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One- and Two-Photon Absorption and Emission Properties of a Zn(II) Chemosensor

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This paper presents the synthesis and two photon-induced absorption (TPA) properties of a functionalized distyrylbenzene (DSB) **1** containing a tetra-azacyclododecane (cyclen) receptor for Zn(II). The influence of Zn(II) on one- and two-photon absorption characteristics of **1** has been investigated in dimethyl sulfoxide. The experiments show that the TPA action spectrum of uncomplexed **1**, at 750 nm employing nanosecond-long excitation pulses, is 5 times more intense than that of the complexed form. This moderate contrast between the bound and unbound species confirms, however, the potential of this design scheme for the development of molecular structures with enhanced sensitivity and contrast to be used as Zn(II) sensors through TPA-induced fluorescence microscopy.

Introduction

The development of new imaging techniques, capable of reaching three-dimensional (3D) submicrometric resolution, has allowed tremendous improvement in the field of biophotonic and materials science. In particular, fluorescence induced by two-photon absorption (TPA)[1] has flourished in the bio-imaging field as the many papers, which recently appeared in the literature, indicate.[2−4] It is a noninvasive, nondestructive technique that employs IR light (usually in the 700−1000 nm range) as pumping source, which is not absorbed by biological tissues. Also, TPA-induced fluorescence allows a better 3D spatial resolution than other imaging methods based on one-photon processes, for the nonlinear nature of the TPA phenomenon [2,4−6] that limits the emitting sample volume to a close vicinity of the focal point of the IR laser beam.

An important area of research relates to the detection of metal ions in biological samples for a better understanding of their influence on important processes involving, for instance, cell membranes or neural connections. Several research papers have appeared in the literature where a wide variety of dyes have been used for TPA-induced fluorescence ion sensing.[3,4,6] However, the majority of the reported dyes, mostly developed for one-photon-induced sensing through fluorescence changes in intensity and/or in spectral position upon ion binding, does not possess high TPA cross section (σ_{TPA}). Nearly all commercial dyes for metal recognition show σ_{TPA} values around 10−100 GM (1 GM = 10^{-50} cm^4·s·photons^{-1}·molecule^{-1}),[7] which is indeed quite low if compared to the 100−1000 GM displayed by a series of recently published new chromophores specifically designed to reach high σ_{TPA} in the wavelength region of interest for the biological field.[8−13] In this connection, the rational design of TPA molecular structures has been greatly advanced by the use of a quadrupolar arrangement of electron donor (D) and acceptor (A) moieties, linked by π-conjugated bridges.[13−15] Among them, the distyrylbenzene (DSB) molecular structure has a distinguished position for the large σ_{TPA} of its derivatives and noticeable fluorescence quantum yield.[9,13] This paper deals with the synthesis and photophysical characterization of a soluble TPA chemosensor, based on the DSB structure, as a Zn(II)-specific probe to be used, after further developmental work, as a basis for ion sensing and mapping within cells.

Zinc is among the most abundant metal ions in the human body.[16] It plays a central role as a regulatory agent in cell metabolism,[17] and as a cofactor in many enzymes[18] including those that are involved in gene expression and DNA repair.[19,20] The zinc role in the regulation of neurological processes is currently a hot topic in the biomedical research.[21,22] Mechanisms of zinc homeostasis are widely studied but still leave several open questions.[23] Cells exert a strict control of Zn(II) regulation. However, several studies have revealed that in many human disorders, including cancer, Alzheimer's disease, diabetes, pools of labile zinc accumulates in cells of various organs such as the brain, pancreas, prostate.[24]

Among Zn(II) ionophores [23,25−36] we selected the tetramethylcyclen (TMC) moiety (cyclen = 1,4,7,9-tetrazacyclododecane). Although TMC presents a little lower affinity for Zn(II)[37] than the parent cyclen (pK_d [(cyclen)Zn(OH$_2$)]$^{2+}$ = 16.2 ± 0.2[38]) for the weaker basicity of tertiary amines, it is easier to handle for synthetic purposes.[39]

