SUPPORTING INFORMATION

Facile functionalization of a fully fluorescent perfluorophenyl BODIPY: Photostable thiol and amine conjugates

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Materials & methods

Reagent-grade tetrahydrofuran (THF) and diethyl ether were predried with Na foil and distilled from sodium–benzophenone under argon immediately before use. All other chemicals were used as received. The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 25 °C with Bruker AC 250 FT, AC 200 FT and Avance II 400 MHz spectrometers at CESAMO (Bordeaux, France). All chemical shifts are referenced to Me_4Si (TMS). Mass spectra were performed at the CESAMO with a QStar Elite mass spectrometer (Applied Biosystems). The instrument was equipped with an ESI source and spectra were recorded in the positive mode. The electrospray needle was maintained at 4500 V and operated at room temperature. Samples were introduced by injection through a 10 μL sample loop into a 200 $\mu L min^{-1}$ flow of methanol from the LC pump. Electronic absorption spectra were recorded with a Varian Cary 5000 spectrophotometer. Steady-state emission spectra were recorded with a Horiba Jobin-Yvon Fluorolog-3 fluorimeter fitted with Hamamatsu R928P, R2658P and H10330-45 detectors and exciting with a 450W Xe-lamp across a double monochromator, and were corrected for instrumental function. Luminescence decays were recorded in single-photon counting mode on the Fluorolog-3, with high frequency pulsed IBH nanoLEDs (λ_{exc} = 460 nm, FWHM = ca. 1.2 ns) as the excitation source. The luminescence quantum yield (Φ) was calculated by using the equation $\Phi = \Phi_r(I/I_r)(A_r/A)(\eta^2/\eta_r^2)$ in which Φ_r refers to the quantum yield reference, I is the integrated emission intensity, A is the absorbance at the excitation wavelength and η is the refractive index of the solvent. An optically dilute solution

fluorescein was used as the standard, $\Phi_f = 0.95$ in 0.1 M NaOH (aq); Brannon, J. H.; Magde, D. *J. Phys. Chem.* **1978**, *82*, 705-709.

Transient absorption/time-resolved fluorescence set-up was built as follows. A frequency tripled Nd:YAG amplified laser system (30 ps, 30 mJ @1064 nm, 20 Hz, Ekspla model PL 2143) output was used to pump an optical parametric generator (Ekspla model PG 401) producing tunable excitation pulses in the range 410 – 2300 nm. The residual of fundamental laser radiation was focused in a high pressure Xe filled breakdown cell where a white light pulse for sample probing was produced. All light signals were analyzed by spectrograph (Princeton Instruments Acton model SP2300) coupled with a high dynamic range streak camera (Hamamatsu C7700). Accumulated sequences (sample emission, probe without and with excitation) of pulses were recorded and treated by HPDTA (Hamamatsu) software to produce two dimensional maps (wavelength vs delay) of transient absorption intensity in the range 300 – 800 nm. Typical measurement error was better than 10^{-3} OD.

Synthesis of 1

F5 BODIPY (1).

In a round bottom flask perfluorobenzaldehyde (0.5 g, 2.5 mmol) was dissolved in freshly distilled dichloromethane (35 mL). 2,4-dimethylpyrrole (0.8 mL, 7.8 mmol) and a drop of TFA were added and the mixture was stirred for 4h. *p*-Chloranil (0.62 g, 2.5 mmol) in solution in CH_2Cl_2 (20 mL) was added dropwise and the mixture was stirred for 1h at rt. Then triethylamine (5.0 mL) and borontrifluoride etherate (5.0 mL) were added and the mixture was stirred at rt two additional hours. Water was added and the product extracted with CH_2Cl_2, the organic phase was dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by chromatography (silica gel Petroleum Ether / EtOAc, 9:1, *v*/*v*) followed by recrystallisation from chlorform, then toluene to give **1** as an orange solid. (347 mg, 0.83 mmol, 33 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (s, 2H), 2.57 (s, 6H), 1.62 (s, 6H). ^{13}C NMR (75 MHz) δ 157.81, 141.70, 141.53, 131.00, 122.77, 122.29, 14.78, 13.58. ^{19}F-NMR (376 MHz, CD_3CN) δ -161.93 (m, 2F), -153.04 (t, 1F, J = 20.2 Hz), -146.69 (q, 2F, J = 32.1 Hz), -143.53 (dd, 2F, J = 22.0 Hz, J = 7,6 Hz). MS: (ESI) *m/z* 437 $[M+Na]^+$; HRMS (ESI): Calculated ($C_{19}H_{14}BF_7N_2Na$) 437.1034 Found: 437.1046.

