NJC

Cite this: *New J. Chem.*, 2011, **35**, 1678–1682

www.rsc.org/njc

PAPER

Bright fluorescence through activation of a low absorption fluorophore: the case of a unique naphthalimide–tetrazine dyad†

Zhou Qing,[ab] Pierre Audebert,*[ab] Gilles Clavier,[a] Rachel Méallet-Renault,[a] Fabien Miomandre[a] and Jie Tang[b]

Received (in Montpellier, France) 7th February 2011, Accepted 4th May 2011
DOI: 10.1039/c1nj20100j

An original fluorescent dyad has been prepared, featuring a 1,8-naphthalimide chromophore linked to a fluorescent tetrazine. This bichromophore benefits from the good absorption coefficient of the imide, and displays a quasi complete energy transfer to the tetrazine, followed by its fluorescence emission. This allows the preparation of remarkable transparent solutions and solids displaying a strong yellow fluorescence with a long life-time.

Introduction

The search for original fluorescent dyes has never stopped and among them, special colours or effect linked to energy transfer between chromophores have for a long time and till now received special attention.[1] This interest has been recently renewed due to multistate molecules and molecular calculators.[2] Bichromophoric dyads have also been widely investigated, especially on the point of view of the mechanisms and efficiency of energy transfer.[3]

However, an especially interesting situation is the activation of a weakly absorbing fluorophore by a more efficient one, which has, up to now, only been scarcely investigated.[3] This is likely due to the shortage of low-absorbing fluorophores, which implies that the transition responsible of the fluorescence is a forbidden or weakly allowed one. Actually, to the best of our knowledge, there are only two examples of low absorption fluorophores in the visible range, biacetyls,[4] and tetrazines[5] (see below). However, this remarkable case could lead to particularly interesting applications, like fluorescence emission from nearly transparent solutions or materials.[6] Another remarkable feature is that fluorescence coming from quasi-forbidden transitions has often a long life-time, which is especially interesting for fluorescence sensing, because it would leave the necessary time for the receptor/analyte interaction. Unfortunately, the sensing efficiency is often hampered by the low absorption coefficient. This problem can typically be overcome using a dyad. This is for example true for the biacetyl family since all its members have an extremely low absorption coefficient ($\varepsilon \approx$ 10–20 L mol^{-1} cm^{-1}). However, they can be activated through energy transfer and this has been extensively studied by Speiser *et al.* These authors nevertheless showed that on many occasions, energy transfer is not complete (may be due to the too low ε value).

We have recently shown that *s*-tetrazines substituted with heteroatoms also display unique fluorescence properties, based on the very same process that biacetyls (the fluorescence stems from a n–π* transition)[5b] featuring among other characteristics a very long lifetime (over 100 ns). Besides, the highly oxidizing character of their excited state makes them especially attractive for sensing electron rich pollutants. Although they absorb light more efficiently than biacetyls, unfortunately, and for a related reason, they still display a relatively low ε in the 500–1000 L mol^{-1} cm^{-1} range[7] which limits the brilliance of these molecules. An attracting development to overcome this drawback was thus to prepare chloroalkoxytetrazines linked to an appropriate strongly absorbing chromophore which is able to absorb light with a much higher efficiency, and transfer its energy to the fluorescent tetrazine. As explained above, and as noticed before by other authors for different chromophores[8] this could lead to a much improved brilliance for the molecule through light harvesting,[9] and thus improved efficiency of any device using this family of molecules. However, the partner chromophore of the tetrazine has to be chosen carefully, because not only the absorption bands have to show some overlap, as usual when energy transfer is envisaged, but also the partner has to be devoid of, even weak, reducing properties, since the excited tetrazine is a very good electron acceptor.

