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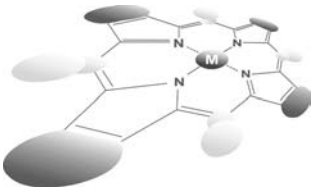

Meso-functionalized aminoporphyrins as efficient agents for photo-antibacterial surfaces

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ABSTRACT: Anionic, neutral and cationic amino porphyrins were synthesized as precursors of photodynamic antimicrobial agents with an aim to functionalize cotton surface through 1,3,5-triazine link. Structures of porphyrin-triazine derivatives were characterized by ^{1}H NMR, MS and UV-vis confirming the feasibility of this novel concept. Porphyrinic cotton fabrics have been developed from these derivatives, and tested *in vitro* against *Staphylococcus aureus*. These novel photodynamic surfaces showed strong and varied antimicrobial activity.

KEYWORDS: cyanuric chloride, amino porphyrin, cotton fabric, antibacterial surface, photodynamic therapy.

INTRODUCTION

Photosensitizing properties of porphyrinic macrocycles and derivatives have been known since the early 20th century. Meyer-Betz described in 1913 the combined action of hematoporphyrin and visible light on cancer cells and introduced the concept of PhotoDynamic Therapy (PDT) [1]. PDT is based upon the selective accumulation of photoreactive compounds (photosensitizers) in tumor tissue and on the production of singlet oxygen by irradiation of the sensitizer-enriched tumor with visible light. Formation of cytotoxic singlet oxygen directly in tumor cells causes cell death and often total tumor necrosis. Since these observations, scientists have wondered if these photosensitizing properties could kill bacterial cells. Nitzan and Malik [2] pioneered the photodestruction of bacteria like *Staphylococcus aureus* and *Escherichia coli* and established the first mechanism of bacteria photokilling by porphyrinic compounds [3]. During the last 30 years new photosensitizers have been synthesized and numerous studies concerning structure-activity relationship have been described [4, 5].

Currently, the field of antimicrobial research is turning toward the development of antimicrobial surfaces for applications in the biomedical, medicine, food-processing and textile industries. In the present case, the strategy consists in incorporating biocidal agents onto synthetic surface structures like nylon, PET [6] or natural surface-like cellulose. For example, antibacterial cellulosic surfaces or fabrics have been developed by grafting different agents like quaternary ammonium salts, *N*-halamines, chitosan or antibiotics [7]. Only few studies have used light-activated antimicrobial agents like photosensitizers to make photoactive surface [8] and results have shown that photosensitizers can keep their antimicrobial properties when grafted to polymers. Recently, we have shown elaboration of photobactericidal cotton fabrics with porphyrinic moieties using "Click-Chemistry" reaction as covalent binding protocol [9].

With the same strategy and in relation with our research programs on photodynamic antimicrobial chemotherapy (PACT) using natural polymeric surfaces, we report in this paper the use of 2,4,6-trichloro-1,3,5-triazine with an aim to graft porphyrin on cotton fabrics using covalent bond. We describe the synthesis and characterization of

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neutral, anionic and cationic amino porphyrins precursors and their 1,3,5-triazinylporphyrin derivatives. Photosensitizing surfaces elaborated from these derivatives were tested *in vitro* against *Staphylococcus aureus*, as a pathogenic agent responsible for numerous nosocomial infections.

RESULTS AND DISCUSSION

Synthesis of porphyrinic precursors

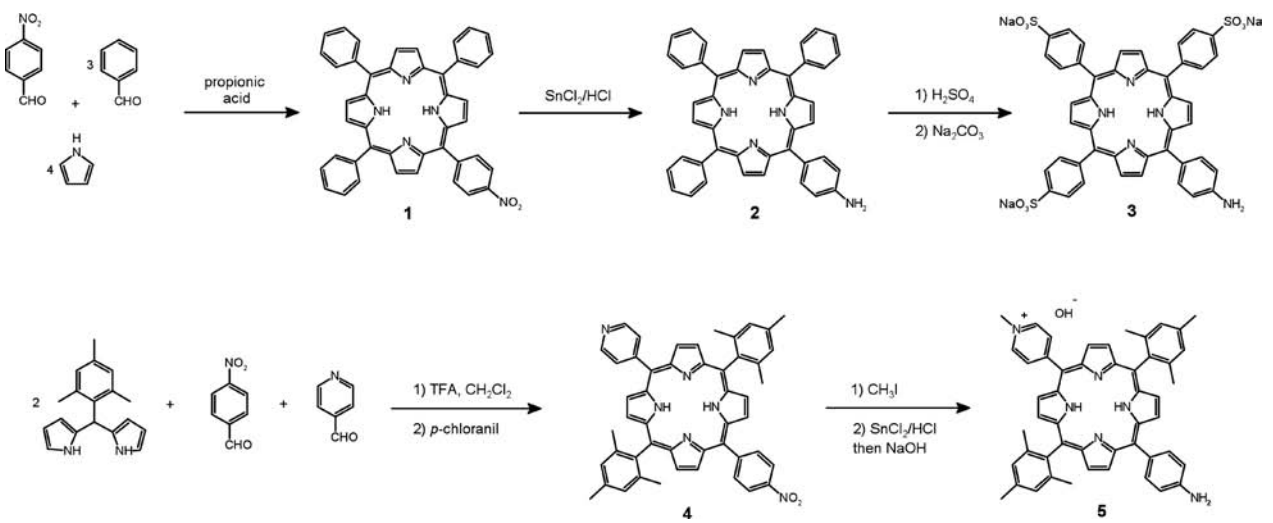
In the aim to graft porphyrins on triazine ring with excellent yield, we substituted chlorine atoms of cyanuric chloride with porphyrin derivatives bearing nucleophilic groups. We chose to prepare 4-aminophenylporphyrin derivatives **2**, **3** and **5** as nucleophilic agents (Scheme 1).

