Photochemically Amplified Detection of Molecular Recognition Events: A Universal Ultra-Sensitive Fluorescence Turn-Off Binding Assay.

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Supporting Information

Table of contents:

General Information

Common solvents were purchased from AAper Alcohol and used as is, except for THF and hexane. THF was refluxed over and distilled from potassium benzophenone ketyl prior to use. Hexane was distilled over calcium hydride before use. All reagents for synthesis were purchased from Alfa Aesar, TCI America, Fisher Scientific, AK Scientific, ChemImpex, or Aldrich. All reagents were used without purification unless otherwise noted. Avidin was purchased from Aldrich. All lipids were purchased from Avanti polar lipids. NMR spectra were recorded at 25 °C on either a Varian Mercury 400 MHz instrument, or a Bruker Biospin 500 MHZ instrument, in $CDCl_3$ with TMS as an internal standard (unless noted otherwise). Column chromatography was performed on silica gel, 32-63μ mesh, and the eluent is noted in the procedure. UV-Vis

spectra were recorded on a Beckman DU-640 Spectrophotometer and bulk solution fluorescence measurements were made on a Varian Cary Eclipse Fluorescence Spectrophotometer.

Photoreactions were carried out using an in house built Nichia UV LED 5x250mW 365nm reactor outfitted with a 300-400 nm bandpass filter. For each photoreaction discussed a bulk solution of fluorophore and adduct was prepared and divided to the appropriate number of samples. Benzophenone was added to each sample, from a stock solution, at the appropriate concentration. The concentrations of each reagent in the photoreactions are stated in the supplemental text where the results are presented. All bulk solution photoreactions were carried out in quartz fluorescence spectroscopy cells from NSG precision cells. The results of photoamplified fluorescence quenching of coumarin 6, for several solvents, are listed in the table below.

Table S1. Coumarin fluorescence "turn-off" photoamplification assay results in chlorinated alkanes

Solvent	Max diff*	Boiling Point °C	Density (g/mL)
Dichloromethane (wet)	90%	40	1.32
1-Chlorocyclohexane	70%	142	1.00
1,3-Dichlorobutane	57%	134	1.12
1,4-Dichlorobutane	50%	162	1.16
1,5-Dichloropentane	14%	65/10 mmHg	1.06

***Difference maximum between normalized emission intensity values for a sample with 10^{-5} M BP and No BP**

Photoreactions carried out in capillaries were irradiated using an in house built mini carousel reactor with two 365 nm LED's from Nichia Corporation and imaged with a custom CCD imager, based on Zeiss Axiovert microscope cube shown below. The CCD Camera used with the imager was a Mead Deep Sky Imager 2 Pro with a Sony ICX429ALL interline monochromatic CCD solid state image sensor. The effective pixels were 7.4x5.95 mm with a pixel size of 8.6x8.3 μm. The fluorescence filter block was from a Zeiss Axiovert 100 microscope and was outfitted with a 45° single-edge dichroic beamsplitter centered at 495 nm with a reflection band from 442 – 488 nm (reflects all light at wavelengths below this band) and a transmission band from 502-730 nm was used (FF495-DiO2-25x36). The excitation source was one 400 nm Ultraviolet LEDs purchased from LED Supply (L3-0-U5TH15-1) which was used without an excitation filter. A 515 nm long pass emission filter was used. TECHSPEC® PCX uncoated lenses, purchased from Edmund optics, were used on both ends of the filter block. A 25x40 mm (NT45-278) lens was used on top of the filter block and a 25x25

mm (NT45-097) lens was used on the bottom of the filter block (see schematic diagram below). The capillaries used to prepare the cassettes were purchased from AM-Systems Inc. ID 0.86 mm and ID 0.4mm.

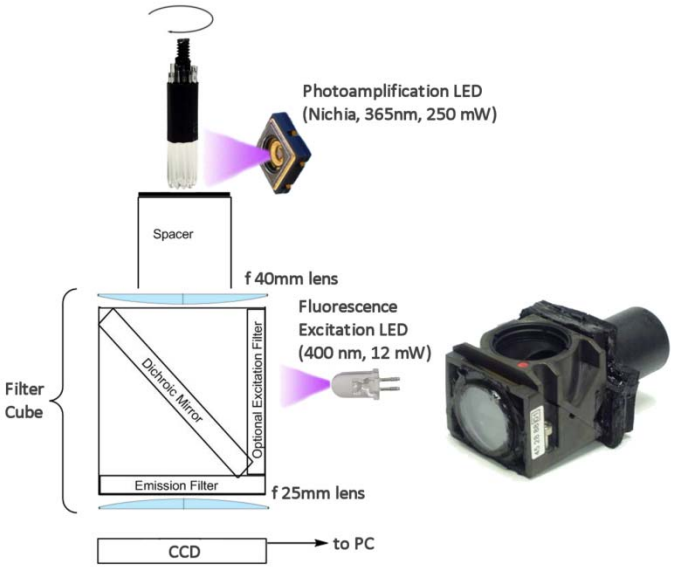


Figure S1

Synthetic Procedures

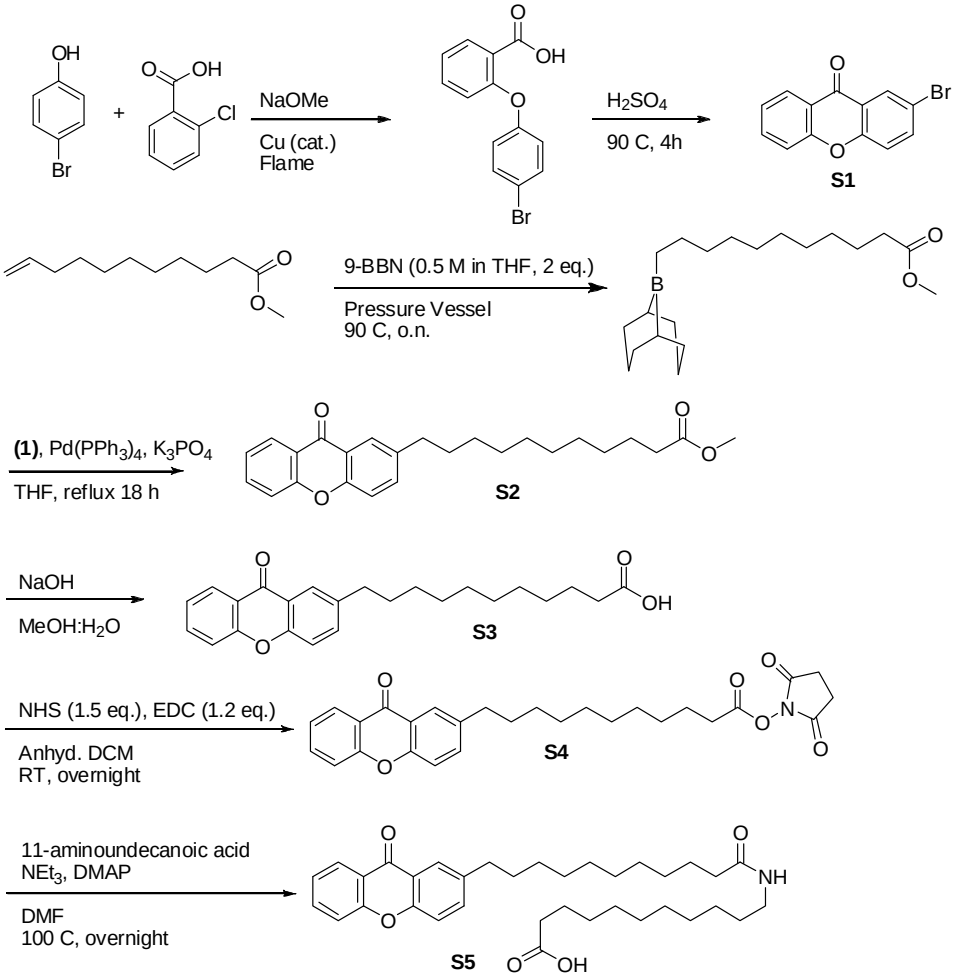
General method for adduct preparation.

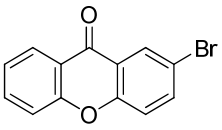
A generic method by Corey and Seebach was modified to prepare the dithiane-benzophenone adducts utilized as masked sensitizers in this study. A dithiane (5.1 mmol) was dissolved in freshly distilled THF (30 mL) and placed under nitrogen. n-Butyllithium (4.3 mL, 6.8 mmol, 1.6 M solution in hexanes) was added at room temperature with stirring. The resulting mixture was stirred for 10 minutes. The appropriate benzophenone (3.4 mmol) was dissolved in freshly distilled THF (10 mL) and added to the anion mixture while stirring. The reaction was left for 2 hours at room temperature, before quenching with a saturated solution of ammonium chloride. The aqueous layer was extracted twice with ethyl acetate, which was collected and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the residue was purified by column chromatography (silica gel, ethyl acetate/hexanes) or recrystallization (DCM in hexanes).

