

One- and Two-Photon Fluorescence Anisotropy of Selected Fluorene Derivatives

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The steady-state excitation anisotropy spectra of fluorene derivatives were measured in viscous solvents, under the one- and two-photon excitation, over a broad spectral range (UV–Visible). The orientation of their absorption transition moments for the first, $S_0 \rightarrow S_1$, and second, $S_0 \rightarrow S_2$, excited states were determined. It was shown experimentally that a decrease in the angle between $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions corresponded to an increased value of two-photon absorption (2PA) cross section for these molecules. Two-photon excitation anisotropy was nearly constant over the spectral region investigated (in contrast to one-photon excitation anisotropy spectra) and can be roughly explained by a simple model of 2PA based on the single intermediate state approximation. For comparison, the same trend in two-photon excitation anisotropy was observed for Rhodamine B in glycerol.

KEY WORDS: Two-photon excitation anisotropy; steady-state fluorescence; transition dipole moments; fluorene derivatives.

INTRODUCTION

Fluorescence anisotropy measurements represent a powerful tool for the investigation of spectroscopic properties of organic molecules and are a subject of a great interest, particularly in the design of materials for photonic and nonlinear optical applications. Steady-state fluorescence anisotropy can be used for determination of the molecular symmetry [1], spectral position of various absorption electronic transitions in complex absorption bands [2], microviscosity of biological membranes [3,4], and directions of the molecular transition moments [5,6]. Additional important information can be obtained from

time-dependent anisotropy decays. These types of experimental techniques have been employed for the investigations of certain fast relaxation processes in the molecules [7,8], intramolecular energy transfer [9], photoinduced proton transfer reactions [10], interaction of a molecule with surrounding solvent [11], and segmental mobility of a biopolymer-bound fluorophore [12].

Fluorescence anisotropy measurements under two-photon excitation are intrinsically more sensitive than one-photon excitation due to its higher anisotropy values [2,13] that, in some cases (especially for biomolecules), can lead to significant advantages when compared to traditional one-photon methods [14]. The comparison of one- and two-photon anisotropy can afford a deeper understanding of the mechanism of two-photon absorption (2PA), providing information not available by conventional fluorescence anisotropy, r_{1PA} [11]. In spite of the merits of two-photon anisotropy (r_{2PA}), these investigations are still very limited. Most of them have been performed over a limited spectral region (typically one electronic band), and their analysis is mostly directed to the comparison r_{1PA} and r_{2PA} .

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It was shown that only in some cases r_{2PA} exceeds r_{1PA} (e.g. [15]), whereas much more often (e.g. the case of tyrosine, phenol [16], indole, and tryptophan [17]) the values of $r_{2PA} \leq r_{1PA}$. This unusual behavior of r_{2PA} is connected with the peculiarities in molecular electronic structures, which determine their 2PA mechanism [18]. However, in many cases, two-photon anisotropy behavior remains unclear and unpredictable [19]. Thus, a detailed investigation of two-photon anisotropy and its comprehensive analysis is very important in developing an understanding of the mechanisms of 2PA.

In this paper, we present the results of a detailed investigation of the steady-state fluorescence anisotropy of four fluorene derivatives, under one- and two-photon excitation over a broad spectral region (UV–Visible) along with the quantitative analysis of their spectral parameters and orientation of the molecular transition moments. The comparison of one- and two-photon fluorescence anisotropy revealed some general peculiarities in 2PA processes that can be attributed not only to the specific fluorene derivatives studied but, perhaps, more generally for other two-photon absorbing fluorophores.

EXPERIMENTAL

The fluorene derivatives (7-benzothiasol-2-yl-9,9-didecylfluorene-2-yl)diphenylamine (**1**), 9,9-didecyl-2,7-bis-(*N,N*-diphenylamino)fluorene (**2**), {4-[2-(7-diphenylamino-9,9-diethylfluorene-2-yl)vinyl]phenyl} phosphoric acid diethyl ester (**3**), and 2,7-bis-[4-(9,9-didecyl-fluorene-2-yl)vinyl] phenylbenzothiazole (**4**), were synthesized previously [20–22] and characterized photo-physically [23,24]. Molecular structures of these compounds are presented in Fig. 1. Excitation anisotropy spectra of **1–4** were measured in viscous silicon oil and poly(tetrahydrofuran) (pTHF, m.w. ~250) obtained from Aldrich. In the viscous solvents, correlation rotational time, θ , for the organic molecules essentially exceeds their fluorescence lifetime, τ , and therefore, anisotropy reaches the maximum value, determined by the molecular structure, and is independent of the rotational movement of the molecule [2].

