

# Singlet oxygen production from fullerene derivatives: effect of sequential functionalization of the fullerene core

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**Singlet oxygen production efficiency of several laser-excited fullerene derivatives is compared by measuring the 1268 nm emissions; the efficiency was independent of the kind of addends, but it decreased with an increase in the number of addends, and addends at adjacent positions caused a greater decrease than remote addends.**

Fullerene and its derivatives produce singlet oxygen ( $^1\text{O}_2$ ,  $^1\Delta_g$ ) by energy transfer with high quantum yield;<sup>1,2,3</sup> [60]fullerene is a more efficient photosensitizer than rose bengal, methylene blue and eosin yellowish (tetrabromofluorescein sodium salt).<sup>4</sup> Singlet oxygen has been postulated to play important roles in many photochemical and photophysical processes in biological systems.<sup>5</sup> For example, it is thought to be responsible for phototoxicity in erythropoietic protoporphyria and for tetracyclin phototoxicity,<sup>5</sup> and it has also been applied for photodynamic therapy of cancer.<sup>5</sup> Thus, it is worthwhile to investigate in detail the photophysical or photochemical properties of fullerene and its derivatives from the standpoint of potential applications to biological systems. In particular, as fullerene derivatives have their UV-VIS absorption maxima at longer wavelengths, and have greater solubility in polar solvents, they may be preferable to the parent compound, [60]fullerene, for pharmaceutical application.<sup>6</sup>

We have already investigated the regiochemistry of several oxidation products of fullerene which were synthesized with the tetraphenylporphyrinatoruthenium(ii) carbonyl-2,6-dichloropyridine-*N*-oxide-HBr system, and reported that [60]fullerene was multiply epoxidized (at least up to pentaepoxide, as determined by FAB mass spectroscopy), and that the second or the third oxygen was introduced at a position adjacent to the existing epoxide (confirmed by  $^{13}\text{C}$  NMR spectroscopy employing  $^{13}\text{C}$ -enriched samples) (Fig. 1).<sup>7</sup> These epoxides might be good models to evaluate the effect of sequential functionalization on the photosensitizing activity of the fullerene structure. Anderson *et al.* have already reported on singlet oxygen production from dihydrofullerene, and discussed the effect of the functionalization.<sup>2</sup> However, they investigated only one derivative, and did not examine the effect of the number of addends, or the regiochemistry of bis or more highly functionalized fullerenes. We have also synthesized diethyl malonate-derivatized [60]fullerenes (see Fig. 1), which were previously reported by Hirsch *et al.*<sup>8</sup> As they have the addends at positions remote from each other (equatorial or *trans*-3),<sup>†</sup> they may have different photophysical properties from those of the corresponding epoxides.

In this work, we measured the near-infrared emission spectra corresponding to the  $\text{O}_2(^1\Delta_g) \rightarrow \text{O}_2(^3\Sigma_g^-)$  transition at 1268 nm, which is highly specific to  $^1\text{O}_2$ , in solutions of fullerene derivatives under argon laser excitation (514.5 nm).<sup>4</sup> This is the most reliable method for  $^1\text{O}_2$  detection developed so far. By this means, the amount of  $^1\text{O}_2$  generated could be compared among the tested samples. We evaluated the efficiency of singlet oxygen production by calculating the value of emission

intensity at 1268 nm/absorption coefficient at 514.5 nm ( $I/\epsilon$ ). As the absorption coefficient is a measure of the efficiency of a transition to an excited state ( $S_1$ ) at a given wavelength, it can be thought that the  $I/\epsilon$  indicates the emission intensity from an unit of excited species ( $S_1$ ) of sensitizer. That is, we evaluated the total efficiency of singlet oxygen production from the excited species by this value, and this value includes the efficiency of intersystem crossing and energy transfer from triplet state to molecular oxygen.

