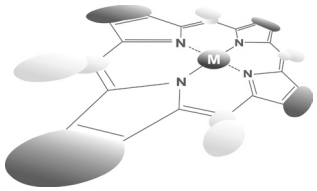
Journal of Porphyrins and Phthalocyanines
J. Porphyrins Phthalocyanines 2012; **16**: 85–92
DOI: 10.1142/S108842461100435X

Published at http://www.worldscinet.com/jpp/



Synthesis, circular dichroism, DNA cleavage and singlet oxygen photogeneration of 4-amidinophenyl porphyrins

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Received 8 July 2011
Accepted 30 July 2011

ABSTRACT: 5,10,15,20-tetrakis(4-amidinophenyl)porphyrin (**Por 1**), its Zn complex (**Por 2**) and its conjugate with a tetraphenylporphyrin to form a bisporphyrin (**Por 3**) were prepared. The monomeric **Por 1** and **Por 2** showed both intercalative and external binding with DNA whereas only external DNA binding was seen in the bisporphyrin, **Por 3** by circular dichroism and UV-vis. The DNA photocleavage activities of these porphyrins followed the order: **Por 1** ~ **Por 2** > **Por 3**, which did not correlate with their measured $^1O_2$ production rates. It suggests 4-amidinophenylporphyrins are promising new photodynamic therapeutic agents.

KEYWORDS: amidinophenylporphyrin, singlet oxygen, DNA cleavage, binding mode.

INTRODUCTION

Photodynamic therapy (PDT), with its inherent selectivity, has received increasing attention as an emerging cancer treatment modality recently. In PDT, a non-toxic photosensitizer, which can be selectively taken up by tumor cells, generates reactive oxygen species, such as singlet oxygen ($^1O_2$), upon photo-activation by visible light. The reactive oxygen species produced causes oxidative damage to the tumor tissues, resulting in cell death [1, 2].

Circular dichroism (CD) and UV-vis spectroscopy were applied to study the porphyrin-DNA interactions. Previous studies showed that the sign of the induced CD in the Soret region of the porphyrins can be used as a signature of their binding modes with DNA under low porphyrin loads ($r < 0.1$): a positive induced CD signal indicates external binding whereas a negative induced CD signal indicates intercalative binding with DNA [3, 4].

Cationic porphyrins, such as 5,10,15,20-tetrakis(4-*N*-methylpyridinium)porphyrin (H_2TMPyP), have been intensively studied as potential PDT agents for decades due to their strong binding affinity towards DNA and their water-solubility which make DNA-binding possible under physiologically relevant conditions [5, 6]. These crucial properties have been attributed to their cationic substituent which facilitates their binding interactions with the anionic DNA in aqueous media [7, 8]. But DNA-binding interactions can be achieved *via* complementary hydrogen bonding with DNA bases as well, particularly at the minor groove region [9, 10]. In this work, a hydrogen bonding functionality, *i.e.* the amidine group, was introduced onto the tetraphenylporphyrin (TPP) by synthesis. Its Zn(II) complex and its conjugate with another TPP *via* the –O–$(CH_2)_3$–O– linker were prepared as well (Schemes 1 and 2). The efficiency of cellular uptake of the drug is very important to play a therapeutic role, which depends on hydrophilic and lipophilic nature of molecular structure [11]. Since the previously prepared bisporphyrin with

