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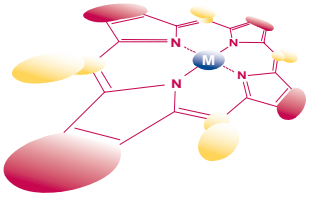

Synthesis of asymmetrically *meso*-substituted porphyrins bearing amino groups as potential cationic photodynamic agents

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ABSTRACT: Novel asymmetrically *meso*-substituted porphyrins bearing amino groups have been synthesized as precursors of cationic photodynamic agents. The amphiphilic character of these porphyrins was increased by the presence of a high lipophilic trifluoromethyl group. Different patterns of porphyrin structures were obtained from *meso*-4-[(3-N,N-dimethylaminopropoxy)phenyl] dipyrromethane **1**, which was formed by the condensation of 4-(3-N,N-dimethylaminopropoxy) benzaldehyde with a large excess of pyrrole. This reaction takes place at high temperature with a yield of 59%. This reaction was also attempted under acid-catalyzed condensation at room temperature. However, under these conditions, the amino group reduces the catalyst and the reaction does not take place. To obtain porphyrins, dipyrromethane **1** was condensed with aldehydes in the presence of trifluoroacetic acid (TFA) under different conditions. First, **1** reacted with 4-(3-N,N-dimethylaminopropoxy)benzaldehyde in dichloromethane catalyzed by TFA (~4 times TFA/**1** molar ratio) to obtain 6.2% of 5,10,15,20-tetrakis(4-[3-N,N-dimethylaminopropoxy]phenyl)porphyrin (A_4-porphyrin). Under similar conditions, reaction of **1** with 4-(trifluoromethyl)benzaldehyde produces 5,15-di(4-[3-N,N-dimethylaminopropoxy]phenyl)-10,20-di(4-trifluoromethylphenyl)porphyrin (A_2B_2-porphyrin) with a 4.8% yield. This procedure also yields a mixture of porphyrins, which were formed due to acidolysis of **1**. When a minor amount of TFA was used in acetonitrile, the yield of A_2B_2-porphyrin was very poor (~0.4%). On the other hand, condensation of **1** with 4-trifluoromethylbenzaldehyde and 4-(3-N,N-dimethylaminopropoxy)benzaldehyde catalyzed by TFA (~2 times TFA/**1** molar ratio) in acetonitrile yields 9.3% of 5-(4-trifluoromethylphenyl)-10,15,20-tris(4-[3-N,N-dimethylaminopropoxy]phenyl)porphyrin (A_3B-porphyrin). A_2B_2 and A_4 porphyrins were also isolated with 6.0 and 2.0%, respectively. Finally, exhaustive methylation of amino porphyrins produces cationic sensitizers (>94% yield). Absorption and fluorescence spectroscopic studies of these sensitizers were compared in N,N-dimethylformamide. In these porphyrins, the cationic centers are isolated from the porphyrin ring by a propoxy bridge. Thus, the cationic charges have minimal influence on the photophysical properties of the sensitizers. In addition, this chain provides a higher mobility of the charge, which could facilitate interaction with the outer membrane of the Gram-negative bacteria. These amphiphilic cationic porphyrins are promising photosensitizers with potential applications in bacterial inactivation by photodynamic therapy.

KEYWORDS: amino porphyrin, dipyrromethane, amphiphilic structure, photosensitizer.

INTRODUCTION

Meso-substituted porphyrins have recently found specific biomedical applications, particularly in the field of detection and treatment of neoplastic tissues [1]. Photodynamic therapy (PDT) consists in the administration of a photosensitizer, which is selectively retained by the neoplastic tissue. The

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subsequent irradiation with visible light in the presence of oxygen specifically inactivates tumor cells [2]. Adequate photosensitizers are deemed to have a high absorption coefficient in the visible region of the spectrum and a long lifetime of the triplet excited state to produce efficiently $O_2(^1\Delta_g)$ [1].

In recent years, cationic porphyrins have shown important applications as sensitizers to photoinduce direct inactivation of multidrug resistant microorganisms [3-10]. These studies indicate that Gram-positive bacteria are susceptible to the photosensitizing action of a variety of sensitizers [5, 6]. However, negatively charged or neutral porphyrins exhibit a low activity in the photoinactivation of Gram-negative bacteria. The low activity of photosensitizers to inactive Gram-negative bacteria has been ascribed to the presence of a highly organized outer membrane, which hinders the interaction of the photosensitizer with the cytoplasmic membrane and intercepts the photogenerated reactive species [9]. On the other hand, cationic porphyrin derivatives have been shown to photoinduce direct inactivation of Gram-negative bacteria even in the absence of additives, which stimulate the membrane translocation of the sensitizers [5, 6, 8]. The positive charge on the photosensitizer molecule appears to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the bacterial cells. This association increases the efficiency of the photoinactivation processes [5, 9, 11].

The combination of hydrophobic and hydrophilic substituents in the sensitizer structure results in an intramolecular polarity axis, which can facilitate membrane penetration and produces a better accumulation in subcellular compartments, enhancing the effective photosensitization [12, 13]. The design of these sensitizers' architecture requires the formation of asymmetrically *meso*-substituted porphyrins. These porphyrins which contain two different types of *meso*-substituents can be prepared by a binary mixed aldehyde and pyrrole condensation. However, this approach is statistical in nature and usually six porphyrins are formed [14]. The isolation requires slow chromatographic separation, the yield is very poor and the isolation of a pure porphyrin is not always possible. A convenient approach for the synthesis of *meso*-substituted *trans*-porphyrins (A_2B_2-porphyrins) has been developed from the condensation of dipyrromethane with an aldehyde in a MacDonald-type 2 + 2 condensation catalyzed by acid [15, 16]. Condensation of dipyrromethane with a binary mixture of aldehydes was also used to obtain *meso*-substituted porphyrins bearing three identical molecular structures B and one different A (AB_3-porphyrins) [17, 18]. In these cases, the structure A bears a functional group which can be used to link the porphyrin with other molecules, while B was used to change the macrocycle properties.

In this paper, we are interested in the synthesis of novel asymmetrically *meso*-substituted porphyrins containing amino groups as precursors of cationic photodynamic agents. The main problem with the synthesis of porphyrins containing amino groups results from the interaction of an acid catalyst with the substrates [19, 20]. In such cases, the necessary amount of acid catalyst for the reaction is difficult to predict. One approach involves protection of amino groups forming amide derivatives [21, 22]. However, this methodology adds two steps to the synthesis process.