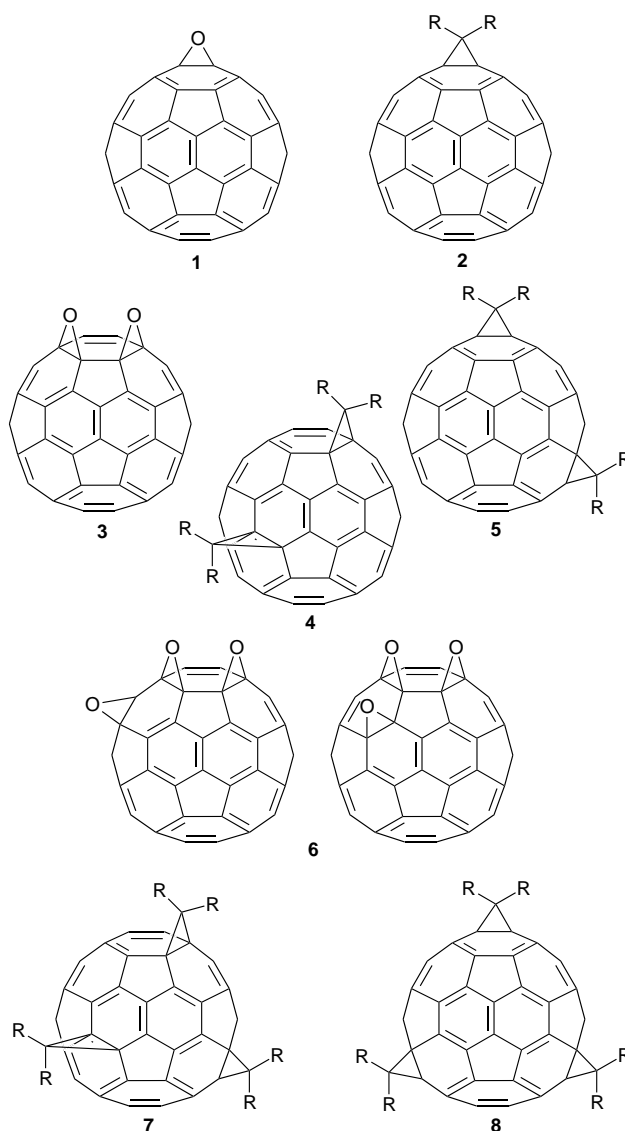


Fig. 1

Emission intensity increased in a concentration-dependent manner with all the compounds. The emission was diminished by the addition of excess  $\beta$ -carotene, a specific  $^1\text{O}_2$  quencher, and was reduced by bubbling  $\text{N}_2$  into the solution for a few minutes. These results confirm that all the compounds used in this study acted as photosensitizers, and that  $^1\text{O}_2$  was responsible for the emission at 1268 nm.

First, we examined the effect of the number of addends on  $^1\text{O}_2$ -producing activity. As shown in Table 1, with both the epoxides and the diethyl malonate derivatives,  $^1\text{O}_2$ -producing ability decreased with an increase in the number of substituents. The size of the decrease was different between the two groups, and this will be discussed later. However, the order of diepoxide **3** and triepoxide **6** was exceptional, and the reason for this is still unknown.

Secondly we compared 1,2-epoxy[60]fullerene **1** and bis(ethoxycarbonyl)[61]fullerene **2**. We did not observe any significant difference between **1** and **2** in  $^1\text{O}_2$ -producing ability. According to Anderson *et al.*,<sup>2</sup> dihydrofullerene had a reduced quantum yield of singlet oxygen production compared with [60]fullerene in  $\text{C}_6\text{D}_6$ . Our results are consistent with their report, even though a different laser wavelength was employed. It appears that functionalization of the fullerene core reduced the photodynamic activity, and the effect was not significantly dependent on the kind of addend, oxygen or diethyl malonate. However, it is noteworthy that the emission intensity itself was much greater with **2** than with **1** or [60]fullerene, because **2** has a greater value of  $\epsilon$  at 514.5 nm (Table 1). This result suggests **2** would produce much more  $^1\text{O}_2$  under given conditions, and could have significant implications for the pharmaceutical application of this class of compounds.