Recently, a few fluorophore sensitive to ions, working on the two-photons excited fluorescence principle, appeared in the literature.[40−45] In particular, Pond and co-workers[42] reported the synthesis and photophysical behavior of TPA chemosensors made of a DSB moiety functionalized, at one or both ends, with the 1-aza-4,7,10,13-tetraoxacyclopentadecyl (aza-15-crown-5) ligand. The authors found a substantial change of the TPA-induced fluorescence upon Mg(II) binding. They observed, for the DSB bearing one aza-15-crown-5, a TPA action spectrum for the uncomplexed molecule that was 50 times more intense than that recorded for the complexed one. The results, corroborated by theoretical calculations, were attributed to a decrease of the σ_{TPA} induced by a substantial reduction of the electron-donating strength of the crown ether ligand, upon ion binding.

It is worth noting that Bazan and co-workers reported in recent publications that the TPA cross section of DSB deriva-

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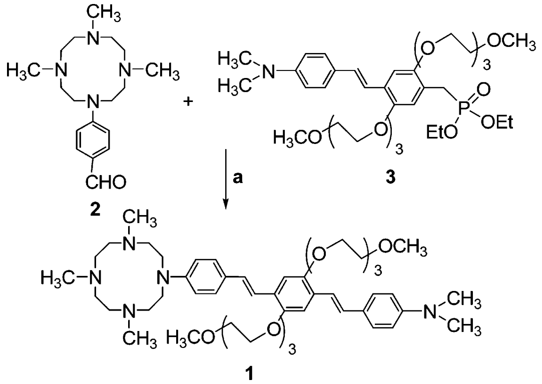
SCHEME 1[a]



[a] Reagents and conditions: (a) *t*-BuOK, DMF, 25 °C, 12 h, 17%.

tives are strongly solvent-dependent, with a reduction by a factor of 5−10 in polar protic solvents, such as ethanol or water. Furthermore, because a large TPA cross section inherently implies a high degree of charge-transfer character in the excited state, the fluorescence emission quantum yield is also affected due to increased solvent−solute interactions.[46]

Experimentals

Materials. All reagents, including dry solvents, were purchased from Aldrich and used without further purification. The aldehyde **2** (Scheme 1) was prepared from *N*-phenyl-4,7,10-trimethyl-1,4,7,10-tetra-azacyclododecane[39] through formylation with $P_2O_3Cl_4$ in dry DMF.[47] Phenylhydrazino−sulfonyl−propionyl−polystyrene resin (PS-TsNHNH$_2$) was purchased from Argonaut Technologies, UK.

Instrumentation. Melting points were measured on a Leitz Laborlux 12 melting point apparatus and are uncorrected. Thin layer (TLC) and column chromatography were performed using a Polygram SilG/UV254 (TLC plates) and silica gel MN 60 (70−230 Mesh) by Macherey-Nagel. ^{1}H (250.1 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on a Bruker AC−F 250 spectrometer. The HR mass spectra were taken on a MALDI-TOF/TOF, 4700 proteomic analyzer from Applied Biosystem equipped with a Nd:YAG pulsed (200 MHz) laser at 355 nm. IR spectra were recorded on a Perkin-Elmer FT-IR model 1720X. Linear absorption (**1**: 2.2×10^{-5} M in DMSO) and emission (**1**: 1.1×10^{-5} M in DMSO) spectra were recorded with a Cary 5 spectrophotometer and a LS50 Perkin-Elmer fluorometer. The fluorescence quantum yield (η) was measured with a LS50 Perkin-Elmer fluorimeter and evaluated using a 1×10^{-5} M solution of fluorescein in water at pH = 11 as an internal standard.[48,49]

