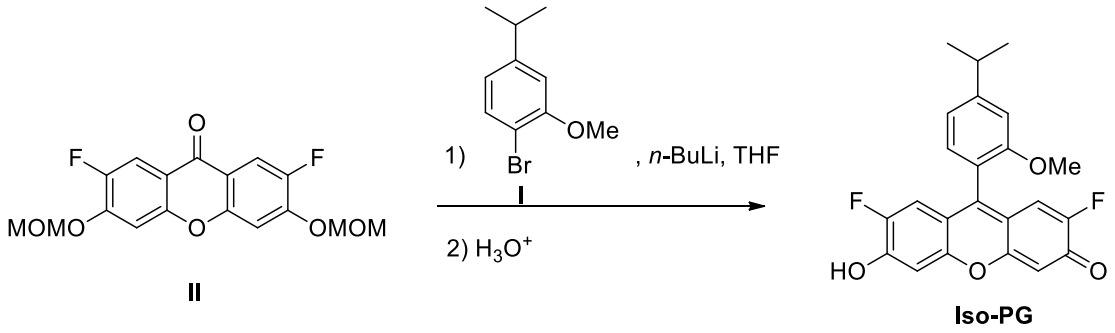
SUPLEMENTARY INFORMATION

Synthetic part

General details

Deoxygenated solvents and reagents were used for the reaction involving *n*-BuLi. THF was freshly distilled from Na. TLC was performed on aluminium-backed plates coated with silica gel 60 (230-240 mesh) with F254 indicator. The spots were visualized with UV light (254 nm) and/or staining with Ce/Mo reagent or phosphomolybdic acid solution and subsequent heating. Products were purified by flash chromatography on Merck silica gel 50. Yields refer to analytically pure samples. ^{1}H NMR spectra were recorded at 400 MHz at room temperature. Chemical shifts are reported in ppm using residual solvent peak as reference (CH_3OH: δ 3.34). Data are reported as follows: chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, m: multiplet, hept: heptuplet), coupling constant (*J* in Hz) and integration. ^{13}C-NMR spectra were recorded at 101 MHz using broadband proton decoupling and chemical shifts are reported in ppm using residual solvent peaks as reference (CD_3OD: δ 49.86). Carbon multiplicities were assigned by DEPT techniques. ^{1}H and ^{13}C NMR of bromide **I**[1] and ketone **II**[2] matched with previously described High resolution mass spectra (HRMS) were recorded on a Micromass AutoSpec using EI at 70eV.

Synthesis of compound Iso-PG



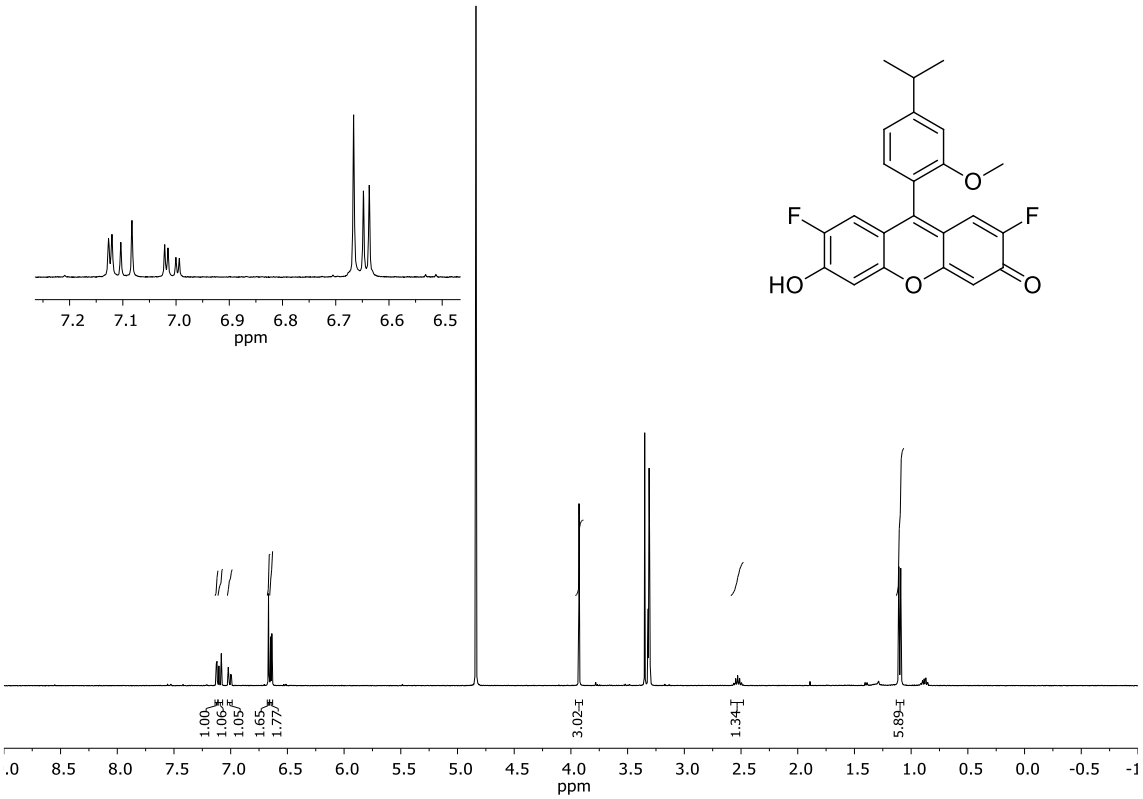
A solution of 2-bromo-5-isopropyl-1-metoxibenzene **I** (46mg, 0.2 mmol, 1 eq) in THF (2 mL) at −78 ºC was treated with *n*-BuLi (0.22 mmol, 1.1 eq). After keeping the reaction at that temperature for 20 minutes, ketone **II** (98.6mg, 0.2 mmol, 1 eq) was added to the reaction. Then, the mixture was stirred at −78ºC for 15 min and after allowed to reach room temperature. The reaction was monitorized by TLC and after consumption of ketone **II** HCl(10%) was added, promoting a color change from pale yellow to orange. Finally, solvent was removed under reduced pressure and residue was submitted to flash chromatography in

