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LETTERS

Fluorescent PET(Photoinduced Electron Transfer) Reagents for Thiols

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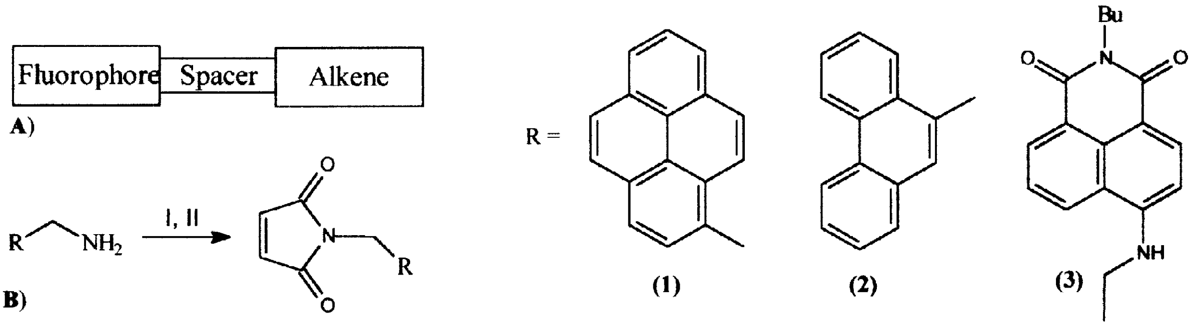
Abstract: Michael addition of thiols to an electron deficient alkene functional group in reagents **(1)**-**(3)** give rise to fluorescence quantum yield enhancements. In the absence of the thiol, photoinduced electron transfer (PET) takes place from the fluorophore to the alkene moiety. Such electron transfer is suppressed after the thiol reaction. © 1998 Elsevier Science Ltd. All rights reserved.

The competition between photoinduced electron transfer (PET) and fluorescence can be sensitively modulated by various chemical interactions.[1] Ion-receptor interactions have received the most attention though redox,[2] solvation,[3] hydrogen bonding,[4] charge transfer[5] and diol-boronate[6] interactions have also generated considerable interest. All these interactions are reversible. In this paper we show for the first time that irreversible organic chemical interactions can also be accommodated within this fluorescence signalling scheme and we demonstrate an example concerning the Michael addition of thiols to electron deficient alkenes. From a practical viewpoint, this opens the way to designed fluorescent PET reagents for thiols and sheds light on the mechanism of action of empirically designed fluorescent reagents previously available.[7]



Scheme 1. **A**) Schematic representation of the model. **B**) I, Maleic anhydride; II, Acetic anhydride/CH_3CO_2Na

Reagents **(1)**-**(3)** (Scheme 1A and B) contain the fluorophore connected to the alkene (maleimide) *via* an aliphatic hydrocarbon spacer, and are thus different from empirically designed thiol reagents which have the maleimide unit directly attached to the fluorophore. Such spacing has been used successfully in reversible PET sensor systems using the fluorophore-spacer-receptor model.[1,8] The short spacer minimises all π-π (or π-n) orbital overlaps between the two main components, and thus largely prevents any ground state interactions. This separation is a requirement for PET, since a fast and an efficient electron transfer can take place over the short spacer. The efficiency of PET is controlled by the free energy of electron transfer (ΔG_{PET}). For **(1)**-**(3)**, PET takes place from the excited state of the fluorophore to the electron deficient alkene, which may be regarded as

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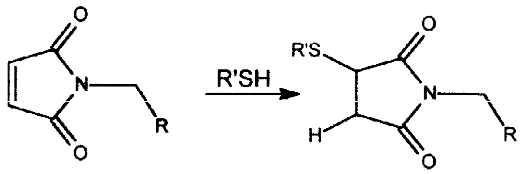
an electron transfer from the lowest unoccupied molecular orbital (LUMO) of the fluorophore after excitation to the LUMO of the alkene.[1,9,10] Upon reacting the thiol with the alkene (Scheme 2) a succinimide derivative is produced which possesses a much higher reduction potential. This renders ΔG_{PET} unfavourable[10] and as a result fluorescence quantum yield enhancements (FE) are observed. Such PET controlled fluorescence have not been previously demonstrated for an irreversible organic chemical reaction.

