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## Synthesis of a hydrophilic and non-ionic anthracene derivative, the N,N'-di-(2,3-dihydroxypropyl)-9,10-anthracenedipropanamide as a chemical trap for singlet molecular oxygen detection in biological systems

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**Abstract**—We report herein the synthesis of a new hydrophilic and non-ionic anthracene derivative, the N,N'-di-(2,3-dihydroxypropyl)-9,10anthracenedipropanamide. The evaluation of this compound as chemical trap of singlet molecular oxygen by using labeling experiments and HPLC–MS analysis showed that it could be efficiently used in biological investigations. © 2006 Elsevier Ltd. All rights reserved.

### 1. Introduction

Considerable evidence supports the involvement of singlet molecular oxygen ( ${}^{1}O_{2}$ ) in biological processes. Enzymatic reactions, lipid peroxidation, photooxidation, and phagocytosis are among the most studied ones.<sup>1,2</sup> Other studies have found tentative evidence that antibodies are able to use  ${}^{1}O_{2}$  as substrate to generate hydrogen peroxide and ozone in phagocytosis.<sup>3,4</sup>

The investigation of  ${}^{1}O_{2}$  generation in biological systems requires sensitivity and specificity. Some studies have employed deuterated solvents and the addition of quenchers of  ${}^{1}O_{2}$ . It is well known that deuterated solvents increase the lifetime of  ${}^{1}O_{2}$ , <sup>5</sup> and quenchers reduce it. Thus, if  ${}^{1}O_{2}$  is involved in a reaction, its lifetime and chemistry will be influenced by the media and additives. Chemical probes to detect  ${}^{1}O_{2}$  may provide the needed data. This approach is particularly interesting considering the reactivity of  ${}^{1}O_{2}$ 

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toward different substances. The products formed can be detected at very low concentrations. However, the efficiency of the overall process depends on the reactivity and the solubility of the trap. $^{6}$ 

For some time, traps used were derived from furans.<sup>7</sup> Furans are oxidized to dicarbonylic products as the result of the formation of ozonide intermediates (Fig. 1). Although furans are highly reactive toward  ${}^{1}O_{2}$ , these traps can also react with other oxidants, such as hydrogen peroxide, generating the same products observed with  ${}^{1}O_{2}$ .<sup>6</sup>

Cholesterol is a known chemical trap of  ${}^{1}O_{2}$  because the product of the reaction is the 5- $\alpha$ -hydroperoxide, which is considered as a fingerprint of  ${}^{1}O_{2}$  oxidation in biological systems; however, there are limitations that include the low reactivity and stability of the product formed. Moreover, 5- $\alpha$ -hydroperoxide can undergo rearrangement to 7- $\alpha$ -hydroperoxides.<sup>8</sup>



Figure 1. Reaction of furan derivative with <sup>1</sup>O<sub>2</sub>. R is any substituent.

*Keywords*: Anthracene; Endoperoxide; HPLC–MS; Singlet oxygen; Trap; Probe.

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The typical reaction of  ${}^{1}O_{2}$  by the [2+2] mechanism generating dioxetane was exploited in the design of a trap-and-trigger chemiluminescent probe, namely 2-[1-(3*tert*-butyldimethylsilyloxy)-phenyl-3,6,9,12-tetraoxa-1-tridecyl-13-hydroxy-methylene]tricyclo[3.3.1.1]decane.<sup>9</sup> This compound forms an intermediate dioxetane that decomposes under treatment with tetra-*n*-butylammonium fluoride for generating a chemiluminescent signal (Fig. 2).

The reversible binding of  ${}^{1}O_{2}$  to aromatic compounds by the [4+2] mechanism has been exploited to produce chemical traps since the endoperoxide formed represents a specific product for the reaction with  ${}^{1}O_{2}$ . Furthermore, the endoperoxides formed with anthracene derivatives are stable at room temperature and their decomposition occurs only at elevated temperatures (around 100 °C).

