ORGANIC LETTERS

2006
Vol. 8, No. 25
5869−5872

Environment-Sensitive Fluorophore Emitting in Protic Environments

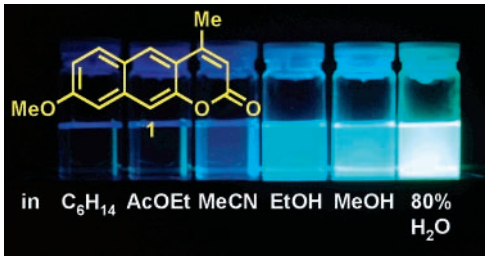
Seiichi Uchiyama,*,† Kazuyuki Takehira,‡ Toshitada Yoshihara,§ Seiji Tobita,§ and Tomohiko Ohwada*,†

Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, Satellite Venture Business Laboratory, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan, and Department of Chemistry, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan

seiichi@mol.f.u-tokyo.ac.jp; ohwada@mol.f.u-tokyo.ac.jp

Received October 9, 2006

ABSTRACT



The unusual fluorescence properties of 8-methoxy-4-methyl-2*H*-benzo[*g*]chromen-2-one (1) are described. The fluorophore 1 is almost nonfluorescent in aprotic solvent (e.g., fluorescence quantum yield $\Phi_f < 0.0003$ in *n*-hexane), whereas it strongly fluoresces at long wavelengths (>450 nm) in protic solvent (e.g., $\Phi_f = 0.21$ in methanol). The fluorophore 1 also shows good applicability in developing a new fluorogenic (fluorescent "off–on") sensor.

Fluorogenic (fluorescent "off−on") molecules have been widely used as sensors[1] and molecular devices.[2] Recently, a new type of fluorogenic sensor/device was developed by the introduction of an environment-sensitive fluorophore into stimulus-responsive macromolecules such as protein and synthetic polymers.[3] In these systems, an environment-sensitive fluorophore, benzofurazan[4] or dansylamine,[5] which fluoresces in a hydrophobic environment, transforms an external stimulus (input) into fluorescence (output) by way of a decrease in the local hydrophilicity of the macromolecules.

Along this line, it would be necessary to have environment-sensitive fluorophores with an opposite fluorescence characteristic, i.e., emitting in a hydrophilic medium when transforming an increase in the local hydrophilicity of

† The University of Tokyo.
‡ Satellite Venture Business Laboratory, Gunma University.
§ Department of Chemistry, Gunma University.

(1) (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnalaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (b) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P. *Acc. Chem. Res.* **1999**, *32*, 846−853. (c) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963−972. (d) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551−8588.

(2) (a) Balzani, V.; Credi, A.; Venturi, M. *Molecular Devices and Machines*; Wiley-VCH: Weinheim, 2003. (b) de Silva, A. P.; McClenaghan, N. D. *Chem.−Eur. J.* **2004**, *10*, 574−586. (c) Leigh, D. A.; Morales, M. Á. F.; Pérez, E. M.; Wong, J. K. Y.; Saiz, C. G.; Slawin, A. M. Z.; Carnichael, A. J.; Haddleton, D. M.; Brouwer, A. M.; Buma, W. J.; Wurpel, G. W. H.; León, S.; Zerbetto, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 3062−3067.

(3) (a) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053−3054. (b) Deo, S.; Godwin, A. *J. Am. Chem. Soc.* **2000**, *122*, 174−175. (c) Uchiyama, S.; Matsumura, Y.; de Silva, A. P.; Iwai, K. *Anal. Chem.* **2003**, *75*, 5926−5935. (d) Wada, A.; Mie, M.; Aizawa, M.; Lahoud, P.; Cass, A. E. G.; Kobatake, E. *J. Am. Chem. Soc.* **2003**, *125*, 16228−16234. (e) Uchiyama, S.; Kawai, N.; de Silva, A. P.; Iwai, K. *J. Am. Chem. Soc.* **2004**, *126*, 3032−3033. (f) Uchiyama, S.; Matsumura, Y.; de Silva, A. P.; Iwai, K. *Anal. Chem.* **2004**, *76*, 1793−1798. (g) Iwai, K.; Matsumura, Y.; Uchiyama, S.; de Silva, A. P. *J. Mater. Chem.* **2005**, *15*, 2796−2800.

(4) (a) Uchiyama, S.; Santa, T.; Imai, K. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2525−2532. (b) Uchiyama, S.; Santa, T.; Okiyama, N.; Fukushima, T.; Imai, K. *Biomed. Chromatogr.* **2001**, *15*, 295−318. (c) Ikeda, H.; Murayama, T.; Ueno, A. *Org. Biomol. Chem.* **2005**, *3*, 4262−4267.