(**1**) and (**2**) (Scheme 1B) were synthesised by reacting, in accordance with literature procedures,[11] 1-amino methylpyrene and 1-aminomethylphenanthrene respectively, with maleic anhydride in refluxing ether to give the acid amide derivatives, which were then treated with acetic anhydride and sodium acetate to give the maleimide reagents (**1**) and (**2**).[11] The 1,8-naphthalimide derivative (**3**), which has an Internal Charge Transfer (ICT) excited state[10b] due to the presence of an electron withdrawing imide and an electron donating amino group, was made by reacting N-butyl-4-chloro-1,8-naphthalimide with excess ethylene diamine, which was then reacted with maleic anhydride and cyclised to give (**3**).[11] The choice of using three different fluorophores was made in order to emphasise the diversity of the system; (**1**)[12] is a PET analogue of 2-pyrenemaleimide[7] whereas (**2**)[12] and (**3**)[12] were chosen for the purposes of addressing different emission wavelengths and concentration ranges.



Scheme 2. Reaction of thiol with the maleimide unit of (**3**)

The ground and excited state properties of (**1**)-(**3**) were investigated in aqueous methanol solution (1:1, v/v) at pH 7.2. In each case the $S_0 \rightarrow S_1$ absorption band was addressed. The sensitivity towards thiols was followed using 2-mercaptoethanol, *n*-butanethiol and L-cysteine. The absorption spectra of the three reagents were similar to those of the parent fluorophores. No changes were observed in the absorption spectra upon addition of thiols, indicating the insulation effect of the spacer. However the fluorescence emission spectra of (**1**)-(**3**) showed thiol-dependent fluorescence enhancements. Figure 1 shows a family of fluorescence emission spectra at various thiol concentrations for reagent (**3**). The maximum intensity in the spectra levelled out at higher thiol concentrations. The FE values (Table 1) depended somewhat on the nature of the thiol, being largest for 2-mercaptoethanol and least for L-cysteine. Furthermore, more than the stoichiometric amount of L-cysteine was required to attain the maximum limiting FE value whereas the other two thiols behaved

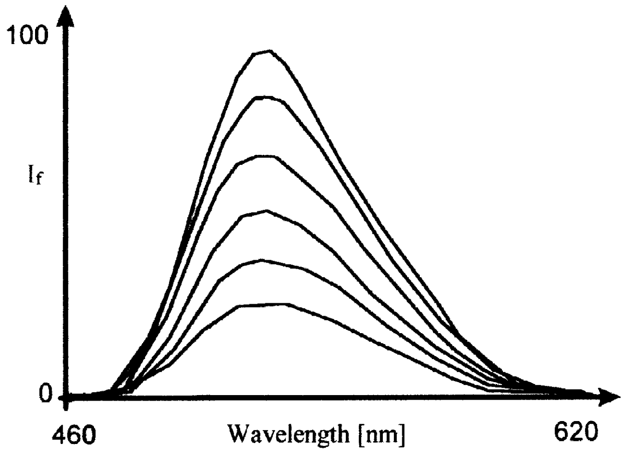


Figure 1. Fluorescence emission spectra excited at 443nm in order of increasing intensity (I_{fmax}) of (**3**), in the presence of 0.00, 0.25, 0.50, 0.75, 1.0, 2.5×10^{-5}M 2-mercaptoethanol

normally. The nature of the fluorescence reagent controlled the experimental results in two ways. Firstly, different concentration ranges of thiol can be accessed by each reagent since the extinction coefficients (ε) of the longest wavelength bands differ widely for (**1**)-(**3**). Secondly the Φ_{Fmax} values differ by nearly an order of magnitude while the FE values are a little lower for (**3**) c.f. (**1**) and (**2**). The Φ_{Fmax} values follow the pattern of Φ_F values of the parent fluorophores, though not proportionately. The latter values are 0.72,[10a] 0.13[10a] and 0.83[13] for pyrene, phenanthrene and N-butyl-4-butylamino-1,8-naphthalimide respectively. The difference in FE values in the three reagents with a given thiol can be attributed to variations in ΔG_{PET} and to the different distances involved in the PET processes.

The demonstrable success of fluorophore-spacer-alkene systems (**1**)-(**3**) for the fluorescence detection of thiols also sheds light on the mode of operation of reagents such as 1-pyrenemaleimide.[7] In the latter case, the maleimide unit is directly attached to the fluorophore unit. Steric forces would be expected to constrain the units to take up an orthogonal geometry. Therefore such reagents can be viewed as fluorophore-alkene systems with a virtual spacer which permits rapid PET between the terminal units.[1d,14] Formation of a non-emissive twisted intramolecular charge transfer (TICT) excited state[15] would be another approach to the understanding of such reagents.

