

***Supplementary Information***

**A Multifunctional Nanoassembly of Mesogen-Bearing Amphiphiles and Porphyrins for the Simultaneous Photodelivery of Nitric Oxide and Singlet Oxygen**

Elisa B. Caruso, Enzo Cicciarella, and Salvatore Sortino\*

*Dipartimento di Scienze Chimiche, Università di Catania, I-95125, Catania, Italy*

E-mail: ssortino@unict.it

**Chemicals.**

All chemicals were purchased by Sigma-Aldrich and used as received. All solvents used were spectrophotometric grade.

**Instrumentation.**

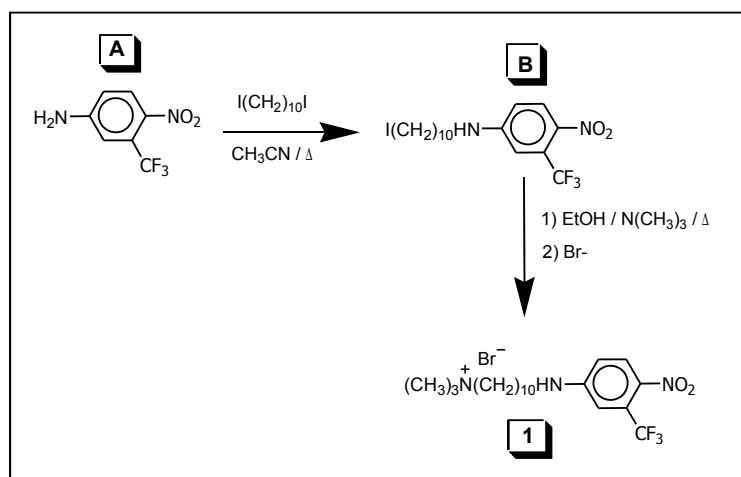
<sup>1</sup>H NMR spectra were recorded on a VARIAN INOVA 200 spectrometer, using TMS as internal standard. ESI-MS spectra were recorded on an Agilent 1100 Series ESI/MSD spectrometer. Experimental conditions were as follows: capillary voltage, 3.5 KV; fragmentor, 100 V; source temperature, 350 °C; drying gas, N<sub>2</sub> (10 L/min), carrier solvent, methanol (0.4 mL/min). The samples were dissolved in a mixture methanol:acetonitrile.

UV/vis absorption spectra were recorded with a Jasco V 560 spectrophotometer. Fluorescence emission spectra were recorded with a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter.

DLS measurements were performed with a Horiba LS 550 apparatus equipped with a diode laser with a wavelength of 650 nm. Conductivity measurements were performed using a TOA Electronics Ltd. electroconductance meter CM-40S

### Synthetic procedures.

*N,N,N*-trimethyl-10-*4*-nitro-3-(trifluoromethyl)phenyl]amino}decan-1-aminium bromide (**1**) was synthesized in a two step synthesis as reported in Scheme 1S. Syntheses were carried out under a low intensity level of visible light.



Scheme 1S

#### *3*-(trifluoromethyl)-*N*-(10-iododecyl)-*4*-nitrobenzenamine (**B**).

Commercial 3-(trifluoromethyl)-4-nitrobenzenamine (**A**) (1.088 g, 5.2 mmol) and 1-10-diiododecane (8.3 g, 20 mmol) were refluxed in 40 ml of acetonitrile for 5 days. The organic solution was concentrated under reduced pressure and purified by column chromatography (dichloromethane:cyclohexane, 70:30) to give **B** (yield 65 %) as a brown-yellow oil. Anal. Calcd (%) for C<sub>17</sub>H<sub>24</sub>F<sub>3</sub>IN<sub>2</sub>O<sub>2</sub>: C, 43.23; H, 5.12; N, 5.93; found: C, 44.12; H, 5.61; N, 6.42;. ESI-MS *m/z*: [M+H]<sup>+</sup> 473.1. <sup>1</sup>H-NMR CDCl<sub>3</sub>-*d*<sub>1</sub> (200 MHz) δ = 7.94 (1H, d, J=9.2 Hz), 6.87 (1H, d, J=2.2 Hz), 6.62 (1H, dd, J<sub>1</sub>=9.2 Hz, J<sub>2</sub>=2.2 Hz), 4.68 (1H, s, broad.), 3.25 (2H, t, J=7.0 Hz), 3.2 (2H, t, J=7.3), 1.71-1.27 (16H, m).

#### *N,N,N*-trimethyl-10-*4*-nitro-3-(trifluoromethyl)phenyl]amino}decan-1-aminium bromide (**1**)

**B** (35 mg, 0.074 mmol) was dissolved in ethanol and heated at 55° C. An excess of trimethylamine was then added and the mixture was kept under continuous stirring for 5 days. After cooling down to ambient temperature, the solvent was distilled under reduced pressure. The solid residue was dissolved in 2 mL of methanol and eluted with methanol on a Dowex 1 x 8 200-400 mesh (Merck) ion-exchange column previously saturated with bromide and washed with water (1 L) and methanol (0.5 L). The eluted fractions which contained product (using a spectrophotometric detection test) were collected and concentrated under reduced pressure to afford **1** (yield 90 %) as a yellow powder. Anal. Calcd (%) for C<sub>20</sub>H<sub>33</sub>BrF<sub>3</sub>N<sub>3</sub>O<sub>2</sub>: C, 49.59; H, 6.87; N, 8.67; found: C, 48.12; H, 6.39; N, 7.96. ESI-MS *m/z*: [M+H]<sup>+</sup> 404.2. <sup>1</sup>H-NMR COC<sub>2</sub>H<sub>6</sub>-*d*<sub>6</sub> (200 MHz) δ = 8.04 (1H, d, J=9.2 Hz), 7.21 (1H, d, J=2.2 Hz), 6.94 (1H, dd, J<sub>1</sub>=9.2 Hz, J<sub>2</sub>=2.2 Hz), 3.66 (2H, t, J=9.5), 3.28 (2H, t, J=9.3 Hz), 3.39 (9H, s), 2.06-1.33 (16H, m).

