Journal of the Korean Chemical Society
2013, *Vol. 57, No. 2*
Printed in the Republic of Korea
http://dx.doi.org/10.5012/jkcs.2013.57.2.246

Synthesis, DNA Photocleavage and Singlet Oxygen Measurement of Cationic Bisporphyrins

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(Received January 24, 2013; Accepted March 13, 2013)

ABSTRACT. With $-OCH_2CO-$ as a linker, a non β-substituted cationic bisporphyrin (**4a**) and a β-substituted cationic bisporphyrin (**4b**) were prepared through methylation of the intermediate which was obtained from β-amino-5,10,15,20-tetra (4-cyanophenyl) porphyrin or 5-hydroxylphenyl-10,15,20-tris(4-cyanophenyl) reacting with 5-hydroxy-10,15,20-trispyridinylporphyrin. Their structures were confirmed by ^{1}H NMR, IR, UV-vis, MS and elemental analysis. DNA photocleavage ability and the singlet oxygen ability of those cationic bisporphyrins were investigated. DNA photocleavage activity of β-substituted cationic bisporphyrin was significantly weaker than that of H_2TMPyP, but similar to that of non β-substituted cationic bisporphyrin. While **4a** and **4b** showed substantial photocleavage activities toward DNA, with 68% and 66% observed at 10 μM. The assessment of indirectly measured $^1O_2$ production rates against H_2TMPyP were described and the relative singlet oxygen production yields were: free cationic bisporphyrins > H_2TMPyP. The results showed the cationic bisporphyrins with β-substitution and non β-substitution could be developed as potential photodynamic agents.

Key words: Cationic bisporphyrin, DNA photocleavage, Singlet oxygen, Photosensitizer

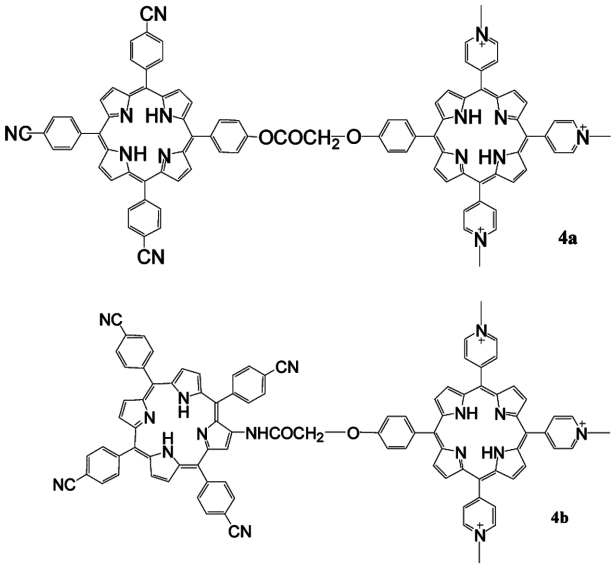
INTRODUCTION

Deoxyribonucleic acid (DNA) is an informational molecule encoding the genetic instructions used in the development and functioning of all known living organisms and many viruses. Along with RNA and proteins, it is also one of the three major macromolecules that are essential to all known forms of life. It exists as the fundamental substance of the gene expression and controls the synthesis of protein. And it is the target molecule of many drugs as well. The research of the interaction between DNA and drug molecules lays the foundation for the design and screen of novel drugs and some pathophysiology research.[1]

Photodynamic therapy (PDT) is an inherently selective novel treatment for cancer, which is used to eliminate abnormal tissues including the tumors. PDT involves in the photosensitizers (such as porphyrin derivatives), visible light illumination and singlet oxygen ($^1O_2$) generation that leads to oxidative damage of target tissues and results in tumor cells death. So it shows a great potential application from its dual-selective mode of this action. A photosensitizer of negligible dark toxicity is introduced into the body and accumulates preferentially in rapidly dividing cells. When the photosensitizer reaches an appropriate concentration accumulating in diseased versus healthy tissue, a regulated dose light is shone onto the diseased tissue and activates the photosensitizer, which elicits the toxic action.[2,3]

An ideal PDT drug has to possess the following properties: strong absorption in the red part of the visible spectrum; high quantum yield of triplet formation; high singlet oxygen quantum yield; low dark toxicity; selectivity for the enrichment in tumor tissue competing with healthy tissue, etc.[3] In this article, the property of singlet oxygen generation was evaluated.