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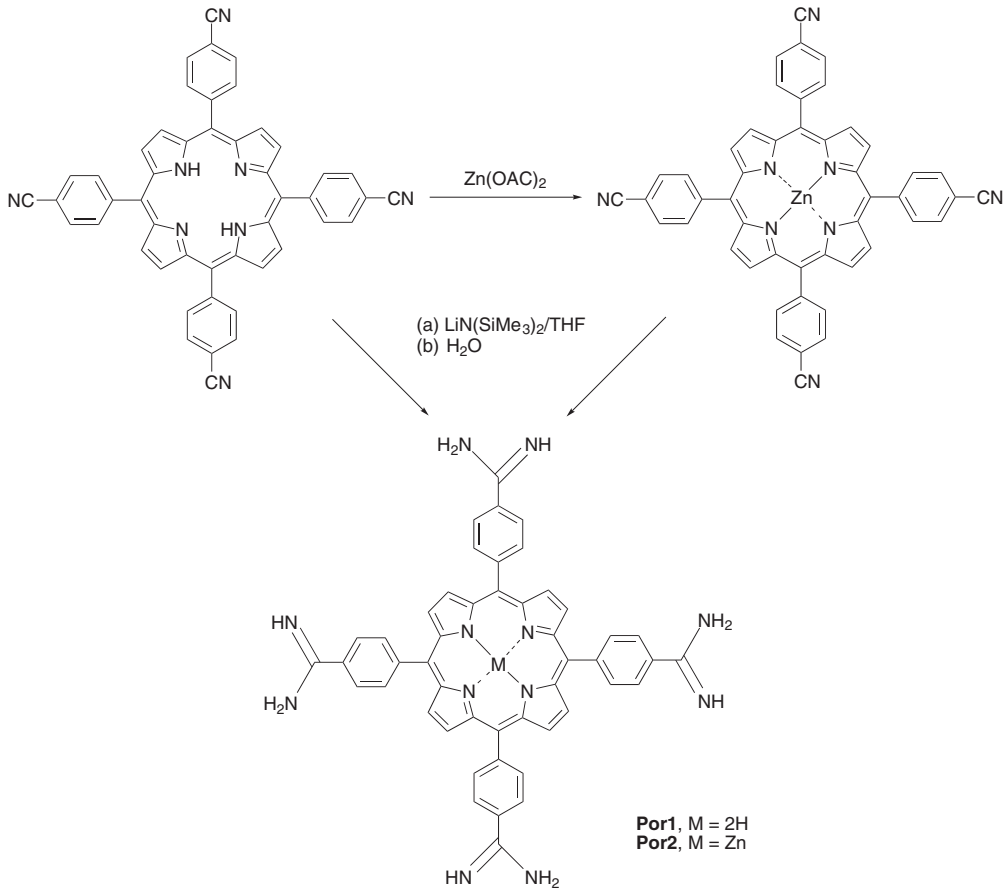
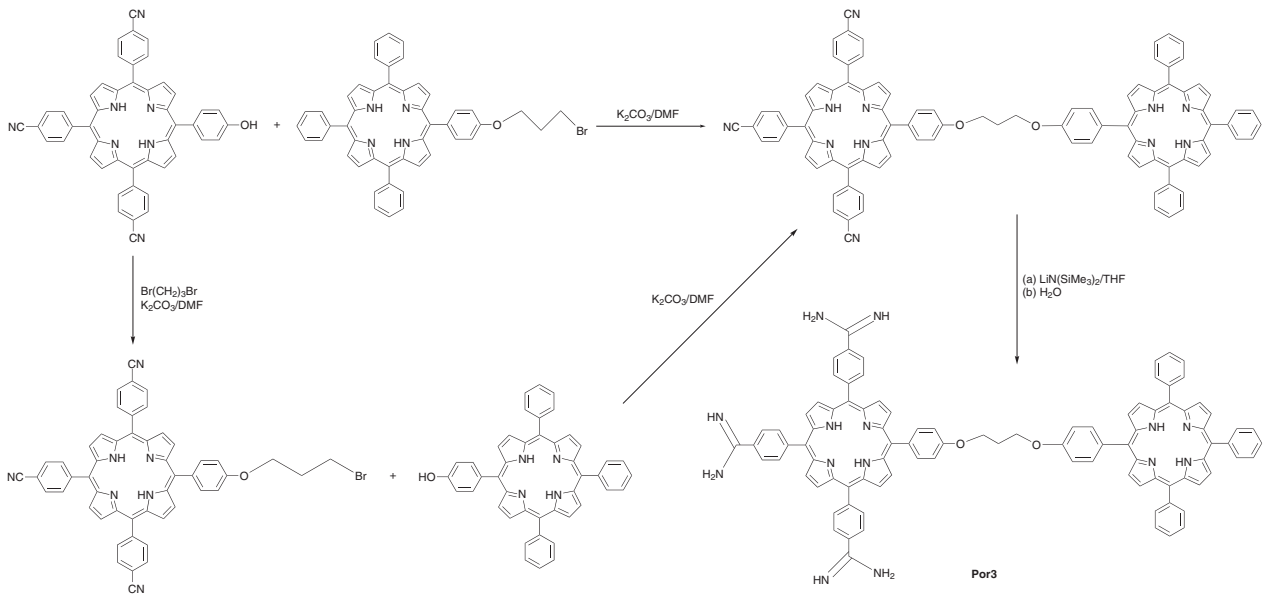


Scheme 1



Scheme 2

hydrophilic (TMPyP) and lipophilic (TPP) structure in our lab had a good cellular uptake [12], amidine group as the hydrophilic part replaced TMPyP in the bisporphyrin structure design. Furthermore, the amidine substituent, in addition to its hydrogen-bonding capability, can also form amidinium-carboxylate salt bridge with biomolecules bearing a pendant carboxylate/carboxylic acid. Such intermolecular amidinium-carboxylate bridges have recently been shown to facilitate photo-excited energy transfer with rates much faster than those predicted by

the Forster mechanism [13]. Herein we reported their binding modes with DNA as studied by circular dichroism spectroscopy and UV-vis. Their DNA photocleavage activities and their relative $^1O_2$ production rates were also investigated.