The first polycyclic aromatic hydrocarbon endoperoxide studied was that derived from rubrene in 1926, when Dufraisse et al. observed that a benzene solution of rubrene became colorless upon exposure to solar light (Fig. 3). They also observed that the peroxide formed was able to regenerate the parent hydrocarbon and oxygen after heating at 150 °C.<sup>10</sup>

In the next two decades, many other endoperoxides were prepared and studies were performed to better understand their behavior. In 1942, Dufraisse and Velluz demonstrated that 1,4-dimethoxy-9,10-diphenylanthracene endoperoxide was able to release oxygen at room temperature. They also reported that liberated oxygen was in an 'activated' state.<sup>11</sup> The precise nature of the reactive oxygen species was established in 1967 by Wasserman and Scheffer who made a crucial observation.<sup>12</sup> It was found that the products of the reaction of 2,5-diphenyl-4-methyloxazole or 1,3-diphenylisobenzofuran with 9,10-diphenylanthracene endoperoxide were the same to those generated by photooxidation.

Non-substituted aromatic compounds are not able to react significantly with  ${}^{1}O_{2}$ . Suitable modification in the polycyclic aromatic structure has to be made in order to increase the reactivity of  ${}^{1}O_{2}$ . Thus, at least one, and preferably two, electron-donating groups must be present at certain positions to allow the [4+2] cycloaddition of  ${}^{1}O_{2}$  and to stabilize the endoperoxide. The substituted compounds exhibit large differences in their reactivity toward  ${}^{1}O_{2}$ . This is related to the electron density of the aromatic core and the steric hindrance induced by either the substituents themselves or by additional groups located on the other positions.  ${}^{13}$  All these features have to be taken into consideration prior to design a new  ${}^{1}O_{2}$  trap.



Figure 3. Reaction of rubrene with  ${}^{1}O_{2}$  and the thermal decomposition of its endoperoxide.

Anthracene traps have indeed demonstrated that  ${}^{1}O_{2}$  is generated from lipid hydroperoxides treated with metals, peroxynitrite or hypochloride using the hydrophobic molecule 9,10-diphenylanthracene (DPA) and  ${}^{18}O$ -labeled lipid hydroperoxides (Fig. 4). The detection of the endoperoxides formed in the reaction was provided by HPLC–MS analysis. The formation of the  ${}^{18}O$ -labeled endoperoxide of DPA indicated the occurrence of reaction between two lipid peroxyl radicals according to the Russell mechanism. ${}^{14-16}$ 

However, as the biological environment is not restricted to the hydrophobic moiety of the membranes and lipoproteins, these traps show some limitations for the investigation of biological systems because of their low water solubility. This problem may be overcome by modifying their structures in order to make them more hydrophilic.<sup>17</sup>

Anthracene derivatives with hydrophilic substituents can serve as chemical traps in aqueous solution. Furthermore, the substituent type and position on the aromatic ring can be modified, thus resulting in different reactivity.

The addition of carboxylic groups is an interesting option. A hydrophilic anthracene derivative, a 9,10-anthracenedipropionic acid<sup>18</sup> or the potassium salt of a rubrene-2,3,8,9-tetracarboxylic acid<sup>17</sup> has been reported (Fig. 5). The former was used for time-resolved laser photolysis experiments to determine the lifetime of  ${}^{1}O_{2}$  in D<sub>2</sub>O. The latter compound showed solubility at around 1 mM in neutral aqueous solution; however, it was largely insoluble in acid media. Moreover, the rubrene-containing endoperoxide was stable for just a few hours in water.

The anthracene-9,10-diyldiethyl disulfate (EAS) has many adequate properties: it reacts with  ${}^{1}O_{2}$  generating the corresponding endoperoxide (Fig. 6), its solubility is not pH dependent, it is stable up to 120 °C, and it is detectable in small amounts by HPLC.<sup>19</sup>

A compound similar to EAS has also been reported, the anthracene-9,10-bisethanesulfonic acid (AES), which is soluble in water and buffer solutions, is stable over a wide range of pH, and has a reactivity constant  $(k_r)$  of



Figure 2. Scheme for the chemiluminescence detection of the stable dioxetane formed by the reaction with  ${}^{1}O_{2}$ .



