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Synthesis of Porphyrin-Polyethylenimine Conjugates as Antimicrobial Agents †

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Abstract: 5,15-di(4-(*N*,*N*-dimethylaminophenyl)-10,20-di(pentafluorophenyl)porphyrin (**1**) and 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (**2**) were synthesized in 24% and 36%, respectively. An aromatic nucleophilic substitution reaction was used to obtain conjugated polymers based in these porphyrins covalently linked to polyethylenimine (**PEI-1** and **PEI-2**). Spectroscopic and photodynamic properties indicate that these polymers are potential phototherapeutic agents for the eradication of bacteria.

Keywords: porphyrin; tetrapyrrolic macrocycle; pentafluorophenyl; photosensitizer

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1. Introduction

Increasing of resistant bacteria to clinical antibiotics has conducted to establish new treatments for infections [1]. One of them includes photodynamic inactivation (PDI) of microorganisms [2]. PDI is founded in the administration of a photosensitizer and the aerobic irradiation produces cell inactivation. In general, the presence of positive charge precursor groups allows improving the photoinactivation due to an increase in interaction with microbial cells [3].

Polyethylenimine is a basic aliphatic polymer that is polycationic due to the presence of primary, secondary and tertiary amino groups [4]. PEI polymers are effective against a variety of Gram-positive and Gram-negative bacteria, including clinical isolates of pathogenic bacteria and bacteria in contaminated water [4]. Both polymers present a numerous amount of free amine groups able to acquire positive charge at physiological pH. This characteristic allows them to interact in aqueous medium with microbial cell membranes. Therefore, this polymer can be used in drug delivery applications, improving solubilization and binding to microorganisms.

In this work, two porphyrin-polyethylenimine conjugates were synthesized as potential phototherapeutic agents to inactivate bacteria.

2. Materials and Methods

2.1. Equipment and Chemical Substances

Proton nuclear magnetic resonance spectra were achieved on a FT-NMR Bruker Avance DPX400 spectrometer (Bruker BioSpin, Rheinstetten, Germany). Mass spectra were attained on a Bruker micrO-TOF-QII (Bruker Daltonics, Billerica, MA, USA) equipped with an ESI source (ESI-MS). Absorption spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan), while fluorescence spectra were carried out on a Spex FluoroMax spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA). Compounds from Sigma-Aldrich (Milwaukee, WI, USA) were used as received. Silica gel thin-layer chromatography (TLC) plates 250 microns were purchased from Analtech (Newark, DE, USA) and silica gel 60 (0.040–0.063 mm, 230–400 mesh) from Merck (Darmstadt, Germany).

2.2. Synthesis

5,15-di(4-(*N,N*-dimethylaminophenyl)-10,20-di(pentafluorophenyl)porphyrin (**1**) and 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (**2**) were synthesized as reported [5,6]. Conjugates **PEI-1** and **PEI-2** were obtained by nucleophilic aromatic substitution reaction following the procedure previously described [7].

2.3. Spectroscopic Studies

Absorption and fluorescence spectra were performed in a quartz cell of 1 cm path length using *N,N*-dimethylformamide (DMF) at room temperature. The fluorescence quantum yields (Φ_F) of the conjugates were calculated by comparison of the area below the corrected emission spectra with that of the reference [5]. Photooxidation of 9,10-dimethylanthracene was used to determine singlet molecular oxygen productions [5].

3. Results and Discussion

3.1. Synthesis of Conjugates

Porphyrin **1** and **2** were synthesized from the condensation of 4-pentafluorobenzaldehyde and 5-(pentafluorophenyl)dipyrromethane or pyrrole catalysed by boron diethyl etherate in dichloromethane. After oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and purification using flash chromatography, porphyrin **1** and **2** were isolated in 24% and 36%, respectively.

An aromatic nucleophilic substitution reaction was used to obtain conjugated polymers based in these porphyrins covalently linked to polyethylenimine (**PEI-1** and **PEI-2**) (Figure 1). The reaction was carried out in *N,N*-dimethylformamide (DMF) giving 100% conversion.

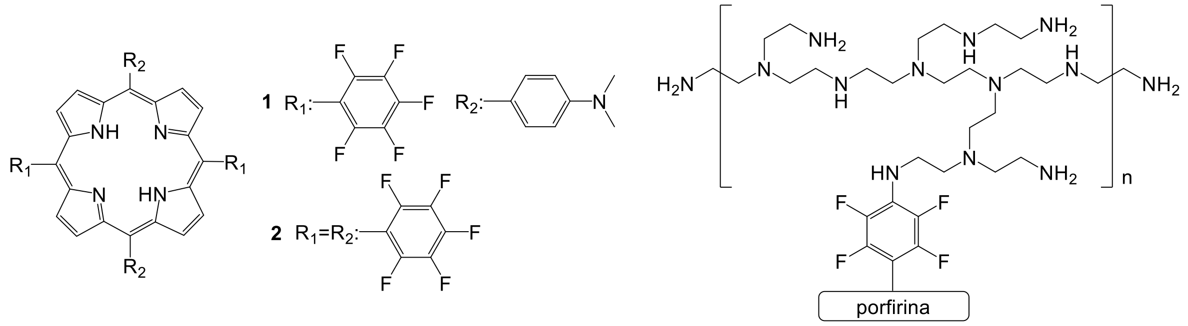

Figure 1. Synthesis of dipyrromethanes **1** and **3**.

3.2. UV-Visible Absorption Spectroscopic Characteristics and Photodynamic Properties

Conjugates **PEI-1** and **PEI-2** showed the typical Soret band at ~420–430 nm and the four Q-bands between 512–653 nm, which are characteristics of *meso*-tetraphenylporphyrin derivatives [5,7]. The spectroscopic properties of the conjugates are summarized in **Error! Reference source not found.**. The steady-state fluorescence emission spectra of these conjugates were obtained in DMF (**Error! Reference source not found.**). The two bands are characteristic for similar *meso*-substituted porphyrin [5]. These bands have been assigned to Q_x(0-0) and Q_x(0-1) transitions. This is a typical behavior for porphyrins with D_{2h} symmetry, like the free bases, and indicates that the porphyrin vibronic structure remains practically unchanged upon excitation. Fluorescence quantum yields (Φ_F) of these conjugates agree with values previously reported by similar porphyrin derivatives [7]. Furtheremore, **PEI-1** and **PEI-2** were able to sensitize the formation of singlet molecular oxygen with appropiated quantum yield (Φ_Δ) (Table 1).

Table 1. Spectroscopic and photodynamic properties of porphyrins **PEI-1** and **PEI-2** in DMF.

PS	$_{max}^{abs}$ **(nm)**	$_{max}^{em}$ **(nm)**	$\Phi_F^{DMF\ a}$	$\Phi_\Delta^{DMF\ b}$
PEI-1	418	643	0.0084 ± 0.0008	0.25 ± 0.02
PEI-2	420	657	0.034 ± 0.003	0.31 ± 0.02

[a] Fluorescence quantum yield, [b] singlet molecular oxygen quantum yield.

4. Conclusions

These conjugates showed the Soret absorption band and the four characteristic Q bands of porphyrins. Moreover, they emit two bands of red light. Photodynamic studies indicated that **PEI-2** is more effective than **PEI-1** to produce singlet molecular oxygen. In vitro PDI studies are in process to demonstrate both polymers are effective phototherapeutic agents for the eradication of bacteria.

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