RESULTS AND DISCUSSION

Binding mode of Por 1, Por 2 and Por 3 with DNA

The absorption titration spectra of these 4-amidinophenylporphyrins with calf thymus DNA (ct-DNA) was given in Fig. 1. Without DNA, λ_{max} of the Soret bands of **Por 1**, **Por 2** and **Por 3** were 414, 423 and 422 nm, respectively. In the presence of low concentrations of ct-DNA (*i.e.,* r = [porphyrin]/[DNA] > 0.6), the Soret bands of **Por 1**, **Por 2** and **Por 3** exhibited substantial hypochromicity (75.9%, 76.1% and 77.1%, respectively) but no significant spectral shift, which suggests an external DNA binding mode with modest effect on the π–π^* Soret absorption. Further addition of ct-DNA to **Por 1**, **Por 2** and **Por 3** (*i.e.,* r < 0.26) resulted in a red shift of 11, 4 and 10 nm, respectively. The substantial bathochromic shifts and hypochromicity observed suggest binding interactions between the 4-amidinophenylporphyrins and ct-DNA [14, 15]. An apparent binding constant, K_{app}, can be calculated from these data using Eq. (1) as follows [16].

$$\frac{[DNA]_{total}}{(|\varepsilon_{app} - \varepsilon_f|)} = \left(\frac{1}{(|\varepsilon_b - \varepsilon_f|)}\right)[DNA]_{total} + \frac{1}{\{K_{app}(|\varepsilon_b - \varepsilon_f|)\}} \quad (1)$$

where ε_{app}, ε_f and ε_b correspond to A_{obsd}/[porphyrin], the molar absorptivity of the free porphyrin, and the molar absorptivity of the DNA-bound porphyrin, respectively. By plotting $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs*. $[DNA]_{total}$, K_{app} of **Por 1**, **Por 2** and **Por 3** were determined to be $(1.53 \pm 0.69) \times 10^6$ M^{-1}, $(7.25 \pm 6.02) \times 10^5$ M^{-1} and $(5.81 \pm 1.14) \times 10^5$ M^{-1}, respectively.

The induced CD spectra in the Soret region of **Por 1**, **Por 2** and **Por 3** (at r([porphyrin]/[DNA]) = 0.05) were given in Fig. 2. From this figure, **Por 1** showed two positive signals at 421 and 443 nm, and one negative signal at 431 nm. **Por 2** showed a strong negative signal at 427 nm and a strong positive signal at 450 nm. These features suggest that both **Por 1** and **Por 2** could bind to ct-DNA *via* both external bounds and intercalation. **Por 3** showed a very weak and broad positive signal centered at 440 nm, indicating that it bond to ct-DNA rather weakly with outside binding mode in the high DNA concentration.

DNA photocleavage ability

DNA photocleavage activities of **Por 1**, **Por 2** and **Por 3** were measured using plasmid DNA relaxation assay [17].

