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Synthesis, Characterization, Singlet-Oxygen Photogeneration, DNA Photocleavage and Two-Photon-Absorption Properties of Some (4-Cyanophenyl)porphyrins

Kai Wang,[a–c] Chun-Ting Poon,[b] Wai-Kwok Wong,*[b] Wai-Yeung Wong,[b] Chun Y. Choi,[b] Daniel W. J. Kwong,*[b] Heng Zhang,[c] and Zao-Ying Li*[a]

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A series of (4-cyanophenyl)porphyrins have been prepared and structurally characterized by ^{1}H NMR, IR and UV/Vis spectroscopy, mass spectrometry, and elemental analysis. The relative singlet-oxygen quantum yields, two-photon absorption cross-sections and DNA photocleavage activities of these porphyrins were measured. The results show that (4-cyanophenyl)porphyrins with a properly designed hydrophobic/hydrophilic balance for better cellular uptake have potential application in two-photon-absorption photodynamic therapy.

Introduction

Porphyrin-based compounds are among the most studied photosensitizers because of their applications in photodynamic therapy (PDT), which is an inherently selective cancer treatment modality rendered by targeted photoirradiation of the cancerous tissues that tend to uptake the porphyrins selectively.[1] Although the mechanism of the selective accumulation of porphyrins in tumours remains unknown, the damage inflicted by these photosensitizers on tumour cells has largely been attributed to the singlet oxygen, $^1O_2$, generated when porphyrins are photoactivated by absorption at the Soret band.[2] The $^1O_2$ is produced by energy transfer from the photoexcited sensitizer (in its triplet state) to the ground-state (triplet) oxygen. This is known as the Type II photosensitization mechanism. In contrast, when the superoxide radical anion, $O_2^{\cdot-}$, is produced or a vital biological substrate is damaged by electron transfer from the excited photosensitizer, it occurs by the Type I mechanism. Although both processes can potentially occur, the Type II mechanism is considered to be more important in PDT.[3]

[a] College of Chemistry and Molecular Sciences, Wuhan University,
Wuhan 430072, Hubei, P. R. China
Fax: 86-27-68754067
E-mail: zyliwuc@whu.edu.cn
[b] Department of Chemistry, Hong Kong Baptist University,
Kowloon Tong, Hong Kong, P. R. China
Fax: 852-3411-7348
E-mail: wkwong@hkbu.edu.hk
dkwong@hkbu.edu.hk
[c] Key Laboratory for Green Chemical Process of the Ministry of Education, Wuhan Institute of Technology,
Wuhan 430073, Hubei, P. R. China

However, one major drawback associated with the clinical application of porphyrin-based sensitizers lies in their absorption wavelengths (the Soret band at 420–500 nm and the weaker Q-bands at 500–600 nm), which are also absorbed significantly by vascularized tissues (e.g., skin), leading to photoallergy and therefore limiting their PDT applications to mostly topical lesions.[4] Thus, a new generation of photosensitizers that absorb at $\lambda > 650$ nm has been designed by various approaches, for example, porphyrin core-modification and expansion,[4,5] to circumvent this problem.

Recently, a new approach to the photogeneration of $^1O_2$ by non-linear two-photon excitation of a sensitizer has been demonstrated.[6–8] In this process, an excited-state S_m is populated as a result of the less probable simultaneous absorption of two lower-energy photons. This transition proceeds via a virtual state, $S_0 \rightarrow S_{virt} \rightarrow S_m$, and can follow selection rules distinct from those corresponding to a one-photon transition.[9] For example, photoexcitation of a sensitizer that absorbs at 400 nm in a one-photon transition can be accomplished by laser light with $\lambda > 800$ nm by two-photon absorption (2PA), allowing for maximum depth penetration through tissues.[10] Furthermore, the use of a focused laser light, required for compensating the less probable two-photon absorption, affords spatial resolution to $^1O_2$ generation at target sites with pinpoint accuracy, which, in addition to minimizing collateral photodamage to neighbouring tissues,[10] allows one to study spatially resolved cellular responses to $^1O_2$ as well.[11] Successful application of 2PA-PDT in vivo has recently been demonstrated.[12] Moreover, if some of the two-photon excitation energy was channelled into a radiative process of the sensitizer, such emission from the sensitizer would inform on its localiza-

tion and uptake by the target tumour cells, allowing it to act as both an imaging as well as a PDT agent.[13,14]

