Synthesis and Antibacterial Activity of New Poly-*S*-lysine−Porphyrin Conjugates

João P. C. Tomé,[†,‡] Maria G. P. M. S. Neves,[†] Augusto C. Tomé,[†] José A. S. Cavaleiro,*[,†] Marina Soncin,[‡] Michela Magaraggia,[‡] Stefania Ferro,[‡] and Giulio Jori*[,‡]

Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal, and Department of Biology, University of Padova, 35131 Padova, Italy

Received March 2, 2004

Studies on the synthesis, structural elucidation, and biological evaluation of new conjugates of poly-*S*-lysine with meso-substituted porphyrins are described. The new conjugates were used in the photoinactivation of antibiotic-resistant Gram-positive bacteria (*Staphylococcus aureus* strains ATCC 25923 and MRSA 110) and Gram-negative bacteria (*Escherichia coli* strain O4). The results show that the cationic conjugates are able to photosensitize the efficient inactivation of both types of bacteria.

Introduction

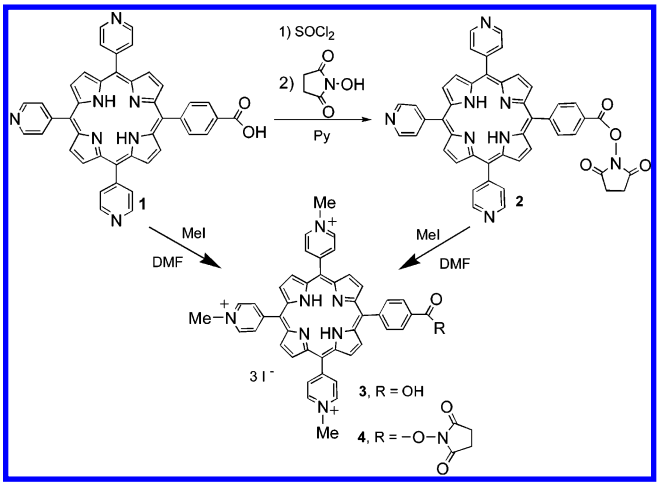
With the increasing number of antibiotic resistant strains of microorganisms, it is extremely important to find alternative treatments for microbial infections. The possibility of using photodynamic therapy (PDT) as a nonantibiotic approach for killing pathogenic microorganisms seems to be very promising.[1,2] The photodynamic destruction of microorganisms is based on the ability of certain photosensitizers, when activated by light, to generate reactive oxygen species that are able to destroy or affect bacterial membranes.[3]

Recent works[4,5] have shown that cationic photosensitizers are able to induce the photoinactivation of Gram-positive [Gram (+)] and Gram-negative [Gram (−)] bacteria without the addition of membrane-permeabilizing agents, such as polymixin or Tris-EDTA. These results are extremely important, especially due to the resistance of Gram (−) bacteria to the direct action of neutral and anionic photosensitizers.[6]

Following our interest in the development of porphyrins with potential use in medicine,[7] we report here the synthesis of new conjugates of poly-*S*-lysine with neutral and cationic meso-tetra-substituted porphyrins. The photosensitizing efficiency of the newly synthesized derivatives was tested against antibiotic-resistant Gram (+) bacteria (*Staphylococcus aureus* strain ATCC 25923 and methycillin-resistant *Staphylococcus aureus* strain MRSA 110) and Gram (−) bacteria (*Escherichia coli* strain O4).