This work reports the synthesis of porphyrins with different patterns of substitution from the condensation of *meso*-4-[(3-N,N-dimethylamino-propoxy)phenyl]dipyrromethane **1** with 4-(trifluoromethyl)benzaldehyde catalyzed by acid. The influence of the trifluoromethyl group in biologically active molecules is often associated with the increased lipophilicity that this substituent imparts [23-25]. Fluorine porphyrin derivatives are also suitable candidates for application in diagnosis and therapy of cancer [26, 27]. In previous work, we have investigated a porphyrin derivative substituted by a trifluoromethyl group on Hep-2 and HeLa cell lines as an interesting photosensitizer [24, 25]. An attractive photobiological feature is its ability to inactivate cultured tumor cells with high efficiency by apoptotic or necrotic modes depending on the light dose. On the other hand, the photodynamic activity of 5-(4-(trimethylammonium)phenyl)-10,15,20-tris(2,4,6-trimethoxyphenyl)porphyrin iodide was evaluated in different biomimetic media and *in vitro* on the Hep-2 human larynx carcinoma cell line [28]. The presence of a cationic charge in the porphyrin structure produces an increase of about three times in the uptake of this asymmetrically substituted porphyrin into Hep-2 cells with respect to a non-ionic porphyrin model. In this case, a higher cellular incorporation is also accompanied by a higher photocytotoxic activity on Hep-2 cells. Recently, a tricationic porphyrin substituted by 4-(trimethylammonium)phenyl groups was studied as active photosensitizer in *Escherichia coli* bacteria [29]. In this work, combinations of positive charges incorporated at the peripheral positions were used to increase the amphiphilic character of the porphyrin structures. This effect could help porphyrin derivatives to pass through or accumulate in biomembranes. However, this must be evaluated in cellular walls of bacteria. In these porphyrins, the cationic centers are isolated from the porphyrin ring by a propoxy bridge. Thus, the charges have minimal influence on the electronic density of the tetrapyrrolic macrocycle. This addresses the consistency of the photophysical properties of the sensitizer. Also, this chain provides

a higher mobility of the charge, which could facilitate the interaction with the outer membrane of the Gram-negative bacteria.

EXPERIMENTAL

General

UV-visible and fluorescence spectra were recorded on a Shimadzu UV-2401PC spectrometer and on a Spex FluoroMax fluorometer, respectively. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a FT-NMR Bruker Avance DPX400 multinuclear spectrometer at 400 MHz. Mass spectra were taken with a Varian Matt 312 operating in EI mode at 70 eV. FAB mass spectra were taken with a ZAB-SEQ Micromass equipment. Silica gel thin-layer chromatography (TLC) plates 250 microns from Aldrich (Milwaukee, WI, USA) were used. All chemicals from Aldrich were used without further purification. Solvents (GR grade) from Merck were distilled. Semiempirical molecular orbital calculations (AM1) were carried out using HyperChem software. Fluorescence quantum yields (ϕ_F) were calculated by comparison of the area below the corrected emission spectrum in N,N-dimethylformamide (DMF) solution with that of tetraphenylporphyrin (TPP, ϕ_F = 0.12) as a fluorescence standard, exciting at λ_{exc} = 550 nm [13].

Synthesis

***meso*-[4-(3-N,N-dimethylaminopropoxy)phenyl]dipyrromethane 1.** A solution of 4-(3-N,N-dimethylaminopropoxy) benzaldehyde (2.0 mL, 10 mmol) and pyrrole (15.0 mL, 216 mmol) was degassed by bubbling with argon for 15 min. The solution was stirred for 48 h at 85 °C under an argon atmosphere. The unreacted pyrrole was removed by vacuum distillation at room temperature. The product was purified by flash chromatography (silica gel, ethyl acetate/triethylamine (TEA) 1%/methanol 5-10% gradient) yielded 1.92 g (59.0%) of the pure dipyrromethane **1**. TLC analysis (ethyl acetate/methanol 5%/TEA 1%) R_f 0.71. ^{1}H NMR (DMSO-d_6, TMS): δ, ppm 1.83 (m, 2H), 2.14 (s, 6H) 2.34 (t, 2H, J = 7.1 Hz), 3.95 (t, 2H, J = 6.3 Hz), 5.31 (s, 1H, *meso*-H), 5.66 (m, 2H, pyrrole-H), 5.91 (q, 2H, pyrrole-H), 6.61 (d, 2H, pyrrole-H), 6.83 (d, 2H, J = 8.4 Hz, Ar 3,5-H), 7.07 (d, 2H, J = 8.6 Hz, Ar 2,6-H), 10.54 (s, brs, 2H, pyrrole NH). MS: *m/z* 323 $[M]^{+\bullet}$ (323.1998 calculated for $C_{20}H_{25}N_3O$). Anal. calcd. C 74.27, H 7.79, N 12.99; found C 74.18, H 7.85, N 13.06.

5,10,15,20-tetrakis[4-(3-N,N-dimethylaminopropoxy)phenyl]porphyrin (A_4). A solution of 4-(3-N,N-dimethylaminopropoxy)benzaldehyde (161 μL, 0.80 mmol) and dipyrromethane **1** (258 mg, 0.80 mmol) in 86 mL of dichloromethane was purged with argon for 15 min. Then, trifluoroacetic acid (TFA, 270 μL, 3.5 mmol) was slowly added and the solution was stirred for 30 min at room temperature. After that, the mixture was treated with TEA (500 μL, 3.6 mmol) and the volatile compounds were removed under reduced pressure. The solid was re-dissolved in 250 mL of dichloromethane. Then, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DDQ (390 mg, 1.7 mmol) was added and the mixture was stirred for an additional 3 h at reflux. The complete reaction mixture was poured onto a pad of florisil 60-100 mesh (30 mm diameter × 30 mm) and eluted with dichloromethane/methanol 15%/TEA 2% (250 mL). The solvent was removed under vacuum and flash column chromatography (silica gel, dichloromethane/methanol 10-20% gradient/TEA 0-3% gradient) yielded 26 mg (6.2%) of the pure porphyrin A_4. TLC analysis (dichloromethane/methanol 15%/TEA 2%) R_f 0.2. ^{1}H NMR ($CDCl_3$, TMS): δ, ppm -2.88 (br, 2H, pyrrole N-H), 2.05 (m, 8H), 2.28 (s, 24H, CH_3-N), 2.56 (t, 8H, J = 7.0 Hz), 4.36 (t, 8H, J = 6.1 Hz), 7.38 (d, 8H, J = 8.2 Hz, 5,10,15,20-Ar 3,5-H), 8.13 (d, 8H, J = 8.2 Hz, 5,10,15,20-Ar 2,6-H), 8.86 (br, 8H, pyrrole). MS: *m/z* 1019 $[M]^{+\bullet}$ (1018.5833 calculated for $C_{64}H_{74}N_8O_4$). Anal. calcd. C 75.41, H 7.32, N 10.99; found C 75.33, H 3.28, N 11.05.

