3H, s, CH_3), 3.95 (3H, s, CH_3), 2.21 (3H, s, CH_3), 1.42 (9H, s, CH_3).

***Tert*-Butyl-2-acetoxy-2-(4,5-dimethoxy-2-nitrophenyl)-2-hydroxyacetate (4).** Compound (**3**) (0.11 g, 0.31 mmol) was dissolved in water/methanol (1 mL / 3 mL) and cesium carbonate (0.1 g, 0.3 mmol) was added. The reaction mixture was stirred for 2 hours at room temperature. Solvents were removed under reduced pressure and the reaction mixture was dissolved in chloroform and filtered through a silica gel plug. The solvent was removed under reduced pressure to obtain the title compound as a light brown oil (**4**, 96 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.61 (1H, s, Ph), 7.13 (1H, s, Ph), 5.92 (1H, s, CH), 3.98 (3H, s, CH_3), 3.96 (3H, s, CH_3), 1.39 (9H, s, CH_3). ^{13}C NMR (100.56 MHz, CD_3OD): δ = 170.9, 153.7, 148.5, 140.3, 129.9, 110.0, 108. 1, 82.0, 70.3, 55.6, 55.6, 26.9.

***Tert*-Butyl 2-(chlorocarbonyloxy)-2-(4,5-dimethoxy-2-nitrophenyl)acetate (5).** Compound (**4**) (0.05 g, 0.16 mmol) and 2,4–lutidine (0.02 mL, 0.16 mmol) were dissolved in anhydrous tetrahydrofuran (5mL), the mixture was purged with argon for 15 minutes and phosgene (20% in toluene, 0.33 mL, 0.64 mmol) was added. The reaction mixture was stirred for 6 hours at room temperature and the solvents were removed by reduced pressure using a safety trap containing potassium hydroxide. The residue was purified by flash chromatography (dichloromethane). The fractions contaning the product were combined and solvent was removed under reduced pressure at room temperature to obtain the title compound as a light yellow oil (**5**, 30 mg, 50% yield). The compound was stored at -80 °C under argon until further use. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (1H, s, Ph), 6.95 (1h, s, Ph), 6.86 (1H, s, CH), 3.96 (3H, s, CH_3), 3.95 (3H, s, CH_3), 1.43 (9H, s, CH_3).

1,2,3,4-Tetrahydroquinolin-7-ol (6). 10 % palladium on carbon (0.1 g) was added to a solution of 7-hydroxyquinoline (1 g, 6.5 mmol) in ethanol/acetic acid (35 mL / 5 mL). The reaction mixture was placed in a hydrogenation vessel for 12 hours with a hydrogen pressure of 45 psi. The catalyst was filtered through a filtering agent (Celite®545, Aldrich), and the solvents were removed under reduced pressure. The resulting reddish-brown oil slowly crystallized to afford the title compound **6** (0.97 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.79 (d, *J* = 8Hz, 1H), 6.1 (dd, *J* = 8.4 Hz and *J* = 2.8 Hz 1H), 5.98 (d, *J* = 2.8 Hz,1H), 4.2 (br), 3.27 (t, *J* = 6 Hz, 2H), 2.69 (t, *J* = 6 Hz, 2H), 1.93 (m, 2H). m/z (MS APCI) 150.3 ($[M+H]^+$).