Chemistry. Synthesis of Porphyrin−Poly-*S*-lysine Conjugates. Porphyrin **1** was obtained from a crossed Rothemund reaction using 4-pyridinecarbaldehyde, 4-formylbenzoic acid, and pyrrole in refluxing acetic acid and nitrobenzene.[8] Attempts to couple porphyrin **1** directly to poly-*S*-lysine did not lead to the desired conjugate **5**. This fact prompted us to prepare the activated porphyrin **2** (Scheme 1) and then to use it in the coupling reaction. Porphyrin **2** was obtained in two steps, in 91% global yield. Treatment of porphyrin **1** with thionyl chloride afforded the corresponding acyl chloride; addition of *N*-hydroxysuccinimide to this intermediate yielded the desired compound **2**. The cationic porphyrins **3** and **4** were obtained from the reaction of porphyrins **1** and **2** with a large excess of methyl iodide in dry DMF (Scheme 1). The products were obtained pure and in excellent yields (higher than 90%) from the reaction mixture by precipitation with diethyl ether, filtration, and recrystallization.

Scheme 1

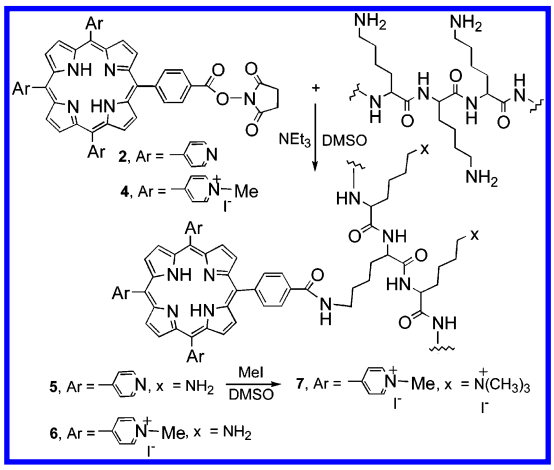


The porphyrin−polylysine conjugates **5** and **6** (Scheme 2) were prepared by reacting poly-*S*-lysine hydrobromide oligomers, with molecular weights between 1000 and 4000, with porphyrins **2** and **4**, respectively. After 24 h at 40 °C all the porphyrin derivative **2** was consumed. The reaction of polylysine with porphyrin **4** was less efficient than that with **2** since after 24 h some unreacted porphyrin **4** and/or its corresponding acid **3** could be detected by TLC. To remove unreacted porphyrins, the reaction mixtures were dialyzed during 48 h using membranes of 1000 MW cutoff successively against three aqueous solutions of hydrochloric acid (1%) in methanol (1:1). Using TLC it was then concluded that all the remaining porphyrin was covalently bound to the poly-*S*-lysine. The red nondiffusible material solutions were concentrated to a few milliliters under reduced pressure, at 35 °C. The resulting solutions were frozen in liquid nitrogen and lyophilized to obtain the

* Correspondence to Prof. Giulio Jori, Department of Biology, University of Padova, via Ugo Bassi 58/B, I-35131 Padova, Italy. Phone: +39 049 827 6333. Fax: +39 049 827 6344. E-mail: jori@bio.unipd.it.
† University of Aveiro.
‡ University of Padova.

10.1021/jm040802v CCC: $27.50
Published on Web 11/18/2004

Scheme 2



conjugates **5** and **6** . The degree of porphyrin linked to the polylysine chains was estimated as ca. 30% (w/w) by UV−vis using the Soret molar extinction coefficients of porphyrin **1** (log $\epsilon_{418\ nm} = 5.61$) and of porphyrin **3** (log $\epsilon_{425\ nm} = 5.34$), respectively, for polymers **5** and **6**. The porphyrin−polylysine conjugate **7** with fully quaternized ϵ-amino groups (Scheme 2) was obtained by treating the derivative **5**, before dialysis, with a large excess of methyl iodide. The reaction was carried out at 40 °C for 7 h. The polymer was purified by dialysis using the protocol referred above.

Biological Evaluation. Photosensitizers. The stock solutions of the photosensitizers were prepared as follows: the neutral porphyrin **1** in DMSO, the poly-*S*-lysine conjugate **5** in DMSO with 10% of water, and the cationic derivatives **3**, **6**, and **7** in PBS (10 μM phosphate-buffered saline, pH 7.4). The concentration of each photosensitizer in the stock solutions was determined by UV−vis using the Soret molar extinction coefficient of porphyrin **1** for polymer **5** and the one of porphyrin **3** for polymers **6** and **7**.

