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Dicyanostilbene-Based Two-Photon Thermo-Solvatochromic Fluorescence Probes with Two-Photon Triple Fluorescence

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Abstract

The unusually sensitive solvatochromism of the two-photon fluorescence fluorophore of 2,5-dicyano-4-methyl-4’-dimethylaminostilbene (P1) whose emission maximum varies from 445 nm in cyclohexane to 641 nm in DMSO are described. P1 with remarkably large two-photon cross sections exhibits very strong polarity-, viscosity-, and temperature-dependence of fluorescence, and can be used to detect polarities, viscosities, and temperature. P1 presents a two-photon triple fluorescence.

Keywords: Dicyanostilbene; Solvatochromism; Triple fluorescence; Fluorescence probe

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Introduction

For the development of Two-Photon (TP) and Two-Photon Fluorescence (TPF)-optimized molecular probes, it is important to have a significant modulation of the photophysical properties of the chromophore in response to external stimuli, such as polarity, pH, ion concentration, and temperature. At the same time, the chromophore should retain a significant Two-Photon Absorption (TPA) cross section (δ) in a suitable excitation window for biological imaging (700–1000 nm, corresponding to an optimum combination of reduced scattering and absorption in biological samples). In contrast to PET-based fluorescence probes, which only show increasing or decreasing emission intensity at fixed wavelength, solvatochromic probes may indicate the presence of an analyte by a significant change of emission wavelength, which is unequivocally detectable. Although a small number of TPF probes for polarity were reported, and two among them were used for lipid rafts imaging, their applications can’t be extended from polarity detection to viscosities and temperature. Furthermore, in them, small molecule-size always is accompanied by narrow solvatochromic range and small δ [1-5]. So they are not ideal candidates for TP solvatochromic probes. An ideal TP solvatochromic probe should have wide solvatochromic range, small molecule-size, and large δ and excellent photostability. Herein, we report two TP solvatochromic probes (P1 and P2) (**Scheme 1**) derived from 4-methyl-2,5-dicyano-4'-amino stilbene (DCS) with remarkably large δ, which has been successfully employed in the design of TPF probes for metal ions [6-9]. DCS is a push-pull chromophore with a donor-π-acceptor (D-π-A) architecture. It is studied that these two probes are applied to the detection of solvent polarities, viscosities and temperature, especially two-photon triple fluorescence is found for the first time.

The stilbene derivative P1 (P2) was obtained in 65 % (62%) yield by the reaction of dimethylaminobenzaldehyde (diphenylaminobenzaldehyde) with (2,5-dicyano-4-methylbenzyl) phosphonic acid diethyl ester in the presence of sodium hydride (Supporting Information (SI) and **Scheme 2**). The absorption properties of P1 are almost independent on the solvent (**Table 1**). However, with respect to the emission properties, P1 exhibits an unexpected strong solvatochromism (**Figures 1, Supplementary Figures S1 and S2**). Only a few previous examples of solvatochromic behavior for stilbene derivatives (10^{-14}) which just possess one substituent in the single aromatic ring and are not ideal candidates for solvatochromic probes had been reported. Thus the Emission Maximum (EM) for P1 (P2) varies from 445 (452) nm in cyclohexane to 641 (604) nm in DMSO. The reason why the solvatochromic shifts of P2 are somewhat smaller (**Tables S1**) is that both decreasing transition energy and increasing sensitivity to solvent polarity for the weak electron donor diphenylamino group are inferior to ones for the strong electron donor dimethylamino group.

Protic solvents (Hydrogen-Bonding-Donor, HBD) can interact with the donor (dimethylamino) for the ground state of P1 to stabilize the ground state more than the excited state, and thus, lower energy for the ground state results in a relative blue shift as compared with strong polar aprotic solvents (Non-Hydrogen-Bonding-Donor, NHD) (λ_{ex} (EtOH) (606 nm) <λ_{ex} (DMF) (634 nm)). The fitting between EM in wavenumbers and the solvent parameters such as the ET (30) data, the Kosower parameter, Z, the Gibbs energy of activation of the solvolysis (δΔG≠) , the index of solvent dipolarity/polarizability (π*) and the Lippert-Mataga scale (Δf) gave an almost linear relationship (r^2=0.90, 0.90, 0.90, 0.85, 0.82, respectively) (**Figure 2**) [10-18].

