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# Synthesis and two-photon absorption spectrum of fluorenone-based molecules



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#### ABSTRACT

The two-photon absorption (2PA) of five symmetrical fluorenone-based molecules is studied by femtosecond wavelength-tunable Z-scan, as well as quantum-chemical calculations. The molecules are transparent for wavelengths greater than 500 nm and two main one-photon absorption bands are observed in the blue region; one weak, centered at 450 nm, and a stronger one at approximately 360 nm. We observed a strong 2PA band located around 720 nm with maxima 2PA cross-sections between 100 and 230 GM. Quantum chemical calculations employing the response function formalism were performed at the Density Function Theory level to support the interpretation of the experimental nonlinear spectra. © 2016 Published by Elsevier B.V.

#### 1. Introduction

Fluorene compounds are known for their large two-photon absorption (2PA) cross-sections [1]. As an alternative to fluorene compounds, fluorenone-based compounds, originated from the oxidation of the methylene bridge of the fluorine, have been advancing as an interesting option. Even though such family of molecules presents, in general, lower 2PA cross-sections than the fluorene-based ones [2,3], they may present other interesting features that can make them interesting candidates for applications in biological systems, for instance, as fluorescent molecular probes to indicate pH, nucleic acids and nitric oxide, as well as to investigate specific organelles.

Two-photon fluorescence microscopy [4] has been exploited as a valuable tool to study cell organelles, since it provides high resolution three-dimensional images. Besides, two-photon fluorescence microscopy is a non-invasive method, i.e., it allows great image quality of the studied cells while they are still functional. In this method, cells are stained by chromophores that present 2PA in the spectral range of 700–1000 nm. Therefore, the 2PA cross-section of the molecules used for this purpose should present high values within this range. As mentioned before, Fluorenonebased compounds have shown to present low cytotoxicity [5] and good 2PA cross-section in the near infra-red region [6], interesting features for two-photon microscopy. Within this context, we present a study on the 2PA crosssection spectrum of five different symmetrical fluorenone derivatives. The 2PA spectra present a peak at approximately 720 nm, with values ranging from 100 to 230 GM, corresponding to the strong one-photon absorption band at 360 nm. The experimental results for the different molecules were compared to theoretical ones, obtained by quantum chemical calculations; the same trend was observed for the 2PA cross-section values within the family of molecules, although the measured ones are smaller than the theoretical.

#### 2. Experimental

#### 2.1. Chemical syntheses

The Fluorenone derivatives studied here were synthesized by coupling a fluorenone molecule with a halogen, specifically Iodine with terminal alkynes. Subsequently, the replacement of iodine atoms of the fluorenone ring by terminal acetylenes is performed. The catalysis is carried out in the presence of the Pd(II) or Pd(0) complexes, copper iodide and a base, via the Sonogashira reaction [7]. The synthesis of the 2,7-di-iodine-9H-fluorenone was made from fluorenone and *N*-iodinesuccinimide, in the presence of sulfuric acid, according to procedures reported in the literature [8] and shown schematically in Fig. 1.

After the syntheses of the double substituted fluorenonederivatives, the hydrogen in positions 2 and 7 were replaced by



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Fig. 1. Synthesis of 2,7-di-iodine-9H-fluoren-9-one.

groups with a triple bond connecting them to the main fluorenone structure. Five different groups (benzonitrile, benzomethoxy, benzene, thiophene and benzothiophene) were coupled to the fluorenone with a triple bond via the Sonogashira reaction. The five obtained molecules, with the substituents 2-benzotiophene (F-benzothiophene), 4-cyanophenyl (F-cyano), benzene (F-phenyl), 4-benzomethoxy (F-methoxy) and, 2-thiophene (F-thiophene) are shown in Fig. 2.