We describe here the preparation of a dyad made of a chloroalkoxytetrazine linked to a naphthalimide, *N*-(2-(6-chloro-*s*-tetrazin-3-yloxy)ethyl)-naphthalimide (that will be designed later as NITZ, Fig. 1). Both partners in the dyad are electro-deficient (the only available report states that the oxidation potential of naphthalimide is higher than +2 V).[10] This ensures that no electron transfer can take place between

---

[a] *PPSM, ENS Cachan, CNRS, UniverSud, 61 av President Wilson, F-94230 Cachan, France. E-mail: audebert@ppsm.ens-cachan.fr; Fax: +33 1 47 40 24 54; Tel: +33 1 47 40 53 39*
[b] *East China Normal University, Department of Chemistry, Shanghai, 200062, China*

† Electronic supplementary information (ESI) available: Voltammogram of NITZ and NMR spectra of compounds. See DOI: 10.1039/c1nj20100j

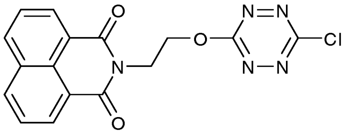


Fig. 1 Formula of NITZ.

the excited state of the tetrazine and the naphthalimide, since the redox potential of the former has been estimated between 1.2 V and 1.4 V, based on the optical gap.[5a] The photochemical behaviour of this new original molecule, along with the demonstration of its improved brilliance (about 10 times the one of a standard tetrazine) is presented along with its electrochemical properties. Also, we show that the compound can be inserted into a polymer and lead to a transparent and yellow fluorescent object, a unique feature that should find application in the realization of decorative objects.

Experimental

Materials and methods

All reagents were purchased from Sigma-Aldrich or Fluka and used as received. All solvents were obtained from Carlo-Erba. Synthesis grade ones have been dried prior to use according to standard literature procedures. All reactions were carried out under an inert argon atmosphere. Photophysical and electrochemical studies have been done in spectroscopic grade solvents. Solution NMR spectroscopy was performed on a Bruker AMX 500 MHz instrument. Mass spectrometric analyses were carried out on an Agilent 5973N apparatus. Dichloro-*s*-tetrazine was prepared as previously described.[5b]

Synthesis

Synthesis of *N*-(2-hydroxyethyl)-1,8-naphthalimide.[11] 1,8-Naphthalimide (0.2g, 1 mmol) was reacted with 2-bromoethanol (0.125g, 1 mmol) in dimethylformamide (DMF, 15 ml) in the presence of potassium carbonate for 10 h (previous workers used acetonitrile but in our hands the yields were unsatisfactory). Then the resulting solution was poured in water (10 ml) and extracted with ethyl acetate; the product was purified by chromatography on silica gel using dichloromethane (DCM) as an eluant to give *N*-(2-hydroxyethyl)-1,8-naphthalimide (yield: 73%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.36 (t, 1H, J = 5.5 Hz, OH), 3.98 (dt, 2H, J_1 = 5.5 Hz, J_2 = 5 Hz, CH_2-OH), 4.47 (t, 2H, J = 5 Hz, CH_2-N), 7.76 (t, 2H, J = 7.5 Hz), 8.23 (d, 2H, J = 7.5 Hz), 8.62 (d, 2H, J = 7.5 Hz).

Synthesis of NITZ. The reaction of dichloro-*s*-tetrazine and *N*-(2-hydroxyethyl)-1,8-naphthalimide was conducted under previously described standard conditions[12] (1 eq. dichloro-*s*-tetrazine, 1 eq. alcohol, 2 eq. collidine in DCM, RT, 2 h) to give NITZ in 56% yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 4.75 (t, 2H, J = 5 Hz), 5.06 (t, 2H, J = 5 Hz), 7.75 (t, 2H, J = 7.5 Hz), 8.23 (d, 2H, J = 7.5 Hz), 8.56 (d, 2H, J = 7.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 38.1 (CH_2-O), 67.6 (CH_2-N), 122.3, 127.1, 128.9, 131.8, 132.3, 134.5 (naphthalene core), 163.7, 164.5, 166.7 (C=O and tetrazine). High-res ESI MS (positive ion): 355, 357 m/z (M^+).