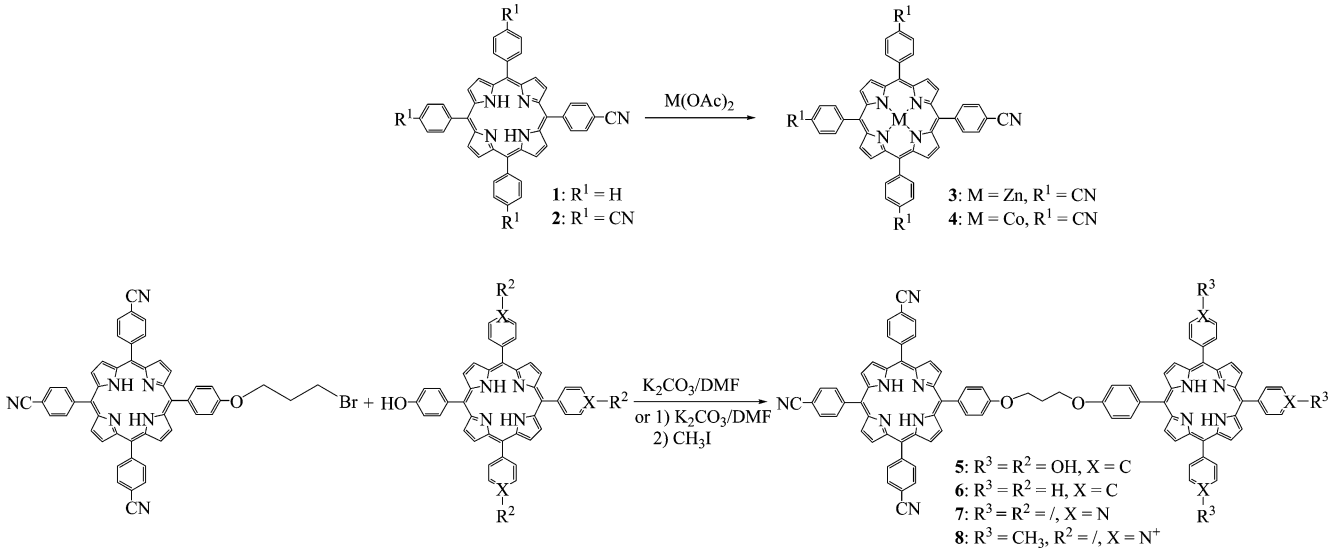
Most porphyrin-based photosensitizers used in PDT have distinct hydrophobic and hydrophilic ends to afford an optimal hydrophobic/hydrophilic balance for better cellular uptake and antitumour efficacy relative to those porphyrins possessing a single hydrophobic or hydrophilic substituent.[15] Previously, we have synthesized an amphiphilic bis(porphyrin) consisting of a tetraphenylporphyrin (H_2TPP) and a tris(*N*-methyl-4-pyridyl)porphyrin linked by an –O–$(CH_2)_3$–O– spacer group, which showed a 10- and 5-fold increase relative to tetrakis(*N*-methyl-4-pyridyl)porphyrin (H_2TMPyP) in cellular uptake and PDT efficacy towards Sarcoma 180 cells, respectively.[14]

Results and Discussion

In this work we have synthesized a series of (4-cyanophenyl)porphyrins, **1**–**4**, and investigated their photophysical properties, particularly their $^1O_2$ quantum yields and two-photon-absorption (2PA) cross-sections, and their DNA photocleavage activities. Furthermore, to modify the cellular uptake and/or photophysical properties of the (4-cyanophenyl)porphyrin, we have also synthesized a series of bis(porphyrins), **5**–**8**, in which the hydrophilic (4-cyanophenyl)porphyrin is connected to a more hydrophobic (e.g., H_2TPP) or hydrophilic porphyrin moiety [e.g., tris(*N*-methyl-4-pyridyl)porphyrin] through a 1,3-propanediyl diether linker (see Scheme 1). The relative $^1O_2$ quantum yields, 2PA cross-sections and DNA photocleavage activities of the bis(porphyrins) **5**–**8** were measured as well.

The (4-cyanophenyl)porphyrins and -bis(porphyrins) were structurally characterized by ^1H NMR, IR, and UV/Vis spectroscopy, mass spectrometry, and elemental analysis. The solid-state structure of one metalloporphyrin, namely, cobalt(II) 5,10,15,20-tetrakis(4-cyanophenyl)porphyrinate (**4**) was determined by X-ray crystallography (see Figure 1). Detailed analysis of this crystal structure revealed that the four pyrrole nitrogen atoms of the porphyrin ring are not coplanar but are displaced [see Figure 1(b)], in contrast to the coplanarity commonly observed for these four nitrogen atoms in most metalloporphyrins, including another known example of a metallated (4-cyanophenyl)porphyrin, that is, mercury(II) tetrakis(4-cyanophenyl)porphyrinate,[16] in which the Hg^{II} ion sits atop of the planar porphyrin ring as a result of its large ionic radius (>1.10 Å). This unusual structural feature is not at present understood. Nonetheless, the four Co–N distances, averaged at 1.95 Å, are typically those of low-spin cobalt(II) porphyrinates.[17]

To evaluate their possible application in photodynamic therapy, the singlet-oxygen quantum yields, Φ_Δ, of these (4-cyanophenyl)porphyrins were determined by measuring the near-IR phosphorescence intensity of the $^1O_2$ (at 1270 nm) produced from these compounds upon photoexcitation. These spectra are shown in Figure 2. By using 5,10,15,20-tetraphenylporphyrin (H_2TPP) as a reference (Φ_Δ = 0.55),[18] the relative $^1O_2$ quantum yields of **1**–**8** were calculated and are given in Table 1. Figure 2 shows that porphyrins **1**, **2** and **5**–**8** show similar $^1O_2$ quantum yields (experimental uncertainty ca. 20%) to that of H_2TPP, which indicates that (i) the introduction of a CN group into H_2TPP (i.e., **1** and **2**) and (ii) linkage through a diether group to a second porphyrin moiety, regardless of its hydrophobic (e.g., **6**) or hydrophilic (e.g., **8**) properties, does not affect its $^1O_2$ production capacity. For cobalt(II) (cyanophenyl)porphyrinate **4** no photogeneration of $^1O_2$ was seen. This lack of photosensitized $^1O_2$ production has previously been observed in other paramagnetic metalloporphyrins with partially filled d orbitals, for example, Ag^{II}–TPP,[19] presumably due to a metal-facilitated relaxation of the porphyrin excited triplet state that precludes bimolecular quenching by oxygen. In contrast, zinc(II) (cyanophenyl)porphyrinate **3** showed a very high $^1O_2$ quantum yield of



Scheme 1. Synthetic routes to (4-cyanophenyl)porphyrins **1**–**4** and -bis(porphyrins) **5**–**8**.