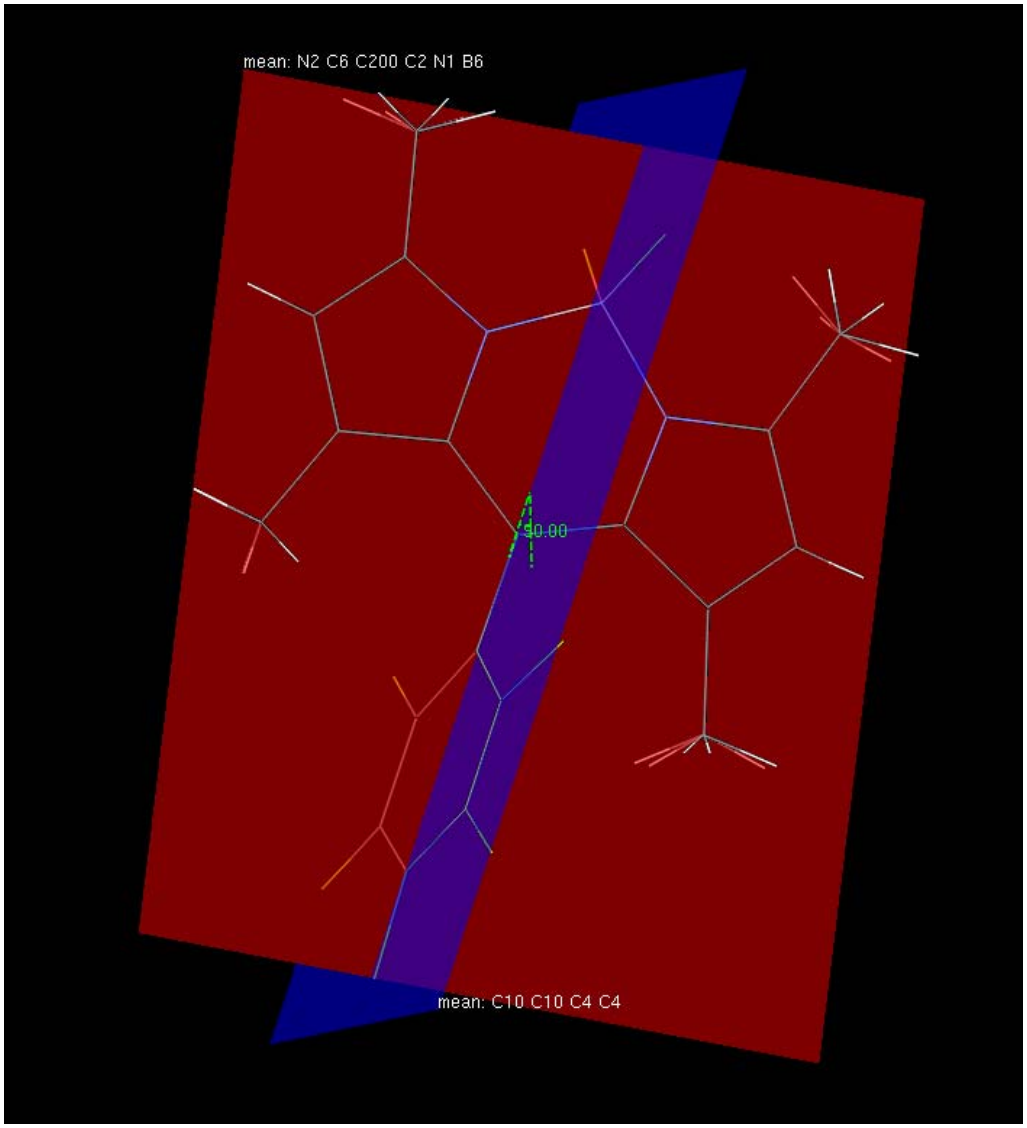


Figure S1 X-ray crystal structure of **1** showing orthogonality of molecular planes (90.0°).

Coupling reactions

Coupling procedure for Thiols (2)

1 (10 mg, 24 µmol) was dissolved in DMF (1mL) and ethanethiol (10 µl, 6 eq.) and K_2CO_3 (2 mg, 15 µmol) were added. The reaction was followed by means of normal phase HPLC using a Kromasil SI-100 column (250mm x 4,6mm, 5µm). Water was added and the aqueous phase extracted with Et_2O. The organic phase was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure yielding crude **2** (9 mg). ^{19}F-NMR was recorded with the crude product. The rest of the sample was dissolved in a mixture of Cyclohexane / Ethylacetate (95/5; *v*/*v*) and passed through a short SiO_2 plug to obtain pure **2**. ^{1}H-NMR (300

MHz, CD_3CN) δ 6.21 (s, 2H), 3,08 (q, J = 7.3 Hz, 2H), 2.54 (s, 6H), 1.68 (s, 6H), 1.26 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz) δ 206.73, 123.40, 122.61, 78.52, 78.19, 77.87, 30.13, 28.68, 14.57, 14.18, 13.04. ^{19}F-NMR (376 MHz, CD_3CN) δ -134.13 (m, 2F), -143.65 (m, 2F), -147.69 (q, 2F, J = 32.0 Hz). MS: (ESI) *m/z* 457.1 $[M+H]^+$ 479.1 $[M+Na]^+$; HRMS (ESI) Calculated ($C_{21}H_{19}B\ N_2F_6SNa$): 479.1158; found: 479.1179

Coupling procedure for primary amines (3)

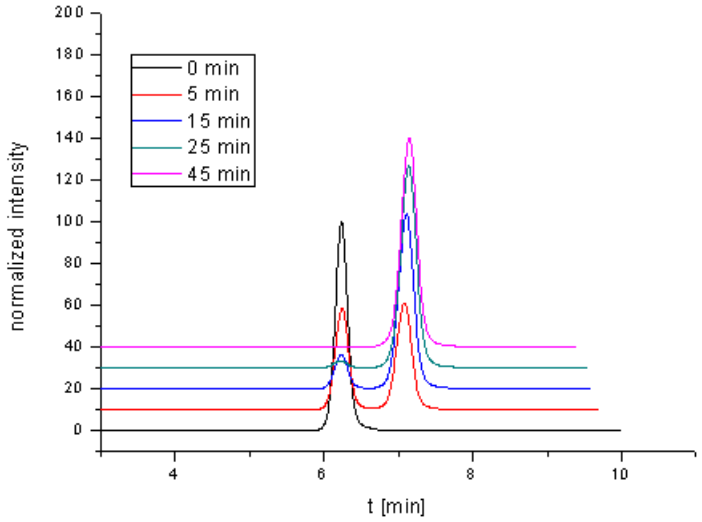
1 (10 mg, 24 µmol) was dissolved in DMF (1 mL) and Bu-NH (20 µl, 8eq.) were added. The reaction was followed by means of normal phase HPLC using a Kromasil SI-100 column (250mm x 4,6mm, 5µm). Water was added and the aqueous phase extracted with Et_2O. The organic phase was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure yielding crude **4** (10 mg). ^{19}F-NMR was recorded using the crude product. The rest of the sample was dissolved in a mixture of Cyclohexane / Ethylacetate (95/5; *v*/*v*) and passed through a short SiO_2 plug to obtain pure **4**. ^{1}H-NMR (300 MHz, CD_3CN) δ 6.20 (s, 2H), 4.95 (s, br, 1H), 3.43-3.51 (m, 2H), 2.53 (s, 6H), 1.73 (s, 6H), 1.58-1.68 (m, 2H), 1.36-1.49 (m, 2H), 0.97 (t, J = 7.3, 3H). ^{13}C NMR (75 MHz) δ 193.54, 157.33, 143.12, 122.17, 78.49, 78.16, 77.83, 44.96, 32.54, 19.72, 14.11, 13.32, 13.03. ^{19}F-NMR (376 MHz, CD_3CN) δ -146.74 (q, 2F, J = 32.3 Hz), -147,60 (m, 2F), -161,61 (m, 2F). MS (Positive Ion mode): MS: (ESI) *m/z* 468.2 $[M+H]^+$, 490.2 $[M+Na]^+$. HRMS (ESI) Calculated ($C_{23}H_{24}B\ N_3F_6Na$): 490.1859; found: 490.1860