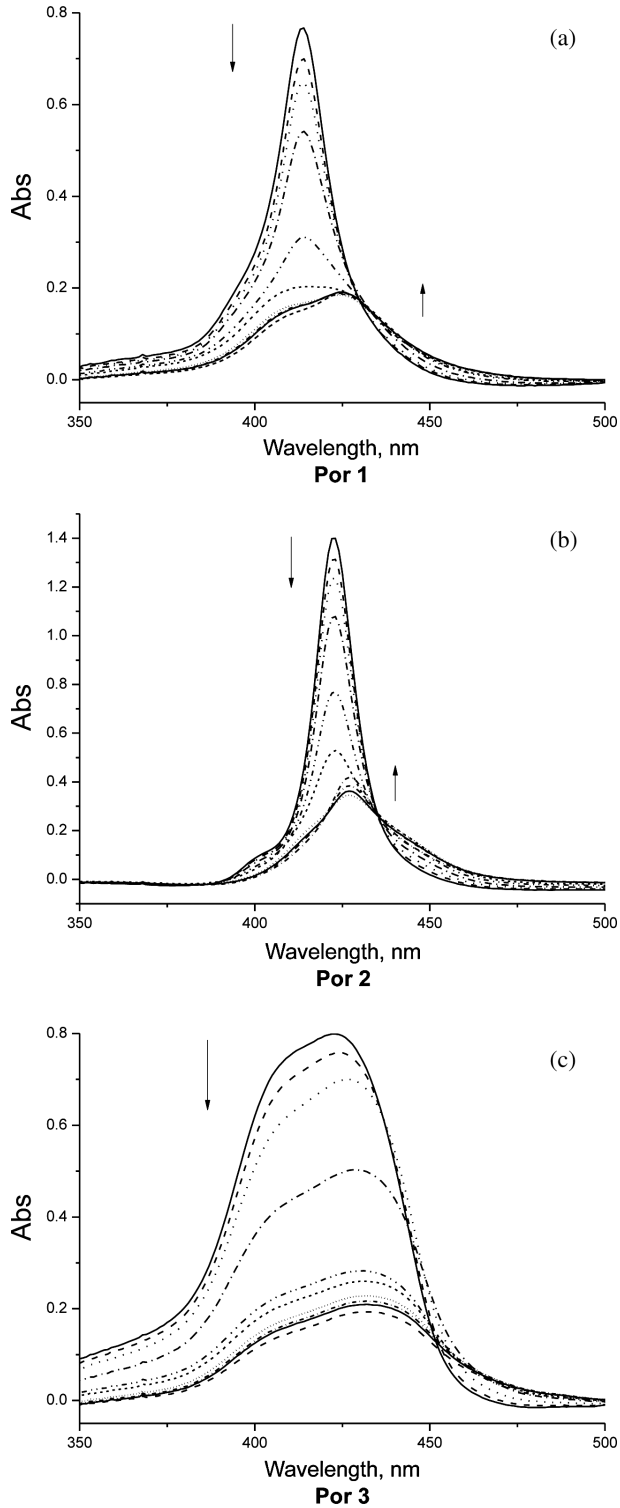


Fig. 1. Absorption titration spectra of the amidinophenylporphyrins (4.0 μM), **Por 1** (a), **Por 2** (b) and **Por 3** (c), with increasing concentrations of ct-DNA (r = [porphyrin]/[DNA base pairs] = ∞, 7.14, 3.57, 1.80, 0.91, 0.62, 0.26, 0.20, 0.17, 0.11) in buffered solution (pH = 7.4, 5 mM tris-HCl, 50 mM NaCl)

The gel images are given in Fig. 3. For **Por 1** and its Zn(II) complex (**Por 2**), their photocleavage activities were comparable and were *ca*. 4-fold lower than that of H_2TMPyP. At 10 μM, their DNA photocleavage activities

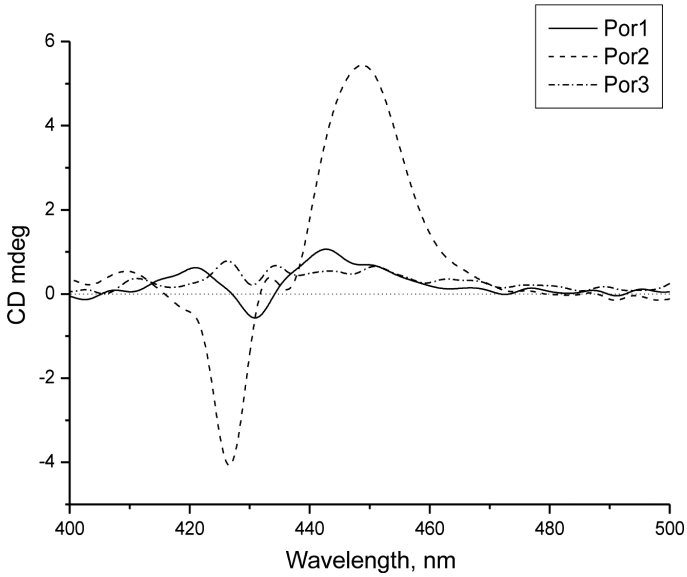


Fig. 2. Induced CD spectra of **Por 1**, **Por 2** and **Por 3** in the tris-HCl buffer solution with the [porphyrin] = 10 μM and [ct-DNA] = 200 μM, $r = 0.05$

were 69% and 74%, respectively, which was *ca*. 4-fold higher than that of **Por 3** (19%).

For **Por 3**, its maximum photocleavage activity of *ca*. 36% was seen at 75 μM. The significant DNA photocleavage activities were likely the result of a binding interaction with DNA and a more effectively oxidative attack from close range with $^1O_2$ [18–20]. It also suggests that **Por 1**, **Por 2** and **Por 3** with hydrogen-bonding capability have relatively weaker electrostatic interaction with DNA compared with H_2TMPyP.

Photogeneration of $^1O_2$

Since the DNA photocleavage activity of a photosensitizer depends on its $^1O_2$ yield as well as its DNA-binding property, we measured the relative $^1O_2$ production rates of **Por 1**, **Por 2**, **Por 3** and H_2TMPyP based on the $^1O_2$-induced bleaching of 1,3-diphenylisobenzofuran (DPBF, λ_{max} = 418 nm) [21, 22]. According to the relationship between A/A_0 absorbed by DPBF and illumination time, one may indirectly obtain the $^1O_2$ yield of those porphyrins compared with H_2TMPyP. All three 4-amidinophenylporphyrins produced $^1O_2$ more efficiently than H_2TMPyP in the following order: **Por 2** > **Por 1** ~ **Por 3** > H_2TMPyP (Fig. 4).

In contrast, Zn-porphyrin **2** showed a greater yield of $^1O_2$, as other Zn(II)-porphyrin derivatives, and this is attributed to its correspondingly high triplet quantum yield, Φ_T [23]. The lack of correlation between the observed DNA photocleavage activities of these porphyrins and their relative $^1O_2$ production rates suggests that their DNA-binding properties must be a more important factor. Presumably, the intercalative binding exhibited by H_2TMPyP, **Por 1** and **Por 2** with DNA was crucial to their higher DNA photocleavage activities than **Por 3** which binds to DNA only externally.