As shown in Scheme 1, 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin **1** was prepared according to classical porphyrin synthesis methods [10], by condensation of 4-nitrobenzaldehyde, benzaldehyde and pyrrole in propionic acid. Compounds **2** and **3** were synthesized according to the literature [11] with reasonable yields: reduction of the nitro group by $SnCl_2$/HCl resulted in 80% yield of amino porphyrin **2** and hydrosoluble product **3** was obtained by sulfonation of **2** in the presence of concentrated sulfuric acid (86% yield). Cationic aminoporphyrin derivative **5** was synthesized by Lindsey's method [12], a 2+2 Mc Donald type condensation: 5,15-bis(mesityl)-10-(4-nitrophenyl)-20-(4-pyridyl)porphyrin **4** was obtained after condensation of *meso*-(mesityl)-dipyrromethane, pyridine-4-carbaldehyde and 4-nitrobenzaldehyde (in stoichiometric proportions) catalyzed by TFA (TFA/aldehyde molar ratio = 4). After oxidation of porphyrinogen by *p*-chloranil (tetrachloro-*p*-benzoquinone) and chromatographic purification, good yield of *trans* unsymmetrical precursor **4** was obtained (18.2%). Methylation with iodomethane, followed by reduction with $SnCl_2$/HCl led to the final cationic compound **5**. Methylation and reduction resulted in good yield (overall 85%). All compounds and intermediates were characterized by ^{1}H NMR, UV-visible and mass spectroscopies.

