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## Synthesis, spectroscopic properties and photodynamic activity of a fulleropyrrolidine bearing a basic amino group and its dicationic analog against *Staphylococcus aureus*



Maximiliano L. Agazzi, Mariana B. Spesia, Natalia S. Gsponer, M. Elisa Milanesio, Edgardo N. Durantini\*

Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal Nro 3, X5804BYA Río Cuarto, Córdoba, Argentina

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### ABSTRACT

*N*-Methyl-2-[4-(3-*N,N*-dimethylaminopropoxy)phenyl]fulleropyrrolidine (MPC<sub>60</sub>) was synthesized by 1,3-dipolar cycloaddition reaction of 4-(3-*N,N*-dimethylaminopropoxy)benzaldehyde, *N*-methylglycine and fullerene C<sub>60</sub> in 43% yield. The amine groups of MPC<sub>60</sub> were methylated with dimethyl sulfate to obtain a dicationic fullerene DPC<sub>60</sub><sup>2+</sup> in 96 % yield. Absorption spectra of these fullerenes in *N,N*-dimethylformamide (DMF) and toluene/sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/water reverse micelles showed strong absorptions in the UV region, with a peak at 430 nm and broader range of absorption up to 710 nm. Fluorescence quantum yields of about 10<sup>-4</sup> were calculated for these compounds in DMF. A higher singlet molecular oxygen, O<sub>2</sub>(<sup>1</sup>Δ<sub>g</sub>), generation was found for MPC<sub>60</sub> than DPC<sub>60</sub><sup>2+</sup> in DMF. The photodynamic activity of these photosensitizers remained high in a simple biomimetic AOT system. Also, the formation of superoxide anion radical induced by MPC<sub>60</sub> and DPC<sub>60</sub><sup>2+</sup> was detected in presence of NADH. Decomposition of L-tryptophan in DMF mediated by both fullerenes indicated a possible contribution of type I photoprocess. Photosensitized inactivation of *Staphylococcus aureus* was investigated using different conditions. Cell suspensions of 10<sup>8</sup> cells/mL incubated with 0.5 μM fullerene and irradiated for 30 min exhibited a 4.4 and 5.0 log decrease of cell survival by MPC<sub>60</sub> and DPC<sub>60</sub><sup>2+</sup>, respectively. Therefore, these fullerene derivatives can be used as effective photosensitizers for the photodynamic inactivation of *S. aureus* cells.

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### 1. Introduction

In recent years, new approaches to the treatment of microbial infections have become necessary due to the changing patterns of infectious diseases and the emergence of antibiotic-resistant microbes. This is mainly because of the inappropriate prescription of antibiotics, the application in prophylaxis and the systemic use that also affect the normal flora and the failure of patients to complete the treatments. The antibiotic era was perhaps largely expected to eliminate *Staphylococcus aureus* and other bacterial pathogens as a leading cause of human infections [1]. However, *S. aureus* has extraordinary ability to develop resistance to antibiotics, which have been the impetus for waves of antimicrobial resistance over the past 60 years [2]. In this sense, new alternative therapies have been proposed [3]. In particular, photodynamic

inactivation (PDI) of microorganisms has been proposed to controlling bacterial infections [4]. PDI involves the administration of a photosensitizer that is accumulated in the microbial cells and the subsequent irradiation with visible light. In the presence of oxygen, the photodynamic activity produces cell inactivation. The main advantages of this approach are: (i) fast eradication of microorganisms, (ii) double selectivity, specific accumulation of photosensitizer in microbial cells and light delivered only to affected area and (iii) similar photoinactivation regardless of the antibiotic resistance and lack of induction of resistance to photodynamic treatments [5].

Appropriate photosensitizers have specific chemical and biological properties. Thus, the combination of visible light absorption and a long lifetime of triplet excited state allow fullerenes to act as photosensitizers [6]. However, fullerenes are hydrophobic molecules with low solubility in polar solvents and consequently fullerenes form aggregates in aqueous solutions [7]. Thus, the lack of solubility in biological environments is the major obstacle in the development of this field. Different approaches

\* Corresponding author. Tel.: +54 358 4676157; fax: +54 358 4676233.  
E-mail address: [edurantini@exa.unrc.edu.ar](mailto:edurantini@exa.unrc.edu.ar) (E.N. Durantini).