The porphyrins are one of the photosensitizers that meet multiple requirements of being a PDT drug. Cationic porphyrins are known not only to bind and cleave DNA, but to be used for the cancer treatment.[4] Cationic porphyrins, especially meso-tetrakis (N-methylpyridinium-4-yl) porphyrin (H_2TMPyP) and its metal complexes, possess significant and wide research value for the reason that they possess good water solubility, which makes DNA-binding possible under physiological conditions and interact with DNA strongly.[5,6] Meanwhile, tris-, tetra- and multiple charge cationic porphyrins are also known as good photosensitizers for DNA photocleavage.[7,8] So we prepared two cationic bisporphyrin compounds (seen in *Scheme* 1) and evaluated their DNA photocleavage activity and singlet oxygen yield.



Scheme **1.**

EXPERIMENTAL

Apparatus and Reagents

β-amino-5,10,15,20-tetra (4-cyanophenyl)porphyrin[9], 5-hydroxyphenyl-10,15,20-trispyridinylporphyrin (*p*-OH-TPyP),[7] 5-hydroxylphenyl-10,15,20-tris(4-cyanophenyl)-porphyrin)[10] and H_2TMPyP[11] were prepared according to the literature procedures. Other reagents were purchased from Acros company and used without further purification. Silica gel 60 (0.04–0.063 mm) for column chromatography was obtained from Merck. DNA was obtained from Pharmacia Biotech company.

NMR spectra were recorded on a Varian INOVA 400 NMR spectrometer; HRMS were recorded on QSTAR mass spectrometer or Finnigan TSQ710 mass spectrometer; UV-vis absorption spectra were recorded with a Varian Cary 100 UV-vis spectrophotometer; fluorescence spectrum were recorded with a Photon Technology International Apphas-scan spectrofluorimeter; IR spectra (KBr pellets) were recorded with a Nicolet Nagba-IR 550 spectrometer. Elemental analysis were performed on an instrument of VarioEL III.

Synthesis

Bromoacetyl chloride was used to react with β-amino-5,10,15,20-tetra (4-cyanophenyl) porphyrin or 5-hydroxylphenyl-10, 15, 20-tris (4-cyanophenyl) porphyrin to gain intermediate **2b** or **2a**. And they condensed with 5-hydroxylphenyl-10, 15, 20-tripyridinyl porphyrin to gain porphyrin **3b** or **3a**. A methylation using CH_3I was performed to obtain non β-substituted bisporphyrin **4a** and β-substituted bisporphyrin **4b**. The synthetic routes of non β-substituted bisporphyrin (**4a**) and β-substituted bisporphyrin (**4b**) are shown below as *Fig.* 1 and 2.

Preparation of 2a, 2b

Monobromo-acetic acid (100 mg, 0.72 mmol) was dissolved in 2 mL thionyl chloride. The mixture was stirred under reflux for 3h and then removed under vacuum. The residue was bromoacetyl chloride with colorless oil. The compound was directly used in the following reaction without any purification.

(**1a**) (50 mg, 0.071 mmol) was dissolved in 20 mL dichloromethane (DCM), to which 1 mL pyridine was added. The bromoacetyl chloride mixed with 5 mL DCM

