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LETTER

Bimolecular photoactivation of NBD fluorescence†

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The concatenation of a photochemical transformation with a chemical reaction allows the activation of nitrobenzoxadiazole (NBD) fluorescence under optical control. Specifically, the coupling of a photoinduced deprotection with a nucleophilic substitution converts a nonemissive NBD chromophore into a fluorescent product. These operating principles can evolve into a general mechanism to implement fluorescent switches based on the attractive photophysical properties of NBDs.

Conventional fluorophores emit electromagnetic radiations upon illumination at an appropriate excitation wavelength (λ_{Ex}).[1] The absorption of incoming photons at λ_{Ex} excites them from the ground to one of the accessible excited electronic states. The subsequent relaxation and radiative deactivation of the excited species back to their ground state results in fluorescence. Photoactivatable fluorophores, instead, emit only after irradiation at a given activation wavelength (λ_{Ac}) first and then illumination at λ_{Ex}.[2–7] The absorption of incoming photons at λ_{Ac} initiates a photochemical transformation that either enables subsequent absorption at λ_{Ex} or prevents quenching of the excited state responsible for emission. Both mechanisms ensure significant fluorescence only after activation and excitation events. Under these conditions, the interplay of two light sources operating at λ_{Ac} and λ_{Ex} can be exploited to switch fluorescence on within a defined region of space at a given interval of time. Such spatiotemporal control permits the monitoring of the translocation of activated fluorophores across a sample of interest in real time with the sequential acquisition of fluorescence images.[8] Similarly, it allows the sequential localization of closely-spaced fluorophores activated at different intervals of time and the reconstruction of images with subdiffraction resolution.[9] As a result, photoactivatable fluorophores offer the opportunity to probe dynamic processes and structural features that would, otherwise, be inaccessible with the sole aid of their conventional counterparts.

The potential applications of photoactivatable fluorophores are stimulating fundamental studies aimed at the identification of viable structural designs to switch the emission of members of the main families of organic dyes under optical control. Several photoactivatable borondipyrromethenes, coumarins, dihydrofurans, fluoresceins and rhodamines have already been reported in the literature.[2–7] Instead, only one NBD with photoactivatable fluorescence has been developed so far,[10] in spite of the attractive combination of structural and photophysical properties that this particular class of organic fluorophores has to offer. Indeed, the synthetic accessibility of NBD derivatives, the possibility to excite them with visible photons and their high fluorescence quantum yields are encouraging their use in a diversity of analytical applications.[11–14]

Literature data demonstrate that the nature of the substituents on the NBD platform has a dramatic influence on the fluorescence quantum yield of this particular chromophore.[15] Derivatives with a pair of electron withdrawing groups in positions 4 and 7 are generally not emissive. The transformation of one of the two substituents into an electron donating group, however, enables the radiative deactivation of the excited state. In fact, this general mechanism can be exploited to transduce the presence of a given reactant into a fluorescence signal and implement valuable sensing schemes.[16–24] For example, compound **1** has electron withdrawing nitro and sulfonamide substituents on its NBD skeleton and is not fluorescent.[22] Reaction with an appropriate nucleophile converts the sulfonamide substituent into an electron-donating amine group to form **2** and switch fluorescence on. These observations suggest that the photoinduced generation of a nucleophile can be exploited to convert **1** into **2** and activate emission under optical control. The resulting concatenation of photochemical and chemical steps is conceptually analogous to schemes developed in our and other laboratories to activate the fluorescence of coumarin[25] and rhodamine[26] derivatives with photoacid generators. In turn, such bimolecular strategies for fluorescence photoactivation permit the monitoring of the diffusion of

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† Electronic Supplementary Information (ESI) available: Experimental procedures and X-Ray Diffraction Data for **6**. See DOI: 10.1039/#

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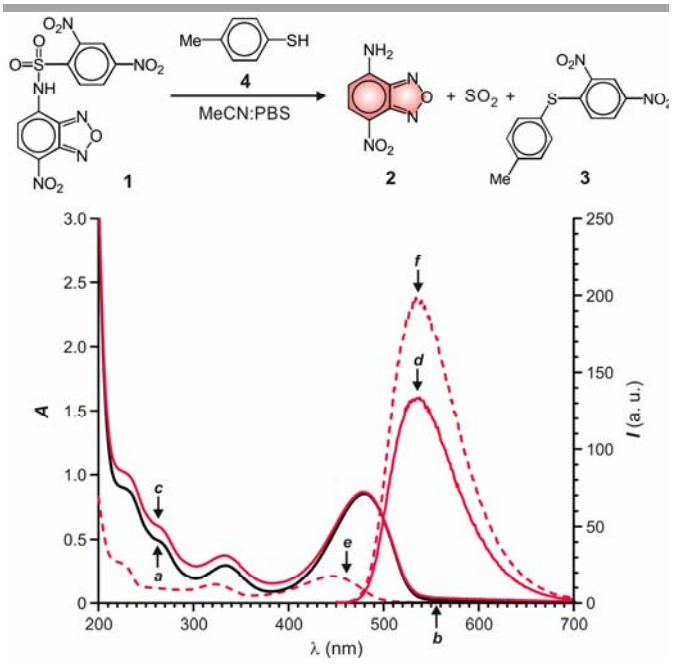