5,10,15-tris[4-(3-N,N-dimethylaminopropoxy)-phenyl]-20-(4-trifluoromethylphenyl)porphyrin (A_3B). A solution of 4-(3-N,N-dimethylaminopropoxy)benzaldehyde (210 μL, 1.0 mmol), 4-(trifluoromethyl)benzaldehyde (174 mg, 1.0 mmol) and dipyrromethane **1** (807 mg, 2.5 mmol) in 1 L of acetonitrile was purged with argon for 15 min. Then, TFA (400 μL, 5.2 mmol) was slowly added and the solution was stirred for 2 h at room temperature. The mixture was treated with TEA (1.37 mL, 10 mmol) and the solvents were removed under reduced pressure. The solid was re-dissolved in 500 mL of dichloromethane and stirred for an additional 3 h at reflux in the presence of DDQ (420 mg, 1.9 mmol). The solvent was removed under vacuum and flash column chromatography (silica gel, dichloromethane/methanol 5-10% gradient/TEA 1%) afforded 95 mg (9.3%) of the pure porphyrin A_3B. TLC analysis (dichloromethane/methanol 10%/TEA 1%) R_f 0.30. ^{1}H NMR ($CDCl_3$, TMS): δ, ppm -2.90 (br, 2H, pyrrole N-H), 2.04 (m, 6H), 2.26 (s, 18H, CH_3-N), 2.53 (t, 6H, J = 7.1 Hz), 4.29 (t, 6H, J = 6.2 Hz), 7.36 (d, 6H, J = 8.3 Hz, 5,10,20-Ar 3,5-H), 8.10 (d, 6H, J = 8.3 Hz, 5,10,20-Ar 2,6-H), 8.19 (d, 2H, J = 8.1 Hz, 20-Ar 2,6-H), 8.44 (d, 2H, J = 8.1 Hz, 20-Ar 3,5-H), 8.80-8.92 (brs, 8H, pyrrole). MS: *m/z* 985 $[M]^{+\bullet}$ (985.4866 calculated for $C_{60}H_{62}F_3N_7O_3$). Anal. calcd. C 73.07, H 6.34, N 9.94; found C 73.15, H 6.27, N 9.87.

5,15-di[4-(3-N,N-dimethylaminopropoxy)phe-

nyl]-10,20-di(4-trifluoromethylphenyl)porphyrin (A_2B_2). A solution of 4-(trifluoromethyl)benzaldehyde (0.804 g, 4.7 mmol) and dipyrromethane **1** (1.285 g, 3.98 mmol) in 390 mL of dichloromethane was purged with argon for 15 min. Then, TFA (1.22 mL, 15.9 mmol) was slowly added and the solution was stirred for 30 min at room temperature. The mixture was treated with TEA (2.2 mL, 16 mmol) and the solvents were removed under reduced pressure. The solid was re-dissolved in 250 mL of dichloromethane and refluxed for 3 h in the presence of DDQ (1.82 g, 8 mmol). The complete reaction mixture was poured onto a pad of florisil 60-100 mesh (30 mm diameter × 30 mm) and eluted with dichloromethane/methanol 15%/TEA 2% (400 mL). The solvent was removed under vacuum and flash column chromatography (silica gel, dichloromethane/methanol 7-20% gradient/TEA 2%) afforded 92 mg (4.8%) of the pure A_2B_2-porphyrin. TLC analysis (dichloromethane/methanol 10%/TEA 1%) R_f 0.49. ^{1}H NMR ($CDCl_3$, TMS): δ, ppm -2.90 (br, 2H, pyrrole N-H), 2.12 (m, 4H), 2.30 (s, 12H, CH_3-N), 2.67 (t, 4H, J = 7.1 Hz), 4.33 (t, 4H, J = 6.1 Hz), 7.39 (d, 4H, J = 8.1 Hz, 5,15-Ar 3,5-H), 8.13 (d, 4H, J = 8.1 Hz, 5,15-Ar 2,6-H), 8.21 (d, 4H, J = 8.1 Hz, 10,20-Ar 2,6-H), 8.47 (d, 4H, J = 8.1 Hz, 10,20-Ar 3,5-H), 8.82 (br, 4H, pyrrole), 8.90 (br, 4H, pyrrole). MS: *m/z* 952 $[M]^{+\bullet}$ (952.3899 calculated for $C_{56}H_{50}F_6N_6O_2$). Anal. calcd. C 70.58, H 5.29, N 8.82; found C 70.64, H 5.35, N 8.74.

This reaction was also analyzed using 4-(trifluoromethyl)benzaldehyde (21 mg, 0.125 mmol) and dipyrromethane **1** (40 mg, 0.125 mmol) in 50 mL of acetonitrile. The mixture was purged with argon for 15 min and then TFA (12 μL, 0.15 mmol) was added. After different times, 2 mL of the reaction was treated with DDQ (45 mg, 0.20 mmol) for 2 h. After that, 1 mL of methanol was added and the solution analyzed. The concentration of porphyrin was measured by spectrofluorimetry (λ_{exc} = 420 nm, λ_{em} = 650 nm). The amount of porphyrin in the sample was estimated by comparison with a calibration curve obtained with standard solutions of the sensitizer.

5-[4-(3-N,N-dimethylaminopropoxy)]-10,15,20-tris(4-trifluoromethylphenyl)porphyrin (AB_3). This porphyrin was purified from the reaction described above for porphyrin A_2B_2, obtaining 99 mg (5.4%). TLC analysis (dichloromethane/methanol 10%/TEA 1%) R_f 0.62. ^{1}H NMR ($CDCl_3$, TMS): δ, ppm -2.92 (br, 2H, pyrrole N-H), 2.07 (m, 2H), 2.29 (s, 6H, CH_3-N), 2.58 (t, 2H, J = 7.1 Hz), 4.31 (t, 2H, J = 6.2 Hz), 7.39 (d, 2H, J = 8.1 Hz, 5-Ar 3,5-H), 8.12 (d, 2H, J = 8.1 Hz, 5-Ar 2,6-H), 8.20 (d, 6H, J = 8.1 Hz, 10,15,20-Ar 2,6-H), 8.46 (d, 6H, J = 8.1 Hz, 10,15,20-Ar 3,5-H), 8.80-9.92 (br, 8H, pyrrole). MS: *m/z* 919 $[M]^{+\bullet}$ (919.2933 calculated for $C_{52}H_{38}F_9N_5O$). Anal. calcd. C 67.90, H 4.16, N 7.61; found C 67.82, H 4.09, N 7.65.

