COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Two-photon induced blue fluorescent emission of heterocycle-based organic molecule†

Z. L. Huang,[a] N. Li,[a] H. Lei,[a] Z. R. Qiu,[a] H. Z. Wang,*[a] Z. P. Zhong[b] and Z. H. Zhou[b]

[a] *State Key Laboratory of Optoelectronic Materials and Technologies, Zhongshan University, Guangzhou 510275, P. R. China. E-mail: stswhz@zsu.edu.cn; Fax: +86-20-84037423*

[b] *School of Chemistry and Chemical Engineering, Zhongshan University, Guangzhou 510275, P. R. China*

Received (in Cambridge, UK) 11th July 2002, Accepted 10th September 2002
First published as an Advance Article on the web 20th September 2002

An organic molecule based on a heterocycle acceptor has been found to exhibit an intensive two-photon induced blue emission and a large two-photon absorption cross section, which implies that the molecule is a promising candidate for an application such as multi-channel two-photon microscopy.

In 1990, Denk *et al.* introduced the two-photon excitation method into laser scanning fluorescence microscopy, achieving highly axial resolution and the possibility of imaging at an increased penetration depth in tissues with reduced photodamage.[1–3] After that, two-photon fluorescence (TPF) has been focused widely. As the development of TPF microscopy would greatly benefit from the synthesis of efficient TPF molecules with large two-photon absorption (TPA) cross sections and desired emitting characteristic, large numbers of TPF molecules have been reported.[4–6] However, the emitting wavelengths of these molecules locate mostly in the 500–700 nm region. The study of TPF molecules with efficient blue emission is still lacking, which blocks the development of multi-channel TPF microscopy because efficient blue TPF molecules are absolutely necessary for tagging some specific cellular components. Furthermore, for the probing of some natural molecules, efficient blue emitting molecules are helpful in reducing background light scattering and auto-fluorescence.[2]

In this communication, an efficient two-photon induced blue emission molecule based on benzimidazole acceptor is reported. The chemical structure of the TPF dye, named as *trans*-2-[*p*-formylstyryl]benzimidazole (abbreviated as ASBM), is shown in Fig. 1. To our knowledge, this kind of molecule based on benzimidazole acceptor has never been studied as a TPA material. Chloroform was used as solvent in the experiment.

The linear absorption and single-photon fluorescence spectra of ASBM at the concentration of 1×10^{-5} mol L^{-1}, recorded by a Shimadzu UV-3101PC UV-Vis-NIR scanning spectrophotometer and a Hitachi model F-4500 fluorescence spectrophotometer, respectively, are shown in Fig. 1. Two absorption peaks around 270 nm and 360 nm are observed in the linear absorption spectrum. As shown in Fig.1, the peak of the single-photon induced fluorescence of ASBM is located at 453 nm. The quantum-yield η of ASBM is 0.38, which was measured by the standard comparison method[7] using Coumarin120 as the reference standard.

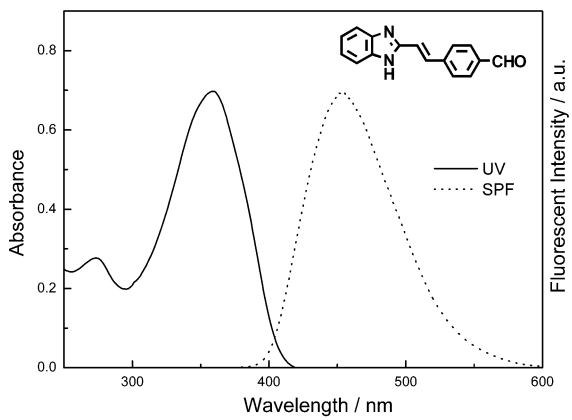

Fig. 1 Molecular structure, linear absorption and single-photon fluorescence spectra of ASBM.

The two-photon induced fluorescence experiment was performed with a mode-locked femtosecond Ti:Sapphire laser (Spectra-physics, 100 fs, 82 MHz) as a pump source (λ_{pump} = 750 nm) and an optical multi-channel analyzer as a recorder. The peak of the two-photon induced fluorescence of the ASBM solution (5×10^{-4} mol L^{-1}) is around 470 nm and the profile is similar to that of the single-photon induced fluorescent spectrum of ASBM.

The dependence of the up-converted fluorescence on the incident intensity for 5×10^{-4} mol L^{-1} ASBM solution is shown in Fig. 2. The nearly perfect quadratic dependence indicates that the up-converted fluorescence is induced by TPA.

