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Novel Y-type two-photon active fluorophore: synthesis and application in fluorescent sensor for cysteine and homocysteine

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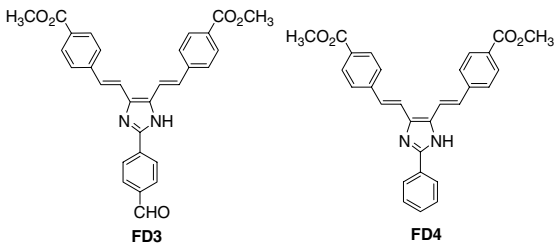
Abstract—A novel Y-shaped two-photon active material FD3 based on the imidazole core has been synthesized and exhibited intense two-photon excited fluorescence with two-photon absorption cross-section of more than 9000 GM. Importantly, FD3 could be used as a potential two-photon excited fluorescent sensor for cysteine and homocysteine.

Molecular two-photon absorption (TPA) has attracted a lot of interest recently for its applications in the field of both biological imaging and materials science, including two-photon laser scanning microscopy imaging (TPLSM), optical power limitation, localized photodynamic therapy, three-dimensional microfabrication, and optical data storage.[1] This has driven the study of in the structure–property correlation to establish guidance for molecular design since the ability of organic molecules to simultaneously absorb two photons to reach the excited state depends on molecular structures. Most designing strategies for large TPA materials mainly focus on D–π–A dipoles[2] and D–π–D/A–π–A quadrupoles[3] (D: electron donating group, A: electron accepting group, and π: conjugated pathway). Further developments involve the stretch of the charge transfer and the length of the π-bridge, such as multibranched octupoles or dendrimers.[4]

On the other hand, in comparison with single-photon fluorescent probes, two-photon fluorescent probes excited in the near-infrared (NIR) region show very low background light, weak photodamage, highly transmission at the low incident intensity. These characters can make such two-photon chromophores suitably act as fluorescent labels in bioimage, and the cases of two-photon excited fluorescence (TPEF) sensor for ions have been reported.[5]

Recently, using Z-scan technique, Bu and co-workers have reported that Y-type chromophores based on the imidazole core combining dipolar branch exhibited high two-photon absorption cross-section of 2000 GM.[6] Herein, using 4-(methylbenzoate)phenylethenyl group as the two branches, a novel Y-type chromophore FD3 (Scheme 1) based on the imidazole core, with an electron-withdrawing group of 4-formylphenyl at the other branch end-group was synthesized by a two-step procedure in good yield.[7] Interestingly, FD3 exhibited intense TPEF with high two-photon absorption cross-section of more than 9000 GM. More importantly,



Scheme 1. Chemical structures of FD3 and FD4.

Keywords: Two-photon; Fluorescent sensor; Cys/Hcy.
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Table 1. Single- and two-photon properties of FD3, FD3-Cys, and FD4 in DMF

	λ_{max}^{SPA} (nm)	ε (M^{-1} cm^{-1})	λ_{max}^{SPEF}[b] (nm)	λ_{max}^{TPEF} (nm)	Φ[a]	σ_{max}[b] (GM)	σ[c] (GM)
FD3	410	528,000	535	534	0.66	9163	1556
FD3-Cys	410	530,000	498	505	0.31	—	246
FD4	412	450,000	490	505	0.33	1249	590

[a] Fluorescence quantum yields in DMF using fluorescein as a standard.
[b] TPA cross-sections maximum were measured by the comparative TPEF methods.
[c] TPA cross-sections under the excitation of fs laser at 787 nm.

FD3 could be acted as a single- and two-photon excited fluorescent probe for the selective detection of cysteine (Cys) and homocysteine (Hcy). For comparison, another Y-type fluorophore FD4 (Scheme 1) with a similar structure was also investigated.