[1] A. Martínez-Peragón, D. Miguel, A. Orte, A. J. Mota, M. J. Ruedas-Rama, J. Justicia, J. M. Alvarez-Pez, J. M. Cuerva, L. Crovetto *Org. Biomol. Chem.* **2014**, *12*, 6432−6439.

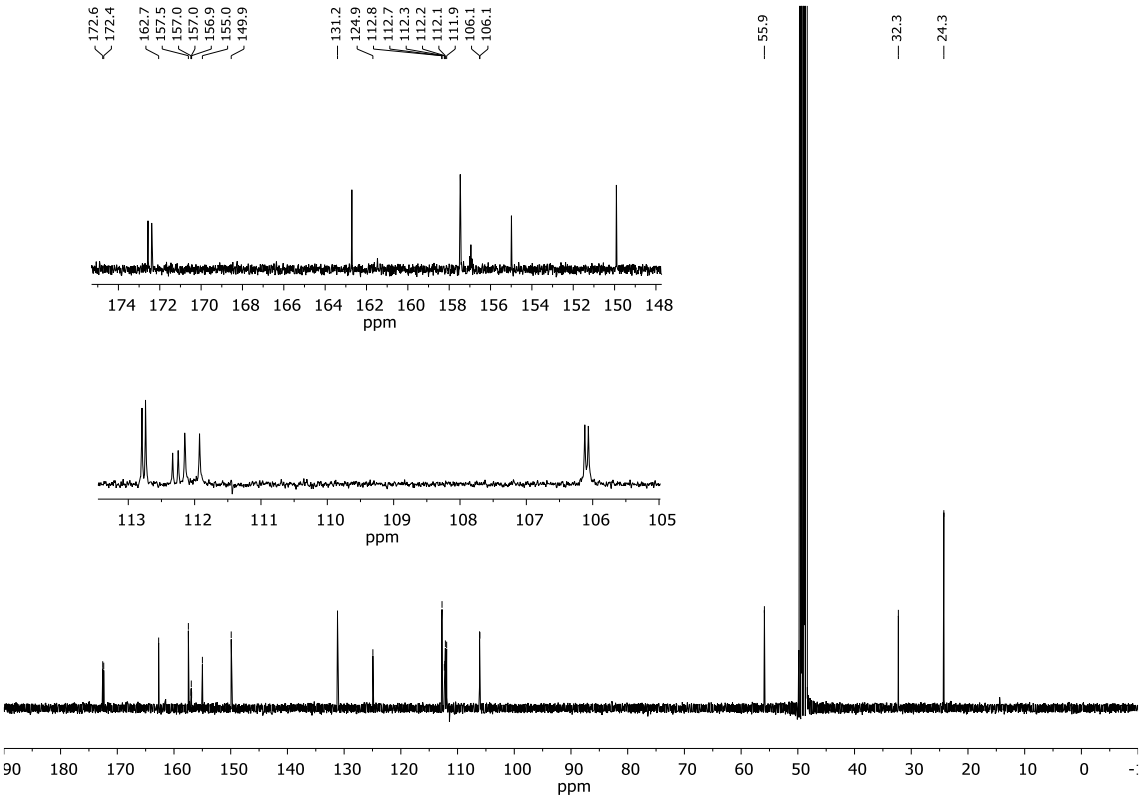
[2] V. Puente-Muñoz, J. M. Paredes, S. Resa, A. M. Ortuño, E. M. Talavera, D. Miguel, J. M. Cuerva, L. Crovetto *Sensors and Actuators B* **2017**, *250*, 623–628.