TPA-induced fluorescence excitation spectra were collected with the following experimental setup. An excimer pumped dye laser delivering 10 ns long pulses in the wavelength range 690−840 nm at 10 Hz repetition rate was used as the two photon excitation source. The fluorescence, collected at 90°, was detected by a photomultiplier tube and averaged using a Labview computer program. The stray-light from the pump pulses was filtered by a solution of CuSO$_4$ in water, placed just in front of the detector and used as a cutoff filter at 580 nm. The fluorescence spectral window detected by the experimental setup was 350−580 nm. Care was taken in the data analysis to account for the presence of the cutoff filter. At each excitation wavelength the fluorescence intensity was measured as a function of input pump energy, to find the region where a quadratic behavior was established. This procedure is necessary to guarantee that the fluorescence is produced by a TPA process. The TPA action spectra, given by the product of the molecular TPA cross section, σ_{TPA}, and the fluorescence quantum yield η of the investigated probe, have been measured using a solution of fluorescein in water at pH = 11, whose TPA spectrum is available in the literature,[7] as a standard. The TPA spectra can then be computed, if the fluorescence quantum yield of the sample under investigation and of the molecular reference are known. Because of the limited sensitivity of our experimental setup, we have been able to work only with relatively concentrated solutions, typically in the range of 10^{-3} M for both the reference and the investigated chromphore. No aggregate formation was observed in solution, even at such a high concentration.

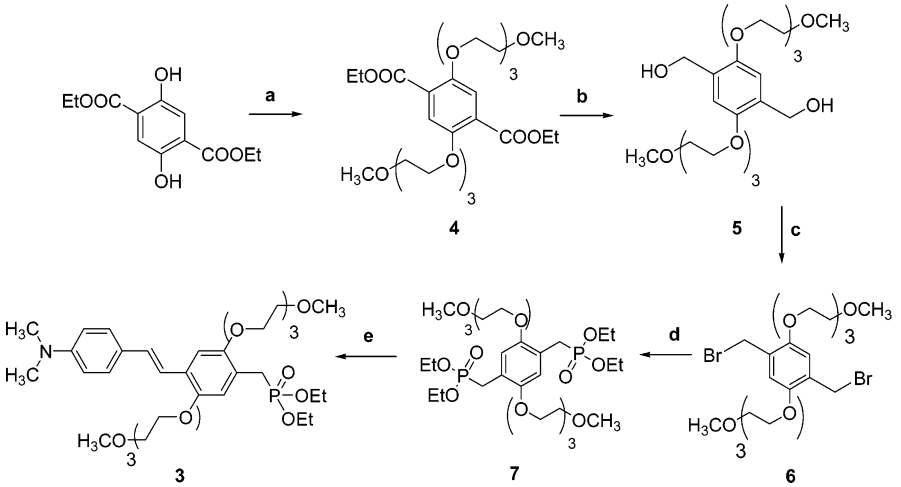
Spectrophotometric Titrations. Solutions for spectrophotometric measurements and titrations were prepared using HPLC-grade DMSO. The binding constant determination for **1-Zn(II)** was performed by plotting the fluorescence changes as a function of [Zn(II)]. Fitting of the titration curves was carried out with the software Scientist 2.01.[50] A model involving the formation of a 1:1 complex of Zn(II) with **1** was used. Total concentration of **1** was set as invariable parameter.