The data of the photophysical properties of FD3 and FD4 are summarized in Table 1. Both novel fluorophores showed intense single-photon absorption (SPA) bands with a maximum absorption wavelength λ_{max}^{SPA} = ~410 nm, corresponding to molar absorption coefficients of 10^5 M^{-1} cm^{-1}. FD3 in DMF showed the intense single-photon excited fluorescence (SPEF) emission with the maximum wavelength of 535 nm, corresponding to a high quantum yield of 66%. However, the SPEF emission band (λ_{max}^{SPEF} = 490 nm) of FD4 was blue-shifted in comparison to that of FD3. This fact indicated that the introduction of the CHO group resulted in a red-shift of the emission band.

The two-photon related photophysical properties of FD3 and FD4 were investigated by the method of two-photon induced fluorescence using Rhodamine B as a standard with known two-photon absorption (TPA) cross-section (Φ) (Supplementary data).[8] The two-photon absorption (TPA) and two-photon excited fluorescent (TPEF) emission spectra of FD3 (as an example) in DMF are shown in Figure 1. The shape and emission maximum of TPEF for FD3 are similar to those of SPEF. Furthermore, we noted that the TPA maximum wavelength occurred at less than half the wavelength of the single-photon absorption maximum wavelength, indicating that there was a significant

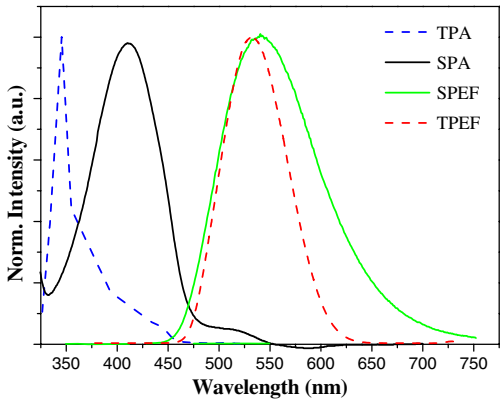


Figure 1. Normalized single-photon and two-photon relative absorption and emission spectra of FD3 in DMF. The wavelength data of TPA are divided by 2, corresponding the TPA characteristic.

excitonic coupling between the dipolar branches.[9] The maxima of TPA cross-section of FD3 and FD4 were measured to be 9163 GM (1 GM = 10^{-50} cm s photo^{-1}) at 691 nm and 1249 GM at 741 nm, respectively. Then, the TPEF emission cross-section ($\sigma\Phi$) of FD3 was calculated to be 6045 GM, which was obviously higher than that of FD4 ($\sigma\Phi$ = 412 GM). This fact also implied that the aldehyde (CHO) group played an important role in the SPEF and TPEF of FD3.

To further understand the dependence of the CHO group on photophysical properties, the calculation based on density functional theory (DFT) for FD3 and FD4 was performed. Figure 2 shows the lowest unoccupical molecular orbitals (LUMOs) of FD3 and FD4. Herein, we denoted the electron-withdrawing groups of 4-(methyl benzoate)phenyl and 4-formylpheny as A and A′, respectively. No obvious change was observed between the HOMO distributions of FD3 and FD4. However, the LUMO orbital distributions of FD3 were located on A–π–A′ structure, which was strikingly different from that of FD4. The LUMO orbital distributions of FD4 were mainly located on A–π–A section. The corresponding energy gaps between HOMO and LUMO were calculated to be 3.20 and 2.96 eV for FD3 and FD4, respectively, which was in agreement with a bathochromic shift of the emission peak for FD3 in comparison to that of FD4.