EXPERIMENTAL

General

5-[*p*-(3-bromopropoxy)phenyl]-10,15,20-tris-phenylporphyrin, 5-(*p*-hydroxyphenyl)- 10,15,20- trisphenylporphyrin and *meso*-tetrakis (*N*-methylpyridinium-4-yl)porphyrin (H_2TMPyP) were prepared according to literature procedures [24, 25]. Other chemicals were obtained from Sigma-Aldrich Company and used without further purification. Silica gel 60 (0.04–0.063 mm) for

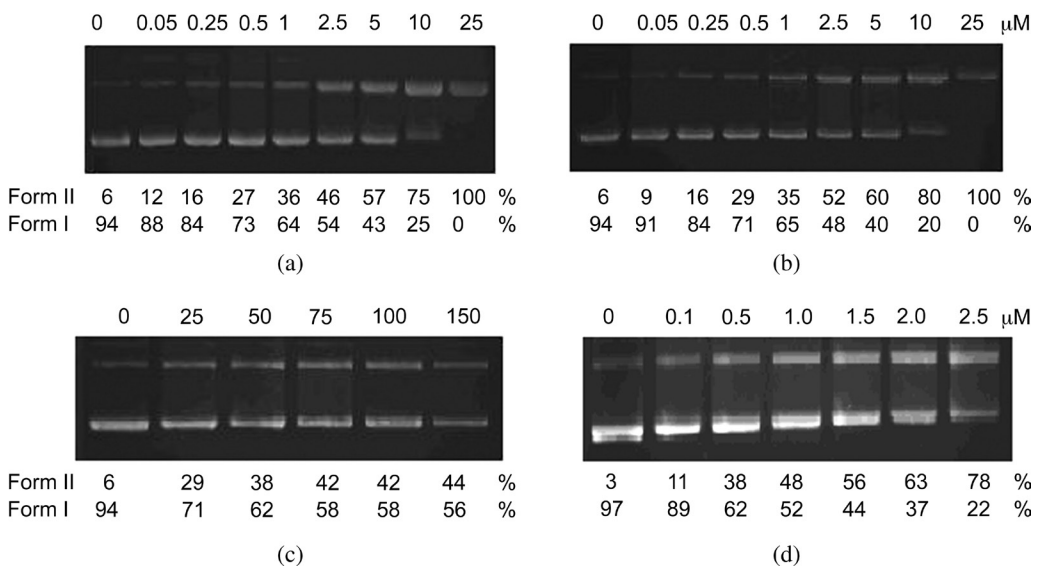


Fig. 3. Agarose gel electrophoresis images of DNA photocleavage assay of (a) **Por 1**, (b) **Por 2**, (c) **Por 3** and (d) H_2TMPyP at different concentrations. Photo-irradiation was conducted using a transilluminator at 455 nm for 45 min

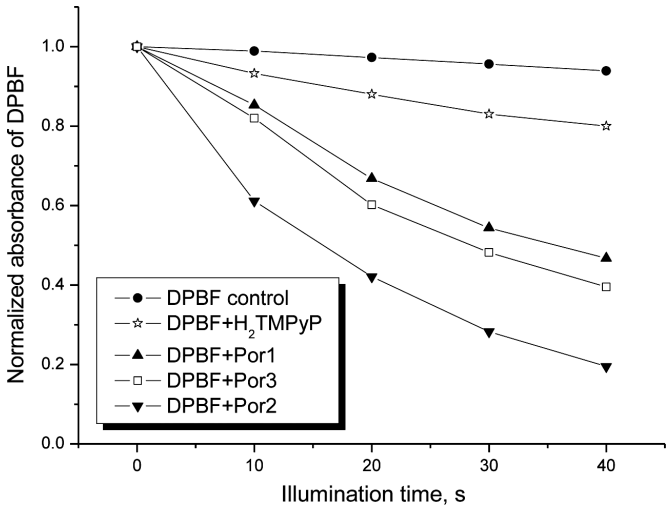


Fig. 4. Normalized absorbance of DPBF (50 μM in DMSO) at 418 nm as a function of photo-irradiation time in the absence (control) and presence of 1 μM of H_2TMPyP, **Por 1**, **Por 2** and **Por 3**

column chromatography was obtained from Merck. Calf thymus DNA (ct-DNA) was obtained from Pharmacia Biotech Company. The concentration of ct-DNA in base-pairs was determined spectrophotometrically using the extinction coefficient $\varepsilon_{260} = 6600\ M^{-1}.cm^{-1}$ [26].

NMR spectra were recorded on a Varian INOVA 400 NMR spectrometer. High-resolution matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were recorded on an Autoflex Bruker MALDI-TOF mass spectrometer. FAB-mass spectra were obtained on a Finnigan TSQ710 mass spectrometer. Electronic absorption spectra in the UV-vis region were recorded with a Varian Cary 100 UV-vis spectrophotometer. Elemental analyses were performed on an instrument of VarioEL III. The IR spectra (KBr pellets) were recorded on a Nicolet Magna-FTIR 550 spectrometer. CD spectra were recorded using a JASCO J-720 spectropolarimeter. The CD spectral and UV-vis measurements were performed in 5 mM Tris-HCl buffer (pH 7.4) containing 50 mM NaCl.

Synthesis routes and procedures of the 4-amidinophenylporphyrins

The synthetic routes for the preparation of porphyrins with tris- and tetra-amidino groups are outlined in Schemes 1 and 2. The detailed procedures for the preparation of all the involved compounds, together with their characterization data, are given in the following sections.