The solutions of the two neutral compounds **1** and **5** to be used were then prepared in PBS/DMSO (95:5) and in PBS for the cationic ones (**3**, **6**, and **7**).

Bacterial Strains. *Staphylococcus aureus* strain ATCC 25923, methycillin-resistant *Staphylococcus aureus* strain MRSA 110, and *Escherichia coli* strain O4 were grown aerobically at 37 °C in brain heart agar (BHA). The cells in the stationary phase of growth were harvested to a tube with brain heart infusion (BHI) at 37 °C, centrifuged (3000 rpm for 10 min), washed two times with PBS, and diluted with the same buffer to an optical density of ca. 0.7 at 650 nm, corresponding to 10^8 to 10^9 cells/mL.

Cellular Uptake of Porphyrins. The cellular uptake was determined by incubating suspensions of bacteria (10^8 to 10^9 cells/mL) for 10 min in the dark at room temperature with the same photosensitizer concentrations as used in the inactivation studies. Then, those suspensions were centrifuged (3000 rpm for 10 min), and the photosensitizer solution was removed by suction and the cell pellet was dissolved by digesting it in 0.5 mL of 2% aqueous SDS to give a homogeneous solution.

The concentration of the porphyrin was determined by spectrophotofluorimetric analysis, and the protein content of the cell extract was determined by a modified Lowry method with bovine serum albumin to obtain calibration curves. Recoveries were expressed as nmol of photosensitizer per mg of cell protein.

Table 1. Photostability Percentage of the Photosensitizers after Irradiation with White Light for Different Periods of Time

	irradiation time (min)						
compd	0	1	3	5	10	20	30
1	100	99	97	96	94	90	85
3	100	93	86	83	77	72	67
5	100	98	98	97	97	95	95
6	100	100	98	94	93	91	89
7	100	92	88	86	84	80	75

Cell Survival Assays at Different Concentrations of the Photosensitizer. The phototoxicity of the various photosensitizers was determined by incubating suspensions of bacteria (10^8 to 10^9 cells/mL) with appropriate concentrations of photosensitizer for 10 min, in the dark and at room temperature, followed by irradiation with white light, at a fluence rate of 50 mW/cm^2 for 30 min. The dark toxicity was determined under the same conditions but without irradiation. Typically, the suspensions were serially diluted with PBS, each dilution was plated on BHA, and the colonies formed after 16−20 h incubation at 37 °C were counted. Percent survival was calculated by dividing the number of colony forming units (cfu) from cells incubated with a photosensitizer by the number of cfu from controls (without photosensitizer).

Irradiation of the cells in the absence of a photosensitizer caused no detectable decrease in cell survival.

Cell Survival Assays at Different Irradiation Times. The cell survival studies at different irradiation times were accomplished by incubation of suspensions (10^8 to 10^9 cells/mL) of *S. aureus* ATCC 25923 and MRSA 110 with 0.1 μM solutions of photosensitizer, as well as of *E. coli* O4, with 1 μM solutions of photosensitizer. The incubations were carried out for 10 min in the dark and at room temperature. The suspensions were irradiated for different periods of time with white light at a fluence-rate of 50 mW/cm^2. The determination of cell survival, as a function of the irradiation time, was performed by counting the cfu and dividing it by the number of cfu from controls (without photosensitizer).

Results and Discussion

The antibacterial efficiencies of the polylysine derivatives **5**, **6**, and **7**, and their porphyrinic precursors **1** and **3**, were tested against the Gram (+) bacteria *S. aureus* ATCC and MRSA, and against the Gram (−) bacterium *E. coli*.