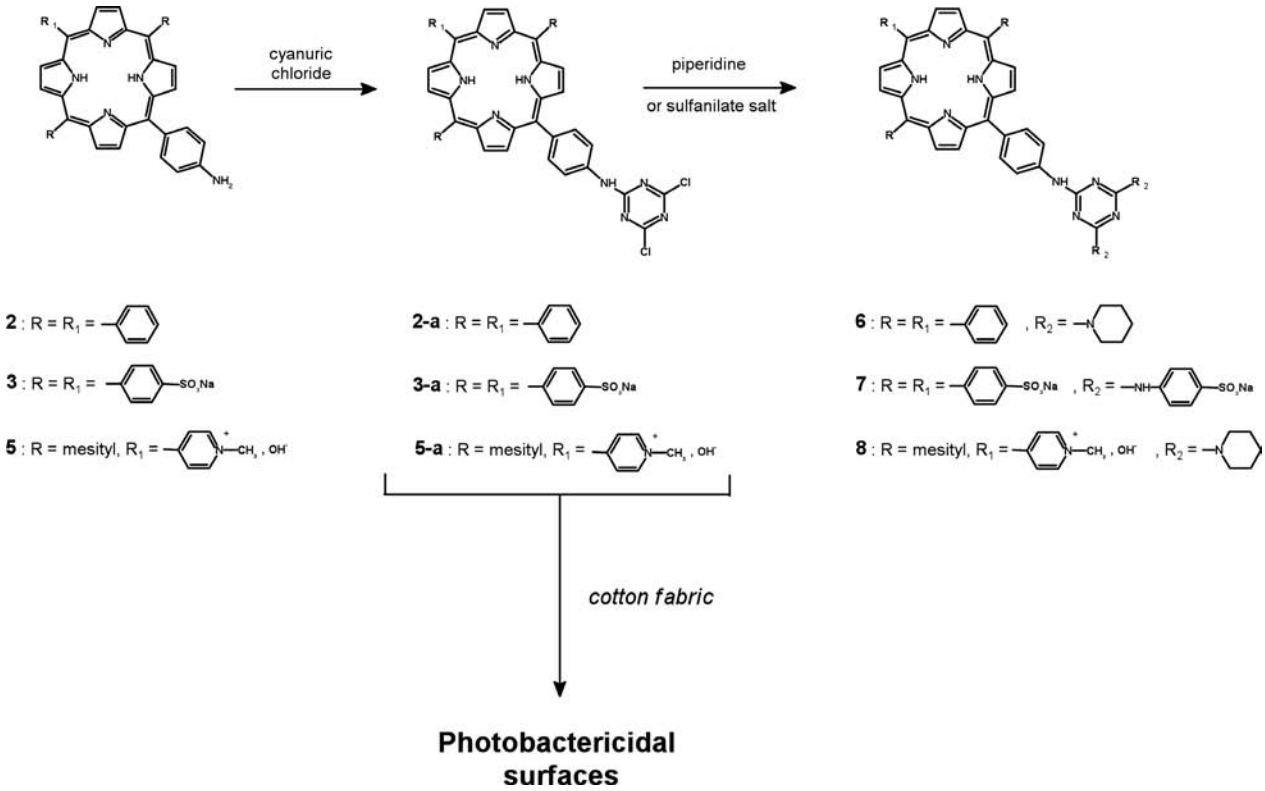
Porphyrin-triazine link

In the aim to elaborate new antibacterial cotton fabrics, we have chosen to use the 1,3,5-triazine derivative cyanuric chloride (2,4,6-trichloro-1,3,5-triazine). This compound has been used as an initiator for the solution phase synthesis of dendrimers [13], macrocycles [14], and combinatorial libraries [15], taking advantage of temperature-dependent stepwise substitution of its three chlorine atoms by different nucleophiles. Furthermore, near quantitative yields are routine for these reactions, which could allow a one-pot protocol. Moreover, this reactant has been frequently used in textile industries, is inexpensive and possesses high reactivity. For these reasons, this triazine derivative was used as grafting agent between porphyrins and cellulose fabrics. Initially, we devoted our attention to the reaction between 4-aminophenylporphyrin derivatives with triazine (Scheme 2) to verify the grafting of one photosensitizer [16]. Moreover, reaction between 1,3,5-triazine derivatives and tetrapyrrolic macrocycles like porphyrins and phthalocyanines has been already reported [17].

Each 4-aminophenylporphyrin derivative was linked to triazine at 0 °C, then addition of piperidine (for neutral and cationic compound **2** and **5**) or sulfanilate salts (for anionic compound **3**) motif allowed us to isolate, purify and characterize triazine derivatives bearing one photosensitizer and also confirmed the feasibility of this method (Scheme 2). The first step between each aminoporphyrins and cyanuric chloride was carried out in the presence of a base at 0 °C. After only 15 min, TLC analysis showed the complete disappearance of the initial



Scheme 1. Synthetic route of neutral (2), anionic (3) and cationic (5) aminoporphyrin derivatives



Scheme 2. Triazinyl porphyrinic derivatives

amino product and the formation of the derivative (**2a**, **3a** or **5a**). These intermediates were very reactive, so they were neither isolated nor characterized. They were used *in situ* to react with an excess of piperidine (**2a** and **5a**) or sulfanilate salts (**3a**) at reflux for 24 h. Good yields of products **6**–**8** were obtained (78, 90 and 52% respectively). Analysis of these substituted triazine porphyrins by ^{1}H NMR and mass spectrometry allowed us to confirm the presence of triazine link with each porphyrin. Then porphyrin-triazine derivatives **2a**, **3a** and **5a** were grafted onto cellulose fabrics (3.5 × 3.5 squares) according to a routine protocol [18].

Spectroscopic studies of porphyrinic derivatives

The absorption spectra of the amino anionic and cationic porphyrins were recorded in $CHCl_3$ or MeOH and compared to reference neutral compound **2**. The spectra showed typical Soret and Q-bands characteristic of free-base porphyrin derivatives. The maximum absorption is summarized in Table 1. With regard to anionic sulfonated derivatives, porphyrin **3** and porphyrin-triazine **7**, a blue shift was observed (intensified effect in MeOH), which is the consequence of aggregation or stacking phenomenon H-type or H-*aggregate* (face to face); contrary to cationic pyridinium derivatives **5** and **8** (red shift) where the stacking effect is of J-type or J-*aggregate* [19]. These differences of stacking effects are due to the *trans* functionalized property of cationic compounds. All shifts were measured and compared with neutral compounds.