Q-Rhodamine (5 and 6 isomers) (7). Compound (**6**) (0.59 g, 4 mmol), phthalic anhydride (0.77 g, 4 mmol) and anhydrous zinc chloride (0.27 g, 2 mmol) were combined in a dry flask. The reaction mixture was heated for 2 hours under an argon atmosphere at 150°C. The reaction mixture was cooled to room temperature and washed with water. The residue was crystallized from 1M HCl upon cooling the solution to 4˚C for 48 hours. The title compound, a red-purple solid, was filtered and dried until constant weight was reached under high vacuum (7, 0.41 g, 45% yield). ^{1}H NMR (400 MHz, D_2O/NaOH): δ = 8.0 (1H, s), 7.89 (1H, d, *J* = 7.6 Hz,), 7.81 (1H, d, *J* = 7.2 Hz), 7.68 (1H, s), 7.62 (3H, m), 7.26 (1H, d, *J* =8 Hz), 6.95 (1H, d, *J* =7.2 Hz), 6.66 (4H, d, *J* =13.2 Hz), 5.96 (4H, d, *J* =4 Hz), 3.03 (8H, br m), 2.34 (8H, br m), 1.54 (8H, br m). m/z (MS APCI) 455.3 ($[M+H]^+$). UV-Vis λ_{max}= 536 nm.

Double caged Q-Rhodamine (8). Compound (**7**) (20 mg, 44 μmol) was dissolved in DMF (0.45 mL) to obtain a final concentration of 0.1M. Diisopropylethylamine (38 μL, 220 μmol) was added to the solution. The mixture was cooled in an ice-bath and purged with argon for 20 minutes. Chloroformate **5** (44 mg, 132 μmol) was dissolved in tetrahydrofuran (1.3 mL) to a final concentration of 0.1M and added to the reaction mixture in one portion. The reaction was protected from the light and stirred at room temperature for 12 hours. The reaction was purified by semi-preparative HPLC. Rt = 14.5 minutes. Fractions containing the title compound were combined to obtain a purple-red semisolid (2.5 mg). m/z (MS APCI) 1134.09 ($[M+H]^+$).

***N*-succinimidyl ester of double caged Q-Rhodamine (9,10).** Compound (**8**) (2.5 mg, 2.2 μmol), *N*-hydroxysuccinimide (2 mg in 50 μL of dichloromethane, 7.6 μL, 2.4 μmol) and triethylamine (0.5 μL) were dissolved in dichloromethane (0.2 mL). *N,N'*-Dicyclohexylcarbodiimide (2 mg in 50 μL of dichloromethane, 12.4 μL, 2.4 μmol) was added to the reaction mixture and stirred at room temperature overnight. The solvents were removed under vacuum and the reaction was taken to the next step without purification. (**9**) m/z (MS APCI) 1231.14 ($[M+H]^+$). Trifluoroacetic acid (50 μL) and dichloromethane (100 μL) were added to the reaction and stirred for 4 hours at room temperature. The solvents were removed under reduced pressure and dried under high vacuum. The mixture was redissolved in acetonitrile (50 μL) and purified by semi-preparative HPLC with a Rt = 13.75 minutes. Fractions containing the title compound were combined and solvents were removed under reduced pressure to obtain the red title compound. Amber glassware and aluminum foil were used to protect the compound from light. (**10**) m/z (MS APCI) 1119.03 ($[M+H]^+$).

4-Nitrosoresorcinol (11) was prepared according literature[4]. A solution of resorcinol (100 g, 0.9 mol) in anhydrous ethanol (250 mL) was cooled in an ice-bath. A solution of potassium hydroxide (72 g, 1.28 mol) in ethanol (400 mL) was added. The reaction mixture was stirred in an ice-bath for 15 minutes, followed by a dropwise addition of isoamylnitrite (120 g, 1.1 mol) over a 1 hour period at 0°C. The reaction mixture was allowed to stir for 4 hours at 0.5 °C. The precipitate was filtered and washed with ethanol (4 x 100 mL) and ether (3 x 100 mL). The title compound **11** (a light-brown solid) was dried under vacuum (141 g, 88 % yield). m/z MS APCI ($[M+H]^+$) 140.1.