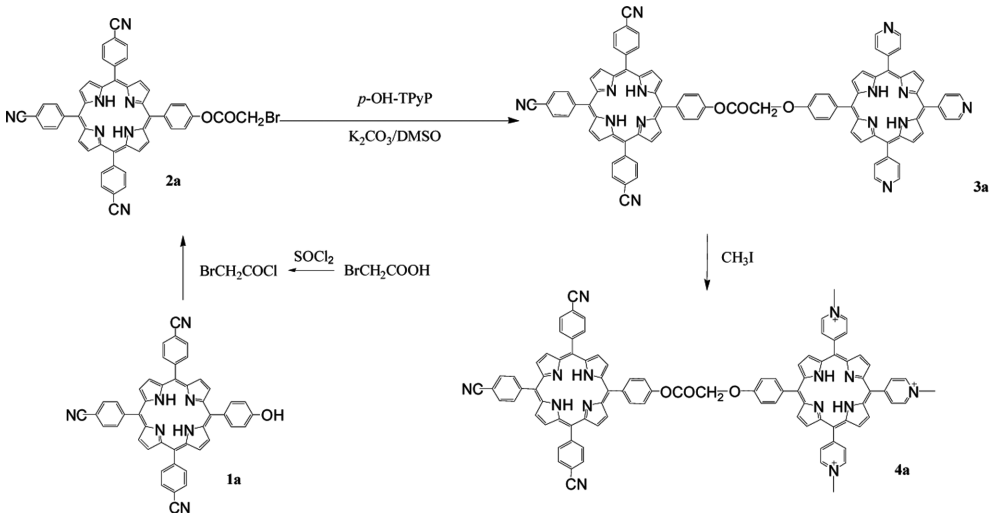


***Fig.* 1.** The synthesis of non β-substituted bisporphyrin.

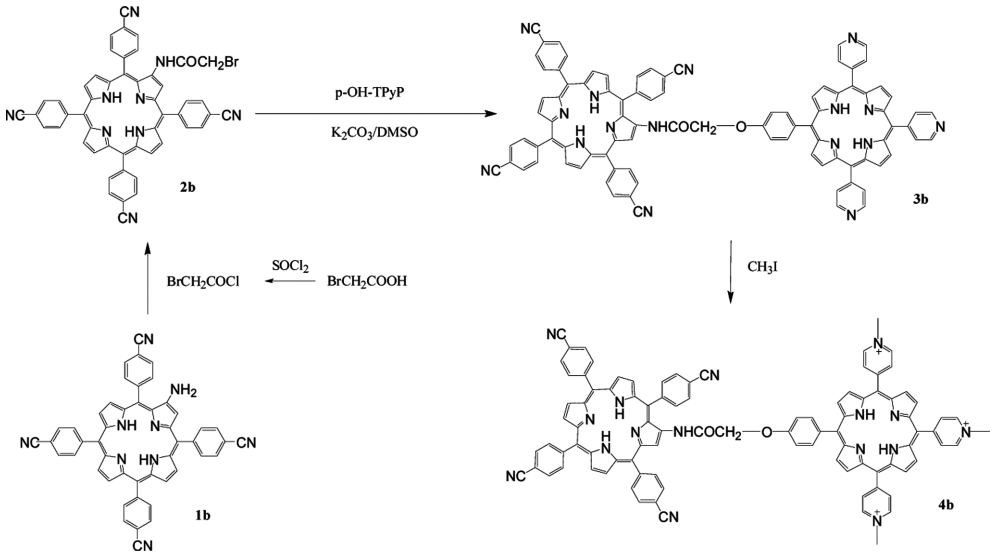


***Fig.* 2.** The synthesis of β-substituted bisporphyrin.

was added dropwise to the above solution in ice bath. After stirring 6h, the mixture was poured into ice water. The DCM layer was collected, and washed by 2% saturated sodium bicarbonate and deionized water in turn. Then it was dried with anhydrous sodium sulfate. The solvent was removed and a purple solid was gained. The solid was purified by column chromatography with chloroform as an eluent. 48 mg of the target product was obtained in the yield of 82.3%, m.p. >300 ºC, MS (FAB, +ve mode): m/z = 826.1 $[M]^+$.

The intermediate **2b** was prepared by the above similar method. 38 mg, the yield was 65.7%, m.p. >300 ºC, MS (FAB, +ve mode): m/z = 850.3 $[M]^+$.

Preparation of 3a, 3b

5-hydroxylphenyl-10, 15, 20-tripyridyl porphyrin (30 mg, 0.048 mmol) and **2a** (40 mg, 0.048 mmol) were dissolved in 15 mL DMSO, to which 1 g anhydrous potassium carbonate was added. The mixture was stirred under 80 ºC for 8h and then poured into saturated salt water. A purple solid was yielded after being filtrated and purified by column chromatography with chloroform/ methanol (V/V = 20:1) as an eluent. 38 mg of **3a** was obtained and the yield was 57.4%, m.p. >300 ºC, UV/Vis ($CHCl_3$, 20 ºC): λ_{max} (log ε) = 422 (5.52), 517 (4.10), 556 (3.81), 592 (3.71), 645 nm (3.42 $dm^3mol^{-1}cm^{-1}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = –2.87 [s, 2 H, NH from tri-pyridyl porphyrin], –2.84 [s, 2 H, NH of pyrrole on tricyanophenyl porphyrin], 4.57 (s, 2 H, CH_2O), 7.44–7.45 (m, 6 H), 8.11–8.17 (m, 6 H), 8.15–8.16 (m, 6 H), 8.28–8.30 (d, J = 8.0 Hz,2 H), 8.65–8.72 (m, 6 H), 8.74–8.90 (m, 16 H), 9.04–9.05 (d, J = 5.8 Hz, 6 H) ppm. IR (KBr): ν = 3336, 3316, 2924, 2227 (CN), 1725 (C=O), 1629 (C=C) cm^{-1}. MS (FAB, +ve mode): m/z = 1378.9 $[M]^+$; $C_{90}H_{54}N_{14}O_3$ (1378.5): calcd. C 78.36, H3.95, N 14.22; found C 78.18, H 4.12, N 14.29.