Photostability of Compounds. The photostability of the various porphyrin derivatives is shown in Table 1.[9] In all cases, exposure of the porphyrins to visible light caused a decrease in the intensity of the visible absorption bands, which is indicative of an irreversible destruction of the tetrapyrrolic macrocycle. Such a process is generally defined as photobleaching.[2] The data in Table 1 clearly suggest that all our compounds show a high photostability under the conditions used for the PDT tests. Other widely used porphyrin derivatives, such as hematoporphyrin and protoporphyrin-IX are photobleached far more than 50% when irradiated under the same experimental conditions (unpublished data from our laboratories). The reduced extent of

Table 2. Porphyrin Dose (μM) Causing a 4-log Decrease in the Survival of Selected Bacterial Strains upon White Light Irradiation for 30 min at a Fluence-Rate of 50 mW/cm^2

porphyrin	*S. aureus* ATCC	*S. aureus* MRSA	E. coli
1	0.25	1.00	10.00
3	2.50	2.50	5.00
5	0.50	2.50	10.00
6	1.00	2.50	2.50
6*	1.00	1.00	-
7	0.50	2.50	10.00

photodegradation exhibited by compounds such as **1**, **5**, and **6** would guarantee that the concentration of the photoactive principle undergoes no important changes during the required irradiation time in case of phototherapeutic applications.

Cellular Uptake Results. The uptake of the various porphyrin samples by the three bacterial strains was found to increase with the porphyrin dose in the 0.1−10 μM range. The amount recovered was of the order of porphyrin nanomoles per mg of cell protein. No plateau value was apparently reached. In particular, the porphyrins exhibited an almost identical affinity for the ATCC and MRSA *S. aureus* strains. On the other hand, the neutral derivatives **1** and **5** showed a higher and similar uptake in both cell types [Gram (+) and Gram (−)] than the cationic derivatives. This behavior is partially at a variance with that displayed by the cationic derivatives **3**, **6**, and **7**, which showed a similar profile of uptake for the three strains studied, but the accumulation by *E. coli* was approximately four times smaller than by *S. aureus.*

Cell Survival Assays at Different Photosensitizer Concentrations. The results obtained upon studying the cytotoxic action of the various photosensitizers on bacterial cells in the dark[9] and upon illumination are summarized in Table 2. No detectable decrease in cell survival was observed upon dark incubation. All porphyrin derivatives proved to be efficient photosensitizers for the damage of both strains of *S. aureus,* and, under the most favorable experimental conditions, drastic inhibitions (>6 log) of cell survival were obtained. Remarkably, derivatives **1** and **5**, which lack any positive charge in the tetrapyrrolic macrocycle, appear to be especially active against the ATCC and, to a lesser extent, the MRSA strain of *S. aureus* in the low (0.1−0.5 μM) concentration range, even though under these conditions the amount of cell-bound photosensitizer was similar with that observed for the cationic porphyrins (see Table 2). For concentrations larger than 1 μM the cationic derivatives appeared to yield more pronounced cytotoxic effects despite the smaller binding affinity, while there was essentially no difference in the degree of photosensitivity between the two *S. aureus* strains. These findings confirm earlier observations[10] showing that the response of bacteria to porphyrin photosensitization is independent of their antibiotic resistance spectrum.

Apparently, *E. coli* is markedly less photosensitive than the Gram (+) bacterial cells. Only the cationic derivatives **3**, **6**, and **7** induced a significant (4−5 log) cell lethality at concentrations in the 1−5 μM range, in agreement with the hypothesis that electrostatic interactions between the positive charges in the photosensitizer and negative charges at the cell surface represent a driving force in promoting the photosensitivity of Gram (−) bacteria.[11] The phototoxic effect was more pronounced in the presence of the polylysine moiety. A similar enhancing action was observed for polylysine−porphycene conjugates,[12] and it was ascribed to the ability of the polylysine chain to interfere with the highly organized structure of the outer wall typical of Gram (−) bacteria, thereby increasing its permeability.[6] Interestingly, for noncationic porphyrin derivatives, such as **1** and **5**, a kind of threshold concentration value appears to exist in the 10 μM range, endowing them with an overall phototoxicity against *E. coli* which is quite similar with that shown by the cationic derivatives. The binding of derivatives **1** and **5** with bacterial cells at a 10 μM concentration is significantly larger than that measured for their cationic counterparts, but the same result obtains also at lower porphyrin concentrations. Therefore, the explanation of this unexpected phototoxic effect of neutral porphyrins **1** and **5** on *E. coli* requires further investigations.