From the above result, it can be seen that the existence of the electron-withdrawing group (CHO) significantly affected the SPEF and TPEF of FD3. The selective reaction of aldehyde with Cys and Hcy to form thiazolidines has been extensively applied in Cys and Hcy detection.[10] Herein, we reasoned that the two-photon active fluorophore FD3 with a functional group of aldehyde also has the same recognizing mechanism for Cys and Hcy. In light of the important roles of Cys and Hcy in a variety of fundamental physicological processes in organisms,[11–13] the search for selective and sensitive fluorescent sensors for Cys and Hcy has gained tremendous attention.[14] To the best of our knowledge, no

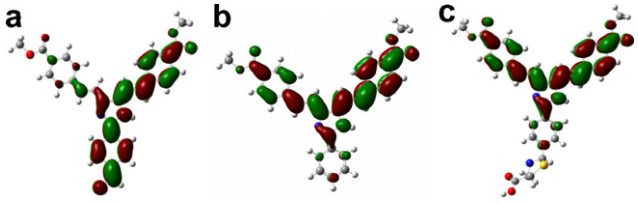


Figure 2. The LUMOs of FD3 (a), FD4 (b), and FD3-Cys (c).

sensitive TPEF sensor for Cys and Hcy has been reported up to date.

Spectroscopic measurements of FD3 in sensing Cys and Hcy were performed in a DMF solution. As shown in Figure 3, the single-photon excited fluorescence (SPEF) emission spectra of FD3 also displayed obvious changes when Cys was added. Upon addition of Cys, the emission band of FD3 at 535 nm was blue-shifted gradually to 498 nm, corresponding to a fluorescent color change from green to cyan (Fig. 4). And the quantum yield of FD3 in the presence of Cys was decreased from 0.66 to 0.31. Figure 3 (inset) shows the TPEF emission spectrum of FD3 in the absence and presence of 40-fold Cys under the excitation of fs laser 787 nm. The intensity of the TPEF of FD3 was about over 10 times stronger than that of the mixture of FD3 and Cys (FD3-Cys), and concomitantly about 30 nm blue-shift. The σ value of FD3-Cys was 246 GM at 787 nm, which was less than 1556 GM for FD3. The TPEF spectrum of FD3-Cys was almost the same as SPEF of FD3-Cys, which provided an interesting possibility that FD3 could be acted as efficient TPEF probe for Cys/Hcy.

To further confirm the transformation of FD3 to thiazolidine derivative FD3-Cys, ^{1}H NMR experiments were carried out (Fig. 4). When excess Cys (in D_2O) was added into DMSO-d_6 solution of FD3, the imidazole NH proton signal in the downfield (12.85 ppm) disappeared for the proton transfer of D_2O. The aldehyde resonances (10.04 ppm) of FD3 disappeared gradually whereas two new peaks centered at 5.73 and 5.55 ppm

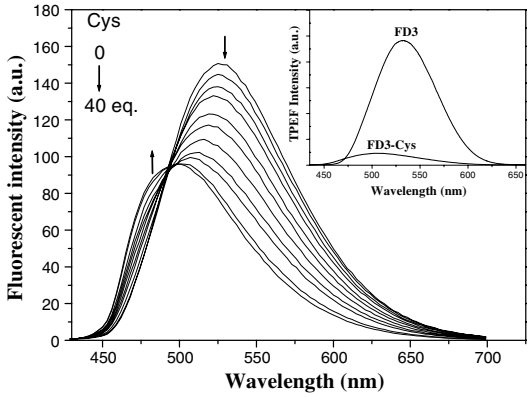


Figure 3. Fluorescent emission spectral change of FD3 (10 μM) upon addition of Cys (0–400 μM) in DMF at 25 °C. $\lambda_{ex} = 410$ nm. Each spectrum is acquired 10 min after Cys (0–400 μM) addition. Inset: TPEF spectra of FD3 and FD3-Cys.