Figure 4. <sup>18</sup>O-labeled lipid hydroperoxides treated with metals, peroxynitrite or hypochloride generates  ${}^{18}[{}^{1}O_{2}]$  that was detected by using the hydrophobic chemical trap 9,10-diphenylanthracene (DPA) and HPLC–MS analysis.



rubrene-2,3,8,9-tetracarboxylic acid 9,10-anthracenedipropionic acid

Figure 5. Hydrophilic aromatic compounds with carboxylic groups.

 $1.15 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  (Fig. 6).<sup>20</sup> AES was prepared from 9,10dibromoanthracene (DBA) by a three-step synthesis. An alternative method for the synthesis of AES has also been demonstrated.<sup>21</sup> This was achieved by the conversion of DBA into anthracene-9,10-divinylsulfonate (AVS) through a Pd-catalyzed Heck reaction based on the use of aqueous sodium vinylsulfonate followed by hydrogenation catalyzed by Pd/C catalyst. Interestingly, it was shown that the intermediate AVS could be a suitable  ${}^{1}O_{2}$  chemical trap.

The characteristic  ${}^{1}O_{2}$  reaction with the anthracene moiety was the basis for the development of fluorescein-based fluorescence probe, the 9-[2-(3-carboxy-9,10-dimethyl)-anthryl]-6-hydroxy-3*H*-xanthen-3-one (DMAX) described by Nagano et al. (Fig. 6).<sup>22</sup> It exhibits a weak fluorescence in the native state, but becomes highly fluorescent upon reaction with  ${}^{1}O_{2}$ .

A possible disadvantage of these anionic traps is the interaction with cationic photosensitizers, such as methylene blue, or the interaction with some cations that are important to



Figure 6. Hydrophilic aromatic compounds.

some chemical reactions that generates  ${}^{1}O_{2}$ . In order to avoid these problems, it has been reported the synthesis of an anthracene derivative with a cationic substituent, the bis-9,10-anthracene-(4-trimethylphenylammonium)dichloride (BPAA) (Fig. 6), this compound is hydrophilic and has an overall quenching constant of  $2.0 \times 10^{7}$  M<sup>-1</sup> s<sup>-1</sup>.<sup>23</sup>

It should be added that the main limitation of anthracenic compounds is light absorption particularly in photochemical studies. In this way, it was reported that disodium 1,3-cyclohexadiene-1,4-diethanoate is more appropriate in such situations.<sup>24</sup> It was shown that the measurement of the amount of <sup>1</sup>O<sub>2</sub> generated in aqueous solution by irradiation above 310 nm is well suited when CHDDE was used as the chemical trap and the quantum yield for photosensitizers in D<sub>2</sub>O may be easily determined.<sup>25</sup>

Naphthalene endoperoxides are generally employed as  ${}^{1}O_{2}$ generators. Their stability and solubility follow the same rules of anthracene ones, however, their decomposition to the respective naphthalene derivative and <sup>1</sup>O<sub>2</sub> occurs at room temperature. A fact of great importance in this field was the preparation of N,N'-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPN) by Aubry et al.<sup>26</sup> The compound has a diol group attached to a propionic arm by an amide linkage. Its hydrophilic and non-ionic character is of fundamental importance for suitable utilization in biological systems. The corresponding endoperoxide (DHPNO<sub>2</sub>) named N-(2,3-dihydroxy-propyl)-3-{8-[2-(2-,3-dihydroxypropylcarbamoyl)-ethyl]-9,10-dioxa-tricyclo[6.2.2.0\*2,7\*]dodeca-2,4,6,11-tetraen-1-yl}-propionamide) may be prepared at low temperature by photosensitization with methylene blue and, after removal of the sensitizer from the solution, it is ready to be used. Its decomposition at 37 °C follows first order kinetics and 60% of the oxygen is released as <sup>1</sup>O<sub>2</sub>.<sup>26</sup> We had chosen the naphthalene derivative DHPN to prepare a <sup>18</sup>O-labeled endoperoxide (DHPN<sup>18</sup>O<sub>2</sub>).<sup>27</sup> This water-soluble naphthalene endoperoxide acts as a chemical source for [<sup>18</sup>O] isotopically labeled singlet oxygen  $\binom{18}{10}$  and it is particularly interesting to assess the reactivity of  ${}^{1}O_{2}$  toward biological targets (Fig. 7).