#### 2.2. General methods

All commercially available reagents were used without any further purification and the reactions were monitored by TLC analysis with TLC plates containing  $GF_{254}$  (E. Merck). Triethylamine and



**Fig. 2.** Structures of F-benzothiophene (2,7-bis(benzo[b]thiophen-2-etinil)-9H-fluorenone), F-cyano (4,4'-(9-oxo-9H-fluorenone-2,7-diil)bis(ethine-2,1-diil)dibenzonitrile), F-phenyl (2,7-bis(feniletinil)-9H-fluorenone), F-methoxy (2,7-bis((4methoxyfenil)etinil)-9H-fluorenone) and F-thiophene (2,7-bis(thiophen-2-etinill)-9H-fluorenone). dimethylformamide were dried under potassium hydroxide and calcium hydride, respectively, and distilled before use. Melting points were determined on a Büchi apparatus and are uncorrected. Column chromatography was performed on Silica Gel 60 (70–230 mesh, E. Merck). NMR spectra were recorded with a Varian Unity Plus 300 MHz or a Varian UNMRS 400 MHz spectrometer. Infrared spectra were obtained on a Brucker IFS66 FT-IR, using KBr pellets. Elemental analysis was performed with a Carlo Erba instrument model E-1110. 2,7-Diiodofluorenone was prepared according to earlier literature [8].

## 2.2.1. Synthesis of 2,7-disubstituted fluorenones via Sonogashira coupling reaction

In a 50 mL Schlenk flask, under argon atmosphere, it was dissolved 0.1 g (0.23 mmol, 1.0 eq) of 2,7-diiodo-9H-fluoren-9-one, 8 mg (0.011 mmol, 0.05 eq) of  $PdCl_2(PPh_3)_2$ , 4.4 mg (0.023 mmol, 0.1 eq) of Cul in a mixture of 2:1 v/v of dried and degassed triethylamine: DMF. Then it was added, dropwise, 0.5 mmol (2.5 eq) of the corresponding terminal alkyne. The mixture was allowed to react at room temperature for 24 h. At the end of the reaction, it was added concentrated HCl until the pH became acidic, and then the mixture was poured into a beaker containing iced water. The solid formed was filtered in vacuum and redissolved in ethyl acetate. To this organic phase, it was added anhydrous sodium sulfate which was vacuum filtered over celite in a sintered glass funnel. The solvent was evaporated and the residue chromatographed on silica gel.2.3.

#### 2.2.2. Characterization

**2,7-Bis-phenylethynyl-fluoren-9-one:** yellow solid; m.p. 183–186 °C; Rf: 0,43 (chloroform:hexanes, 3:7, v/v); Yield: 41%; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$ : 7,35–7,37 (m, 6H, H<sub>arom</sub>); 7,48–7,56 (m, 6H, H<sub>arom</sub>); 7,64 (dd, 2H, *J* = 8,7 Hz, 1,2 Hz, H<sub>arom</sub>); 7,79 (s, 2H, H<sub>arom</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$ : 77,0; 88,5; 91,4; 120,5; 122,7; 124,5; 127,3; 128,4; 128,6; 131,6; 134,4; 137,7; 143,1; 192,2. IR (KBr pellet) v<sub>max</sub>/cm<sup>-1</sup>: 1717; 1492; 753; Calculated elemental analysis for C<sub>29</sub>H<sub>16</sub>O: C, 91,07%; H, 4,74%; found: C, 91,35%; H, 4,52%.

2,7-Bis-(4-methoxy-phenylethynyl)-fluoren-9-one: yellow solid; m.p.: 193–195 °C; Rf: 0.5 (hexanes:ethyl acetate, 9:1, v/v); Yield: 51%. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 3.84 (s, 6H, OCH<sub>3</sub>); 6.89 (d, 4H, *J* = 8.4 Hz); 7.47 (dd, 2H, *J* = 8.4 Hz, 2.4 Hz); 7.50 (dd, 4H, *J* = 8.4 Hz, 2.4 Hz); 7.62 (d, 2H, *J* = 8.4 Hz); 7.78 (s, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$ : 30.3; 87.4; 91.5; 114.1; 114.8; 120.5; 124.8; 127.2; 133.2; 134.4; 137.5; 142.9; 159.9; 192.4; 194.7. IR (KBr pellet)  $\nu_{max}/cm^{-1}$ : 1510; 1717; 2837; 2958. Calculated elemental analysis for C<sub>31</sub>H<sub>20</sub>O<sub>3</sub>: C, 84.14%; H, 5.48%; found: C, 83.79%; H, 5.01%.