Electrochemistry

Electrochemical studies were performed using DCM as a solvent and *N*,*N*,*N*,*N*-tetrabutylammonium hexafluorophosphate (TBAFP) as the supporting electrolyte. The substrate concentration was *ca.* 5 mM. A 1 mm diameter Pt or glassy carbon electrode was used as the working electrode, along with a Ag^+/Ag (10^{-2} M) reference electrode and a Pt wire counter electrode. The cell was connected to a CH Instruments 600B potentiostat monitored by a PC computer. The reference electrode was checked *vs.* ferrocene as recommended by IUPAC. In our case, $E^0(Fc^+/Fc)$ = 0.097 V. All solutions were degassed by argon bubbling prior to each experiment.

Photophysical measurements

Steady-state spectroscopy. All the spectroscopic experiments were carried out in DCM and at concentrations *ca.* 10 μmol L^{-1} for absorption spectra and *ca.* 1 μmol L^{-1} for fluorescence spectra. UV-vis absorption spectra were recorded on a Varian Cary 500 spectrophotometer. Fluorescence emission and excitation spectra were measured on a SPEX fluorolog-3 (Horiba-Jobin-Yvon). For emission fluorescence spectra, the excitation wavelengths were set equal to the maximum of the corresponding absorption spectra. Only dilute solutions with an absorbance below 0.1 at the excitation wavelength λ_{ex} were used. For the determination of the relative fluorescence quantum yields (ϕ_F), sulforhodamine 101 in ethanol (ϕ_F = 0.9) was used as a fluorescence standard.

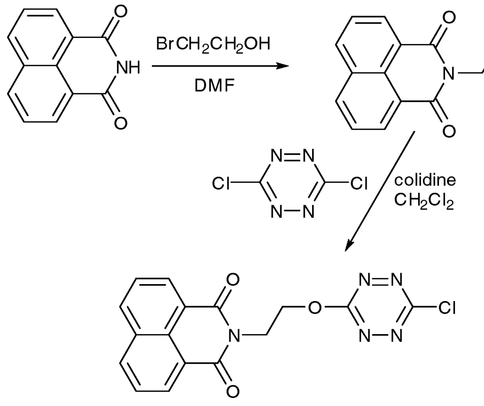
Time-resolved spectroscopy. The fluorescence decay curves were obtained with a time-correlated single-photon-counting method using a titanium–sapphire laser (82 MHz, repetition rate lowered to 4 MHz thanks to a pulse-peaker, 1 ps pulse width, a doubling crystal is used to reach 495 and 355 nm excitations) pumped by an argon ion laser. Data were analyzed by a nonlinear least-squares method (Levenberg–Marquardt algorithm) with the aid of Globals software (Globals Unlimited, University of Illinois at Urbana-Champaign, Laboratory of Fluorescence Dynamics). Pulse deconvolution was performed from the time profile of the exciting pulse recorded under the same conditions by using a Ludox solution. In order to estimate the quality of the fit, the weighted residuals were calculated.

Results and discussion

The following synthetic route (Scheme 1) was used for the preparation of NITZ.

The synthesis is relatively straightforward and allows the preparation of appreciable quantities of compound if needed. We have performed the spectroscopic and electrochemical study of both *N*-(2-hydroxyethyl)-1,8-naphthalimide and NITZ in order to evaluate the properties of the imide alone, and further to be able to analyze the energy transfer in the bichromophoric NITZ. Table 1 gathers the physicochemical characteristics of *N*-(2-hydroxyethyl)-1,8-naphthalimide and NITZ, in the latter case focusing, respectively, on the imide and the tetrazine characteristics.

Regarding absorption and fluorescence, the *N*-(2-hydroxyethyl)-1,8-naphthalimide behaves like a standard naphthalimide,



Scheme 1 Synthesis of NITZ.

as shown in Fig. 2.[10,13] It should be noted that the fluorescence yields are usually not very high with this type of compounds. However, the situation with NITZ is more interesting. The absorption is very close to the sum of the contributions from the imide and the tetrazine behaving as independent chromophores (Fig. 2, left). NITZ when excited at 517 nm displays a classical fluorescence spectrum (λ_{max} = 567 nm) characteristic of all chloroalkoxytetrazines associated with a long fluorescence lifetime and a relatively high quantum yield. The electrochemistry data also show that the reduction potentials are in the same range, so that, as expected electron transfer is not possible upon photochemical excitation.

