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Synthesis of a tricationic subphthalocyanine with potential use in photoinactivation of bacteria

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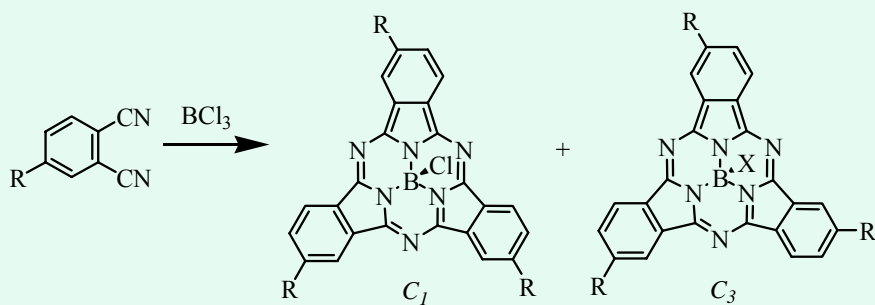
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Abstract. A novel chloro[2,9,16(2,9,17)-trikis-4-(N-methylpyridyloxy)]subphthalocyaninato boron(III) iodide (SubOPc³⁺) has been synthesized by boron trichloride-induced cyclotrimerization of 4-(4-pyridyloxy)phthalonitrile in 1-chloronaphthalene. This approach produced a regioisomers mixture of chloro[2,9,16(2,9,17)-trikis-4-(pyridyloxy)]subphthalocyaninato boron(III) (SubOPc) with 19% yield. Exhaustive methylation of SubOPc with methyl iodide yielded 92% of tricationic SubOPc³⁺. The spectroscopic and photodynamic properties of the SubOPc³⁺ were compared with its homologous non-charged SubOPc and with subphthalocyanine (SubPc). The cationic SubOPc³⁺ is essentially aggregated in solution of different solvents and it is partially dissolved as monomer in N,N-dimethylformamide (DMF)/water (10% v/v)/HCl (1.2 mM). The singlet molecular oxygen, O₂(¹Δ_g), production was evaluated using 9,10-dimethylanthracene (DMA). The photodynamic effect was strongly dependent of the medium, diminishing where the sensitizer is aggregated but it increases in an appropriated surrounding microenvironment. The studies show that SubOPc³⁺ can be an interesting agent with potential applications in photodynamic inactivation of bacteria.

Introduction

Subphthalocyanines are composed of three diiminoisoindole rings N-fused around a boron core. Their 14 π -electron aromatic core along with their nonplanar cone-shaped structure make them attractive compounds due to their chemical and physical properties [1].

Subphthalocyanines are synthesized by cyclotrimerization reaction of phthalonitrile precursors in the presence of a boron trihalide. Symmetrically substituted phthalonitriles produce a single subphthalocyanine, while asymmetric phthalonitriles yield a mixture of two subphthalocyanine regioisomers with C_1 and C_2 symmetries (Scheme 1). When electronic or steric interactions are not influencing the regioisomeric distribution, the ratio $C_3:C_1$ follows a statistical distribution 1:3, respectively [1].



Scheme 1. Synthesis of subphthalocyanine regioisomers

UV-Visible absorption spectra of subphthalocyanines are comparable to the ones obtained for phthalocyanines in that they both show a Soret and Q-bands. However, in the case of subphthalocyanines there is a tendency for both the Soret band (~ 300 nm) and the Q-band (~ 560 nm) to shift to shorter wavelength with respect to phthalocyanines as a consequence of the decrease of the π -conjugation system. The absorption coefficients (ϵ) at Q-band are $\sim 5 \times 10^4$ $M^{-1}cm^{-1}$ [2]. Also, subphthalocyanines are fluorescent with quantum yields of $\phi_F \sim 0.25$ and they have a long lifetime of triplet excited state to produce efficiently singlet molecular oxygen, $O_2(^1\Delta_g)$ with quantum yields of $\Phi_\Delta \sim 0.2-0.7$ [2].

Therefore, subphthalocyanines are interesting candidates for use in photosensitization processes, especially in situations where absorption in the red part of the spectra is not required. This can be the case of pathogenic microorganisms growing *in vivo* as localized foci of infection, on skin or on accessible mucous membrane, which can be treated by photodynamic inactivation (PDI) [3]. In previous works, we have investigated the photodynamic activity of photosensitizers with different number of cationic charges *in vitro* as agents to eradicate Gram-negative bacteria [4-7]. Porphyrin derivatives bearing three cationic charges showed to be active photosensitizers to inactivate *Escherichia coli* cells [6,7].

