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First Fluorescent Photoinduced Electron Transfer (PET) Reagent for Hydroperoxides

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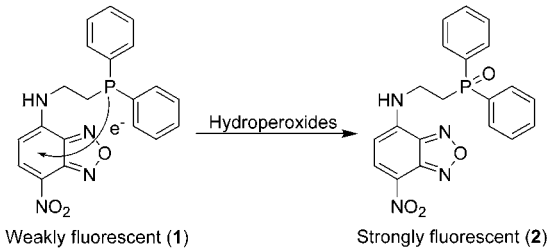
ABSTRACT



Weakly fluorescent (**1**) Strongly fluorescent (**2**)

A novel fluorescent reagent for hydroperoxides, 4-(2-diphenylphosphinoethylamino)-7-nitro-2,1,3-benzoxadiazole (1), was developed on the basis of the method for designing photoinduced electron transfer (PET) reagents having a benzofurazan skeleton. Compound 1 was quantitatively reacted with hydroperoxides to give its fluorescent derivative, 2. In acetonitrile, the Φ value (0.44) of 2 was 31 times greater than that of 1. The long excitation (458 nm) and emission (520 nm) wavelengths of 2 are suitable for the determination of hydroperoxides, especially in biosamples.

The sensitive detection of hydroperoxides is important in a broad range of fields such as analytical, biological, and biomedical sciences, since lipid peroxidation reduces food quality[1] and is related to aging[2] and some diseases,[3] including cancer, Alzheimer's disease, and atherogenesis. Among the several techniques for detecting hydroperoxides in foods and biological materials,[4] the method using a fluorescent "off−on" derivatization reagent, diphenyl-1-pyrenylphosphine (DPPP),[4,5] has been the most useful and provides sensitivity at a picomole level using flow injection and the HPLC postcolumn methods. DPPP, however, is not always satisfactory with respect to the detectability of hydroperoxides,[6] since the maximum excitation and emission wavelengths of its oxidized derivative are so short (ca. 352 and 380 nm in methanol,[5] respectively) that the detection of hydroperoxides is often affected by fluorescent compounds contained in

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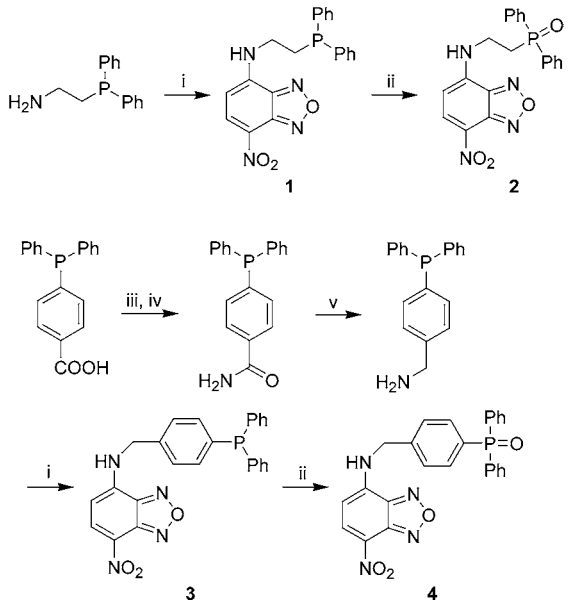
biomatrices. We now report the development of a more useful reagent for hydroperoxides utilizing our general procedure for designing fluorescent "off−on" reagents[7,8] based on the most favored device for fluorescence switching in recent years, i.e., photoinduced electron transfer (PET)[9,10] (so-called "PET reagents"[11]).