5-(*p*-hydroxyphenyl)-10,15,20-tris(4-cyanophenyl)-porphyrin and 5,10,15,20-tetrakis(*p*-cyanophenyl)-porphyrin (H_2TCNPP). Pyrrole (5.36 g, 0.08 mmol) was added dropwisely to a solution of 4-cyanobenzaldehyde (2.62 g, 0.06 mmol) and 4-hydroxybenzaldehyde (2.44 g, 0.02 mmol) in refluxing propionic acid (200 mL). The mixture was refluxed for 1 h and then evaporated to dryness. After adding chloroform (300 mL) to the residue and mixing for 3 h, it was filtered, and chloroform layer was washed with water (300 mL × 3), which was dried by anhydrous sodium sulfate. After it had been filtered and concentrated, the residue was purified by silica gel column chromatography using dichloromethane as eluent. The first fraction was 5,10,15,20-tetrakis(*p*-cyanophenyl)porphyrin. Yield 220 mg (1.5%), mp > 300 °C. MS (FAB): *m/z* 714.6 (calcd. for $[M]^+$ 714.2); the second fraction was the title blue-violet product. Yield 665 mg (4.7%) after recrystallization from chloroform/methanol, mp > 300 °C. IR (KBr): ν, cm^{-1} 3310.9, 2227.6, 1604.3. UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 422 (5.62), 517 (4.24), 553 (3.90), 591 (3.73), 648 (3.68). MS (FAB): *m/z* 706.3 (calcd. for $[M + H]^+$ 705.2).

5,10,15,20-tetrakis(*p*-cyanophenyl)-Zn-porphyrin(ZnTCNPP). The complex was prepared by refluxing H_2TCNPP (100 mg, 0.14 mmol) with an excess amount of zinc acetate in $CHCl_3$/methanol for 5 h and purified by column chromatography on silica gel using chloroform as eluent. Yield 103 mg (95%), mp > 300 °C. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm 8.09–8.11 (8H, d, J = 8.0 Hz, porPH$_m$), 8.33–8.35 (8H, d, J = 8.0 Hz, porPH$_o$), 8.90 (8H, s, pyrrole H). IR (KBr): ν, cm^{-1} 2228, 1603, 996 (Zn^{II}, OSMB). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 427 (5.76), 557 (4.35), 597 (3.74). HRMS (MALDI-TOF, in chloroform): *m/z* 776.1421 (calcd. for $[M]^+$ 776.1409, Δ_m = 1.4288 ppm).

5-[*p*-(3-bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)porphyrin. In the presence of anhydrous potassium carbonate (1.0 g), 5-(*p*-hydroxyphenyl)-10,15,20-tris(4-cyanophenyl)porphyrin (100 mg, 0.14 mmol) and 1,3-dibromopropane (0.57 g, 2.83 mmol) in dry DMF (10 mL) was heated to 65 °C for 4 h with stirring under nitrogen. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and then filtered. The precipitate was purified by silica gel column chromatography using chloroform as eluent. The first fraction was the title blue-violet product. Yield 82 mg (70.8%) after recrystallization from chloroform/methanol. mp > 300 °C. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -2.88 (2H, s, pyrrole ring NH), 2.50–2.52 (2H, m, -CH_2- **CH_2**- CH_2-), 3.77–3.80 (2H, t, J = 6.4 Hz, CH_2Br), 4.39–4.42 (2H, t, J = 5.6 Hz, OCH_2), 7.29–7.31 (2H, m, O-PH$_o$), 8.06–8.10 (8H, m, porPH$_m$), 8.31–8.33 (6H, d, J = 7.2 Hz, porPH$_o$), 8.73–8.76 (8H, m, pyrrole H). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 420 (5.65), 517 (4.22), 553 (3.85), 591 (3.70), 647 (3.66). MS (FAB): *m/z* 827.3 (calcd. for $[M]^+$ 826.7).

Free CN-bisporphyrin. In the presence of anhydrous potassium carbonate (1.0 g), 5-(*p*-hydroxyphenyl)-10,15,20-tris(4-cyanophenyl)porphyrin (100.0 mg, 0.14 mmol) and 5-[*p*-(3-bromopropoxy)phenyl]-10,15,20-trisphenylporphyrin (106.5 mg, 0.14 mmol) [or 5-[*p*-(3-bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)-

porphyrin (100 mg, 0.12 mmol) and 5-(*p*-hydroxyphenyl)-10,15,20-trisphenylporphyrin (76 mg, 0.12 mmol)] in dry DMF (15 mL) was heated to 65 °C for 5 h with stirring under nitrogen. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (50 mL) and then filtered. The precipitate was purified by silica gel column chromatography using chloroform as eluent. The second fraction was the title blue-violet product. Yield 135 mg (70.4%) after recrystallization from chloroform/methanol, mp > 300 °C. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -2.86 (2H, s, trisphenyl pyrrole ring NH), -2.79 (2H, s, tris(cyanophenyl) pyrrole ring NH), 2.63–2.66 (2H, m, -CH_2- **CH_2**- CH_2-), 4.60–4.64 (4H, m, CH_2), 7.37–7.42 (4H, m, O-PhH_o), 7.64–7.77 (9H, m, portris$PhH_{m,p}$), 8.31–8.33 (22H, m, porPhH_o and tris$CNPhH_o$), 8.68–8.99 (16H, m, pyrrole H). IR (KBr): ν, cm^{-1} 3311, 2227, 1604. UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 418 (5.53), 517 (4.18), 553 (3.88), 592 (3.65), 647 (3.62). MS (FAB): *m/z* 1376.6 (calcd. for $[M + H]^+$ 1375.5).