(1,3-dithian-2-yl)diphenylmethanol[1]: 1.8 g, 60% yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56-7.63 (d, 4H, J = 7.2 Hz), 7.30-7.37 (d, 4H, J = 7.5 Hz), 7.22-7.27 (m, 2H), 3.28 (s, 1H), 2.84-2.99 (m, 4H), 2.04-2.12 (m, 1H), 1.82-1.90 (m, 1H).

Preparation of Xanthone Derivatives for coupling to Avidin:

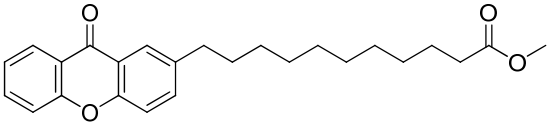
Scheme S1



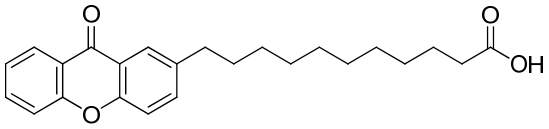


2-Bromo-9H-xanthen-9-one (S1)[2]: Sodium metal (~2.4 g) was dissolved in methanol (60 mL). 4-bromophenol (10 g, 58 mmol) and 2-chlorobenzoic acid (9.2 g, 59 mmol) were added and methanol was removed under vacuum to give a cake like solid. Copper powder (cat.) was added, the reaction was heated with an open flame (bunsen burner) , which was applied until dense white smoke spread over the entire solid for

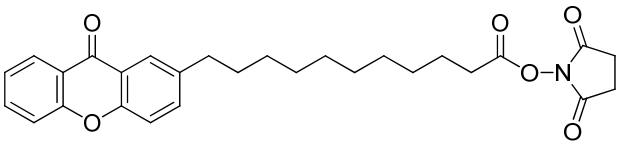
several minutes. The reaction was cooled for 30 min. Concentrated sulfuric acid (100 mL) was added and the mixture was heated for 4 hours at 90 °C, cooled to room temperature, poured over ice, extracted with dichloromethane (2 x 150 mL), and washed with 5% aq. NaOH solution. The organic layer was collected, dried over anhydrous sodium sulfate, and removed under vacuum. The resulting solid was purified by gel filtration (eluted by DCM) and the solvent was removed to give the product (1.4 g, 9% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46-8.47 (d, 1H, J = 2.5 Hz), 8.32-8.35 (dd, 1H, J = 1.7, 8.0 Hz), 7.77-7.82 (ddd, 1H, J = 0.6, 2.5, 8.9 Hz), 7.73-7.76 (m, 1H), 7.48-7.51 (d, 1H, J = 8.5 Hz), 7.39-7.43 (m, 2H).



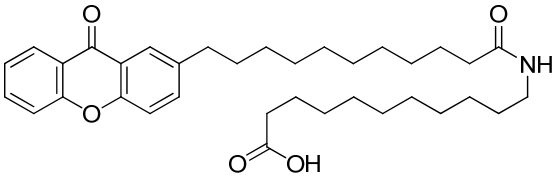
methyl 11-(9-oxo-9H-xanthen-2-yl)undecanoate (S2): To a pressure vessel containing methyl-10-undecenoate (2.45 mL, 10 mmol) was added 9-BBN (0.5 M in THF, 22 mL, 10 mmol). The reaction mixture was heated to 85-90 °C with stirring for 18 hrs. After cooling to room temperature water was added (0.15 mL). This mixture was combined with **(S1)** (1.4 g, 5.1 mmol), $Pd(PPh_3)_4$ (0.6 g, 0.5 mmol), and potassium phosphate tribasic (7 g) in a round bottom flask with stirring. The reaction was heated at reflux for 18h, filtered, and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate and purified via gel filtration (eluted with hexane:ethyl acetate (20:1)) to give **S2** as a white solid (0.877 g, 41 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34-8.36 (dd, 1H, J = 1.7, 8.0 Hz), 8.13 (s, 1H), 7.70-7.74 (ddd, 1H, J = 1.8, 7.1, 8.7 Hz), 7.54-7.57 (dd, 1H, J = 2.3, 8.6 Hz), 7.48-7.50 (d, 1H, J = 8.6 Hz), 7.41-7.43 (d, 1H, J = 8.6 Hz), 7.36-7.41 (ddd, 1H, J = 1.0, 7.1, 8.0 Hz), 3.66 (s, 3H), 2.71-2.75 (m, 2H), 2.28-2.31 (t, 2H, J = 7.6 Hz), 1.55-1.67 (qd, 4H, J = 7.6, 15.1 Hz), 1.25-1.35 (m, 12H).



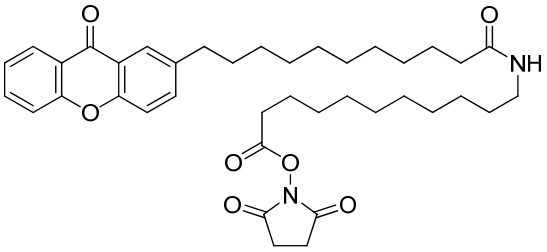
11-(9-oxo-9H-xanthen-2-yl)undecanoic acid (S3): **S2** (0.877 g, 2.2 mmol) was suspended in MeOH (50 mL) with stirring. NaOH (2 g) dissolved in H_2O (10 mL) was added to the suspension. This mixture was refluxed for 4 h, poured over ice, and made acidic using concentrated HCl. The solid was collected via filtration to give acid **S3** (0.714 g, 86% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34-8.36 (dd, 1H, J = 1.6, 8.0 Hz), 8.14 (d, 1H, J = 2.2 Hz), 7.70-7.74 (ddd, 1H, J = 1.7, 7.2, 8.7 Hz), 7.54-7.57 (dd, 1H, J = 2.3, 8.6 Hz), 7.48-7.50 (d, 1H, J = 8.0 Hz), 7.41-7.43 (d, 1H, J = 8.5 Hz), 7.35-7.39 (t, 1H, J = 8.0 Hz), 2.71-2.75 (m, 2H), 2.33-2.37 (t, 2H, J = 7.5 Hz), 1.59-1.69 (m, 4H), 1.23-1.37 (m, 12H). HRMS (ESI) calcd for $C_{24}H_{27}O_4^-$ ($[M-H]^-$) 379.1915, found 379.1919



11-(9-oxo-9H-xanthen-2-yl)undecanoic acid N-hydroxysuccinamide ester (S4): **S3** (0.75 g, 1.97 mmol), DIPEA (0.1 mL), DMAP (cat.), N-hydroxysuccinimide (0.45 g, 3.9 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, (0.60 g, 3.15 mmol) were dissolved in THF:CH_2Cl_2 (2:1, 75 mL) and stirred for 24 hours. The solution was washed with water (60 mL) and saturated aq. sodium bicarbonate (60 mL), followed by NaCl (60 mL). The organic layer was dried over anhydrous sodium sulfate and removed under vacuum to give the product (0.94 g, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34-8.36 (dd, 1H, J = 1.7, 8.0 Hz), 8.13 (d, 1H, J = 2.2 Hz), 7.70-7.74 (ddd, 1H, J = 1.7, 7.1, 8.6 Hz), 7.55-7.57 (dd, 1H, J = 2.2, 8.6 Hz), 7.48-7.50 (d, 1H, J = 8.5 Hz), 7.42-7.43 (d, 1H, J = 8.6 Hz), 7.36-7.39 (m, 1H), 2.85 (m, 4H) 2.71-2.74 (m, 2H), 2.58-2.61 (t, 2H, J = 7.5 Hz), 1.64-1.76 (m, 4H), 1.25-1.43 (m, 12H).



11-(11-(9-oxo-9H-xanthen-2-yl)undecanamido)undecanoic acid (S5): **S4** (1.31 g, 5.8 mmol) was dissolved in DMF (30 mL), 11-aminoundecanoic acid (2.36 g, 11.7 mmol), NEt_3 (0.2 mL), and DMAP (cat.) were added and the reaction was heated at 100 °C overnight with stirring. The reaction was poured over ice and the product was collected by filtration and purified by gel filtration (eluted with 7% MeOH in DCM) to give acid **S5** (0.62 g, 20 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25-8.27 (d, 1H, J = 8.0 Hz), 8.04 (s, 1H), 7.67-7.70 (t, 1H, J = 7.8 Hz), 7.51-7.53 (dd, 1H, J = 1.9, 8.6 Hz), 7.44-7.46 (d, 1H, J = 8.5 Hz), 7.38-7.39 (d, 1H, J = 8.6 Hz), 7.31-7.34 (t, 1H, J = 7.5 Hz), 3.11-3.14 (t, 2H, J = 7.2 Hz), 2.65-2.68 (m, 2H), 2.20-2.23 (t, 2H, J = 7.5 Hz), 2.07-2.10 (t, 2H, J = 7.6 Hz), 1.51-1.62 (m, 8H),1.35-1.43 (m, 4H) 1.10-1.25 (m, 20H).

