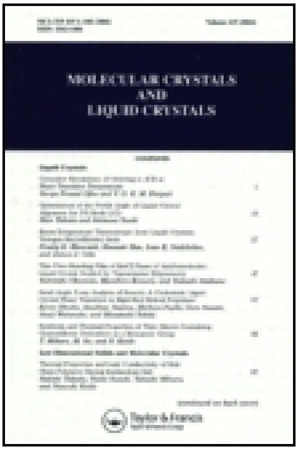

Molecular Crystals and Liquid Crystals

Water-Soluble Two-Photon Fluorophores Based on Paracyclophane

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Version of record first published: 31 Aug 2012.

To cite this article: Han Young Woo & Guillermo C. Bazan (2008): Water-Soluble Two-Photon Fluorophores Based on Paracyclophane, Molecular Crystals and Liquid Crystals, 485:1, 928-933

To link to this article: http://dx.doi.org/10.1080/15421400801925075

Mol. Cryst. Liq. Cryst., Vol. 485, pp. 180/[928]–185/[933], 2008
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ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400801925075

Water-Soluble Two-Photon Fluorophores Based on Paracyclophane

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We report the synthesis, linear and nonlinear spectroscopy of the paracyclophane-based two-photon absorption (TPA) fluorophores. Two-photon induced fluorescence (TPIF) measurements in water show a substantial decrease in TPA cross section (δ) and fluorescence quantum yield (η), relative to toluene. We note that to obtain optimum two-photon action cross sections ($\eta\delta$) in water, one needs to fine tune the intramolecular charge transfer (ICT). Microenvironment modulation using the micellar structure in water increases $\eta\delta$ values similar to those obtained in toluene. The measured $\eta\delta$ values in water are the highest among reported so far, suggesting important guidelines for designing of optimized TPIF molecular-labels.

Keywords: bioimaging; paracyclophane; TPA molecular label; two-photon absorption

INTRODUCTION

Since the two-photon absorption (TPA) phenomenon was theoretically predicted by M. Göppert-Mayer in 1931 [1], two-photon induced fluorescence (TPIF) materials have received considerable attention [2] for molecular labels in two-photon fluorescence microscopy (TPM) which provides the best non-invasive means of biological imaging [3]. The advantages of TPM relative to single-photon counterpart include reduced photodamage, improved depth penetration, the ability to

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-331-D00139).

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image turbid samples and reduced background cellular autofluorescence [4]. The application of TPM in understanding biological processes is increasing exponentially and TPM-specific fluorophores for biomolecular tags are an important consideration.

Best signal to noise is obtained with chromophores having a large two-photon action cross section defined by $\eta\delta$, where η is the fluorescence quantum yield and δ is the two-photon absorption (TPA) cross section. Molecular design and TPA properties in organic solvents have been extensively studied [5,6]. However, the effect of the medium, and particularly water, on the magnitude of δ remains less precisely understood. There are conflicting theoretical expectations on the solvent effects [7]. The currently used fluorescent reporters, which are developed for single-photon microscopy, have $\eta\delta$ values of 1–50 GM in water ($\mathrm{GM} = 1 \times 10^{-50} \cdot \mathrm{cm}^4 \cdot \mathrm{s} \cdot \mathrm{photon}^{-1} \cdot \mathrm{molecule}^{-1}$). These values are much lower than those (more than 1000 GM) in organic solvent. There has been no systematic study on water-soluble TPA fluorophores and TPIF properties in water.

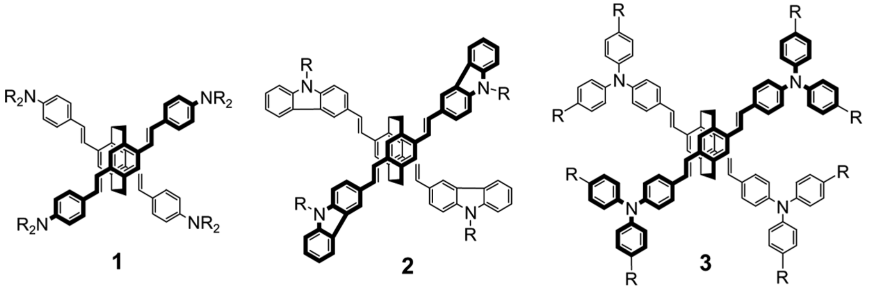
In this contribution, we report the synthesis, linear and TPA spectroscopy of water-soluble TPA chromophores based on the [2.2]paracyclophane. We also discuss the molecular design strategies to get efficient TPA fluorophores in water.