Absorption spectra of **1–4** were recorded with Cary-3 UV–Visible spectrophotometer. Steady-state fluorescence and one-photon excitation anisotropy spectra

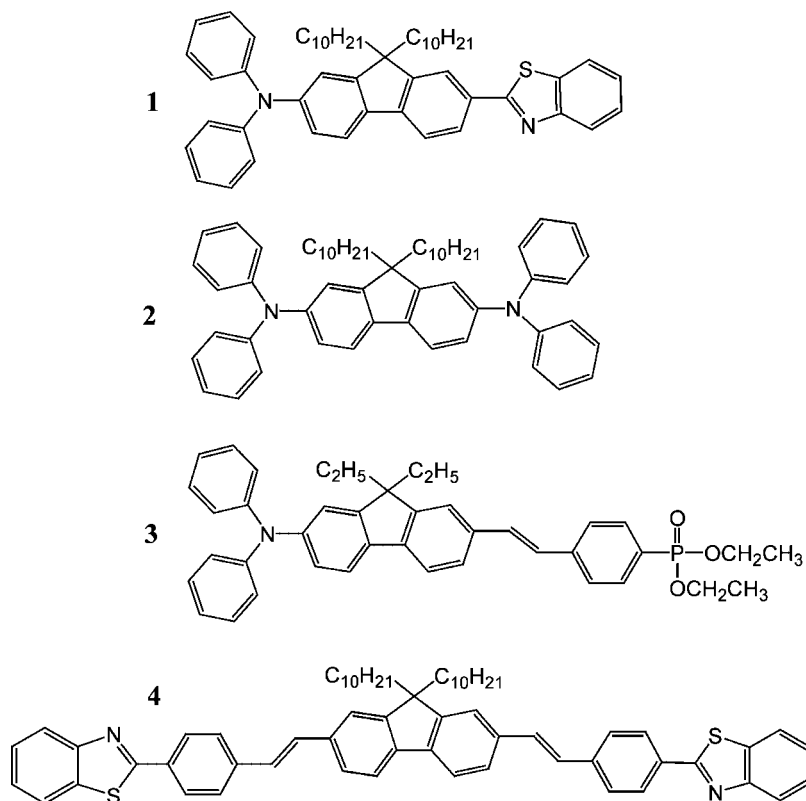


Fig. 1. Structures of compounds 1–4.

were measured in 10 mm quartz cuvettes with a Photon Technologies, Inc. (PTI). Quantamaster spectrofluorimeter in the photon-counting regime of the photomultiplier tube (PMT) using an L-format method for anisotropy measurements [2]. Concentrations (C) of **1–4** in silicon oil and pTHF were $\approx 10^{-6}$ M for one-photon excitation measurements.

Two-photon linear polarized excitation of **1–4** were performed using a Clark-MXR 2001 Ti:Sapphire amplified, second harmonic of an erbium-doped fiber ring oscillator system (output 775 nm) pumped an optical parametric generator/amplifiers (TOPAS, Light Conversion), with pulse duration, ≈ 120 fs, (FWHM), 1 kHz, repetition rate, tuning range 560–2100 nm, and maximum average power ≈ 25 mW. Upconverted fluorescence and excitation anisotropy spectra of **1–4** under two-photon excitation were measured in 10 mm quartz cuvettes ($C \approx 10^{-5}$ M) with a PTI Quantamaster spectrofluorimeter in the analog regime of PMT using an L-format method for anisotropy studies. Fluorescence lifetimes of **1–4** in silicon oil and pTHF were obtained with a PTI Timemaster spectrofluorimeter. Experimental details of these measurements were previously reported [24]. All spectra were background subtracted using the corresponding solvent in 10 mm quartz cuvettes. Measurements of the upconverted fluorescence intensity on the incident laser power confirmed the quadratic dependence for all excitation wavelengths, characteristic of 2PA.