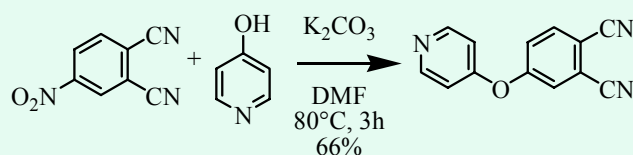
Taking into account these considerations, in this paper we report the synthesis a novel chloro[2,9,16(2,9,17)-trikis-4-(N-methylpyridyloxy)]subphthalocyaninato boron(III) iodide (SubOPc³⁺). The spectroscopic and photodynamic properties of the SubOPc³⁺ was compared with its homologous non-charged chloro[2,9,16(2,9,17)-trikis-4-(pyridyloxy)]subphthalocyaninato boron(III) (SubOPc) and with subphthalocyanine (SubPc). The results indicate that SubOPc³⁺ can be an interesting agent with potential applications in PDI of bacteria.

Synthesis of subphthalocyanines

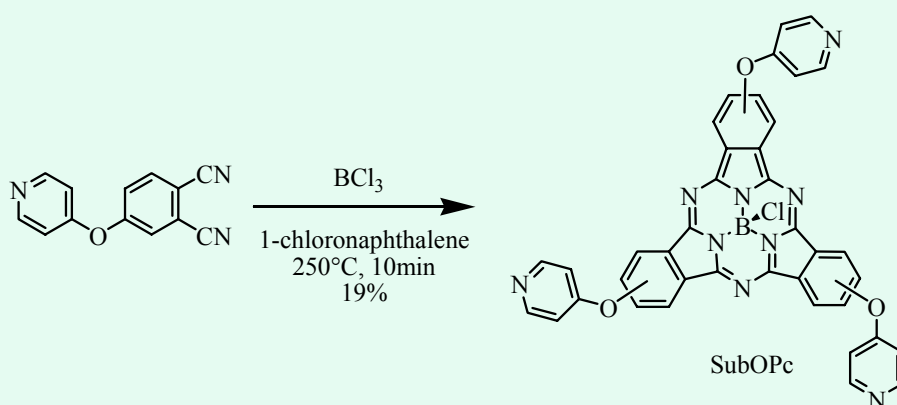
A three-step method was used to synthesize SubOPc³⁺. First, 4-(4-pyridyloxy)phthalonitrile was formed by a nucleophilic aromatic substitution reaction of 4-nitrophthalonitrile with 4-

hydroxypyridine in the presence of K_2CO_3 (Scheme 2). Phthalonitrile derivative was isolated by flash chromatography with 66 % yield. The boron trichloride-induced cyclotrimerization of 4-(4-pyridyloxy)phthalonitrile was performed in 1-chloronaphthalene (Scheme 3). After mixture, the reaction was heated to 250 °C for 10 min and then cooled to room temperature. The subphthalocyanine was isolated by flash chromatographic column (silica gel, dichloromethane/methanol 5%). The product was re-crystallized from dichloromethane/cyclohexane to obtain SubOPc as a regioisomeric mixture with 19% yield [1H NMR (DMSO- d_6 , TMS) δ [ppm] 7.13 (6H), 7.25 (3H), 7.42 (3H), 7.73 (3H), 7.93 (6H); MS [m/z] 709 (M^+) (709,1549 calculated for $C_{39}H_{21}BClN_9O_3$); elemental analysis: calcd. C 65.98, H 2.98, N 17.76 found C 65.91, H 2.96, N 17.81].