Carboxyresorufin (12) was synthesized according to the published protocol[5]. A suspension of 4-nitrosoresorcinol (2.25 g, 16.3 mmol) and 2-carboxyresorcinol (2.74 g, 17.8 mmol) in 95 % sulfuric acid (16 mL) was heated for 35 minutes at 110 °C. The reaction mixture was cooled to room temperature, 50 mL of ice-cold water was added, and the mixture was placed on ice for 15 min. The solid was collected by filtration and dried to afford a dark-red solid (**12**, 1.56 g, 60% yield). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.69 (d, 1H), 7.67 (d, 1H), 6.93 (d, 1H), 6.86 (d, 1H), 6.67 (s, 1H). m/z (MS APCI) 257.2 ($[M+H]^+$).

***Tert*-Butyl 3-(7-hydroxy-3-oxo-3*H*-phenoxazine-4-carboxamido)propanoate (13).** *N*-Hydroxysuccinimide (3 g, 26 mmol) was dissolved in trifluoroacetic anhydride (15 mL) under argon. The reaction mixture was stirred for 2 hours at room temperature. The solvent was removed under reduced pressure and the product dried under vacuum to constant weight to afford a white solid (5.5 g). The active ester was stored under argon and used in the next step without purification. Carboxyresorufin (0.2 g, 0.78 mmol) was dissolved in anhydrous tetrahydrofuran (6 mL) and pyridine (0.19 mL). Trifluoroacetate *N*-succinimide ester (0.217 g, 1.03 mmol) was added to the solution, and stirred under an argon atmosphere for 3 hours. The solvent was removed under reduced pressure and the solid dried under vacuum. Activated carboxyresorufin was dissolved in dimethylformamide (2 mL). β-Alanine *t*-butyl ester hydrochloride salt (0.142 g, 0.78 mmol) was dissolved in 200 mM triethyl ammonium bicarbonate buffer (2 mL, pH 8.5) and slowly added to the carboxyresorufin solution. The reaction mixture was stirred at room temperature overnight and extracted with chloroform (3x200 mL). The organic fractions were combined and the volume was reduced to 5-10 mL. Silica gel (1.5 g) was added to the residue and the solvents were removed under vacuum. The residue, now loaded on silica gel, was purified by flash chromatography (12 g column, chloroform and 1% acetic acid:methanol from 10:0 to 8:2). The fractions containing the product were combined and the solvents were removed under reduced pressure. The title compound, **13**, was obtained as a dark red semi-solid (90 mg, 30% yield over two steps). ^{1}H NMR (400 MHz, CD_3OD/NaOH): δ = 7.49 (s, 1H), 7.47 (s, 1H), 6.76 (d, *J* =2.4 Hz, 0.5H), 6.73 (d, *J*=2.4Hz, 0.5H), 6.71 (s, 0.5H), 6.69 (s, 0.5H), 6.50 (d, *J*=2Hz, 1H), 3.64 (t, *J*= 6.4Hz, 2H), 2.59 (t, *J*=6.4Hz, 2H), 1.43 (s, 9H). m/z (MS APCI) 384.3 ($[M+H]^+$).

***Tert*-Butyl 3-(7-(2-nitrobenzyloxy)-3-oxo-3*H*-phenoxazine-4-carboxamido)propanoate (14).** Compound (**13**) (50 mg, 0.13 mmol) was dissolved in anhydrous acetonitrile (2 mL) and silver oxide (50 mg, 0.26 mmol) was added. The reaction was heated for 10 minutes at 70°C and *ortho*-bromomethylnitrobenzene (85 mg, 390 mmol) was added. The reaction mixture was then heated for 2 hours at 70°C in the dark. The reaction was cooled to room temperature, filtered and silica gel (0.4 g) was added to the residue. The residue, loaded on silica gel, was purified by flash chromatography using an 8 g silica column (dichloromethane: methanol from 10:0 to 9:1). The fractions containing the product were combined and solvents were removed under reduced pressure. The title compound, **14**, was obtained as red-brown semi-solid (47 mg, 70% yield). ^{1}H NMR (400 MHz, d_6-DMSO/CD_3OD): δ = 8.58 (t, J=5.6Hz, 1H), 8.14 (dd, J=8 Hz and J=1Hz, 1H), 7.78-7.71 (m, 3H), 7.59-7.54 (m, 1H), 7.47 (d, J=10Hz, 1H), 7.21 (d, J =8.8 Hz, 1H), 6.74 (dd, *J*=7.6Hz and *J*=2Hz, 1H), 6.20 (d, *J*=2.4Hz, 1H), 5.63 (s, 2H), 3.61 (t, *J*= 6.4Hz, 2H), 2.52 (t, *J*=6.4Hz, 2H), 1.32 (s, 9H). m/z (MS APCI) 520.2 ($[M+H]^+$).