Table 1. UV-vis absorption data (a: $CHCl_3$, b: MeOH)

Porphyrins	Absorption λ_{max}, nm				
2[a]	421	517	554	591	648
3[b]	416	514	552	590	648
5[a]	424	523	572	595	656
6[a]	420	516	552	591	647
7[b]	415	519	557	586	641
8[a]	423	521	567	592	654

Photoinactivation of bacterial cells

The photodynamic activity of the porphyrin-triazine derivatives (**2a**, **3a** and **5a**) grafted on cotton fabrics was tested *in vitro* against *Staphylococcus aureus* (S2375), as model of Gram positive bacteria. The experimental results are reported in Table 2. First of all, it appears necessary to justify the different controls. All untreated cotton samples (in darkness or irradiated) and treated cotton samples (in darkness), permit bacterial growth of 4 log compared with reference (t = 0, CFU number initially present on textile squares). These controls demonstrate that either chemical modification of cotton alone or light dose (9.5 J/cm²) alone have no influence on bacterial growth. Furthermore, these three controls allowed us to distinguish between bacteriostatic or bactericidal properties of the photosensitive fabrics.

Experimental data show that all surfaces cause a photobactericidal effect at quantity as low as ~10 μmol of

Table 2. Bacterial counts (log_{10} CFU) of *Staphylococcus aureus*; (A) neutral cotton fabric, (B) anionic cotton fabric and (C) cationic cotton fabric

Cotton fabrics	t = 0	Control		Darkness	Light
		Darkness	Light		
2a grafted: **A**	6.60 ± 0.03	10.16 ± 0.04	10.44 ± 0.03	9.18 ± 0.15	5.40 ± 0.5
3a grafted: **B**	6.30 ± 0.02	10.27 ± 0.09	10.3 ± 0.03	10.26 ± 0.04	6.10 ± 0.05
5a grafted: **C**	6.43 ± 0.01	10.36 ± 0.09	10.47 ± 0.04	5.73 ± 0.06	0

active compound per fabric square sample. After 24 h exposure to light at fluence dose of 9.5 J/cm^2 (h = 10 cm, T = 37 °C), percentages of surviving bacteria were 63%, 6.3% and 0% for anionic, neutral and cationic surfaces respectively. Values of photoinactivation (obtained by comparison treated light *vs*. t = 0) depend on the global charge of the photosensitizer. These results suggest a structure-activity relationship on photoinactivation of bacteria cells. For cationic cotton fabric, toxicity in the dark is probably due to the presence of quaternary ammonium, known for disorganizing cell walls [20]. Due to the insoluble and immobilized property of the photosensitizers, mechanistic interpretations of these experiments must take into account the generation of a reactive species, such as singlet oxygen, on the material surface [8], followed by its diffusion and eventual interaction with the target cell. Midden and co-workers [21] have already shown that such photoinhibition is due to the type II photochemical process implying singlet oxygen ($^1O_2$) that ultimately damages the cell envelope since the photosensitizer does not penetrate the bacterial cell.

From these preliminary results, we can conclude that all tested surfaces exhibit good Gram positive bacteria photoinactivation and seem to be promising for antibacterial application.

EXPERIMENTAL

All solvents and reagents were purchased from Aldrich, Prolabo or Acros. Analytical thin layer chromatography (TLC) was performed on Merck 60F254. Column chromatography was carried out with Merck silica gel (60 ACC; 15–40 μm).

^{1}H NMR spectroscopy was performed with a Bruker DPX 400 spectrometer. Chemical shifts are reported as δ (ppm), downfield from internal TMS and are listed according to the standard numbering of *meso*-arylporphyrins. UV-vis spectra were recorded on a Perkin Elmer Lambda 25 double-beam spectrophotometer using 10-mm quartz cells. MALDI-TOF mass spectra were recorded with a Voyager Elite (Framingham MA, USA) time-of-flight mass spectrometer equipped with a 337 nm nitrogen laser, VSL 337ND (Université Pierre et Marie Curie, Paris).

Photosensitizer synthesis

5-(4-nitrophenyl)-10,15,20-triphenyl porphyrin (1). To a solution of 4-nitrobenzaldehyde (302 mg, 2 mmol, 1 equiv.) and benzaldehyde (613 μL, 6 mmol, 3 equiv.) in propionic acid (60 mL) under magnetic stirring at 120 °C for 1 h, freshly distilled pyrrole (555 μL, 8 mmol, 4 equiv.) was added dropwise. The mixture was kept under magnetic stirring at 120 °C for 1 h. After removing of solvent, the crude product was purified by chromatography on silica gel ($CHCl_3$/petroleum ether: 6/4), to give 96 mg of **1**, purple solid (7.2% yield). TLC ($CHCl_3$/petroleum ether: 6/4): R_f = 0.62. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^{-3}) 420 (241), 516 (12.2), 552 (7.1), 591 (4.4), 647 (3.1). ^{1}H NMR ($CDCl_3$): δ, ppm 8.89 (d, 2H, *J* = 4.8 Hz, $H_{β\text{-pyrrolic}}$), 8.86 (br s, 4H, $H_{β\text{-pyrrolic}}$), 8.73 (d, 2H, *J* = 4.8 Hz, $H_{β\text{-pyrrolic}}$), 8.62 (d, 2H, *J* = 8.5 Hz, $H_{3,5\text{-aryl}}$), 8.39 (d, 2H, *J* = 8.5 Hz, $H_{2,6\text{-aryl}}$), 8.21 (d, 6H, *J* = 6.8 Hz, $H_{2,6\text{-phenyl}}$), 7.76 (d, 9H, *J* = 7.3 Hz, $H_{3,4,5\text{-phenyl}}$), -2.78 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{44}H_{29}N_5O_2$, calcd. 659.23, found 660.07 $[M + H]^+$.