Table 1. Results from ground and excited state investigation of (**1**)-(**3**)[a]

No.	**λ_{Abs}[b](log ε)**	**λ_{Flu} [b]**	**Φ_{Fmin} [c]**	**Φ_{Fmax} [d]**	**Φ_{Fmax} [e]**	**Φ_{Fmax} [f]**	**FE[d,g]**	**FE[e,g]**	**FE[f,g]**
(**1**)	264(4.35), 275(4.65), 312(4.06), 325(4.42), 343(4.58)	379, 395, 409	0.042	0.18	0.17	0.14	4.3	4.1	3.4
(**2**)	324(2.53), 332(2.53), 339(2.45), 347(2.43)	368, 388, 409	0.020	0.079	0.065	0.065	4.0	3.3	3.3
(**3**)	443(4.15)	525	0.19	0.61	0.58	0.54	3.3	3.1	2.9

a) The measurements were carried out in MeOH:H_2O (1:1, v/v) with 0.1 M KCl for ionic strength control and 0.02 M MOPS (4-morpholinopropane sulfonic acid) buffer at pH 7.2, using 7.0×10^{-6}M of (**1**) and (**3**), and 3.0×10^{-4} M of (**2**). b) In nm units. c) The fluorescence quantum yield measured before addition of any thiol. Fluorescence quantum yields are obtained by comparison with protonated N-butyl-4-(2'-dimethylaminoethylamino)-1,8-naphthalimide and N-butylaminomethyl-anthracene as secondary standards. d) For 2-mercaptoethanol. e) For *n*-butanethiol. f) For L-cysteine. g) The FE was determined from the ratio of maximum fluorescence intensity (after thiol addition) and minimum fluorescence intensity (thiol-free).

In conclusion, the three PET reagents (**1**)-(**3**) represent an important extension to the PET sensor principle for the detection of an organic species using irreversible interactions.

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12. All were obtained in satisfactory yields; Calculated for (**1**) $C_{21}H_{13}NO_2$; C, 81.0; H, 4.2; N, 4.5, found: C, 79.8; H, 4.4; N, 4.5; *m/z* = 311.095046, found 311.094629; δ_H(300 MHz, $CDCl_3$) 8.56(d, 1H, J = 9.32, Ar-$\underline{H}$); 8.11 (m, 8H, Ar-$\underline{H}$); 6.72 (s, 2H, -C$\underline{H}$=C$\underline{H}$-); 5.43 (s, 2H, Ar-C$\underline{H}_2$-maleimide); Calculated for (**2**) $C_{19}H_{13}NO_2$; C, 79.4; H, 4.6; N, 4.9, found: C, 79.1; H, 4.4; N, 4.2; *m/z* = 287.3172, found 287.0946; δ_H(500 MHz, $CDCl_3$) 8.75 (d, 1H, J = 2.5Hz, Ar-$\underline{H}$); 8.74 (d, 1H , J 5.5Hz, Ar-$\underline{H}$); 8.66 (d, H, J = 7.6Hz, Ar-$\underline{H}$), 8.26 (dd, J = 1.66Hz, J = 3.5Hz, Ar-$\underline{H}$); 7.74 (m, 5H, Ar-$\underline{H}$); 6.77 (s, 2H, -C$\underline{H}$=C$\underline{H}$-); 5.22 (s, 2H, Ar-C$\underline{H}_2$-maleimide); Calculated for (**3**) $C_{22}H_{21}N_3O_4$: *m/z* = 391.154143, found 391.153206; δ_H(300 MHz, $CDCl_3$) 8.59 (d, 1H, J = 8.0Hz, Ar-$\underline{H}$), 8.45 (d, 1H, J = 8.6Hz, Ar-$\underline{H}$), 8.15 (d, 1H, J = 8.6Hz, Ar-$\underline{H}$), 7.68 (t, 1H, J = 7.3Hz, Ar-$\underline{H}$), 6.80 (s, 2H, -C$\underline{H}$=C$\underline{H}$-), 6.64 (d, 1H, J = 8.0 Hz, Ar-H), 6.08 (bs, 1H, Ar-N$\underline{H}$-CH_2-), 4.15 (t, 2H, J = 7.75Hz, Ar-NH-C$\underline{H}_2$-CH_2-maleimide), 4.06 (t, 2H, J = 7.75Hz, Ar-NH-C$\underline{H}_2$-CH_2-maleimide), 3.75 (m, 2H, imide-C$\underline{H}_2$-CH_2-), 1.69 (m, 2H, imide-CH_2-C$\underline{H}_2$-), 1.44 (m, 2H, -C$\underline{H}_2$-CH_3), 0.97 (t, 3H, J = 7.5Hz, CH_2-C$\underline{H}_3$).
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