4,4'-(9-Oxo-9*H*-fluorene-2,7-diyl)-bis-(ethyne-2,1-diyl)-dibenzonitrile: orange solid, m. p.: 269–272 °C; Rf: 0.6 (hexanes:ethyl acetate 9:1, v/v); Yield: 42%. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 7.58 (d, 4H, *J* = 7.8 Hz, *J* = 1.8 Hz); 7.66 (dd, 4H, *J* = 8.1 Hz, 1.8 Hz); 7.69 (dd, 4H, *J* = 7.8 Hz, 1.5 Hz); 7.83 (s, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$ : 89,7; 92.5; 111.9; 118.3; 120.8; 123.6; 127.5; 127.6; 132.1; 134.5; 138.1; 143.7; 191.7. IR (KBr pellet) v<sub>max</sub>/cm<sup>-1</sup>: 1601; 1715; 2226. Calculated elemental analysis for  $C_{31}H_{14}ON_2$ : C, 86.09%; H, 3.73%; found: C, 85.56%; H, 4.03%.

2,7-Bis-thiophen-2-yl-ethynyl-fluoren-9-one: red solid; m.p.: 227–230 °C; Rf: 0.4 (hexanes:dichloromethane 2:1, v/v); Yield: 40%. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 7.03 (dd, 2H, *J* = 5.1 Hz, 5.1 Hz); 7.31–7.34 (m, 4H); 7.50 (d, 2H, *J* = 7.8 Hz); 7.64 (dd, 2H, *J* = 7.8 Hz, 1.5 Hz); 7.78 (s, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$ : 29.6; 84.7; 92.2; 120.6; 122.7; 124.2; 127.1; 127.2; 127.8; 132.4; 134.4; 137.5; 143.2; 192.0. IR (KBr pellet) v<sub>max</sub>/cm<sup>-1</sup>: 1466; 1718; 2850; 2918. Calculated elemental analysis for C<sub>25</sub>H<sub>12</sub>OS<sub>2</sub>: C, 76.09%; H, 3.58%; found: C, 75.85%; H, 3.29%.

2,7-Bis-benzo[b]thiophen-5-yl-ethynyl-fluoren-9-one: red solid; m.p.: 267–270 °C; Rf: 0.3 ((hexanes:dichloromethane 2:1, v/v); Yield: 54%. <sup>1</sup>H NMR (DMSO<sub>d6</sub>, 400 MHz): 7.41–7.46 (m, 6H); 7.78–7.96 (m, 8H); 7.98 (s, 2H). <sup>13</sup>C NMR (DMSO d<sub>6</sub>, 100 MHz)  $\delta$ : 85.2; 94.4; 121.9; 122.7; 122.8; 123.3; 124.7; 125.6; 126.9; 130.5; 134.4; 138.7; 139.2; 140.2; 143.8; 191.5. IR (KBr pellet)  $v_{max}/cm^{-1}$ : 1025; 1646; 3417. Calculated elemental analysis for C<sub>33</sub>H<sub>16</sub>OS<sub>2</sub>: C, 80.46%; H, 3.27%; found: C, 79.96%; H, 3.52%.

#### 2.3. Two-photon absorption measurements

Each molecule was diluted in Dimethylformamide (DMF) in concentrations ranging from 10<sup>17</sup> to 10<sup>18</sup> molecules/cm<sup>3</sup>. The samples were placed in a 2 mm quartz cell to obtain its linear and 2PA absorption spectra. Linear absorption spectra were obtained using a UV-1800 Shimatzu spectrometer. The 2PA cross-section spectrum was obtained through the open-aperture Z-Scan technique [9]. In these measurements, a 775 nm Ti:Sapphire laser amplifier, delivering pulses with 150 fs of duration and operating at a 1 kHz repetition rate, is used as the excitation source for an Optical Parametric Amplifier (OPA) that provides 120 fs pulses in the wavelength range of 560–1000 nm. The beam spatial profile is made Gaussian by using a spatial filter before the Z-Scan setup.