5-(4-aminophenyl)-10,15,20-triphenyl porphyrin (2). Porphyrin **1** (80 mg, 121 μmol) was dissolved in 20 mL of chloroform and a solution (20 mL) of $SnCl_2$ (3 equiv./NO_2) in concentrated HCl was added. Acetic acid (20 mL) was added to form homogeneous solution and the resulting mixture was heated at 70–80 °C, under stirring overnight. Reaction was stopped by neutralization with 2 M NaOH (150 mL). Mixture solution was washed with water (2 × 100 mL) then dried on $MgSO_4$, and filtered; after purification by chromatography on silica gel ($CHCl_3$), compound **2** (purple solid) was obtained (61 mg, 80%). TLC ($CHCl_3$): R_f = 0.50. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^{-3}) 421 (369), 517 (14.0), 554 (7.6), 591 (4.4), 648 (4.0). ^{1}H NMR ($CDCl_3$): δ, ppm 8.94 (d, 2H, *J* = 4.7 Hz, $H_{β\text{-pyrrolic}}$), 8.83 (br s, 6H, $H_{β\text{-pyrrolic}}$), 8.21 (br d, 6H, *J* = 7.6 Hz, $H_{2,6\text{-phenyl}}$), 7.99 (d, 2H, *J* = 8.2 Hz, $H_{2,6\text{-aryl}}$), 7.74 (br d, 9H, *J* = 7.4 Hz, $H_{3,4,5\text{-phenyl}}$), 7.04 (d, 2H, *J* = 8.2 Hz, $H_{3,5\text{-aryl}}$), 3.99 (s, 2H, NH_2), -2.75 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{44}H_{31}N_5$, calcd. 629.26, found 630.11 $[M + H]^+$.

5-(4-aminophenyl)-10,15,20-tri(4-sulfonatophenyl) porphyrin trisodium (3). Porphyrin **2** (75 mg, 119 μmol) was dissolved in 5 mL of 95% H_2SO_4 and heated at 70 °C, under stirring overnight. Water (100 mL) was added and the mixture was neutralized by saturated Na_2CO_3. After

water removal, sodium salts (containing hydrosoluble product **3**) were precipitated by an excess of methanol. This operation was carried out twice. Salts were filtered and dissolved in minimum water. After 4 days of dialysis (cut-off: 1000 Da), pure product **3** (brown-green solid) was lyophilized and obtained with 86% yield (95.6 mg). TLC ($CHCl_3$/MeOH/H_2O: 60/45/12): R_f = 0.34. UV-vis (MeOH): λ_{max}, nm ($\varepsilon \times 10^{-3}$) 416 (354), 514 (16.6), 552 (10.1), 590 (5.8), 648 (4.8). ^{1}H NMR (DMSO): δ, ppm 8.98 (d, 2H, *J* = 4.5 Hz, $H_{\beta\text{-pyrrolic}}$), 8.83 (br s, 6H, $H_{\beta\text{-pyrrolic}}$), 8.20 (d, 6H, *J* = 8.0 Hz, $H_{3,5\text{-phenyl}}$), 8.07 (d, 6H, *J* = 8.0 Hz, $H_{2,6\text{-phenyl}}$), 7.89 (d, 2H, *J* = 8.2 Hz, $H_{2,6\text{-aryl}}$), 7.02 (d, 2H, *J* = 8.2 Hz, $H_{3,5\text{-aryl}}$), 5.61 (br s, 2H, NH_2), -2.86 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{44}H_{28}N_5S_3O_9Na_3$, calcd. 935.07, found 936.09 $[M + H]^+$, 1871.26 $[M_2 + H]^+$.