Since the relatively low water solubility of compounds **1** and **5** required the use of a binary PBS/DMSO (95:5) solvent mixture, we checked whether the presence of 5% DMSO could affect the photoprocess in any way, by repeating the studies of *S. aureus* photoinactivation in the presence of compound **6** dissolved in PBS/DMSO (95:5). Marginal differences were observed.

Cell Survival Assays as a Function of the Irradiation Time. These experiments were performed by using cell suspensions with an overall volume of 3 mL containing 0.1 or 1 μM photosensitizer for Gram (+) and Gram (−) bacteria, respectively. Such a protocol allowed us to utilize one sample for each photokinetic experiment, which generally required cell viability assays at different irradiation times up to a maximum of 30 min.

Such experiments, while confirming the order of porphyrin photoactivity observed in the previous photosensitization studies pointed out that *S. aureus* MRSA is more photoresistant than *S. aureus* ATCC to the photosensitizing action of all porphyrins studied by us. Under most favorable circumstances, the survival of the wild and antibiotic-resistant *S. aureus* strains decreased by about 5 and 3−4 log, respectively. In particular, porphyrin **1** appears to be more efficient in promoting the inactivation of *S. aureus* ATCC, while conjugate **6** exhibits a high phototoxicity against *S. aureus* MRSA.

The results obtained for the survival of *E. coli* cells in the presence of 1 μM photosensitizers **3**, **6**, and **7** underlined the unusually high photoefficiency displayed by derivative **6** which caused a 7 log drop in cell survival just after 5 min of irradiation. Conjugate **7** also proved to be very efficient after a 20 min irradiation. These results and the negligible phototocicity of tricationic porphyrin **3** confirmed the importance of the polylysine moiety for inducing an extensive inactivation of this Gram (−) bacterium.[13]

Conclusions

New conjugates of poly-*S*-lysine with neutral and cationic meso-substituted porphyrins were efficiently prepared, and the antibacterial efficiency of the new polymers and of their porphyrinic precursors was tested on the photoinactivation of antibiotic-resistant Gram (+) and Gram (−) bacteria. The results show that all

compounds studied were able to photoinactivate directly both strains of *S. aureus*.

The photoinactivation of *E. coli* occurred only in the presence of the cationic derivatives **3**, **6**, and **7**. The highest efficiency was obtained with conjugates **6** and **7**; thus, positive charges are required to photoinactivate Gram (−) cells and that polylysine significantly increases the activity of the photosensitizers. However, the extraordinary efficiency of **6** after 5 min of irradiation when compared with **7** shows that the conversion of the free amino groups into quaternary ammonium ones in polylysine induces a smaller efficiency. From these data we can conclude that the use of conjugate **6** represents the most convenient choice in order to achieve the most efficient photosensitized inactivation of both Gram (+) and Gram (−) bacteria.

These results are also promising for the progress of any research work on the synthesis of novel porphyrin derivatives and their potential application for the inactivation of highly antibiotic-resistant Gram (+) and Gram (−) bacteria. Potentially, this opens the possibility to obtain a drug with a large spectrum of antimicrobial activity.