In the Z-scan technique, the 2PA cross-section is determined by translating the sample around the focal plane of a Gaussian beam, while obtaining the transmittance change in the far field. For a sample presenting nonlinear absorption, its absorption coefficient depends on the intensity as  $\alpha = \alpha_0 + \beta I$ , where  $\alpha_0$  is the linear absorption coefficient,  $\beta$  is the 2PA absorption coefficient and I is the intensity of the beam. The power transmitted through a nonlinear absorptive material, for a Gaussian beam, can be integrated over time, for each wavelength, giving the normalized transmittance as

$$T(z) = \frac{1}{\sqrt{q}q_0(z,0)} \int_{-\infty}^{\infty} ln [1+q_0(z,0)e^{-\tau^2}] d\tau$$
(1)

where

$$q_0 = \beta I_0 L \left( 1 + \left( \frac{z^2}{z_0^2} \right) \right)^{-1}, \tag{2}$$

L is the sample thickness, I<sub>0</sub> is the laser beam intensity at the focus,  $z_0$  is the Rayleigh length and z is the sample position. The 2PA absorption coefficient  $\beta$  is obtained by fitting the experimental transmittance data with Eq. (1). Then, the 2PA cross-section is calculated using  $\sigma_2 = \hbar \omega \beta / N$ in which  $\hbar \omega$  is the excitation photon energy and N is the number of molecules/cm<sup>3</sup>. 2PA cross-section values are generally presented in GM (Göpert-Meyer) units, where 1GM =  $10^{-50}$  cm<sup>4</sup> s/photon.

#### 2.4. Fluorescence and quantum yield measurements

The fluorescence spectrum measurements were carried in a HITACHI F7000 Fluorimeter. To determine the fluorescence quantum yield ( $\varphi$ ) of the compounds, disodium fluorescein was used

as the reference material. By knowing the quantum yield for the disodium fluorescein in methanol (0.97) [10],  $\phi$  for other compounds of interest can be calculated using

$$\varphi_f = \varphi_{f_{ref}} \frac{\int_{\lambda_0}^{\lambda_f} F(\lambda) d\lambda}{\int_{\lambda_0}^{\lambda_f} F_{ref}(\lambda) d\lambda} \frac{f_{ref}}{f} \frac{n^2}{n_{ref}^2},$$
(3)

in which  $F(\lambda)$  is the fluorescence spectrum, f is the absorption factor given by  $f = 1 - 10^{-A(\lambda_{exc})}$ , in which  $A(\lambda_{exc})$  is the absorbance at the excitation wavelength (450 nm) and n is the refractive index of the solvent.

#### 3. Theoretical approach

All the quantum-chemical calculations in this study were carried out at the density functional theory (DFT) level [11,12]. The Gaussian 09 package [13] was used to determine the equilibrium geometry of the molecules with the aid of the CAM-B3LYP functional [14] and the standard 6-311G(d,p) basis set [15]. Subsequently, to compute the molecular parameters related to the one-photon and two-photon electronic transitions of the molecules, the response functions formalism,[16] within the DFT framework and as implemented in the DALTON 2011 program [17], was employed. In this approach, the oscillator strengths and twophoton transition probabilities are calculated analytically as single residues of the linear and quadratic response functions of the molecular electronic density respectively.

A preliminary assessment of the most popular DFT functionals revealed that the standard hybrid B3LYP functional and also the standard long-range corrected CAM-B3LYP functional fail to well reproduce the experimental UV–vis spectra of the molecules investigated. Therefore, in this study the strategy of Okuno et al. [18] of employing a tuned CAM-B3LYP functional was employed. In the tuned CAM-B3LYP functional the default value of the functional parameters, that is,  $\mu = 0.33$ ,  $\alpha = 0.19$ , and  $\beta = 0.46$ , are tuned to  $\mu = 0.150$ ,  $\alpha = 0.0799$  and  $\beta = 0.9201$ .

