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Synthesis and photophysical properties of novel pyrimidine-based two-photon absorption chromophores

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Abstract—Three new two-photon absorption chromophores based on a pyrimidine core were synthesized by Aldol condensation in the absence of any organic solvents. Their single-photon spectroscopic characterization as well as their two-photon absorption properties is reported. In addition, strong modulation of single-photon and two-photon fluorescent spectra of these molecules by (de)protonation is also discussed.

Although two-photon process was theoretically predicted by Maria Göppert-mayer in 1931 and experimentally observed by Kaiser et al. in 1961, not many reports on the practical applications of two-photon absorption (2PA) were found before 1990s, which is mainly due to the lack of high peak power lasers and appropriate materials with large 2PA cross-sections.[1] A seminal study of Denk et al. on two-photon fluorescence microscopy (2PM) was published in 1990,[2] and it demonstrated that 2PM images with excellent optical sectioning could be obtained without killing cells,[3] and thereafter attracted considerable attentions on the two-photon process for a wide range of applications.[4–9] Large amount of organic chromophores with symmetrical D–π–D or A–π–A conjugation (D: donor, π: π-conjugation, A: acceptor), asymmetrical D–π–A conjugation and multibranch structures were synthesized and reported, where many of them were found to have attractive 2PA characteristics.[10–15] Recently, different types of heterocycle-based chromophores were also proved to have excellent 2PA performance.[16–19] However, only few of these reported molecules have the potentials of being two-photon fluorescence (2PF) probes, mainly due to the synthetic difficulties of the molecules (such as complicated molecular structure, long synthetic routes and harsh conditions), and are less favorable for large-scale production. Therefore, investigating simple and efficient synthesis of 2PA molecules in environmentally friendly condition would be beneficial to the development of 2PF probes.

Pyrimidine derivatives were intensively investigated as electroluminescent materials in the past.[20,21] However, to the best of our knowledge, they were never modified to become 2PA materials. On the other hand, it is well known that styryl group is an effective building block for 2PA chromophores.[22] Therefore, pyrimidine derivatives with styryl group as π-conjugation may serve as a kind of effective 2PA materials. In this Letter, we report the design, green synthesis and spectroscopic characterization of three pyrimidine-based organic chromophores (molecules **1**–**3**).

The synthetic route of molecules **1**–**3** is shown in Scheme 1. In the presence of hot aqueous solution of sodium hydroxide (5 M) and tetrabutylammonium hydrogen sulfate (10%), an Aldol reaction of aromatic aldehydes with 2-amino-4,6-dimethylpyrimidine gave the condensation products (**1**–**3**). Compounds **1**–**3** could be obtained after short reaction times (1 h, 1.5 h and 0.5 h for molecules **1**–**3**, respectively) and high yields (84%, 75% and 94% for molecules **1**–**3**, respectively). The structures of **1**–**3** were identified by NMR, MS and

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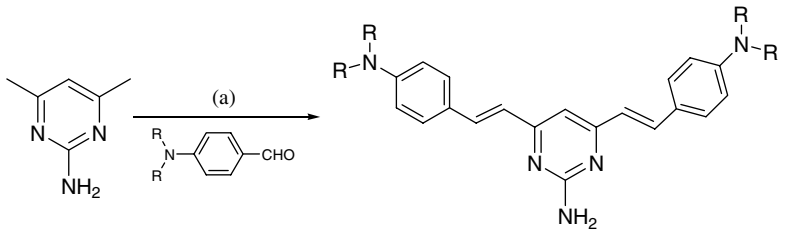

Scheme 1. Reagents and conditions: (a) Tetrabutylammonium hydrogen sulfate, NaOH (5 M), reflux 1 h (84%), 1.5 h (75%) and 0.5 h (94%) for molecules **1**–**3**, respectively.