5,15-bis(mesityl)-10-(4-nitrophenyl)-20-(4-pyridyl) porphyrin (4). *Meso*-(mesityl)dipyrromethane (600 mg, 2.27 mmol, 2 equiv.) solubilized in anhydrous CH_2Cl_2 (227 mL), 172 mg of 4-nitrobenzaldehyde (1.135 mmol, 1 equiv.) and 108 µL of pyridine-4-carbaldehyde (1.135 mmol, 1 equiv.) were added. The mixture was bubbled under argon for 15 min then 315 µL of trifluoroacetic acid (4.1 mmol, 3.6 equiv.) was introduced dropwise. The reaction was stirred under argon for 45 min. 560 mg of *p*-chloranil (2.27 mmol, 2 equiv.) were added and the reaction was kept under stirring for 90 min at room temperature. After purification by chromatography on silica gel ($CHCl_3$), 155 mg of porphyrin **4** were collected (purple solid, 18.2%). TLC ($CHCl_3$/EtOH: 98/2): R_f = 0.45. UV-vis ($CHCl_3$): λ_{max}, nm ($\varepsilon \times 10^{-3}$) 419 (363), 515 (18.6), 550 (7.6), 590 (5.6), 648 (3.9). ^{1}H NMR ($CDCl_3$): δ, ppm 8.96 (d, *J* = 5.1 Hz, 2H, $H_{2,6\text{-pyridyl}}$), 8.68 (br s, 6H, $H_{\beta\text{-pyrrolic}}$), 8.63 (d, *J* = 4.6 Hz, 2H, $H_{\beta\text{-pyrrolic}}$), 8.55 (d, *J* = 8.4 Hz, 2H, $H_{3,5\text{-aryl}}$), 8.33 (d, *J* = 8.4 Hz, 2H, $H_{2,6\text{-aryl}}$), 8.11 (d, *J* = 5.1 Hz, 2H, $H_{3,5\text{-pyridyl}}$), 7.18 (s, 4H, $H_{mesityl}$), 2.56 (s, 6H, $CH_{3,p\text{-mesityl}}$), 1.76 (s, 12H, $CH_{3,o\text{-mesityl}}$), -2.73 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{49}H_{40}N_6O_2$, calcd. 744.32, found 745.33 $[M + H]^+$.

5-(4-aminophenyl)-10,20-bis(mesityl)-15-(4-*N*-methylpyridinium)porphyrin (5). To a solution of **4** (76 mg, 0.1 mmol, 1 equiv.) in anhydrous DMF (10 mL), an excess of iodomethane (62 µL, 1 mmol, 10 equiv.) was added under argon atmosphere. The mixture was kept under magnetic stirring at room temperature for 24 h. After precipitation with diethyl ether and filtration, the corresponding cationic nitro porphyrin was obtained with 94% yield (87 mg). Then, the procedure was the same as for synthesis of product **2**. To a solution of cationic nitro porphyrin (71 mg, 80 µmol, 1 equiv.) in $CHCl_3$ (10 mL), a solution of $SnCl_2$ (54 mg, 240 µmol, 3 equiv.) in 10 mL 37% HCl was added. Acetic acid (10 mL) was added to form homogeneous solution and resulting mixture was heated at 70–80 °C, overnight under stirring. Reaction was stopped by neutralization with 1 M NaOH (100 mL). The mixture solution was washed with water (2 × 100 mL), dried on $MgSO_4$ and filtered. 54 mg of porphyrin **5**, purple solid, were obtained (90%). TLC ($CHCl_3$/MeOH: 8/2): R_f = 0.43. UV-vis ($CHCl_3$): λ_{max}, nm ($\varepsilon \times 10^{-3}$) 424 (76), 523 (7.1), 572 (7.2), 595 (5.4), 656 (4.7). ^{1}H NMR ($CDCl_3$): δ, ppm 9.67 (s, broad, 2H, $H_{2,6\text{-pyridyl}}$), 8.84 (d, *J* = 4.7 Hz, 2H, $H_{\beta\text{-pyrrolic}}$), 8.73 (d, *J* = 4.4 Hz, 2H, $H_{3,5\text{-pyridyl}}$), 8.68 (br s, 4H, $H_{\beta\text{-pyrrolic}}$), 8.61 (d, *J* = 4.7 Hz, 2H, $H_{\beta\text{-pyrrolic}}$), 7.91 (d, *J* = 8.2 Hz, 2H, $H_{2,6\text{-aryl}}$), 7.18 (s, 4H, $H_{mesityl}$), 6.99 (d, *J* = 8.2 Hz, 2H, $H_{3,5\text{-aryl}}$), 5.03 (s, 3H, N_{methyl}), 2.54 (s, 6H, $CH_{3,p\text{-mesityl}}$), 1.73 (s, 12H, $CH_{3,o\text{-mesityl}}$), -2.58 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{50}H_{46}N_6O$, calcd. 746.37, found 729.20 $[M - OH]^+$.

