Pergamon

Tetrahedron Letters 41 (2000) 9143–9147

TETRAHEDRON LETTERS

Synthesis of a new water-soluble octa-cationic phthalocyanine derivative for PDT

Maria Paola De Filippis, Donata Dei, Lia Fantetti and Gabrio Roncucci*

Molteni Farmaceutici, S.S 67 Loc. Granatieri, 50018 Scandicci, Florence, Italy

Received 22 June 2000; revised 13 September 2000; accepted 20 September 2000

Abstract

The synthesis and characterization of a new octa-cationic Zn–phthalocyanine is described. This compound is water-soluble, not aggregated under a wide range of solvent conditions and is a powerful photosensitizer for the inactivation of microorganisms by using a strategy based on photodynamic therapy.

Keywords: phthalocyanines; PDT; cationic photosensitizers.

In the last few years, a great deal of interest has been focused on the synthesis of phthalocyanine derivatives (PCs) due to their applications in many fields such as liquid crystals,[1] chemical sensors,[2] and non-linear optics.[3] One of the most promising fields is the use of phthalocyanine derivatives as photosensitizers for photodynamic therapy (PDT), an emerging new bimodal strategy for treating a large variety of pathologies such as psoriasis, cancer, dysplastic and infectious diseases.[4]

In order to be used for PDT, a phthalocyanine derivative must have a long wavelength absorption in the red region of the visible spectrum, within the so called 'therapeutic window',[5] a large molar extinction coefficient (ε), photostability, low dark toxicity and rapid clearance.

One important issue related to phthalocyanine derivatives is their water solubility and aggregation properties, which may have a strong influence on the bioavailability, the in vivo distribution and the singlet oxygen production efficiency.

While lipophilic phthalocyanine derivatives are reported to have a higher tumor affinity, but associated with cutaneous phototoxicity,[6] probably due to overall lower excretion, water soluble phthalocyanines are considered the best targets for a new generation of photosensitizers.[7] Among water soluble compounds, great attention has initially been focused on the synthesis of anionic derivatives, such as sulfo-, carboxy- etc. and phosphono- derivatives,[8] mainly evaluated

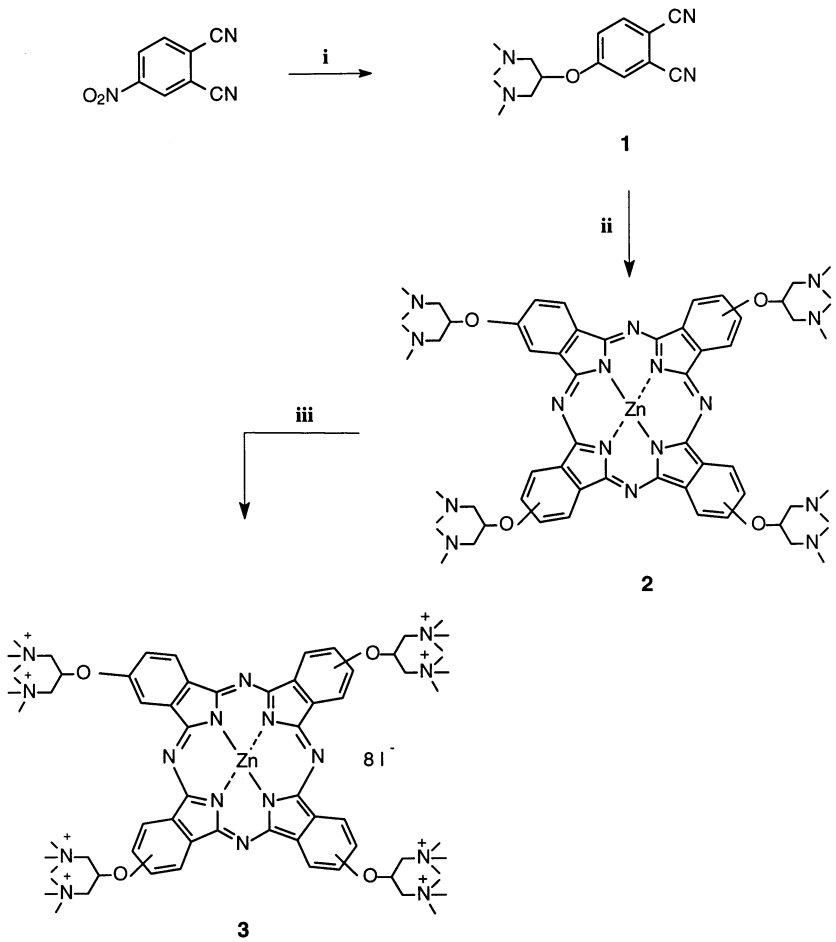
* Corresponding author.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01638-5

for tumor treatment and cationic phthalocyanine derivatives for the treatment of both tumor and infectious diseases.[9] Among the latter, however, only tetracationic constitutional isomers or poorly structurally defined dicationic phthalocyanines[9,10] have been obtained and described so far.