Fig. 1 Absorption (***a*** and ***c***) and emission (***b*** and ***d***) spectra of a solution [10 μM, MeCN:PBS (95:5 v/v), 25 °C, λ_{Ex} = 440 nm] of **1** before (***a*** and ***b***) and after (***c*** and ***d***) the addition of **4** (2 equiv.) and stirring for 25 min in the dark. Absorption (***e***) and emission (***f***) spectra of **2** [10 μM, MeCN:PBS (95:5 v/v), 25 °C, λ_{Ex} = 440 nm].

the activating reactant in real time[25a] as well as allow the reconstruction of images with subdiffraction resolution.[25]

The absorption spectrum (***a*** in Fig. 1) of **1** shows an intense band at 479 nm. Illumination at a λ_{Ex} positioned within this absorption does not cause any detectable fluorescence (***b*** in Fig. 1), in agreement with literature data.[22] After the addition of **4** under experimental conditions that ensure the formation of the corresponding thiolate,[22] noticeable changes can be observed in the absorption spectrum (***c*** in Fig. 1). Furthermore, an intense band appears at 540 nm in the corresponding emission spectrum (***d*** in Fig. 1). This band is essentially identical to that observed in the spectrum (***f*** in Fig. 1) of **2**. Thus, the treatment of **1** with **4** produces **2** and activates fluorescence, consistently with literature precedents.[22]

The transformation of **1** into **2** is a consequence of the nucleophilic displacement of the sulfonyl group of one species with the concomitant formation of the other. If the nucleophile is protected with an appropriate photocleavable group, then the conversion of **1** into **2** can be achieved under photochemical control. Specifically, treatment of **4** with **5**, in the presence of potassium carbonate, produces **6** in a yield of 94%. The electrospray ionization mass spectrum of the product together with its ^{1}H and ^{13}C nuclear magnetic resonance spectra confirm the covalent incorporation of a 2-nitrobenzyl group. Furthermore, single crystals of **6** could be obtained from a dichloromethane/hexane solution of the compound, after the slow evaporation of the solvent. The corresponding structure[§] (Fig. 2) clearly reveals the presence of a 2-nitrobenzyl group within the covalent skeleton of the molecule.

The absorption spectrum (***a*** in Fig. 3) of **6** shows a band at 346 nm for the 2-nitrobenzyl chromophore. In agreement with extensive literature data on this particular photocleavable protecting group,[Error! Bookmark not defined.] illumination at a λ_{Ac} of 350 nm cleaves the bond between the benzylic carbon atom and the adjacent sulphur atom to

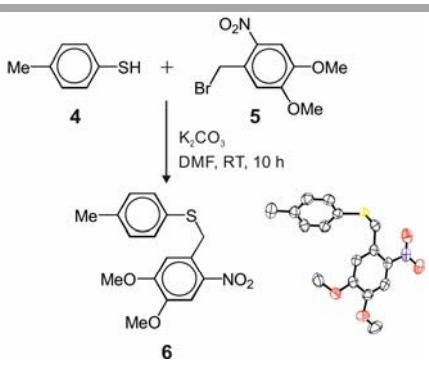

Fig. 2 Synthesis of **6** and ORTEP representation of its geometry in single crystals (50% thermal ellipsoid probability).

convert **6** into **4**. Consistently, new absorption bands develop at *ca.* 240 and 280 nm (***b***–***e*** in Fig. 3) during the photolytic transformation. These absorptions resemble those observed in the spectrum (***f*** in Fig. 3) of **4** and confirm the photoinduced formation of this species.