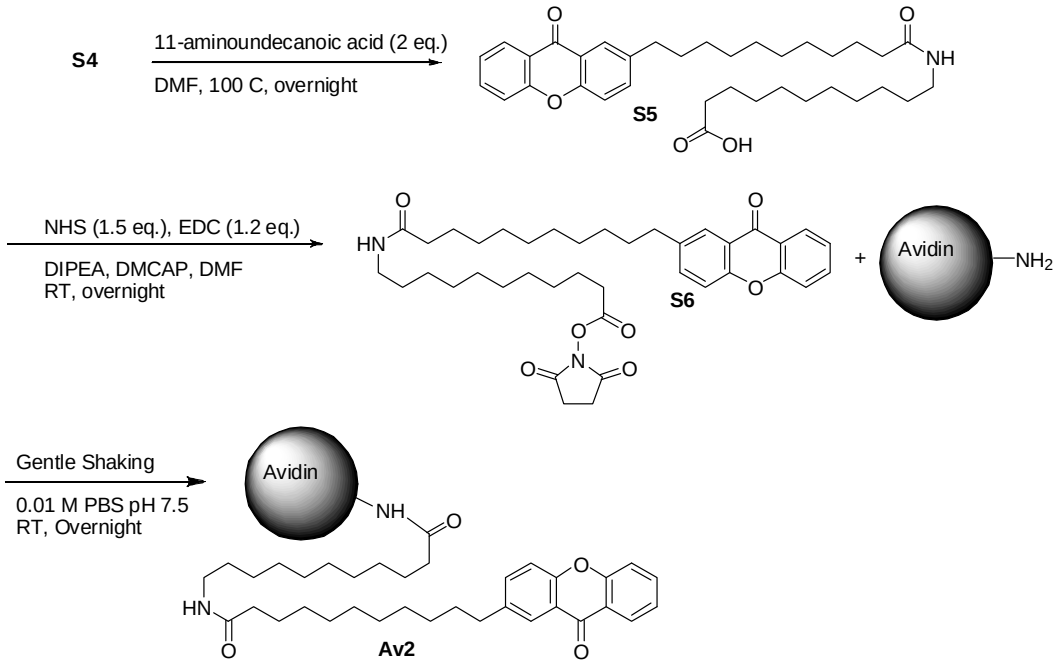


11-(11-(9-oxo-9H-xanthen-2-yl)undecanamido)undecanoic acid N-hydroxysuccinamide ester (S6): A mixture of acid **S5** (0.5 g, 0.89 mmol), NEt_3 (0.05 mL), DMAP (cat.), N-hydroxysuccinimide (0.154 g, 1.3 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, (0.204 g, 1.1 mmol) was

dissolved in DMF (15 mL) and stirred for 24 hours. The solvent was removed under vacuum. The product was dissolved in DCM and purified by gel filtration eluted with DCM (50 mL) and then 10% MeOD in DCM (100 mL) to give pure NHS ester **S6** (0.135 g, 23% Yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34-8.36 (d, 1H, J = 1.7, 8.0 Hz), 8.14 (d, 1H, J = 2.2 Hz), 7.70-7.74 (ddd, 1H, J = 1.7, 7.1, 8.6 Hz), 7.55-7.57 (dd, 1H, J = 2.2, 8.5 Hz), 7.49-7.50 (d, 1H, J = 8.4 Hz), 7.42-7.44 (d, 1H, J = 8.5 Hz), 7.36-7.39 (t, 1H, J = 7.5 Hz), 3.21-3.25 (dd, 2H, J = 7.0, 13.1 Hz), 2.71-2.74 (m, 2H), 2.58-2.61 (t, 2H, J = 2.5, 7.5 Hz), 2.13-2.16 (m, 2H), 1.70-1.76 (dt, 2H, J = 7.5, 15.2), 1.60-1.68 (m, 6H),1.46-1.50 (m, 2H) 1.22-1.43 (m, 22H).

General procedure for avidin-xanthone conjugation:

Scheme S2



Avidin (5 mg, 74 nmol), purchased from Invitrogen or Pierce Biotechnology (now ThermoScientific) was dissovled in PBS (0.01 M, pH 8.0, 1 mL). The appropriate xanthone-NHS ester was dissolved in DMSO (0.5 mg/1 mL) over 1 hour at room temperature and 100 μL was added to the avidin solution. The resulting mixture was shaken gently for one hour at room temperature, a second 100 μL aliquot of xanthone-NHS in DMSO was added, and the reaction was left with shaking for 48 hours. It was then purified on a Sephadex G-25 column (to 2.5 g of Sephadex G-25, purchased from Aldrich, was added a 0.01 M PBS to make a slurry, this slurry was poured into the appropriate column, the mixture was added and eluted with the phosphate buffer). 16 fractions (1 mL each) were collected and tested by UV/Vis. The conjugated avidin was generally found between

fractions 3 and 8 containing the product with an average of 2 to 3 xanthone molecules per avidin. The Solver in Excel was used to carry out a least squares analysis between a model system (a fit the sum of OD's for free ketone and protein, at known concentrations) and the actual UV/Vis spectrum of a fraction of the conjugated protein. The solver determined the relative concentrations of ketone and avidin present in the conjugate, from which the ratio could be taken to determine the number of coupled xanthone molecules per avidin. Each avidin generally carried between 3 and 5 xanthones, depending on the experiment and the fraction. The actual concentration of xanthone (ε_{365} = 635) within the conjugate was determined and from this the approximate concentration of avidin was calculated for each fraction. Avidin for each binding experiment was then diluted from these fractions.

Avidin-(S5) conjugation: Prepared with **S4**. Each avidin tetramer carries approximately 2 to 3 xanthone molecules. The concentration of xanthone was calculated to be 1.4×10^{-4} M, from this it was determined the approximate concentration of avidin was 8×10^{-5} M. This fraction was diluted to the appropriate concentrations as indicated in the text before use. Below is the fitting of OD's for free avidin and free xanthone at 10^{-4} M to the actual avidin-xanthone conjugate spectrum.

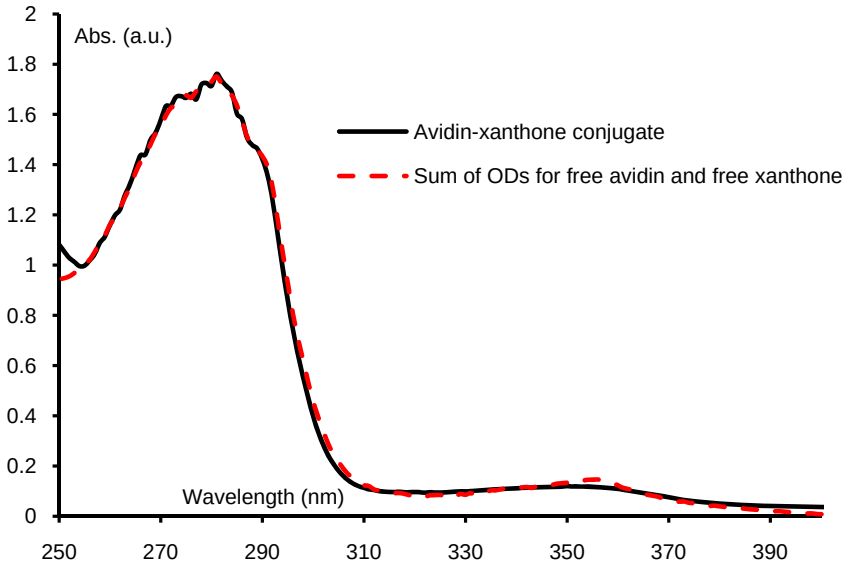


Figure S2.

Avidin-(S6) conjugation: Prepared with **S5**. Each avidin tetramer carries approximately 3 to 4 xanthone molecules. The concentration of xanthone was calculated to be 1.4×10^{-4} M, from this it was determined the approximate concentration of avidin was 8×10^{-5} M. This fraction was diluted to the appropriate concentrations

as indicated in the text before use. Below is the fitting of OD's for free avidin and free xanthone at 10^{-4} M to the actual avidin-xanthone conjugate spectrum.