The TPA cross section was measured by the two-photon induced fluorescence method.[8] Femtosecond laser passed first through a couple of Nicol's prisms, which were used as an attenuator to obtain a tunable excitation intensity, then the laser was split into two beams. The weaker one was used as a reference beam, which entered directly into the detector of a power meter (Coherent, FieldMaster NO.33-0506) in order to monitor the intensity of the excitation beam. The intense beam was focused into the sample by a lens. The fluorescence was collected at a direction perpendicular to the pump beam, and then passed through a liquid barrier filter (1 cm path-length 1 mol L^{-1} $CuSO_4$ solution) with O.D. > 10.0 for wavelengths > 690 nm to exclude excitation illumination.[8] To minimize the reabsorption effect, the excitation beam was focused as closely as possible to the front wall of the quartz cell . A photomultiplier tube (Hamamatzu R928) was used as the detector for TPF and the signal was read out by a Lock-in Amplifier (EG&G 5210).

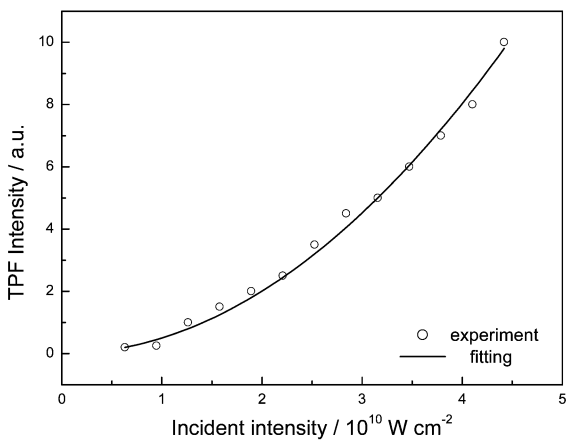

Fig. 2 The dependence of the up-converted fluorescence intensity on the incident intensity.

† Electronic supplementary information (ESI) available: preparation of ASBM. See http://www.rsc.org/suppdata/cc/b2/b206715c/

DOI: 10.1039/b206715c

 This journal is © The Royal Society of Chemistry 2002

The TPA cross section σ_2 of ASBM was determined by comparing its TPF to that of Rhodamine 6G (both at a concentration of 5×10^{-4} mol L^{-1}) according to[8,9]

$$\sigma_2\eta = \sigma_{2cal}\eta_{cal}\cdot(C_{cal}/C)\cdot(n_{cal}F/nF_{cal})$$

where C is the concentration, n is the refractive index and F is the time-averaged fluorescence. The subscript cal refers to the standard reference solution. In the experiment, methanol was used as solvent for Rhodamine 6G and the fluorescent quantum-yield of Rhodamine 6G was assumed to be 0.98. The time-averaged laser power at 750 nm is kept the same for the unknown and the standard.

The TPA cross section of ASBM was measured to be 180×10^{-50} cm^4 s at 750 nm, which is far away from the TPA peak, because as shown in ref. 10, the twice frequency of TPA peak should be on the blue side of the corresponding peak of the linear absorption. A significant enhancement of the σ_2 value is expected at the TPA peak.

We acknowledge support from the National Natural Science Foundation of China, National Key Basic Research Special Foundation, Natural Science Foundation of Guangdong Province and Natural Science Foundation of Education Ministry of China.

Notes and references

1 W. Denk, J. H. Strickler and W. W. Webb, *Science*, 1990, **248**, 73.
2 J. D. Bhawalkar, A. Shih, S. J. Pan, W. S. Liou, J. Swiatkiewicz, B. A. Reinhardt, P. N. Prasad and P. C. Cheng, *Bioimaging*, 1996, **4**, 168.
3 L. Ventelon, S. Charier, L. Moreaux, J. Mertz and M. Blanchard-Desce, *Angew. Chem., Int. Ed.*, 2001, **40**, 2098.
4 M. Albota, D. Beljonne, J. L. Brédas, J. E. Ehrlich, J. Y. Fu, A. A. Heikal, S. E. Hess, T. Kogej, M. D. Levin, S. R. Marder, D. McCord-Maughon, J. W. Perry, H. Rökeel, M. Rumi, G. Subramaniam, W. W. Webb, X. L. Wu and C. Xu, *Science*, 1998, **281**, 1653.
5 S. J. Chung, T. C. Lin, K. S. Kim, G. S. He, J. Swiatkiewicz, P. N. Prasad, G. A. Baker and F. V. Bright, *Chem. Mater.*, 2001, **13**, 4071.
6 A. Abbotto, L. Beverina, R. Bozio, A. Facchetti, C. Ferrante, G. A. Pagani, D. Pedron and R. Signorini, *Org. Lett.*, 2002, **4**, 1495.
7 J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.
8 C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481.
9 M. A. Albota, C. Xu and W. W. Webb, *Appl. Opt.*, 1998, **37**, 7352.
10 H. Lei, Z. L. Huang, H. Z. Wang, X. J. Tang, L. Z. Wu, G. Y. Zhou, D. Wang and Y. B. Tian, *Chem. Phys. Lett.*, 2002, **352**, 240.