elemental analysis.[23] Although the longest reaction time was needed for **2**, the yield was the lowest under almost identical conditions. Further experiments revealed that, with increasing the length of the straight carbon chain located at the end of the aromatic aldehyde, the yields of disubstituted products reduced drastically. Particularly, when a butyl side chain was used in the reaction, only 5% of the expected disubstituted product was obtained. In this case, the main product was monosubstituted pyrimidine derivatives. This is probably due to less solubility of longer alkyl groups in the aqueous solution. In addition, when the $N(R)_2$ side group of aromatic aldehydes was replaced by a nitryl group, no product was detected under the same condition. Similar phenomena could be observed when the amine group in the 2-amino-4,6-dimethylpyrimidine ring was replaced by a hydroxyl group.

It is interesting to investigate why the methyl group attached to the pyrimidine ring can easily undergo reactions with an aromatic aldehyde at the absence of any organic solvents. One possible mechanism is that, under a strong base condition, the methyl groups at α position (positions 4 and 6) of pyrimidine ring may form carbanions, which can de-localize their negative charges to the nitrogen atoms of the pyrimidine ring.[24,25] Then, intermediate products may form by an aldol reaction of the aromatic aldehyde (electrophilic reagents) with the carbanions. Finally, with increasing the temperature, the intermediate may get dehydrated and form molecules **1**–**3**. Phase transfer catalyst (tetrabutylammonium hydrogen sulfate) increases the kinetics of the reaction process. If no catalyst was added, no product could be detected under the experimental conditions. It is worthy to notice that the advantages of this reaction are: (1) it is an organic solvent free synthesis (green chemistry), (2) it can be performed in a large-scale, and (3) only filtration and re-crystallization are necessary in the purification.

Single-photon (linear) absorption (1PA) and single-photon fluorescence (1PF) emission peaks of molecules **1**–**3** in solvents of different polarity are summarized in Table 1. It can be seen that, with increasing the solvent polarity for each molecule (polarity: $CHCl_3$ > toluene),[26] the maximum peaks of both the 1PA and 1PF spectra red-shift notably. This result suggests an effective intramolecular charge transfer (ICT) in these molecules.[27,28] ICT increases with increasing solvent polarity and results in a significant red-shifted 1PF spectrum. Molecules **1**–**3** have high quantum yields ranging from 0.55 to 0.88, which decrease with the increasing solvent polarity. These phenomenons might imply twisted intramolecular charge transfer (TICT) geometries between the amino donor and the π-conjugation in the pyrimidine derivatives.[28]

The up-converted fluorescence spectra of all molecules were measured in toluene and $CHCl_3$ at a concentration of 1×10^{-5} M using femtosecond laser as excitation

Table 1. Single-photon and two-photon spectroscopic properties of molecules **1**–**3**[a]

Molecule	Solvent	1PA		1PF		2PA	
		λ_{max}/nm	$\varepsilon/10^4$[b]	λ_{max}/nm	η[c]	σ_{2PA}[d]	$\eta\sigma_{2PA}$
1	Toluene	414	4.4	466	0.65	475	309
	$CHCl_3$	426	3.6	511	0.55	366	201
2	Toluene	418	5.9	468	0.72	596	429
	$CHCl_3$	433	4.5	518	0.61	461	281
3	Toluene	417	7.4	467	0.88	819	721
	$CHCl_3$	432	5.4	526	0.73	625	456
Fluorescein	Water	—	—	—	—	36[e]	—

[a] Single-photon absorption (1PA), single-photon fluorescence (1PF), two-photon absorption (2PA).
[b] Molar absorption coefficient (unit: $M^{-1}\,cm^{-1}$).
[c] Fluorescence quantum yield. Fluorescein in 0.1 M NaOH ($\eta = 0.90$) was used as reference standard.[29]
[d] Two-photon absorption (2PA) cross-section at 800 nm for toluene and 840 nm for $CHCl_3$, respectively. Fluorescein with the same concentration in NaOH (pH 13) was used as reference standard. 1GM (Göppert-Mayer) = 10^{-50} cm^4 s $photon^{-1}$.
[e] Maximal 2PA cross-section (800 nm).[30]