Synthesis of DSB 1. Sodium hydride (21 mg, 0.53 mmol) was added, under a nitrogen atmosphere, to a solution of phosphonate **3** (94 mg, 0.13 mmol) in 5 mL of dry THF kept at the temperature of an ice-bath. After 30 min, a solution of aldehyde **2** (43.5 mg, 0.13 mmol) in 3 mL of dry THF was added. When the addition was complete, the solution was warmed to room temperature and stirred for 12 h. Traces of unreacted aldehyde **2** were removed by addition of the scavenger PS-TsNHNH$_2$ resin (160 mg, 2.70 mmol/g) followed by the addition of a few drops of acetic acid. The residue, after filtration of the resin, was purified by flash chromatography (SiO$_2$, ethyl acetate/petroleum ether/EtOH/NH$_3$ 3:3:3:1). 19 mg (17%) of pure **1** were isolated as an orange oil. ^{1}H NMR (250 MHz, CDCl$_3$) δ (ppm): 7.36−7.30 (m, 4H), 7.22−7.16 (d, 1H, $^3J_{\mathrm{HH}} = 16.0$ Hz), 7.19−7.13 (d, 1H, $^3J_{\mathrm{HH}} = 16.0$ Hz), 7.05 (s, 2H), 6.97−6.91 (d, 1H, $^3J_{\mathrm{HH}} = 16.0$ Hz), 6.95−6.89 (d, 1H, $^3J_{\mathrm{HH}} = 16.0$ Hz), 6.66−6.55 (m, 4H), 4.15−4.11 (m, 4H), 3.87−3.81 (m, 4H), 3.70−3.47 (m, 20H), 3.28 (s, 6H), 2.91 (s, 6H), 2.82−2.78 (m, 4H), 2.52 (m, 8H), 2.32 (s, 6H), 2.19 (s, 3H). ^{13}C NMR (62.9 MHz, CDCl$_3$) δ (ppm): 151.24, 150.71, 149.98, 147.35, 132.12, 129.98, 128.66, 128.43, 127.81, 127.55, 127.16, 126.41, 118.86, 112.42, 111.78, 111.26, 110.74, 71.86, 70.87, 70.85, 70.79, 70.63, 70.52, 69.93, 69.88, 69.36, 69.26, 55.58, 55.45, 54.75, 49.94, 43.79, 43.53. IR (KBr) ν (cm^{-1}): 2926 ($\nu_{\mathrm{C-H}}$), 2874 ($\nu_{\mathrm{C-H}}$), 1351 ($\nu_{\mathrm{C-N}}$), 1060 ($\nu_{\mathrm{sC-O-C}}$), 965 ($\delta_{\mathrm{C=C-H}}$). HR-ESI MS: ($C_{49}H_{75}N_5O_8$) 862.5634 [M + H]$^+$. $\Delta = 6.94$ ppm.

Results and Discussion

We considered the nonsymmetrical substituted distyrylbenzene **1** in which the electron density shifts, upon photoexcitation, from the side aromatic rings toward the center of the molecule (push−push system). This was accomplished through the use of the cyclen and *N*,*N*-dimethylamino electron donating moieties in the DSB structure. DSB **1** was synthesized through a Horner-Emmons Wittig coupling reaction of aldehyde **2** with diethylphosphonate **3** in 17% yield (Scheme 1). The yield refers to the isolated compound after column chromatography that turned out to be a rather difficult task because the highly polar **1** tends to irreversibly bind to the stationary phase (either silica gel or alumina). The trans−trans configuration of the double bonds in DSB **1** was established via NMR spectroscopy, through the measurement of the $^3J_{\mathrm{HH}}$ of the olefin protons which was found to be around 16 Hz, as reported in the literature.[51]

SCHEME 2[a]



[a] Reagents and conditions: (a) 2-[2-(2-methoxyethoxy)ethyl]-*p*-toluenesulfonate, K_2CO_3, DMF, 60 °C, 48 h, 96%; (b) $LiAlH_4$, THF, 0 °C-reflux temperature, 0.5 h, 93%; (c) PBr_3, THF, 0 °C-reflux temperature, 5 h, 71%; (d) $P(OEt)_3$, Δ, 3 h, 84%; (e) 4-(*N*,*N*-dimethylamino)benzaldehyde, *t*-BuOK, DMF, 0 °C, 12 h, 24%.

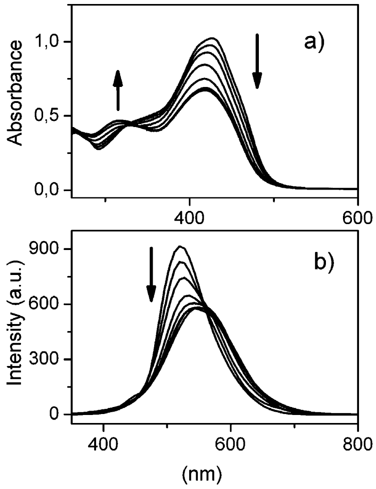


Figure 1. UV−vis spectral changes (a) and fluorescence spectral changes (b) observed upon the addition of $Zn(ClO_4)_2{\cdot}6H_2O$ to 2.2 × 10^{-5} M (a) and 1.1 × 10^{-5} M (b) of **1** in DMSO. The arrows point in the direction of increasing amount of Zn(II).