All spectroscopic computations were carried out employing the tuned-CAMB3LYP functional [8] and the standard 6-31+G(d) basis set [15]. The 10 lowest-energy electronic transitions were determined and characterized. For a consistent comparison between experimental data and theoretical results, the quantum-chemical calculations took into account the solvent environment by employing the polarizable continuum model (PCM) approach, as implemented in Gaussian 09 package for geometry optimization calculations [19] and as implemented in Dalton 2011 program for response function calculations [20]. For consistency reasons, the van der Waals surface used in the response function calculations was defined by adopting the set of van der Waals radius and atomic centers determined at the geometry optimization stage using the Gaussian 09 package.

The two-photon transition probabilities computed in the quadratic response function calculations assumes the degenerate  $(\omega_1 = \omega_2 = \omega)$  and resonant  $\omega_{gf} = 2\omega$  conditions. The degenerate two-photon transition probability  $(\langle \delta_{gf} \rangle)$  for the transition from the ground (g) state to a final (f) excited state in an isotropic medium using a linearly polarized laser beam is given by [21,22]

$$\langle \delta_{gf} \rangle = \frac{1}{30} \sum_{\alpha,\beta} 2S_{\alpha\alpha}^{gf} \left( S_{\beta\beta}^{gf} \right)^* + 4S_{\alpha\beta}^{gf} \left( S_{\alpha\beta}^{gf} \right)^* \tag{4}$$

in which the subscripts  $\alpha$  and  $\beta$  represent the Cartesian coordinates and  $S_{\alpha\beta}^{gf}$  is the two-photon matrix element, which for a single beam 2PA experiment is defined as [23–25]

$$S_{\alpha\beta}^{gf} = \frac{1}{2\hbar} \sum_{k} \left[ \frac{\langle g|\hat{e} \cdot \hat{\mu}_{\alpha}|k\rangle \langle k|\hat{e} \cdot \hat{\mu}_{\beta}|f\rangle}{\omega_{gk} - \omega} + \frac{\langle g|\hat{e} \cdot \hat{\mu}_{\beta}|k\rangle \langle k|\hat{e} \cdot \hat{\mu}_{\alpha}|f\rangle}{\omega_{gk} - \omega} \right]$$
(5)

Assuming that the normalized line-shape of the excited states is represented by the Lorentzian function, the 2PA cross-section ( $\sigma_{gf}$ ) of the degenerate process is written as [23,24,26]

$$\sigma_{gf}(\omega) = \frac{16\pi^3 \alpha a_0^5}{c} \frac{(\hbar\omega)^2}{\pi (\Gamma_f/2)} \langle \delta_{gf} \rangle \tag{6}$$

in which  $\alpha$  is the fine structure constant, c is the speed of light,  $a_0$  is the Bohr's radius and  $\hbar\omega$  is the photon energy.  $\Gamma_f$  is the damping constant describing the full width at half-maximum (FWHM) of the final state line width. In Eq. (6), one must use  $a_0 = 5.291772108 \times 10^{-9}$  cm,  $c = 2.99792458 \times 10^{10}$  cm/s and the values of  $\hbar\omega$ ,  $\Gamma_f$  and  $\langle \delta_{gf} \rangle$  in atomic units to obtain the 2PA cross-section ( $\sigma_{gf}$ ) in Göppert–Mayer units (1GM =  $1 \times 10^{-50}$  cm<sup>4</sup>sphoton<sup>-1</sup>).

#### 4. Results and discussion

The linear absorption spectra of the studied molecules in DMF are displayed in Fig. 3. All compounds present similar features; two intense absorption bands located at approximately 300 and 360 nm, and a weaker one around 450 nm. The band around 350 nm is related to the fluorenone group, while the one centered at approximately 450 nm is an intra-molecular charge-transfer (ICT) band [27,28]. For F-benzothiophene, the absorption band at 375 nm is red shifted by about 25 nm in comparison to F-phenyl, which can be explained by the increase in the conjugation length. The other three molecules, F-methoxy, F-thiophene and F-cvano are all located at approximately 360 nm, 10 nm red-shifted when compared to F-phenyl. The molar absorptivity of the transition at 360 nm presents almost the same value for all molecules, around  $5 \times 10^4 \,\text{L}\,\text{mol}^{-1}\,\text{cm}^{-1}$ , while for the one at 450 nm the value is ten times smaller. It indicates that the molecules in DMF solution present almost the same quasi-planar geometry, as was expected due to the triple bonds.