RESULTS AND DISCUSSION

The fluorescence lifetimes, τ , and rotational correlation times, θ [2], for fluorenes **1–4** in silicon oil and pTHF were obtained in order to demonstrate that the fluorescence anisotropy was independent of rotational diffusion, and mainly determined by the electronic structure of the molecules (see Table I). It was found that $\tau \ll \theta$ for all investigated compounds. In this case, the anisotropy, r_{1PA} , reached the maximum value, r_{1PA}^{\max} , and can be expressed as [2]:

$$r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp}) = r_{1PA}^{\max} / (1 + \tau/\theta) \approx r_{1PA}^{\max} \quad (1)$$

where I_{\parallel} and I_{\perp} are the fluorescence intensities polarized parallel and perpendicular to the excitation light, respectively; $\theta = \eta V / (RT)$; η is the solvent viscosity ($\eta \geq 200$ cP for both silicon oil and pTHF); V is the volume of the molecule; R is the gas constant; T is the temperature in Kelvin.

It is known that r_{1PA}^{\max} depends on the angle between the absorption and emission transition moments, β , and range from $-0.2 \leq r_{1PA}^{\max} \leq 0.4$ for one-photon excitation and $-0.29 \leq r_{2PA}^{\max} \leq 0.57$ for two-photon excitation (parallel absorbing oscillators), according to the Eqs. 2a and 2b, respectively [2]:

$$r_{1PA}^{\max} = (3 \cos^2 \beta - 1) / 5 \quad (2a)$$

and

$$r_{2PA}^{\max} = (6 \cos^2 \beta - 2) / 7 \quad (2b)$$

The UV-Visible absorption and corrected fluorescence spectra of **1–4** in silicon oil and pTHF are presented in Figs. 2a–d. The absorption spectra (curves 1, 1') are nearly independent of solvent polarity, Δf , while their fluorescence spectra (curves 2, 2') exhibit a strong dependence on Δf . The Stokes' shifts of the compounds were shown to be proportional to Δf in accordance to the Lippert equation [2]. The nature of the absorption spectra over the spectral range 250–450 nm was revealed from the one-photon excitation anisotropy measurements (curves 3, 3'). The constant anisotropy values within the long wavelength absorption bands (350–450 nm) for all compounds **1–4** correspond to the first electronic transition, $S_0 \rightarrow S_1$. For $\lambda_{\text{exc}} \approx 310$ nm (Figs. 2a–c, curves 3, 3') and $\lambda_{\text{exc}} \approx 330$ nm (Fig. 2d, curves 3, 3') the excitation anisotropy decreased and reached a minimum, indicating the position of the second electronic transition, $S_0 \rightarrow S_2$. For compound **4**, the intensity of the transition at $\lambda_{\text{exc}} \approx 330$ nm (Fig. 2d, curve 3, 3') was relatively small (Fig. 1), compared to other fluorene derivatives.

As mentioned above, the absorption spectra of **1–4** were nearly solvent-independent. This trend was observed for **1–4** in silicon oil, pTHF, and other solvents [23]. Therefore, we can assume that the orientation of the absorption transition moments $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ are also nearly solvent-independent, and are primarily determined by the

Table I. Fluorescence Lifetimes, τ , and Rotational Correlation Times, θ , for **1–4** in Silicon Oil and pTHF

Solvent compound	Silicon oil				pTHF			
	1	2	3	4	1	2	3	4
τ (ns)	1.7 ± 0.15	1.07 ± 0.15	1.5 ± 0.15	0.4 ± 0.1	2.0 ± 0.2	0.8 ± 0.1	1.8 ± 0.2	0.85 ± 0.15
θ (ns)	≥ 35	≥ 38	≥ 40	≥ 42	≥ 35	≥ 38	≥ 40	≥ 42

