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Bioorganic & Medicinal Chemistry 14 (2006) 2956–2965
Bioorganic & Medicinal Chemistry

Dicationic pyridium porphyrins appending different peripheral substituents: Synthesis and studies for their interactions with DNA

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Received 26 September 2005; revised 4 December 2005; accepted 6 December 2005
Available online 27 December 2005

Abstract—Twelve *trans*-dicationic pyridium porphyrins appending different peripheral substituents were synthesized and their abilities to bind and cleave DNA under irradiation have been investigated. Their binding modes to DNA were studied by UV–vis spectroscopy, circular dichroism. The apparent constants were measured by EB competitive fluorescence method and most of them were in the range of 10^4–10^5 M^{-1}. We found that both the position of positive charges and steric hindrance could greatly influence their binding affinities and modes to DNA, and then affect their photocleaving abilities to DNA.

1. Introduction

Water-soluble cationic porphyrins have long been interested for their interactions with DNA because of their potential clinical applications in photodynamic therapy[1,2] and many unique physicochemical properties in their interactions with nucleic acids.[3,4] Among of them, *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (T4MPyP) is a typical representative of this family and has been extensively studied in biological and medicinal applications.[5–14] There are three major binding modes between cationic porphyrins and DNA, namely intercalation like T4MPyP, outside groove binding, and outside stacking binding.[15]

When porphyrin's structures are modified, their binding modes to DNA will be changed. A typical example was reported and directly observed by Neidle's group.[16] They found that Ni^{2+}-T4MPyP is of highly nonplanar conformation, whereas T4MPyP is a typical planar one. They found that the binding mode to DNA was changed from intercalation (for planar T4MPyP) to minor groove binding (for NiT4MPyP). These results encouraged us to modify the structures of porphyrin's periphery because it had been reported that introduction to periphery of porphyrin could totally change porphyrin's structures.[16–20] We could assume that interactions with DNA by hindrance porphyrin will exert different binding modes to DNA. Some research groups have obtained interesting results for the interactions between modified porphyrins and DNA.[21–23] However, few have concentrated on both the influence of electric and steric effects of peripheral substituents of dicationic porphyrins on binding with DNA previously.[24] Our preliminary results reported that the peripheral substituents of di(pyridiumyl)porphyrins affected their interactions with DNA.[25] Herein, we shall fully report our design and synthesis of water-soluble cationic *meso*- and β-substituted porphyrins (Scheme 1) and studied their interactions with DNA.

2. Results and discussion

The strategy to synthesize *meso*-substituted porphyrins **17**, **18**, **19**, and **20** (Scheme 2) was based on Alder–Longo's method.[26] Pyrrole and two corresponding aldehydes were mixed and refluxed in propionic acid and then separated the desired product from among the six porphyrins by flash chromatography. Synthesis of β-substituted porphyrins was finished by reacting with

Keywords: Dicationic porphyrins; DNA; Interaction; Photocleavage.
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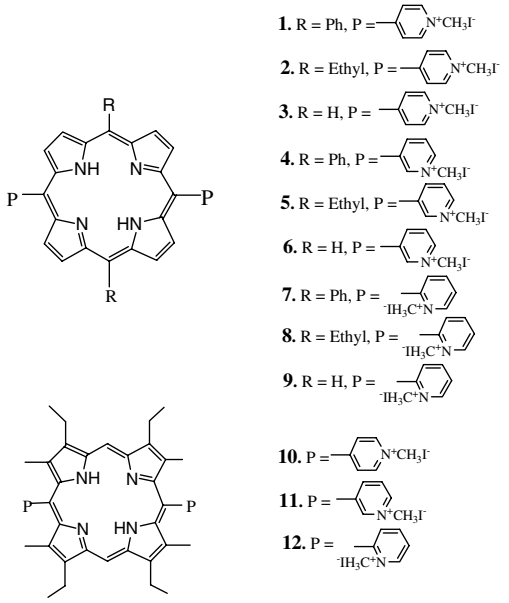
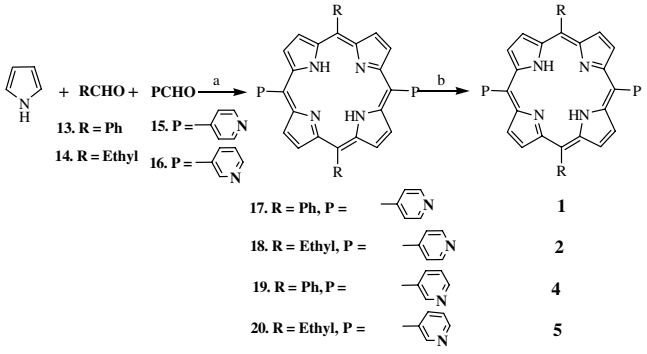
doi:10.1016/j.bmc.2005.12.011

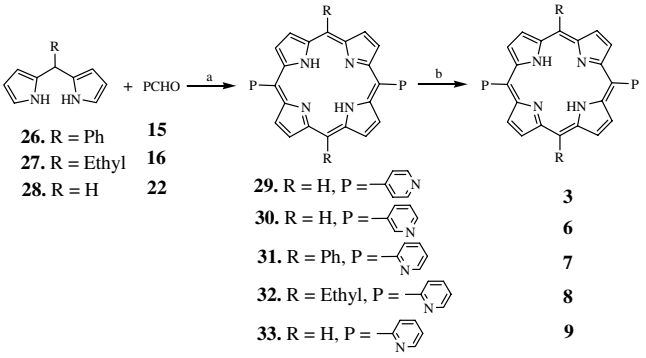


Scheme 1. Structures of dicationic pyridium porphyrins.



Scheme 2. Synthesis routes of porphyrins. Reagents and conditions: (a) propionic acid, reflux 1 h; (b) CH_3I, $CHCl_3$/DMF (4:1), rt, overnight.

dipyrromethane **21** and aldehyde (Scheme 3), and then obtained in good yields over 18%. Meanwhile, we had synthesized *trans*-di(2-pyridyl)-porphyrins **31** by the route shown in Scheme 4. We modified the methods reported by MacMillin and co-workers[24,27], and porphyrins **29**, **30**, and **33** were synthesized by the following procedures. Dipyrromethane **28** and 2, or 3, or 4-pyridinecarbaldehyde were dissolved in propionic acid, respectively, and then dropped into the hot propionic acid with almost the same speed slowly. The desired products were obtained in good yields. Methylation of all *trans*-dipyridyl porphyrins was performed in cosolvent $CHCl_3$/DMF and the porphyrins **1**–**12** were obtained with very good yields (>90%).