CH_2Cl_2: MeOH mixtures, affording **Iso-PG** in a 68% yield. Its spectroscopic data are: **^{1}H NMR (400 MHz, MeOD)** δ 7.12 (d, *J* = 2.5 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 7.01 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.67 (s, 2H), 6.64 (d, *J* = 4.4 Hz, 2H), 3.93 (s, 3H), 2.53 (hept, *J* = 6.8 Hz, 1H), 1.10 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR (101 MHz, MeOD)** δ 172.5 (d, *J* = 17.8 Hz, C), 162.7 (s, C), 156.97 (d, *J* = 11.2 Hz, C), 156.95 (s, C), 156.2 (d, *J* = 249.3 Hz, C), 149.9 (s, C), 131.2 (s, CH), 124.9 (s, C), 112.8 (d, *J* = 5.6 Hz, CH), 112.3 (d, *J* = 8.5 Hz, C), 112.2 (s, CH), 111.9 (s, CH), 106.1 (d, *J* = 5.5 Hz, CH), 55.9 (s, CH_3), 32.3 (s, CH), 24.3 (s, CH_3). **HRMS (ESI):** *m/z* $[M\text{-}Na]^+$ calcd for $C_{23}H_{18}F_2O_4$: 419.1065; found: 419.1060.

^{1}H NMR (400 MHz, MeOD):



^{13}C NMR (101 MHz, MeOD):



Materials and methods

Reactive

The chemical products used in this work have been: MilliQ water, acetic acid and sodium acetate, sodium hydroxide, perchloric acid, sodium chloride, potassium chloride and disodium phosphate salt. All reactive were acquired from Sigma-Aldrich.

Sample preparation

A stock solution of Iso-PG (4×10^{-4} M) was prepared in water adding 10 µL of NaOH (0.1 M). This stock solution was kept in dark and 4°C. Stock solutions (0.7M) of acetic acid and sodium acetate were prepared using MilliQ as solvent. Work solutions were prepared adding the adequate volume from stock solutions to obtain the desired acetate concentration and pH. NaOH and $HClO_4$ were used to adjust the pH value in the acetate absence solutions. Iso-PG was added to all samples to obtain a final concentration of 4×10^{-5} M for the absorption experiments and 1×10^{-6} M for the fluorescence experiments.

Instrumentation

Absorption spectra were acquired in a Perkin-Elmer Lambda 650 UV/Vis double-beam spectrophotometer. Steady-state fluorescence spectra were acquired using a JASCO FP-8300 spectrofluorimeter using as excitation a 150W xenon lamp. Time resolved fluorescence decays were acquired through the time-correlated-single-photon-counting (TCSPC) in a FluoTime200 fluorimeter (PicoQuant, Inc). As excitation sources we used a LDH-485 and LDH-440 head lasers (PicoQuant, Inc) and the emission wavelength selected by a monochromator were 500-510, 520 and 530 nm. The pulse repetition rate was 20 MHz and the histograms were obtained in 1320 channels with a resolution of 37ps. Laser response functions were acquired using LUDOX as scatterer. All decay histograms were obtained with 2×10^4 counts in the maximum. All measurements were realized at room temperature using 10×10 mm cuvettes.

FLIM images were acquired using a Microtime 200 confocal fluorescence lifetime microscope (PicoQuant, Inc.) through the time-tagged time resolved (TTTR) methodology, which allows reconstruct the fluorescence decay histograms. The excitation source was the LDH-485 nm pulse laser. The excitation light beam was directed to a dichroic mirror (510 dcxr) and afterwards to the oil immersion objective (1.4 NA, 100×) of an inverted microscope system IX-71 (Olympus). The fluorescence was filtered by a long-pass HP500LP filter (AHF/Chroma) and focus in a 75µm pinhole. Finally, the fluorescence arrived to a single photon avalanche diode SPCM-AQR 14 (Perkin-Elmer). Raw images were recorded scanning an area of 80 × 80 µm with a resolution of 512 × 512 pixels.

Data analysis

Data graphical representation and fitting was realized using Originpro 8.5 software (OriginLab, corp). Fluorescence decay curves were analyzed individually and globally through an interative deconvolution method with exponential models using the software FluoFit (PicoQuant, Inc.). FLIM images data were analyzed using SymphoTime software (PicoQuant, Inc.) and *FIJI is just image j*.[1] The fitted FLIM instrument response function was reconstructed to analyze the histogram of each pixel using a monoexponential model. All decay histograms were fitted applying the maximum likelihood estimator (MLE) which yield correct parameter sets for low count rates.[2] The FLIM images obtained were exported and analyzed in *FIJI is just image j*[1] using home-made macros. This instruction include a 10×10 binning process and a Gaussian smoothing function (s.d. = 1 pixel).

1 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nature Methods* **9**, 676-682, doi:10.1038/nmeth.2019 (2012).

2 Maus, M. *et al.* An experimental comparison of the maximum likelihood estimation and nonlinear least squares fluorescence lifetime analysis of single molecules. *Analytical Chemistry* **73**, 2078-2086, doi:10.1021/ac000877g (2001).