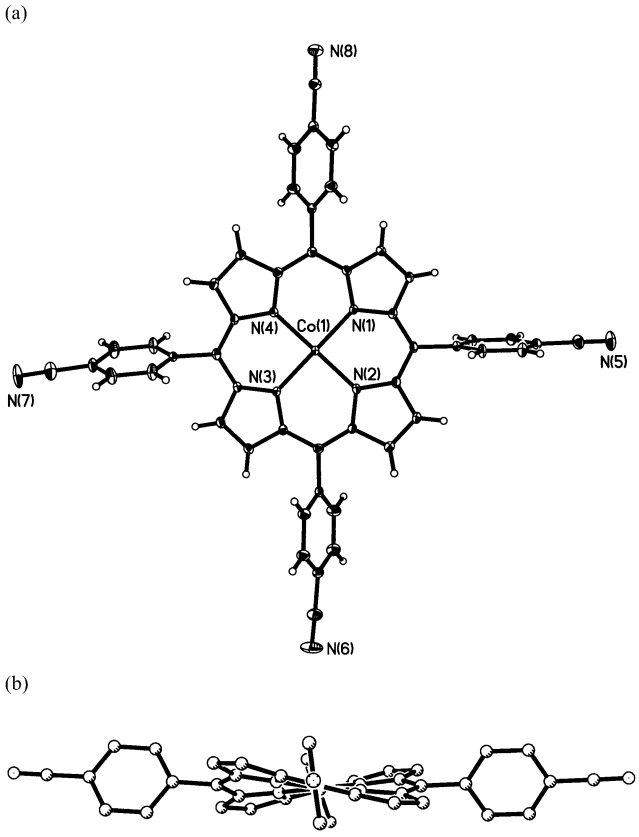


Figure 1. Perspective drawing of **4**: (a) top view and (b) side view. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and bond angles (°) are as follows: N(1)–Co(1) 1.948(4), N(2)–Co(1) 1.950(4), N(3)–Co(1) 1.950(4), N(4)–Co(1) 1.955(3); N(1)–Co(1)–N(2) 90.19(14), N(1)–Co(1)–N(3) 172.97(15), N(2)–Co(1)–N(3) 90.17(14), N(1)–Co(1)–N(4) 90.48(14), N(2)–Co(1)–N(4) 170.62(15), N(3)–Co(1)–N(4) 90.30(14).

0.94, which is almost twice that of the other (cyanophenyl)-porphyrins studied. Such behaviour has also been observed in other Zn^{II}–TPP derivatives, with reported Φ_Δ values ranging from 0.68 to 0.99, and has been attributed to their correspondingly high triplet quantum yields, Φ_T, which range from 0.86 to 1.02.[20] In summary, all the (4-cyanophenyl)porphyrins, except the Co^{II}-metallated porphyrin, exhibit substantial $^1O_2$ quantum yields, which makes them excellent potential PDT agents.

Table 1. Relative singlet-oxygen quantum yields, Φ_Δ, and absolute two-photon-absorption (2PA) cross-sections, $\sigma^{(2)}$, of (4-cyanophenyl)porphyrins **1**–**8**.

Porphyrin	Φ_Δ[a]	$\sigma^{(2)}$ [GM][b]
1	0.51	37.1
2	0.53	39.0
3	0.94	57.8
4	nd[c]	33.8
5	0.51	180
6	0.65	85.4
7	0.51	74.7
8	0.49	157

[a] The singlet-oxygen quantum yields of these compounds were measured in $CHCl_3$ relative to H_2TPP (Φ_Δ = 0.55). The experimental uncertainty was ca. 20%. [b] 2PA cross-sections are expressed in units of GM, where 1 GM = 10^{-50} cm^4 s photon^{-1}. [c] nd = non-detectable.

The two-photon-absorption (2PA) cross-sections, $\sigma^{(2)}$, of the (4-cyanophenyl)porphyrins **1**–**8** were measured at 800 nm by using 100 fs laser pulses with the open-aperture *Z*-scan method.[21] Figure 3 shows the *Z*-scan traces of these porphyrins, from which the absolute $\sigma^{(2)}$ values can be determined (see Table 1). From Table 1 the following observations were made. (i) The measured $\sigma^{(2)}$ values of the monomeric (4-cyanophenyl)porphyrins **1**–**4**, which range from 33.8 to 57.8 GM, are consistent with those reported for other monomeric porphyrins such as H_2TPP ($\sigma^{(2)}$ = 28 GM).[22,23] (ii) When we compare this value with those of **1** (37.1 GM) and **2** (39.0 GM), the presence of a 4-cyanophenyl group, a charge-transfer chromophore, appears to enhance the 2PA cross-section of the tetraphenylporphyrin. This is consistent with a previous observation made for other two-photon photosensitizers that more charge transfer in the chromophore facilitates their two-photon absorption.[9] (iii) This comparison also shows that the presence of more CN groups in H_2TPP does not further enhance its $\sigma^{(2)}$ value. (iv) Comparison of the 2PA cross-sections of the