Phosphonate **3** was obtained by the monofunctionalization of bisphosphonate **7** with 4-(*N*,*N*-dimethylamino)benzaldehyde in 24% yield (Scheme 2). Compound **7** was synthesized from dibromobenzyl derivative **6** that, in turn, was prepared from bisalcohol **5** and PBr_3 in 71% yield, followed by a Michaelis−Arbuzov reaction with triethyl phosphite in 84% yield. Bisalcohol **5**, bearing the oxyethylene solubilizing chains, was obtained in 89% overall isolated yield through nucleophilic displacement of 2-[2-(2-methoxyethoxy)ethyl]-*p*-toluensulfonate and commercial ethyl 1,4-dihydroxy-2,5-terephthalate, followed by $LiAlH_4$ reduction of the ester functions. The details for the synthesis and characterization of phosphonate **3** and intermediates **4**−**7** will be reported elsewhere.[52]

Linear Absorption and Emission. Figure 1 shows the evolution of the absorption (a) and emission (b) spectra of **1** as the titration with Zn(II) proceeds.

As it can be noticed, the absorption spectrum of uncomplexed **1** shows a broad band whose maximum falls at 426 nm, and a pronounced shoulder around 336 nm. Upon addition of Zn-$(ClO_4)_2$ there is a decrease of absorbance for the 426 nm band, whose maximum shifts to 420 nm, and a new peak is formed at 314 nm. These two peaks were attributed to the complex of **1** with Zn(II) (**1-Zn(II)**). Analysis of the absorption as well as the fluorescence data, performed with the Scientist software package[50] considering a model involving the formation of a 1:1 complex **1-Zn(II)**, yielded a lower limit of 10^6 for the binding constant (K_d). This value points out that the TMC ring has a high affinity for Zn(II), as already reported in the literature.[37]

A somewhat different behavior is observed for the fluorescence spectra of compound **1** upon titration with Zn(II), as depicted in Figure 1b. All the fluorescence spectra were excited at 330 nm. At this wavelength the solution absorbance was almost constant throughout the whole titration. Although there was not a noticeable change in the overall fluorescence intensity during the titration, there was a marked change in the shape of the spectra. Although compound **1** shows a maximum at 519 nm, when Zn(II) was added the 519 nm maximum shifted toward longer wavelengths and the spectrum broadened. When excess Zn(II) was added, the fluorescence maximum sets at 548 nm. The fluorescence quantum yield η turns out to be 0.31 ± 0.05 and 0.32 ± 0.05 for compounds **1** and **1-Zn(II)** respectively.

The behavior observed in the absorption and emission spectra, upon Zn(II) complexation, can be attributed to a lowering of the effective overall symmetry of **1** upon Zn(II) addition. Before the addition of Zn(II), chromophore **1** has a D−π−D′ structure. Complexation with Zn(II) induces a higher localization of the electrons of the cyclen ring. The nitrogen atom of the cyclen moiety, which is directly bound to the DSB structure, changes its nature going from an electron donor to a weak electron acceptor group, with respect to the central phenylene group. The molecule thus becomes a D−π−A system with a nonsymmetrical electronic structure. The redistribution of oscillator strength in the absorption spectrum of compound **1** upon Zn-(II) complexation and the appearance of a new peak at 314 nm is likely a manifestation of such a symmetry loss.

The shift of the maximum in the fluorescence spectrum toward lower wavelengths, upon Zn(II) complexation, can be attributed to a stronger stabilization of the D−π−A molecule with respect to the D−π−D′ one, through dielectric interactions with the highly polar DMSO solvent.