(5) Li, Y.-H.; Chan, L.-M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118−3126.

10.1021/ol062490r CCC: $33.50
Published on Web 11/16/2006

Table 1. Photophysical Properties of **1**: Fluorescence Quantum Yield (Φ_f), Maximum Absorption Wavelength (λ_{abs}), Molar Absorption Coefficients (ϵ), Maximum Emission Wavelength (λ_{em}), Fluorescence Lifetime (τ_f), Quantum Yields of Intersystem Crossing (Φ_{isc}), and Internal Conversion (Φ_{ic})

solvent	α^a	D^b	Φ_f	λ_{abs} (nm)	ϵ (M^{-1} cm^{-1})	λ_{em} (nm)	τ_f (ns)	$\Phi_{isc}{}^c$	Φ_{ic}
n-hexane	0.00	1.9	<0.0003[d]	337	17500	ND[d]	ND[d]	0.021	0.98
ethyl acetate	0.00	6.0	0.0006	340	17800	435	0.066	0.051	0.95
acetonitrile	0.19	37.5	0.0071	340	17600	453	0.35	0.071	0.92
chloroform	0.44	4.8	0.011	344	18900	443	0.57	0.31	0.68
ethanol	0.83	24.6	0.13	343	18600	464	4.2	0.33	0.54
methanol	0.93	32.7	0.21	343	18600	469	6.7	0.34	0.45
water−methanol[e] (4:1, v/v)	(1.17)[f]	70.7	0.27	345	17500	500	9.2	0.17	0.56
trifluoroethanol	1.51	26.5	0.33	344	18000	496	13	0.41	0.26

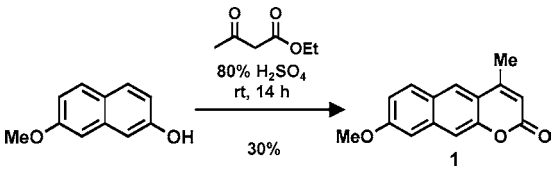
[a] Hydrogen-bond donor acidity of solvent (ref 15). [b] Dielectric constant of solvent. [c] Determined by using the E_T value (19 000 cm^{-1}) in a mixture of methanol−ethanol (1:1) at 77 K. [d] Below the detection limit. ND: Fluorescence signal was too weak to be detected. [e] Insoluble in 100% water. [f] Value for water.

macromolecules (e.g., due to unfolding) into a fluorescence signal. However, only acridine,[6] pyrene-3-carboxaldehyde (PCA),[7] and 7-methoxy-4-methylcoumarin (MMC)[8] were reported as fluorophores with this characteristic and the applicability of these fluorophores is limited because of fluorescence quenching by protonation,[9] difficulty of structural modification,[10,11] or short emission wavelengths.[12] Here, we propose the structure **1** (8-methoxy-4-methyl-2*H*-benzo[*g*]chromen-2-one) as a novel environment-sensitive fluorophore which strongly emits in the visible wavelength region, specifically in a protic environment.

The fluorophore **1** was designed by referring the chemical structures of the conventional fluorophores (i.e., acridine, PCA, and MMC). The structural key points in **1** are the lactone ring and the absence of a strong electron-donating substituent such as a dimethylamino group. The former can provide the desired sensitivity to environments by involving an $n \rightarrow \pi^*$ state in excited **1**.[8] On the other hand, the latter can control the intramolecular charge-transfer (ICT) character[1a] of the fluorophore to be moderate, otherwise fluorescence quenching would be observed in hydrophilic environments as seen for benzofurazan and dansylamine.

The fluorophore **1** was synthesized by the simple condensation of 7-methoxy-2-naphthol and ethyl acetoacetate in the presence of sulfuric acid (Scheme 1).[13,14] Then, the

Scheme 1. Synthesis of the New Fluorophore **1**



fluorescence characteristics of **1** were examined in various solvents. Table 1 summarizes the absorption and fluorescence characteristics of **1**, and Figure 1 displays representative spectra. These results clearly demonstrate high sensitivity of **1** to its environment. The fluorophore **1** is nonfluorescent (Φ_f < 0.0003) in *n*-hexane but strongly fluorescent (Φ_f = 0.27) in aqueous solution. This increase in the Φ_f value of **1** is well correlated with the hydrogen-bond donor acidity (α)[15] of the solvents used rather than with the dielectric constant (*D*, see Table 1). Therefore, it can be concluded that the fluorescence of **1** requires the hydrogen bonding between the solvent as a donor and **1** (essentially, its carbonyl group) as an acceptor; i.e., the fluorophore **1** strongly emits specifically in a protic environment.