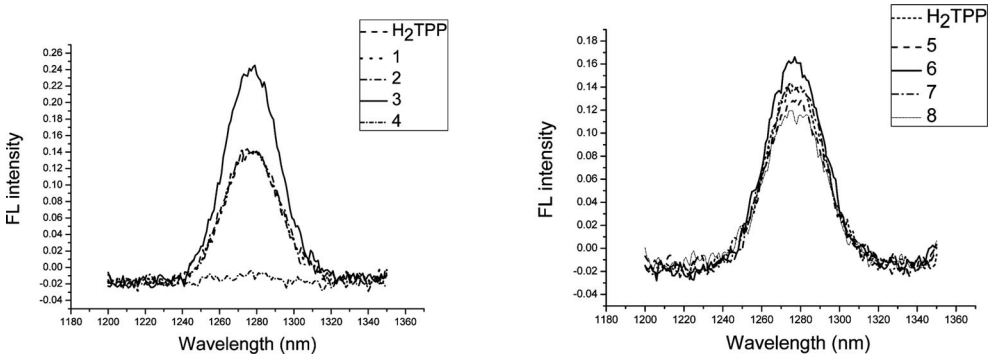


Figure 2. Near-IR phosphorescence spectra of $^1O_2$ produced from (4-cyanophenyl)porphyrins **1**–**8** and tetraphenylporphyrin (H_2TPP) in $CHCl_3$ under identical photoirradiation conditions [λ_{exc} = 420 nm, abs(λ_{exc}) = 0.03].

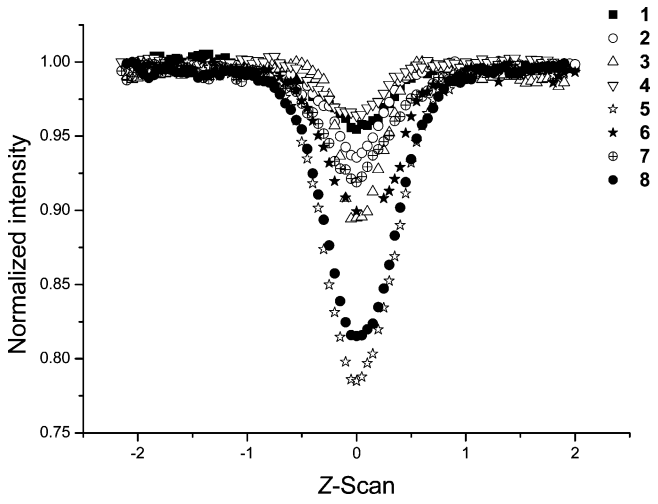


Figure 3. Open-aperture *Z*-scan traces of **1**–**8** (2 mM) excited at 800 nm. The solvent was DMSO except for **1**, **6** and **7**, for which $CHCl_3$ was used instead. The average power of the laser beam used was 0.262 mW except for **7**, for which the average power was 0.206 mW.

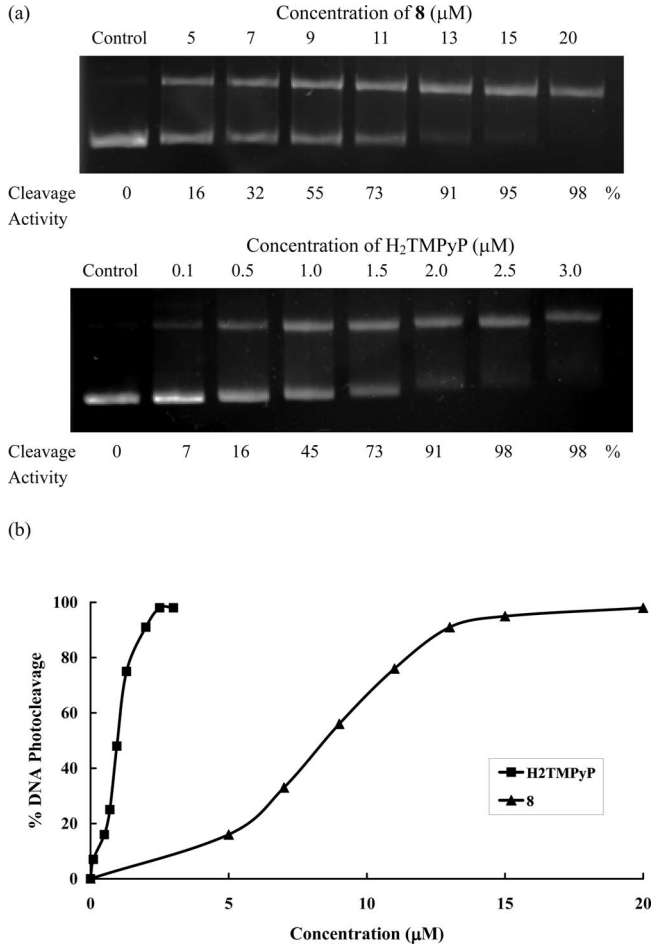


Figure 4. (a) Agarose gel electrophoresis images of DNA photocleavage assays of **8** and H_2TMPyP as a function of their concentrations. The control sample contained only supercoiled DNA (Form I). Photoirradiation was conducted by using a transilluminator at 455 nm for 45 min. (b) Plot of the DNA photocleavage activities of **8** and H_2TMPyP as a function of their concentration.

monomeric porphyrins **1**–**4** with those of the bis(porphyrins) **5**–**8**, which range from 74.7 to 180 GM, show the $\sigma^{(2)}$ values of the latter were around two- to four-fold higher. The photophysical reason behind this observation is not at present understood. Nonetheless, the substantial 2PA cross-sections observed in some of the (4-cyanophenyl)bis(porphyrins), particularly **5** and **8**, together with their relatively high $^1O_2$ quantum yields, qualify them as potential 2PA-PDT agents.