Figure 7. DHPN<sup>18</sup>O<sub>2</sub> as a chemical source of  ${}^{18}[{}^{1}O_{2}]$ .

Oxidation products thus formed will be labeled with, at least, the incorporation of one 18-oxygen atom. Therefore, the oxidation products that contain the labeled oxygen can be detected and quantified using appropriate methods, as HPLC coupled to mass spectrometry. It was known that  ${}^{1}O_{2}$  is able to oxidize the guanine bases of DNA. However, it was not possible to distinguish between two possible processes: the direct reaction of  ${}^{1}O_{2}$  within cellular DNA or the oxidative stress induced by the intracellular production of  ${}^{1}O_{2}$ . In order to clarify this issue, the labeled endoperoxide was incubated with cells; then nuclear DNA was extracted and HPLC–MS/MS analysis of the labeled product allowed to demonstrate the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGua) in DNA by the direct reaction with  ${}^{1}O_{2}$ .<sup>28</sup>

In addition, the labeled endoperoxide DHPN<sup>18</sup>O<sub>2</sub> was successfully used to study the reaction of  ${}^{1}O_{2}$  with 8-oxodGua inserted into oligodeoxynucleotides<sup>29</sup> or as the free nucleoside in aqueous solution.<sup>30</sup> Interestingly, when analyzing the products in those studies some amount of non-labeled products were also detected. Using the chemical trap EAS, we were able to show that an energy transfer from labeled  ${}^{1}O_{2}$  to ground state molecular oxygen occurs in aqueous solution. The products of the reaction were analyzed by ESI-MS measurement at the output of a HPLC column.<sup>31</sup>

We report in the present work, the synthesis of a new hydrophilic and non-ionic anthracene derivative containing the same group found in DHPN, the N,N'-di-(2,3-dihydroxy-propyl)-9,10-anthracenedipropanamide (DHPA, Fig. 8). The evaluation of this compound as a chemical trap of  ${}^{1}O_{2}$  showed that it could be efficiently used in biological investigations, since it is soluble in water, has no charge on its structure, its solubility does not depend on pH, and the product is stable at temperatures above 37 °C, and it may be quantified by appropriate means.

### 2. Results and discussion

## **2.1.** Synthesis of *N*,*N*<sup>'</sup>-di(2,3-dihydroxypropyl)-9,10-anthracenedipropanamide (DHPA)

The first step of the DHPA synthesis was the bromination of DMA resulting in the DBMA (yield of 71%). DBMA structure was assigned by <sup>1</sup>H NMR analysis. In the next step, malonic synthesis resulted in the derivative tetracid, which was characterized by its typical fragmentation in the ESI<sup>-</sup> mass spectrum. In the same way, the structure of the expected decarboxylated product (ADPA) was confirmed by ESI<sup>-</sup> mass spectrometry. After esterification, HPLC–MS analysis of DEADP showed the presence of a molecular ion [M+H]<sup>+</sup> at m/z=379 and the sodium adduct [M+Na]<sup>+</sup> at m/z=396. In the final step, DEADP amidation resulted in DHPA with a yield of 41%. DHPA was characterized by its <sup>1</sup>H and <sup>13</sup>C NMR features and HPLC–MS analysis in the ESI<sup>+</sup> mode.

## 2.2. Formation of DHPA endoperoxide

After 3 h of photosensitization about 75% of DHPA (retention time=15 min, Fig. 9A) was consumed and a new product was detected (retention time=13 min, Fig. 9B). The



Figure 8. DHPA synthesis.