The normalized fluorescence spectra of the compounds are also shown in Fig. 3 (right axis), for excitation at 450 nm. All compounds display similar fluorescence spectra, with peaks around 550 and 600 nm. By using disodium fluorescein diluted in methanol as reference ( $\varphi = 0.97$ ), the quantum yield of the compounds were obtained, accordingly to Eq. (3) and using the measured fluorescence spectra F( $\lambda$ ) of each sample. The absorbances of all samples were kept lower than ~0.1 to avoid fluorescence reabsorption. The obtained fluorescence quantum yields are: 0.19 for F-benzotiophene; 0.31 for F-cyano; 0.06 for F-tiophene; 0.05 for F-methoxy and 0.17 for F-phenyle.

For the nonlinear optical measurements, DMF solutions of the compounds were prepared in concentrations of the order of  $10^{-3}$  M. Open-aperture Z-Scan measurements were performed between 560 and 800 nm, to locate the maximum position of the 2PA cross-section for each molecule. As observed in Fig. 3, samples do not present linear absorption for wavelengths greater than 500 nm. Therefore, to avoid inducing resonant effects that appear upon single photon excitation, we initiated Z-scan experiments at 560 nm. A typical Z-Scan transmittance curve for F-benzothiophene is shown in the inset of Fig. 4(a) for an excitation wavelength of 710 nm. The solid line in the inset represents the fit obtained using Eq. (1), from which we can determine the 2PA cross-section.

From Z-Scan curves similar to the one displayed in the inset of Fig. 4(a), obtained at distinct excitation wavelengths, the 2PA cross-section spectrum can be obtained. The circles in Fig. 4 (a) and (b) (right axes) represent, respectively, the experimental 2PA spectrum for F-benzothiophene and F-methoxy. For comparison purposes the linear absorption spectra of F-benzothiophene and F-methoxy are also plotted (left-axis – dashed line). The 2PA spectra present a monotonic increase of the 2PA cross-section as the excitation wavelength approaches the one-photon absorption region (resonant enhancement of the nonlinearity), and a 2PA band at around 720 nm, with a peak value of approximately 220 GM. A similar behavior was observed for the other molecules. Table 1 summarizes the 2PA cross-section peak values experimentally determined, as well as its spectral positions for each compound.

The fitting of the 2PA spectrum, obtained using a sum-over essential states (SOS) approach, is also shown in Fig. 4 (red solid line) for the F-benzothiophene. Within this approach, to model the 2PA spectra of the fluorenone molecules we considered a



Fig. 3. Linear absorption (left axis) and normalized fluorescence (right axis) spectra of the studied molecules.



**Fig. 4.** The dashed line (left-axis) shows the linear absorption spectrum for F-benzothiophene (a) and F-methoxy (b). The solid line (red) represents the theoretical fitting of the experimental 2PA cross-section spectra (circles) obtained using the SOS approach. The inset in (a) shows a Z-Scan curve for F-benzothiophene obtained at 710 nm for a pulse energy of 8 μJ. The four-energy-level diagram employed in the SOS approach is presented as an inset in (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Spectroscopic parameters used/obtained in the sum-over-states model.

Molecule	F-phenyl	F-benzotiophene	F-cyano	F-methoxy	F-tiophene
$\lambda_{01}$ (nm)	430	444	410	441	434
$\lambda_{02}$ (nm)	366	366	361	359	360
$\lambda_{03}$ (nm)	326	300	323	300	311
$\Gamma_{01} (10^{14} \text{ Hz})$	1.2	1.5	1.7	2.1	1.5
$\Gamma_{02} (10^{13} \text{ Hz})$	9.7	14.5	9	9.5	10.5
$\Gamma_{03}$ (10 <sup>13</sup> Hz)	8.0	14.0	9.6	18.0	9.0
μ <sub>01</sub> (D)	3.3	3.3	3.2	3.05	3.09
$\Delta \mu_{01}$ (D)	1.56	1.25	1.42	1.29	1.42
$\mu_{02}$ (D)	20.0	35.5	29.0	29.2	29.0
μ <sub>03</sub> (D)	19.0	27	30.0	26.9	19.6