Next we compared 1,2,3,4-diepoxyl[60]fullerene **3**, and tetra(ethoxycarbonyl)[62]fullerenes (equatorial) **4** and (*trans*-3) **5** in order to evaluate the effect of addend position on the fullerene structure. All three derivatives are bisadducts, but **3** has addends at adjacent positions, while **4** and **5** have them at remote positions. The emission intensity of **4** or **5** was stronger than that of [60]fullerene owing to the high  $\epsilon$  value at 514.5 nm but, as expected the photodynamic efficiency was decreased after normalization, as described above. The results in Table 1 show that all of the derivatives have decreased  $^1\text{O}_2$ -producing

ability ( $I/\epsilon$ ) compared with **1** or **2**, but the extent of the decrease was different between the two groups. That is, while **4** and **5** had 20–25% lower activity than **2**, **3** retained only 15% activity compared with that of **1**. This result suggests that a second addend introduced at an adjacent position to the existing one caused a greater perturbation of the electronic structure of the fullerene core than a second addend at a remote position. In the case of trisadducts **6**, **7** and **8**, the result was similar, that is to say, the photodynamic activity of **6** was greatly decreased compared with those of **7** and **8**, because of the proximity of the three addends on the fullerene core.

We also examined multihydroxylated fullerene (fullerenol) prepared by the literature method.<sup>9</sup> No peak was detected in  $\text{D}_2\text{O}$  at concentrations up to about 10 mM (data not shown). This concentration is 10 000 times that of other fullerene derivatives used in this study. Thus, we concluded that extensive derivatization of the fullerene core results in loss of photodynamic activity. This is in accordance with the results reported by Cardullo *et al.* In the report, they showed that a fullerene hexaadduct had no photodynamic activity.<sup>10</sup>

As the results obtained in this study were relative efficiency of  $^1\text{O}_2$  production of the fullerene derivatives, further investigation will be required for the clarification of their detailed photophysical properties, such as quantum yield of the energy transfer from triplet state to molecular oxygen, in the future.

## Footnote

† The nomenclature follows the proposals of Hirsch *et al.* (ref. 8).

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**Table 1** Photodynamic efficiency of fullerene and its derivatives<sup>a</sup>

Compound <sup>b</sup>	$I^c$	$I/\epsilon^d$
$\text{C}_{60}$	1.0	1.0
$\text{C}_{60}\text{O}$ <b>1</b>	1.21	0.74
BECC <sub>61</sub> <b>2</b>	1.59	0.77
$\text{C}_{60}\text{O}_2$ <b>3</b>	0.29	0.11
TECC <sub>62</sub> (equatorial) <b>4</b>	2.09	0.59
TECC <sub>62</sub> ( <i>trans</i> -3) <b>5</b>	2.15	0.53
$\text{C}_{60}\text{O}_3$ <b>6</b>	0.42	0.22
HECC <sub>63</sub> (equatorial, equatorial) <b>7</b>	1.71	0.42
HECC <sub>63</sub> ( <i>trans</i> -3, <i>trans</i> -3) <b>8</b>	1.73	0.40
$\text{C}_{60}\text{O}_4$	0.12	0.06
$\text{C}_{60}\text{O}_5$	0.06	0.03

<sup>a</sup> The emission intensity at 1268 nm was measured in  $\text{CS}_2$  solution for photosensitizers excited by Ar laser light at 514.5 nm with 25 mW output power. <sup>b</sup> Abbreviations: BECC<sub>61</sub> = bis(ethoxycarbonyl) $\text{C}_{61}$ ; TECC<sub>62</sub> = tetra(ethoxycarbonyl) $\text{C}_{62}$ ; HECC<sub>63</sub> = hexa(ethoxycarbonyl) $\text{C}_{63}$ . The nomenclature follows the proposals of Hirsch *et al.* (ref. 8). <sup>c</sup> Emission intensity at 1268 nm. <sup>d</sup> Emission intensity at 1268 nm/absorption coefficient at 514.5 nm (relative value to that of [60]fullerene).