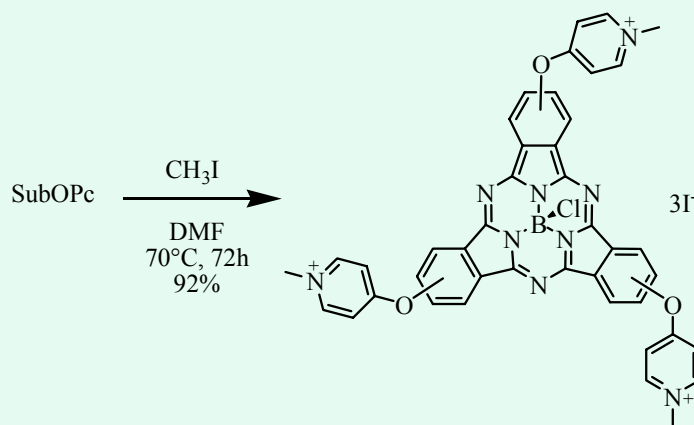
Cationic SubOPc $^{3+}$ was obtained treating the SubOPc with an excess of methyl iodide for 72 h in N,N-dimethylformamide (DMF) (Scheme 4). The exhaustive methylation produces SubOPc $^{3+}$ with 92 % yield [1H NMR (DMSO- d_6 , TMS) δ [ppm] 4.21-4.26 (9H), 7.24 (3H), 7.42 (3H), 7.77 (3H), 7.95 (6H), 8.93 (6H); MS [m/z] 754 (M^+-3I) (754,2253 calculada para $C_{42}H_{30}BClN_9O_3$); elemental analysis: calcd. C 44.42, H 2.66, N 11.10 found C 44.36, H 2.73, N 11.16].



Scheme 2. Synthesis of phthalonitrile derivative



Scheme 3. Synthesis of SubOPc



Scheme 4. Synthesis of SubOPc³⁺

Spectroscopic studies

Non-charged subphthalocyanines, SubOPc and SubPc, are soluble in several organic solvents, such as dichloromethane and DMF, as indicated by the intense absorption Q-band around 565 nm. The spectrum of tricationic SubOPc³⁺ in different media shows a low intensity and broadening Q-band (Figure 1A). This behavior is characteristic of the aggregated form and is not fully dissolved as monomer. The monomeric form increases in DMF/water(10%) moderately acidified with HCl 1.2 mM (Figure 1B). However, as it can be observed in Figure 1B the fluorescence excitation spectrum of SubOPc³⁺ shows that even in this condition the cationic subphthalocyanine is not completely unaggregated. The spectroscopic characteristics are summarized in Table 1. The Q-band of substituted subphthalocyanines presents a small bathochromic shift (~4 nm) when compared with that of SubPc.

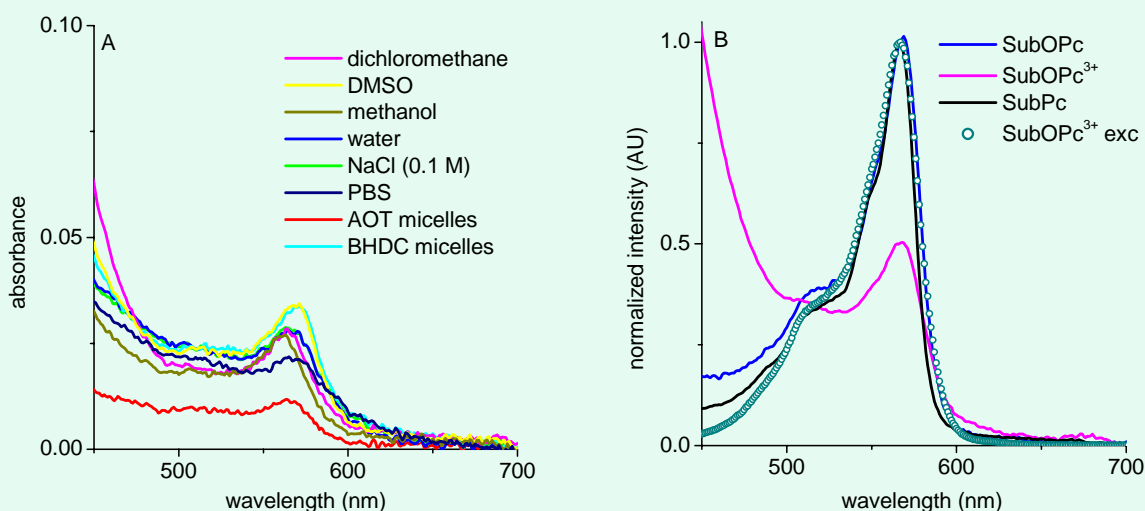


Figure 1. Absorption spectra of (A) SubOPc³⁺ in different media and (B) SubPc, SubOPc and

SubOPc³⁺, and fluorescence excitation spectrum of SubOPc³⁺ ($\lambda_{em}=625$ nm) in DMF/water (10% v/v)/HCl (1.2 mM); dimethylsulphoxide (DMSO), phosphate-buffered saline (PBS), *n*-heptane/sodium bis(2-ethylhexyl)sulfosuccinate (0.1 M)/water ($W_0=10$) (AOT micelles), benzene/benzyl-*n*-hexadecyldimethyl ammonium chloride (0.1 M)/water ($W_0=10$) (BHDC micelles).