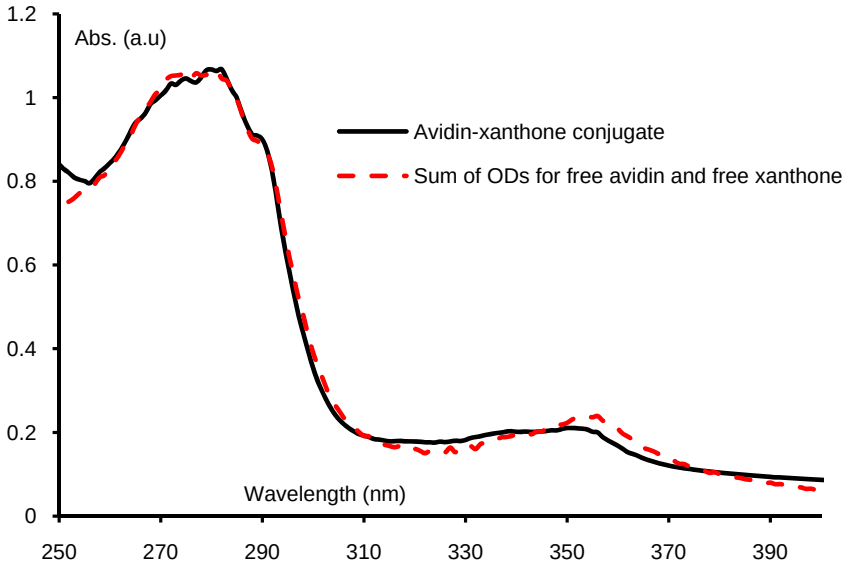


Figure S3.

Photobleaching of diethylaminocoumarin.

We hypothesize that the major channel for diethylaminocoumarin photobleaching with benzophenone is two oxidative de-ethylations, first into mono-ethylaminocoumarin, and then into unsubstituted aminocoumarin, which is quickly quenched or further oxidized. This is supported by NMR data which shows the formation of a new coumarin with only one ethylamine group by integration (see below).

Below we demonstrate that a diethylaminocoumarin (S9) is photo-de-ethylated by benzophenone into mono-ethylaminocoumarin (**S10**). We found that while **S10** is a fluorophore itself, it is efficiently photobleached by irradiation in the presence of benzophenone into a non-emitting state.

Our hypothesis is that coumarin-6 undergoes similar transformation, i.e. it is doubly de-ethylated upon irradiation in the presence of benzophenone and then is finally photobleached into a non-emitting state.

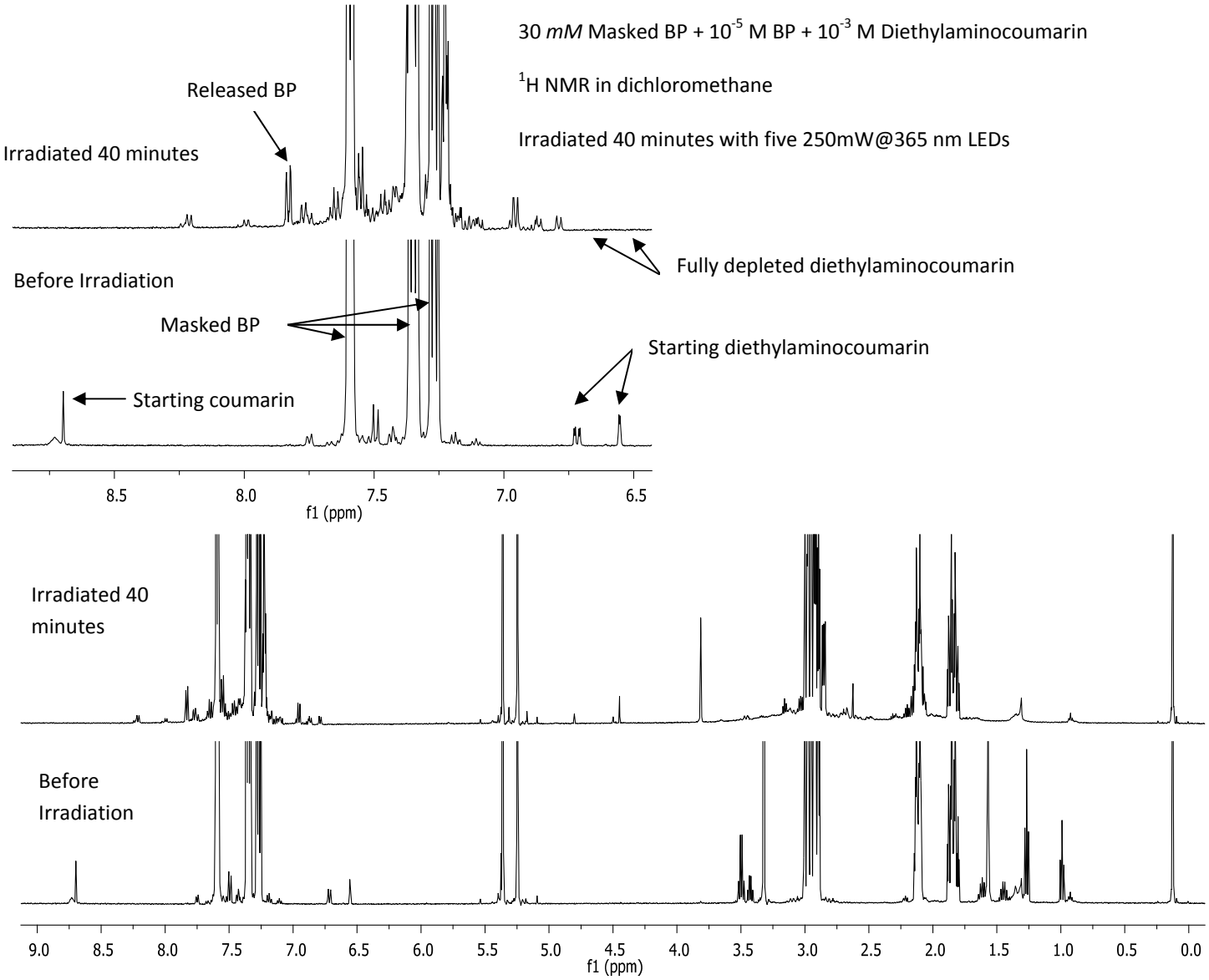
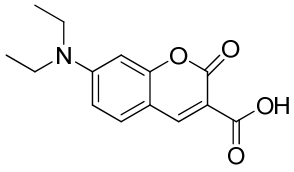
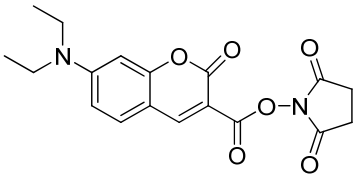


Figure S4. NMR monitoring of the amplification process with concomitant photochemical bleaching of the diethylaminocoumarin

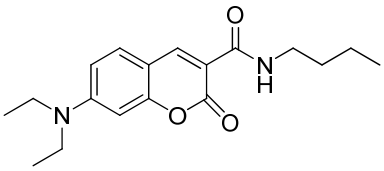


7-diethylaminocoumarin-3-carboxylic acid (S7): A mixture containing ethanol (20 mL), 4-diethylamino salicylaldehyde (5.0 g, 26 mmol), Meldrum's Acid (3.7 g, 26 mmol), piperidine (0.40 mL), and acetic acid (0.80 mL) was allowed to react at room temperature for 30 min and then refluxed for 4 hours with stirring. The mixture was cooled to room temperature and chilled in an ice bath for 1.5 h to precipitate the product, a bright orange solid. The solid was filtered and washed with ice cold ethanol to give pure coumarin (5.10 g, 75% yield).

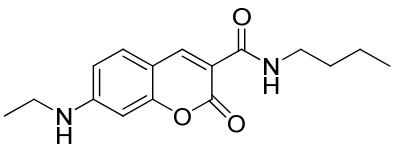
^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.66 (s, 1H), 7.44-7.46 (d, 1H, J=9.08 Hz), 6.69-6.72 (dd, 1H, J=2.48, 9.03 Hz), 6.53 (d, 1H, J=2.47 Hz), 3.46-3.51 (q, 4H, J=7.16, 14.30 Hz), 1.24-1.28 (t, 6H, J=7.15 Hz).



7-diethylaminocoumarin-3-carboxylic acid N-hydroxysuccinamide ester (S8): S7 (1.0 g, 3.8 mmol) was dissolved in dichloromethane (50 mL), N-hydroxysuccinimide (0.66 g, 5.7 mmol) and EDC (0.87 g, 4.6 mmol) were added. The reaction mixture was stirred overnight at room temperature. The solution was dilluted with water (30 mL), extracted with ethyl acetate (2 x 30 mL), washed with sodium bicarbonate solution (2x30 mL), and brine (30 mL). The organic layer was collected and evaporated under vacuum to give a bright yellow solid (1.41 g, 99% yield)**.** ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.58 (s, 1H), 7.36-7.38 (d, 1H, J=9.04 Hz), 6.62-6.64 (dd, 1H, J=2.5, 9.0Hz), 6.46 (d, 1H, J=2.42 Hz), 3.44-3.51 (q, 4H, J=7.14, 14.29 Hz), 2.88 (s, 4H), 1.55 (s, 4H), 1.24-1.28 (t, 6H, J=7.15 Hz).