The addition of **6** to a solution of **1** does not affect the absorption band (***a*** in Fig. 4) of the NBD chromophore and does not produce any fluorescence (***b*** in Fig. 4). Furthermore, the absorption and emission spectra of the mixture remain unchanged even after storage for hours in the dark. These observations indicate that **6** cannot react with **1** to produce **2** and activate fluorescence. Upon illumination of the mixture at a λ_{Ac} of 350 nm, however, the 2-nitrobenzyl group of **6** cleaves to generate **4**. The corresponding thiolate then disconnects the sulfonamide group of **1** to release **2**. In fact, the absorption and emission spectra (***c***–***l*** in Fig. 4), recorded during the course of the photolytic transformation, show essentially the same changes observed after the physical addition of **4** to a solution of **1** (Fig. 1).[¶] Specifically, the emission of the product develops into an intense band (***h***–***l*** in Fig. 4), demonstrating that the fluorescence of the NBD

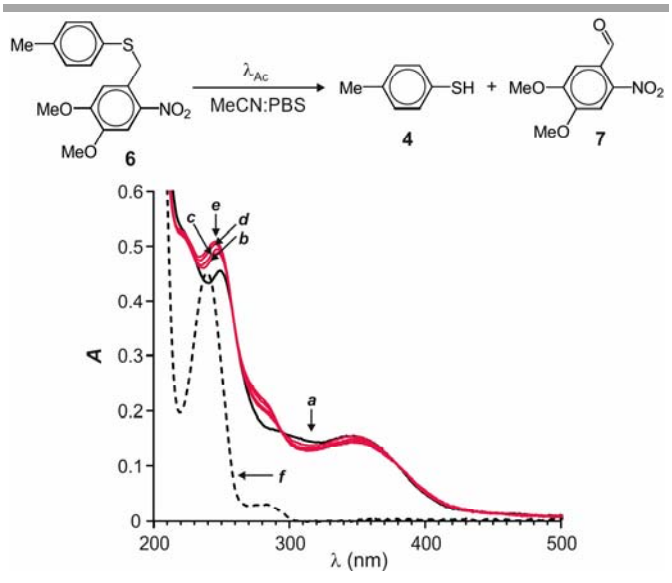

Fig. 3 Absorption spectra of a solution [20 μM, MeCN:PBS (95:5, v/v), 25 °C] of **6** before (***a***) and after illumination at λ_{Ac} (350 nm, 2.48 mWcm^{-2}) for 5 (***b***), 10 (***c***), 15 (***d***) and 20 min (***e***). Absorption spectrum (***f***) of **4** [20 μM, MeCN:PBS (95:5, v/v), 25 °C].

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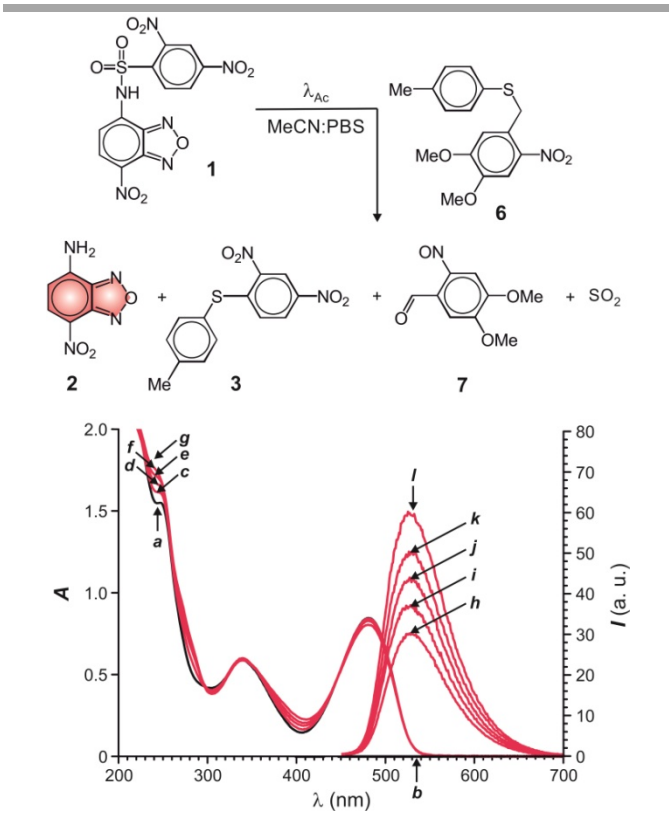

Fig. 4 Absorption (***a***) and emission (***b***) spectra of a solution of **1** [10 μM, MeCN:PBS (95:5 v/v), 25 °C, λ_{Ex} = 440 nm] after the addition of **6** (2 equiv.). Absorption (***c***–***g***) and emission (***h***–***l***) spectra of the same solution after illumination at λ_{Ac} (350 nm, 2.48 mW cm^{-2}) for 5 (***c*** and ***h***), 10 (***d*** and ***i***), 15 (***e*** and ***j***), 20 (***f*** and ***k***) and 30 min (***g*** and ***l***).

chromophore can, indeed, be activated on the basis of these operating principles.