Coupling procedure for secondary amines (4)

In a schlenk tube under nitrogen, **1** (10 mg) was dissolved in DMF (1 mL) and Et_2NH (0.1 mL) was added. The mixture was heated at 120°C overnight. Water was added and the crude product was extracted with Et_2O and further washed with water. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to yield the crude product (6 mg). The crude sample was dissolved in a mixture of Cyclohexane / Ethylacetate (95/5; *v*/*v*) and passed through a short SiO_2 plug to obtain pure **5**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.04 (s, 2H), 4.33 (q, J = 7.1 Hz, 4H), 2.56 (s, 6H), 1.65 (s, 6H), 1.12 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz) δ 206.12, 123.40, 194.08, 155.78, 30.13, 26.85, 12.92, 12.72. ^{19}F-NMR (376 MHz, CD_3CN) δ -143 (m, 2F), -147 (m, 2F), -153 (m, 2F).). MS: (ESI) *m/z* 490.2 $[M+Na]^+$. HRMS (ESI) Calculated ($C_{23}H_{24}B\ N_3F_6Na$): 490.1859; found: 490.1854.

HPLC analysis of coupling reactions:-



Figures S2 HPLC analysis of **2** formation using an isocratic solvent mixture Cyclohexane / Ethylacetate (96/4; *v*/*v*). Absorption measured at 519 nm.

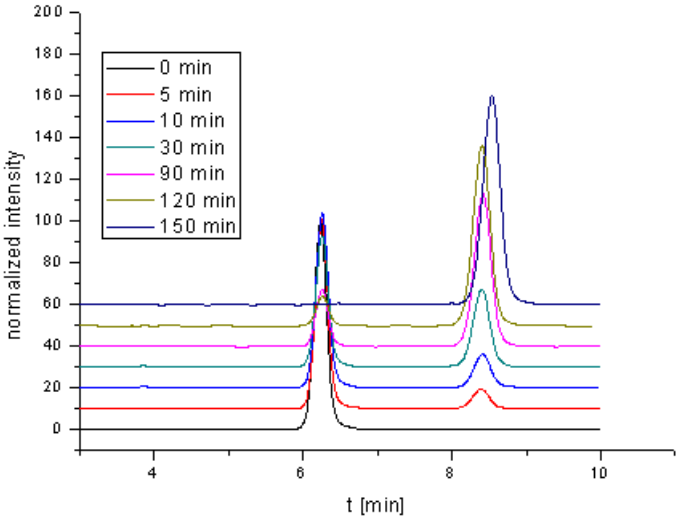


Figure S3 HPLC analysis of **3** formation using an isocratic gradient Cyclohexane / Ethylacetate (96/4; *v*/*v*). Absorption measured at 519 nm.

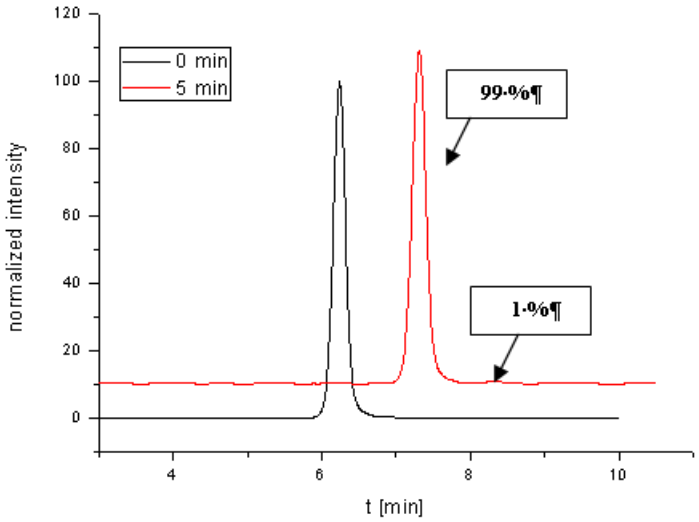


Figure S4 HPLC analysis of BODIPY **1** (black trace) leading to **2** (99 %) and **3** (1 %) upon addition of 5 eq. EtSH and 8 eq. $BuNH_2$ using an isocratic gradient Cyclohexane / Ethylacetate (96/4; *v*/*v*). Absorption measured at 519 nm.

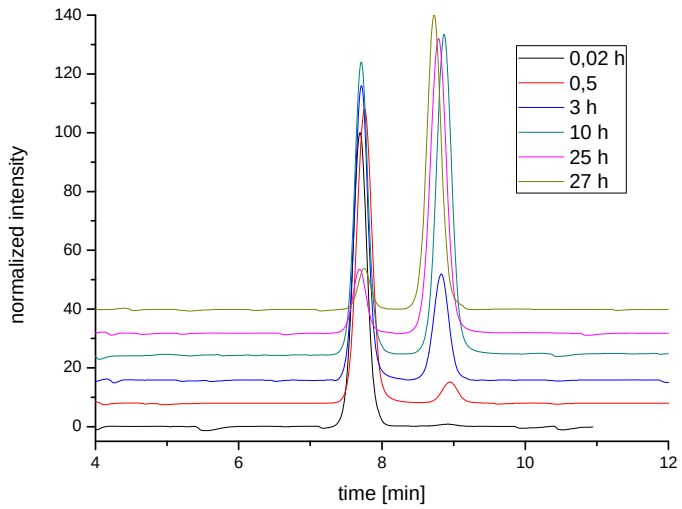


Figure S5 HPLC analysis of BODIPY **1** (retention time 7.7 min) leading to **2** (retention time 8.8 min), of aliquots from reaction of **1** (24 µmol) with EtSH (6eq.) and $BuNH_2$ (8 eq.) in 20mL H_2O/DMF (1:1, *v*/*v*), using an isocratic gradient Cyclohexane / Ethylacetate (96/4; *v*/*v*). Absorption measured at 519 nm.