The finding that cationic compounds may specifically target mitochondria,[11] together with evidence that cationic photosensitizers are indeed effective for gram-negative bacteria[12] and yeast photoinactivation,[13] have prompted us to prepare an octa-cationic phthalocyanine derivative for evaluation as a photosensitizer aimed at the inactivation of antibiotic resistant microorganisms.

The synthesis of compound **3** is reported in Scheme 1.



Scheme 1. i. 1,3-Bis-(dimethylamino)2-propanol, K_2CO_3, DMSO, rt (74%); ii. DBU, $Zn(OAc)_2$, N_2, 150–180°C (18%); iii. MeI, *N*-methyl-2-pyrrolidinone, rt (82%)

The 4-[1,3-bis-(dimethylamino)2-propyloxy]1,2-dicyanobenzene **1** can be easily prepared from 4-nitrophthalonitrile and 1,3-bis-(dimethylamino)2-propanol.[14]

Cyclization of compound **1** was best performed by using neat 1,8 diazabicyclo-[5.4.0]-undec-7-ene (DBU), in the presence of anhydrous zinc(II)acetate at 150–180°C to produce 18% yield of compound **2** by following a procedure already described.[15]

Compound **3** was obtained by reaction of **2** with an excess of methyl iodide in *N*-methyl-2-pyrrolidinone as solvent at rt (yield = 82%). A shorter reaction time was achieved by using this solvent compared to other solvents such as *N*,*N*-dimethylformammide, methanol, chloroform or by using neat methyl iodide at different temperatures.

Compounds **2** and **3** were identified through various spectroscopic techniques such as ^{1}H NMR,[15a,16a] ^{13}C NMR[15b,16b] and MS analysis[15c,16c] and characterized specrophotometrically in the UV–vis region in order to assess the aggregation tendency and in particular the dimerization.[15d,16d]

The complexity of ^{1}H and ^{13}C NMR spectra evidenced the presence of a mixture of at least two constitutional isomers of the four expected from the cyclotetramerization of a mono-substituted phthalonitrile.

As for the MS analysis, it was not possible to determine the mass value of compound **3**. In fact the ESI-MS spectrum of **3** shows a large number of fragments, in which predominates $m/z = 1167.6$ $[M-7CH_3-8I]^+$, probably due to a rapid decomposition of the sample during the analysis.

Compounds **2** and **3** are readily soluble in DMF with strong absorption at 678 ($\varepsilon_{DMF} = 63\ 250$ M^{-1} cm^{-1}) and 679 nm ($\varepsilon_{DMF} = 169\ 810$ M^{-1} cm^{-1}), respectively, according to the ones reported for phthalocyanine derivatives. Compound **3** is very soluble in water, it shows a main absorption band at 677 nm ($\varepsilon_{water} = 109.140$ M^{-1} cm^{-1}) and is, unexpectedly, not aggregated in a wide range of concentrations as judged by the low absorption of the dimer band centered at 632 nm,[17] probably because of the cooperative effect of electrostatic repulsion and bulky substituents.

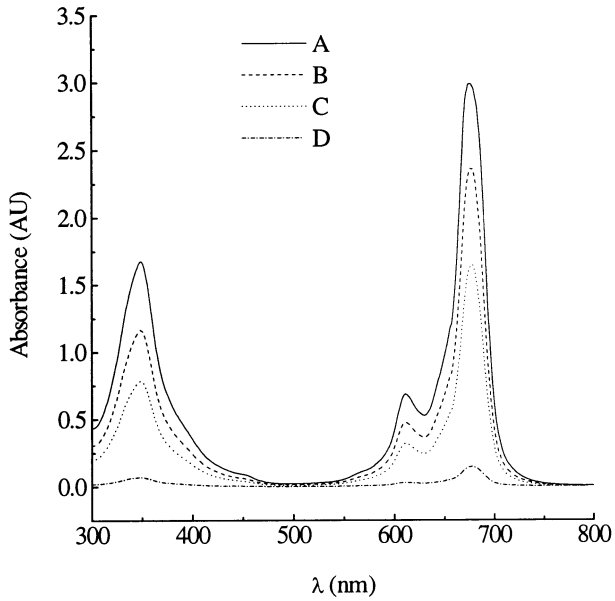


Figure 1. Absorption spectra of **3** in water at different concentrations: 1.92×10^{-5} (A), 1.44×10^{-5} (B), 9.6×10^{-6} (C), 9.6×10^{-7} M (D)

Due to the high value of the *Q* absorption band, we were unable to obtain quantitative data for more concentrated solutions, however we found very low dimerization up to 1.92×10^{-5} M (Fig. 1). Thus, it seems evident that the presence of eight positive charges efficiently prevents the aggregation of this phthalocyanine, unlike neutral compounds such as compound **2** and even strictly related molecules bearing four cationic functional groups on the ring which still have an overall tendency to aggregate in polar solvents and water.[18]

These results encourage us to prompt the preparation of new water-soluble phthalocyanines for PDT.