5,10,15,20-triskis(4-trifluoromethylphenyl)-porphyrin (B_4). This porphyrin was purified from the reaction described above for porphyrin A_2B_2, obtaining 58 mg (2.8%). TLC analysis (dichloromethane) R_f 0.85. ^{1}H NMR ($CDCl_3$, TMS): δ, ppm -2.93 (br, 2H, pyrrole N-H), 8.19 (d, 8H, J = 8.2 Hz, 5,10,15,20-Ar 2,6-H), 8.46 (d, 8H, J = 8.2 Hz, 5,10,15,20-Ar 3,5-H), 8.87 (br, 8H, pyrrole). MS: *m/z* 886 $[M]^{+\bullet}$ (886.1966 calculated for $C_{48}H_{26}F_{12}N_4$). Anal. calcd. C 65.02, H 2.96, N 6.32; found C 65.11, H 3.03, N 6.28.

General procedure of porphyrins methylation. A solution of porphyrin (30 mg) and 3 mL of methyl iodide in 3 mL acetone was refluxed under an argon atmosphere for 24 h. After that, 3 mL of methyl iodide was added and the mixture was refluxed for another 72 h. The solvent was removed under reduced pressure and the solid washed with *n*-heptane. Porphyrin A_4 yielded 44 mg (94%) of 5,10,15,20-tetrakis[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]porphyrin iodide (A_4^{4+}), FAB-MS: *m/z* 1079 $[M]^{+\bullet}$ (1078.6750 calculated for $C_{68}H_{86}N_8O_4$). ^{1}H NMR (DMSO-d_6, TMS): δ, ppm -2.90 (br, 2H, pyrrole N-H), 2.14 (m, 8H), 3.31 (s, 36H, CH_3-N), 3.49 (t, 8H, J = 7.0 Hz), 4.37 (t, 8H, J = 6.0 Hz), 7.39 (d, 8H, J = 8.1 Hz, 5,10,15,20-Ar 3,5-H), 8.16 (d, 8H, J = 8.1 Hz, 5,10,15,20-Ar 2,6-H), 8.90 (br, 8H, pyrrole). Anal. calcd. C 51.46, H 5.46, N 7.06; found C 51.37, H 5.38, N 7.14. Absorption spectrum UV-vis (DMF): λ_{max}^{Soret}, nm (ε, $M^{-1}.cm^{-1}$) 421 (164000). Porphyrin A_3B yielded 41 mg (95%) of 5,10,15-tris[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]-20-(4-trifluoromethylphenyl)porphyrin iodide (A_3B^{3+}), FAB-MS: *m/z* 1031 $[M]^{+\bullet}$ (1030.5554 calculated for $C_{63}H_{71}F_3N_7O_3$). ^{1}H NMR (DMSO-d_6, TMS): δ, ppm -2.91 (br, 2H, pyrrole N-H), 2.14 (m, 6H), 3.30 (s, 27H, CH_3-N), 3.51 (t, 6H, J = 6.9 Hz), 4.33 (t, 6H, J = 6.1 Hz), 7.38 (d, 6H, J = 8.1 Hz, 5,10,20-Ar 3,5-H), 8.15 (d, 6H, J = 8.1 Hz, 5,10,20-Ar 2,6-H), 8.20 (d, 2H, J = 8.0 Hz, 20-Ar 2,6-H), 8.46 (d, 2H, J = 8.0 Hz, 20-Ar 3,5-H), 8.82-8.94 (brs, 8H, pyrrole). Anal. calcd. C 53.59, H 5.07, N 6.94; found C 53.51, H 4.96, N 6.99. Absorption spectrum UV-vis (DMF): λ_{max}^{Soret}, nm (ε, $M^{-1}.cm^{-1}$) 420 (169000). Porphyrin A_2B_2 yielded 37 mg (95%) of 5,15-di[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]-10,20-di(4-trifluoromethylphenyl)porphyrin iodide ($A_2B_2^{2+}$), FAB-MS: *m/z* 982 $[M]^{+\bullet}$ (982.4369 calculated for $C_{58}H_{56}F_6N_6O_2$). ^{1}H NMR (DMSO-d_6, TMS): δ, ppm -2.92 (br, 2H, pyrrole N-H), 2.15 (m, 4H), 3.31 (s, 18H, CH_3-N), 3.50 (t, 4H, J = 7.0 Hz), 4.34 (t, 4H, J = 6.0 Hz), 7.39 (d, 4H, J = 8.0 Hz, 5,15-Ar 3,5-H), 8.16 (d, 4H, J = 8.0 Hz, 5,15-Ar 2,6-H), 8.21 (d, 4H, J = 8.2 Hz, 10,20-Ar 2,6-H), 8.48 (d, 4H, J = 8.2 Hz, 10,20-Ar 3,5-H), 8.84 (br, 4H, pyrrole), 8.92 (br, 4H, pyrrole). Anal. calcd. C 56.32, H 4.56, N 6.79; found C 56.23, H 4.47, N 6.83. Absorption spectrum UV-vis (DMF):

λ_{max}^{Soret}, nm (ε, $M^{-1}.cm^{-1}$) 418 (167000). Porphyrin AB_3 yielded 33 mg (94%) of 5-[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]-10,15,20-tris(4-trifluoromethylphenyl)porphyrin (AB_3^+), FAB-MS: *m/z* 934 $[M]^{+\bullet}$ (934.3167 calculated for $C_{53}H_{41}F_9N_5O$). ^{1}H NMR (DMSO-d_6, TMS): δ, ppm -2.91 (br, 2H, pyrrole N-H), 2.14 (m, 2H), 3.30 (s, 9H, CH_3-N), 3.49 (t, 2H, J = 6.9 Hz), 4.34 (t, 2H, J = 6.1 Hz), 7.40 (d, 2H, J = 8.0 Hz, 5-Ar 3,5-H), 8.15 (d, 2H, J = 8.0 Hz, 5-Ar 2,6-H), 8.20 (d, 6H, J = 8.3 Hz, 10,15,20-Ar 2,6-H), 8.47 (d, 6H, J = 8.3 Hz, 10,15,20-Ar 3,5-H), 8.83-9.94 (br, 8H, pyrrole). Anal. calcd. C 59.95, H 3.89, N 6.60; found C 59.85, H 3.74, N 6.65. Absorption spectrum UV-vis (DMF): λ_{max}^{Soret}, nm (ε, $M^{-1}.cm^{-1}$) 417 (171000).