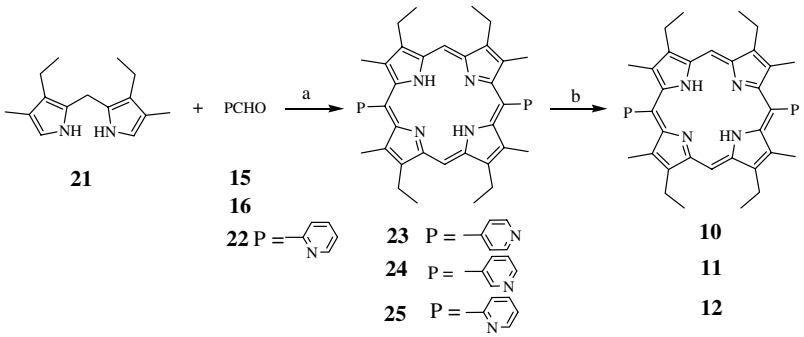


Scheme 4. Synthesis of porphyrins. Reagents and conditions: (a) propionic acid, for compounds **29**, **30**, and **33**, 70 °C, overnight; for compounds **31** and **32**, 110 °C, 4 h; (b) CH_3I, $CHCl_3$/DMF (4:1), rt, overnight.

2.1. Binding studies

Affinity to calf thymus (CT DNA) was evaluated via measuring apparent constants by competition with ethidium bromide (EB), a well-known DNA intercalator.[28] Binding modes of porphyrins and CT DNA could be estimated and apparent constants (K_{app}) could be calculated by the difference of the slopes of Scatchard plot which were constructed by the decrease of fluorescence intensity of EB bound to DNA in the absence and in the presence of increasing concentrations of porphyrins. The procedure of measurement was undertaken according to the literature reported.[15,28]

All the binding constants of most of porphyrins were calculated and are listed in Table 1. Apparently, K_{app} of all porphyrins showed a tendency that *trans*-di(pyridium-4-yl)porphyrin > *trans*-di(pyridium-3-yl)porphyrin > *trans*-di(pyridium-2-yl)porphyrin. It suggested that the positions of positive charges were the essential factor to have an affect their binding abilities to DNA. Meanwhile, no appreciable difference was observed



Scheme 3. Synthesis of porphyrins. Reagents and conditions: (a) CF_3COOH, CH_2Cl_2/EtOH (95:5), N_2, rt, 15 h; then *p*-chloranil, THF, rt, 4 h; (b) CH_3I, $CHCl_3$/DMF (4:1), rt, overnight.

Table 1. Interactions of various porphyrins with CT DNA[a]

Porphyrin	Soret band (nm)	Δλ		K_{app}	CD[b]		Binding mode[c]
		(nm)	% H		+	–	
1	419	7	15.7	4.35×10^5	438	462	A(major) + B
2	417	2	23.1	4.76×10^5	411	454	A + B (major)
10	466	1	5.5	1.06×10^5	483	429	C
3	407	19	23.2	—	—	428	A
4	416	0	39.7	8.39×10^4	421	447	C
5	416	1	21.2	9.70×10^4	414	438	C
11	407	0	16.2	3.37×10^4	404	—	B
6	403	16	42.0	—	—	418	A
7	415	0	16.2	2.50×10^4	421	—	B
8	412	1	17.4	1.97×10^4	416	—	B
12	399	0	18.9	3.70×10^3	412	—	B
9	400	15	51.8	—	—	428	A

[a] All measurements in 0.05 M Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl at room temperature. The ratio of [b.p]/[porhyrin] was up to 20:1 eventually in the UV titration experiment. The apparent binding constant of EB with CT DNA was 5.54×10^5 M^{-1}.
[b] All the ratio of [b.p]/[porphyrin] remained-5:1, and '+' represents positive peak, whereas '–' represents negative peak.
[c] Binding modes were analyzed by CD. A represents intercalation, B outside binding, and C outside stacking, respectively.

between *meso*-substituted porphyrins **1** and **2** as well as their corresponding *trans*-di(pyridium-3-yl)porphyrins (**4** and **5**) and *trans*-di(pyridium-2-yl)porphyrins (**7** and **8**) isomers, indicating electronic effect had slight influence on binding with DNA. However, β-substituted porphyrins **10**, **11**, and **12** showed dramatically decreasing binding abilities comparing with all the other *meso*-substituted porphyrins with the same charged positions, indicating that steric hindrance might be an important factor to affect their interactions between bulky porphyrins and DNA.

To further study their interactions between porphyrins and DNA, we investigated the interactions between all dicationic porphyrins synthesized and CT DNA by UV absorbance. It is widely admitted that the extent of shift and hypochromism or hyperchromism counted on the nature of DNA, porphyrin, and the binding modes between them. Generally speaking, for a given DNA, intercalation exhibits large change, while small change occurs in the groove binding or the stacking mode.

The UV titration spectrum of the series of *trans*-di(pyridium-3-yl)porphyrins **4**, **5**, **6**, and **11** was selected to illustrate how binding modes alter with the influence of peripheral substituents (Fig. 1).[9] The spectrum of porphyrin **6** showed the largest change of hypochromity and redshift among the *trans*-di(pyridium-3-yl)porphyrins. With the concentration of CT DNA increasing, hyperchromity could be observed and reached its highest mount at $R = 0.042$. Hypochromism maximizes at a high ratio of $R = 0.105$ (R = ([porphyrin]/[DNA base pair])), and this may be ascribed to the porphyrin aggregation on the DNA substrate at the higher porphyrin-to-base pair ratios.[27] However, large hypochromity of 42% and red shift of 16 nm match the criterion for the intercalative mode.[9] The spectra of other porphyrins were very different, and small redshift (or no) and hypochromity from 16.2% to 39.7% indicated the porphyrins adopt an outside binding or a combination of outside binding with intercalation mode. All physical data about UV-titration experiments are listed in Table 1.

2.2. Circular dichroism (CD) studies

CD studies were also carried out to investigate the binding modes and the results of the series of *trans*-di(*N*-methyl-pyridium-3-yl)porphyrins **4**, **5**, **6**, and **11** were demonstrated for their binding alteration (Fig. 2).

Apparently, three kinds of shapes of the induced CD spectra could be observed. Porphyrin **4** upon binding to calf thymus DNA showed a strong positive peak centered at 418 nm. A strong negative peak centered at 440 nm and the intensity of the two peaks were almost identical. Similar curve of porphyrin **5** could be observed with slight difference of the positive peak centered at 423 nm. The negative peak centered at 450 nm and intensity of the peaks were weaker than those of **4**. The shapes of **4** and **5** suggested that both of them formed a long-range helical structure with CT DNA.[29] The β-substituted porphyrin **11** showed a very broad weak positive peak centered at 416 nm, indicating that **11** binds to CT DNA very weakly with outside binding mode.[29] For compound **6**, a very strong negative peak centered at 415 nm was obtained, suggesting **6** was an excellent DNA intercalator. By analysis of binding modes of structurally related *trans*-(pyridium-3-yl)porphyrins **4**, **5**, **6** and **11**, we might know that steric effect played an essential role in the course of porphyrins binding to DNA, while electronic effect contributed little. The binding modes of all the porphyrins to CT DNA were obtained in this way and are shown in Table 1. We found no significant difference in the binding mode between *meso*-phenyl and *meso*-ethyl substituted porphyrins (**1** and **2**, **4**, and **5**, and **7** and **8**) and we estimated that ethyl groups at *meso*-positon were not bulky enough to greatly influence their binding modes. Meanwhile, because of the lack of rotation of *meso*-(*N*-methylpyridium) rings, all *trans*-(*N*-methylpyridium-2-yl)porphyrins might bind externally to DNA, nevertheless, as could be seen in Table 1, porphyrin **9**