However when the excitation is set at 355 nm where the naphthalimide absorbs almost exclusively, the NITZ fluorescence spectrum (Fig. 3) shows that the intrinsic fluorescence of the imide moiety has almost disappeared, at the expense of the one of the tetrazines, evidencing the occurrence of energy transfer between the two chromophores. We have quantified the efficiency of the energy transfer, on the basis of the data reported in Table 1. On the assumption that all the fluorescence lost by the donor is transferred to the acceptor, the efficiency of the energy transfer is calculated to be:[9]

$$\phi_{\mathrm{ET}} = 1 - \frac{\phi_{\mathrm{donor}}}{\phi^0_{\mathrm{donor}}} = 1 - \frac{0.003}{0.061} = 0.95$$

This shows that the energy transfer is quite efficient. Molecular modelization shows that the average distance between the imide donor and the tetrazine ring acceptor is about 8.5 Å. The spectral overlap between the fluorescence of the donor

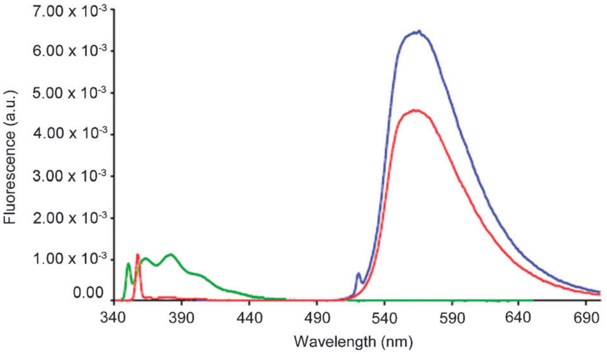


Fig. 3 Fluorescence spectra of *N*-(2-hydroxyethyl)-1,8-naphthalimide (green), NITZ with λ_{ex} = 518 nm (blue) and NITZ with λ_{ex} = 355 nm (red).

Table 1 Photophysicochemical and electrochemical characteristics of compounds in dichloromethane

Compound	λ_{max}/nm	ε/L mol^{-1} cm^{-1}	λ_{em}/nm	ϕ_F	$\varepsilon_{\lambda(ex)} \times \phi_F$	τ_F/ns	E^0_{red}/V
N-(2-Hydroxyethyl)-1,8-naphthalimide	335	8700	363 382 402^a	0.06^a	522	0.37^c	−1.70
NITZ (tetrazine data)	517	400	562^b	0.32^b	130	158^{c,d}	−0.86
NITZ (imide data)	334	9100 (5000 at 355 nm)	378 400^c	0.003^c	12	0.03^c	−1.70

^{a} λ_{ex} = 350 nm. ^{b} λ_{ex} = 517 nm. ^{c} λ_{ex} = 355 nm. ^{d} λ_{ex} = 495 nm.