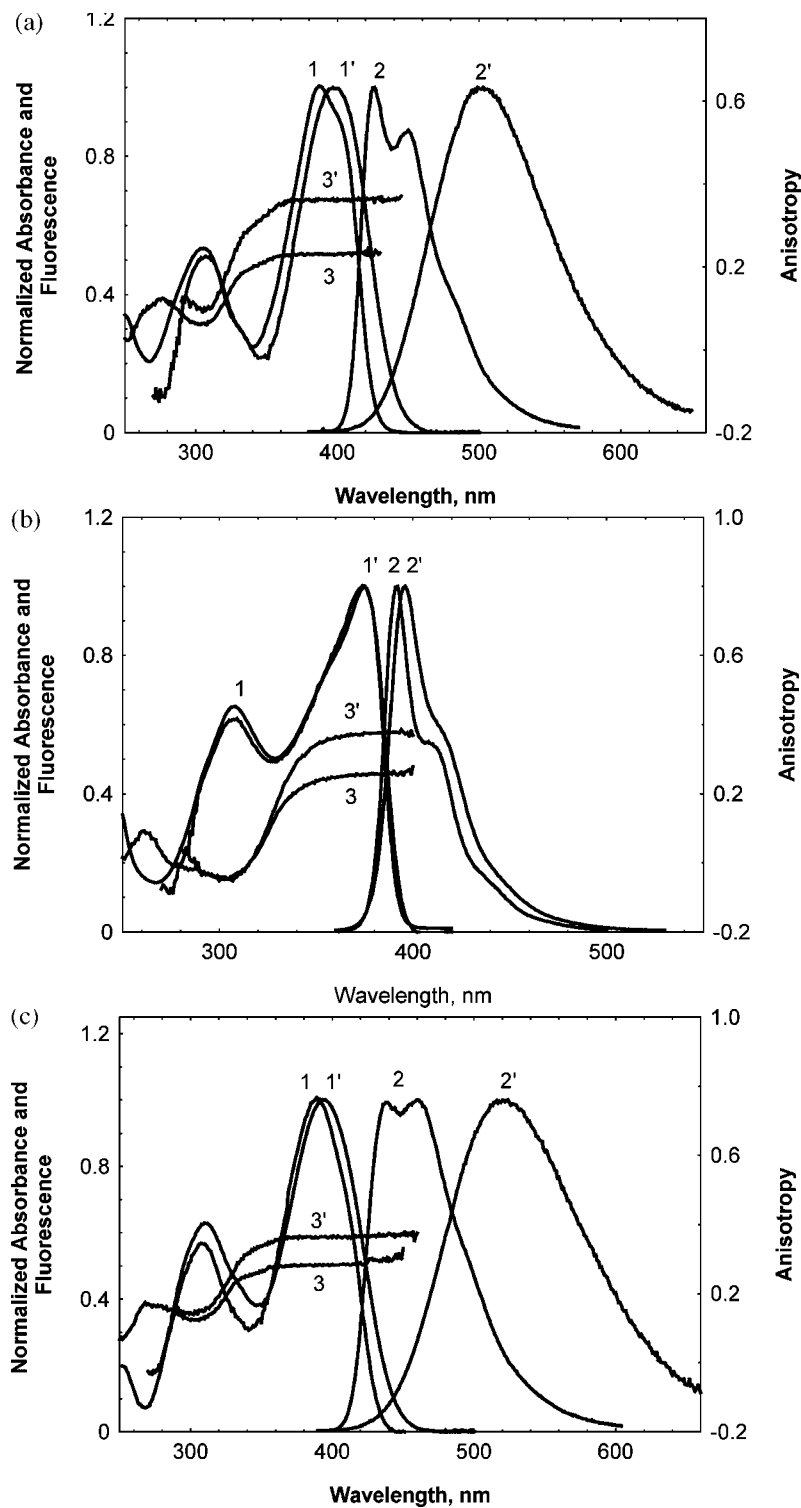


Fig. 2. (a) Absorption (1, 1'), fluorescence (2, 2'), and one-photon excitation anisotropy (3, 3') spectra of **1** in silicon oil (1, 2, 3) and pTHF (1', 2', 3'). (b) Absorption (1, 1'), fluorescence (2, 2'), and one-photon excitation anisotropy (3, 3') spectra of **2** in silicon oil (1, 2, 3) and pTHF (1', 2', 3'). (c) Absorption (1, 1'), fluorescence (2, 2'), and one-photon excitation anisotropy (3, 3') spectra of **3** in silicon oil (1, 2, 3) and pTHF (1', 2', 3'). (d) Absorption (1, 1'), fluorescence (2, 2'), and one-photon excitation anisotropy (3, 3') spectra of **4** in silicon oil (1, 2, 3) and pTHF (1', 2', 3').