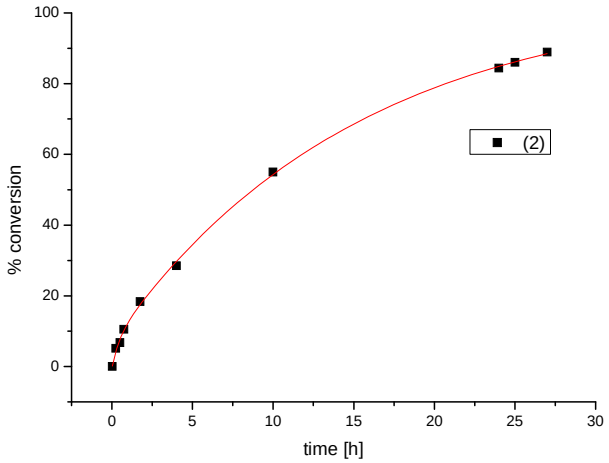


Figure S6 Progression of reaction forming **2** as a function of time (see figure S5 legend for details) in aqueous mixture.

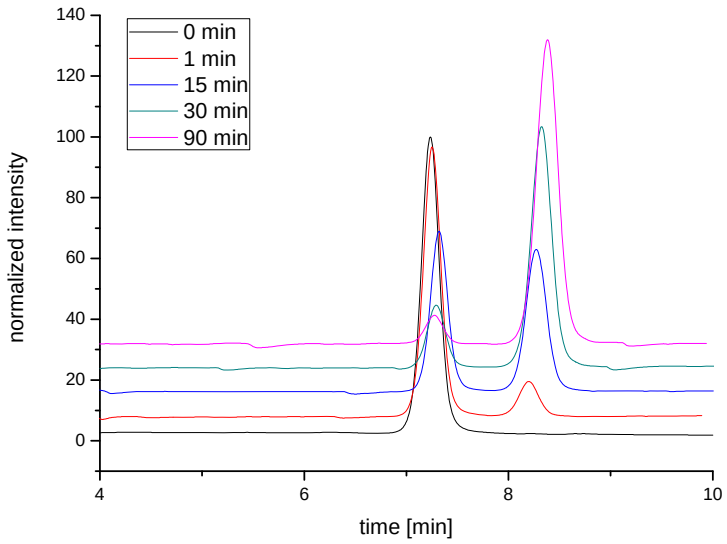


Figure S7 HPLC analysis of BODIPY **1** (retention time 7.7 min) leading to **2** (retention time 8.8 min), of aliquots from reaction of **1** (24 µmol) with EtSH (6eq.) and K_2CO_3 (30 µmol) in 20 mL H_2O/DMF (1:1, *v*/*v*), using an isocratic gradient Cyclohexane / Ethylacetate (96/4; *v*/*v*). Absorption measured at 519 nm.

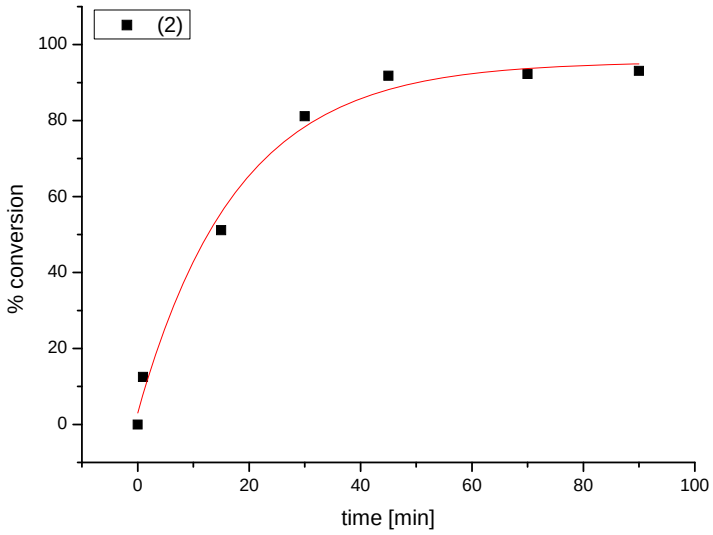


Figure S8 Progression of reaction forming **2** as a function of time (see figure S7 legend for details) in aqueous mixture..

^{19}F-NMR Spectra of 1, 2 & 3:

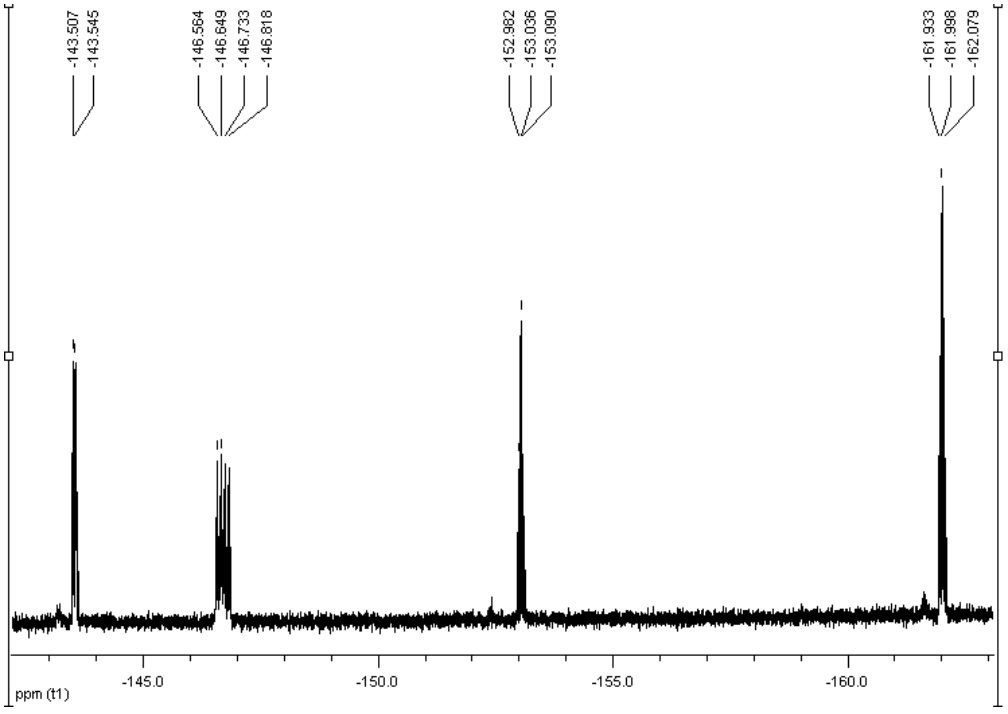


Figure S9 ^{19}F NMR spectrum of **1** in CD_3CN.