UV-vis spectra of these compounds are shown in Fig. 9C and D. Contrasting with DHPA (Fig. 9C), the UV-vis spectrum of 13-min product peak did not show the characteristic band of anthracene (Fig. 9D).

In order to further evaluate the ability of DHPA to react with  ${}^{1}O_{2}$ , DHPNO<sub>2</sub> was used as a chemical source of  ${}^{1}O_{2}$ . For comparison, the reaction with DHPNO<sub>2</sub> was also carried out with EAS, a frequently used  ${}^{1}O_{2}$ -trap. Spectra in



Figure 9. HPLC analysis of DHPA irradiation. (A) Chromatogram of DHPA in methanol, (B) chromatogram of DHPA in methanol after 1 h of irradiation, (C) UV-vis spectrum of the 15-min eluting product peak in chromatogram A, and (D) UV-vis spectrum of the 13-min eluting product peak in chromatogram B.



Figure 10. UV-vis spectra during incubation with DHPNO2. (A) DHPA spectra and (B) EAS spectra.

the visible region show that the products lose the characteristic anthracene moiety absorption, suggesting the formation of respective endoperoxides with a similar reactivity (Fig. 10).

After the incubation of DHPA with DHPNO<sub>2</sub> for 2 h at 37 °C, three product peaks were formed with retention times of 10, 12 and 13 min in an HPLC elution profile ( $\lambda$ =215 nm, Fig. 11A). The mass spectra of the compounds reveal that the observed peaks correspond to DHPN (*m*/*z*=419 [M+H]<sup>+</sup>, Fig. 11B), DHPAO<sub>2</sub> (*m*/*z*=501 [M+H]<sup>+</sup>, Fig. 11C) and DHPA (*m*/*z*=469 [M+H]<sup>+</sup>, Fig. 11D), respectively. These results suggest that DHPA effectively traps the <sup>1</sup>O<sub>2</sub> produced by thermolysis of DHPNO<sub>2</sub>, since the product with

m/z=501 (DHPAO<sub>2</sub>) corresponds to an increment of one O<sub>2</sub> molecule in DHPA structure (m/z=469+32).

Further insights into DHPA-trapping ability of  ${}^{1}O_{2}$  were gained upon incubation with  ${}^{18}O$ -labeled DHPNO<sub>2</sub>. We observed the formation of a product with the same retention time as that of DHPA ${}^{16}O_{2}$  (data not shown), that exhibits a molecular weight and molecular ion at m/z=505 (Fig. 12). This new product corresponds to DHPA ${}^{18}O_{2}$ , since it shows an increment of 4 units in the m/z of DHPA ${}^{16}O_{2}$ . A small amount of non-labeled product was also observed resulting from energy transfer to residual O<sub>2</sub> in solution as previously demonstrated.<sup>31</sup> The results obtained are summarized in Figure 13.



**Figure 11**. HPLC–MS (ESI<sup>+</sup>) analysis of DHPA reaction with DHPNO<sub>2</sub>. (A) UV detection at  $\lambda$ =215 nm, (B) ESI<sup>+</sup> mass spectrum of the 10-min eluting product peak (DHPA), (C) ESI<sup>+</sup> mass spectrum of the 12-min eluting product peak (DHPAO<sub>2</sub>), and (D) ESI<sup>+</sup> mass spectrum of 13-min eluting product peak (DHPA).



Figure 12.  $\text{ESI}^+$  mass spectrum of DHPA<sup>18</sup>O<sub>2</sub>.



Figure 13. <sup>1</sup>O<sub>2</sub>-trapping ability of DHPA.

