Communication

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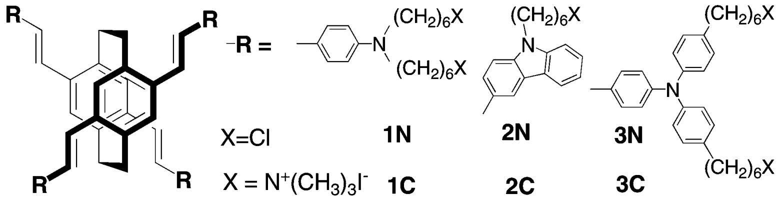

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Water-Soluble [2.2]Paracyclophane Chromophores with Large Two-Photon Action Cross Sections

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Two-photon fluorescence microscopy (TPM),[1] and higher multiphoton variations, provide the best noninvasive means of fluorescence microscopy for biological imaging. The advantages of TPM have been described in detail elsewhere[2] and include reduced photodamage, improved depth penetration, the ability to image turbid samples, and reduced background cellular autofluorescence. The application of multiphoton microscopy in elucidating biological processes is increasing exponentially.[3]

TPM-specific fluorophores for biomolecular tags are an important consideration, since these provide a means to further reduce photodamage and enable the use of more economical laser sources.[2] Two parameters need to be optimized for best performance. One is the two-photon absorption cross section (δ, expressed in GM = 1×10^{-50} cm^4·s·photon^{-1}·molecule^{-1}), which provides the probability of two-photon absorption (TPA) at a particular frequency. A large fluorescence quantum yield (η) is also desired for better signal-to-noise. The product $\eta\delta$ defines the two-photon action cross section of a molecule.

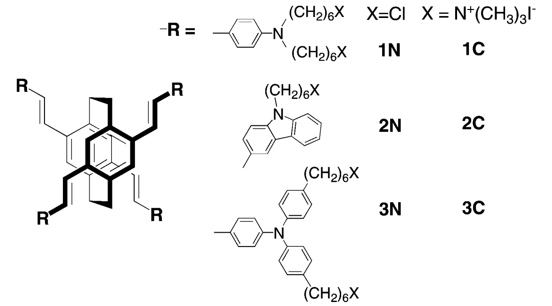
Commonly used fluorescent reporters have δ values in the 1−100 GM range, which give $\eta\delta$ products of 1−50 GM.[2,4,5] Recent molecular guidelines have shown how to obtain $\delta > 1000$ GM.[6] One approach is a quasi linear D−π−D structure, where D is a donor group and π is a π-conjugated bridge. The magnitude of δ depends on the degree of intramolecular charge transfer (ICT) upon excitation.[7] However, in polar aqueous media, necessary for biological samples, the ICT leads to reduced η values and lower overall $\eta\delta$.[8,9]

In this contribution, we report the synthesis of water-soluble TPA chromophores based on the [2.2]paracyclophane (pCp) core which display exceptionally large $\eta\delta$ values. The specific molecular structures are shown in Scheme 1. Each molecule can be considered to contain two distyrylbenzene fragments held together by their internal rings via the pCp core and was expected to have a δ value approximately twice that of the constituent distyrylbenzene chromophore.[10]

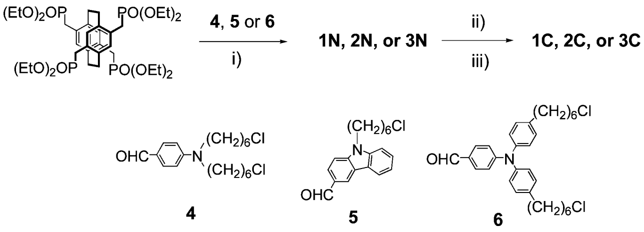
The terminal groups determine the donor ability of the nitrogen atom[6b] and whether the molecule is neutral (**N** series) and soluble in nonpolar organic solvents, or cationic (**C** series) and soluble in water. The overall set of compounds allows the examination of the solvent influence on δ and the effect of donor strength on η.

The synthetic routes are given in Scheme 2. Four-fold Horner−Emmons Wittig coupling reactions between 4,7,12,15-tetra(diethylphosphonatemethyl)-[2.2]paracyclophane[11] and excess **4**, **5**, or **6** using potassium *tert*-butoxide in THF at 0 °C give **1N**, **2N**, or **3N**, respectively, in yields close to 60%. Chloride/iodide exchange of **1N**, **2N**, and **3N**, via the Finkelstein reaction, followed by quaternization with a large excess trimethylamine, provides **1C**, **2C**, and **3C**, respectively, in 85−90% yields.

Scheme 1



Scheme 2 [a]



[a] Reagents and conditions: (i) *t*-BuOK, THF, 0 °C, 6 h; (ii) NaI, acetone/CH_2Cl_2, reflux, 2 days; (iii) NMe_3, THF/H_2O, room temperature, 24 h.

Table 1. Spectroscopy Summary

	solvent	λ_{abs}	λ_{em}	η^a	λ_{TPA}	δ (GM)^b
1N	toluene	434	486	0.92	725	1290
1C	water	435	553	0.04	725	370
2N	toluene	420	468	0.95	700	1690
2C	water	410	505	0.42	700	700
3N	toluene	441	492	0.92	770	2080
3C	water	431	537	0.52	750	690

[a] Measured at 10^{-6} M relative to fluorescein. [b] Peak TPA cross section at λ_{TPA}.