Porphyrin-triazine link

5-[4-((4,6-(bis)piperidyl)-1,3,5-triazinyl)-amino-phenyl]-10,15,20-triphenyl porphyrin (6). Porphyrin **2** (35 mg, 56 µmol, 1 equiv.) in THF (5 mL) was cooled at 0 °C, then cyanuric chloride (10.3 mg, 56 µmol, 1 equiv.) and *N*,*N*-diisopropyl ethylamine, DIPEA (12 µL, 67 µmol, 1.2 equiv.) were added. The mixture was kept under magnetic stirring at room temperature. After 15 min, TLC analysis (petroleum ether/ethyl acetate: 2/1) showed a complete disappearance of porphyrin **2** and the formation of derivative **2a** (R_f = 0.7). Product **2a** was not isolated, but it was further reacted with an excess of piperidine (28 µL, 280 mmol, 5 equiv.) and 2.4 equiv. of DIPEA were added then the reaction was heated at reflux under stirring for 24 h. THF was removed under vacuum and compound **6** was obtained after purification by preparative TLC, petroleum ether/ethyl acetate: 8/2, (purple solid, 39 mg, 78%). TLC (petroleum ether/ethyl acetate: 8/2): R_f = 0.46. UV-vis ($CHCl_3$): λ_{max}, nm ($\varepsilon \times 10^{-3}$) 420 (723), 516 (18.4), 552 (11.8), 591 (6.1), 647 (4.6). ^{1}H NMR ($CDCl_3$): δ, ppm 8.95 (d, 2H, *J* = 4.7 Hz, $H_{\beta\text{-pyrrolic}}$), 8.84 (d, 6H, *J* = 5.1 Hz, $H_{\beta\text{-pyrrolic}}$), 8.21 (d, 6H, *J* = 7.0 Hz, $H_{2,6\text{-phenyl}}$), 8.14 (d, 2H, *J* = 8.4 Hz, $H_{3,5\text{-aryl}}$), 8.0 (d, 2H, *J* = 8.4 Hz, $H_{2,6\text{-aryl}}$), 7.76 (m, 9H, $H_{3,4,5\text{-phenyl}}$), 7.07 (s, broad, 1H, NH), 3.94 (s, 8H, $H_{\alpha\text{-piperidyl}}$), 1.66 (s, 12H, $H_{\beta,\gamma\text{-piperidyl}}$), -2.75 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{57}H_{50}N_{10}$, calcd. 874.42, found 875.14 $[M + H]^+$.

5-[4-((4,6-(bis)sulfanilatyl)-1,3,5-triazinyl)-amino-phenyl]-10,15,20-tri(4-sulfonatophenyl)porphyrin (7). To a solution of porphyrin **3** (30 mg, 32 µmol) in ultra pure water (10 mL) cooled at 0 °C were added THF (1 mL), a solution of cyanuric chloride (7.1 mg, 38 µmol, 1.2 equiv.) and a saturated solution of Na_2CO_3 (2 mL). After 30 min, the reaction was complete, as witnessed by TLC analysis ($CHCl_3$/MeOH/H_2O: 6/4/1), showing the formation of derivative **3a** (R_f = 0.25). Product **3a** was not isolated, but it reacted with an excess of sulfanilic acid (55 mg, 10 equiv.), prepared with 5 mL of saturated solution of Na_2CO_3. The resulting mixture was stirred at reflux for 24 h and the solution was dialyzed (cut-off: 1000 Da) for 48 h. Compound **7** (40.4 mg, 90%), red solid, was obtained after lyophilization. TLC ($CHCl_3$/MeOH/H_2O: 6/4/1): R_f = 0.19. UV-vis (MeOH): λ_{max}, nm ($\varepsilon \times 10^{-3}$) 415 (254), 519 (10.4), 557 (7.0), 586 (4.8), 641

(3.4). ^{1}H NMR (DMSO): δ, ppm 8.93 (d, 2H, J = 3.9 Hz, $H_{\beta\text{-pyrrolic}}$), 8.86 (br s, 6H, $H_{\beta\text{-pyrrolic}}$), 8.20 (d, 12H, J = 7.6 Hz, H_{phenyl}), 8.07 (d, 8H, J = 7.9 Hz, $H_{sulfanilic}$), 7.85-7.60 (m, 4H, H_{aryl}), -2.89 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{59}H_{37}N_{10}Na_5O_{15}S_5$, calcd. 1400.05, found 1401.09 $[M + H]^+$.