## 3. Conclusion

Recent findings suggest the involvement of  ${}^{1}O_{2}$  in biological processes. However, adequate strategies must be employed to detect this transient species. The development of traps, with controllable properties in their structure, e.g., solubility, reactivity, and specificity, represents important strategies to detect <sup>1</sup>O<sub>2</sub>. Thus, the reported synthesis of a new hydrophilic and non-ionic chemical trap may contribute to a better evaluation of <sup>1</sup>O<sub>2</sub> participation in biological processes. We showed that DHPA reacts with <sup>1</sup>O<sub>2</sub> generated by photosensitization or by thermolysis of DHPNO<sub>2</sub> or DHPN<sup>18</sup>O<sub>2</sub>. The DHPA endoperoxide product was detected by the loss of the absorbance from the anthracene moiety and by mass spectrometry confirming the incorporation of two oxygen atoms on the DHPA structure. The main advantage of this compound is the absence of charge and its hydrophilicity that may allow an easier traffic inside the cell.

#### 4. Experimental

#### 4.1. General

Malonic ester, 3-amino-1,2-propanediol, 9,10-dimethylanthracene, sodium bicarbonate, sodium hydroxide, bromine, and sodium were obtained from Sigma (St. Louis, MO). Benzene, methanol, isopropanol, carbon tetrachloride, toluene, ethanol, acetone, *n*-hexane, hydrochloric acid, sulfuric acid, chloroform and acetonitrile (HPLC grade) were acquired from Merck (Rio de Janeiro, Brazil). The water used in the experiments was treated with the Nanopure Water System (Barnstead, Dubuque, IA). <sup>1</sup>H NMR spectra were recorded on Bruker DRX 500 series Advance (Bruker–Biospin, Germany). Mass spectra were determined on a Quattro II instrument (Micromass, UK) in the positive or negative ESI mode (cone voltage was set to 25 V). HPLC analyses were recorded on a Shimadzu LC-10AD/ VP system (Shimadzu, Japan) with a Supelcosil<sup>TM</sup> (Supelco, PA) LC-18 column (5 µm, 150×4.6 mm) and UV detection ( $\lambda$ =260 nm). A gradient of water–acetonitrile was used, starting from 15% acetonitrile, reaching 30% acetonitrile in 10 min, and 80% in the next 5 min, followed by an isocratic elution for 5 min, ending with a decreasing gradient back to 15% within 5 min, at a flow rate of 0.6 mL/min. At the output of the UV detector, the eluent was split and a small fraction was directed into the MS spectrometer at a flow rate of 150 µL/min.

## 4.2. Synthesis of 9,10-dibromomethylanthracene (DBMA)

The route employed was adapted from previous works that describe the synthesis of hydrophilic naphthalene derivatives.<sup>19,32–34</sup> In the first step, 300 µL of Br<sub>2</sub> (5.8 mmol) was added to 0.5 g (2.43 mmol) of 9,10-dimethylanthracene (DMA) dissolved in 50 mL of CCl<sub>4</sub>. The solution was refluxed and irradiated with an Hg lamp (500 W) for 4 h prior to stand overnight at room temperature. The reaction was followed by measuring the pH from the fume released and DBMA formation was checked by thin-layer chromatography (80% CHCl<sub>3</sub> and 20% *n*-hexane). The solvent was removed by rotatory-evaporation under reduced pressure and the solid was purified by recrystallization in chloroform (yield: 0.63 g, 71%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.52 (4H, s), 7.68 (4H, dd, *J*=6.9, 3.3 Hz), 8.36 (4H, dd, *J*=6.9, 3.3 Hz).

## 4.3. Synthesis of 9,10-anthracenedipropionic acid (ADPA)

To synthesize ADPA, 1.5 g of sodium (0.0625 mol) was added to 250 mL of ethanol in a dry 1 L flask and then 20 mL of malonic ester (0.132 mol) was introduced. During the course of the reaction, the system was kept warm (~50 °C) to prevent precipitation. After that, 350 mL of dry benzene and 0.55 g (1.51 mmol) of DBMA were added to the reaction mixture. The system was maintained under reflux for 4 h. The mixture was neutralized with 200 mL of 10% HCl aqueous solution. The organic phase was separated and the solvents were removed by rotatory-evaporation under reduced pressure. The saponification was performed with the residue obtained, by adding 100 mL of 6 M NaOH, 100 mL of methanol, and 5 mL of CHCl<sub>3</sub>. The system was kept under reflux for 3 h. Then, HCl was added until pH ~1 and the solid was separated by filtration. MS (ESI<sup>-</sup>): m/z=409 [M–H]<sup>-</sup>, 365 [M–CO<sub>2</sub>–H]<sup>-</sup>, 321 [M–2CO<sub>2</sub>–H]<sup>-</sup>, and 277 [M–3CO<sub>2</sub>–H]<sup>-</sup>. The decarboxylation was done by keeping the solid at 120 °C for 5 days to get ADPA. MS (ESI<sup>-</sup>): m/z=321 [M–H]<sup>-</sup> and 277 [M–CO<sub>2</sub>–H]<sup>-</sup>.