Our results prove that the photoinduced generation of a nucleophile can initiate a chemical reaction to convert a nonemissive reactant into a fluorescent product. In particular, the established photochemistry of the 2-nitrobenzyl group together with the photophysical properties of the NBD chromophore translate into the opportunity to activate the fluorescence of the latter with the photocleavage of the former. The resulting bimolecular mechanism for fluorescence activation can evolve into a general strategy for the implementation of photoresponsive ensembles of molecules based on the coupling of photochemical transformations with chemical reactions.

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Experimental

Chemicals were purchased from commercial sources and used as received with the exception of MeCN, which was distilled over CaH_2. Compounds **1** and **2** were prepared according to literature procedures.[10,22] Electrospray ionization mass spectra (ESIMS) were recorded with a Bruker micrOTO-Q II spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 spectrometer. Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path length of 1.0 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Solutions were irradiated at 350 nm (2.48 mW cm^{-2}) with a Luzchem Research LZC-4V photoreactor.

4 (124 mg, 1 mmol) was added dropwise to a mixture of **5** (276 mg, 1 mmol) and K_2CO_3 (179 mg, 1.3 mmol) in dimethylformamide (DMF, 25 mL) maintained at ambient temperature under Ar. The mixture was stirred for 10 hours, diluted with H_2O (20 mL) and extracted with EtOAc (40 mL). The organic phase was washed with brine (3 × 15 mL), dried over $MgSO_4$ and the solvent was distilled off under reduced pressure. The resulting oil solidified upon standing in air to give **6** (300 mg, 94%) as a yellow crystalline solid. ESIMS: *m*/*z* = 342.0784 $[M + Na]^+$ (*m*/*z* calcd. for $C_{16}H_{17}NNaO_4S$ = 342.0776); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.31 (3H, s), 3.73 (3H, s), 3.92 (3H, s), 4.39 (2H, s), 6.50 (1H, s), 7.06 (2H, d, 8 Hz), 7.20 (2H, d, 8 Hz), 7.64 (1H, s) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 21.0, 21.2, 38.4, 56.1, 56.3, 108.4, 113.2, 128.7, 129.6, 129.8, 131.1, 132.8, 133.4, 137.7, 138.6, 140.2, 147.7, 152.5 ppm.

Notes and references

§ Crystal data for **6**: $C_{16}H_{17}NO_4S$, M_r = 319.37, monoclinic, space group $P2_1/c$, a = 15.7805(9) Å, b = 5.0908(3) Å, c = 21.0600(13) Å, β = 110.851(1)°, V = 1468.32(17) Å^3, Z = 4, T = 296 K, Mo K_α = 0.71073 Å. GOF = 1.038, No. Parameters = 202, $2\Theta_{max}$ = 56°. The final $R1(F^2)$ was 0.0380 for 2912 reflections I>2σ(I). CCDC No. 1033127.

¶ Irradiation of **1** in the absence of **6**, under otherwise identical conditions, does not cause any change in the absorption and emission spectra (Fig. S1).

1 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
2 T. J. Mitchison, K. E. Sawin, J. A. Theriot, K. Gee and A. Mallavarapu, *Methods Enzymol.*, 1998, **291**, 63–78.
3 L. M. Wysocki and L. D. Lavis, *Curr. Op. Chem. Biol.*, 2011, **15**, 752–759.
4 D. Puliti, D. Warther, C. Orange, A. Specht and M. Goeldner, *Bioorg. Med. Chem.*, 2011, **19**, 1023–1029.
5 W.-H. Li and W.-H. Zheng, *Photochem. Photobiol. Sci.*, 2012, **11**, 460–471.
6 P. Klán, T. Šolomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov and J. Wirz, *Chem. Rev.*, 2013, **113**, 119−191
7 F. M. Raymo, *Phys. Chem. Chem. Phys.*, 2013, **15**, 14840-14850.
8 F. M. Raymo, *ISRN Phys. Chem.*, 2012, 619251-1–15.
9 F. M. Raymo, *J. Phys. Chem. Lett.*, 2012, **3**, 2379–2385.
10 S. J. Lord, H. D. Lee, R. Samuel, R. Weber, N. Liu, N. R. Conley, M. A. Thompson, R. J. Twieg and E. W. Moerner, *J. Phys. Chem. B*, 2010, **114**, 14157–14167.
11 (*a*) K. Imai, S. Uzu and T. Toyo'oka, *J. Pharm. Biomed. Anal.*, 1989, **7**, 1395–1403. (*b*) K. Imai, S. Uzu, S. Kanda and W. R. G. Baeyens, *Anal. Chim. Acta* 1994, **290**, 3–20. (*c*) T. Santa, T. Fukushima, T. Ichibangase and K. Imai, *Biomed. Chromatogr.*, 2008, **22**: 343–353.
12 A. Chattopadhyay, *Chem. Phys. Lipids.*, 1990, **53**, 1–15.
13 S. Uchiyama, A. P. de Silva and K. Iwai, *J. Chem. Educ.*, 2006, **83**, 720–727.
14 T. Toyo'oka, *Chromatography*, 2012, **33**, 1–17.