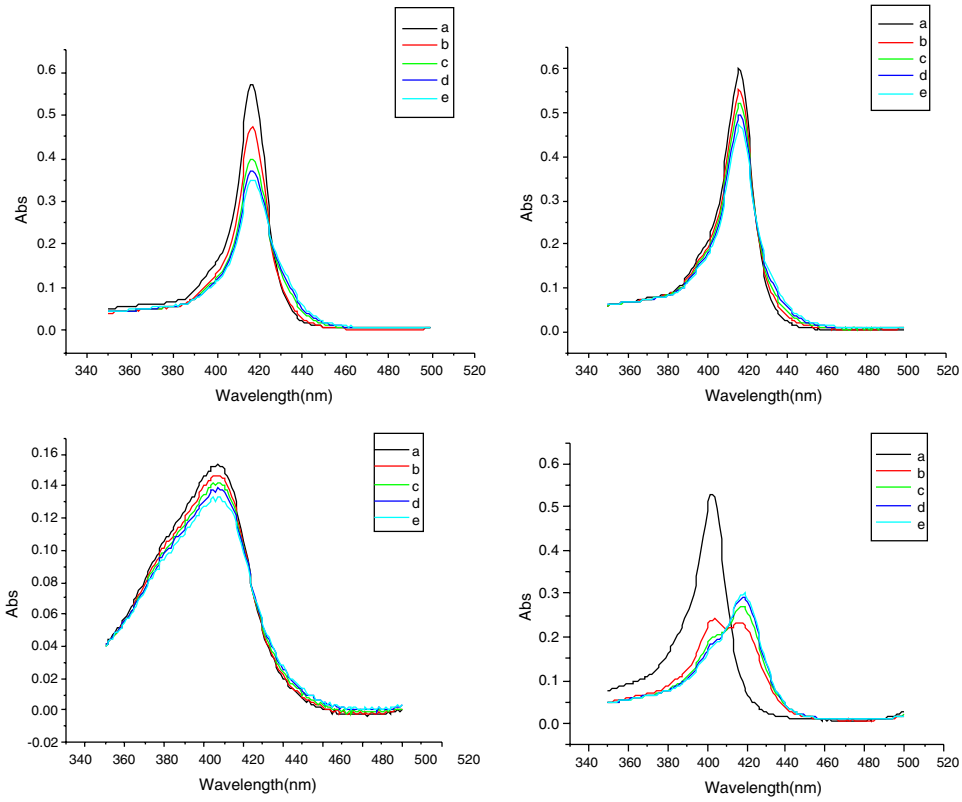


Figure 1. Absorption spectra of porphyrin **4** (upper left), **5** (upper right), **11** (lower left), **6** (lower right), all [porphyrin] = 2 μM, and the ratio of porphyrin/CTDNA—(a) 0; (b) 0.105; (c) 0.07; (d) 0.053; (e) 0.042 in 0.5 M Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl.

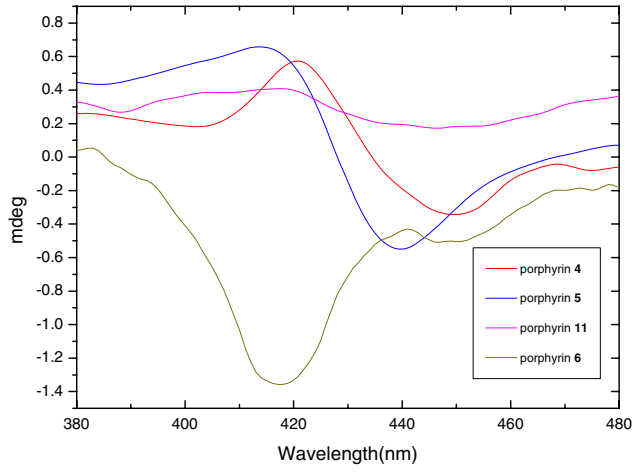


Figure 2. Visible CD spectra of CT DNA with porphyrins, all [porphyrin] = 5 μM, and the ratio of [b.p]/[porphyrin] = 5, in 0.05 M Tris–HCl, 0.5% DMF, containing 0.1 M NaCl.

could intercalate into CT DNA. Constant opening and closing of the double helix of DNA due to thermal agitation made it possible to let the bulky porphyrins intercalate and porphyrin **9** adopted a twisted conformation to intercalate CT DNA.[27]

All three measurements showed higher consistency, for example, as discussed above. The characteristics of UV titration spectrum of porphyrin **6** indicated that the porphyrin binds to CT DNA with intercalation and the same result could be obtained by analysis of CD spectrum and the higher affinity to CT DNA.

2.3. Photoclevage studies

To intensively study the relationship between the ability to photocleave DNA and binding affinities including binding modes and binding constants, DNA photocleavage abilities were determined. *trans*-(Pyridium-3-yl)porphyrins **4**, **5**, **6**, and **11** were mixed with plasmid DNA pBR322 in the absence or in the presence of irradiation (Fig. 3). All experiments were performed in the buffer (0.5 M Tris–HCl, pH 7.3, 0.5% DMF containing 0.1 M NaCl) and the irradiation was carried out by a high pressure mercury lamp 50 W at room temperature and the distance between the samples and the filament of the mercury lamp was 20 cm. No cleavage occurred without irradiation, indicating that cleavage of DNA was caused by singlet oxygen raised from irradiation of the porphyrins. The converted amount of nicked

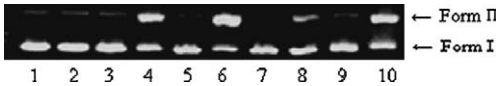


Figure 3. Cleavage of supercoiled pBR322 DNA by *trans*-di(pyridium-3-yl)porphyrins in 0.05 M Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl. Reaction mixture (10 μL) containing 0.15 μg DNA and all [porphyrin] = 1 μM, lane 1, DNA alone; lane 2, DNA + hν (1 h); lane 3, DNA + **4**; lane 4, DNA + **4** + hν (1 h); lane 5, DNA + **5**; lane 6, DNA + **5** + hν (1 h); lane 7, DNA + **11**; lane 8, DNA + **11** + hν (1 h); lane 9, DNA + **6**; lane 10, DNA + **6** + hν (1 h).

Table 2. Percentage of DNA formation

Porphyrin	4	5	11	6
% form II	52.0	67.7	24.6	47.1
% form I	48.0	32.3	75.4	52.9

closed circular DNA (form II) transferred from supercoiled DNA (form I) represented the photocleavage ability of porphyrins (Table 2). The order of cleavage ability was: **5** > **4** > **6** > **11**, and we found that photocleavege ability of the porphyrins had a good relationship with their binding constants, except for porphyrin **6** which intercalated supercoiled DNA deeply. Meanwhile, the same experiments were carried out on *trans*-diethyl-dipyridium porphyrins **2**, **5**, and **8** with various positions of positive charges (Fig. 4). The photocleavage abilities of porphyrin isomers were consistent with their binding abilities with CT DNA (Table 1), indicating that the photocleavage abilities might be closely related to the positions of positive charges, which affected the binding ability as well.