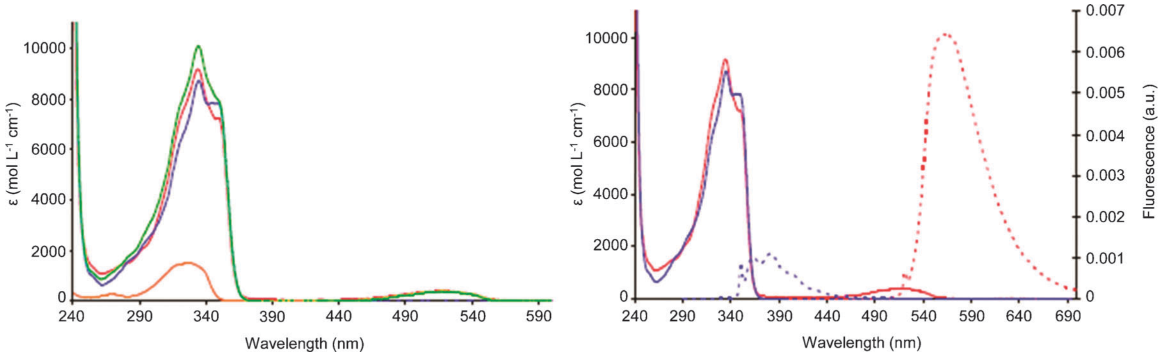


Fig. 2 Left: absorption spectra of *N*-(2-hydroxyethyl)-1,8-naphthalimide (blue), chloromethoxy-*s*-tetrazine (orange), NITZ (red) and the sum of *N*-(2-hydroxyethyl)-1,8-naphthalimide and chloromethoxy-*s*-tetrazine (green); right: absorption (full lines) and fluorescence (dotted lines) spectra of *N*-(2-hydroxyethyl)-1,8-naphthalimide (blue) and NITZ (red). The fluorescence of NITZ was obtained by direct excitation of the *s*-tetrazine (λ_{ex} = 517 nm).

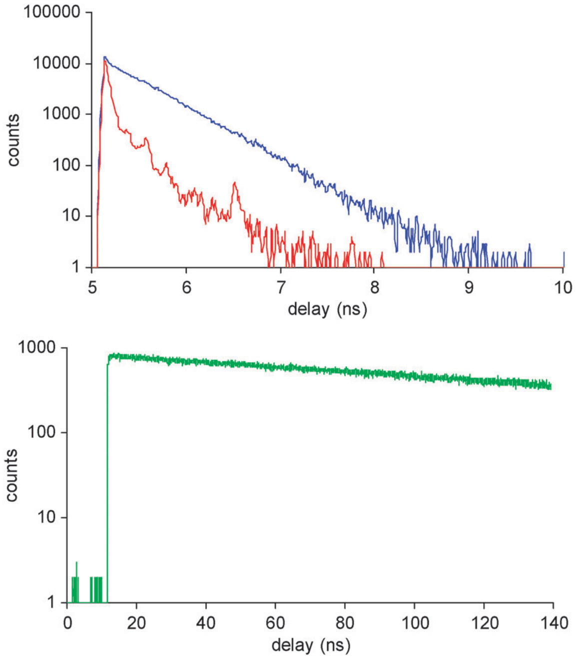


Fig. 4 Fluorescence decay profiles upon excitation at 355 nm. Up: *N*-(2-hydroxyethyl)-1,8-naphthalimide (blue) and NITZ (red) for λ_{em} = 365 nm; down: NITZ (green) for λ_{em} = 562 nm.

and the absorption of the acceptor being small a short Förster radius of R = 9.3 Å is calculated, and therefore the efficiency of the energy transfer should be only around 63%. The discrepancy between the calculated and the experimental value therefore inclines to think that the energy transfer mechanism would rather be of the Dexter type or a mixed one.

In order to refine our investigations, we recorded the fluorescence decay of the *N*-(2-hydroxyethyl)-1,8-naphthalimide and NITZ. The results (Fig. 4) give a fluorescence lifetime for the naphthalimide of 0.37 ns in the first case and 0.03 in the second, and therefore it is possible to calculate the efficiency and the rate of the energy transfer:

$$\phi_{ET} = 1 - \frac{\tau_{donor}}{\tau^0_{donor}} = 1 - \frac{0.03}{0.37} = 0.92 \quad k_{ET} = \frac{1}{\tau_{donor}} - \frac{1}{\tau^0_{donor}} = 3.06 \times 10^{10}\ \mathrm{s}^{-1}$$

which is two orders of magnitude higher than the radiative lifetime of the *N*-(2-hydroxyethyl)-1,8-naphthalimide (k_R = 1.65 × 10^8 s^{-1}). It is noteworthy that the two calculated values of ϕ_{ET} are in good agreement. On the other hand, the tetrazine fluorescence decay is more complex and displays a rising time. The decay could be fitted by a bi-exponential function giving a rising time of 0.06 ns similar to the fluorescence lifetime of the naphthalimide in NITZ and a decay of 158 ns typical of the tetrazine.