Table 1: Photophysical properties of P1.

Solvent^a	λ_{max}^{b}	λ_{max}^{c}	Φ^d	λ_{max}^{e}	δ_{max}^{f}
c-hexane	401	445	0.236	790	5560
Toluene	405	510	0.504	790	2750
Benzene	406	515	0.715	790	3150
Dioxane	401	537	0.812	790	4480
THF	404	577	0.563	790	2820
$CHCl_3$	404	554	0.456	790	3650
Acetone	401	622	0.038	790	1120
DMF	402	634	0.112	790	130
DMSO	409	641	0.013	790	650
MeCN	396	632	0.019	790	980

^{a}Solvents arranged in order of decreasing E_T (30) value. ^{b}Absorption maximum with the lowest energy in nm, *c* (**P1**)=10^{-5} M. ^{c}Emission maximum in nm, *c* (**P1**)=10^{-5} M, λ_{ex}=410 nm. ^{d}Relative to quinine sulfate 10^{-6} M in 0.05 mol L^{-1} H_2SO_4; estimated error, ± 10 % of the given values. ^{e}Two-photon excitation maximum in nm. ^{f}The peak TPA cross-sections in 10^{-50} cm^4 s/photon (GM).

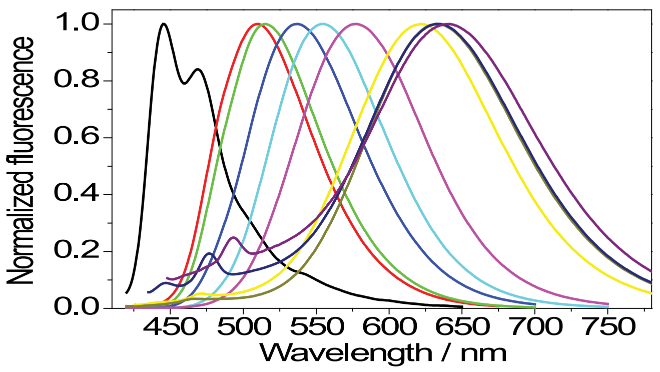
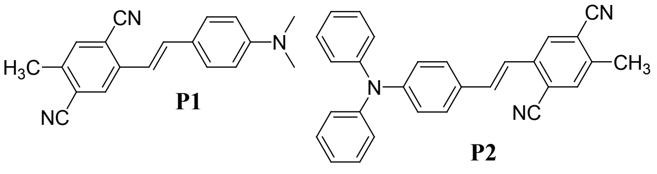
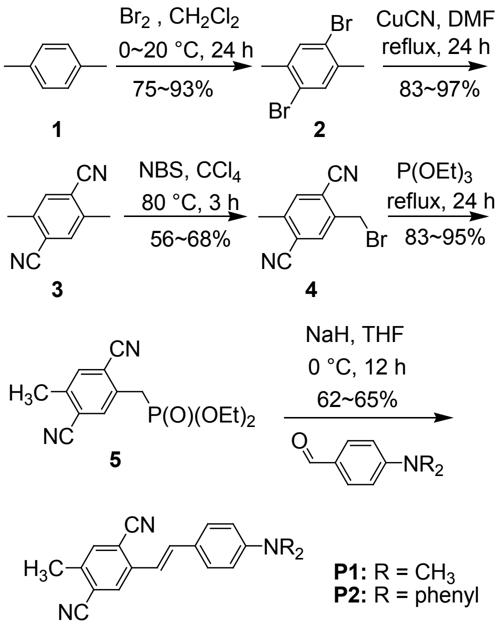


Figure 1 Normalized one-photon emission spectra (left to right: C-hexane, toluene, benzene, dioxane, $CHCl_3$, THF, acetone, MeCN, DMF, DMSO).