Since the photocleavage abilities of porphyrins might have two factors involved, one is binding with DNA and the other the production of singlet oxygen, it is necessary to compare the difference in the production of singlet oxygen induced from irradiation of porphyrins. Measurement of singlet oxygen production was performed by measuring the reduction of absorbance of 1,3-diphenylisobenzofuran (DPBF) whose decomposition was caused by singlet oxygen.[30–33] The tested results of porphyrins **2**, **4**, **5**, **6**, **8**, and **11** are shown in Figure 5 and the values of the slopes, which were proportional to the production of singlet oxygen, are listed in Table 3. No significant difference was observed among these porphyrins and it implied that photocleavage abilities of the porphyrins were controlled by their binding modes with DNA. Steric hindrance effects, positions of positive charges of porphyrins might be the key factors to influence their interactions with DNA.

Comparison of compounds **2**, **5**, and **8** with T4MPyP, which is a typical porphyrin, was investigated. We found that T4MPyP had a better binding ability ($6.68 \times 10^6\ M^{-1}$) than those of other porphyrins. However, their photocleaving abilities of porphyrins to DNA were **2** > **5** > T4MPyP > **8** (Fig. 6). It suggested that more numbers of positive charges might favor porphyrin to bind to DNA (T4MPyP), and numbers of positive charges, binding modes, and conformation of porphyrins might affect the cleaving abilities to DNA.

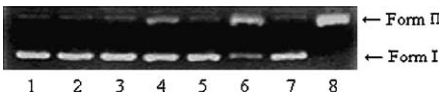


Figure 4. Cleavage of supercoiled pBR322 DNA by *trans*-diethyl-dipyridium porphyrins in 0.05 M Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl. Reaction mixture (10 μL) containing 0.15 μg DNA, and all [porphyrin] = 2 μM, lane 1, DNA alone; lane 2, DNA + hν (45 min); lane 3, DNA + **8**; lane 4, DNA + **8** + hν (45 min); lane 5, DNA + **5**; lane 6, DNA + **5** + hν (45 min); lane 7, DNA + **2**; lane 8: DNA + **2** + hν (45 min).

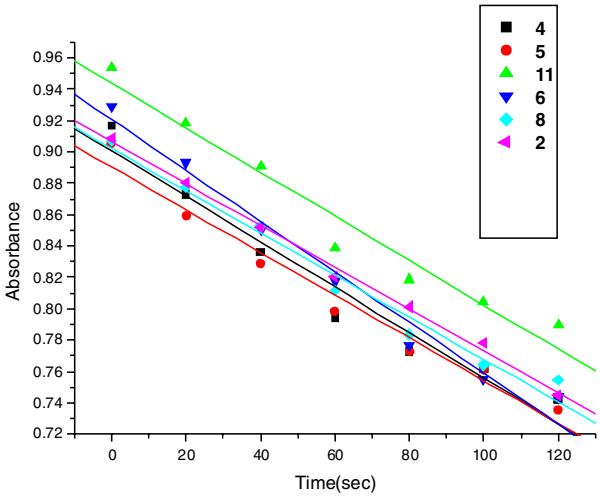


Figure 5. Decomposition of DPBF by compounds **2**, **4**, **5**, **6**, **8**, and **11**. Porphyrins (1 μM) and DPBF (100 μM) were irradiated in 0.05 mM Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl.

Table 3. The slopes(s) of the plots of the absorption by porphyrins **2**, **4**, **6**, **8**, and **11**

Porphyrin	2	4	5	6	8	11
S ($\times 10^{-3}$)	−1.45	−1.36	−1.41	−1.52	−1.35	−1.33

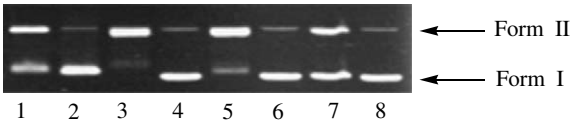


Figure 6. Cleavage of supercoiled pBR322 DNA by *trans*-diethyl-dipyridium porphyrins and T4MPyP in 0.05 M Tris–HCl buffer, pH 7.4, 0.5% DMF, containing 0.1 M NaCl at 37 °C. Reaction mixture (10 μL) containing 0.05 μg DNA, and all [porphyrin] = 0.5 μM, lane 1, DNA + T4MPyP + hν (15 min); lane 2, DNA + T4MPyP; lane 3, DNA + **2** + hν (15 min); lane 4, DNA + **2**; lane 5, DNA + **5** + hν (15 min); lane 6, DNA + **5**; lane 7, DNA + **8** + hν (15 min); lane 8: DNA + **8**.

3. Conclusion

The UV–vis absorption, circular dichroism, and measurement of the apparent binding constants from the present study supported the opinion that steric effects of porphyrins with the same position played the crucial role in the binding of DNA, while electronic effects contribute little. These results were supported by the similar binding modes, binding constants between *trans*-bisphenyl-dipyridium-porphyrins with appending weak electron-withdrawing phenylgroup at *meso*-position and *trans*-diethyl-dipyridium-porphyrins bearing an electron-donating group at *meso*-position. Meanwhile, we found that with the increase of steric hindrance, the binding modes could be altered from intercalation to external binding. The second important factor affecting binding affinity was the position of positive charges, positive charges located at *para*-position of pyridyl rings possessing the highest binding ability and strongest photocleaving ability, and then *meta*-charged

porphyrins and *ortho*-charged porphyrins in turn. Since all the porphyrins have the similar abilities to produce singlet oxygen (see Fig. 5 and Table 3), the photocleaving abilities of the dicationic porphyrins were directly related to the hindrance effects and the position of positive charges. Correlations between binding mode (intercalation or outside binding) of dicationic porphyrins and their DNA cleavage abilities did not fit well enough. We found that porphyrin **6** with intercalation mode to DNA had a better ability to cleave DNA than porphyrin **11**, which has an outside binding mode to DNA. However, porphyrin **6** had a weaker ability to cleave DNA than porphyrins **4** and **5**, which have the outside binding mode to DNA as well (Fig. 3). T4MPyP with intercalation mode to DNA has a better binding ability to DNA because of its four positive charges. However, comparing with compounds **2** and **5**, it had a weaker ability to cleave DNA. It suggested that the abilities to photocleave DNA by porphyrins might depend on several factors such as binding modes, numbers of charges, and conformation of structures.