Photosensitized inactivation of bacteria cells

Escherichia coli strain (EC7) recovered from clinical urogenital material was used as previously described [29]. Cell suspensions of *E. coli* (2 mL, ~10^6 CFU/mL) in PBS were incubated with 1 μM of porphyrin for 30 min in the dark at 37 °C. The sensitizer was added from a stock solution of porphyrin (4.0×10^{-4} M) in DMF. After that, cell suspensions were serially diluted with PBS, each solution was plated in triplicate on tryptic soy agar. Then the plates were irradiated for 10 min with visible light and the number of colonies formed after over night incubation at 37 °C was counted. The light source used was a Novamat 130 AF slide projector equipped with a 150 W lamp. The light was filtered through a 2.5 cm glass cuvette filled with water to absorb heat. The light intensity at the treatment site was 90 mW/cm^2 (Radiometer Laser Mate-Q, Coherent). Control experiments were carried out without illumination in the absence and in the presence of sensitizer.

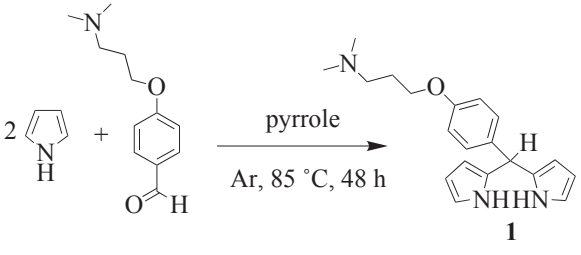
RESULTS AND DISCUSSION

Synthesis

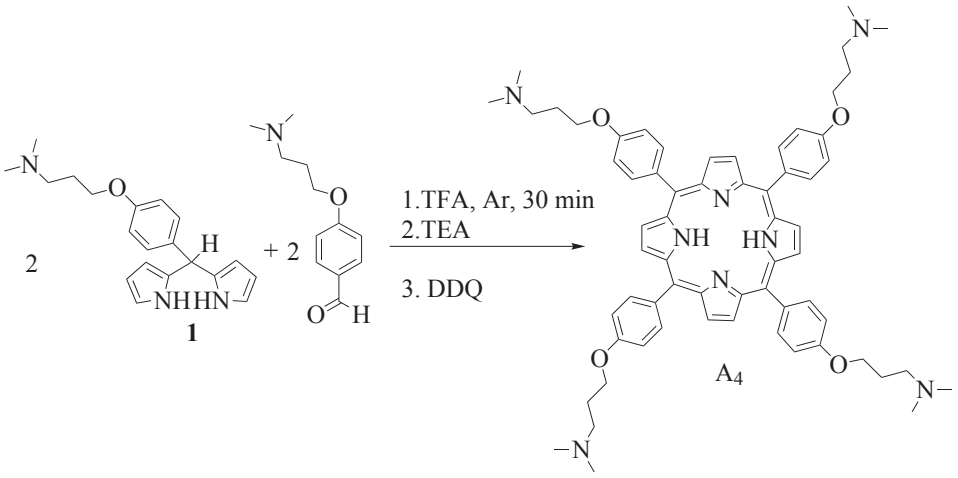
Dipyrromethane formation. One approach to obtain *meso*-substituted dipyrromethane involves aldehyde and pyrrole acid-catalyzed condensation in the absence of any solvent at room temperature [15-18]. This reaction has been used to prepare dipyrromethanes bearing a wide variety of electron-donor or electron-withdrawing substituents. Upon application of this procedure at room temperature with 4-(3-N,N-dimethylaminopropoxy)benzaldehyde and a large excess of pyrrole (1:45 aldehyde/pyrrole mol ratio), catalyzed by TFA (30%), dipyrromethane was not obtained after 20 min of stirring in atmosphere of argon. Under these conditions, the amino group reduces the catalyst and the reaction does not take place [19, 20]. The dipyrromethane-forming reaction also can be performed at elevated temperature in the absence of acid [19, 20]. Thus, when the aldehyde and pyrrole mixture was heated at 85 °C for 48 h without added acid (Scheme 1), the desired *meso*-[4-(3-N,N-dimethylaminopropoxy)phenyl]dipyrromethane **1** was obtained in an appreciable yield of 59%. Under these reaction conditions, pyrrole serves as the reactant in excess and as the solvent for the reaction, giving direct formation of dipyrromethane **1**. The dipyrromethane **1** was purified by removing the excess pyrrole under high-vacuum distillation and by flash chromatography on silica gel in a mildly basic medium, using ethyl acetate/methanol/triethylamine as eluent. In this case, triethylamine (1%) was added to prevent decomposition of the dipyrromethane on the silica column, which is slightly acidic [17, 15]. Dipyrromethane **1** is stable in the purified form upon storage at -18 °C under a nitrogen atmosphere and in the absence of light. Thus, this result shows that the procedure using high temperature is also useful to prepare dipyrromethane from an aromatic aldehyde bearing an amine group.

Synthesis of porphyrin. In the first attempt to evaluate the reactivity of dipyrromethane **1**, it was reacted with aldehyde under conditions where the formation of only one porphyrin is expected. Thus, a mixture of **1** (~10 mM) and 4-(3-N,N-dimethylaminopropoxy)-benzaldehyde was treated with TFA (~4 times TFA/reactant molar ratio) in dichloromethane for 30 min at room temperature (Scheme 2). After oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), the predictable A_4-porphyrin was purified by flash chromatography affording 6.2% yield.