N-butyl-7-diethylaminocoumarin-3-carboxamide (S9): **S8** (0.30 g, 0.84 mmol) was added to a solution of n-butylamine (0.11 mL, 1.1 mmol) and NEt_3 (0.15 mL) in dichloromethane (15 mL). The reaction was left overnight at room temperature before being poured into 5% aq. HCl (25 mL), extracted with dichloromethane (2 x 30 mL), and washed with saturated aq. $NaHCO_3$ (25 mL). The organic layer was collected, dried over anhydrous sodium sulfate, and removed under vacuum to give the product (0.20 g, 75% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.79-8.83 (t, 1H, J = 5.86 Hz), 8.73 (s, 1H), 7.45-7.46 (d, 1H, J = 8.95 Hz), 7.29 (s, 1H), 3.44-3.53 (m, 6H), 1.60-1.66 (m, 2H), 1.41-1.48 (dq, 2H, J = 7.31, 7.35, 14.66 Hz), 1.25-1.28 (t, 6H, J = 7.13 Hz), 0.96-0.99 (t, 3H, J = 7.36 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 163.20, 162.98, 157.63, 152.63, 148.14, 131.25, 110.78, 110.07, 108.61, 96.78, 45.25, 39.58, 31.85, 20.44, 14.00, 12.63.



N-butyl-7-ethylaminocoumarin-3-carboxamide (S10): Coumarin **S9** (0.30 g, 0.95 mmol) and benzophenone (0.364 g, 2.0 mmol) were dissolved in CH_2Cl_2 (150 mL) and then irradiated with stirring using 4 - 365nm LED's. The reaction mixture was reacted for 72 hours. The product was purified by column chromatography (gradient of methanol in DCM from 0 to 7%). 27 mg of the ethyl product was obtained. ^{1}H NMR (CD_3CN, 500 MHz): δ 8.65 (s, 1H), 8.63 (s, 1H), 7.46-7.48 (d, 1H, J = 8.68 Hz), 6.63-6.65 (d, 1H, J = 8.69 Hz), 6.45 (s, 1H), 3.33-3.37 (dd, 2H, J = 6.9, 12.9 Hz), 3.21-3.23 (m, 2H), 1.52-1.55 (m, 2H), 1.35-1.40 (dd, 2H, J = 7.5, 14.9 Hz), 1.21-1.24 (t, 3H, J = 7.2 Hz), 0.92-0.95 (t, 3H, J = 7.4 Hz).

Spectra Comparison for diethyl (S8) and mono-ethyl (S9) aminocoumarins:

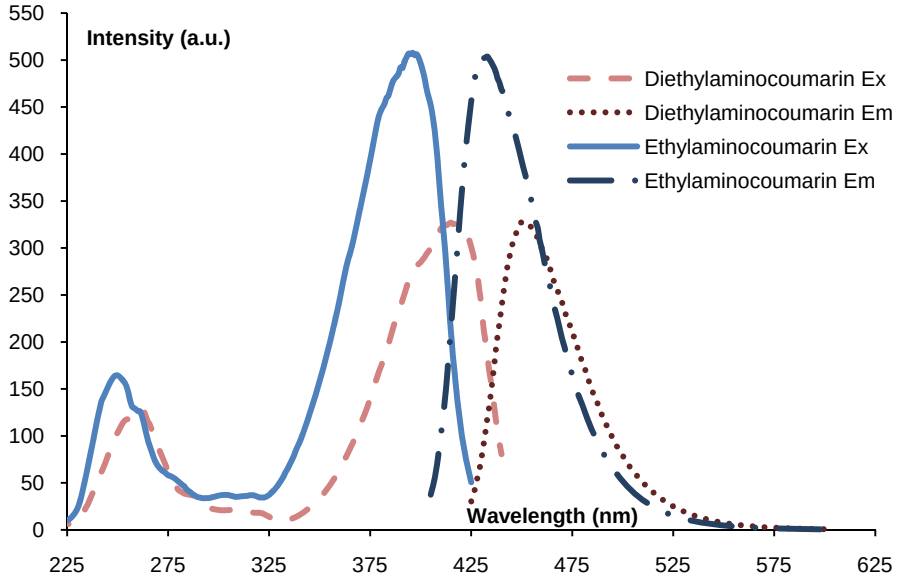


Figure S5.

Photoamplified Fluorescence Quenching in Bulk Solution

A 10^{-6} M solution of coumarin 6 in 1,4-dichlorobutane was prepared with 30 mM of 1,3-dithiane-benzophenone adduct (DT-BP Adduct) (10 mL). 0.5 mL of this solution was added to six 0.7 mL fluoresence cells to give samples A-F. BP was added to each sample as follows: (A) No BP, (B) 10^{-5} M BP, (C) 10^{-6} M BP, (D) 10^{-7} M BP, (E) 10^{-8} M BP, (F) 10^{-9} M BP. An initial FL spectrum was taken for each sample and then they were irradiated using five 365 nm LEDs outfitted with a 300-400 nm long pass filter. FL spectra were taken at various intervals and the data was analyzed to determine the results.

Biotin-Avidin Binding in Bulk Solution (Fig.3 of the main text)

To test initiation of photoamplified fluorescence quenching via biotin-avidin binding, and to determine if POPS works as a control lipid, the adduct was recrystallized 8 times in Hexane:DCM (3:1). A bulk solution of 10^{-5} M coumarin 6 and 30 mM DT-BP adduct was prepared in 1,4-dichlorobutane. The bulk solution was divided to create two 1 mL solutions. Too one solution was added $9.5x10^{-5}$ M biotin capped lipid and too the other the same concentration of POPS. 0.3 mL of each solution was added to two 0.7 mL FL cells giving four samples. 0.3 mL of **S6** (10^{-5} M) was added to one cell containing biotin and one containing POPS. 0.3 mL of 0.01 M PBS buffer pH 7.5 was added to the other two cells. An initial FL spectrum was taken for each sample and then they were irradiated using five 365 nm LEDs outfitted with a 300-400 nm long pass filter. FL spectra were

Biotin-Avidin Binding in Capillary Cassettes to Determine the Avidin Detection Limit

0.86 mm ID Capillary Cassettes: Sealed capillary reactor cassettes were prepared by drawing OD: 1.5 x ID: 0.86 mm capillary tubes from AM Systems glass to a 15 μm point on a Sutter Instrument Co. flaming brown micropipette puller model #P97 using a 3 mm box filament with program P=500 (Heat = 515, Vel = 20, Del = 1). The 15 μm end was then flame sealed for approximately 20 seconds and the capillaries were cut to 2.5 cm in length. 10 sealed capillaries were assembled around a center rod and then bound by heat shrink tubing to create a cassette. Each pore of the cassette was filled with the solution of interest by hand using a Hamilton 10 μL gas-tight syringe. The buffer was then added to the top of the solution by putting the tip of the syringe just below the solvent line and then injecting the buffer solution containing avidin.

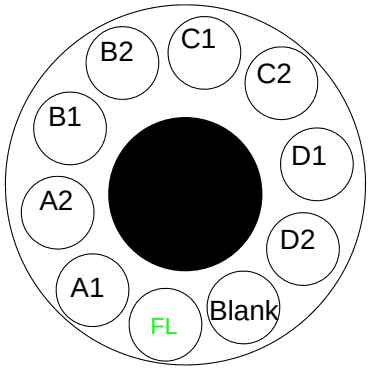
Figure S6.

0.4 mm ID Capillary Cassettes: Open capillary reactor cassettes were prepared by cutting OD: 0.6 x ID: 0.4 mm capillary tubes from AM Systems glass to 2 cm in length. 13 capillaries were assembled around a Steel 13T: RS4 Micro pinion gear from hobby products international using IC gel super glue to create a cassette.

Each pore of the cassette was filled first with the buffer containing the avidin-xanthone conjugate **S5** and then with the solution of interest, both by capillary forces.