TPA Spectra. The two photon absorption properties of **1** and **1-Zn(II)** have been investigated by means of the TPA-induced

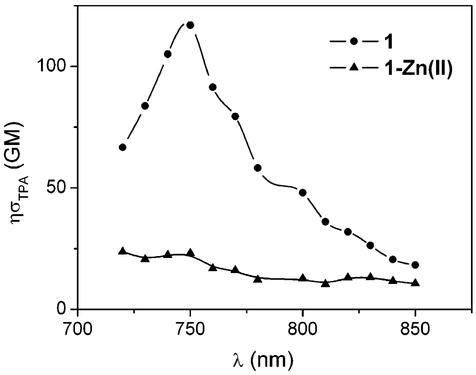


Figure 2. Two photon action spectra of **1** (circles) and of **1-Zn(II)** (triangles) in DMSO (1.0 × 10^{-3} M). The full lines are a guide to the eye.

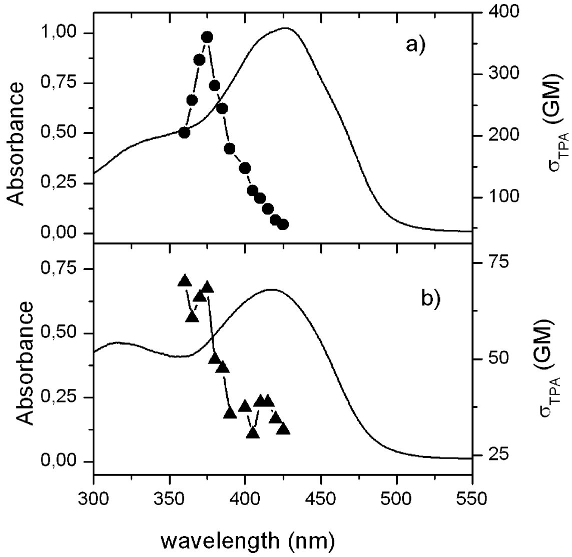


Figure 3. Comparison of the linear (full line) and two-photon (line and symbols) absorption spectra of **1** (a) and of **1-Zn(II)** (b) in DMSO.

fluorescence excitation technique. It has been already demonstrated that, under suitable experimental conditions, this technique is only sensitive to TPA coherent processes, and therefore it gives the same TPA cross section values even if pulses with different time duration are employed.[7,53] Other techniques, such as for example z-scan experiments, show a marked dependence of the experimental TPA cross section on the pulse duration of the exciting laser source.[54] This phenomenon is attributed to the fact that the fluorescence method is solely influenced by the population coherently excited in the two photon allowed state, whereas it is insensitive to sequential absorption processes starting form this excited state, or other excited state populated through relaxation from the initially excited state.

The measurements were performed in DMSO (1.0 × 10^{-3} M for both **1** and **1-Zn(II)**).

At each excitation wavelength, the fluorescence intensity was measured as a function of input pump energy. These data were fitted with a power law whose exponent was a free fitting parameter. Typically, the power law exponent showed a value of 1.9 ± 0.1 in the whole range of wavelengths investigated for the fluorescein reference solution. This was very close to the theoretical value of 2.0, as appropriate for a pure TPA process with no saturation or other concurrent effects. For excitation wavelengths above 780 nm, this exponent was equal to 1.8 ± 0.1 for both compounds **1** and **1-Zn(II)**, whereas it decreased to 1.6 ± 0.1 at wavelengths below 780 nm. This behavior suggests that, in the short wavelength range, **1** and **1-Zn(II)** tend to give rise to saturation starting at lower pump intensities, and hence show stronger deviations toward exponents smaller than 2, because they have larger TPA coefficients than fluorescein.

Figure 2 shows the TPA action spectrum of **1** and **1-Zn(II)** in the 720−850 nm range. It has been found that TPA-induced fluorescence of **1** at 750 nm is 5 times stronger than that of **1-Zn(II)**. These findings revealed an interesting discrimination potential of **1** as an ion sensor: the free ligand shows an intense fluorescence induced by TPA, whereas the same molecule bound to Zn(II) is almost nonfluorescent when excited by a TPA process.

This behavior is not unprecedented for TPA-ionophores. Those proposed by Pond et al.[42] display a quenching of the TPA action spectrum upon Mg(II) binding that is stronger than what we could observe with DSB **1** upon Zn(II) binding.