four-energy-level diagram, presented in the inset of Fig. 4(b), based on the one and two-photon absorption spectrum. The SOS expression used to fit the 2PA spectra is given by

$$\sigma_{ng}^{(2)}(\omega) = \frac{4(2\pi)^{5}}{5(hc)^{2}} L^{4} \omega^{2} \left\{ \frac{|\vec{\mu}_{01}|^{2} |\Delta \vec{\mu}_{01}|^{2}}{\omega^{2}} g_{01}(2\omega) + \frac{|\vec{\mu}_{01}|^{2} |\vec{\mu}_{13}|^{2}}{(\omega_{01} - \omega)^{2} + \Gamma_{01}^{2}(\omega)} g_{02}(2\omega) + \frac{|\vec{\mu}_{01}|^{2} |\vec{\mu}_{13}|^{2}}{(\omega_{01} - \omega)^{2} + \Gamma_{01}^{2}(\omega)} g_{03}(2\omega) \right\}$$
(7)

where *h* is the Planck's constant, c is the speed of light and  $\omega$  is the excitation laser frequency.  $\omega_{nm}$ ,  $\Gamma_{nm}$  and  $\mu_{nm}$ , represent, respectively, the transition angular frequency, damping constant and transition dipole moment, corresponding to the  $n \rightarrow m$  electronic transition.  $\Delta\mu_{\rm 01}$  is the difference between the first excited state and the ground permanent dipole moments. L is the Onsager local field factor,  $L = \frac{3n^2}{2n^2} + 1$  with n = 1.4305 (refractive index for DMF), considered to take into account effects of the medium;  $g_{0m}(2\omega)$  is the lineshape of the two-photon absorption to a given final state *m*, which is assumed a Lorentzian. In this model, the 2PA spectrum of the studied compounds are described by a dipolar contribution (first term inside the curly brackets) related to the two-photon transition of the first excited state, 1, (first term inside the curly brackets) and contributions arising from two-photon transitions to higher electronic states (|2 and |3). Also, the resonance enhancement in this model is given by the first excited state (|1), as can be seen in Eq. (7). The solid line in Fig. 4 represents the fitting obtained using Eq. (7), with  $\Gamma_{01}$  and  $\omega_{01}$  taken from the linear absorption spectra displayed in Fig. 3 and  $\Delta \mu_{01}$  obtained from quantum chemical calculations.  $\mu_{01}$  is obtained from the linear absorption spectrum (Fig. 3) using

$$|\vec{\mu}_{01}|^2 = \frac{3 \times 10^3 \ln(10) hc}{(2\pi)^3 N_A} \frac{n}{L^2} \frac{\varepsilon^{max}}{\omega_{01} \theta_{01}}$$
(8)

in which  $\varepsilon^{max}$  is the maximum molar absorptivity,  $N_A$  is the Avogradós number and  $\theta_{01}=\sqrt{4\ln(2)/\pi G_{01}^2}$  is the maximum value of the normalized Gaussian line-shape width, with  $G_{01}$  describing the FWHM (Full Width at Half Maximum) of the line width. Therefore, from the fitting of the 2PA data with Eq. (7) shown in Fig. 4, we were able to determine  $\Gamma_{02}$  and  $\Gamma_{03}$ , as well as the transition dipole moments ( $\mu_{12}$  and  $\mu_{13}$ ). Table 1 summarizes the parameters used/ determined in the SOS modeling. As shown in Fig. 4, the SOS model describes very well the observed nonlinear spectrum, indicating that the energy diagram considered is consistent with the experimental results. It is interesting to mention that no 2PA band was observed at about 900 nm. This observation, which is in agreement with the results obtained by the quantum-chemical calculations, indicates that the excited state accessed via the weak one-photon transition, at approximately 450 nm, is basically two-photon forbidden.