have been developed for the transfer of fullerenes to water. Perhaps, the most versatile methodology to resolve this problem is based on the chemical modification of the fullerene by the attachment of functional hydrophilic cationic groups. These amphiphilic fullerenes can produce an increase in the biological activity and therefore act as efficient photosensitizer to inactivate microbial cells. The development of covalent chemistry of  $C_{60}$  has opened the possibility to attach this sphere structure with several groups, which allows increase in the biological activity [8]. Thus, chemical modification of the fullerene can be used to attach functional hydrophilic cationic groups. These amphiphilic fullerenes can be efficient photosensitizer in biological media. Interesting results using cationic fullerenes as photosensitizers have been found to photoinactivate microorganisms [9–11].

Under aerobic conditions, the triplet excited state of fullerene ( ${}^3C_{60}^*$ ) can interact with ground state molecular oxygen to form reactive oxygen species (ROS). This process can occur by energy transfer from the  ${}^3C_{60}^*$  to produce singlet molecular oxygen,  $O_2({}^1\Delta_g)$  or by electron transfer to form superoxide anion radical ( $O_2^{\bullet-}$ ) [12]. Fullerenes are extremely efficient  $O_2({}^1\Delta_g)$  generators with a quantum yield that is near unity. On the other hand, fullerenes can be easily reduced to  $C_{60}$  radical anion ( $C_{60}^{\bullet-}$ ) by electron transfer. Thus, the  ${}^3C_{60}^*$  or  $C_{60}^{\bullet-}$  can transfer an electron to molecular oxygen forming  $O_2^{\bullet-}$ . In contrast to  $O_2({}^1\Delta_g)$  generation, the electron transfer type of reaction preferentially occurs in polar solvents, particularly in the presence of reducing agents such as NADH. These pathways, yielding  $O_2({}^1\Delta_g)$  and  $O_2^{\bullet-}$ , are analogous to the two main photochemical reaction types known as type II and type I mechanisms, respectively [6].

In the present work, a novel fulleropyrrolidine  $C_{60}$  derivative (MPC<sub>60</sub>) was synthesized, which contains an aliphatic chain with an amino group at the end (Scheme 1). The nitrogen atoms in the amine groups were used to obtain a dicationic fulleropyrrolidinium (DPC<sub>60</sub><sup>2+</sup>). The formation of cationic amphiphilic photosensitizers has several interesting features that make these compounds attractive photosensitizers for a variety of biological systems [6]. The spectroscopic and photodynamic properties of these fullerenes were studied in organic solution and in a simple biomimetic medium formed by reverse micellar system. Also, photodynamic activity mediated by these photosensitizers was evaluated *in vitro* for inactivation of *S. aureus* cells.

## 2. Materials and methods

### 2.1. General

Proton nuclear magnetic resonance spectra were performed on a FT-NMR Bruker Avance DPX400 spectrometer at 400 MHz. Mass spectra were recorded on a Bruker micrOTOF-QII (Bruker

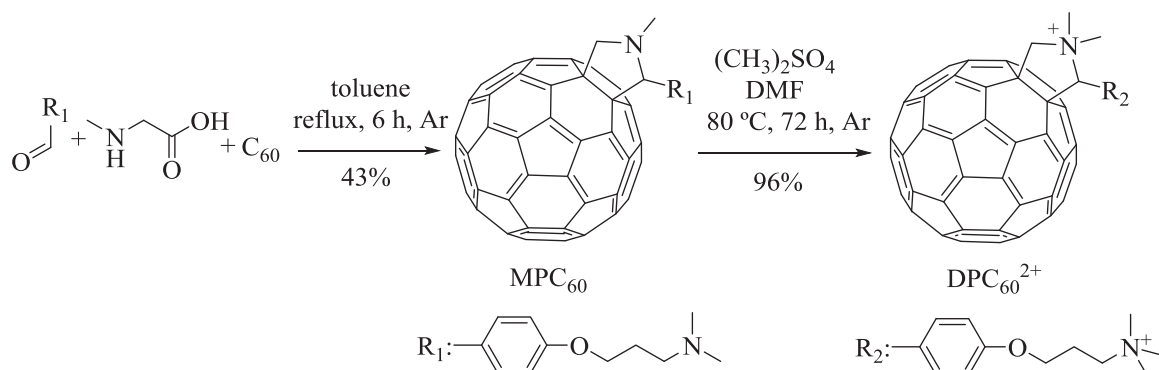
Daltonics, MA, USA) equipped with an atmospheric pressure photoionization (APPI) source. Absorption and fluorescence spectra were carried out in a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan) and on a Spex FluoroMax spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA), respectively. Experiments of photooxidation of substrates were carried out with a Cole-Parmer illuminator 41,720-series (Cole-Parmer, Vernon Hills, IL, USA) with a 150 W halogen lamp through a high intensity grating monochromator (Photon Technology Instrument, Birmingham, NJ, USA). The visible light source used to irradiate cell suspensions was a Novamat 130 AF (Braun Photo Technik, Nürnberg, Germany) slide projector containing a 150 W lamp. A 2.5 cm glass cuvette filled with water was used to remove the heat from the lamp. A wavelength range between 350 and 800 nm was selected by optical filters. The fluence rate was determined as was 90 mW/cm<sup>2</sup> (Radiometer Laser Mate-Q, Coherent, Santa Clara, CA, USA).