Finally, we have performed visual evaluation of the brilliance of our molecule, by simply comparing the brilliance of a 5 × 10^{-6} M solution of NITZ and the one of a solution of 3-(adamant-1-ylmethoxy)-6-chloro-*s*-tetrazine, which comprises the same tetrazine emitter but without the presence of a donor. Fig. 5A shows the pictures of both solutions in white light; at these concentrations the solutions are almost colourless. Fig. 5B displays the fluorescence of the solution, excited at *ca.* 365 nm. It is clear that, due to the efficiency of the imide absorbance and the energy transfer, the brilliance of the NITZ solution is much higher than the one of the standard tetrazines. The quantitative evaluation of the brilliance (column $\varepsilon_{\lambda(ex)} \times \phi_F$ in Table 1) shows that it is 7.5 times higher when NITZ is excited at 355 nm (selective of the imide moiety) rather than 517 nm (selective of the tetrazine moiety) as is evidenced in Fig. 5.

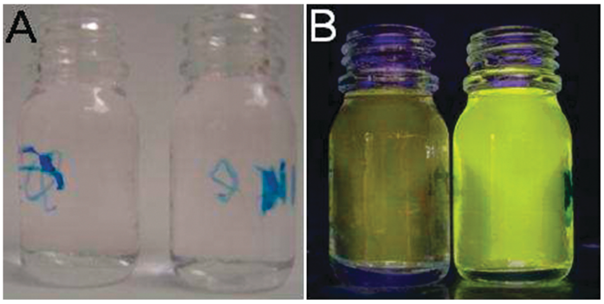


Fig. 5 Pictures of two 5 × 10^{-6} M solutions of, right, 3-(adamant-1-yl-methoxy)-6-chloro-*s*-tetrazine and, left, NITZ, both in (A) standard white light and (B) 365 nm UV light.

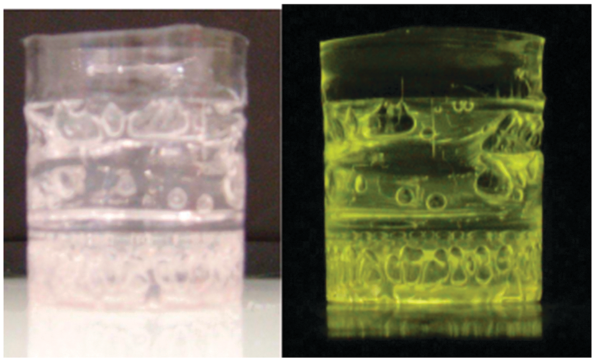


Fig. 6 Pictures of a block of polystyrene incorporating NITZ under white (left) and UV (right) light. The dye concentration in the polymer was *ca.* 10^{-5} M.

All tetrazines, including NITZ are soluble in most organic polymers because of their moderate molecular weight. Fig. 6 shows the pictures of a block of polystyrene into which NITZ has been dispersed at a 10^{-5} M concentration. Such a low amount of dye gives a perfectly transparent object under ambient light while it exhibits a nice yellow fluorescence when exposed to UV light. In addition, the picture has been taken three weeks after the object fabrication, which demonstrates that the fluorophore does not degrade in normal conditions.

Conclusions

We have presented a new fluorescent dyad made of a naphthalimide absorber and a tetrazine emitter. The fluorescence properties of this new molecule have been studied. The results show that, due to the efficiency of the energy transfer, the dyad is much more brilliant than a simple tetrazine irradiated in the same conditions. Application of this new molecule to fluorescent sensors is ongoing.