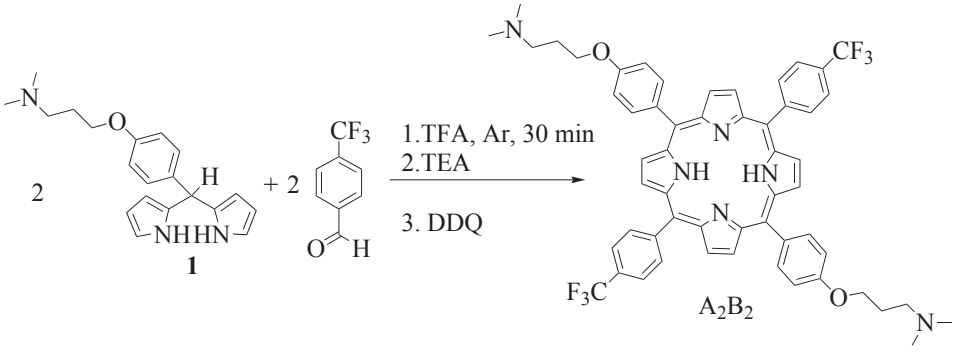
To obtain porphyrins with different patterns of substitution, dipyrromethane **1** was condensed with benzaldehydes under different conditions. First, dipyrromethane **1** (~10 mM) was reacted with 4-(trifluoromethyl)benzaldehyde in 1:1 mol ratio using dichloromethane as solvent (Scheme 3). The reaction was treated with trifluoroacetic acid (TFA) (~4 times TFA/reactant molar ratio) at room temperature for 30 min. This amount of acid was used to neutralize the amine groups and to act as a catalyst [19]. After that, the reaction mixture was subjected to oxidation with DDQ. Condensation under these conditions produces



Scheme 1.



Scheme 2.



Scheme 3.

a mixture of porphyrins, which were separated by flash chromatography in high purity using dichloromethane/methanol/TEA gradient. The first purple band yielded 2.8% of 5,10,15,20-tetrakis(4-trifluoromethylphenyl)porphyrin (B_4). Then, 5-(4-[3-N,N-dimethylaminopropoxy]phenyl)-10,15,20-tris(4-trifluoromethylphenyl)porphyrin (AB_3), 5,15-di(4-[3-N,N-dimethylaminopropoxy]phenyl)-10,20-di(4-trifluoromethylphenyl)porphyrin (A_2B_2), 5,10,15-tris[4-(3-N,N-dimethylaminopropoxy)phenyl]-20-(4-trifluoromethylphenyl)porphyrin (A_3B) and 5,10,15,20-tetrakis[4-(3-N,N-dimethylaminopropoxy)phenyl]-porphyrin (A_4) were sequentially obtained with 5.4, 4.8, 3.0 and 2.0% yields, respectively. Therefore, although only one porphyrin is also expected in this experiment, a mixture of porphyrin derivatives was found with different patterns of substitution. In this case, excess acid was used to overcome the basicity of the amine group in the dipyrromethane **1**. However, this amount of TFA produces acidolysis of dipyrromethane **1**, which induces scrambling in the reaction [19, 30].

To evaluate the effect produced by the acid, the reaction between dipyrromethane **1** (2.5 mM) and 4-(trifluoromethyl)benzaldehyde (1:1 mol ratio) was also investigate using 3 mM TFA (1.2 times TFA/**1** molar ratio). The condensation was performed at room temperature in acetonitrile as solvent. This polar solvent was previously used in the reaction of a dipyrromethane bearing a nitrogen heterocyclic and dipyrromethane-dicarbinol [19]. After different reaction times, an aliquot (2 mL) of reaction mixture was treated with DDQ for 2 h and then with 1 mL of methanol. Porphyrin formation was analyzed by fluorescence spectroscopy. The results show that under these conditions no significant amount of porphyrin was observed after 1 h of reaction. The overnight reaction yielded ~0.4% of porphyrin and prolonging the reaction time for 48 h with stirring under an Ar atmosphere did not improve the yield.

On the other hand, a pathway to prepare porphyrins bearing two different types of *meso*-substituents, such as A_3B-porphyrin, involves condensation of a binary mixture of aldehyde and dipyrromethane [17]. Thus, taking into account the previous results, the condensation of dipyrromethane **1** (2.5 mM) with 4-trifluoromethylbenzaldehyde and 4-(3-N,N-dimethylaminopropoxy)benzaldehyde (2.5:1:1 molar ratio) was performed using 5 mM TFA (Scheme 4). The reaction was carried out in acetonitrile at room temperature for

2 h. After that, the acid was neutralized with TEA and the volatile compounds were evaporated. The solid was dissolved in dichloromethane and the solution treated with DDQ for 3 h. This procedure produces a mixture of the three expected porphyrins *i.e.* A_4, A_3B and A_2B_2 porphyrins, which were isolated by flash chromatography using dichloromethane/methanol/TEA gradient as eluent. Under these conditions, the reaction yields 2.0% of A_4-porphyrin, 9.3% of A_3B-porphyrin and 6.0% of A_2B_2-porphyrin. This procedure also produces 0.3% of AB_3-porphyrin.

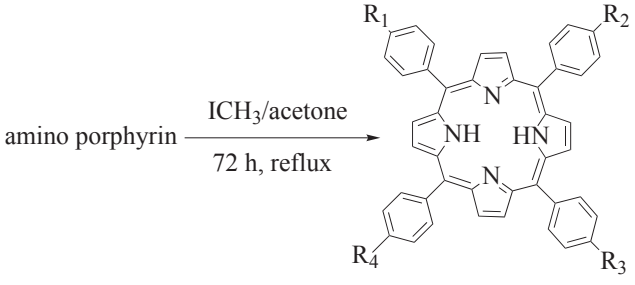
Finally, cationic sensitizers were obtained by treating the amino porphyrins with methyl iodide for 72 h at reflux in acetone (Scheme 5). The exhaustive methylation produces 5,10,15,20-tetrakis[4-(3-N,N,N-trimethylammoniumpropoxy)-phenyl]porphyrin iodide (A_4^{4+}), 5,10,15-tris[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]-20-(4-trifluoromethylphenyl)porphyrin iodide (A_3B^{3+}), 5,15-di[4-(3-N,N,N-trimethylammoniumpropoxy)-phenyl]-10,20-di(4-trifluoromethylphenyl)porphyrin iodide ($A_2B_2^{2+}$) and 5-[4-(3-N,N,N-trimethylammoniumpropoxy)phenyl]-10,15,20-tris(4-trifluoromethylphenyl)porphyrin (AB_3^{+}) with >94% yields.

Porphyrin properties, spectroscopic studies and antibacterial activity

These cationic porphyrins contain a combination of trimethylammonium and trifluoromethyl groups with different patterns of peripheral distribution. To evaluate the effect produced by this distribution of different polarity groups upon the intramolecular polarity, the dipole moments of the porphyrins were estimated. The semi-empirical method for molecular modeling (AM1) was used in structure geometry optimization calculations. Values of 21.0, 36.7, 16.7 and 60.8 D were found for A_4^{4+}, A_3B^{3+}, $A_2B_2^{2+}$ and AB_3^{+}, respectively. These values are considerably higher than those found for the corresponding porphyrin derivatives substituted by amino groups (~7 D). As expected, the presence of cationic groups in the periphery of the porphyrins considerably enhances the dipole moment with respect to the non-charged structure. This effect is mainly enhanced in the AB_3^{+}-porphyrin. In particular, for the A_3B^{3+}-porphyrin the presence of a trifluoromethyl group instead of a methyl group produces an increase from 24.0 D to 36.7 D.