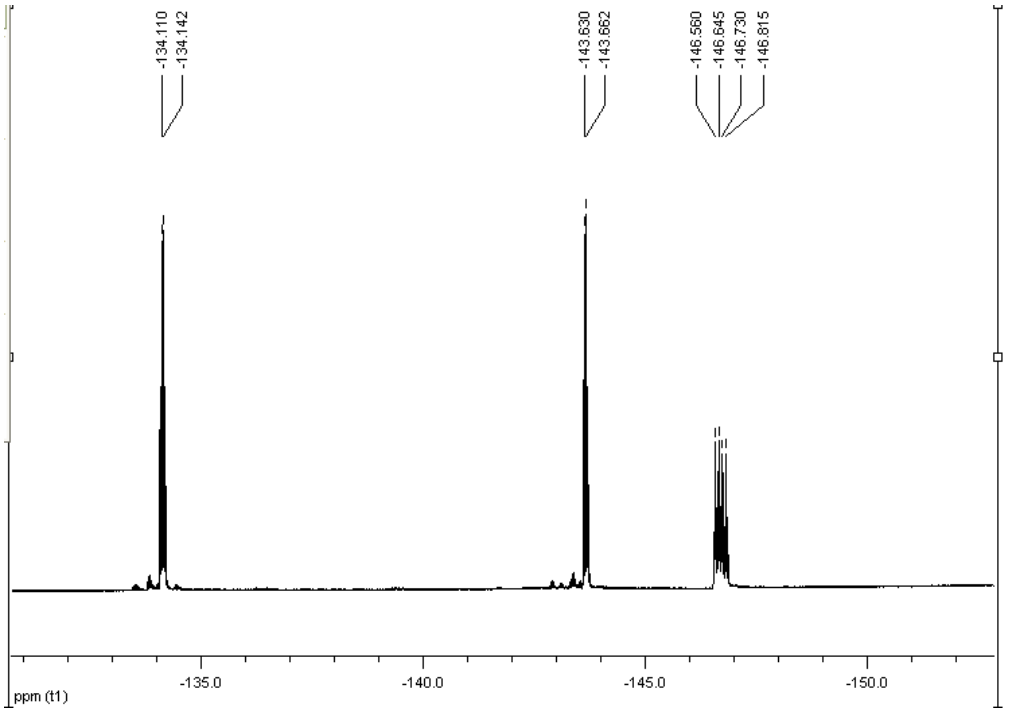


Figure S10 ^{19}F NMR spectrum of **2** in CD_3CN.

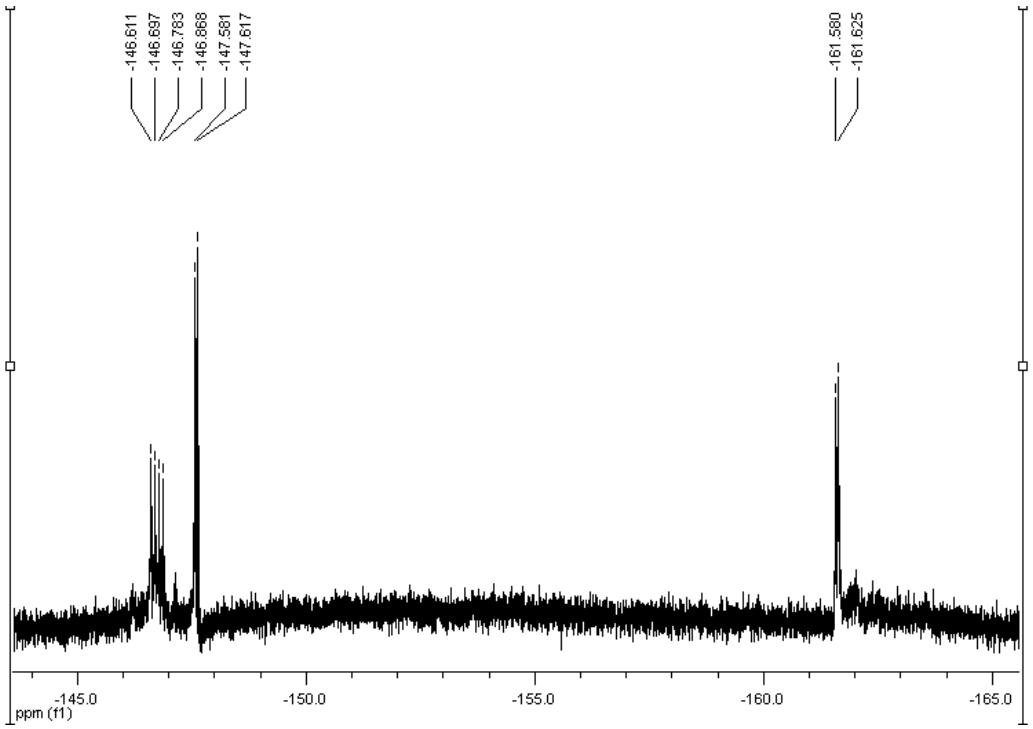


Figure S11 ^{19}F NMR spectrum of **3** in CD_3CN.