First, we designed **1** and **3** as PET reagents for hydroperoxides according to the following three-step procedure (the structures of **1** and **3**, and the corresponding oxidized derivatives, **2** and **4**, are shown in Scheme 1). Triphenylphosphine and methyldiphenylphosphine were chosen as the reactive moieties, since they are stable and react with hydroperoxides to give the corresponding phosphine oxides under mild conditions.[12] 4-Methylamino-7-nitro-2,1,3-benzoxadiazole (NBD-NHMe), on the other hand, was chosen as the fluorophore,[13] since it strongly fluoresces and its excitation and emission wavelengths are sufficiently long to avoid any interference from biomatrices ($\Phi = 0.38$, excitation = 458 nm, and emission = 524 nm in acetonitrile[14]) (step 1). The electron-donating ability of the reactive moiety to the fluorophore was examined by the HOMO energy and/or the *K* value of the Stern−Volmer plots. The PM3/COSMO calculation indicated that the HOMO energies of triphenylphosphine (−9.488 eV) and methyldiphenylphosphine (−9.029 eV) in acetonitrile were greater than those of the corresponding phosphine oxides (−9.916 and −9.910 eV, respectively). The Stern−Volmer plots for NBD-NHMe gave relatively high *K* values for triphenylphosphine (67.1) and methyldiphenylphosphine (81.6) in acetonitrile, whereas those for the corresponding oxides were negligible, thus showing that the oxides were unable to quench NBD-NHMe fluorescence (step 2). These results indicate that the phosphine moieties possess a high electron-donating ability to NBD-NHMe, whereas the phosphine oxide moieties do not. Accordingly, **1** and **3** have been designed by connecting the phosphine and NBD-NHMe moieties with a methylene spacer (step 3).[15]

Scheme 1. Synthesis of the Reagent (**1** and **3**) and Their Oxidized Derivatives (**2** and **4**)[a]



[a] Reagents and conditions: (i) 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), MeCN, rt, 30 min (37% for **1**, 42% for **3**); (ii) MCPBA, MeCN, rt, 30 min (93% for **2**, 91% for **4**); (iii) $(PNCl_2)_3$, benzene, rt, 1 h; (iv) aqueous NH_3, MeCN, rt, 40 min (56%; two steps); (v) $LiAlH_4$, THF, reflux, 1 h (89%).

Compounds **1** and **3**, and their oxidized products were then synthesized in a straightforward manner as depicted in Scheme 1. Coupling reaction of commercially available diphenylphosphinoethylamine and NBD-F by addition−elimination type reaction furnished **1**, which was readily oxidized by treatment with MCPBA. Preparation of **4**, on the other hand, commenced with conversion of the commercially available benzoic acid derivative to its amide. After reduction of amide to benzylamine derivative, **3** was obtained by addition to NBD-F. The corresponding oxidized product was prepared by oxidation with MCPBA.

Next, the fluorescence characteristics of **1**−**4**[16] synthesized according to Scheme 1 were determined. The data in acetonitrile, methanol, and benzene are summarized in Table 1. As expected, in acetonitrile, **1** and **3** were weakly fluorescent, whereas **2** and **4** were strongly fluorescent with long excitation and emission wavelengths ($\lambda_{ex} = 454-458$, $\lambda_{em} = 520$ nm). In particular, the Φ value of **2** was quite large ($\Phi = 0.44$) and 31 times greater than that of **1**, indicating that **1** would be a sensitive PET reagent for hydroperoxides. Similar behavior of **1**−**4** was also observed in methanol. In benzene, however, **3** unexpectedly fluoresced. The high Φ value of **3** in benzene is along the lines of a previous report[17,18] in which the PET process occurred more efficiently in polar solvents than in nonpolar solvents.

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(15) This is the first utilization of a phosphorous atom for PET reagents despite at least two hundred previous reports on the various PET reagents.

(16) For detailed synthetic procedures and spectral data, see Supporting Information.

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Table 1. Fluorescence Characteristics of **1**−**4**

	in acetonitrile				in methanol				in benzene			
	λ_{ex} (nm)	λ_{em} (nm)	Φ	ratio[a]	λ_{ex} (nm)	λ_{em} (nm)	Φ	ratio[a]	λ_{ex} (nm)	λ_{em} (nm)	Φ	ratio[a]
1	461	522	0.014	31	466	527	0.0073	29	451	508	0.051	8.2
2	458	520	0.44		459	525	0.21		449	505	0.42	
3	456	521	0.050	7.2	461	527	0.027	7.0	446	507	0.30	0.7
4	454	520	0.36		457	526	0.19		444	504	0.22	

[a] Equal to the Φ value of the derivative (**2** or **4**) divided by that of the reagent (**1** or **3**).