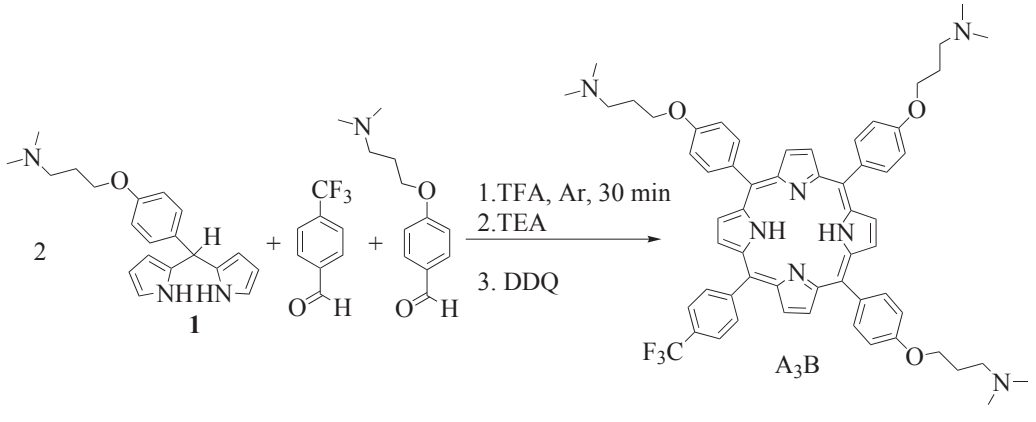
The absorption spectra of the cationic porphyrins were recorded in DMF. The spectra show typical Soret and Q-bands characteristic of free-base porphyrin derivatives [31]. The absorption maxima are summarized in Table 1.

Steady-state fluorescence emission spectra of the cationic porphyrins were analyzed in DMF (Table 1). Two bands are characteristic for porphyrin derivatives and have been assigned to Q(0,0) and Q(0,1) transitions [32]. Also, a small Stokes shift is expected for tetraphenylporphyrin derivatives indicating that the spectroscopic energy is nearly identical to the relaxation energy of the singlet state. By comparison with tetraphenylporphyrin (TPP) as a reference, the values of fluorescence quantum yields (ϕ_F) were calculated for the porphyrins in DMF. In all cases, values



A_4^{4+}	$R_1 = R_2 = R_3 = R_4$: $-O(CH_2)_3N^+(CH_3)_3I^-$	
A_3B^{3+}	$R_1 = R_2 = R_3$: $-O(CH_2)_3N^+(CH_3)_3I^-$	R_4: $-CF_3$
$ABAB^{2+}$	$R_1 = R_3$: $-O(CH_2)_3N^+(CH_3)_3I^-$	$R_2 = R_4$: $-CF_3$
A_3B^{+}	R_1: $-O(CH_2)_3N^+(CH_3)_3I^-$	$R_2 = R_3 = R_4$: $-CF_3$

Scheme 5.



Scheme 4.

Table 1. Absorption and fluorescence emission data for cationic porphyrins in DMF

Porphyrin	Absorption λ_{max}, nm					Emission λ_{max}, nm		ϕ_F
AB_3^+	417	512	549	586	644	650	715	0.10
$A_2B_2^{2+}$	418	514	550	592	646	652	718	0.12
A_3B^{3+}	420	515	552	592	649	655	720	0.13
A_4^{4+}	421	516	554	595	650	659	724	0.12

of ~0.1 were obtained, which are in agreement with that previously reported for free-base porphyrins and they are appropriated for quantification and detection of the sensitizer in biological media [13].

Preliminary studies to evaluate the photodynamic activity of these porphyrins were performed using a typical Gram-negative bacterium *E. coli* [29]. The cultures (~1 × 10^6 cells/mL) were incubated with 1 μM of sensitizer for 30 min, after which the cells were plated and irradiated for 10 min. Viable bacteria were monitored and their number calculated by counting the number of colony forming units. Under these conditions, no colony formation was detected using A_3B^{3+}, while the cell survivals were 37, 78 and 82% for A^{4+}, $A_2B_2^{2+}$, AB_3^+, respectively. These results indicate that the photodynamic activities follow the order: $A_3B^{3+} > A^{4+} > A_2B_2^{2+} \sim AB_3^+$. Therefore, the tricationic porphyrin structure bearing a trifluoromethyl group presents interesting properties to be used as agent in the photodynamic bacterial control.

CONCLUSION

Asymmetrically *meso*-substituted porphyrins bearing amino groups have been synthesized with different patterns of peripheral substitution from dipyrromethane **1**. Usually, dipyrromethane bearing a wide variety of substituents can be prepared from a one-flask room temperature condensation of an aldehyde with excess of pyrrole in the presence of TFA of BF_3-etherate [15-22]. However, under this acid catalytic condition, the presence of an amino group in the aldehyde reduces the catalyst and the reaction does not take place. An attractive option to avoid this inconvenience is amino group protection but this approach enlarges the process of synthesis in two steps [17, 18, 22]. On the other hand, in previous studies dipyrromethanes containing one nitrogen heterocyclic group were synthesized using elevated temperature in the absence of acid [19, 20]. Thus, this work shows that direct condensation at high temperature is also possible to form dipyrromethane **1** with appreciable yield (59%). This result enlarges the scope of this reaction to aromatic aldehydes substituted by an aliphatic chain bearing an amine group. The second step to obtain the porphyrins involves condensation of dipyrromethane **1** with different mixtures of aldehydes catalyzed by TFA. Also, in these cases the presence of amine groups makes it difficult to estimate the amount of acid catalyst necessary for the reaction to proceed effectively. Therefore, we tried different reaction conditions. First, dipyrromethane **1** was reacted with 4-(3-N,N-dimethylaminopropoxy)benzaldehyde in dichloromethane catalyzed by TFA (~4 times TFA/**1** molar ratio) to obtain 6.2% of A_4-porphyrin. Under similar conditions, reaction of dipyrromethane **1** with 4-(trifluoromethyl)benzaldehyde produces a mixture of porphyrins, which were formed due to acidolysis of dipyrromethane **1**. On the other hand, when minor amounts of TFA (~1.2 times TFA/**1** molar ratio) was used in acetonitrile, the yield of A_2B_2-porphyrin was very poor (~0.4%) still after 48 h of reaction. Therefore, when a large excess of acid is used the reaction is accompanied by an increase in the scrambling whereas a low amount of TFA produces a diminishing in the porphyrin yield still after long reaction time. Taking into account these results, to synthesize the A_3B-porphyrin, dipyrromethane **1** was condensed with a binary mixture of 4-trifluoromethylbenzaldehyde and 4-(3-N,N-dimethylaminopropoxy)-benzaldehyde catalyzed by TFA (~2 times TFA/**1** molar ratio) in acetonitrile. This condition appears to be appropriate to obtain the A_3B-porphyrin with a 9.3% yield. Although this yield is lower than for the formation of other porphyrins bearing protected amino groups (~15-17%) [17, 18, 22], the present pathway diminishes the number of reaction steps.