4. Experiments

4.1. General

Pyridine-4-carbaldehyde and its isomers pyridine-3-carbaldehyde and pyridine-2-carbaldehyde were purchased from Acros.Com. without purification before use. Pyrrole, methyl iodide, trifluoroacetic acid (TFA), trifluoroboronate, *p*-chloranil, and all organic solvents including dichloromethane, methanol, ethanol, acetone, and DMF were from Shanghai Medical Com, Ltd. Plasmid DNA (pBR322) and calf thymus DNA were purchased from Sino-American Biotechnology Company. Pyrrole and TFA were freshly distilled before use. Dichloromethane used as solvent in Lindsey synthesis and DMF were stirred with calcium hydride for 2 h and then carefully distilled to avoid moisture and kept with 4 Å molecular sieves before use. Absolute ethanol was obtained by refluxing reagent grade ethanol with magnesium and catalytic amount of iodine for 2 h and distilling afterward.

NMR spectra were recorded on a Varian Mercury-VX300 spectrometer at 300 MHz. MS were recorded on a Brucker Daltonics APE XII 47e and VG-707V-HF mass spectrometer. UV–vis spectra were carried out on a UV–vis Spectrophotometer (Shimadzu). Fluorescence of binding constant measurement was performed on a PE LS55 fluorescence spectrometer. Circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter.

4.2. General procedure for the synthesis of porphyrins 17, 18, 19, and 20

Pyridine-4-carbaldehyde (or pyridine-3-carbaldehyde) and equivalent of benzaldehyde (or propionaldehyde) were mixed in 200 mL of hot propionic acid. Pyrrole contained in a 20 mL dropping tunnel was dropped into the aldehyde mixture over 20 min. After refluxed for 1 h and cooled down to room temperature, the sticky dark products were evaporated under vacuum to remove the excess acid. TLC indicated that six porphyrin products were formed and the desired porphyrin was in the third position. The crude product was isolated on silica gel with a solvent of CH_2Cl_2/CH_3OH (100:3) and the desired band was collected. After evaporated, the isolated product was further purified with silica gel chromatography with an eluent of CH_2Cl_2/acetone, 4:1. The desired band was collected and solvents were evaporated under reduced pressure. The pure products were obtained by recrystallization with $CHCl_3$/ether.

4.3. General procedure for the synthesis of porphyrins 23, 24, and 25

3,3′-Diethyl-4,4′-dimethyl-2,2′-dipyrrylmethane **21** was obtained via a five-step reaction with ethyl acetoacetate as starting material with a total yield of 10.8% according to the literature.[34,35] A standard procedure in the Lindsey method was used to produce the desired porphyrins.[36] Dipyrromethane and equivalent of pyridinecarbaldehyde were dissolved in cosolvent CH_2Cl_2/EtOH, 95:5, and argon was purged in for 10 min to remove the oxygen that remained. Catalytic amount of TFA was added with argon purged for continuous 2 min and the mixture was stirred for 15 h with aluminum foil wrapped to avoid light. Then a solution of THF (25 mL) containing *p*-chloranil (492 mg, 2 mmol) was added and the mixture was stirred for a further 4 h. After solvents evaporated, the residue was chromatographed on silica gel with eluent CH_2Cl_2/CH_3OH, 100:3, and the pigmented fraction was collected and the solvents were evaporated under reduced pressure. The desired products were obtained by recrystallization with $CHCl_3$/ether.

4.4. General procedure for the synthesis of compounds 31, 32

5-Phenyl-2,2′-dipyrrylmethane **26** was synthesized according to the literature.[37] Benzaldehyde (2.0 mL, 20 mmol) and 20-fold of pyrrole (28 mL, 400 mmol) were mixed in the presence of 0.5 mL TFA. The mixture was stirred in the darkness under argon purged with 20 min. After finishing the reaction, excess pyrrole was distilled under reduced pressure. The crude product was purified by silica gel chromatography with an eluent of CH_2Cl_2/cyclohexane/Et_3N, 100:50:1 and obtained 1.98 g (44.6%) of the colorless crystalline product after evaporation of solvents.

5-Ethyl-2,2′-dipyrrylmethane **27** was synthesized in the similar method as **26**.[38,39] Propionaldehyde (2 mL, 27.5 mmol) and 20-fold of pyrrole (39 mL, 558 mmol) were mixed in 1 L of CH_2Cl_2 in the presence of 0.5 mL $BF_3 \cdot Et_2O$ as catalytic Lewis acid and the reaction was run for 5 h. The same purification procedure described above and obtained the desired product (1.70 g, 35.5%) as a thick light yellow sticky liquid.

During the synthesis of porphyrins **31** and **32**, dipyrromethane and equivalent of pyridine-2-carbaldehyde

were mixed in propionic acid and the mixture was reacted under 110 °C for 4 h. TLC monitored the reaction and showed five porphyrin derivatives existence. The products were purified by silica gel chromatography with a solvent of CH_2Cl_2/CH_3OH (100:2). The second pigmented fraction (main product) was collected and the solvent was evaporated. Further purification was finished by flash chromatography with a solvent of CH_2Cl_2/acetone, 5:1. After removal of solvents, the product was recrystallized with $CHCl_3$/ether and obtained the desired purple porphyrins **31** and **32**, respectively.

4.5. General procedure for the synthesis of porphyrins 29, 30, and 33

Dipyrromethane **28** was produced with slight modification according to the reported literature.[40] Paraformaldehyde (0.9 g, 30 mmol in formaldehyde) was suspended in 20 mL of absolute ethanol and the mixture was refluxed until clear solution appeared. After cooling down, 20-fold of pyrrole (42 mL, 601 mmol) and 1 L of freshly distilled CH_2Cl_2 were added and the mixture was stirred for 5 min to assure that oxygen was removed. Catalyst $BF_3 \cdot Et_2O$ (0.5 mL) was then added and stirred at room temperature under inert atmosphere in the darkness overnight. The same purification procedure was described above and obtained 2.54 g (yield: 58.0%) of the desired product in colorless crystalline.

Dipyrromethane **28** and equivalent of pyridine-carbaldehyde were dissolved in 20 mL of cooled propionic acid and moved into two dropping tunnels, respectively. Then the mixture was dropped into 200 mL of hot propionic acid (70 °C) slowly over 2 h at approximately the same speed and stirred at the same temperature overnight. At least three porphyrin products could be detected by TLC. The excess acid was removed under vaccum and left dark tan mixture. Most of pyrrole polymers were removed by chromatography with 5 cm height silica with a cosolvent of CH_2Cl_2/CH_3OH (20:1). The desired porphyrin appeared at the second pink band by subsequent flash chromatography on silica (CH_2Cl_2/CH_3OH, 100:3) and was collected. After removal of solvents, the crude products were recrystallized with $CHCl_3$/ether and obtained the pure products **29**, **30**, and **33**, respectively.

4.6. General procedure for methylation of *trans*-dipyridylporphyrins

Each porphyrin was dissolved in 50 mL $CHCl_3$/DMF (4:1), and large excess of methyl iodide (>500-fold) was added. The reactant mixture was stirred at room temperature overnight and monitored by TLC. After finishing the reaction, the solution was concentrated to about 3 mL and then 50 mL of ether was poured into the mixture. The precipitate was obtained with a centrifuge and dried under vaccum.