The DNA photocleavage activities of the water-soluble (4-cyanophenyl)porphyrins and -bis(porphyrins), namely, **2**, **3**, **5** and **8**, were also measured, as well as the well-studied cationic porphyrin, H_2TMPyP.[24] Only the cationic porphyrins **8** and H_2TMPyP showed substantial photocleavage activities towards the anionic DNA, with over 90% cleavage activities observed at 13 and 2.5 μM, respectively (see Figure 4). As for porphyrins **2**, **3** and **5**, their insignificant DNA photocleavage activities (data not shown) are likely the result of a lack of a binding interaction with DNA, precluding a more effective oxidative attack from close range despite their relatively high $^1O_2$ quantum yields. This result underscores the importance of a close-range interaction with the target in effecting the desired outcome.

In PDT, the efficacy of a photosensitizer depends more on its cellular uptake (i.e., lipophilic) properties than on its $^1O_2$ quantum yield.[25] For example, even though H_2TMPyP showed strong DNA-binding and photocleavage activity, its PDT efficacy was poor due to its poor cellular uptake. Hence, more hydrophobic porphyrins, such as **5**, which are expected to be more membrane-permeable, can be an effective PDT agent because, in addition to DNA, there are other more hydrophobic intracellular targets for PDT action as well.[26]

Conclusions

A series of (4-cyanophenyl)porphyrins and -bis(porphyrins) have been synthesized, characterized and shown to possess substantial singlet-oxygen quantum yields (i.e., Φ_Δ = 0.49–0.94). Their two-photon-absorption cross-sections $\sigma^{(2)}$, which range from 33.8 to 180 GM, were also measured. Two (4-cyanophenyl)bis(porphyrins), **5** and **8**, which show substantial two-photon-absorption cross-sections as well as $^1O_2$ quantum yields, can potentially be developed as prime 2PA-PDT agents as they possess an additional porphyrin that allows for various substituent modifications to achieve an optimal hydrophobic/hydrophilic balance for cellular uptake. The in vitro PDT activities and cellular uptake properties of these (4-cyanophenyl)bis(porphyrins) are currently under investigation.

Experimental Section

Materials and Methods: DMF was distilled from calcium hydride, and THF was distilled under nitrogen in the presence of sodium chips by using benzophenone as indicator. 5-(4-Cyanophenyl)-10,15,20-triphenylporphyrin (**1**), 5-(4-hydroxyphenyl)-10,15,20-

tris(4-pyridyl)porphyrin, 5,10,15,20-tetrakis(4-cyanophenyl)porphyrin (**2**) and 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin were prepared according to literature procedures.[27] Other chemicals were purchased from Sigma–Aldrich Company and used as received. Silica gel 60 (0.04–0.063 mm) for column chromatography was purchased from Merck. NMR spectra were recorded with a Varian INOVA 400 NMR spectrometer. High-resolution matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were recorded with a Bruker Autoflex MALDI-TOF mass spectrometer. Low-resolution FAB mass spectra were obtained with a Finnigan TSQ710 mass spectrometer. Electronic absorption spectra in the UV/Vis region were recorded with a Varian Cary 100 UV/Vis spectrophotometer, and singlet-oxygen near-IR (NIR) emission spectra were recorded with a Photon Technology International (PTI) QM4 luminescence spectrometer equipped with an InGaAs detector. The filter was LG-697-F from Corion Company. The IR spectra (KBr pellets) were recorded with a Nicolet Magna 550 FTIR spectrometer. Elemental analyses were performed by using a VarioEL III elemental analyzer at the School of Chemical Technology & Pharmacy, Wuhan Institute of Technology, P. R. China. All measurements were performed at ambient temperature (20 ± 2 °C) under atmospheric pressure. The UV/Vis absorption and NIR emission spectra of all solution samples were measured in a 10 mm quartz cell.

Singlet-Oxygen Measurements: Singlet oxygen was detected directly by its phosphorescence emission at 1270 nm with an InGaAs detector. The singlet-oxygen quantum yields, Φ_Δ, of the (4-cyanophenyl)-porphyrins were determined in chloroform relative to the reference compound H_2TPP ($\Phi_\Delta = 0.55 \pm 0.11$)[18] by using Equation (1), where Φ_Δ is the singlet-oxygen quantum yield, AUC is the integrated area under the $^1O_2$ emission spectrum and A is absorbance at the excitation wavelength. Superscripts R and S correspond to the reference and sample, respectively. In all cases the $^1O_2$ emission spectra were measured after excitation at 420 nm with the absorbance set at 0.03 to minimize reabsorption of the emitted light.

$$\Phi_\Delta^S = \Phi_\Delta^R \frac{AUC^S(1 - 10^{-A^R})}{AUC^R(1 - 10^{-A^S})} \quad (1)$$