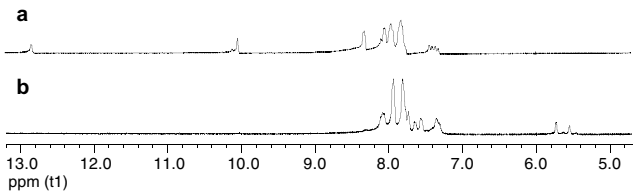
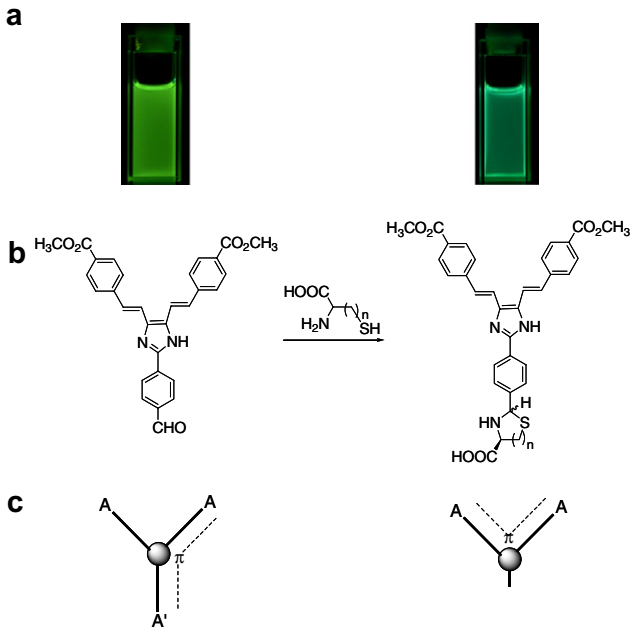


Figure 4. ^{1}H NMR spectra of **FD3** in DMSO-d_6 in the absence (a) and presence (b) of 40 equiv of Cys at 25 °C.

appeared which could be assigned to the methine protons of the thiazolidine diastereometer.[10a] This fact indicated thiazolidine was formed by the interaction of aldehyde with Cys. Therefore, a possible mechanism was proposed as shown in Scheme 2. When Cys was added, the CHO group reacted with Cys and thiazolide was formed. Complexation with Cys disturbed the π–π conjugation of the 4-formylphenyl group, and led to a high localization change of the LUMO orbital distributions from A–π–A′ system (A: -$COOCH_3$, A′: –CHO) to A–π–A system in this system (Scheme 2). This mechanism was also verified by theoretical calculation on the molecular orbital (Fig. 2), the corresponding energy gap between HOMO and LUMO for FD3-Cys was 2.94 eV, which was almost equal to that of FD4. This result also explained the blue-shift of fluorescent emission maximum wavelength for FD3 upon addition of Cys.

For an excellent chemosensor, high selectivity is a matter of necessity. Fluorescent spectra of FD3 in DMF were also investigated upon addition of other amino acids (alanine, arginine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tyrosine, and valine). As shown in Figure 5, no obvious fluorescent changes were observed upon addition of other amino acids compared with the hypsochromic shift of FD3 with Cys and Hcy, indicating that the reaction of the CHO group with Cys and Hcy was a key for the selective recognition of Cys and Hcy. Furthermore, no response of FD3 was observed upon addition of reduced glutathione (GSH). All but, the competitive experiments were tested in the presence of Cys or Hcy, as well as in a mixture of 40-fold other amino acids or GSH, respectively. No significant variation in fluorescent intensity was found by comparison with that only containing Cys or Hcy (Fig. 5). These



Scheme 2. The change in fluorescent color (a), possible reaction (b), and sensing mechanism (c) of FD3 with Cys.