Experimental Section

5-(4-Carboxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin (1). 4-Formylbenzoic acid (1.27 g, 8.46 mmol, 1.2 equiv) and 4-pyridinecarbaldehyde (2.00 mL, 20.9 mmol, 2.9 equiv) were added to a refluxing mixture of glacial acetic acid (200 mL) and nitrobenzene (150 mL). After the dissolution of the 4-formylbenzoic acid, pyrrole (2.00 mL, 28.9 mmol, 4 equiv) was added dropwise (ca. 4 min) to the mixture. The reaction mixture was then refluxed for 1 h. The solvents were distilled under reduced pressure, and the crude material was taken into chloroform/methanol (85:15) and directly chromatographed on a silica column using a mixture of chloroform/methanol (85:15) as eluent. The first fraction was identified by TLC as 5,10,15,20-tetrakis(4-pyridyl)porphyrin (93 mg, 3%). The second fraction gave porphyrin **1** (257 mg, 6% yield) after evaporation ofthe solvent and recrystallization from chloroform/methanol(85:15)/acetone.

General Procedure for the Methylation of Porphyrins 1 and 2. To a stirred solution (or suspension) of porphyrins **1** and **2** in dry DMF (5 mL for 25 mg of porphyrin) was added a large excess of methyl iodide, and the reaction mixture was heated at 40 °C in one flask equipped with a condenser for 3−4 h. The progress was monitored by TLC using a mixture of acetic acid/methanol/water (5:2:1) as eluent. When the reactions were complete, the cationic porphyrins **3** or **4** were precipitated with diethyl ether, filtered, and washed several times with the same solvent. The solids were dissolved in acetone/water (1:1) and reprecipitated with acetone. The products were dried under reduced pressure.

Porphyrin−Polylysine Conjugate 5. Poly-*S*-lysine hydrobromide (29 mg) was dissolved in dry DMSO (19 mL), and triethylamine (0.5 mL) was added to this solution. The porphyrinic activated ester **2** (24.0 mg, 31.7 μmol), in dry DMSO (10 mL), was then added dropwise at room temperature. The reaction mixture was stirred in the dark and under nitrogen for 24 h at 40 °C. The coupling reaction was monitored by TLC using chloroform or acetic acid/methanol/water(5:2:1) as eluents. The reaction mixture was then dialyzed using membranes of 1000 MW cutoff for 48 h against a 1:1 solutions of aqueous hydrochloric acid (1%) and methanol (3 × 300 mL). The retentate solution was concentrated to a few milliliters under reduced pressure at a maximum temperature of 35 °C. The resulting solution was frozen in liquid nitrogen and lyophilized to give **5**·*n*HCl (25.5 mg) as a green flocculated solid.

Porphyrin−Polylysine Conjugate 6. Poly-*S*-lysine hydrobromide (64 mg) was dissolved in dry DMSO (50 mL), and triethylamine (1.5 mL) was added to this solution. The porphyrinic activated ester **4** (80.0 mg, 67.6 μmol), dissolved in dry DMSO (14 mL), was added dropwise at room temperature. The mixture was stirred in the dark and under nitrogen for 24 h at 40 °C. The progress of the reaction was monitored for TLC using acetic acid/methanol/water(5:2:1) as eluent. After the workup (as described for the preparation of conjugate **5**), the conjugate **6**·*n*HCl (68.0 mg) was obtained as a green flocculated solid.

Porphyrin−Polylysine Conjugate 7. The activated ester **2** (21.0 mg, 27.7 μmol) dissolved in dry DMSO (10 mL) was added to a solution of poly-*S*-lysine hydrobromide (26 mg) in dry DMSO (16 mL) and triethylamine (0.5 mL). After 24 h at 40 °C, the mixture was cooled to room temperature, and methyl iodide (3 mL) was added. The mixture was stirred for 7 h at 40 °C. After the workup (as described for conjugates **5**), the mixture was dialyzed against (1%) hydrochloric acid aqueous solutions (3 × 300 mL). The conjugate **7**·*n*HCl (21.5 mg) was obtained as a green flocculated solid. The degree of homogeneity of the sample was assayed by HPLC analysis (see Supporting Information).

Acknowledgment. Thanks are due to FCT − Fundação para a Ciência e a Tecnologia − FEDER (projects POCTI/38750/FCB/2001 and POCTI/32851/QUI/1999) and to Universities of Aveiro and Padova, for funding this work. J. P. C. Tomé is also grateful to FCT for a Post-Doc grant.