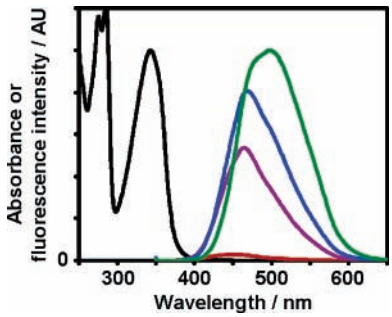


Figure 1. Representative absorption (black) and fluorescence (colored) spectra of **1** (10 μM); in acetonitrile (red), ethanol (purple), methanol (black and blue), and water−methanol (4:1, v/v) (green). Excitation: λ_{abs}.

The maximum emission wavelength (λ_{em}) of **1** is also sensitive to the environment (Table 1). The longer emission wavelength in a hydrophilic solvent (e.g., 469 nm in methanol) compared with that in a hydrophobic solvent (e.g., 435 nm in ethyl acetate) is due to an ICT character of the

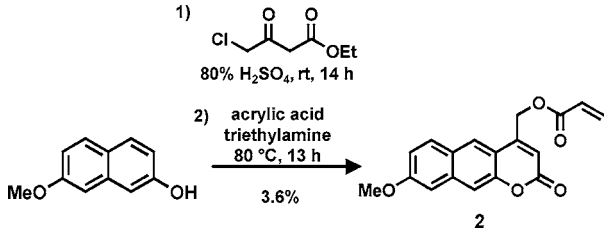
(6) Kellmann, A. *J. Phys. Chem.* **1977**, *81*, 1195−1198.
(7) Kalyanasundaram, K.; Thomas, J. K. *J. Phys. Chem.* **1977**, *81*, 2176−2180.
(8) de Melo, J. S. S.; Becker, R. S.; Maçanita, A. L. *J. Phys. Chem.* **1994**, *98*, 6054−6058.
(9) Nakamaru, K.; Niizuma, S.; Koizumi, M. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 255−277.

excited state.[1a] In a mixture of water and methanol (4:1, v/v), the maximum emission wavelength reached 500 nm. This long emission wavelength and the large Stokes shift (8900 cm^{-1}) are favorable for biorelevant applications. It should also be noted that **1** is stable even under severe conditions. No decomposition (i.e., photolysis or hydrolysis) was observed during excitation (345 nm) in an aqueous basic solution of pH 9 at 60 °C.

Table 1 also lists the τ_f, Φ_{isc}, and Φ_{ic} values of **1** in various solvents.[16] In *n*-hexane, internal conversion was observed almost exclusively as the relaxation pathway of excited **1**. The efficiency of the internal conversion (Φ_{ic}) decreased as that of the fluorescence (Φ_f) increased in protic solvents (e.g., ethanol, methanol, and water−methanol (4:1)). These data indicate that internal conversion is the dominant pathway competing with fluorescence in **1**, and protic solvents reduce the contribution of the former process.[17] Such a solvent effect on the relaxation pathways of **1** is, interestingly, different from that for acridine[6,18] or PCA,[19] in which intersystem crossing is the main competing process with fluorescence.

Finally, we present a new fluorescent polymeric thermometer emitting at lower temperature as an example of the potential applications of the new fluorophore.[20] We have recently reported sensitive fluorescent thermometers based on incorporation of an environment-sensitive fluorophore into a thermoresponsive poly(*N*-alkylacrylamide).[3c] With heating of the thermometer in aqueous solution, water molecules become separate from nanospaces near the main chain of the polymer, and the fluorophore senses the change in the local environment. Thus, the fluorophore **1** should afford a fluorescent thermometer emitting at lower temperature (i.e., behavior opposite to the previous report using benzofurazan[3c]). The fluorescent monomer **2** was synthesized (Scheme 2),[21] and subsequently, the random copolymer **3** (Figure 2a) was obtained.[13] The fluorescence behavior of the copolymer **3** at various temperatures in aqueous solution is shown in Figure 2b,c. As expected, stronger emission was observed at lower temperature. The blue shift of the maximum emission wavelength of **3** from 500 nm (at 5 °C) to 471 nm (at 50 °C) is consistent with the removal of water molecules from local spaces near the polymeric structure. Random copolymers prepared from other *N*-alkylacrylamides also showed sharp responses to the decrease in temperature (see Supporting Information). The fluorescent polymeric thermometers prepared in this study are capable of detecting a small temperature decrease in an aqueous phase.