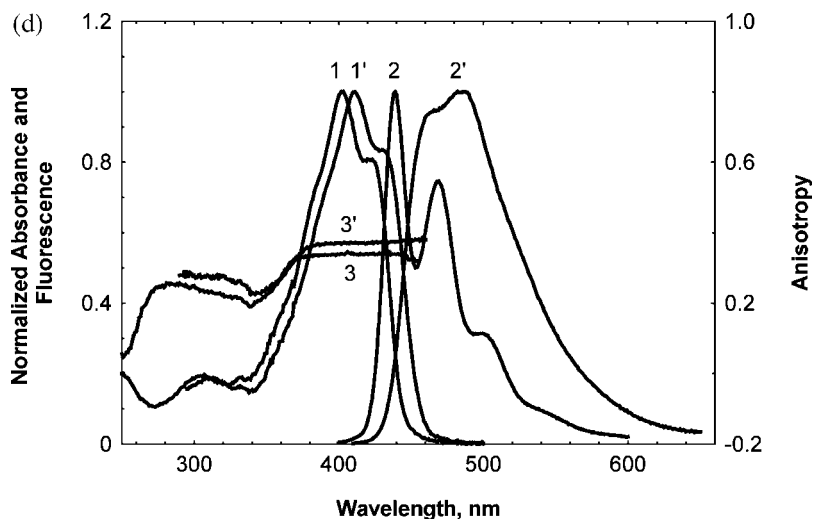


Fig. 2. Continued

electronic structure of the molecules. This orientation may affect the efficiency of one- and two-photon absorption processes and is a subject of great interest [2,25].

In general, one is unable to determine the relative orientation between different ground state transition moments from the excitation anisotropy spectra if $r_{1PA}^{\max} \neq 0.4$ in the $S_0 \rightarrow S_1$ transition. Only the angles between $S_0 \rightarrow S_1$ absorption and $S_1 \rightarrow S_0$ emission (β_1), as well as between $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_0$ (β_2), can be calculated from Eq. (2a) at known values of one-photon excitation anisotropy r_{1PA}^{\max} and r'_{1PA}^{\max} , respectively (see Table II). From this data, it was found that the value of β_1 strongly depended on the solvent properties and can be altered more than 5-fold, for example, see the data for fluorene **4**. In contrast, the value of β_2 was nearly solvent-independent for **1–4**. This behavior facilitated the determination of the angles, α , between the orientations of $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions.

As follows from the above discussion and illustrated in Fig. 3, the angles β_1 and β_2 are placed in perpendicular planes and, therefore, $\beta_2 \approx \alpha$. Thus, in this case angle α , for all compounds **1–4**, can be estimated from the excita-

tion anisotropy data. These data are presented in Table III along with the corresponding 2PA cross sections, σ_{2PA} , obtained by the upconverted fluorescence method [26]. Table III also contains the values of another important parameter, $\Delta\mu$, i.e. the change in the permanent dipole moments obtained from the Lippert equation [2].

Analysis of the data presented in Table III made it reasonable to assume that the angle α (between $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transition moments) can play an important role in 2PA processes. For example, the symmetrical fluorenes **2** and **4** have nearly the same values of $|\Delta\mu| \approx 5\text{--}10$ D but exhibit a considerable difference in σ_{2PA} . It follows from the data in Table III that a decrease in the angle α (from 59–60 to 25–29°) leads to an increase in σ_{2PA} . This trend was found for σ_{2PA} relating to both absorption bands with the peak positions at 390 nm (S_1 is a final state) and 310 nm (S_2 is a final state). A systematical increase in σ_{2PA} at 310 nm, corresponding to the absorption into S_2 -state, is known to be associated with the quantum mechanical symmetry rules governing one- and two-photon absorption processes [27]. Thus, our experimental data show that a more parallel orientation of $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$

Table II. The Values of One-Photon Excitation Anisotropy, r_{1PA}^{\max} , r'_{1PA}^{\max} , and Corresponding Angles, β_1 and β_2 , for **1–4** in Silicon Oil and pTHF

Solvent compound	Silicon oil				pTHF			
	1	2	3	4	1	2	3	4
r_{1PA}^{\max}	0.23 ± 0.002	0.255 ± 0.005	0.283 ± 0.003	0.34 ± 0.003	0.363 ± 0.003	0.377 ± 0.003	0.38 ± 0.004	0.398 ± 0.003
$\beta_1, ^\circ$	32.2 ± 0.5	29.4 ± 0.5	26.2 ± 0.5	18.4 ± 0.5	14.4 ± 0.5	11.3 ± 0.5	11.5 ± 0.5	3.3 ± 0.5
r'_{1PA}^{\max}	0.062 ± 0.005	-0.042 ± 0.004	0.125 ± 0.003	0.254 ± 0.003	0.096 ± 0.005	-0.047 ± 0.005	0.15 ± 0.006	0.29 ± 0.02
$\beta_2, ^\circ$	48.6 ± 0.5	59.1 ± 0.5	42.8 ± 0.5	29.6 ± 0.5	45.4 ± 0.5	59.2 ± 0.5	40.2 ± 0.5	25.4 ± 0.5

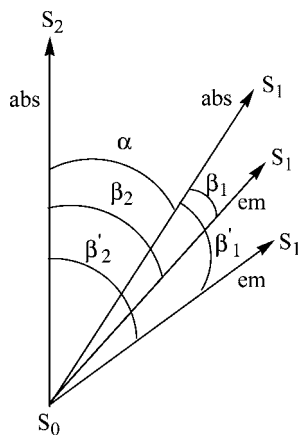


Fig. 3. The directions of the absorption $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$ and emission $S_1 \rightarrow S_0$ transition moments of fluorene derivatives in pTHF (corresponding angles β_1 , β_2) and silicon oil (corresponding angles β'_1 , β'_2).

absorption transition moments can lead to an increase in σ_{2PA} (discussed further below).