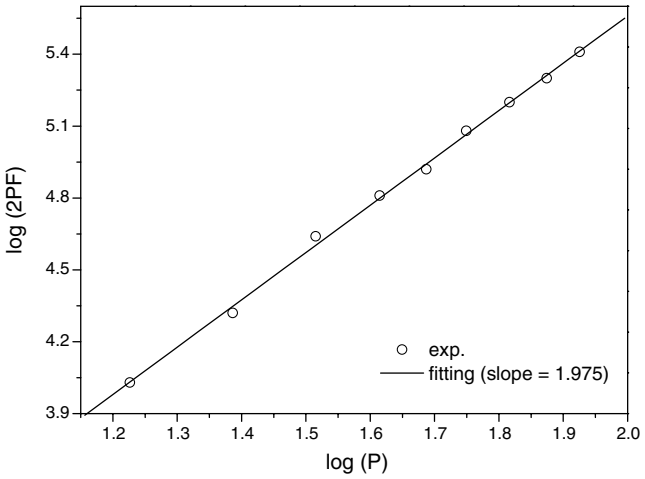

Figure 1. Input intensity dependent up-converted fluorescence of molecule **3** (1×10^{-5} M in $CHCl_3$) excited at 840 nm.

source. The data were recorded in the excitation wavelength ranging from 740 nm to 960 nm with a step size of 20 nm. To further confirm that the up-converted emission underwent 2PA under these conditions, the dependence of the up-converted emission fluorescence intensity on the excitation intensity of these molecules in different solvents was measured, and a representative dependence curve for molecule **3** in $CHCl_3$ is shown in Figure 1. The quadratic dependence indicates that the up-converted fluorescence is induced by 2PA.

The 2PA cross-sections of molecules **1**–**3** were obtained by a comparison of the 2PF spectra of target molecules with fluorescein calibration standard (both at a concentration of 1×10^{-5} M). Detailed procedures were similar to Ref. 30 and had been described in our previous works.[31,32] The 2PA cross-section values for molecules **1**–**3** in $CHCl_3$ and toluene were calculated and are shown in Figure 2. Molecule **3** was found to have the largest 2PA cross-section value (819 GM) in toluene, which is 22 times larger than that of the fluorescein standard.[30] As large 2PA cross-section value is always associated with efficient π de-localization,[33] the above findings imply that these pryimidine-based derivatives with Y-shaped structure may have a fairly good coplanar configuration. It can also be seen from Figure 2 that the 2PA cross-section values gradually increase from molecule **1** to molecule **3**, which is probably attributed to the ICT ability of these molecules.[27,28]