3-(7-(2-Nitrobenzyloxy)-3-oxo-3*H*-phenoxazine-4-carboxamido)propanoic acid (15). Compound **14** (10 mg, 0.02 mmol) was dissolved in dichloromethane and trifluoroacetic acid (1 mL / 0.1 mL) and stirred at room temperature for 2 hours in the dark. The solvents were removed under reduced pressure and dried under high vacuum to obtain the final monocaged resorufin as a dark, red-brown solid. Amber glassware and aluminum foil were used to protect the compound from light. m/z (MS APCI) 464.2 ($[M+H]^+$).

Double caged Q-rhodamine IgG conjugate. The antibody was provided in lyophilized form as 2 mg in 10 mM phosphate buffered saline, pH 7.4, containing 0.1% sodium azide as a preservative. To the lyophilized antibody was added 200 µL of water to give a final antibody concentration of 10 mg/mL (13.65 nmol of goat anti-mouse IgG and 13.58 nmol of goat anti-rabbit IgG). While protecting from light, the double caged Q-rhodamine NHS ester was dissolved in dimethylformamide (DMF) at a concentration of 1 mg/mL. To the antibody was then added 45 µL (40nmol) of the Q-rhodamine NHS solution and mixed well. The mixture was then allowed to react for 2 hours on ice and then overnight at 4˚C. The conjugated antibody was then purified from any excess dye by gel filtration HPLC, affording a clear liquid. The purified,

caged Q-rhodamine-IgG conjugate showed a single peak at 8.6 minutes and 8.38 minutes for the anti-mouse and anti-rabbit conjugates, respectively. *m/z* (MALDI-TOF) 147,858.25 for the anti-mouse conjugate and 148,774.54 for the anti-rabbit conjugate.

Mono caged resorufin IgG conjugates. While protecting from light, the caged resorufin (**15**) was dissolved in dimethylformamide (DMF) at a concentration of 1 mg/mL. To that solution (31 μL, 66.7 nmol) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in water (10.5 μL, 66.7 nmol) and *N*-hydroxysuccinimide in dimethylformamide (73 μL, 333 nmol). The mixture was allowed to react for 30 minutes at room temperature and used without any further purification.

The antibody was provided in lyophilized form as 2 mg in 10 mM phosphate buffered saline, pH 7.4, containing 0.1% sodium azide as a preservative. To the lyophilized antibody was added 400 μL of water to give a final antibody concentration of 5 mg/mL (13.65 nmol of goat anti-mouse IgG and 13.58 nmol of goat anti-rabbit IgG). To the antibody was added succinimide ester of monocaged resorufin (34 μL, 333 nmol) and mixed well. The mixture was allowed to react for 2 hours on ice and then overnight at 4˚C. The conjugated antibody was purified from any excess dye by gel filtration HPLC, affording a clear liquid. The purified, mono-caged resorufin-IgG conjugate showed a peak at 8.57 minutes and 8.65 minutes for the anti-mouse and anti-rabbit conjugates, respectively. *m/z* (MALDI-TOF) 147,799.54 for the anti-mouse conjugate and 147,639.54 for the anti-rabbit conjugate.

