Fluorescence enhancement by hydroperoxides based on a change in the intramolecular charge transfer character of benzofurazan†

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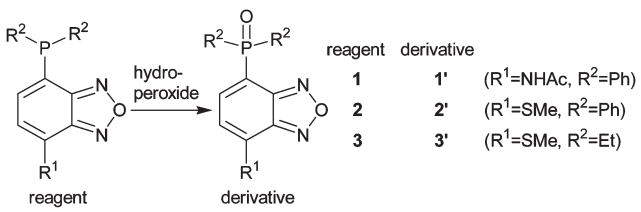
Strong fluorescence signals were observed after the reaction of novel reagents with hydroperoxides.

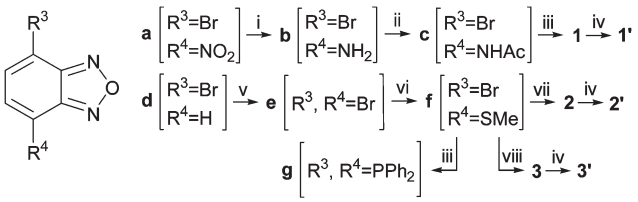
Lipid peroxidation is closely related to aging,[1] and a number of diseases[2] including cancer, Alzheimer's disease, and atherogenesis. Thus, the development of a method to detect hydroperoxides is an important task in the area of biological and biomedical sciences.[3] The use of a fluorescent reagent[4] has been one of the most practical methods for the detection of hydroperoxides in biological samples because it offers high sensitivity and selectivity. Especially, the significant fluorescence enhancement assists the detection of low concentrations of hydroperoxides. Up until now, two fluorescent derivatisation reagents for hydroperoxides, diphenyl-1-pyrenylphosphine (DPPP)[5] and 4-(2-diphenylphosphinoethylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-DPP),[6] have been developed. In both cases, a 30-fold fluorescence enhancement was seen after reaction with hydroperoxides. Interestingly, their fluorescence switching mechanisms are different. The former fluorescence is controlled by the electron withdrawing/donating features of substituents. The ICT (intramolecular charge transfer) character of the fluorophore changes on reaction with hydroperoxides. On the other hand, the latter fluorescence is controlled by the PET (photoinduced electron transfer) process. Comparing the two types of fluorescent derivatisation reagents, the design of the latter is much easier because the efficiency of the PET process has been predictable.[7] Nevertheless, the former type of fluorescent reagent can afford higher sensitivity since it has a very low intrinsic fluorescence, as seen in dansyl chloride.[8] Now we are able to design sensitive fluorescent derivatisation reagents even with control of the ICT character by a recently established method.[9] In this paper, we describe the design, synthesis, and properties of novel fluorescent derivatisation reagents for hydroperoxides, which give an over fourteen times larger fluorescence enhancement than conventional reagents as the maximum.

The benzofurazan (2,1,3-benzoxadiazole) skeleton was chosen as the fluorophore,[10,11] since it possesses long excitation and emission wavelengths, which avoid interference due to biomatrices. Furthermore, the fluorescence intensities of the 4,7-disubstituted benzofurazans are predictable using the Hammett substituent constants at the 4- and 7-positions, as has been shown in developing some fluorescent reagents for other targets.[12] Next, the phosphino group was chosen as a reactive moiety at the 4-position of the benzofurazan skeleton because its electron withdrawing/donating character can be varied by reaction with hydroperoxides, which dramatically helps the fluorescence switching. Finally, by choosing a substituent at the 7-position based on the method previously reported[9] (see ESI for details†), we designed compounds **1–3** as fluorescent derivatisation reagents for hydroperoxides, which react with hydroperoxides to form the oxidised derivatives **1′–3′** (Scheme 1).

As shown in Scheme 2, we adopted an addition–elimination reaction for the introduction of the phosphine unit to the benzofurazans.[13] Bromobenzofurazan derivative **c**, which was readily prepared from nitro derivative **a**, was treated with lithium diphenylphosphide to afford the desired compound **1** in modest yield. In the case of the reaction with compound **f**, however, the sulfide group behaved as a leaving group and the doubly substituted compound **g** was obtained as the main product. Thus, we then utilised a palladium-mediated coupling reaction of bromobenzofurazan with (trimethylsilyl)diphenylphosphine.[14] Although the initial trial using bis(acetonitrile)palladium dichloride as a catalyst resulted in recovery of the starting compounds, the reaction in the presence of palladium acetate and



Scheme 1 The structures of the novel fluorescent derivatisation reagents for hydroperoxides and their hydroperoxide derivatives.



Scheme 2 Synthesis of **1–3** and **1′–3′**. *Reagents and conditions*: (i) Fe, HCl, MeOH, CH_2Cl_2, rt, 25 min (72%); (ii) Ac_2O, pyridine, 50 °C, 4 h (68%); (iii) Ph_2PLi, THF, −10 °C, 10 min (21% for **1**); (iv) *tert*-butyl hydroperoxide, $CHCl_3$, rt, 20 min (83% for **1′**, 82% for **2′**, 86% for **3′**); (v) Br_2, Fe, CH_2Cl_2, 100 °C, 1 h (72%); (vi) NaSMe, aqueous $NaHCO_3$, MeCN, rt, 1.5 h (89%); (vii) Ph_2PSiMe_3, $Pd(OAc)_2$, P(*o*-tol)$_3$, DMF, 90 °C, 4 h (32%); (viii) Et_2PSiMe_3, $Pd(OAc)_2$, P(*o*-tol)$_3$, DMF, 90 °C, 4 h (35%).