The steady-state fluorescence emission spectra of subphthalocyanines were performed in DMF/water (10%)/HCl (1.2 mM) (Figure 2). The spectra show one intense band at ~575 nm, which are characteristic for similar subphthalocyanines [1]. By comparison with SubPc as a reference, the values of fluorescence quantum yields (ϕ_F) were obtained. Values of ϕ_F are shown in Table 1. A small Stokes shift (~10 nm) was observed indicating that the spectroscopic energy is nearly identical to the relaxed energy of the singlet state. Taking in account the energy of the 0-0 electronic transitions, the energy levels of the singlet excited stated (E_s) were calculated (Table 1). These results are in agreement with those previously reported for this family of photosensitizers [1].

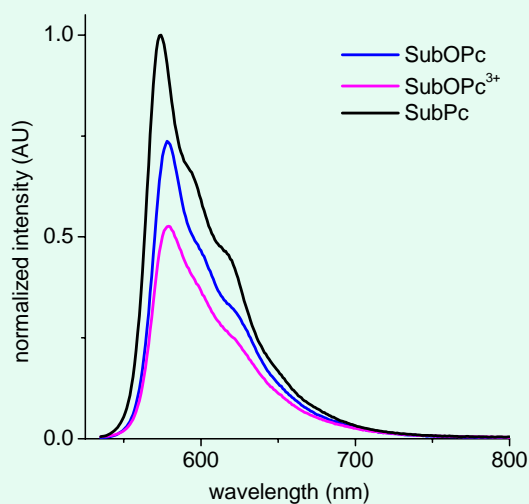


Figure 2. Fluorescence emission spectra ($\lambda_{exc}=530$ nm) of SubPc, SubOPc and SubOPc³⁺ in DMF/water (10% v/v)/HCl (1.2 mM).

Table 1. Spectroscopic characteristics of subphthalocyanines in DMF/water (10%)/HCl (1.2 mM)

Subphthalocyanine	λ_{max}^{abs} (nm) ^a	λ_{max}^{em} (nm) ^b	ϵ (M ⁻¹ cm ⁻¹)	ϕ_F	E_s (kJ mol ⁻¹)
SubPc	566	574	63000 ^c	0.18±0.01 ^c	210

SubOPc	569	578	63500	0.14±0.01	211
SubOPc ³⁺	569	579	31000	0.10±0.01	211

^aQ-band, ^b $\lambda_{exc}=530$ nm, ^c $\phi_F=0.25$ (benzene) ref. [2].

Photodynamic activity

9,10-Dimethylanthracene (DMA) was used to evaluate the ability of these subphthalocyanines to produce $O_2(^1\Delta_g)$ [4]. This substrate quenches $O_2(^1\Delta_g)$ by exclusively chemical reaction. Solutions of the photosensitizers ($A^{570}=0.1$) were irradiated with monochromatic light ($\lambda_{irr}=570$ nm) in the presence of DMA (35 μ M). A time-dependent decrease in the DMA concentration was observed by following a decrease in its absorbance. The photooxidative process was studied in different media: DMF, aqueous sodium dodecyl sulfate (SDS) 2% w/v and DMF/water(10%)/HCl (1.2 mM). Figure 3 shows representative results in acidified DMF/water. From first-order kinetic plots the values of the observed rate constant (k_{obs}^{DMA}) were calculate for DMA (Table 1). In DMF, non-charged subphthalocyanines, SubPc and SubOPc, photodecompose DMA with identical rate, indicating that both sensitizers present the same $O_2(^1\Delta_g)$ production in this solvent. However, DMA reaction was not detected using SubOPc³⁺ in DMF since in this media the cationic sensitizer is highly aggregate. In SDS, only SubOPc and SubOPc³⁺ were evaluated due to the low solubility of SubPc in this micellar system. In this case, a higher rate of DMA decomposition was obtained with SubOPc³⁺ probably due to a better solubilization as monomer than the non-charged sensitizer. The ability of the tree sensitizers were compared in acidified DMF/water. In this condition, the quantum yield of $O_2(^1\Delta_g)$ production (Φ_{Δ}) were calculated comparing the slope for subphthalocyanines with the corresponding slope obtained for the reference, SubPc. The results are gathered in Table 2. Thus, it can be note that $O_2(^1\Delta_g)$ production are very dependent of the medium where the sensitizer is localized and considerably diminishes when the subphthalocyanine is aggregated.