Notes and references

1 (*a*) G. Weber, *Nature*, 1957, **180**, 1409; (*b*) M. Sirish, R. Kache and B. G. Maiya, *J. Photochem. Photobiol., A*, 1996, **93**, 129; (*c*) G. Hinze, M. Haase, F. Nolde, K. Mullen and Th. Basché, *J. Phys. Chem. A*, 2009, **109**, 6725; (*d*) Ch. Scharf, K. Peter, P. Bauer, Ch. Jung, M. Thelakkat and J. Köhler, *Chem. Phys.*, 2006, **328**, 403; (*e*) S. Diring, R. Ziessel, f. Barigelletti, A. Barbieri and B. Ventura, *Chem.–Eur. J.*, 2010, **16**, 9226.

2 (*a*) D. Margulies, G. Melman and A. Shanzer, *J. Am. Chem. Soc.*, 2006, **128**, 4871; (*b*) O. Kunetz, H. Salman, Y. Eichen, F. Remacle, R. D. Levine and S. Speiser, *J. Photochem. Photobiol., A*, 2007, **191**, 176; (*c*) O. Kunetz, D. Davis, H. Salman, Y. Eichen and S. Speiser, *J. Phys. Chem. C*, 2007, **191**, 176; (*d*) J. M. Tour, *Acc. Chem. Res.*, 2000, **33**, 79.

3 S. Speiser, *Chem. Rev.*, 1996, **96**, 1953.

4 (*a*) S. Speiser, R. Kataro, S. Welner and M. B. Rubin, *Chem. Phys. Lett.*, 1979, **61**, 199; (*b*) D. Getz, A. Ron, M. B. Rubin and S. Speiser, *J. Phys. Chem.*, 1980, **84**, 768; (*c*) S. Hassoon, S. Lustig, M. B. Rubin and S. Speiser, *J. Phys. Chem.*, 1984, **88**, 6367.

5 (*a*) P. Audebert, F. Miomandre, G. Clavier, M. C. Vernieres, S. Badré and R. Méallet-Renault, *Chem.–Eur. J.*, 2005, **11**, 5667; (*b*) Y.-H. Gong, F. Miomandre, R. Méallet-Renault, S. Badré, L. Galmiche, J. Tang, P. Audebert and G. Clavier, *Eur. J. Org. Chem.*, 2009, 6121; (*c*) P. Audebert and G. Clavier, *Chem. Rev.*, 2010, **110**, 3299.

6 This situation can only be achieved in the rare case when a fluorophore can interconvert between two structures after photon absorption. For example it has been shown through a very fast proton transfer in the remarkable paper: S. Park, J. E. Kwon, S. H. Kim, J. Seo, K. Chung, S.-Y. Park, D.-J. Jang, B. M. Medina, J. Gierschner and S. Y. Park, *J. Am. Chem. Soc.*, 2009, **131**, 14043.

7 Y.-H. Gong, P. Audebert, G. Clavier, F. Miomandre, J. Tang, S. Badré, R. Méallet-Renault and E. Naidus, *New J. Chem.*, 2008, **32**, 1235.

8 S. E. Weber, *Chem. Rev.*, 1990, **90**, 1469.

9 (*a*) J. E. Guillet, *Polymer Photophysics and Photochemistry*, Cambridge Univ. Press, Cambridge, 1985; (*b*) B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, 2001.

10 B. Ramachandram, G. Saroja, N. B. Sankaran and A. Samanta, *J. Phys. Chem. B*, 2000, **104**, 11824.

11 L. D. Van Vliet, T. Ellis, P. J. Foley, L. Liu, F. M. Pfeffer, R. A. Russell, R. N. Warrener, F. Hollfelder and M. J. Waring, *J. Med. Chem.*, 2007, **50**, 2326.

12 Y.-H. Gong, P. Audebert, G. Clavier, F. Miomandre, J. Tang, S. Badré, R. Méallet-Renault and E. Naidus, *New J. Chem.*, 2008, **32**, 1235.

13 (*a*) A. Demeter, T. Berces, L. Biczok, V. Wintgens, P. Valat and J. Kossanyi, *J. Phys. Chem.*, 1996, **100**, 2001; (*b*) U. C. Yoon, S. W. Oh, S. M. Lee, S. J. Cho, J. Gamlin and P. S. Mariano, *J. Org. Chem.*, 1999, **64**, 4411.