Two-Photon-Absorption (2PA) Measurements: The two-photon-absorption spectra (i.e., *Z*-scan traces) were measured at 800 nm by the open-aperture *Z*-scan method using 100 fs laser pulses with a peak power of 276 GW cm^{-2} from an optical parametric amplifier operating at a repetition rate of 1 kHz generated from a Ti:sapphire regenerative amplifier system. The laser beam was split into two parts by a beam splitter. One was monitored by a photodiode (D1) as the incident intensity reference, I_0, and the other was detected as the transmitted intensity by another photodiode (D2). After passing through a lens with $f = 20$ cm, the laser beam was focused and passed through a quartz cell. The position of the sample cell, z, was moved along the direction of the laser beam (z axis) by a computer-controlled translatable table so that the local power density within the sample cell could be changed under the constant incident intensity laser power level. Finally, the transmitted intensity from the sample cell was detected by the photodiode D2. The photodiode D2 was interfaced to a computer for signal acquisition and averaging. Each transmitted intensity datum represents the average of over 100 measurements. Assuming a Gaussian beam profile, the non-linear absorption coefficient, β, can be obtained by curve-fitting to the observed open-aperture traces, $T(z)$, with Equation (2),[28] where a_0 is the linear absorption coefficient, l is the sample length (the 1 mm quartz cell) and z_0 is the diffraction length of the incident beam. After obtaining the nonlinear absorption coefficient, β, the 2PA cross-section, $\sigma^{(2)}$, of the sample molecule (in units of 1 GM = 10^{-50} cm^4 s photon^{-1}) can be determined by using Equation (3), where N_A is Avogadro's constant, d is the concentration of the sample compound in solution, h is Planck's constant and v is the frequency of the incident laser beam.

$$T(z) = 1 - \frac{\beta I_0(1 - e^{-a_0 l})}{2a_0[1 + (z/z_0)]^2} \quad (2)$$

$$\sigma^{(2)} = \frac{1000\beta h v}{N_A d} \quad (3)$$

DNA Photocleavage Assay: The DNA photocleavage activities of the porphyrins were measured by using the plasmid DNA relaxation assay. Briefly, the plasmid DNA (pBluescript), enriched with the covalently closed circular (supercoiled) conformer, and one-phor-all plus buffer (10 mM Tris/acetate, 10 mM magnesium acetate, 50 mM potassium acetate, pH = 7.5) were vortexed. Aliquots of the DNA were added by pipette to different Eppendorf tubes. Various amounts of autoclaved water (control sample) or porphyrins (test sample) were added to the Eppendorf tubes to give a final volume of 20 μL in each sample tube. The sample mixtures were then photoirradiated at 420–480 nm for 45 min by using a transilluminator (Vilber Lourmat) containing 4 × 15 W light tubes (Aqua Lux) with a maximum emission at 455 nm. After photoirradiation, a 6X sample dye solution (2 μL, 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 (13 cm × 10 cm), with Tris/borate/EDTA (TBE) buffer (89 mM Tris/borate, 2.5 mM EDTA, pH = 8) used as the supporting electrolyte, and subjected to electrophoresis at 1.3 V cm^{-1} for 3 h by using a mini gel set (CBS Scientific Co., Model No. MGU-502T). After electrophoresis, the gel was stained with 0.5 μg mL^{-1} ethidium bromide solution for 20 min and then destained by using deionized water for 5 min. The resulting gel image was viewed under 365 nm light, captured digitally and analysed by using a gel documentation system (BioRad).

Preparation and Characterization of the (4-Cyanophenyl)porphyrins

Zinc(II) 5,10,15,20-Tetrakis(4-cyanophenyl)porphyrinate (3): The complex was prepared by heating **2** (100 mg, 0.14 mmol) at reflux with an excess amount of zinc(II) acetate in $CHCl_3$/methanol for 5 h; it was then purified by column chromatography on silica gel with chloroform as eluent. Yield: 103 mg, 95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09–8.11 (d, J = 8.0 Hz, 8 H), 8.33–8.35 (d, J = 8.0 Hz, 8 H), 8.90 (s, 8 H) ppm. IR (KBr): $\tilde{\nu}$ = 3320, 2228 (CN), 1603, 1519, 1340, 1206, 996 (Zn^{II}, OSMB), 810 cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 776.1421 [M]$^+$ ($C_{48}H_{24}N_8Zn$: calcd. 776.1409, Δ_m = 1.55 ppm). UV/Vis ($CHCl_3$, 20 °C): λ_{max} (log ε) = 427 (5.76), 557 (4.35), 597 nm (3.74 dm^3 mol^{-1} cm^{-1}). $C_{48}H_{24}N_8Zn$ (778.15): calcd. C 74.23, H 3.09, N 14.43; found C 74.11, H 3.12, N 14.34.

Cobalt(II) 5,10,15,20-Tetrakis(4-cyanophenyl)porphyrinate (4): The complex was prepared by heating **2** (100 mg, 0.14 mmol) at reflux with an excess amount of cobalt(II) acetate in $CHCl_3$/methanol for 5 h; it was then purified by column chromatography on silica gel with chloroform as eluent. Yield: 93 mg, 94%. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.99–9.44 (br. m, 16 H), 12.88 (br. s, 8 H) ppm. IR (KBr): $\tilde{\nu}$ = 3312, 2227 (CN), 1605, 1500, 1350, 1002 (Co^{II}, OSMB), 811 cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 771.1480 [M]$^+$ ($C_{48}H_{24}N_8Co$: calcd. 771.1507, Δ_m = –3.50 ppm). UV/Vis ($CHCl_3$, 20 °C): λ_{max} (log ε) = 412 (5.91),

529 nm (4.59 $dm^3 mol^{-1} cm^{-1}$). $C_{48}H_{24}CoN_8$ (771.69): calcd. C 74.71, H 3.11, N 14.53; found C 74.82, H 3.03, N 14.61.