4.6.1. 5,15-Bisphenyl-10,20-di(4-pyridyl)-porphyrin (17)[15]. Pyrrole (4 mL, 57.2 mmol), pyridine-4-carbaldehyde (2.74 mL, 28.6 mmol), and benzaldehyde (2.91 mL, 28.6 mmol) were mixed. The crude product was purified according to procedure in Section 4.2. Porphyrin **17** was obtained with a yield of 0.93% (58 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.84 (s, 2H), 7.75 (m, 6H), 8.16 (m, 8H), 8.77 (d, J = 4.5 Hz, 4H), 8.87 (d, J = 4.2 Hz, 4H), 9.01 (d, J = 5.4 Hz, 4H).

4.6.2. 5,15-Bisphenyl-10,20-di(*N*-methyl-pyridium-4-yl)-porphyrin (1)[15]. Porphyrin **17** (26 mg, 42 μmol) and methyl iodide (2 mL, 32.1 mmol) were mixed in 50 mL $CHCl_3$/DMF (4:1). Reaction was finished and purified according to procedure in Section 4.6. Porphyrin **1** was obtained in 37 mg (98.4%). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.97 (s, 2H), 4.67 (s, 6H), 7.84 (m, 6H), 8.18 (d, J = 5.4 Hz, 4H), 8.85–8.97 (m, 12H), 9.39 (s, 4H).

4.6.3. 5,15-Diethyl-di(4-pyridyl)-porphyrin (18). Pyrrole (4 mL, 57.2 mmol), pyridine-4-carbaldehyde (2.74 mL, 28.6 mmol), and propionaldehyde (2.08 mL, 28.6 mmol) were mixed. The reaction and purification were finished according to procedure in Section 4.2. The desired porphyrin **18** was obtained in a yield of 1.33% (69 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.76 (s, 2H), 2.13 (t, J = 7.4 Hz, 6H), 5.0 (m, 4H), 8.11 (d, J = 5.7 Hz, 4H), 8.79 (d, J = 3.0 Hz, 4H), 9.0 (d, J = 4.8 Hz, 4 H), 9.45 (d, J = 4.8 Hz, 4H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 417 (5.58), 516 (4.14), 551 (3.80), 592 (3.60), 650 (3.72); ESI HRMS for $C_{34}H_{28}N_6$ $(M+H)^+$: found 521.2454, calcd 521.2448.

4.6.4. 5,15-Diethyl-di(*N*-methyl-pyridium-4-yl)-porphyrin (2)[25]. Porphyrin **18** (30 mg, 57.6 μmol) and methyl iodide (3 mL, 48.2 mmol) were mixed in 50 mL $CHCl_3$/DMF (4:1). After reaction, the crude product was purified according to procedure in Section 4.6 and porphyrin **2** was obtained in 44 mg (95.8%). Fully characterized data were reported in our previous paper.[25]

4.6.5. 5,15-Di(4-pyridyl)-porphyrin (29). Dipyrromethane **28** (1.46 g, 10 mmol) and pyridine-4-carbaldehyde (0.95 mL, 10 mmol) were mixed and purified according to procedure in Section 4.5. The final product **29** (66 mg, 2.84%) was obtained. ^{1}H NMR ($CDCl_3$, 300 MHz): −3.21 (s, 2H), 8.25 (s, 4H), 9.07 (d, J = 4.5 Hz, 8H), 9.48 (d, J = 4.2 Hz, 4H), 10.41 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 406 (5.40), 500 (4.14), 536 (3.81), 574 (3.78), 628 (3.48); ESI HRMS for $C_{30}H_{20}N_6$ $(M+H)^+$: found 465.1816, calcd 465.1822.

4.6.6. 5,15-Di(*N*-methyl-pyridium-4-yl)-porphyrin (3). Porphyrin **29** (30 mg, 64.6 μmol) and 2 mL of methyl iodide were mixed in 50 mL of the cosolvent $CHCl_3$/DMF (4:1) and then the reacted mixture was purified according to procedure **4. 5**. The desired porphyrin **3** was obtained and yielded 98.8% (48 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −3.35 (s, 2 H), 4.73 (s, 6H), 9.08 (s, 4H), 9.24 (s, 4H), 9.48 (s, 4H), 9.85 (s, 4H), 10.83 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 410 (5.41), 503 (4.22), 542 (4.10), 575 (3.88), 632 (3.80); ESI MS for $C_{32}H_{26}N_6$ $[(M^+-2I)/2]$: found 247.4, calcd 274.1.

4.6.7. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(4-pyridyl)-porphyrin (23). 3,3′-Diethyl-4,4′-dimethyl-2,2′-dipyrrylmethane (**21**) (460 mg, 2 mmol) and pyridine-4-carbaldehyde (190 μL, 2 mmol) were mixed in 500 mL of CH_2Cl_2/C_2H_5OH (95:5). The reaction mixture was treated and purified according to procedure in Section 4.2. The desired porphyrin **23** was obtained in a yield of 18.4% (116 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.52 (s, 2H), 1.76 (m, 12H), 2.52 (s, 12H), 4.0 (d, J = 7.2 Hz, 8H), 8.05 (d, J = 5.1 Hz, 4H), 8.99 (d, J = 4.2 Hz, 4H), 10.23 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 408 (5.16), 508 (4.13), 542 (3.80), 576 (3.88), 627 (3.51); ESI HRMS for $C_{42}H_{44}N_6$ $(M+H)^+$: found 633.3708, calcd 633.3700.

4.6.8. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(*N*-methyl-pyridium-4-yl)-porphyrin (10)[25]. Porphyrin **23** (40 mg, 63.3 μmol) and methyl iodide (2 mL) were mixed. After reaction, the reaction mixture was treated as per procedure **4.5** described to yield 94.5% (55 mg) of porphyrin **10**. Fully characterized data were reported in our previous paper.[25]

4.6.9. 5,15-Bisphenyl-10,20-di(3-pyridyl)-porphyrin (19)[15]. Pyrrole (4 mL, 57.2 mmol), pyridine-3-carbaldehyde (2.74 mL, 28.6 mmol), and benzaldehyde (2.91 mL, 28.6 mmol) were mixed and crude the product was purified according to procedure in Section 4.2 to yield 76 mg (1.23%) of porphyrin **19**. ^{1}H NMR ($CDCl_3$, 300 MHz): −2.79 (s, 2H), 7.70 (m, 8H), 8.21 (d, J = 6 Hz, 4H), 8.51 (d, J = 7.5 Hz, 2H), 8.79 (d, J = 4.8 Hz, 4H), 8.91 (d, J = 4.8 Hz, 4H), 9.04 (t, J = 2.4 Hz, 2H), 9.47 (s, 2H).