# 4.4. Synthesis of diethyl 9,10-anthracenedipropionate (DEADP)

The ADPA was subsequently used for the synthesis of DEADP by acid-catalyzed esterification.<sup>35</sup> In this way, 150 mg of ADPA (0.47 mmol) was refluxed in 23 mL of ethanol and 0.1 mL of H<sub>2</sub>SO<sub>4</sub> (95%) during 2 h. Dean–Stark trap was settled in the presence of 6 mL of toluene and the reflux was left for 4 h. The organic phase was washed with 5% aqueous NaHCO<sub>3</sub>, dried, and evaporated to yield DEADP as a oil. MS (ESI<sup>+</sup>): m/z=379 [M+H]<sup>+</sup> and 396 [M+Na]<sup>+</sup>.

# **4.5.** Synthesis of *N*,*N*′-di(2,3-dihydroxypropyl)-9,10-anthracenedipropanamide (DHPA)

The amidation of the diester DEADP with 3-amino-1,2-propanediol was made by stirring, under reflux, a solution of DEADP (80 mg, 0.21 mmol) and 3-amino-1,2-propanediol (0.2 g, 2.2 mmol) in 7 mL methanol and 4 mL of isopropanol for 24 h. After evaporation of the solvent, the residue was triturated with 20 mL of acetone. The colorless precipitate was filtered by suction, rinsed with acetone, and recrystallized in methanol (yield: 40.5 mg, 41%). MS (ESI+): m/z=491 [M+Na]<sup>+</sup>. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  2.66 (4H, t, J=8.3 Hz), 3.17 (2H, dd, J=13.8, 6.8 Hz), 3.31 (2H, dd), 3.38 (2H, dd, J=11.5, 4.9 Hz), 3.59 (2H, m), 3.95 (4H, t, J=8.3 Hz), 7.54 (4H, dd, J=6.9, 3.3 Hz), 8.41 (4H, dd, J=6.9, 3.2 Hz). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 24.8 (CH<sub>2</sub>), 38.0 (CH<sub>2</sub>), 43.2 (CH<sub>2</sub>), 64.5 (CH<sub>2</sub>), 71.5 (CH), 125.8 (CH), 126.2 (CH), 130.4 (C), 133.2 (C), 175.4 (C=O). The solubility in water is around 0.8 mM.

## **4.6.** Endoperoxide of *N*,*N*′-di(2,3-dihydroxypropyl)-9,10-anthracenedipropanamide

The chemical trap capacity of DHPA was evaluated by the reaction with  ${}^{1}O_{2}$ , generated by photooxidation or by naphthalene endoperoxide thermolysis. The photooxidation was performed in methanol without sensitizer and it was followed by the loss of UV–vis absorption in 372 nm and by HPLC analysis. In order to further confirm the identity of the product formed, the reaction was performed with DHPA (0.8 mM) and DHPNO<sub>2</sub> (0.5 mM) or  ${}^{18}O_{-1}$ abeled naphthalene endoperoxide (DHPN ${}^{18}O_{2}$ , 0.5 mM) under argon atmosphere at 37 °C for 2 h. The products were submitted to HPLC separation and ESI<sup>+</sup>-MS analysis. The DHPA UV–vis spectrum was followed along the time of incubation. For comparison, a known chemical trap (EAS, 0.8 mM) was also submitted to the same conditions of reaction and its spectrum was also recorded.

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