To determine the incubation time and biotin capped lipid concentration necessary for biotin-avidin binding fluoresceine labeled avidin was purchased from Pierce and diluted to $3x10^{-8}$ M in DI water. Solutions of biotin capped lipid were prepared at $9.5x10^{-4}$ M, $9.5x10^{-5}$ M, $9.5x10^{-6}$ M, and $9.5x10^{-7}$ M in 1,4-dichlorobutane. The biotin lipid solution (5 μL) was added to the bottom of a 0.86 mm capillary tube and under 5 mL of the aqueous solution of avidin using a 10 mL Hamilton syringe. The capillary tube was imaged every 15 minutes over 24 hours to determine the optimal lipid concentration and incubation time for binding, which was $9.5x10^{-5}$ M biotin capped lipid incubated with avidin for 2-4 hours. We hypothesize that the necessary concentration and incubation time is likely due to the lipids solubility in 1,4-dichlorobutane.

The detection limit of avidin (**S6**) was determined by preparing two solutions: (1) contained Coumarin 6 (10^{-5} M), DT-BP adduct recrystallized 7 times in DCM:Hexane (1:3) (30 mM) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl)(sodium salt) (biotin capped lipid) in 1,4-dichlorobutane; (2) contained Coumarin 6 (10^{-5} M), DT-BP adduct recrystallized 7 times in DCM:Hexane (1:3) (30 mM) and L-α-phosphatidylcholine (POPS) in 1,4-dichlorobutane. Solution #2 is a control in which no biotin avidin binding can take place to initiate photoamplification. Solutions (1) and (2) were added to the 0.86 mm ID array (shown above) as described using a Hamilton syringe. The solutions were added in alternating order as pairs where each pair contained a different concentration of avidin-xanthone conjugate (example below, A1-2 contains 10^{-9} M avidin-xanthone conjugate, B1-2 contains 10^{-11} M avidin-xanthone conjugate). The results (main text, Figure 4A) show solution 1 on the left and solution 2 on the right. The results using 0.4 mm ID capillaries were obtained in an identical fashion using the described capillary array. Figure 4A&B are a compilation of our most representative results obtained from multiple experiments. The two adjacent capillaries containing (1) and (2) were taken from a larger image to represent each avidin-xanthone concentration in a complied image for ease of comparison in this publication. These results are compiled from several experiments run to determine the avidin detection limit for this assay.



1 = Biotin
2 = POPS
A,B,C,D have different avidin-xanthone conjugate concentrations (i.e. A=10^{-9} M, B= 10^{-11} M, etc.)
FL = C6
Blank = solvent

Figure S7.

Assay Control Experiment

To verify that the "positive hits" were in fact due to the binding of the biotin-avidin pair control experiments were run to show that non-specific binding was not the cause of photoamplified fluorescence quenching. In the control a lysozyme-xanthone conjugate was used in place of the avidin-xanthone conjugate. The lysozyme conjugate was prepared using the avidin-xanthone coupling procedure where lysozyme purchased from Sigma was used in place of avidin. The purified conjugate was generally found between fractions 3 and 8 containing the product with an average of 2 to 3 xanthone molecules per lysozyme. The Solver in Excel was used to carry out a least squares analysis as described for avidin. Lysozyme for each binding experiment was then diluted from these fractions.

Lysozyme-(S11) conjugation: Prepared with **S5**. Each lysozyme carries approximately 2 to 3 xanthone molecules. The concentration of xanthone was calculated to be 8.1×10^{-5} M, from this it was determined the approximate concentration of lysozyme was 8.8×10^{-6} M. This fraction was diluted to the appropriate concentrations as indicated in the text before use. Below is the fitting of OD's for free lysozyme and free xanthone at 10^{-4} M to the actual lysozyme-xanthone conjugate spectrum.

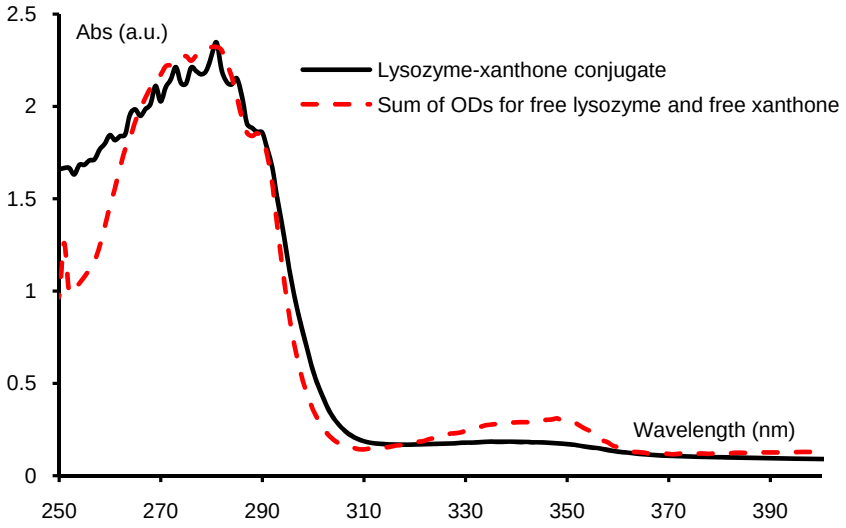


Figure S8.

To verify biotin-avidin binding initiated photoamplified fluorescence quenching, and that non specific binding of a protein to the lipid surface was not affecting the results of the assay a control experiment was run using a 0.86 mm ID capillary cassette and lysozume-xanthone conjugate **S11**. Two solutions were prepared: (1) contained Coumarin 6 (10^{-5} M), DT-BP adduct recrystallized 7 times in DCM:Hexane (1:3) (30 mM) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl)(sodium salt) (biotin capped lipid) in 1,4-dichlorobutane; (2) contained Coumarin 6 (10^{-5} M), DT-BP adduct recrystallized 7 times in DCM:Hexane (1:3) (30 mM) and L-α-phosphatidylcholine (POPS) in 1,4-dichlorobutane. Solution #2 is a control in which no biotin avidin binding can take place to initiate photoamplification. Solutions (1) and (2) were added to the 0.86 mm ID array (shown above) as described using a Hamilton syringe. Avidin, lysozyme, or a mixture of the two in 0.01 M PBS pH 7.5 was added as a concentration of 10^{-8} M and incubated before irradiation (A schematic of the actual cassette loading is shown below). An initial FL image was taken then the cassette was irradiated using 2-365 nm LEDs outfitted with a 300-400 nm long pass filter. Following irradiation a distinct difference in fluorescence is seen in those capillaries where biotin-avidin binding took place as compared to those where only lysozyme was present

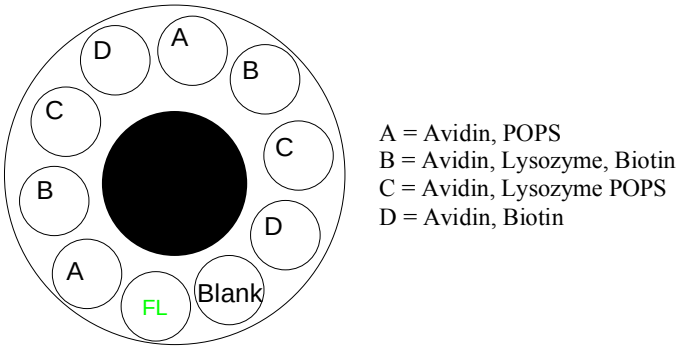


Figure S9.

Biotin-avidin binding control with lysozyme: - The figure below is representative of our results obtained from multiple experiments. Four adjacent capillaries two with (1) and two with (2) incubated with Avidin, lysozyme, or a mixture of the two in 0.01 M PBS pH 7.5, as indicated in the schematic diagram of cassette loading, were taken from a larger image for ease of comparison in this publication.

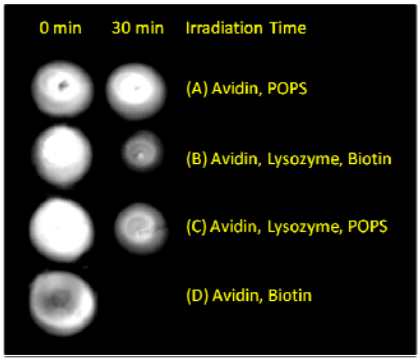


Figure S10.

Camera Phone Imaging

The imaging of the capillaries prior to the amplified fluorescence turn-off assay can be realized with such low sensitivity devices and cell phone cameras. A standard, off the shelf Motorola Droid with a 5.0 MP Bayer coated (Color) CMOS sensor was used for imaging. The contrast ratio was achieved by setting the phone into "night time" mode and the focus set to macro mode. To determine approximate detection limit for C6 (i.e. the capability to confidently image/detect the pre-amplification spot), both 0.86 and 0.4 mm ID capillaries were filled with decreasing volumes of 10^{-5} M C6 in 1,4-dichlorobutane: (i) 0.86 mm capillary with 4 μL of the fluorophore solution; (ii) 0.4 mm capillary with 0.4 μL fluorophore solution; (iii) 0.4 mm capillary with 0.1μL fluorophore solution.