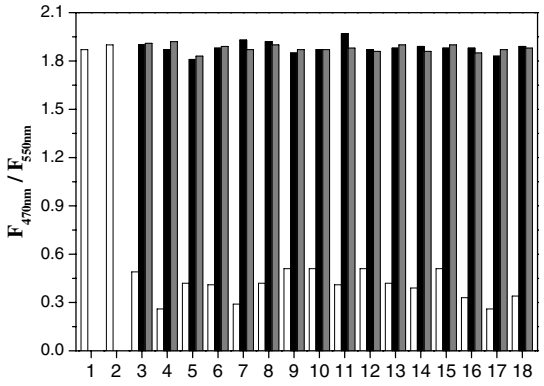


Figure 5. Fluorescent responses of FD3 in DMF (10 μM) to various amino acids (40 equiv) at 25 °C. Bars represent the ratio of fluorescence intensity at 470 and 550 nm. White bars represent the addition of 40-fold various amino acids to a 10 μM solution of FD3. Black and gray bars represent the addition of Cys and Hcy (400 μM) to the above solution, respectively. ($\lambda_{ex} = 410$ nm). 1, Cys; 2, Hcy; 3, Ala; 4, Arg; 5, Asp; 6, Glu; 7, Gly; 8, GSH; 9, His; 10, Iso; 11, Leu; 12, Lys; 13, Met; 14, Pro; 15, Ser; 16, Thr; 17, Tyr; 18, Val.

facts suggested that FD3 displayed a high selectivity in sensing Cys and Hcy.

In summary, we have described a novel two-photon excited fluorescent (TPEF) sensor FD3 for cysteine and homocysteine. Based on the imidazole core with the elongated arms, the Y-type fluorophore FD3 showed very large two-photon absorption cross-section of more than 9000 GM. An obvious blue-shift (~30 nm) of TPEF upon addition of Cys/Hcy indicated that FD3 could be used as a potential TPEF sensor for Cys/Hcy. To the best of our knowledge, this is the first example of the TPEF sensor for Cys/Hcy. This preliminary understanding on the sensing mechanism may help to find possible structural modification to potentially achieve new TPEF probes for Cys/Hcy.

Acknowledgments

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Supplementary data

Synthetic and experimental details (PDF) are available in the supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tetlet.2007.01.158.

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7. (a) Data for FD3: mp 161–163 °C; IR (KBr): *v* 3032, 2962, 1718, 1691, 1598, 1433, 1278, 1109, 958 cm^{-1}; ^1H NMR (400 Hz, DMSO-d_6): δ 12.85 (s, 1H), 10.04 (s, 1H), 8.32–8.34 (d, $J = 8.0$ Hz, 2H), 8.098 (s, 1H), 8.050 (d, $J = 8.0$ Hz, 2H), 7.93–7.99 (m, 4H), 7.78–7.86 (m, 5H), 7.32–7.44 (m, 2H), 3.85 (s, 6H); ^{13}C NMR (100 Hz, DMSO-d_6): δ 52.7, 117.6, 122.1. 126.1, 127.1, 127.2, 130.1, 130.6, 135.2, 136.6, 141.2, 142.8, 147.4, 166.7, 193.1; ESI-MS: *m*/*z* (%) 492 (100); Anal. Calcd for $C_{30}H_{24}N_2O_5$: C, 73.16; H, 4.91; N, 5.69. Found: C, 73.25; H, 4.69; N, 5.77; (b) Data for FD4: mp 209–211 °C; IR (KBr): *v* 3039, 2944, 1721, 1602, 1436, 1286, 1112, 957 cm^{-1}; ^1H NMR (400 Hz, DMSO-d_6): δ 12.60 (s, 1H), 8.11 (d, $J = 7.2$ Hz, 2H), 7.92–7.98 (m, 4H), 7.76–7.85 (m, 6H), 7.52 (t, $J = 7.6$ Hz, 2H), 7.28–7.45 (m, 3H), 3.85 (s, 6H); ^{13}C NMR (100 Hz, DMSO-d_6): δ 52.4, 117.7, 122.3, 125.7, 126.3, 126.9, 129.9, 130.1, 130.5, 140.6, 142.5, 142.9, 148.6, 166.5; ESI-MS: *m*/*z* (%) 464 (100); Anal. Calcd for $C_{29}H_{24}N_2O_4$: C, 74.98; H, 5.21; N, 6.03. Found: C, 74.77; H, 5.54; N, 6.15.
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