4.6.10. 5,15-Bisphenyl-10,20-di(*N*-methyl-pyridium-3-yl)-porphyrin (4)[15]. Porphyrin **19** (30 mg, 48.7 μmol) and methyl iodide (2 mL) were mixed. After reaction, the crude product was treated and purified according to procedure in Section 4.6 to yield 92.7% (41 mg) of porphyrin **4**. ^{1}H NMR (DMSO-d_6, 300 MHz): −3.02 (s, 2H), 4.65 (s, 6H), 7.88 (t, J = 8.6 Hz, 6H), 8.21 (d, J = 5.4 Hz, 4H), 8.58 (t, J = 6.9 Hz, 2H), 8.94 (d, J = 3.9 Hz, 4H), 9.09 (s, 4H), 9.37 (s, 2H), 9.50 (d, J = 5.7 Hz, 2H), 10.0 (d, J = 6 Hz, 2H).

4.6.11. 5,15-Diethyl-di(3-pyridyl)-porphyrin (20). Pyrrole (4 mL, 57.2 mmol), pyridine-4-carbaldehyde (2.74 mL, 28.6 mmol), and propionaldehyde (2.08 mL, 28.6 mmol) were mixed. After reaction, the mixture was treated and purified according to procedure **4.1** to yield 62 mg (1.09%) of porphyrin **20**. ^{1}H NMR ($CDCl_3$, 300 MHz): −2.73 (s, 2H), 2.11 (t, J = 7.5 Hz, 6H), 5.01 (m, 4H), 7.74 (m, 2H), 8.47 (d, J = 6 Hz, 2H), 8.87 (d, J = 4.8 Hz, 4H), 9.05 (t, J = 2.4 Hz, 2H), 9.46 (t, J = 7.2 Hz, 6H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 418 (5.51), 516 (4.19). 552 (3.93), 592 (3.70), 651 (3.81); ESI HRMS for $C_{34}H_{28}N_6$ $(M+H)^+$: found 521.2454, calcd 521.2448.

4.6.12. 5,15-Diethyl-di(*N*-methyl-pyridium-3-yl)-porphyrin (5). Porphyrin 20 (25 mg, 48.1 μmol) and 2 mL of methyl iodide were mixed. After reaction, the mixture was treated and purified according to procedure in Section 4.6 described and porphyrin **5** was obtained in 34 mg (87.0%). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.97 (s, 2H), 2.06 (t, J = 7.1 Hz, 6H), 4.65 (m, 6H), 5.15 (d, J = 7.2 Hz, 4H), 8.60 (t, J = 6.9 Hz, 2H), 9.09 (s, 4H), 9.35 (d, J = 6 Hz, 2H), 9.52 (d, J = 5.7 Hz, 2H), 9.91 (d, J = 3.6 Hz, 4H), 10.0 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 422 (5.47), 516 (4.25), 552 (3.74), 592 (3.65), 651 (3.81); SIMS HRMS for $C_{36}H_{34}N_6$ (M^+−2I): found 550.2834, calcd 550.2839.

4.6.13. 5,15-Di(3-pyridyl)-porphyrin (30). Dipyrromethane **28** (1.46 g, 10 mmol) and pyridine-3-carbaldehyde (0.95 mL, 10 mmol) were reacted. After reaction, the mixture was treated and separated according to procedure in Section 4.5 and porphyrin **30** was obtained in a yield of 2.5% (58 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −3.17 (s, 2H), 7.83 (m, 2H), 8.58 (m, 2H), 9.06 (m,6H), 9.48 (d, J = 4.2 Hz, 4H), 9.52 (s, 2 H), 10.41 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 407 (5.42), 503 (4.16), 536 (3.83), 574 (3.78), 628 (3.60); ESI HRMS for $C_{30}H_{20}N_6$ $(M+H)^+$: found 465.1833, calcd 465.1827.

4.6.14. 5,15-Di(pyridium-3-yl)-porphyrin (6). Porphyrin **30** (30 mg, 64.7 μmol) and 3 mL of methyl iodide were mixed. After reaction, the crude was treated and purified according to procedure in Section 4.6 described. Porphyrin **6** was obtained in a yield of 90.7% (44 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −3.41 (s, 2H), 4.70 (s, 6H), 8.63 (s, 2H), 9.30 (s, 4H), 9.45 (s, 2H), 9.52 (s, 2H), 9.84 (s, 4H), 10.07 (m, 4H), 10.82 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 408 (5.57), 502 (4.37), 536 (3.95), 574 (3.88), 628 (3.68); ESI MS for $C_{32}H_{26}N_6$ [(M^+−2I)/2]: found 247.4, calcd 274.1; SIMS HRMS for $C_{32}H_{26}N_6$ (M^+−2I–CH_3): found 479.1978, calcd 479.1979.

4.6.15. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(3-pyridyl)-porphyrin (24). 3,3′-Diethyl-4,4′-dimethyl-2,2′-dipyrrylmethane (**21**) (460 mg, 2 mmol) and pyridine-3-carbaldehyde (190 μL, 2 mmol) were mixed in 500 mL CH_2Cl_2/C_2H_5OH (95:5). After reaction, the mixture was treated and purified according to procedure in Section 4.3. Purified porphyrin **24** was obtained in 135 mg (21.4%). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.28 (s, 2H), 1.77 (m, 12H), 2.66 (m, 12H), 4.23 (m, 8H), 7.93 (s, 2H), 8.55 (s, 2H), 9.28 (d, J = 4.2 Hz, 2H), 9.57 (d, J = 6.6 Hz, 2H), 10.46 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 409 (5.25), 508 (4.19), 542 (3.81), 576 (3.86), 626 (3.48); ESI HRMS for $C_{42}H_{44}N_6$ $(M+H)^+$: found 633.3708, calcd 633.3700.

4.6.16. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(*N*-methyl-pyridium-3-yl)-porphyrin (11). Porphyrin **24** (50 mg, 79.1 μmol) and 3 mL of methyl iodide were mixed. After reaction, the mixture was treated and purified according to procedure in Section 4.6. Compound **11** was obtained in a yield of 97.4% (71 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.56 (s, 2H), 1.76 (t, J = 7.4 Hz, 12H), 3.40 (s, 12H), 4.11 (d, J = 7.5 Hz, 8H), 4.72 (s, 6H), 8.64 (t, J = 7.4 Hz, 2H), 9.49 (t, J = 7.4 Hz, 2H), 9.57 (d, J = 6.0 Hz, 2H), 9.93 (d, J = 10.2 Hz, 2H), 10.40 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 412 (5.03), 509 (4.15), 542 (3.97), 576

(3.90), 630 (3.76); SIMS HRMS for $C_{44}H_{50}N_6$ (M^+-2I): found 662.4087, calcd 662.4091.

4.6.17. 5,15-Bisphenyl-10,20-di(2-pyridyl)-porphyrin (31)[15]. 5-Phenyl-2,2′-dipyrrylmethane **26** (0.65 g, 2.93 mmol) and pyridine-2-carbaldehyde (278 μL, 2.93 mmol) were mixed in 50 mL of propionic acid. After reaction, the mixture was treated and purified according to procedure in Section 4.4 described. Porphyrin **31** was obtained in a yield of 18.4% (166 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.80 (s, 2H), 7.74 (m, 8H), 8.10 (t, J = 6.9 Hz, 2H), 8.23 (m, 6H), 8.88 (d, J = 9.9 Hz, 8H), 9.14 (d, J = 3.9 Hz, 2H); ESI HRMS for $C_{42}H_{28}N_6$ $(M+H)^+$: found 617.2454, calcd 617.2448.