Electronic absorption spectroscopy, fluorescence emission and transient absorption spectra:

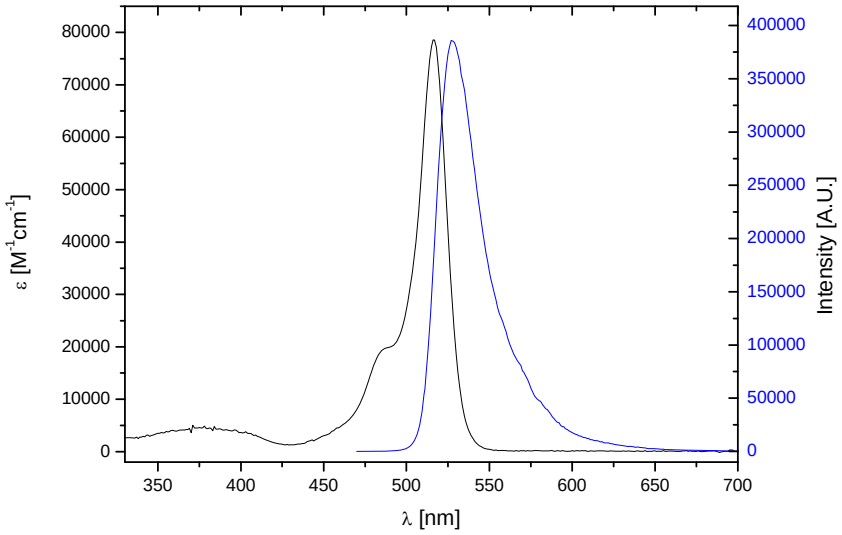


Figure S12 Electronic absorption spectrum and fluorescence emission spectrum of **1** in THF. λ_{ex} = 460 nm.

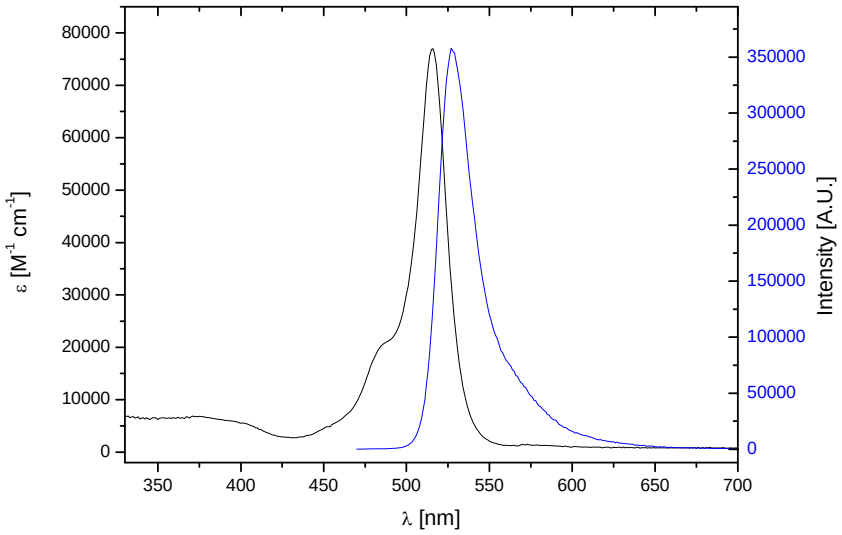


Figure S13 Electronic absorption spectrum and fluorescence emission spectrum of **2** in THF. λ_{ex} = 460 nm.

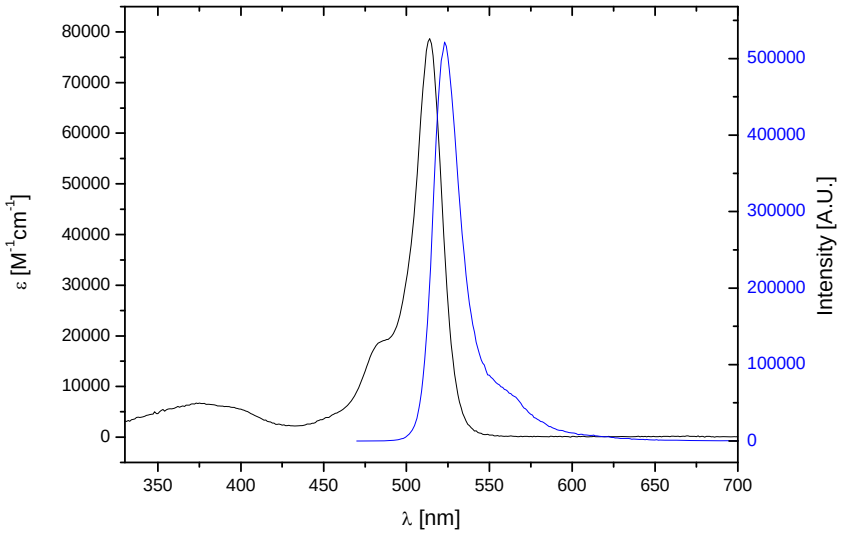


Figure S14 Electronic absorption spectrum and fluorescence emission spectrum of **3** in THF. λ_{ex} = 460 nm.

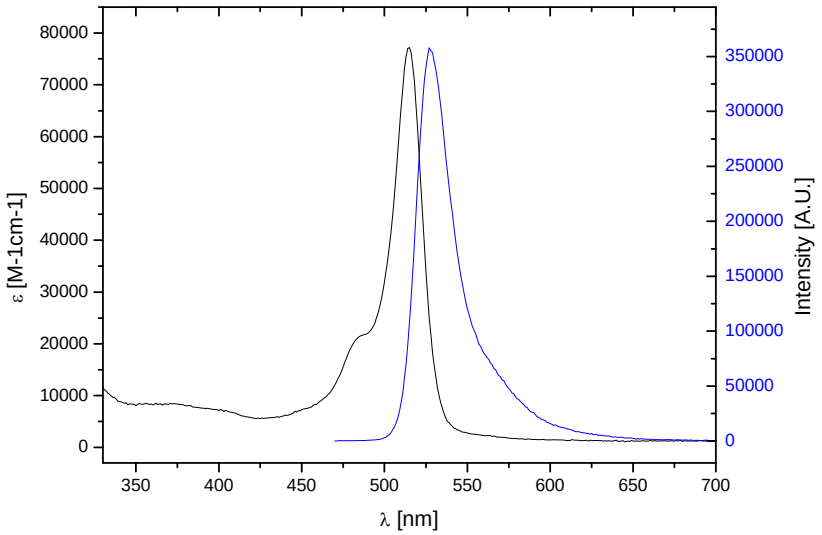


Figure S15 Electronic absorption spectrum and fluorescence emission spectrum of **4** in THF. λ_{ex} = 460 nm.