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)porphyrin: In the presence of anhydrous potassium carbonate (1.0 g), a mixture of 5-(4-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 with stirring under nitrogen for 4 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The first fraction was the blue-violet title product. Yield: 82 mg, 71%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = –2.88 (s, 2 H, pyrrole ring NH), 2.50–2.52 (m, 2 H, $CH_2CH_2CH_2$), 3.77–3.80 (t, J = 6.4 Hz, 2 H, CH_2Br), 4.39–4.42 (t, J = 5.6 Hz, 2 H, OCH_2), 7.29–7.31 (m, 2 H), 8.06–8.10 (m, 8 H), 8.31–8.33 (d, J = 7.2 Hz, 6 H), 8.73–8.76 (m, 8 H) ppm. IR (KBr): $\tilde{\nu}$ = 3321, 2923, 2228 (CN), 1605, 1244, 1176, 967, 803 cm^{-1}. MS (FAB, +ve mode): m/z = 827.3 $[M]^+$. UV/Vis ($CHCl_3$, 20 °C): λ_{max} (log ε) = 420 (5.65), 517 (4.22), 553 (3.85), 591 (3.70), 647 nm (3.66 $dm^3 mol^{-1} cm^{-1}$).

Porphyrin 5: In the presence of anhydrous potassium carbonate (1.0 g), a mixture of 5-[4-(3-bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)porphyrin (100 mg, 0.12 mmol) and $H_2T_{OH}PP$ (81 mg, 0.12 mmol) in dry DMF (15 mL) was heated to 65 °C with stirring under nitrogen for 6 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (50 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The second fraction was the blue-violet title product. Yield: 43 mg, 25%. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = –2.97 [s, 2 H, tris(phenyl), pyrrole ring NH], –2.88 [s, 2 H, tris(cyanophenyl), pyrrole ring NH], 2.73–2.80 (m, 2 H, $CH_2CH_2CH_2$), 4.57–4.60 (m, 4 H, OCH_2), 7.04–7.06 (m, 4 H), 7.20–7.44 (m, 6 H), 7.82–7.84 (m, 6 H), 8.00–8.02 (m, 2 H), 8.05–8.12 (m, 8 H), 8.28–8.40 (m, 6 H), 8.75–8.89 (m, 16 H) ppm. IR (KBr): $\tilde{\nu}$ = 3328, 2925, 2854, 2230 (CN), 1606, 1509, 1262, 801 cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 1424.4905 $[M]^+$ ($C_{94}H_{61}N_{11}O_5$: calcd. 1424.4883, Δ_m = 1.54 ppm). UV/Vis (DMSO, 20 °C): λ_{max} (log ε) = 422 (5.85), 517 (4.50), 553 (4.28), 590 (3.96), 646 nm (3.89 $dm^3 mol^{-1} cm^{-1}$). $C_{94}H_{61}N_{11}O_5$ (1424.56): calcd. C 79.27, H 4.29, N 10.82; found C 79.35, H 4.39, N 10.74.

Porphyrin 6: In the presence of anhydrous potassium carbonate (1.0 g), a mixture of 5-[4-(3-bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)porphyrin (100 mg, 0.12 mmol) and 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin (76 mg, 0.12 mmol) in dry DMF (15 mL) was heated to 65 °C with stirring under nitrogen for 5 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (50 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The second fraction was the blue-violet title product. Yield: 135 mg, 70% after recrystallization from chloroform/methanol. ^{1}H NMR (400 MHz, $CDCl_3$): δ = –2.86 (s, 2 H, triphenyl, pyrrole ring NH), –2.79 [s, 2 H, tris(cyanophenyl), pyrrole ring NH], 2.63–2.66 (m, 2 H, $CH_2CH_2CH_2$), 4.60–4.64 (m, 4 H, OCH_2), 7.37–7.42 (m, 4 H), 7.64–7.77 (m, 9 H), 7.97–8.33 (m, 22 H), 8.68–8.99 (m, 16 H) ppm. IR (KBr): $\tilde{\nu}$ = 3311, 2924, 2851, 2227 (CN), 1604, 1506, 1242, 966, 799 cm^{-1}. MS (FAB, +ve mode): m/z = 1376.6 $[M]^+$. UV/Vis ($CHCl_3$, 20 °C): λ_{max} (log ε) = 418 (5.53), 517 (4.18), 553 (3.88), 592 (3.65), 647 nm (3.62 $dm^3 mol^{-1} cm^{-1}$). $C_{94}H_{61}N_{11}O_2$ (1376.56): calcd. C 81.98, H 4.43, N 9.59; found C 81.88, H 4.54, N 9.70.