Scheme 2. Synthesis of the Fluorescent Monomer **2**



2

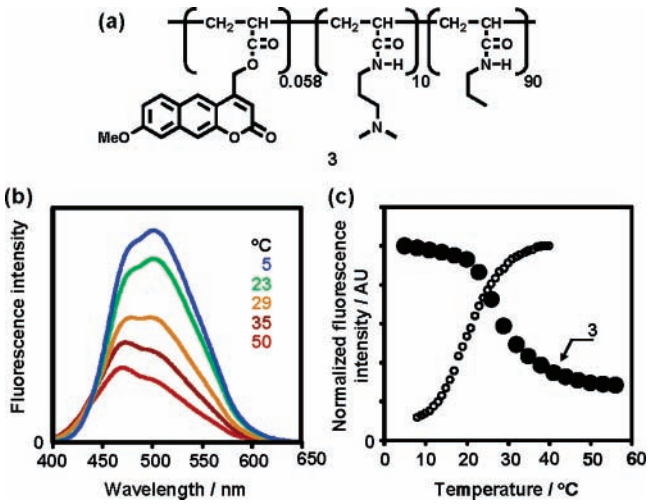


Figure 2. Fluorescent polymeric thermometer emitting at lower temperature as an example of potential applications of **1**. (a) Chemical structure of the random copolymer **3**. (b) Fluorescence spectra of **3** (0.01 w/v %) in buffer (pH 4) in different temperatures. The high solubility of **3** in water is due to the ionizable acrylamide units. The response to temperature change was reversible. Excitation: 345 nm. (c) Relationship (●) between fluorescence intensity of **3** at 500 nm and temperature. The Φ_f value at 10 °C was 0.23. The open circle (○) indicates the previously reported fluorescence behavior of the polymeric thermometer containing a benzofurazan unit as an environment-sensitive fluorophore (see ref 3e).

(10) The fluorescence signal from the compound bearing the PCA structure is dramatically reduced if the unstable aldehyde group is replaced by the stable ketone group. See: Armbruster, C.; Knapp, M.; Rechthaler, K.; Schamschule, R.; Parusel, A. B. J.; Köhler, G.; Wehrmann, W. *J. Photochem. Photobiol. A: Chem.* **1999**, *125*, 29−38. For photolysis of PCA, see also ref 18.

(11) It is noteworthy that fluorescent nucleobases with the PCA structure can distinguish types of single nucleotide polymorphism. See: Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820−4827.

(12) The short maximum excitation and emission wavelengths of MMC (330 and 392 nm in water, respectively) are not suitable for biorelevant applications. See: Muthuramu, K.; Ramamurthy, V. *J. Photochem.* **1984**, *26*, 57−64.

(13) For details of the synthetic procedure, see Supporting Information.

(14) The major product of the reaction was a regioisomer of **1**, which was less sensitive to environments than **1**.

(15) Kamlet, M. J.; Abboud, J.-L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877−2887.

(16) To determine these values, measurements of the fluorescence lifetime, optoacoustic signal, and phosphorescence spectra were carried out. For details of the experimental procedures and results, see Supporting Information.

(17) The hydrogen bonding may increase an energy gap between the S_1 and S_2 states in **1** and diminish a "proximity effect". For a related discussion, see: de Melo, J. S.; Becker, R. S.; Elisei, F.; Maçanita, A. L. *J. Chem. Phys.* **1997**, *107*, 6062−6069.

(18) (a) Noe, L. J.; Degenkolb, E. O.; Rentzepis, P. M. *J. Chem. Phys.* **1978**, *68*, 4435−4438. (b) Kasama, K.; Kikuchi, K.; Yamamoto, S.; Uji-ie, K.; Nishida, Y.; Kokubun, H. *J. Phys. Chem.* **1981**, *85*, 1291−1296.

(19) Kumar, C. V.; Chattopadhyay, S. K.; Das, P. K. *Photochem. Photobiol.* **1983**, *38*, 141−152.

(20) For reviews on fluorescent thermometers, see: (a) Chandrasekharan, N.; Kelly, L. A. In *Reviews in Fluorescence 2004*; Geddes, C. D., Lakowicz, J. R., Eds.; Kluwer Academic/Plenum: New York, 2004; Vol. 1, pp 21−40. (b) Uchiyama, S.; de Silva, A. P.; Iwai, K. *J. Chem. Educ.* **2006**, *83*, 720−727.

(21) The low yield was mainly due to the production of a regioisomer (yield, 46%) in the first step.

In closing, we have reported the unusual fluorescence properties of the new fluorophore **1**, i.e., emitting strongly in protic environments. The fluorophore **1** also possesses high stability, a long emission wavelength, a large Stokes shift, and facility in structural modification. These unique features are convenient for developments of new fluorogenic sensors and devices.

Acknowledgment. S.U. thanks The Sumitomo Foundation for financial support. We thank Dr. J. Kondo (Daiichi Sankyo Co. Ltd.) for his valuable comments and discussions. T.O. is grateful for a Grant-in-Aid for Scientific Research (Category S, No. 17109001) from the Japan Society for the Promotion of Science.

Supporting Information Available: Experimental details and supplementary figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL062490R