The images were acquired through a 550±25 nm bandpass filter by simply illuminating the capillaries with a 400nm (12mW) excitation LED from the side as shown in Figure SX. A control, with one capillary filled with the solution of the fluorophore and another – with blank solvent, showed no image of the blank solvent solution, indicating that in this simplified design scattering is not an issue and the dichroic mirror is not required.

The results (Figure 4C in the text) show that the cell phone camera can image as little as 1 pmole of fluorophore in 0.1μL volume.

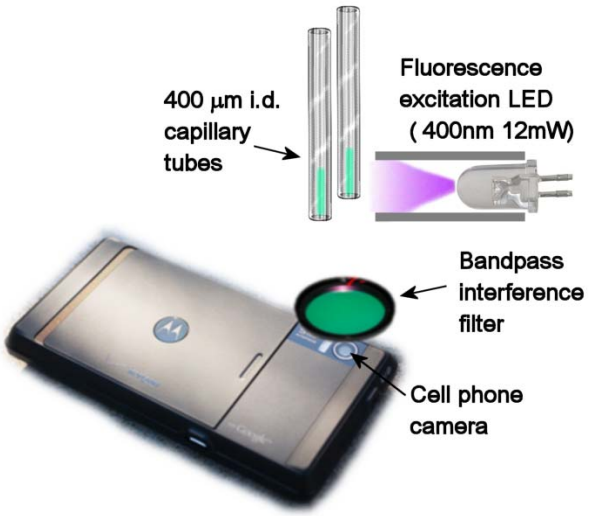


Figure S11. Schematic representation of the set up for cell phone imaging.

With the interference filter and added single plano-convex lens (f25mm) the following images of .4mm capillaries loaded with 0.6μL (left) and 0.2μL (right) of the fluorophore solution were obtained *with an old 2 megapixel cell phone camera.*

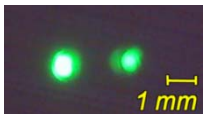
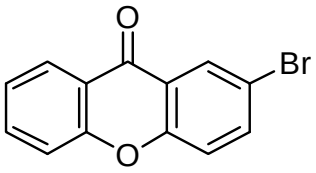


Figure S12. 0.4 mm capillaries with 0.6μL (left) and 0.4μL (right) of 10^{-5} *M* C6 imaged with a motorolla 2 megapixel camera

References

S1. Gustafson, T. P.; Kurchan, A. N.; Kutateladze, A. G., Externally sensitized mesolytic fragmentations in dithiane-ketone adducts. *Tetrahedron* **2006**, *62*, (27), 6574-6580.
S2. Zhao, J.; Larock, R. C., Synthesis of Xanthones, Thioxanthones, and Acridones by the Coupling of Arynes and Substituted Benzoates. *The Journal of Organic Chemistry* **2006**, *72*, (2), 583-588.

NMR SPECTRA



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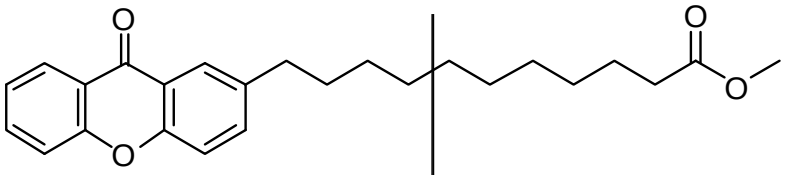

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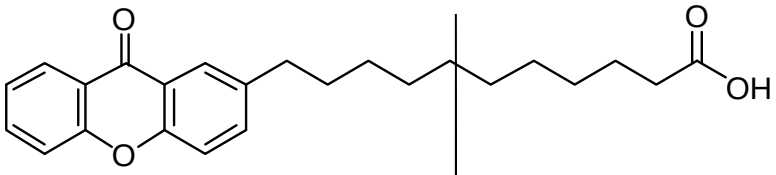

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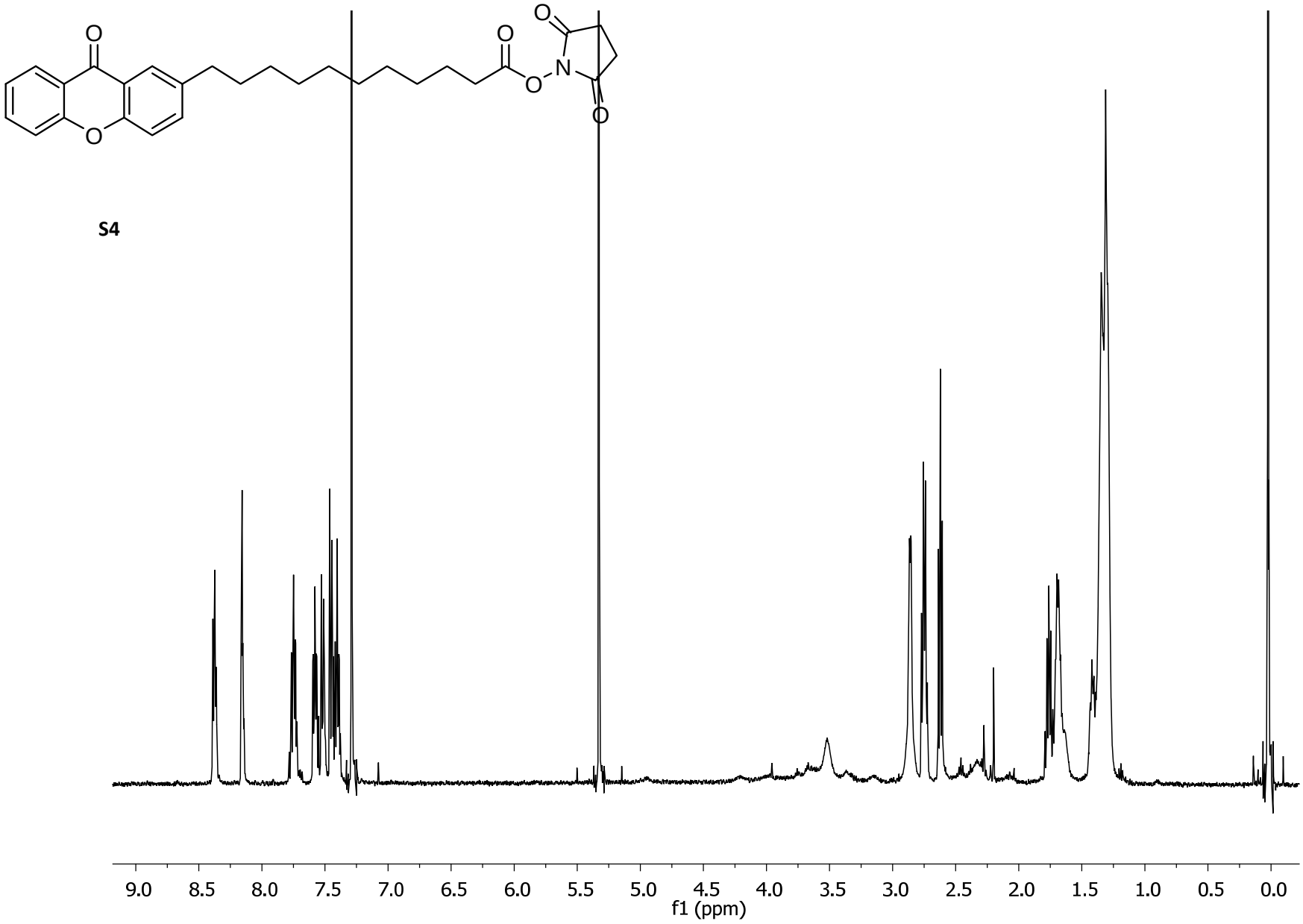