5,10,15,20-tetrakis(4-amidinophenyl)porphyrin (Por 1). A slurry of H_2TCNPP (150 mg, 0.21 mmol) and $LiN(SiMe_3)_2$ (0.16 mmol/mL in THF solution, 0.32 mmol) in dry THF (20 mL) was sonicated at room temperature for 5 h [27]. The solvent was removed under vacuum. The residue was then washed with distilled water and chloroform, purified by recrystallization from methanol and acetonitrile. The resulting title compound was characterized by NMR, UV-vis, MS and elemental analysis. Yield 164 mg (91%), mp > 300 °C. Anal. calcd. for $C_{48}H_{38}N_{12}$: C, 73.53; H, 4.85; N, 21.45. Found: C, 73.60; H, 4.79; N, 21.50. ^{1}H NMR (400 MHz; DMSO-d_6; Me_4Si): δ_H, ppm -2.95 (2H, s, pyrrole ring NH), 8.24 (8H, s, porPhH_m), 8.26 (8H, s, porPhH_o), 8.87 (8H, s, pyrrole H). IR (KBr): ν, cm^{-1} 3318, 3201, 1667, 1607, 1507, 1440, 966, 863, 799, 502. UV-vis (DMSO): λ_{max}, nm (log ε) 421 (5.43), 515 (4.07), 551 (3.72), 589 (3.51), 645 (3.44). HRMS (MALDI-TOF, in methanol): *m/z* 783.3456 (calcd. for $[M]^+$ 783.3420, Δ_m = 1.1489 ppm).

Zn(II)-5,10,15,20-tetrakis(4-amidinophenyl)-porphyrin (Por 2). A slurry of ZnTCNPP (50 mg, 0.06 mmol) and $LiN(SiMe_3)_2$ (0.16 mmol/mL in THF solution, 0.32 mmol) in dry THF (20 mL) was sonicated at room temperature for 5 h. The solvent was removed in vacuum. The residue was then washed with distilled water and chloroform and then purified by recrystallization from methanol and acetonitrile. Yield 51 mg (94%), mp > 300 °C. Anal. calcd. for $C_{48}H_{36}N_{12}Zn$: C, 68.14; H, 4.26; N, 19.86. Found: C, 68.07; H, 4.31; N, 19.79. ^{1}H NMR (400 MHz; methanol-d_4; Me_4Si): δ_H, ppm 8.10–8.12 (8H, d, *J* = 8.0 Hz, porPhH_m), 8.28–8.30 (8H, d, *J* = 8.0 Hz, porPhH_o), 8.82 (8H, s, pyrrole H). IR (KBr): ν, cm^{-1} 3372, 3326, 1633, 1606, 992 (Zn^{II}, OSMB). UV-vis (DMSO): λ_{max}, nm (log ε) 430 (5.55), 561 (4.16), 602 (3.83). HRMS (MALDI-TOF, in methanol): *m/z* 845.2529 (calcd. for $[M]^+$ 845.2550, Δ_m = -2.4986 ppm).

4-amidinophenylbisporphyrin (Por 3). A slurry of free CN-bisporphyrin (50 mg, 0.036 mmol) and $LiN(SiMe_3)_2$ (0.16 mmol/mL in THF solution, 0.32 mmol) in dry THF (15 mL) was sonicated at room temperature for 5 h. The solvent was removed in vacuum. The residue obtained was washed by water. Yield 50 mg (97%), mp > 300 °C. Anal. calcd. for $C_{94}H_{70}N_{14}O_2$: C, 78.96; H, 4.90; N, 13.72. Found: C, 78.82; H, 4.92; N, 13.63. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -2.76 (4H, s, pyrrole ring NH), 2.57–2.67 (2H, m, -CH_2- **CH_2**- CH_2-), 4.50–4.63 (4H, m, CH_2), 7.37–7.40 (4H, m, O-PhH_o), 7.61–7.77 (15H, m, porPhH_m and portrisPhH_p), 8.09–8.22 (16H, m, porPhH_o), 8.83–8.85 (16H, m, pyrrole H). IR (KBr): ν, cm^{-1} 3318, 3036, 2918, 1674, 1598, 1482. UV-vis (DMSO): λ_{max}, nm (log ε) 421 (5.77), 516 (4.44), 552 (4.14), 591 (3.88), 647 (3.89). HRMS (MALDI-TOF, in methanol): *m/z* 1428.5971 (calcd. for $[M]^+$ 1428.5909, Δ_m = 4.2923 ppm).