Supporting Information Available: Detailed information on the synthesis of porphyrin derivatives **2** and **4**, analytical and spectroscopic data, photostability studies, and biological results (graphics) of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *The Porphyrin Handbook − Applications: Past, Present and Future*; Kadish, K. M., Smith, K. M., Guilard, R., Eds; Academic Press: New York, 2000; Vol. 6. (b) Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science Publishers: London, 2000. (c) Jori, G., Perrin, C., Eds *Photodynamic Therapy of Tumors and Other Diseases*; Libreria Progetto Editore: Pádua, 1985. (d) Stojiljkovic, I.; Evavold, B. D.; Kumar, V. *Expert Opin. Inv. Drug*. **2001**, *10*, 309−320. (e) Koning, K.; Teschke, M.; Sigusch, B.; Glockmann, E.; Eick, S.; Pfister, W. *Cell. Mol. Biol*. **2000**, *46*, 1297−1303.

(2) *Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use*; Bock, G., Harnett, S., Eds.; John Wiley: Chichester, 1989.

(3) Bonnett, R.; Buckley, D. G.; Burrow, T.; Galia, A. B. B.; Saville, B.; Songca, S. P. *J. Mater. Chem*. **1993**, *3*, 323−324.

(4) (a) Reddi, E.; Ceccon, M.; Valduga, G.; Jori, G.; Bommer, J. C.; Elisei, F.; Latterini, L.; Mazzucato, U. *Photochem. Photobiol*. **2002**, *75*, 462−470. (b) Polo, L.; Segalla, A.; Bertoloni, G.; Jori, G.; Schaffner, K.; Reddi, E. *J. Photochem. Photobiol. B: Biol*. **2000**, *59*, 152−158. (c) Merchat, M.; Spikes, J. D.; Bertoloni, G.; Jori, G. *J. Photochem. Photobiol. B: Biol*. **1996**, *35*, 149−157.

(5) (a) Hamblin, M. R.; O'Donnell, D. A.; Murthy, N.; Rajagopalan, K.; Michaud, N.; Sherwood, M. E.; Hasan, T. *J. Antimicrob. Chemother*. **2002**, *49*, 941−951. (b) Hamblin, M. R.; O'Donnell, D. A.; Murthy, N.; Contag, C. H.; Hasan, T. *Photochem. Photobiol*. **2002**, *75*, 51−57. (c) Soukos, N. S.; Ximenez-Fyvie, L. A.; Hamblin, M. R.; Socransky, S. S.; Hasan, T. *Antimicrob. Agents Chemother*. **1998**, *42*, 2595−2601.

(6) Malik, Z.; Ladan, H.; Nitzan, Y. *J. Photochem. Photobiol., B: Biol*. **1992**, *14*, 262−266.

(7) Faustino, M. A. F.; Neves, M. G. P. M. S.; Vicente, M. G. H.; Cavaleiro, J. A. S.; Neumann, M.; Brauer, H.-D.; Jori, G. *Photochem. Photobiol*. **1997**, *66*, 405−412; *Ibid.* **2000**, *72*, 217−225.

(8) *The Porphyrin Handbook − Synthesis and Organic Chemistry*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: New York, 2000; Vol. 1.

(9) The data are available at Supporting Information.

(10) Wainwright, M. *J. Antimicrob. Chemother*. **1998**, *42*, 13−28.

(11) Merchat, M.; Bertoloni, G.; Giocomoni, P.; Villanueva, A.; Jori, G. *J. Photochem. Photobiol., B: Biol*. **1996**, *32*, 153−157.

(12) Lauro, F. M.; Covolo, L.; Jori, G.; Bertoloni, G. *Photochem. Photobiol. Sci*. **2002**, *1*, 468−470.

(13) Jori, G. *Photodyn. News* **1999**, *2*, 2−4.

JM040802V