Strickler-Berg analysis:

The strongly allowed nature of the $S_1 \leftarrow S_0$ transition, as denoted by the strong molar extinction coefficients allows estimation of the excited-state lifetime using a Strickler-Berg analysis, equation 1, where n is the refractive index of the solvent (assumed to be the same in all spectral regions), $\langle \nu_F^{-3} \rangle$ is the reciprocal of the mean value of ν^{-3} across the fluorescence spectrum, ε is the molar absorptivity, allows estimation of the natural lifetime (τ_{rad}).[1] Substituting the reciprocal of the natural lifetime (k_r) into eq. 2 gives estimated fluorescence lifetimes on the nanosecond scale which are in good agreement with the measured values (see table 1). This suggests that the excited parent and derivatives are all structurally reminiscent to that of the respective ground-state species and that solvent interactions are unchanging.

$$\frac{1}{\tau_{rad}} = 2.88 \times 10^{-9} n^2 \left\langle \nu_F^{-3} \right\rangle^{-1} \int \frac{\varepsilon(\nu)}{\nu} d\nu \qquad \text{eq. 1}$$

$$k_r = \frac{\phi_f}{\tau_0} \qquad \text{eq. 2}$$

1 a) S. J. Strickler, R. A. Berg, J. Chem. Phys. 1962, 37, 814; b) J. B. Birks, Photophysics of aromatic molecules, Wiley Interscience, London, 1970.

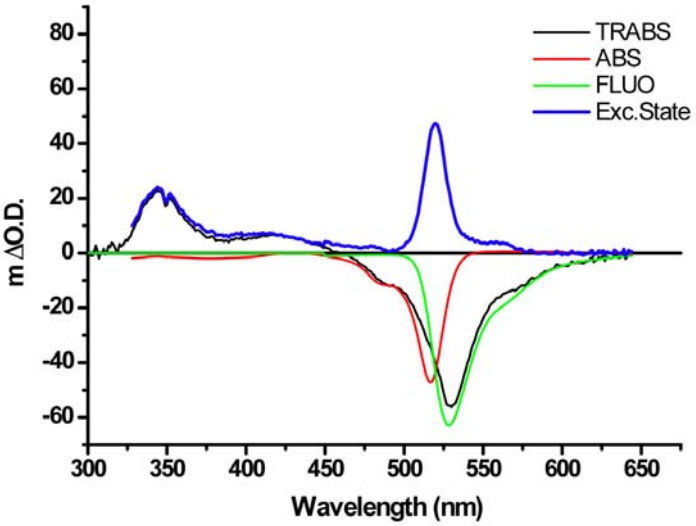
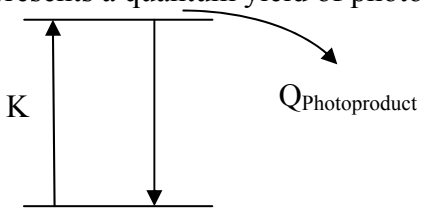


Figure S16 Measured transient absorption spectrum (TRABS, black) in THF. Steady-state ground state absorption (red) and steady state fluorescence (green). The excited state spectrum of **1** (blue) was obtained on subtracting absorption, fluorescence/stimulated emission contributions from TRABS . λ_{exc} = 510 nm.

Photostability experiments:-

The reaction scheme to study the photodegradation is presented below. We suppose that all photoexcited states relax *in fine* to the ground state except a part denoted $Q_{photoproduct}$ which represents a quantum yield of photodegradation or other irreversible photoreaction:



The Stark-Einstein law, states that for each photon of light absorbed by a chemical system, only one molecule is activated for subsequent reaction. According to this "photoequivalence law", equation 1 can be written. Number of excited molecules N_{exc} is equal to number of absorbed photons K_{abs} :

$K_{abs}= K(1-10^{-\varepsilon c}{}_0)=N_{exc}$ (1)

K is incident photon number. It can be found from

$$K = \frac{Wt}{h\upsilon}, \quad (2)$$

where W.t (power by time, Watts by second) multiplication gives the energy dose (Joules) of radiation. If we divide it to one photon energy, result is the number of photons.

According to our photoreaction scheme, some molecules undergo an irreversible photoreaction (photodegradation) with a quantum yield of Q. This photoreaction yields that number of molecules reaching the ground state after being excited can be find as follows:

$$N_{relax} = N_{exc}(1 - Q) \quad (3)$$

or

Nrelax-Nexc=-Nexc.Q . (4)

According to the equation 4, the changes of the ground state population in time can be written as

dNg/dt=-dNexc/dt.Q (5).

Substituting equation 1 into equation 4 and having in mind the photon flux (equation 2), one obtains for slowly varying steady state absorption (1-10^(- $N_G\varepsilon/V$))

$$\frac{dN_G}{dt} = -K\left(1 - 10^{-\frac{N_G\varepsilon}{V}}\right)Q = -\frac{W}{h\upsilon}\left(1 - 10^{-\frac{N_G\varepsilon}{V}}\right)Q \quad (6)$$

Initial condition is N_G=N at the beginning of the experiment (t=0), and $c_0 = \frac{\varepsilon}{V}$ for 1cm cell

If the absorption ($N_G\varepsilon/V$) of initial solution is small, we can develop the exponent into Taylor series

$$10^{ax} = e^{xa\ln 10} = 1 + xa\ln 10 + \ldots \quad (7)$$

Taking the first term from the series 7, the differential equation 6 becomes

$$\frac{dN_G}{dt} = -\frac{W}{h\upsilon}(1-(1-\frac{N_G\varepsilon}{V}\ln 10))Q = -\frac{W\varepsilon\ln 10Q}{h\upsilon V}N_G \quad (8)$$