It is interesting to notice that, though a clear maximum at 750 nm was observed for **1**, **1-Zn(II)** showed a steady increase of the TPA-induced fluorescence as the excitation wavelength decreased. The value of σ_{TPA} for **1** and **1-Zn(II)** can be extrapolated from the action spectra if the fluorescence quantum yield, η, of the reference solution (fluorescein in water at pH = 11) as well as those of the solutions of **1** and **1-Zn(II)** in DMSO were known. If one assumes that η does not change when pumping by either one-photon or two-photon, then the estimates for η quoted above, i.e., 0.31 and 0.32 for compounds **1** and **1-Zn(II)**, respectively, can be used. The σ_{TPA} values, calculated making use of such an approximation, are 360 ± 15 GM for **1** at 750 nm and 68 ± 10 GM for **1-Zn(II)** at 720 nm. The absolute values and relative errors are the result of two sets of independent measurements. The strong difference observed is likely a result of the different electronic structure of **1** and of **1-Zn(II)**, prompted by the TMC ring changing from a D group to an A group when it is bound to Zn(II). It is well-known that D−π−D molecular system show a higher σ_{TPA} than D−π−A ones.[8,9,14,53]

Figure 3 shows one- (full line) and two-photon (data points) absorption spectra of molecule **1** (Figure 3a) and **1-Zn(II)** (Figure 3b). Notice that the TPA spectra are plotted against half the excitation wavelength value to allow comparison of the transition wavelengths for the one-photon and two-photon allowed electronic transitions. For both molecules the TPA peak does not coincide with the one-photon absorption maximum. Although for **1**, this could be expected because it is an almost symmetric D−π−D′ system; this was less obvious for **1-Zn(II)**, where peaks falling at the same wavelengths, though with different amplitudes, could be expected for the linear absorption and the TPA spectra. It has to be pointed out, however, that there is not a clear maximum in the TPA spectrum of compound **1-Zn(II)** (see Figure 3b). To ascertain the presence of a relative maximum of TPA around 420 nm, where the first linear absorption peak falls, one should extend the TPA measurements at wavelengths longer than 840 nm. Unfortunately, our experimental setup did not allow us to extend the measurements to higher wavelengths. It also appears clearly from Figure 3b that the relative intensities of the one- and two-photon allowed transitions are very different, because the transition falling at 314 nm in the linear absorption spectrum is weaker than the one at 420 nm, whereas the opposite appears to be true for the TPA spectrum. This behavior is attributed to an enhancement of the TPA absorption cross section induced by preresonance with the lower energy one photon transition.

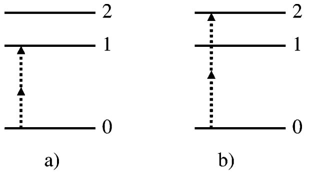


Figure 4. Three-level model showing the enhancement of the σ_{TPA} promoted by the one-photon preresonace effect, which is less effective in (a), where the pump beam is two-photon resonant with the lowest excited state, than in (b), where the pump beam is two-photon resonant with the highest excited state.

If the molecule is depicted as a three-level system:[55]

$$\sigma_{TPA} \propto \frac{\langle|\vec{\mu}_{10}\cdot\vec{e}|^2|\vec{\mu}_{12}\cdot\vec{e}|^2\rangle\Gamma_{20}}{(\omega_{10}-\omega)^2[(\omega_{20}-2\omega)^2+\Gamma_{20}{}^2]} \tag{1}$$

where the subscripts 0, 1, and 2 refer to the ground state, the first and second excited states; $\vec{\mu}_{ij}$, ω_{ij}, Γ_{ij} are the transition dipole moment, the transition frequency and the HWHM relative to transition between states *i* and *j* respectively; ω is the frequency of the laser beam; $\vec{e}$ is a unitary vector describing the polarization of the applied laser, and the angle brackets indicates average over all the possible orientations of the active chromophores. This equation can be used when $(\omega_{10} - \omega) \gg \Gamma_{10}$, $\Gamma_{12} \approx \Gamma_{10}$ and $(\omega_{10} - \omega) \gg [\omega_{20} - (2\omega)]$.