Porphyrin 7: In the presence of anhydrous potassium carbonate (1.0 g), a mixture of 5-[4-(3-bromopropoxy)phenyl]-10,15,20-tris(4-cyanophenyl)porphyrin (83 mg, 0.10 mmol) and 5-(4-hydroxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin (63 mg, 0.10 mmol) in dry DMF (15 mL) was heated to 65 °C with stirring under nitrogen overnight. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (50 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform/methanol. The second fraction was the blue-violet title product. Yield: 77 mg, 56% after recrystallization from chloroform/methanol. ^{1}H NMR (400 MHz, $CDCl_3$): δ = –2.87 [s, 2 H, tris(pyridinium-4-yl), pyrrole ring NH], –2.84 [s, 2 H, tris(cyanophenyl), pyrrole ring NH], 2.65–2.71 (m, 2 H, $CH_2CH_2CH_2$), 4.65–4.68 (m, 4 H, OCH_2), 7.43–7.45 (m, 4 H), 8.10–8.15 (m, 6 H), 8.17–8.19 (m, 6 H), 8.33–8.35 (d, J = 8.1 Hz, 2 H), 8.63–8.73 (m, 6 H), 8.72–8.89 (m, 16 H), 9.06–9.07 (d, J = 5.8 Hz, 6 H) ppm. IR (KBr): $\tilde{\nu}$ = 3314, 2923, 2228 (CN), 1653, 1593, 1506, 1243, 799 cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 1381.5105 $[M]^+$ ($C_{91}H_{58}N_{14}O_2$: calcd. 1381.5048, Δ_m = 4.13 ppm). UV/Vis ($CHCl_3$, 20 °C): λ_{max} (log ε) = 420 (5.62), 516 (4.28), 552 (3.83), 590 (3.69), 646 nm (3.38 $dm^3 mol^{-1} cm^{-1}$). $C_{91}H_{58}N_{14}O_2$ (1379.53): calcd. C 79.25, H 4.21, N 14.22; found C 79.17, H 4.27, N 14.30.

Porphyrin 8: Porphyrin **7** (30 mg, 0.02 mmol) was added to CH_3I (10 mL). After heating at reflux overnight, the reaction mixture was filtered, and the precipitate was washed with $CHCl_3$/CH_3OH (20:1). The title blue-violet product was obtained in 35 mg (91%) yield after recrystallization from chloroform/methanol. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = –2.98 [s, 2 H, tris(cyanophenyl), pyrrole ring NH], –2.96 [s, 2 H, tris(*N*-methyl-4-pyridyl), pyrrole ring NH], 2.59–2.62 (m, 2 H, $CH_2CH_2CH_2$), 4.61–4.64 (m, 4 H, OCH_2), 4.71 (s, 9 H, CH_3), 7.52–7.59 (m, 4 H), 8.18–8.21 (m, 4 H), 8.26–8.32 (m, 6 H), 8.39–8.41 (m, 6 H), 8.81–8.85 (m, 6 H), 8.97–8.99 (m, 8 H), 9.10–9.16 (m, 8 H), 9.44–9.48 (m, 6 H) ppm. IR (KBr): $\tilde{\nu}$ = 3322, 2924, 2227 (CN), 1638, 1604, 1507, 1241, 801 cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 1424.5683 $[M]^+$ ($C_{94}H_{67}N_{14}O_2^+$: calcd. 1424.5632, Δ_m = 3.49 ppm). UV/Vis (DMSO, 20 °C): λ_{max} (log ε) = 423 (5.76), 517 (4.49), 553 (4.12), 590 (3.99), 646 nm (3.77 $dm^3 mol^{-1} cm^{-1}$). $C_{94}H_{67}I_3N_{14}O_2$ (1804.34): calcd. C 62.53, H 3.71, N 10.86; found C 62.64, H 3.65, N 10.78.

X-ray Crystal Structure Determination of 4: Pertinent crystallographic data and other experimental details are summarized in

Table 2. Crystallographic data for cobalt(II) 5,10,15,20-tetrakis(4-cyanophenyl)porphyrinate–2chloroform.

Empirical formula	$C_{50}H_{26}Cl_6CoN_8$
Formula mass	1010.42
Crystal size [mm]	0.32 × 0.25 × 0.22
Crystal system	monoclinic
Space group	$P2_1/c$
a [Å]	9.0341(5)
b [Å]	30.4580(16)
c [Å]	15.7403(8)
β [°]	96.1070(10)
V [Å^3], Z	4306.5(4), 4
$\rho_{calcd.}$ [mg m^{-3}]	1.558
μ [mm^{-1}]	0.820
F(000)	2044
θ range for data collection [°]	2.39–25.00
Reflections collected	20698
Independent reflections	7470 (R_{int} = 0.0290)
Goodness-of-fit on F^2	0.992
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0674, wR_2 = 0.1854
R indices (all data)	R_1 = 0.0876, wR_2 = 0.2024

Table 2. Crystals of cobalt(II) 5,10,15,20-tetrakis(4-cyanophenyl)-porphyrinate–2chloroform suitable for X-ray diffraction studies were grown by slow concentration in air of a chloroform/methanol solution of the complex. The crystals were wrapped in epoxy glue to prevent them from losing solvent and mounted on a thin glass fibre. Intensity data were collected at 293 K with a Bruker Axs SMART 100 CCD area-detector diffractometer by using graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). The collected frames were processed with the SAINT software[29] and an absorption correction was applied (SADABS)[30] to the collected reflections. The space group of the crystal was determined from the systematic absences and Laue symmetry checks and confirmed by successful refinement of the structure. The structure was solved by direct methods (SHELXTL)[31] and refined against F^2 by full-matrix least-squares analysis. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were generated in their idealized positions and allowed to ride on their respective parent carbon atoms. CCDC-699825 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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