Conjugate Analyses

The concentrations of the antibodies were calculated from their absorbance at 280 nm. The extinction coefficient (ε), where ε (1%)=14.0 at 280 nm was used as provided from the manufacturer (Invitrogen). From the information provided and the UV-VIS data of the antibody conjugates, it was determined that there was 9.91 nmol (1.451 mg/mL) of Resorufin-Goat Anti-Mouse IgG, 13.59 nmol (2 mg/mL) of Resorufin-Goat Anti-Rabbit IgG, 10.68 nmol (1.566 mg/mL) of Q-rhodamine Goat Anti-Mouse IgG and 10.96nmol (1.614 mg/mL) of Q-rhodamine Goat Anti-Rabbit IgG present in the corresponding conjugate solutions. However, the UV-VIS spectra gave no supporting data that can be used for the analysis of the ratio of dye molecules to IgG. This is due to the Resorufin and Q-rhodamine compounds being caged. With the caging

groups attached to the dyes, the absorbances at 480 nm for Resorufin and 560 nm for Q-rhodamine are not detectable in the samples at the low levels of concentration used for the conjugations. The increasing absorbances of these dyes at their respective wavelengths can be seen in the uncaging studies that were done (Figures 1, 2). However, the ratio of caged dye molecules to IgG was calculated from the difference in molecular weights of the conjugated and naked antibodies as determined by MALDI-TOF. According to the MALDI-TOF data, there are approximately 1.08 Caged Resorufin molecules per Goat Anti-Mouse IgG, 2.38 Caged Resorufin molecules per Goat Anti-Rabbit IgG, 1.18 Caged Q-rhodamine molecules per Goat Anti-Mouse IgG and 1.32 Caged Q-rhodamine molecules per Goat Anti-Rabbit IgG.

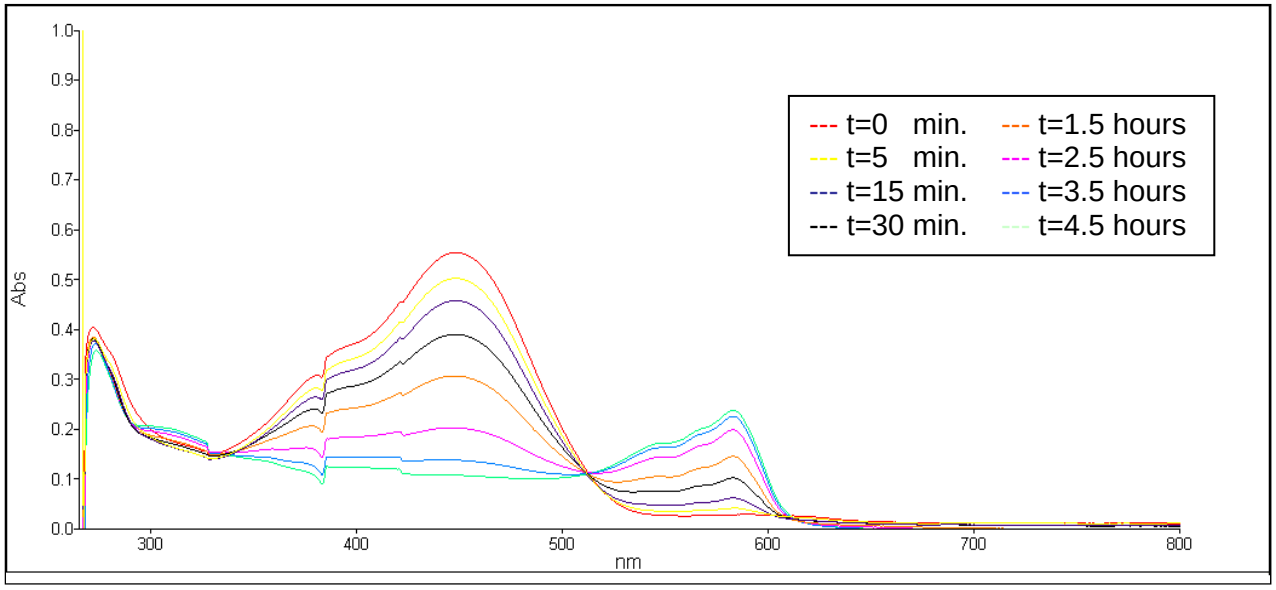


Figure 1. Uncaging Study of Single Caged Resorufin in DMF at 365nm Over a Period of 4.5 hours.