EXPERIMENT

All commercial chemicals were purchased from Aldrich and used as received. All intermediates, including the final water-soluble fluorophores, were characterized by common spectroscopic techniques such as ^{1}H-, ^{13}C-NMR, UV/vis, and mass spectrometry. ^{1}H- and ^{13}C-NMR spectra were collected on a Varian Unity 400 MHz (or 200 MHz) spectrometer. The UV/vis absorption spectra were recorded on a Shimadzu UV-2401 PC diode array spectrometer. Photoluminescence spectra were obtained on a PTI Quantum Master fluorometer equipped with a Xenon lamp excitation source. Mass spectrometry analyses were performed by UC, Santa Barbara, Mass Spectrometry Laboratory. TPA spectra of the compounds were measured using two-photon induced fluorescence spectroscopy [6]. Fluorescein (purchased from Acros Inc.) was used as a reference material for TPA measurements.

RESULTS AND DISCUSSION

The molecular structures of two-photon fluorophores based on [2.2]paracyclophane (pCp) are shown in Figure 1. Different substituents were chosen to modulate the intramolecular charge transfer



N: $R = (CH_2)_6Cl$
C: $R = (CH_2)_6N^+(CH_3)_3I^-$

FIGURE 1 Molecular structures of neutral- and cationic paracyclophane fluorophores.

(ICT) strength from the donor nitrogen groups to the pCp core and to allow the molecules to be neutral (**N** series), and soluble in non-polar organic solvents, or charged and water-soluble (**C** series). Four-fold Horner-Emmons Wittig coupling reactions between the pCp core (4,7,12,15-tetra(diethylphosphonatemethyl)-[2.2]paracyclophane) and three donor structures with aldehyde functionality using potassium tert-butoxide in THF at 0°C give **1N, 2N** and **3N**, respectively, in yields close to 60%. Successive quaternization with a large excess trimethylamine provides **1C, 2C** and **3C**, respectively, in 85 ~ 90% yields.

Table 1 summarizes the absorption and photoluminescence (PL) spectroscopy of the pCp fluorophores. A comparison of the linear spectra of the **C** series in water with those of the **N** series in toluene reveals

TABLE 1 Spectroscopy Summary

	Solvent	λ_{abs} (nm)	λ_{PL} (nm)	η^a	λ_{TPA} (nm)	δ (GM)^b	$\eta\delta$ (GM)
1N	Toluene	434	486	0.92	725	1290	1190
1C	Water	435	553	0.04	725	370	15
1C	SDS (0.05 M)	434	484	0.84	730	1550	1300
2N	Toluene	420	468	0.95	700	1690	1610
2C	Water	410	505	0.42	700	700	290
3N	Toluene	441	492	0.92	770	2080	1910
3C	Water	431	537	0.52	750	690	360
3C	SDS (0.05 M)	440	488	0.95	770	2020	1920

[a] Measured relative to fluorescein at pH = 11 in water.
[b] Peak TPA cross section at λ_{TPA}.

the following trends. The λ_{abs} values are nearly the same (**1N/1C**) or slightly blue shifted (**2N/2C, 3N/3C**). The emissions are broader, with no vibronic structure, and larger Stokes shifts are observed. Most importantly for the design of efficient TPM fluorophores, the η values in water are inversely proportional to the donor strength of the terminal groups ($\mathbf{1N} \sim \mathbf{1C} > \mathbf{2N} \sim \mathbf{2C} > \mathbf{3N} \sim \mathbf{3C}$). A pronounced drop in η is observed for **1C**, while the η for **2C** (0.42) and **3C** (0.52) remain high. The reduced η in water is caused by quenching related to strong ICT in highly polar water. It is also noted that in a solvent of intermediate polarity (DMSO), there are no measurable differences in the linear spectroscopy of the **N** and **C** series. The charged groups therefore are not interacting with the chromophores electronic structures.