† Electronic supplementary information (ESI) available: the method for designing the reagents and their synthetic details. See http://www.rsc.org/suppdata/cc/b5/b500419e/

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Table 1 Fluorescence characteristics of reagents **1–3** and their oxidised derivatives **1′–3′**; maximum absorption wavelength (λ_{ab}), maximum emission wavelength (λ_{em}), fluorescence quantum yield (Φ), extinction coefficient (ε), Stokes shift ($\Delta\nu$) and fluorescence enhancement (FE) equal to the Φ value of the derivative divided by that of the reagent

		Reagents			Oxidised derivatives					
	Solvents	λ_{ab}/nm	λ_{em}/nm	Φ	λ_{ab}/nm	$\varepsilon/10^4$ M^{-1} cm^{-1}	λ_{em}/nm	Φ	$\Delta\nu/10^3$ cm^{-1}	FE
1, **1′**	Acetonitrile	388	480	0.0090	364	1.01	471	0.32	6.2	36
	Methanol	385	470	0.0064	363	1.06	476	0.35	6.5	55
	Methanol : water = 1 : 1	385	—[a]	(<0.0005)[b]	360	0.86	492	0.21	7.5	>420
	Water	—[c]	—[c]	—[c]	360	0.96	496	0.11	7.6	—[d]
2, **2′**	Acetonitrile	401	510	0.0031	384	0.87	495	0.32	5.8	103
	Methanol	400	—[a]	(<0.0005)[b]	385	0.95	494	0.032	5.7	>64
	Water	424	535	0.0038	389	0.77	520	0.022	6.5	6
3, **3′**	Acetonitrile	389	492	0.0072	382	0.82	495	0.42	6.0	58
	Methanol	388	—[a]	(<0.0005)[b]	383	0.91	497	0.045	6.0	>90
	Water	424	518	0.0005	385	0.85	521	0.039	6.8	78

[a] Not detected. [b] Estimated from the detection limit. [c] Insoluble in water. [d] Cannot be calculated.

tris(2-methylphenyl)phosphine took place chemoselectively to afford the desired **2** in 32% yield. In the same way, the corresponding diethylphosphine **3** was obtained from **f** in 35% yield. This is the first report of the direct introduction of a phosphine unit to the benzofurazan skeleton. The oxidised derivatives **1′–3′** were prepared by oxidation with *tert*-butyl hydroperoxide (Scheme 2).

Next, the fluorescence characteristics of the reagents **1–3** and their derivatives **1′–3′** were investigated in polar environments so as to mimic bio-relevant systems. The data are summarised in Table 1. As expected, fluorescence enhancement (FE) was observed when comparing reagents **1–3** and the oxidised derivatives **1′–3′**. High FE values were obtained from **1–3** (max > 420) (*cf.* those in DPPP and NBD-DPP: ~30) showing the success of our strategy. The Stokes shifts of **1′–3′**($\geqslant$5700 cm^{-1}) were larger than those of the derivatives of conventional reagents ($\leqslant$2700 cm^{-1}). Furthermore, **1′–3′** showed longer emission wavelengths (471–521 nm) than the derivative of DPPP (~387 nm). These results indicated that reagents **1–3** have advantages in terms of avoiding interference from real samples. In water, the oxidised derivative **1′** had the highest Φ value (Φ = 0.11) among the three oxidised derivatives, suggesting that reagent **1** could be the most effective for the detection of hydroperoxides under water-rich conditions.

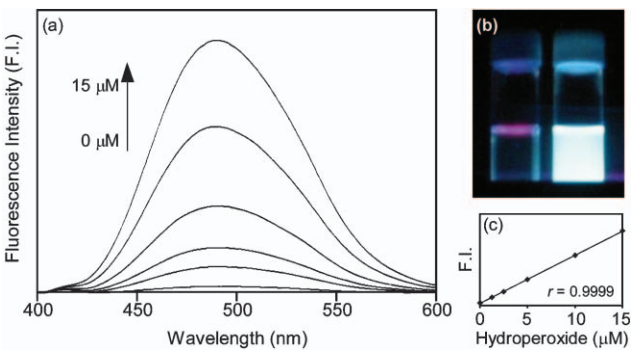

Fig. 1 (a) Fluorescence spectra of the reaction mixtures of **1** (100 μM) and cumene hydroperoxide (0, 1.25, 2.5, 5, 10, 15 μM) exposed at 60 °C for 90 min and then diluted in methanol–water (1 : 1). The excitation wavelength was 360 nm. (b) Fluorescent images of **1** before (left) and after (right) treatment with hydroperoxide. (c) Relationship between the fluorescence intensity (F.I.) at 492 nm and the concentration of hydroperoxide.

Having successfully obtained the desired properties, we then examined the utility of **1** as a representative of the reagents. As shown in Fig. 1, a stronger fluorescence signal was produced by higher concentrations of hydroperoxide (a and b), and a linear relationship was obtained between the fluorescence intensity and the concentration of the hydroperoxide (c). The detection limit (S/N = 3) of hydroperoxide was 1 μM in this system, which overruns the limits of conventional reagents (DPPP: 12 μM, NBD-DPP: 9 μM) under comparable conditions. By using the donor–acceptor choices from this design method,[9] much improved fluorescent derivatisation reagents **1–3** are available.

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