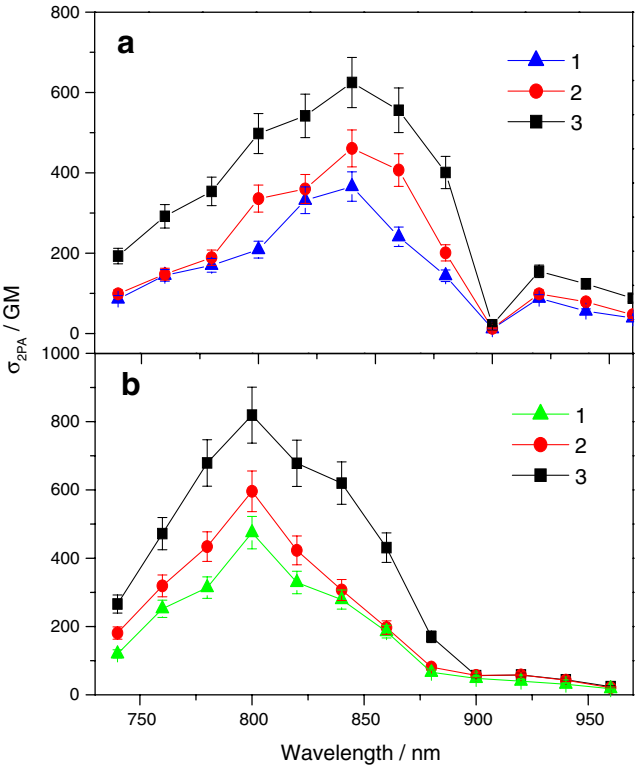

Figure 2. Two-photon excitation spectra for molecules **1**–**3** in $CHCl_3$ (a) and toluene (b).

Experiments on the evolution of the 1PF and 2PF spectra upon protonation and deportation were performed to evaluate the pH probing abilities of these molecules. Representative results for molecule **3** are shown in Figure 3. Upon protonation by trifluoroacetic acid

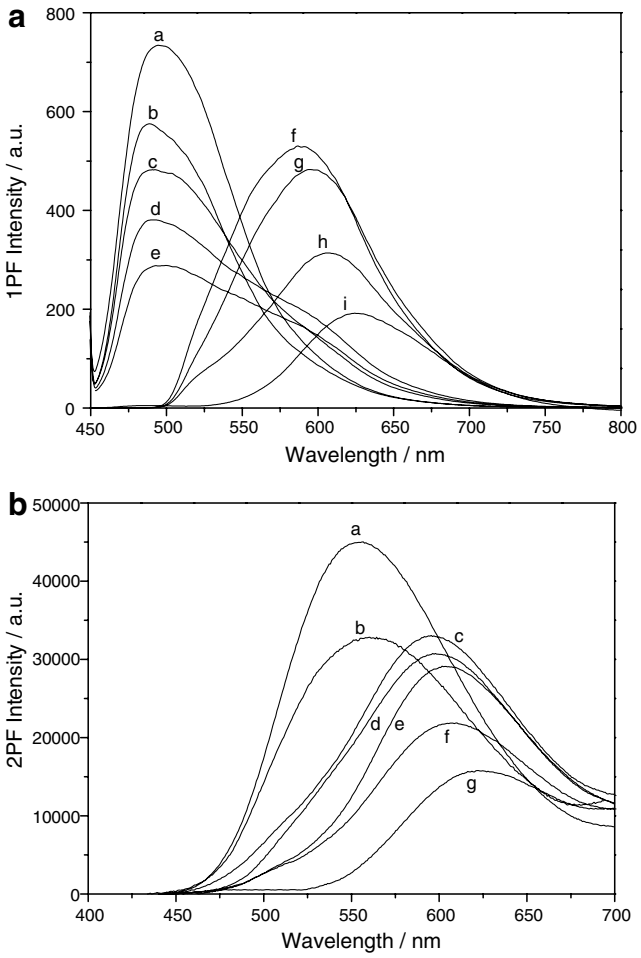

Figure 3. Evolution of single-photon fluorescence (a) and two-photon fluorescence (b) spectra of molecule **3** in DMSO–H_2O (1:1) at a concentration of 1×10^{-5} M, upon protonation or deprotonation. (a–i in (a) pH 10.21, 9.00, 7.00, 6.98, 6.16, 4.98, 4.01, 3.31, 3.26, respectively; a–g in (b): pH 10.21, 8.40, 7.09, 6.21, 5.22, 4.35, 3.32, respectively).

(TFA), a new 1PF peak located at ~590 nm appears with increasing the TFA concentration. This new peak reaches maximal intensity at ~pH 5, and then decreases intensity and shifts to longer wavelength with decreasing the pH value (Fig. 3A). Upon deprotonation by triethylamine, the fluorescence intensity increases with increasing the triethylamine concentration, while no obvious peak shift can be observed (data not shown). Figure 3B shows the 2PF spectra of molecule **3** upon protonation, where an intensity decrease and a peak shift can be observed with decreasing the pH value. The pH-sensitive behavior of these two-photon chromophores makes them promising candidates for biological applications.