In order to help interpret the observed 2PA results, quantum chemistry calculations were performed, as described in Section 3, and the obtained results are also presented in Table 2. As it can be seen from Table 2, the results from the quantum chemistry cal-

culations show that all studied molecules present a strongly 2PA allowed transition in the range of 642–696 nm. These theoretical results are slightly blue-shifted in relation to the experimental ones (0.08–0.12 eV), within 10% difference.

The theoretical 2PA cross-section at the peak position ( $\sigma_{2PA}^{peak}$ ), on the other hand, displays a larger variation, from 66 GM (F-cyano) to 456 GM (F-benzothiophene). To better visualize the comparison between experimental and theoretical results, in Fig. 5 it is plotted the experimental (squares) and theoretical (circles)  $\sigma_{2PA}^{peak}$ , determined for each compound. Even though their absolute values differ, the behavior observed for the experimental and theoretical  $\sigma_{2PA}^{peak}$ , among the studied molecules, are in very good agreement. This result seems to be related to the conjugation length and strength of the different electron-acceptor and electron-donor groups of the molecules.

Fig. 6 presents the main molecular orbitals (highest probability contribution to the transition) involved in the 2PA transition displayed in Table 2. The calculated transitions for F-benzothiophene (a), F-phenyl (c), F-cyano (e) present a charge transfer character, while F-thiophene (b) has a backbone character. For F-methoxy (d), charge transfer and  $\pi \rightarrow \pi^*$ characters are both presented in the orbitals mainly involved.

By analyzing the molecular orbitals of the molecules, we observe that the largest cross-section of F-benzothiophene can be attributed to its larger conjugation length. Since F-thiophene only differs from F-benzothiophene by two aromatic rings, their 2PA cross-sections are comparable. F-methoxy and F-phenyl present similar 2PA cross-section values because they only differ by the OCH<sub>3</sub> groups. Also, F-methoxy presents a higher 2PA cross-section peak value than F-cyano, something that can be explained because the OCH<sub>3</sub> group, as a substituent, is an electron density donor, whereas CN is a strong acceptor.

#### 5. Conclusion

Herein, we reported the syntheses of new fluorenone-derived compounds with high 2PA cross-section values. Five different compounds were prepared via a synthetic procedure using Sonogashira reactions: F-thiophene, F-benzothiophene, F-cyano, F-methoxy and F-phenyl. Subsequently, we studied the 2PA properties of these materials, and obtained their experimental maximum 2PA cross-sections as well as the wavelengths in which they occur. The compound which displayed the largest  $\sigma_{2PA}$  was Fbenzothiophene, with 224 GM, whereas the one with the lowest  $\sigma_{2PA}$  was F-cyano with 129 GM, both at 720 nm. A comparison with theoretical 2PA cross-sections obtained by quantumchemistry calculations was done, reaffirming the ordering of the synthesized molecules in terms of 2PA cross-sections. Since all molecules (except F-phenyl) displayed experimental maximum cross-sections around 700 nm and reasonable fluorescence quantum yields, they can be interesting options, for example, for twophoton microscopy and allow high-resolution imaging of intracellular organelles, even in living tissues.

Table 2

Experimental and theoretical 2PA cross-section peak wavelength and its corresponding magnitude.

	Experimental		Theoretical quantum chemistry	
Molecule	$\lambda_{2PA}^{peak}$ (nm)	$\sigma_{2PA}^{peak}$ (GM)	$\lambda_{2PA}^{peak}$ (nm)	$\sigma_{2PA}^{peak}$ (GM)
F-benzothiophene	710	220	684	456
F-thiophene	720	210	688	340
F-methoxy	710	200	696	279
F-phenyl	655	200	650	289
F-cyano	720	130	642	66



Fig. 5. Experimental (squares - left axis) and theoretical (circles - right axis) 2PA cross-section peak values. The error bars for the experimental values is 10%.



Fig. 6. Molecular orbitals involved in the electronic transitions of (a) F-benzothiophene, (b) F-thiophene, (c) F-phenyl, (d) F-methoxy and (e) F-cyano.

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