Table 1 summarizes the absorption and photoluminescence (PL) spectra. Figure 1 shows the spectra for **3N** and **3C**, which are typical for the entire series of compounds. Neutral compounds were measured in toluene and the charged counterparts in water.

The maxima in absorption (λ_{abs}) and emission (λ_{em}) for **1N** are similar to those reported for 4,7,12,15-tetra(4′-dihexylaminostyryl)-[2.2]paracyclophane,[11] which indicates negligible perturbation by the chloride functionalities. There is a slight blue shift in the λ_{abs} and λ_{em} of **2N**, relative to those of **1N**. The absorption and emission of **3N** are red-shifted relative to those of **1N**. We note that the energy differences between the electronic states of bichromophoric pCp molecules are determined by through-space delocalization (electron exchange) across the pCp core.[12] The main absorption in the UV−vis range corresponds to two closely separated states (IIa and IIb in ref 12a), and the energy difference between these states

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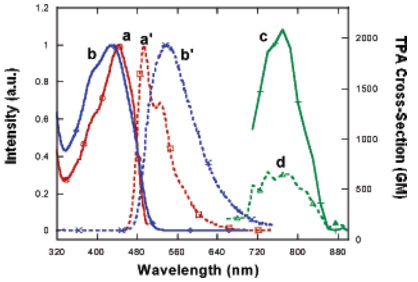

Figure 1. Normalized absorption and PL spectra of **3N** in toluene (a, a′) and **3C** in water (b, b′). PL spectra were collected by exciting at the λ_{abs} of each sample. TPA spectra of **3N** in toluene (c) and **3C** in water (d) (GM = 10^{-50} cm^4•s•photon^{-1}).

determines the overall absorption band shape and ultimately λ_{abs}. A discussion of the relatively small perturbation of solvents on λ_{abs} and λ_{em} is therefore not appropriate at this stage. In toluene, **1N**, **2N**, and **3N** have high η values, in the range of 0.9.

A comparison of the linear spectra of the **C** series in water with those of the **N** series in toluene reveals the following trends (Table 1). The λ_{abs} values are nearly the same (**1C**/**1N**) or slightly blue-shifted (**2C**/**2N**, **3C**/**3N**). The emissions are red-shifted and broader, with no vibronic structure (see Figure 1). 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 (**1N** ∼ **1C** > **2N** ∼ **2C** > **3N** ∼ **3C**).[13] A substantial drop in η is observed for **1C** (0.04), while the η values for **2C** (0.42) and **3C** (0.52) remain high. We also note that, in a solvent of intermediate polarity (DMSO), there are no measurable differences in the linear spectroscopy of the **N** and **C** series (Supporting Information). The charged groups therefore are not interacting with the chromophores.[14]

Two-photon excitation spectra were measured using the two-photon induced fluorescence technique[4,14] using a femtosecond pulsed laser source (see Supporting Information). The TPA maximum (λ_{TPA}) and δ for **1N** in toluene (725 nm and 1290 GM, respectively) are similar to those previously reported for 4,7,12,15-tetra(4′-dihexylaminostyryl)-[2.2]paracyclophane (720 nm and 1410 GM).[10] The λ_{TPA} values for **1N**, **2N**, and **3N** follow the trend of λ_{abs}. Compounds **2N** and **3N** have higher δ than **1N**. Literature precedent shows that arylamine donor groups in D−π−D structures can provide for larger[15] or similar[6b] cross sections relative to their alkylamine counterparts. Higher δ values may be expected on the basis of additional delocalization within the extended π electron system.

Within the range of frequencies accessible with our instrumentation (620−900 nm), the TPA measurements in water show a substantial decrease in δ, relative to those in toluene (Table 1), with little change in the λ_{TPA} or the general band shape (Supporting Information). Combining these results with the determination of η obtained by linear spectroscopy methods provides for $\delta\eta$ values of (in GM) 294 and 359 for **2C** and **3C**, respectively, which are the highest reported action cross sections for chromophores in water.

In summary, we provide a synthetic entry to water-soluble paracyclophane-based TPA chromophores. These molecules have allowed us to measure a substantial decrease in δ when the solvent is changed from toluene to water. It has been predicted theoretically,[16] by looking at the effect of external fields, that δ increases to a maximum point, after which increasing the strength of the field causes a substantial decrease.[7,16b] The larger polarity of water could provide for a similar charge transfer stabilization in the ground and excited states. However, there is no theoretical examination of how hydrogen bonding from the solvent influences the strength of the donor groups at the termini of the π system and how these changes influence δ. A more detailed structure/optical properties relationship analysis therefore awaits theoretical examination. From a practical perspective, the larger $\eta\delta$ values of **2C** and **3C**, relative to that of **1C**, indicate that maximizing the donor strength of the terminal nitrogen atoms is not necessarily a useful design parameter for TPM applications. We propose that weaker donors, such as triarylamine, are a better choice, because the weaker ICT character of the excited state leads to higher η. This search for an optimal balance is likely to be applicable to other molecular systems. Despite the gap in relating molecular structure to optical properties, **2C** and **3C** show exceptionally large action cross sections in water.

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Supporting Information Available: Synthetic details and characterization for all compounds, TPA measurements, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The donor strength order is consistent with the oxidation potential measured by cyclic voltammetry (CV): the measured electrochemical $E(M^+/M)$ is −50 mV for **1N**, 160 mV for **2N**, and 210 mV for **3N** relative to ferrocene. CV was carried out in 0.1 M *n*-Bu$_4$NPF$_6$ in THF with a scan rate of 100 mV/s.

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