Finally, it would be interesting to discuss the role of the amino group in the pyrimidine ring. First of all, the amine group at position 2 of the pyrimidine ring increases the reactive activity of the methyl groups at positions 4 and 6 of the same pyrimidine ring. Secondly, the application of heterocycle-based dyes in pH probing is usually restricted to acid, as they are less sensitive to protons, due to low pK_a values.[34] The amine group at position 2 of the pyrimidine ring can tune the pK_a to higher value. In fact, we found that the pK_a value of one of the molecules reported in this Letter (molecule **1**) in DMSO–H_2O (1:1) (Fig. S3 in Supplementary Information) is about 6.2. Thirdly, the amino at position 2 of the pyrimidine ring can be easily functionalized to a promising amino active fluorescence probe by changing the $-NH_2$ group to an –N=C=S group.[35]

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Supplementary data

Spectroscopic characterizations of molecules **1**–**3** in different solvents are available in the supporting information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.122.

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23. *Experimental procedures and selected data for: General procedure for preparation of the molecules* **1**–**3**: 2-amino-4,6-dimethylpyrimidine (1.24 g, 10 mmol) and 4-[*N*,*N*-di(melthylamino)]benzaldehyde (3.28 g, 22 mmol) were stirred in boiling aqueous solution of sodium hydroxide (5 M, 50 ml) and in the presence of tetrabutylammonium hydrogen sulfate (1 mmol, 0.33 g). The reaction was monitored by TLC (silica gel. $CHCl_3$/methanol 9:1). After cooling the crude product was washed with water and collected by suction filtration. 2-amino-4,6-bis-[(4-*N*,*N*′-dimethylamino)styryl]pyrimidine (**1**): Recrystallized in ethanol/$CHCl_3$ (9:1). Red solid. Yield: 84%; mp 208–210 °C. ESI-MS (*m*/*z*), 386.3 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.71 (d, $J = 15.6$ Hz, 2H), 7.50 (d, $J = 9.0$ Hz, 4H), 6.78 (s, 1H), 6.72 (d, $J = 9.3$ Hz, 6H), 3.02 (s, 12H). Anal. Calcd for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06;

N, 18.17. Found: C, 74.85; H, 7.01; N, 18.23. 2-Amino-4,6-bis-[(4-*N*,*N*′-diethylamino)styryl]pyrimidine (**2**): Recrystallized in ethanol/$CHCl_3$ (9:1). Red solid. Yield: 75%; mp 219–223 °C. ESI-MS (*m*/*z*), 442.5 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.78 (d, $J = 15.6$ Hz, 2H), 7.48 (d, $J = 8.0$ Hz, 4H), 6.70 (s, 1H), 6.66 (d, $J = 9.2$ Hz, 6H), 3.46–3.37 (m, 8H), 1.25–1.18 (m, 12H). Anal. Calcd for $C_{28}H_{35}N_5$: C, 76.15; H, 7.99; N, 15.86. Found: C, 76.09; H, 7.94; N, 15.92. 2-Amino-4,6-bis-[(4-*N*,*N*′-diphenylamino)styryl]pyrimidine (**3**): Recrystallized in DMF. Yellow solid. Yield: 94%; mp 232–234 °C. ESI-MS (*m*/*z*), 634.5 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77 (d, $J = 15.6$ Hz, 2H), 7.47 (d, $J = 8.8$ Hz, 4H), 7.30–7.28 (m, 12H), 7.18–7.14 (m, 8H), 7.11–7.04 (m, 6H), 6.73 (s, 1H). Anal. Calcd for $C_{44}H_{35}N_5$: C, 83.38; H, 5.57; N, 11.05. Found: C, 83.37; H, 5.55; N, 11.09.

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