When the excitation frequency (ω) of the applied electromagnetic field is two-photon resonant with the lowest electronic state 1 ($\omega_{10} = 2\,\omega$), as depicted in Figure 4a, the one photon preresonance is described by ($\omega_{10} - \omega = \omega_{10}/2$) in the denominator of eq 1. When, instead, the excitation frequency is two-photon resonant with the higher electronic state 2 ($\omega_{20} = 2\,\omega$), as depicted in Figure 4b, the one-photon preresonance is described by ($|\omega_{10} - \omega| = |\omega_{10} - \omega_{20}/2|$ (which is most likely smaller than $\omega_{10}/2$) in the denominator of eq 1. In the latter case the detuning from one-photon resonance will be smaller; therefore the enhancement of σ_{TPA} due to one-photon preresonance will be larger.

We compared the TPA spectrum measured for **1** with TPA spectra reported for similar D−π−D symmetric distyrylbenzene dyes, where the cyclen ring and the dimethylamino groups were substituted by two identical dibutylamino groups. The measurements were performed in toluene solution (1.0×10^{-4} [13] and 5.0×10^{-6} M[56]). The reported TPA spectra showed good qualitative agreement with the one presented in Figure 3a. The absorption maximum for TPA fell around 730 nm, which is in good agreement with the 750 nm value found for **1**, if one considers the difference in the structures of the two molecules and the fact that they were dissolved in solvents with different polarity and polarizability. On the other hand, the maximum TPA cross section at 730 nm was 900[13] and 1050−1250 GM,[56] respectively, in the literature data. These values are more than twice the one recorded in the present work for **1**. One of the possible reasons for this striking difference might be the higher concentration of the solutions investigated in this paper, with respect to the one employed in the literature. The fluorescence quantum efficiency, η, of molecules in solution may decrease as the solution concentration increases, because of self-quenching phenomena taking place between excited molecules, because there is a higher probability for the excited molecule to encounter each other and be subject to a self-quenching process.[48] Unfortunately, it was not possible to assess η in the highly concentrated solutions used for two photon induced fluorescence measurements. Anyway, if this would be the case, then the σ_{TPA} value calculated from the action spectra would be higher and likely closer to the literature values.

Conclusions

In this paper we present the synthesis and one- and two-photon absorption and emission properties of a Zn(II) chemosensor (**1**) soluble in polar media. The TPA properties of **1** in DMSO have been investigated by the TPA fluorescence excitation technique, in the 720−850 nm wavelength range. It has been found that the TPA-induced fluorescence for the uncomplexed molecule is stronger than that for the molecule bound to Zn(II), in the whole spectral range investigated. The maximum contrast is attained at 750 nm, where a factor of 5 is reached, with the free ligand **1** being more fluorescent than the Zn(II) complex. This behavior is mainly related to the higher σ_{TPA} of **1** (360 ± 15 GM) with respect to **1-Zn(II)** (68 ± 10 GM). It is reasonable to assume, in line with the findings of Pond and co-workers,[42] that the observed σ_{TPA} decrease could be related to a reduction of the electron-donating strength of the cyclen ligand upon Zn(II) binding, whereas the fluorescence quantum yield of the two compounds is almost the same within experimental errors. Finally, it is important to remind the reader that quadrupolar chormophores, based on a symmetrical DSB structure, show a TPA action cross section in water of ∼100 GM, as reported by Bazan and co-workers.[46] These experimental findings cast some doubts on the improved efficiency expected for this class of dyes in biological applications of TPA induced fluorescence microscopy, with respect to currently used fluorescein based sensors. On the other hand, the present study sheds some light on the influence that Zn(II) can play on the linear and nonlinear optical response of an asymmetrical quadrupolar chemosensor, and therefore it can help in the future design of new chemosensors.

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References and Notes

(1) This technique exploits the simultaneous absorption, by a suitable dye, of two photons whose frequency is lower than the threshold frequency for one-photon absorption. The excited dye then emits at a frequency higher than that of the exciting photons.

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