The second important point of this work is associated with the comparison of the two- and one-photon excitation anisotropy spectra. The two-photon excitation anisotropy spectra of **1–4** in silicon oil and pTHF are shown in Figs. 4a and b. As can be seen, the anisotropy values of **1–3** and **4** (in pTHF) under the two-photon excitation remain nearly constant over the entire spectral region (Figs. 4a and b, curves 1, 1', 2') and slightly increase for **4** in silicon oil at $\lambda_{exc} \leq 330$ nm (Fig. 4b, curve 2). In contrast, the one-photon excitation anisotropy exhibited considerable changes upon excitation into the second excited state S_2 .

This somewhat unusual behavior of two-photon anisotropy can be explained by a simplistic mechanism of 2PA process based on the single intermediate state (SIS) approach [28]. In this case, the molecule can be modeled by the two arbitrary oriented linear oscillators, which simultaneously absorb two-photons and transfer their energy to the emission oscillator. Mutual orientation of these three oscillators (two in absorption and one in emission) determines the value of two-photon anisotropy, r_{2PA}^{max} . The parallel orientation of all oscillators corresponds to

Table III. The Angles α , Two-Photon Absorption Cross Sections, σ_{2PA} , and Changes in the Stationary Dipole moments, $\Delta\mu$, for **1–4**.

Compound	$\alpha, ^\circ$	σ_{2PA} (GM)		$\Delta\mu$ (D)
		$\lambda_{exc} = 390$ nm	$\lambda_{exc} = 310$ nm	
2	59–60	~10	~140	6–10
1	45–48	~100	~350	20–26
3	40–43	~300	~600	20–30
4	25–29	~1400	~6000	4–10

the theoretical limit of $r_{2PA}^{max} \approx 0.57$. In general case, for estimation of r_{2PA}^{max} , its necessary to find out what real molecular oscillators are involved into 2PA process. In SIS approach, the absorption cross sections, σ_{2PA} , into S_1 and S_2 electronic states can be written as [29,30]:

$$\sigma_{2PA}(S_0 \rightarrow S_1) \sim (|\mu_{01}|^2 |\Delta\mu|^2) / [(E_1 - E_0 - h\omega)\Gamma_{01}], \quad (3a)$$

$$\sigma_{2PA}(S_0 \rightarrow S_2) \sim (|\mu_{01}|^2 |\mu_{12}|^2) / [(E_1 - E_0 - h\omega)\Gamma_{02}], \quad (3b)$$

where the subscripts 0, 1, and 2 refer to S_0 , S_1 , and S_2 electronic states, respectively; E_0 , E_1 , E_2 are the energies of the corresponding electronic states; $h\omega = (E_i - E_0)/2$ ($i = 1$ for Eq. (3a), and $i = 2$ for Eq. (3b)); Γ_{01} , Γ_{02} and μ_{01} , μ_{02} are the damping factors and transition dipole moments for $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$, respectively; $\Delta\mu$ is the change in the permanent molecular dipole moment under the excitation $S_0 \rightarrow S_1$. In this approach, the dipoles μ_{01} and $\Delta\mu$ (for two-photon excitation into S_1), and μ_{01} , μ_{12} (for excitation into S_2) can be considered as real molecular oscillators involved in 2PA process, and can determine the behavior of two-photon anisotropy. Therefore, relatively low values of r_{2PA}^{max} for **2** in silicon oil and pTHF can be explained by the large angle between μ_{01} and $\Delta\mu$. In contrast, a large value of $r_{2PA}^{max} \geq 0.5$ for **4** in silicon oil at $\lambda_{exc} \leq 330$ nm (Fig. 4b, curve 2) corresponds to the small angle between μ_{01} and μ_{12} . Constant values of r_{2PA}^{max} over the region of 280–450 nm (Figs. 4a and b, curves 1, 1', 2') reveal a nearly parallel orientation of μ_{12} and $\Delta\mu$, and S_1 as a dominant intermediate electronic state for both $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ two-photon transitions. Additional quantum-chemical calculations and pump-probe polarization experiments [25] are needed to prove this hypothesis.