Finally, exhaustive methylation of amino porphyrins were used to obtain cationic sensitizers (>94% yields). The amphiphilic character of these porphyrins was increased by the presence of a high lipophilic trifluoromethyl group [29]. In addition, the mobility of the charges given by the three-carbon chain could facilitate interaction with the outer membrane of the Gram-negative bacteria. On the other hand, a higher photodynamic activity for inactivation of *E. coli* cells was found for the A_3B^{3+} porphyrin. Thus, the amphiphilic tricationic porphyrin offers a promising molecular architecture with potential applications in photodynamic inactivation of bacteria. Further studies of photosensitization *in vitro* are presently in progress in our laboratory.

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REFERENCES

1. Bonnett R. *Chemical Aspects of Photodynamic Thera*py, Advanced Chemistry Texts, Gordon and Breach Science Publishers, Amsterdam, 2000.
2. Henderson BW and Dougherty T. *J. Photochem. Photobiol*. 1992; **55**: 145-157.
3. Taylor PW, Stapleton PD and Luzio JP. *Drug Discovery Today* 2002; **7**: 1086-1091.
4. Orenstein A, Klein D, Kopolovic J, Winkler E, Malik Z, Keller N and Nitzan Y. *FEMS Immunol. Med. Microbiol*. 1998; **19**: 307-314.
5. Merchat M, Bertoloni G, Giacomini P, Villanueva A and Jori G. *J. Photochem. Photobiol. B: Biol*. 1996; **32**: 153-157.
6. Merchat M, Spikes G, Bertoloni G and Jori J. *J. Photochem. Photobiol. B: Biol*. 1996; **35**: 149-157.
7. Li H, Fedorova OS, Grachev AN, Trumble WR, Bohach GA and Czuchajowski L. *Biochim. Biophys. Acta* 1997; **1354**: 252-260.
8. Nitzan Y, Balzam-Sudakevitz A and Ashkenazi H. *J. Photochem. Photobiol. B: Biol*. 1998; **42**: 211-218.
9. Reddi E, Ceccon M, Valduga G, Jori G, Bommer JC, Elisei F, Latterini L and Mazzucato U. *Photochem. Photobiol*. 2002; **75**: 462-470.
10. Nitzan Y and Ashkenazi H. *Curr. Microbiol*. 2001; **42**: 408-414.
11. Minnock A, Vernon DI, Schofield J, Griffiths J, Parish JH and Brown SB. *Antimicrob. Agents Chemother*. 2000; **44**: 522-527.
12. Weitemeyer A, Kliesch H, Michelsen U, Hirth A and Wöhrle D. *in Photodynamic tumor therapy, 2nd and 3rd genaration photosensitizers*, Moser JG (Ed.), Harwood Academic Publishers: Amsterdam, 1998; 87-99.
13. Milanesio ME, Alvarez MG, Silber JJ, Rivarola V and Durantini EN. *Photochem. Photobiol. Sci*. 2003; **2**: 926-933.
14. Lindsey SJ, Schreiman YC, Hsu HC, Kearney PC and Marguerettaz AM. *J. Org. Chem*. 1987; **52**: 827-836.
15. Lee C-H and Lindsey JS. *Tetrahedron* 1994; **50**: 11427-11440.
16. Littler BJ, Ciringh Y and Lindsey JS. *J. Org. Chem*. 1999; **64**: 2864-2872.
17. Durantini EN and Silber JJ. *Synth. Commun*. 1999; **29**: 3353-3368.
18. Durantini EN. *J. Porphyrins Phthalocyanines* 2000; **4**: 233-242.
19. Gryko D and Lindsey JS. *J. Org. Chem*. 2000; **65**: 2249-2252.
20. Ruzié C, Michaudet L and Boitrel B. *Tetrahedron Lett*. 2002; **43**: 7423-7426.
21. Milanesio ME, Gervaldo M, Otero LA, Sereno L, Silber JJ and Durantini EN. *J. Porphyrins Phthalocyanines* 2003; **7**: 42-51.
22. Lazzeri D and Durantini EN. *Arkivox (Arkat USA)* 2003; **10**: 227-239.
23. McClinton MA and McClinton DA. *Tetrahedron* 1992; **48**: 6555-6666.
24. Milanesio ME, Moran MS, Yslas EI, Alvarez MG, Rivarola V and Durantini EN. *Bioorg. Medicinal Chem*. 2001; **9**: 1943-1949.
25. Villanueva A, Durantini EN, Stockert JC, Rello S, Vidania R, Cañete M, Juarranz A, Arranz R and Rivarola V. *Anti-Cancer Drug Design* 2001; **16**: 279-290.
26. Ando A and Kumadaki I. *J. Fluor. Chem*. 1999; **100**: 135-146.
27. Grancho JCP, Pereira MM, Miguel M da G, Rocha Gonsalves AM and Burrows HD. *Photochem. Photobiol*. 2002; **75**: 249-256.
28. Milanesio ME, Alvarez MG, Silber JJ, Rivarola V and Durantini EN. *Photochem. Photobiol. Sci*. 2003; **2**: 926-933.
29. Lazzeri D, Rovera M, Pascual L and Durantini E. N. *Photochem. Photobiol*. 2004; **80**: 286-293.
30. Gryko DT and Tasior M. *Tetrahedron Lett*. 2003; **44**: 3317-3321.
31. Smith KM. *in Porphyrins and Metalloporphyrins*, Elsevier: Amsterdam, 1975; 20-27.
32. Pineiro M, Carvalho AL, Pereira MM, Rocha Gonsalves AM, Arnaut LG and Formosinho S. J. *Chem. Eur. J*. 1998; **4**: 2299-2307.