5-[4-((4,6-(bis)piperidyl)-1,3,5-triazinyl)-aminophenyl]-10,20-bis(mesityl)-15-(4-*N*-methylpyridinium)porphyrin (8). Porphyrin **5** (43 mg, 58 µmol, 1 equiv.) in THF (10 mL) was cooled at 0 °C, then cyanuric chloride (11 mg, 58 µmol, 1 equiv.) and DIPEA (12 µL, 70 µmol, 1.2 equiv.) were added. The mixture was stirred for 30 min then was left to reach room temperature. TLC analysis ($CHCl_3$/MeOH: 9/1) showed a complete disappearance of porphyrin **5** and the formation of derivative **5a**. An excess of piperidine (57 µL, 580 mmol, 10 equiv.) and 2.4 equiv. of DIPEA were added and the reaction was heated at reflux under stirring for 24 h. THF was removed and compound **8** was obtained after purification by preparative TLC, $CHCl_3$/MeOH: 9/1 (purple solid, 30 mg, 52%). TLC ($CHCl_3$/MeOH: 9/1): R_f = 0.34. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^{-3}) 423 (146), 521 (12.7), 567 (11), 592 (8.2), 654 (6.6). ^{1}H NMR ($CDCl_3$): δ, ppm 9.73 (s, broad, 2H, $H_{2,6\text{-pyridyl}}$), 8.85 (d, J = 4.6 Hz, 2H, $H_{\beta\text{-pyrrolic}}$), 8.73 (s, 2H, $H_{3,5\text{-pyridyl}}$), 8.67 (br s, 4H, $H_{\beta\text{-pyrrolic}}$), 8.62 (d, J = 4.6 Hz, 2H, $H_{\beta\text{-pyrrolic}}$), 8.05 (d, J = 8.2 Hz, 2H, $H_{3,5\text{-aryl}}$), 7.93 (d, J = 8.2 Hz, 2H, $H_{2,6\text{-aryl}}$), 7.20 (s, 4H, $H_{mesityl}$), 5.05 (s, 3H, *N*-methyl), 3.76 (s, 8H, $H_{\alpha\text{-piperidyl}}$), 2.54 (s, 6H, $CH_{3,p\text{-mesityl}}$), 1.73 (s, 12H, $CH_{3,o\text{-mesityl}}$), 1.57 (s, 12H, $H_{\beta,\gamma\text{-piperidyl}}$), -2.60 (br s, 2H, $NH_{int.}$). MS (MALDI): *m/z* for $C_{63}H_{65}N_{11}O$, calcd. 991.54, found 974.67 $[M - OH]^+$.

Grafting of cotton fabrics

Experimental conditions are similar to porphyrin-triazine link characterization. From 10 mg of aminoporphyrins **2**, **3** or **5**, porphyrin derivatives **2a**, **3a** and **5a** were obtained. Then, cotton fabrics (3.5 × 3.5 cm squares), previously soaked in 0.5 M NaOH for 24 h, were introduced. After 24 h of grafting reaction, modified surfaces were washed with DMF under reflux during 24 h then dried at 100 °C for 1 h. Grafting yields (determined by UV-vis titration) have been evaluated to 57, 73 and 54% for aminoporphyrins **2**, **3** and **5** respectively.

Photoinactivation tests

Growth conditions of bacterial cells. *S. aureus* (strain S2375, Institut Pasteur, Paris) was grown in Tryptic Soy agar at 37 °C under aerobic conditions overnight. Cells were taken from the culture during the stationary growth phase and diluted in fresh media in order to obtain about 10^8 cells per mL.

Photodynamic treatment with photoactive cotton surfaces. Sterile photosensitive textiles (3.5 × 3.5 cm), previously autoclaved 15 min at 120 °C, were impregnated with 1 mL of bacterial inoculum at cell density of approximately 10^6 colonies forming units/mL (CFU/mL), deposited in a sterile Petri dish and then incubated at 37 °C for 24 h under white light irradiation (fluence dose = 9.5 J/cm^2, h = 10 cm) in wet atmosphere. Then, each sample was removed and transferred into 20 mL of extraction solution (Triton X-100, 0.5% v/v). After 15 min of gentle stirring at room temperature, serial dilutions of this suspension were prepared. One hundred µL of each dilution were plated on Tryptic Soy agar plates. Colonies were counted after 24 h incubation at 37 °C. Results were expressed as the number of CFU per textile square.

Each test was repeated three times, and was conducted along with necessary controls: after bacterial impregnation, photosensitive textiles were processed immediately (t = 0), another one was incubated 24 h at 37 °C in the dark; unmodified cotton was used in the same conditions (24 h at 37 °C in the dark and under white light irradiation). Moreover, Triton X-100 used was tested in order to determine its influence on bacterial viability; the extracting agent Triton X-100 (solution at 0.5% v/v) had no deleterious effect on *S. aureus*.

CONCLUSION

In summary, we have obtained neutral, anionic and cationic aminoporphyrins with an overall satisfying yield. Moreover cyanuric chloride has been efficient in grafting porphyrins onto cotton fabrics. The photoinactivation preliminary results confirmed the interest in studying these surfaces as potential photoactive materials for antimicrobial applications. Antimicrobial evaluation against other bacterial strains (like Gram negative bacteria) and antifungal activity against *Candida albicans* are in progress in our laboratory.

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