Scheme 1 Structure of P1 and P2.



Scheme 2 Synthetic procedure of P1 and P2.

In one- and two-photon emissions (OPE and TPE), band A (^1L_a) of P1 in glycerol all shift to the red with increasing temperature, and the relative intensities of band B (^1L_b) gradually decreases (**Figure 3a and 3b**). The viscosity dependence of the fluorescence for P1 exhibits differences between A band and B band, and between OPE and TPE (**Figure 3c and 3d**). The intensities of B band in OPE and TPE steadily increase upon the augment of viscosities, whereas the intensities of A band in TPE at first rapidly decline, and then enhance, and a third band at 542 nm, namely Exciplex (E) fluorescence from strong intermolecular donor-acceptor systems [19-21], finally disappears in pure glycerol (**Figure 3d**). Never has this property in two-photon event been reported.

High viscosity preventing intermolecular charges from transfer should be responsible for the disappearance of the third band whose no emergence in the OPE possibly consists in its low intensity and short lifetime. In addition, at the low concentration (10^{-6} M) of P1, the intensity ratio (IR) I_a/I_b of the emission bands A and B decreases with increasing viscosity, but at the high concentration (3 × 10^{-6} M) the IR I_a/I_b increases, irrespective of OPE and TPE (**Supplementary Figures S3-S5**). Furthermore, the concentration dependence [22] is found for the IR I_a/I_b of the emission bands A and B of P1 in alcohol, and the intensities of band E progressively increase with increasing concentrations of P1 (**Figure S6**). The above environmental influences upon triple fluorescence bands B, A and E usually indicate three emissive species, i.e., the localized excited state (LE), a twisted

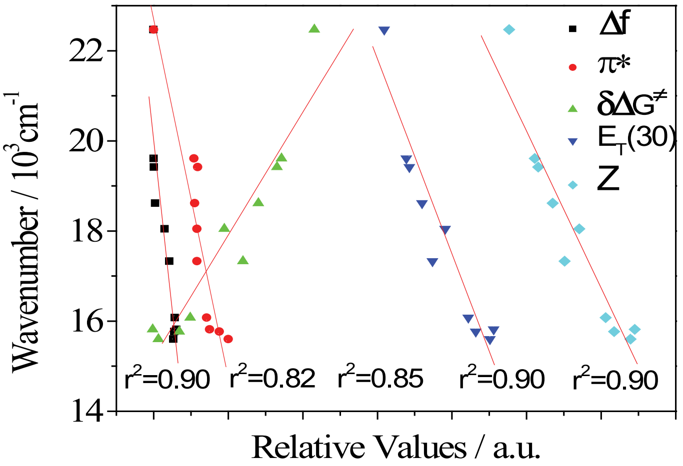


Figure 2 Correlation between the emission maxima (in cm^{-1}) of **P1** ($c=10^{-5}$ M, $\lambda_{ex}=410$ nm) in various solvents and selected solvent parameters such as Δf, π^*, $\delta\Delta G^{\neq}$, $E_T(30)$ and Z.