Absorption titration of Por 1, Por 2 and Por 3 with DNA

The sample solutions were prepared by mixing 2 mM 4-amidinophenylporphyrin stock solution (in DMSO) and 0.169 mM ct-DNA stock solution in appropriate ratios and then diluted with Tris-HCl buffer solution to obtain the final desired concentration ratios (*r* = [porphyrin]/[ct-DNA]). UV-vis absorption spectra of these porphyrin-DNA sample mixtures were recorded from 350–500 nm.

Circular dichroism (CD) measurement of Por 1, Por 2 and Por 3 with DNA

Appropriate amount of calf thymus DNA was added to a Tris-HCl buffered 10 μM porphyrin solution to obtain a porphyrin-DNA mixture with a concentration ratio *r* ([porphyrin]/[ct-DNA]) = 0.05. After an incubation period of 45 min, the sample mixture was scanned in the Soret region of porphyrin (*i.e.,* 400–500 nm). Baseline correction was obtained by scanning the same buffer solution without the porphyrin. The spectral bandwidth and the cell length for the CD measurements were 1 nm and 1 cm, respectively.

DNA photocleavage assay

The DNA photocleavage activities of **Por 1**, **Por 2**, **Por 3** and H_2TMPyP were measured using the plasmid DNA relaxation assay. Briefly, the plasmid DNA (pBluescript, 0.5 μg), enriched with the covalently-closed circular or supercoiled conformer (Form I), and the one-phor-all plus buffer (10 mM tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, pH 7.5) was vortexed. Aliquots of the DNA were pipetted into different Eppendorf tubes. Various amounts of autoclaved water (control sample) or prophyrins (test sample) were added into the Eppendorf tubes to give a final volume of 20 μL in each sample tube.

The sample mixtures were then photo-irradiated at 400–450 nm for 45 min using a transilluminator (Vilber Lourmat) containing 4 × 15 W light tubes (Aqua Lux) with maximum emission at 435 nm. After photo-irradiation, 2 μL of the 6x sample dye solution (which contained 20% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol FF) was added to each Eppendorf tube and mixed well by centrifugation. The sample mixtures were loaded onto a 0.8% (v/v) agarose gel (Gel dimension: 13 cm × 10 cm), with 1x TBE buffer (89 mM tris-borate, 1 mM EDTA, pH 8) used as supporting electrolyte, and electrophoresized at 1.3 $V.cm^{-1}$ for 3 h using a mini gel set (CBS Scientific Co., Model No. MGU-502T). After electrophoresis, the gel was stained with 0.5 μg/mL ethidium bromide solution for 30 min and then destained using deionized water for 10 min. The resulting gel image was viewed under 365 nm and captured digitally using a gel documentation system (BioRad).

The DNA photocleavage activities of **Por 1**, **Por 2** and **Por 3** were measured by monitoring the conversion of supercoiled conformer (Form I) to the open-circular conformer (Form II) upon nicking by a DNA-cleavage agent.

Measurement of singlet oxygen production rate

1,3-diphenylisobenzofuran (DPBF) was used as a selective singlet oxygen ($^1O_2$) acceptor, which was bleached upon reaction with $^1O_2$. Five sample solutions of DPBF in DMSO (50 μM) containing, respectively, no porphyrin (control sample), H_2TMPyP (1 μM), **Por 1** (1 μM), **Por 2** (1 μM) and **Por 3** (1 μM) were prepared in dark. Each sample container was covered with aluminum foil with a yellow filter (with cutoff wavelength < 500 nm) on one side. The samples were then exposed to light (50 watt) through the filter. After irradiation, visible spectra of the sample solutions were measured spectrophotometrically. The normalized absorbances of DPBF at 418 nm in these samples were reported as a function of the photo-irradiation time. From this plot, the $^1O_2$ production rates of **Por 1**, **Por 2** and **Por 3** relative to that of H_2TMPyP can be determined.

CONCLUSION

The monomeric **Por 1** and **Por 2** showed both intercalative and external binding with DNA by the CD spectra whereas only external DNA binding was seen in the bisporphyrin, **Por 3**. The DNA photocleavage activities of these porphyrins followed the order: **Por 1** ~ **Por 2** > **Por 3**. All three 4-amidinophenylporphyrins produced $^1O_2$ more efficiently than H_2TMPyP. It suggests the porphyrins with strong hydrogen bonding capacity are promising new photo-dynamic therapeutic agents. To ascertain whether hydrogen bonding occurred between the amidine group and the DNA bases in the observed porphyrin-DNA binding interactions, ^{1}H NMR study using oligodeoxyribonucleotides as binding substrates is currently underway. Additionally, cellular uptake and cytotoxicity tests are also in progress.

Acknowledgements

The authors are grateful for the financial supports of National Natural Science Foundation of China (Nos. 20902071, 20672082), Hong Kong Research Grants Council (No. HKBU 202407) and Open Funds of Key Laboratory for Green Chemical Process of Ministry of Education in China (No. RGCT201003).

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