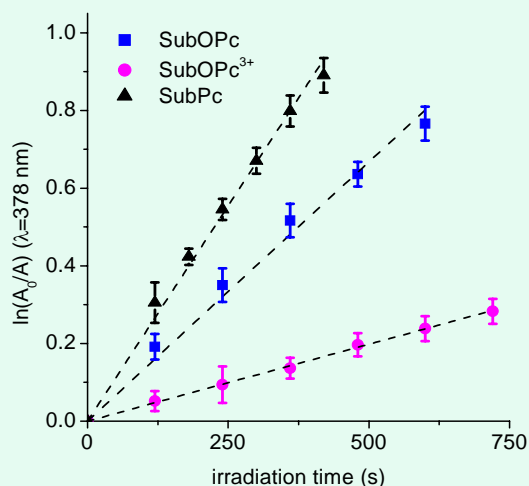


Figure 3. First-order plots for the photooxidation of DMA (35 μM) in DMF/water (10% v/v)/HCl (1.2 mM) photosensitized by SubPc, SubOPc and SubOPc³⁺, $\lambda_{\text{irr}}=570$ nm. Values represent mean \pm standard deviation of three separate experiments.

Table 2. Kinetic parameters (k_{obs}) and quantum yield of $\text{O}_2(^1\Delta_g)$ production (Φ_{Δ}) of subphthalocyanines in different media

Subphthalocyanine	k_{obs} (s^{-1}) DMF	k_{obs} (s^{-1}) SDS ^a	k_{obs} (s^{-1}) DMF/water/HCl ^b	Φ_{Δ}
SubPc	$(1.1 \pm 0.1) \times 10^{-3}$	-	$(2.2 \pm 0.2) \times 10^{-3}$	0.61 ^c
SubOPc	$(1.1 \pm 0.1) \times 10^{-3}$	$(4.9 \pm 0.2) \times 10^{-4}$	$(1.3 \pm 0.2) \times 10^{-3}$	0.36 ± 0.03
SubOPc ³⁺	-	$(7.6 \pm 0.3) \times 10^{-4}$	$(0.4 \pm 0.1) \times 10^{-3}$	0.11 ± 0.01

^aSDS 2% w/v, ^bDMF/water (10%)/HCl (1.2 mM), ^cref. [1].

Conclusions

In summary, a novel tricationic subphthalocyanine has been conveniently synthesized in three-steps: nucleophilic aromatic substitution reaction was used to produce 4-(4-pyridyloxy)phthalonitrile with 66% yield, boron trichloride-induced cyclotrimerization of 4-(4-pyridyloxy)phthalonitrile given 19% of SubOPc and exhaustive methylation of SubOPc with methyl iodide yielded 92% of tricationic SubOPc³⁺. In the structure of SubOPc³⁺, the cationic centers are isolated from the subphthalocyanine macrocycle ring by an alcohoxy bonds. This spacer provides a higher mobility of the charges, which could facilitate the interaction with the outer membrane of the Gram-negative bacteria [5].

Subphthalocyanines with their cone-shaped structure should not be form aggregated in several solvents. That is the case of non-charged subphthalocyanines, such as SubPc o SubOPc. Unfortunately, the cationic SubOPc³⁺ is essentially aggregated in solution of different solvents and it is partially dissolved as monomer in only ones few media (per example acidified DMF/water and SDS solution). The O₂(¹Δ_g) production studies in different media confirm that SubOPc³⁺ can be an efficient sensitizer mainly depending of the microenvironment where it is localized. Thus, its photodynamic efficiency in biological systems is not directly predictable on the basic of photophysical investigations in solution [8]. Studies of PDI *in vitro* to evaluate the activity of these sensitizers on a Gram-negative bacterium *Escherichia coli*, are presently in progress in our laboratory.

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