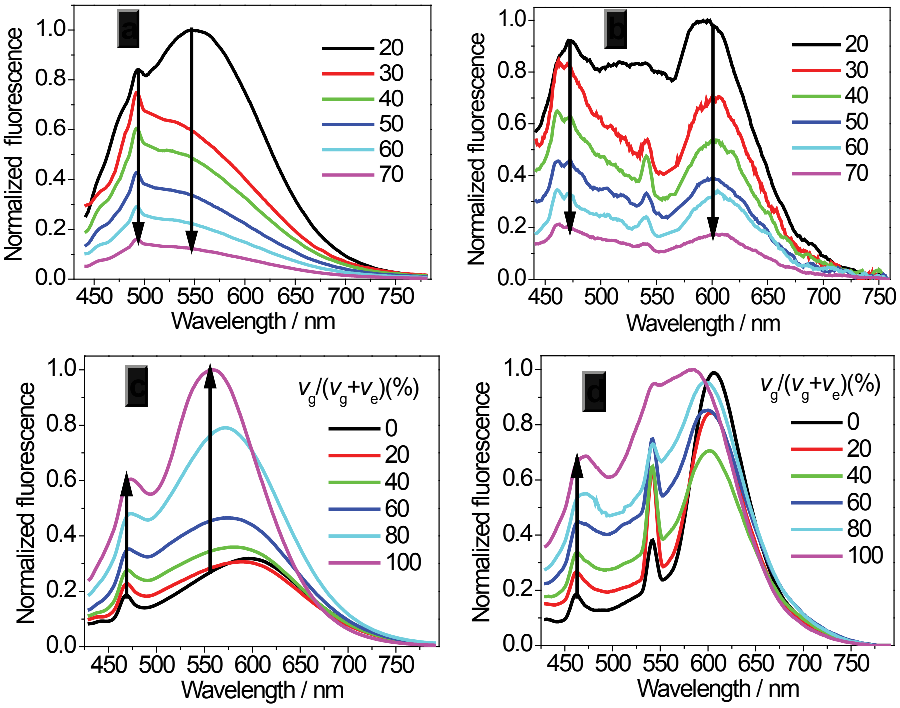


Figure 3 Normalized (c) one- and (d) two-photon emission spectra of **P1** ($c=10^{-6}$ M, λ_{ex}(OP)=410 nm, λ_{ex}(TP)=790 nm) in glycerol vs. temperature (°). Normalized (e) one- and (f) two-photon emission spectra of **P1** ($c=3\times10^{-6}$ M, λ_{ex}(OP)=410 nm, λ_{ex}(TP)=790 nm) in glycerol-ethanol ($v_g/(v_g+v_e)$, %).

intramolecular charge transfer state (TICT) and an intermolecular exciplex [23].

As expected, δ of P1 climbs its maximum of 5560 GM in nonpolar cyclohexane, while in strongly polar aprotic DMF it falls to its minimum of 130 GM. In general, δ values of P1 are much larger in low polarity aprotic solvents (PAS) than in high PAS, which should be attributed to the excited state configuration transformation (**Table 1**). The reduction in emission intensity for P1 in acetone and other more polar solvents together with a near lack of photoreactivity is certainly consistent with decay of the emissive state to one or more of the possible noncoplanar and nonemissive TICT which subsequently decay rapidly to the ground state [12].

As in other solvatochromic systems [24], an Internal Charge Transfer (ICT) due to a strong donor-acceptor interplay between the dimethylamino and the 2,5-dicyano-4-methyl stilbyl moieties can take place in the excited state of P1. On one hand, ortho- and meta-cyano groups of the double bond (C=C) are fairly strong electron acceptor in the ground state, which can remarkably decrease transition energy, stabilize resonance structures and increase both the excited state dipole moment and molecular sensitivity to solvent polarity. On the other hand, two cyano groups can notably extend conjugated system, which consumedly improves δ and the fluorescence quantum yield. Additionally, in the excited state, the adiabatic ICT leads to an intermediate that most likely develops a negative charge for the nitrogen atom of the ortho-cyano group, that is, after solvent relaxation [15,24,25], stabilized not only by polar solvents, especially those with high π*, but also by the meta-cyano group with strong electron-withdrawing capacity for greater diffusion of the concentrated negative charge. This ICT-solvent relaxation sequence explains the significant red shift of the emission spectra in polar solvents as well as the temperature and viscosity dependence of the emission properties [15,25].

In conclusion, we have shown that the covalent attachment of two cyano groups in the single aromatic ring and dimethylamino group to stilbene is a considerably ingenious strategy, which makes the molecule a highly sensitive two-photon thermo-solvatochromic probe with a remarkably large δ and a wide solvatochromic range capable of being used to detect the analytes with high π*, polarities, viscosities, and temperature. It was the first time that TP triple fluorescence was sighted.

Acknowledgments

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