### NO measurements.

NO release was measured with a World Precision Instrument, ISO-NO meter, equipped with a data acquisition system, and based on direct amperometric detection of NO with short response time (< 5 s) and sensitivity range 1 nM – 20 μM. The analog signal was digitalized with a four-channel recording system and transferred to a PC. The sensor was accurately calibrated by mixing standard solutions of NaNO<sub>2</sub> with 0.1 M H<sub>2</sub>SO<sub>4</sub> and 0.1 M KI according to the reaction<sup>18</sup>:  $4\text{H}^+ + 2\text{I}^- + \text{NO}_2^- \rightarrow 2\text{H}_2\text{O} + 2\text{NO} + \text{I}_2$

Irradiation was performed in a thermostated quartz cell (1 cm pathlength, 3 ml capacity) by using the monochromatic radiation ( $\lambda_{\text{exc}} = 450 \text{ nm}$ ) of a fluorimeter Fluorolog-2 (mod. F-111). The sample solutions were previously deoxygenated with N<sub>2</sub> and continuously and gently stirred during the experiments. NO measurements were carried out with the electrode positioned outside the light path in order to avoid false NO signal due to photoelectric interference on the ISO-NO electrode. The quantum yield of NO photogeneration  $\Phi_{\text{NO}}$  was determined by using equation (1):

$$\Phi_{\text{NO}} = \Delta [\text{NO}]V / \Delta t I_0 F \quad (1)$$

where  $\Delta[\text{NO}]/\Delta t$  is the rate of formation NO,  $V$  is the volume of the irradiated solution,  $I_0$  is the intensity of the incident photons and  $F$  is the fraction of the photons absorbed by the NO donor at the excitation wavelength.

### Singlet oxygen measurements.

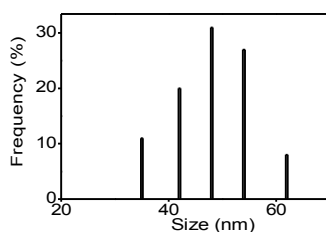
The photogeneration of singlet oxygen ( $^1\Delta_g$ ) was monitored by luminescence measurements in air-saturated water solution. The samples were excited with the second harmonic of a Nd–YAG Continuum Surelite II–10 laser (532 nm, 6 ns), using quartz cells with a path length of 1.0 cm. Upon laser excitation, the emission of singlet oxygen at 1.27 μm was probed orthogonally to the exciting beam with a pre-amplified (low impedance) Ge-photodiode (Hamamatsu EI-P, 300 ns resolution) maintained at –196°C and coupled to a long-pass silicon filter (> 1.1 μm) and an interference filter (1.27 μm). The signals from the photodiode were initially captured by a Tektronix TDS 3032 digitizer, operating in pre-trigger mode and then transferred to a personal computer, controlled by Luzchem Research software operating in the National Instruments LabView 5.1 environment. The energy of the laser pulse was measured at each shot with a SPHD25 Scientech pyroelectric meter. The temporal profile of the luminescence was fitted to a single-exponential decay function with the exclusion of the initial portion of the plot, which is affected by the scattered excitation. The luminescence at initial time ( $L\Delta$  at  $t = 0$ ) was extrapolated from the curve fitting.

Comparative experiments of  $^1\text{O}_2$  ( $^1\Delta_g$ ) photogeneration were performed with aqueous solution of TPPS in the absence and in the presence of micelles. For a more accurate comparison of the  $^1\text{O}_2$  ( $^1\Delta_g$ ) generated by the different samples, time-resolved measurements were performed at different intensity of the laser pulse. The values of  $L\Delta$  at  $t = 0$ , corrected for the slight difference of photons absorbed by the different samples at the excitation wavelength, were then plotted against the laser intensity, and the related slopes ( $\chi$ ) were compared. The quantum yield of singlet oxygen photogeneration in the nanoassembly  $\Phi_{\Delta\text{-TPPS/I}}$  was determined by using equation (2). A standard value  $\Phi_{\Delta\text{-TPPS}} = 0.6$  was taken as quantum yield of singlet

oxygen formed upon excitation of TPPS in water medium in the absence of micelles.<sup>2S</sup> The terms  $\chi_1$  and  $\chi_2$  in equation (2) are the slopes of the plots of the singlet-oxygen luminescence, determined at initial time upon excitation TPPS in the presence and in the absence of **1**, against the energy of the laser pulse.

$$\Phi_{\Delta\text{-TPPS}/\mathbf{1}} = \Phi_{\Delta\text{-TPPS}} \chi_1 / \chi_2 \quad (2)$$

**Size distribution of **1** micellar aggregates in the presence of TPPS ([**1**] = 2 mM), [TPPS] = 20  $\mu$ M)**



**Fig. 1S**

(1S). D. W. Godwin, D. Che, D. M. O'Malley and Q. Zhou, *J. Neurosci. Methods.* **1997**, 73, 91.

(2S). J. Mosinger and Z. Micka, *J. Photochem. Photobiol. A* **1997**, 107, 77.