All the chemicals from Aldrich (Milwaukee, WI, USA) were used as received. Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) from Sigma (St. Louis, MO, USA) was dried under vacuum. Tetrasodium 2,2'-(anthracene-9,10-diyl) bis(methylmalonate) (ABMM) was synthesized as previously described [13]. Silica gel thin-layer chromatography (TLC) Plates 250 microns from Analtech (Newark, DE, USA) were used. Solvents (GR grade) from Merck (Darmstadt, Germany) were distilled. Labconco (Kansas, MO, USA) equipment model 90,901-01 was used to obtain ultrapure water.

### 2.2. Synthesis of fullerene $C_{60}$ derivatives

*N*-Methyl-2-[4-(3-*N,N*-dimethylaminopropoxy)phenyl]fulleropyrrolidine (MPC<sub>60</sub>). A solution of  $C_{60}$  (51 mg, 0.071 mmol), 4-(3-*N,N*-dimethylaminopropoxy) benzaldehyde (25 mg, 0.120 mmol) and *N*-methylglycine (26 mg, 0.292 mmol) in 55 mL of dry toluene was stirred at reflux under an argon atmosphere for 6 h. Then, the solvent was removed under vacuum. Flash column chromatography (silica gel) using toluene/ethyl acetate (100:0–50:50 gradient, 1% triethylamine) as eluent afforded 29 mg (43%) of MPC<sub>60</sub>. TLC (silica gel, toluene/ethyl acetate/triethylamine, 1:1:0.01)  $R_f$  = 0.2.  $\lambda_{max}$  (DMF) [nm] ( $\epsilon$ , M<sup>-1</sup> cm<sup>-1</sup>) 431 (3980). <sup>1</sup>HNMR (CDCl<sub>3</sub>, TMS)  $\delta$  [ppm] 1.97 (m, 2H, –CH<sub>2</sub>–), 2.28 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 2.50 (t, 2H, NCH<sub>2</sub>–,  $J$  = 7.0 Hz), 2.79 (s, 3H, NCH<sub>3</sub> pyrrolidine ring), 4.02 (t, 2H, OCH<sub>2</sub>–,  $J$  = 6.1 Hz), 4.24 (d, 1H, pyrrolidine ring,  $J$  = 9.4 Hz), 4.88 (s, 1H, pyrrolidine ring); 4.97 (d, 1H, pyrrolidine ring,  $J$  = 9.4 Hz), 6.95 (d, 2H, Ar,  $J$  = 8.0 Hz), 7.70 (d, 2H, Ar,  $J$  = 8.0 Hz). APPI-MS [ $m/z$ ] 955.1810 [M + H]<sup>+</sup> (954.1732 calculated for C<sub>74</sub>H<sub>22</sub>N<sub>2</sub>O).

*N,N*-Dimethyl-2-[4-(3-*N,N,N*-trimethylammoniopropoxy)phenyl]fulleropyrrolidinium (DPC<sub>60</sub><sup>2+</sup>). A mixture of MPC<sub>60</sub> (10 mg, 0.010 mmol) and dimethyl sulfate (200  $\mu$ L, 2.11 mmol) in 2 mL of *N,N*-dimethylformamide (DMF) was stirred for 40 h at 90 °C under an



Scheme 1. Synthesis of fullerene derivatives.

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