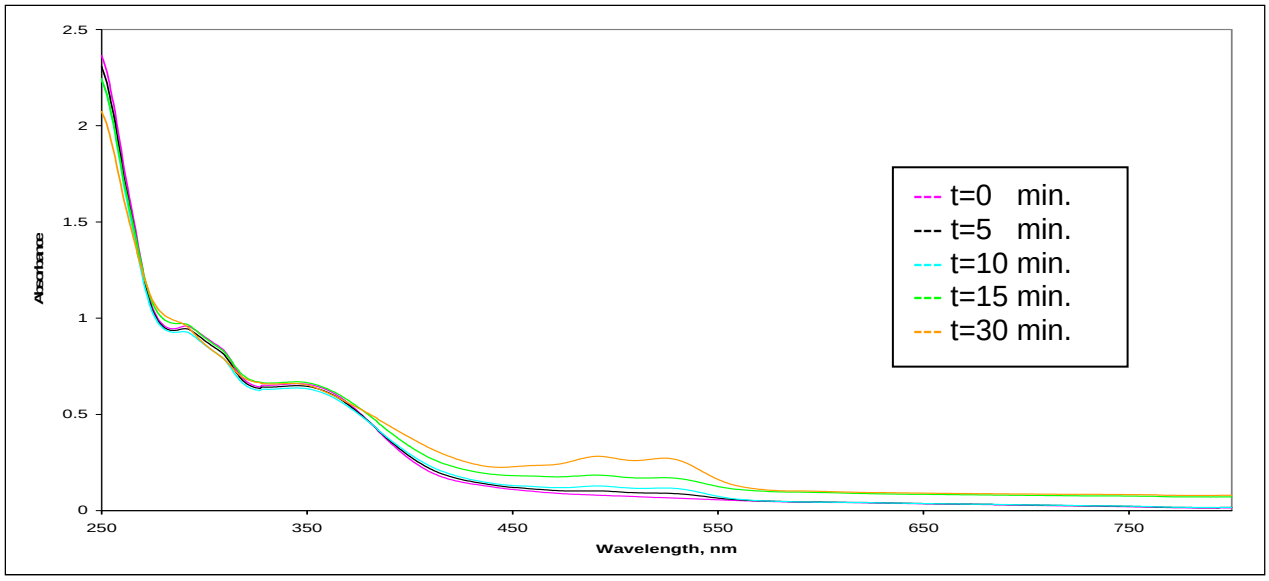


Figure 2. Uncaging Study of Double Caged Q-rhodamine in DMF at 365nm Over a Period of 30 minutes.

References

1. Betzig E, Patterson GH, Sougrat R, et al. Imaging intracellular fluorescent proteins at nanometer resolution. Science 2006; 313:1642-1645.
2. Lippincott-Schwartz J, Patterson GH. Fluorescent proteins for photoactivation experiments. Methods Cell Biol. 2008; 85:45-61
3. Mitchison TJ, Sawin KE, Theriot JA, Gee K, Mallavarapu A. Caged fluorescent probes. Methods Enzymol. 1998; 291: 63-78.
4. Meunier JC, Sealock R, Olsen R, Changeux JP. Purification and Properties of Cholinergic Receptor Protein from Electrophorus-Electricus Electric Tissue. Eur J Biochem. 1974 Jun 15; 45(2):371-94.
5. Corry S, Downey W, Filanowski B, Gee K, Greenfiled I. L, Hirsch J, Johnson I, Rukavishnikov A (2005). WO 2005/071096 A3R6.