In order to find out whether this explanation may be related to other classes of organic molecules, analogous excitation anisotropy measurements were performed for Rhodamine B, under one- and two-photon excitation over a broad spectral region (Fig. 5). As can be seen, the one-photon excitation anisotropy spectrum revealed the nature of absorption bands. Different maxima and minima in this spectrum (curve 2) correspond to the position of the short wavelength absorption bands attributed to the higher excited electronic transitions ($S_0 \rightarrow S_2$, $S_0 \rightarrow S_3$, ...). In contrast, the value of two-photon excitation anisotropy (curve 3) remains nearly constant over the entire spectral region (three electronic transitions) in the same way as for the fluorene molecules **1–4**. Currently, there is not enough information to explain this behavior of r_{2PA}^{max} for

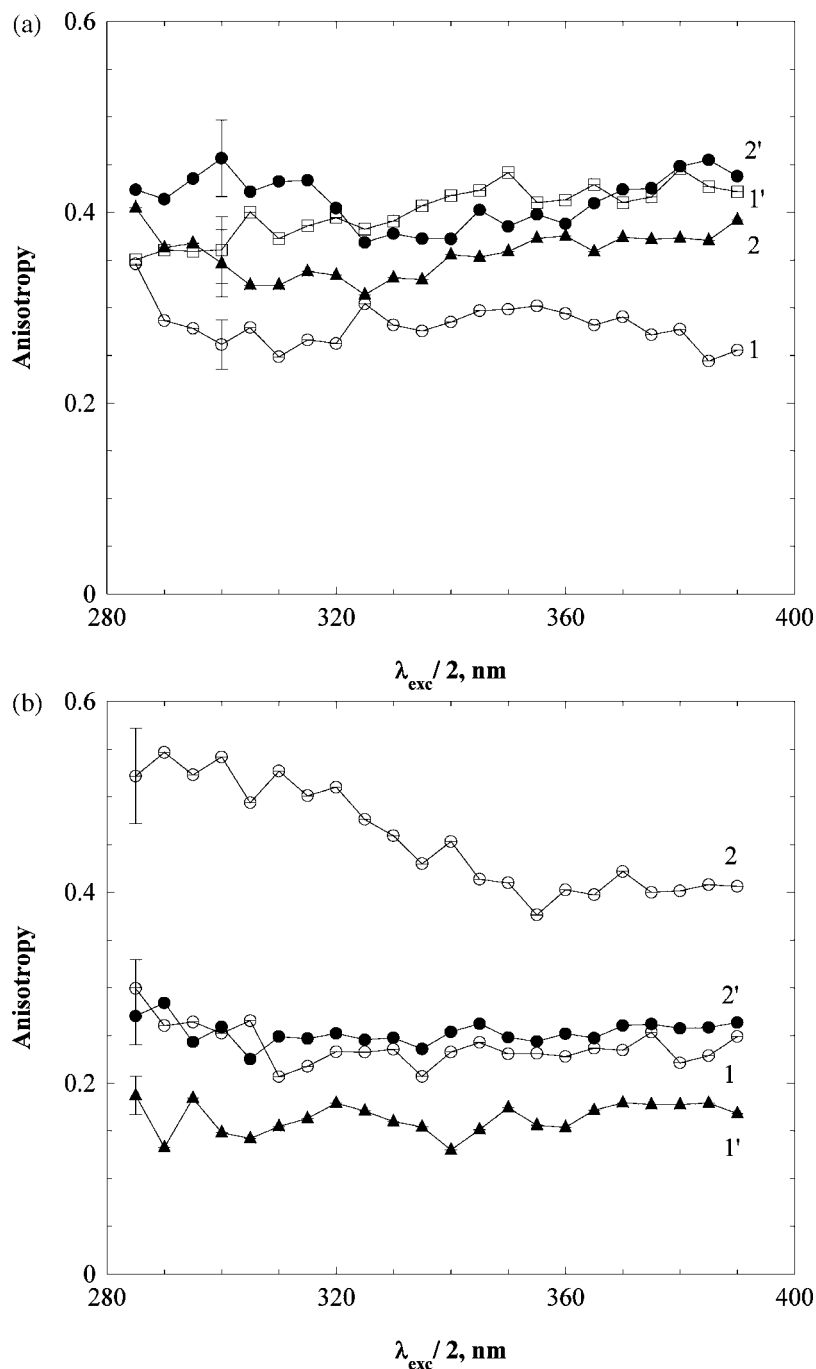


Fig. 4. (a) Two-photon excitation anisotropy spectra of **1** (1, 1') and **3** (2, 2') in silicon oil (1, 2) and pTHF (1', 2'). (b) Two-photon excitation anisotropy spectra of **2** (1, 1') and **4** (2, 2') in silicon oil (1, 2) and pTHF (1', 2').