Comparing the two reagents, **1** should be more sensitive to detect hydroperoxides than **3** since the Φ value of **1** was lower than that of **3** in all solvents (i.e., 0.014 vs 0.050 in acetonitrile, 0.0073 vs 0.027 in methanol, and 0.051 vs 0.30 in benzene). In general, the PET process occurs more efficiently as the distance between the donor and the acceptor becomes shorter.[18,19] These differences in the Φ values could be explained by the distance between the donor and the acceptor of **1** and **3**. According to semiempirical PM3/COSMO calculations, the electron clouds of HOMO for both triphenylphosphine and methyldiphenylphosphine were localized on the phosphorus atom, and a comparison of the stable conformations of **1** and **3** clearly indicated that the distance between the phosphorus atom and the acceptor of **1** was shorter than that of **3**.

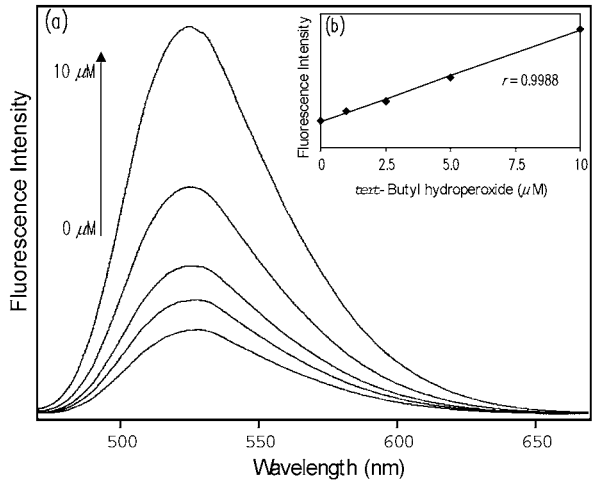

Figure 1. (a) Fluorescence spectra of the diluted reaction mixtures of **1** and *tert*-butyl hydroperoxide in methanol solution. The hydroperoxide (0, 1, 2.5, 5, and 10 μM) was reacted with **1** (100 μM) at 50 °C for 1 h in methanol. The excitation wavelength was 459 nm. (b) Relationship between the concentration of *tert*-butyl hydroperoxide and the fluorescence intensity of the mixture at λ_{em}.

Having successfully designed the reagent, we then examined the utility of **1** for the detection of hydroperoxides. Compound **1** (100 μM) was treated with *tert*-butyl hydroperoxide (0, 1, 2.5, 5, and 10 μM) at 50 °C for 1 h in methanol. The fluorescence spectra of the diluted reaction mixtures were measured with excitation at 459 nm. As shown in Figure 1a, the fluorescence intensity increased with the increase in the concentration of *tert*-butyl hydroperoxide, and a good correlation between the fluorescence intensity and the concentration of *tert*-butyl hydroperoxide was observed (Figure 1b). Similar results were obtained by using cumene hydroperoxide instead of *tert*-butyl hydroperoxide. These results indicated that **1** quantitatively reacted with hydroperoxides and should be applicable to the determination of hydroperoxides.

In summary, we have developed a new fluorescent reagent **1** for hydroperoxides on the basis of our method for designing a PET reagent having a benzofurazan skeleton. In acetonitrile, the Φ value (0.44) of derivative **2** was 31 times greater than that of **1** and the excitation (458 nm) and emission (520 nm) wavelengths of **2** were sufficiently long to be used for biosamples. This is the first description of a highly sensitive PET reagent for hydroperoxides, and reagent **1** should find widespread use in a broad range of fields.

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Supporting Information Available: Synthetic details, spectral data, and fluorescence spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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