TPA spectra were obtained by using the two-photon induced fluorescence (TPIF) technique using a femtosecond pulsed laser source [6]. The wavelength of TPA maximum, λ_{TPA} for **1N, 2N** and **3N** follow the trend of λ_{abs}. Compounds **2N** and **3N** have higher δ than **1N**. Higher δ values may be attributed to additional electronic delocalization within the extended π conjugation. Within the range of frequencies accessible with our instrumentation ($620 \sim 900\,\mathrm{nm}$), the TPA measurements in water show a substantial decrease in δ, relative to toluene (Table 1), with little change in the λ_{TPA} or general band shape. We also measured a clear reduction in η in water owing to ICT related quenching. Combining these results provides for $\eta\delta$ values of 15 GM for **1C**, 294 GM for **2C** and 359 GM for **3C**, respectively, which are much smaller than the values in toluene. The medium effect on δ is not clear at present stage although there have been several theoretical approaches on the solvent effect. Interestingly, the larger $\eta\delta$ values of **2C** and **3C**, relative to **1C**, indicate that maximizing the electron density at the donor nitrogen atom is not necessarily a useful design parameter for TPM applications in water, due to ICT quenching. To improve TPM imaging performance, it is recommended to make the nonpolar microenvironment around the chromophore in water, because typical TPA structures with strong ICT show larger $\eta\delta$ in nonpolar solvents.

Micelles are dynamic microheterogeneous structures containing surfactant molecules and constitute an important research subject [8]. It is possible within their internal environment to include some compounds that are insoluble in water, to perturb the kinetics of many photophysical processes and provide structural mimics of biological membranes. Sodium dodecylsulfate (SDS) is a typical anionic surfactant, displays a critical micelle concentration (cmc) of 8.1 mM at 25°C and forms spherical micelles in aqueous solution.

Table 1 summarizes the PL and absorption spectra of **1C** and **3C** ([**1C**] or [**3C**] = 10^{-5} M) in the presence of SDS at concentrations above the cmc ([SDS] = 0.05 M). Under these conditions the chromophores are incorporated within the micellar core and the probability of finding two chromophores within a single micelle is low. There is virtually no change in the absorption maxima (λ_{abs}), however a significant increase in the molar absorptivity of **3C** is observed in micellar soultion. The PL spectra are blue-shifted, have more pronounced vibronic structures and larger η values are observed (0.84 for **1C** and 0.95 for **3C**), relative to measurements in the absence of SDS. These PL characteristics are similar to those obtained with **1N** and **3N** in toluene.

As shown in Figure 2, there is a significant increase in $\eta\delta$ when one compares the results in water (δ_{max} = 15 GM for **1C**, 360 GM for **3C**) with those in the presence of SDS (1300 GM for **1C** and 1920 GM for **3C**). Indeed, the TPA action spectra under micellar conditions are similar to the results in toluene (1190 GM for **1C**, 1910 GM for **3C**). The combined maximum $\eta\delta$ in SDS aqueous solution is 1300 GM for **1C** and 1920 GM for **3C**, respectively, which are more than 10 times larger than those of the current fluorescent reporters in TPM. These data show a large enhancement of $\eta\delta$ in aqueous micellar solution

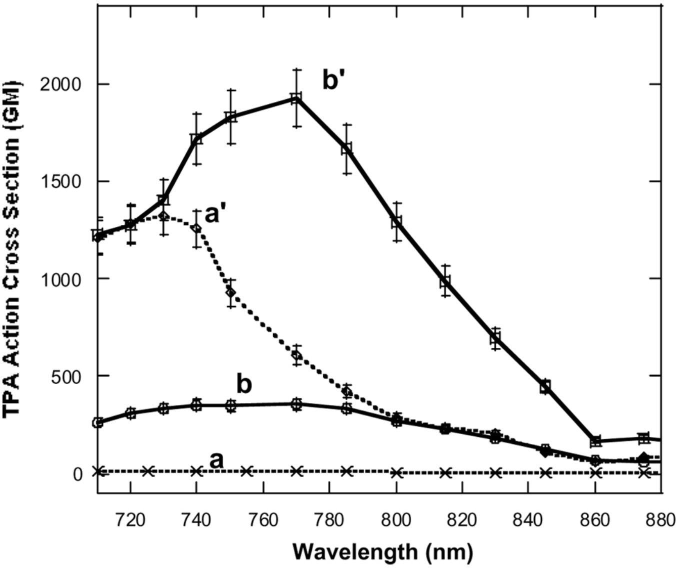


FIGURE 2 TPA action spectra of **1C** and **3C** in water (a, b) and in SDS micellar solution (a′, b′). [**1C**] or [**3C**] = 10^{-5} M, [SDS] = 0.05 M. (GM = $10^{-50}\,\mathrm{cm}^4 \cdot \mathrm{s} \cdot \mathrm{photon}^{-1}$).

by incorporation of the optically active units within hydrophobic microenvironments.

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