Rhodamine, especially taking into account nearly parallel orientation of μ_{01} and μ_{12} [31].

From the theory of 2PA, the largest value of σ_{2PA} into S_2 state (all other parameters being equal) corresponds

to the situation in which both transitions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are parallel. Unfortunately, from our measurements, the angles between $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions could not be determined. However, for fluorenes **1–4**,

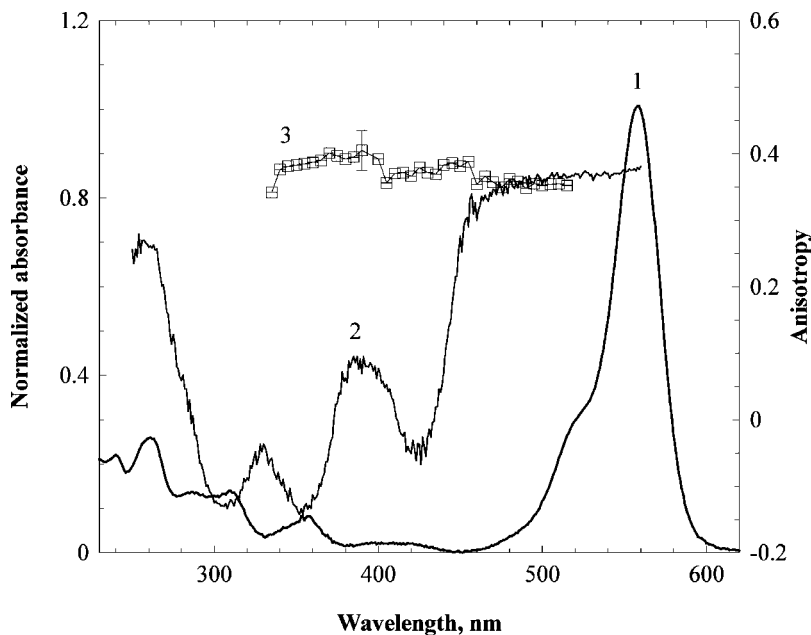


Fig. 5. Absorption (1), one- (2) and two-photon (3) excitation anisotropy spectra of Rhodamine B in glycerol.

the calculated angles α (shown in Table III) may reflect the relative orientation between $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions. Compound **4** can be considered as the analogue of a linear polyene, for which it is known that almost all of the low-lying one-photon absorption bands are aligned along the long molecular axis. Therefore, a relatively small angle α ($<30^\circ$) indicates that not only $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions are close to parallel but $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions are close to parallel as well, leading to an increased value of σ_{2PA} . Certainly, a large increase of σ_{2PA} from **2** to **4** cannot be completely explained by the orientation of their transition dipole moments. Even though the changes in permanent dipole moments (i.e. $\Delta\mu$) for both molecules are similar, the magnitudes of their transition dipole moments (i.e. μ_{01} and μ_{12}) may be quite different. Nonetheless, the effect of the orientation of these transition moments plays an important role in the enhancement of σ_{2PA} .

CONCLUSIONS

The specific electronic structure of the fluorene derivatives **1–4** afforded the determination of the orientation of absorption transition moments $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ from one-photon excitation anisotropy spectra in viscous solutions. The molecules with a more parallel orientation of these transitions exhibited an increased value of 2PA cross sections. This result can be use-

ful for the design of new fluorophores exhibiting strong 2PA.

The values of two-photon excitation anisotropy for **1–4** in pTHF and **1–3** in silicon oil remained nearly constant over the entire spectral region (290–390 nm), and can be roughly explained by a simple model of 2PA process based on a SIS approximation. The same spectral behavior of two-photon excitation anisotropy was found for a representative of another class of organic dyes, Rhodamine B. Additional investigations and quantum-chemical calculations are needed for a deeper understanding of two-photon anisotropy phenomenon.

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