4.6.18. 5,15-Bisphenyl-10,20-di(*N*-methyl-pyridium-2-yl) porphyrin (7)[15]. Porphyrin **31** (51 mg, 82.8 μmol) and 4 mL of methyl iodide were mixed. After reaction, the mixture was treated and purified according to procedure in Section 4.6. Porphyrin **7** was obtained in a yield of 93.5% (70 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.91 (s, 2H), 4.02 (m, 6H), 7.89 (s, 6H), 8.21–8.23 (m, 4H), 8.70 (s, 2H), 8.95–9.02 (m, 12H), 9.59 (s, 2H); SIMS HRMS for $C_{44}H_{34}N_6$(M^+-2I): found 646.2838, calcd 646.2839.

4.6.19. 5,15-Diethyl-di(2-pyridyl)-porphyrin (32). 5-Ethyl-2,2′-dipyrrylmethane (**27**) (1.46 g, 10 mmol) and pyridine-2-carbaldehyde (0.95 mL, 10 mmol) were mixed in 100 mL of propionic acid. After reaction, the mixture was treated and purified according to the procedure in Section 4.4. Porphyrin **32** was obtained in a yield of 5.5% (142 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.71(s, 2H), 2.10 (t, J = 6.9 Hz, 6H), 5.02 (d, J = 7.2 Hz, 4H), 7.74 (d, J = 5.1 Hz, 2H), 8.10 (t, J = 6.9 Hz, 2H), 8.20 (d, J = 7.2 Hz, 2H), 8.85 (d, J = 4.2 Hz, 4H), 9.14 (s, 2H), 9.47 (d, J = 4.2 Hz, 4 H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 418 (5.35), 515 (4.02), 552 (3.60), 593 (3.51), 650 (3.44); ESI HRMS for $C_{34}H_{28}N_6$ $(M+H)^+$: found 521.2454, calcd 521.2448.

4.6.20. 5,15-Diethyl-di(*N*-methyl-pyridium-2-yl)-porphyrin (8). Porphyrin **32** (35 mg, 67.3 μmol) and 2 mL of methyl iodide were mixed. After reaction, the mixture was treated and purified as procedure in Section 4.6 described. Porphyrin **8** was obtained in 50 mg (yield: 91.9%). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.81(s, 2H), 2.08 (s, 6H), 4.04 (d, J = 18.6 Hz, 6H), 5.14 (m, 4H), 7.98 (s, 2H), 8.75 (s, 2H), 9.02 (s, 6H), 9.64 (s, 2H), 9.96 (s, 4H); UV–vis (DMF): λ_{max} (nm, log ε) = 418 (5.34), 515 (4.15), 552 (3.74), 592 (3.57), 650 (3.65); SIMS HRMS for $C_{36}H_{34}N_6$ (M^+-2I): found 550.2844, calcd 550.2839.

4.6.21. 5,15-Di(2-pyridyl)-porphyrin (33). Dipyrromethane **28** (1.46 g, 10 mmol) and pyridine-2-carbaldehyde (0.95 mL, 10 mmol) were mixed and reaction was treated as procedure in Section 4.5. Porphyrin **33** was obtained in 87 mg (3.75%). ^{1}H NMR ($CDCl_3$, 300 MHz): −3.13 (s, 2H), 7.77 (d, J = 6.0 Hz, 2H), 8.18 (d, J = 7.8 Hz, 2H), 8.32 (d, J = 7.2 Hz, 2H), 9.14 (d, J = 4.5 Hz, 4H), 9.21 (s, 2H), 9.45 (d, J = 4.5 Hz, 4H), 10.38 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 407 (5.28), 502 (4.01), 536 (3.70), 576 (3.63), 628 (3.35); ESI HRMS for $C_{30}H_{20}N_6$ $(M+H)^+$: found 465.1824, calcd 465.1822.

4.6.22. 5,15-Di(*N*-methyl-pyridium-2-yl)-porphyrin (9). Porphyrin **33** (25 mg, 53.9 μmol) and 2 mL of methyl iodide were mixed and reaction was treated as procedure **5**. Porphyrin **9** was obtained in a yield of 90.1% (36 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −3.42 (s, 2H), 4.04 (d, J = 10.5 Hz, 6H), 8.70–8.75 (m, 2H), 8.94–9.08 (m, 4H), 9.20 (d, J = 3.9 Hz, 4H), 9.63 (s, 2H), 9.85 (d, J = 4.2 Hz, 4 H), 10.86 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 406 (5.20), 501 (4.00), 535 (3.76), 574 (3.57), 628 (3.57); SIMS HRMS for $C_{32}H_{26}N_6$ (M^+-2I): found 494.2204, calcd 494.2213.

4.6.23. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(2-pyridyl)-porphyrin (25). 3,3′-Diethyl-4,4′-dimethyl-2,2′-dipyrrylmethane **21** (460 mg, 2 mmol) and pyridine-2-carbaldehyde (190 μL, 2 mmol) were mixed in 500 mL CH_2Cl_2/C_2H_5OH (95:5) and reaction was treated according to procedure in Section 4.3. Porphyrin **25** was obtained in a yield of 36.8% (233 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.77 (t, J = 7.5 Hz, 12H), 2.41 (s, 12H), 4.01 (d, J = 7.2 Hz, 8H), 7.76 (s, 2H), 8.05 (d, J = 9.0 Hz, 4H), 9.13 (s, 2H), 10.22 (s, 2H); UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 408 (5.23), 505 (4.20), 542 (3.90), 575 (3.89), 626 (3.63); ESI HRMS $C_{42}H_{44}N_6$ $(M+H)^+$: found 633.3695, calcd 633.3700.

4.6.24. 2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-di(*N*-methyl-pyridium-2-yl)-porphyrin (12). Porphyrin **25** (51 mg, 81 μmol) and 3 mL of methyl iodide were mixed and reacted according to procedure in Section 4.6. Porphyrin **12** was obtained in a yield of 97% (71 mg). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.71 (s, 2H), 1.75 (s, 12H), 2.41 (m,12H), 4.02–4.11 (m, 14H), 8.79 (s, 2H), 9.09–9.15 (m, 4H), 9.61 (s, 2H), 10.46 (s, 2H); UV–vis (DMF): λ_{max} (nm, log ε) = 405 (5.09), 510 (4.13), 544 (4.04), 576 (3.83), 630 (3.93); SIMS HRMS for $C_{44}H_{50}N_6$ (M^+-2I): found 662.4081, calcd 662.4091.

Acknowledgments

X.Z. thanks the NSFC (National Science Fund of China) (No. 20272046) and National Science Fund for Distinguished Young Scholars (No. 20425206) for financial support. X.P.C. thanks for TQ program 20021001 by the National Science of Foundation of China.

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