View Article Online
DOI: 10.1039/C4NJ01983K NJC

15 S. Uchiyama, T. Santa, T. Fukushima, H. Homma, and K. Imai, *J. Chem. Soc. Pekin Trans. 2*, 1998, 2165–2173.
16 M. Onoda, S. Uchiyama, T. Santa and K. Imai, *Anal. Chem.*, 2002, **74**, 4089–4096.
17 (*a*) S. Uchiyama, Y. Matsumura, A. P. de Silva and K. Iwai, *Anal. Chem.*, 2003, **75**, 5926–5935. (*b*) S. Uchiyama, Y. Matsumura, A. P. de Silva and K. Iwai, *Anal. Chem.*, 2004, **76**, 1793–1798. (*c*) S. Uchiyama, N. Kawai, A. P. de Silva and K. Iwai, *J. Am. Chem. Soc.*, 2004, **126**, 3032–3033. (*d*) K. Iwai, Y. Matsumura, S. Uchiyama and A. P. de Silva, *J. Mater. Chem.*, 2005, **15**, 2796–2800. (*e*) E. M. Graham, K. Iwai, S. Uchiyama, A. P. de Silva, S. W. Magennis and A. C. Jones, *Lab on a Chip*, 2010, **10**, 1267–1273.
18 (*a*) M. Onoda, S. Uchiyama, T. Santa and K. Imai, *Anal. Chem.*, 2002, **74**, 4089–4096. (*b*) Y. Numasawa, K. Okabe, S. Uchiyama, T. Santa and K. Imai, *Dyes and Pigments*, 2005, **67**, 189–195.
19 B. Heyne, C. Beddie and J. C. Scaiano, *Org. Biomol. Chem.*, 2007, **5**, 1454–1458.
20 (*a*) G. Ambrosi, S. Ciattini, M. Formica, V. Fusi, L. Giorgi, E. Macedi, M. Micheloni, P. Paoli, P. Rossi and G. Zappia, *Chem. Commun.*, 2009, 7039–7041. (*b*) S. Amatori, G. Ambrosi, M. Fanelli, M. Formica, V. Fusi, L. Giorgi, E. Macedi, M. Micheloni, P. Paoli, R. Pontellini, P. Rossi and M. A. Varrese, *Chem. Eur. J.*, 2012, **18**, 4274–4284. (*c*) S. Amatori, G. Ambrosi, E. Borgogelli, M. Fanelli, M. Formica, V. Fusi, L. Giorgi, E. Macedi, M. Micheloni, P. Paoli, P. Rossi and A. Tassoni, *Inorg. Chem.*, 2014, **53**, 4560–4569.
21 B. Bag, A. Pal and B. Biswal, *J. Lumin.*, 2011, **131**, 1121–1130.
22 W. Jiang, Q. Fu, H. Fan, J. Ho and W. Wang, *Angew. Chem. Int. Ed.*, 2007, **46**, 8445–8448.
23 (*a*) Z. Yang, C. Yan, Y. Chen, C. Zhu, C. Zhang, X. Dong, W. Yang, Z. Guo, Y. Lu and W. He, *Dalton Transactions*, 2011, **40**, 2173-2176. (*b*) Z. Liu, X. Wang, Z. Yang and W. He, *J. Org. Chem.*, 2011, **76**, 10286–10290.
24 P. Xie, F. Guo, S. Yang, D. Yao, G. Yang and L. Xie, *J. Fluorescence*, 2014, **24**, 473–480.
25 (*a*) S. Swaminathan, M. Petriella, E. Deniz, J. Cusido, J. D. Baker, M. L. Bossi and F. M. Raymo, *J. Phys. Chem. A*, 2012, **116**, 9928−9933. (*b*) M. Petriella, E. Deniz, S. Swaminathan, M. J. Roberti, F. M. Raymo and M. L. Bossi, *Photochem. Photobiol.*, 2013, **89**, 1391–1398.
26 A. J. Berro, A. J. Berglund, P. T. Carmichael, J. S. Kim and J. A. Liddle, *ACS Nano*, 2012, **6**, 9496–9502.