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14. Compound **1**: 4-Nitrophthalonitrile (0.30 g–1.73 mmol) and 1,3-bis-(dimethylamino)-2-propanol (0.38 g–2.05 mmol) with K_2CO_3 (2.40 g–3.78 mmol) in anhydrous DMSO (4 mL) were stirred at rt for 96 hours. The product was isolated from the reaction mixture by extraction with acidic water (acetic acid, pH 5), washing of the acidic solution with dichloromethane, addition of $NaHCO_3$ to the aqueous solution until pH 8, and finally extraction of the product from the basic solution with dichloromethane in 74% yield. Yellowish viscous fluid. ^{1}H NMR (200 MHz, 299 K, DMSO-d_6) δ (ppm) 8.00 (d, 1H, H-6, $J_o=8.84$ Hz), 7.79 (d, 1H, H-3, $J_m=2.06$ Hz), 7.48 (dd, 1H, H-5, $J_o=8.84$ Hz, $J_m=2.8$ Hz), 4.91–4.83 (quintet, 1H, CH, $J=5.26$ Hz), 2.54 (d, 4H, 2 CH_2, $J=4.98$ Hz), 2.20 (s, 12H, 4 CH_3).
15. Compound **2**: The phthalonitrile **1** (1.00 g–3.67 mmol), solubilized in neat DBU (1.40 g–9.18 mmol), was heated at 150–180°C for 4 hours in the presence of $Zn(OAc)_2$ (0.34 g–1.84 mmol). The phthalocyanine **2** was then isolated from the crude mixture through protonation of the amino groups by treatment with aqueous acetic acid, washing the aqueous solution with several solvents (Et_2O, AcOEt, CH_2Cl_2, $CHCl_3$), after basification with K_2CO_3, extraction of the neutral compound with Et_2O and slow precipitation of the pure product from this solution (193 mg; yield = 18%). Blue-green solid. (a) ^{1}H NMR (200 MHz, 299 K, DMSO-d_6) δ (ppm) 9.29–9.24 (m, 4H), 8.97 (broad s, 4H), 7.85–7.66 (m, 4H), 5.16–5.13 (m, 4H), 2.86–2.83 (m, 16H; 340 K: 2.92 ppm, d,

$J = 5.26$ Hz), 2.44 and 2.40 (two s, 48 H; 340 K: 2.49 ppm, sharp s); (b) ^{13}C NMR (200 MHz, 299 K, DMSO-d_6) δ (ppm) 159.9, 130.9, 123.8, 118.9, 76.9, 61.1, 60.9, 54.8, 46.24, 46.16; (c) ESI-MS m/z 1153.6 $[M+H]^+$; (d) UV–vis (DMF) λ_{max} (ε, M^{-1} cm^{-1}): 678 (63 250), 613, 354 nm.

16. Compound **3**: Compound **2** (0.017 g–0.0015 mmol) was treated with excess of methyl iodide (2.5 mL–40 mmol) in *N*-methyl-2-pyrrolidinone (2.5 mL) at rt for 8 days. The reaction mixture was diluted with Et_2O (10 mL) and the suspension thus obtained was filtered, washing the solid extensively with organic solvents (Et_2O, AcOEt, CH_2Cl_2, $CHCl_3$, acetone) to afford pure compound **3** as a blue–green solid in 82% yield. (a) ^1H NMR (200 MHz, 299 K, DMSO-d_6) δ (ppm) 9.48 (d, 4H, $J = 8.24$ Hz), 9.33–9.19 (double m, 4H), 8.27 (broad d, 4H, $J = 7.99$ Hz), 6.35–6.12 (m, 4H), 4.22 (broad signal, 16H), 3.45 (s, 72 H); (b) ^{13}C NMR (200 MHz, 299 K, DMSO-d_6) δ (ppm) 155.8, 152.7, 140.2, 133.3, 124.3, 119.7, 110.9, 68.9, 68.7, 66.7, 54.1; (c) ESI-MS m/z 1167.6 $[M-7CH_3-8I]^+$; (d) UV–vis (DMF), λ_{max} (ε, M^{-1} cm^{-1}): 679 (169 810), 613, 354 nm.
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