The solution of the equation 8 is evident

$$N_G = \exp(-\frac{W\varepsilon Q\ln 10}{h\upsilon V}t) \quad (9)$$

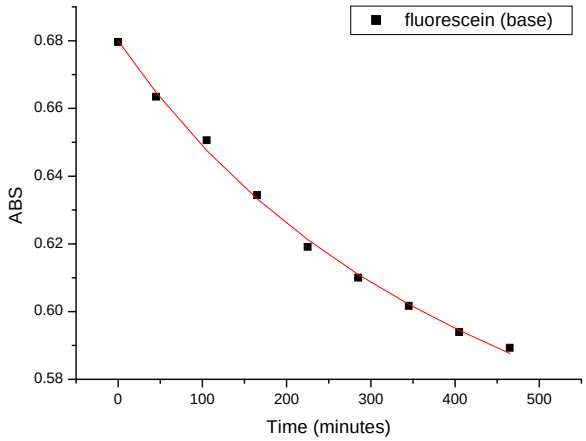
It corresponds to the exponential relaxation of ground state population (and accordingly steady state absorption) with a time constant

$$\tau = \frac{h\upsilon V}{W\varepsilon Q \ln 10} \quad (10)$$

Thus, the quantum yield of irreversible photoreaction (photodegradation) can be found from the exponential kinetic of steady state absorption decay upon irradiation as follows:

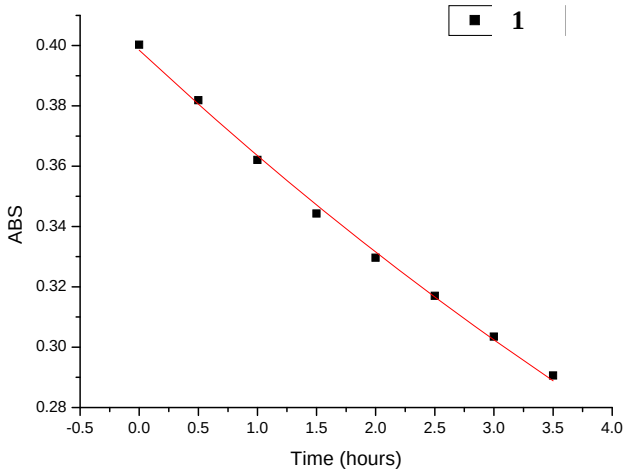
$$Q = \frac{h\upsilon V}{W\varepsilon \ln 10\tau} \quad (11)$$

(In the conditions of real experiment, it is necessary to account for the reflection losses on the input window of the cell as well as permanent stirring of solution is required in order to have an uniform distribution of molecular concentration in the cell)



$\phi_{photodegradation} = 7.55 \times 10^{-5}$

Figure S17 Changing absorption at 464 nm accompanying the photodegradation of fluorescein in air-equilibrated ethanol as function of irradiation time, λ_{exc} = 504 nm. (P=3.5 mW).



$\phi_{photodegradation} = 2.7 \times 10^{-5}$

Figure S18 Changing absorption at 515 nm accompanying the photodegradation of **1** in air-equilibrated acetonitrile as a function of irradiation time, λ_{exc} = 512 nm (P=3.9 mW).

Single Molecule Microscopy:

The samples are prepared as follows: 0.1 nM of **1** were dissolved in deoxygenated THF with PMMA (10 mg/mL) and spin-coated on a glass slide, then placed under a continuous light N_2 gas flow during the measurements. We used a Picoquant Micro-time 200 confocal microscope with an MPD SPAD (QE < 50%) and a frequency doubled Ti-Sa laser chain (Coherent) yielding 4–6 ps pulses at 4.75 MHz (power used: 1.5 kW/cm^2 at 488 nm). A 80%T / 20%R spectrally flat beam splitter is used in combination with a microscope objective (100× UPLSAPO, N.A. 1.4), a 500 nm long-pass interferential filter and a 50 µm pinhole. Considering the N.A. of the objective, it is estimated that it collects about 25% of the light emitted at the focal spot. Point-measurements are performed on a single molecule after a first prescan of the sample used to localize the molecules. These measurements are acquired in a TTTR mode and yield emission trajectories as well as decay times. For emission trajectories a binning of 30 ms is used and all emission trajectories are individually inspected to keep those that display blinking and an abrupt one-step bleaching. The total amount of photons and emission duration before photobleaching, as well as the peak emission intensity, are obtained from the emission trajectory of each single molecule after removing the contribution of the background (weak PMMA emission and instrumental background). In Fig. 4c and 4d, each fluorescence decay time corresponds to the longest lifetime resulting from a biexponential fitting of the decays (no deconvolution was applied; using the Picoquant Symphotime software). Verification of several PMMA samples and emission trajectories of single molecules of **1** (threshold-dependent fluorescence decay analyses) allowed attribution of the very short component to the PMMA film (residual emission, light scattering or Raman), and the longest component to emission from **1**.

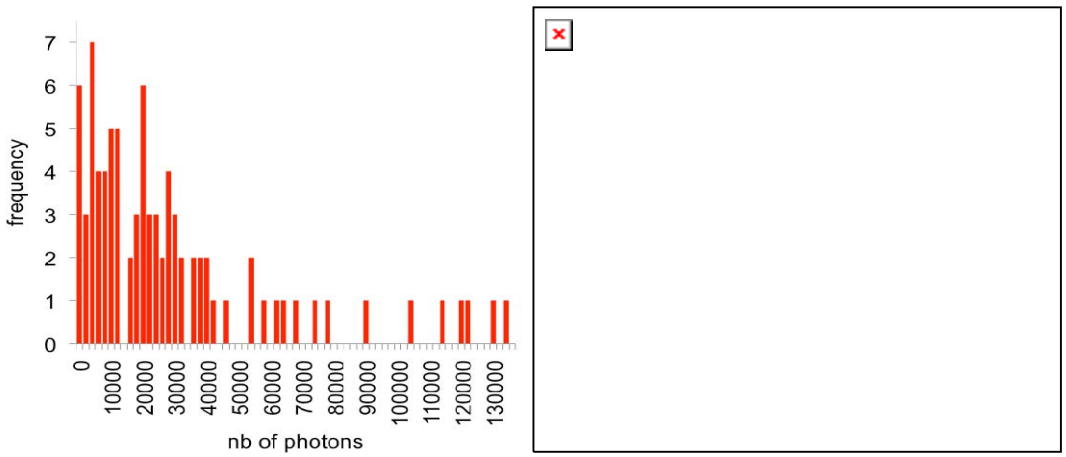


Fig. S19 : (left) Histogram of the total number of photons collected before photobleaching for 82 molecules of **1**. One molecule yielded 2.4×10^5 photons (not shown). The average is 3.5×10^4 photons. (right) Emission trajectory between 1 s and 2 s of the same single molecule as in Figure 4a, using a 1 ms binning.