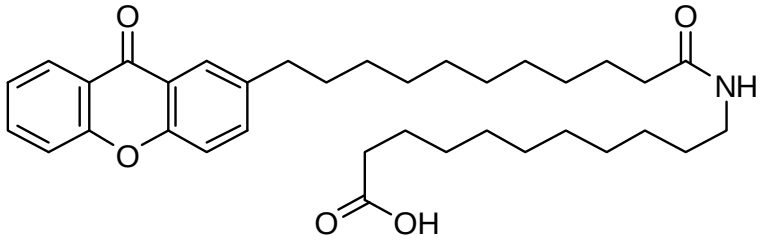

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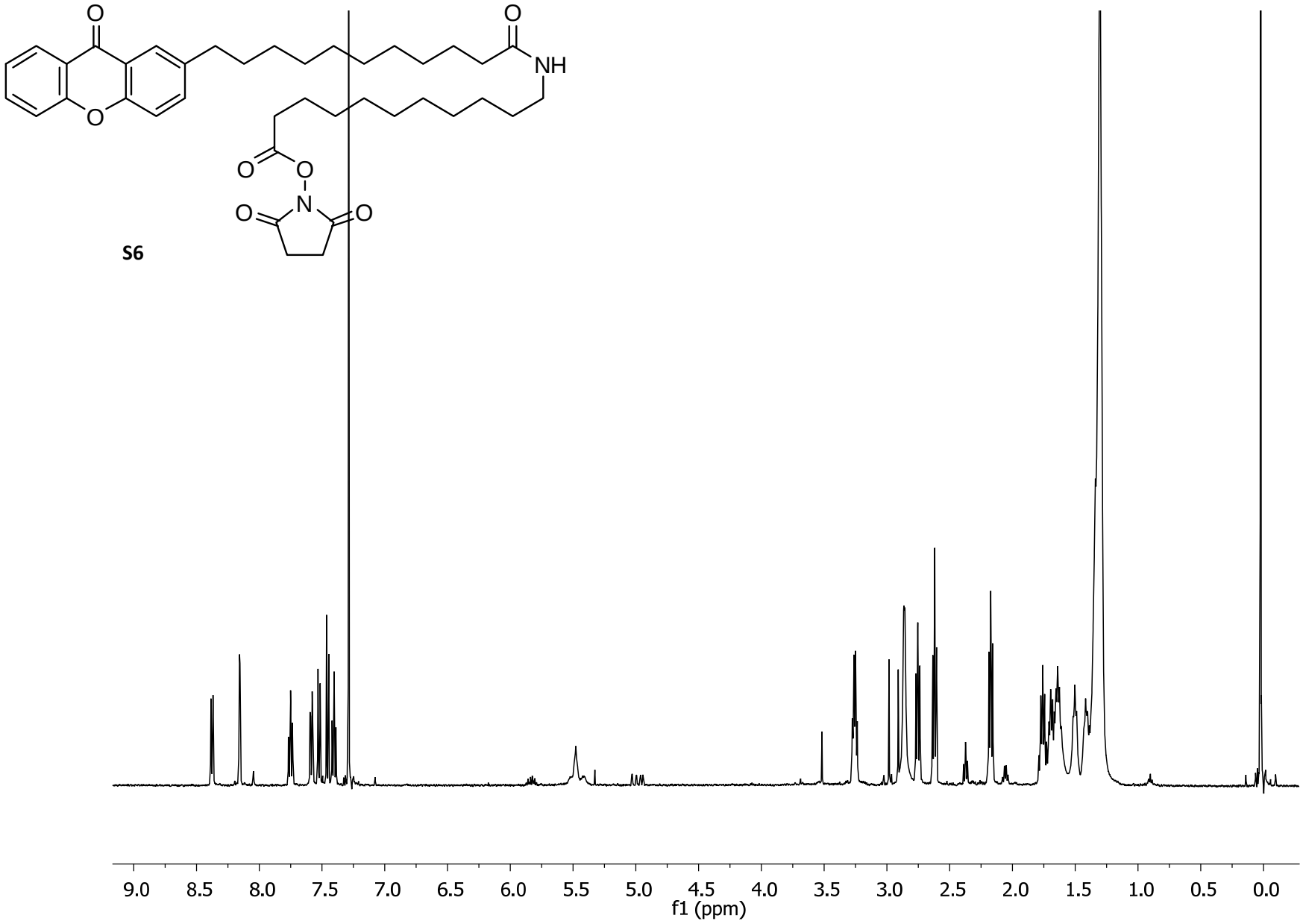

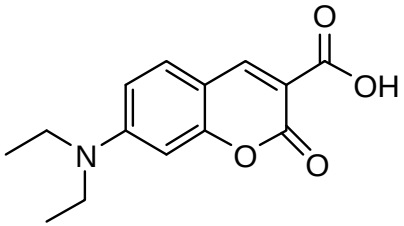

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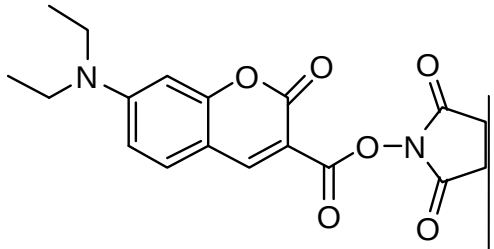

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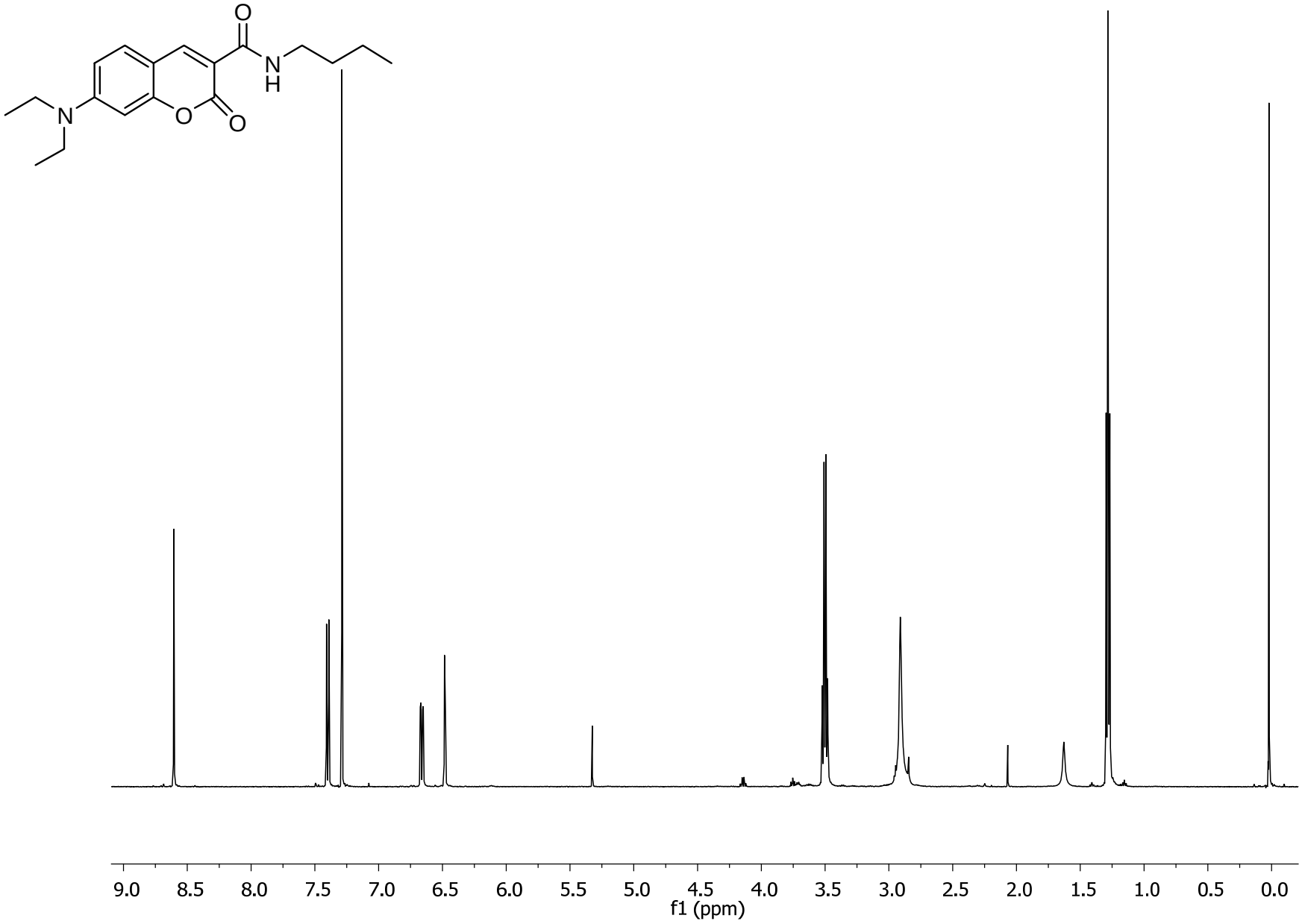

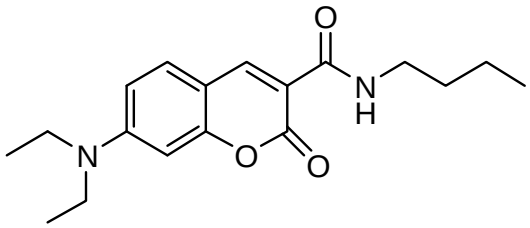

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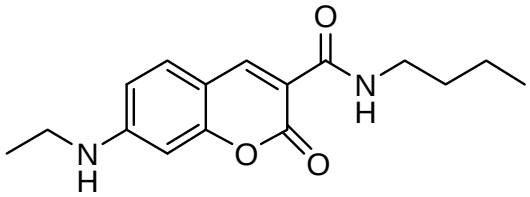

S9