The intermediate **3b** was prepared by the above similar method. The yield was 43.5%, m.p. >300 ºC, UV/Vis ($CHCl_3$, 20 ºC): λ_{max} (log ε) = 422 (5.85), 518 (4.50), 556 (4.28), 593(3.96), 650 nm (3.58 $dm^3mol^{-1}cm^{-1}$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = –2.97 [s, 2 H, NH of pyrrole on tetracyanophenyl porphyrin], –2.88 [s, 2 H, NH of pyrrole on tripyridinyl porphyrin], 4.59 (s, 2H, CH_2O), 7.40–7.41 (m, 6 H), 8.13–8.16 (m, 6 H), 8.25–8.28 (m, 8 H), 8.70–8.72 (m, 6 H), 8.75–8.89 (m, 15 H), 9.07–9.09 (m, 6 H) ppm. IR (KBr): ν = 3435, 3328, 2921, 2230 (CN), 1668 (C=O), 1625 (C=C), 1505 cm^{-1}. MS (FAB, +ve mode): m/z = 1403.1 $[M]^+$; $C_{91}H_{54}N_{16}O_2$ (1402.5): calcd. C 77.87, H3.88, N 15.97; found C 77.79, H 4.07, N 16.07.

Preparation of 4a, 4b

3a (30 mg, 0.022 mmol) was dissolved in 20 mL iodomethane and the fixture was stirred under reflux for 12h. Then CH_3I was removed under vacuum. The residue was recrystallized by acetonitrile and methanol to gain a purple solid, 34 mg, the yield was 85.7%, m.p. >300 ºC, UV/Vis (DMSO, 20 ºC): λ_{max} (log ε) = 424 (5.50), 518 (4.22), 553 (3.82), 590 (3.60), 642 nm (3.49 $dm^3mol^{-1}cm^{-1}$); ^{1}H NMR (400 MHz, d_6-DMSO): δ = –2.94 [s, 2 H, NH of pyrrole on tricyanophenyl porphyrin], –2.92 [s, 2 H, NH of pyrrole on tris-(N-methylpyridinyl) porphyrin], 4.63 (s, 2 H, CH_2O), 4.73 (s, 9 H, CH_3), 7.48–7.54 (m, 4 H), 8.19–8.23 (m, 4 H), 8.30–8.35 (m, 6 H), 8.39–8.41 (m, 6 H), 8.80–8.87

(m, 8 H), 8.95–8.97 (m, 6 H), 9.13–9.16 (m, 8 H), 9.40–9.43 (m, 6 H) ppm. IR (KBr): ν = 3337, 3315, 2926, 2228 (CN), 1726 (C=O), 1621 (C=C) cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 1423.5206 $[M]^+$ ($C_{93}H_{63}N_{14}O_3^+$: calcd. 1423.5191, Δm = 1.05 ppm); $C_{93}H_{63}N_{14}O_3I_3$ (1804.2): calcd. C 61.87, H3.52, N 10.86; found C 61.95, H 3.62, N 10.81.

The intermediate **4b** was prepared by the above similar method. The yield was 90.5%, m.p. >300 °C, UV/Vis (DMSO, 20 °C): λ_{max} (log ε) = 425 (5.75), 519 (4.55), 557 (4.27), 591(3.85), 652 nm (3.48 $dm^3mol^{-1}cm^{-1}$); ^{1}H NMR (400 MHz, d_6-DMSO): δ = –2.96 [s, 2 H, NH of pyrrole on tetracyanophenyl porphyrin], –2.91 [s, 2 H, NH of pyrrole on tris-(N-methylpyridinyl) porphyrin], 4.69 (s, 2H, CH_2O), 4.78 (s, 9 H, CH_3), 7.45–7.50 (m, 4 H), 8.24–8.27 (m, 4H), 8.35–8.37 (m, 6 H), 8.40–8.43 (m, 6 H), 8.80–8.82 (m, 6 H), 8.85–8.88 (m, 7 H), 9.12–9.16 (m, 8 H), 9.43–9.47 (m, 6 H) ppm. IR (KBr): ν = 3436, 3320, 2929, 2227 (CN), 1668 (C=O), 1628 (C=C) cm^{-1}. HRMS (MALDI-TOF, positive mode, chloroform): m/z = 1447.5338 $[M]^+$ ($C_{94}H_{63}N_{16}O_2^+$: calcd. 1447.5303, Δm = 2.41 ppm); $C_{94}H_{63}N_{16}O_2I_3$ (1828.3): calcd. C 61.72, H3.47, N 12.25; found C 61.81, H 3.57, N 12.17.

DNA Photocleavage

The ability of DNA photocleavage was evaluated with plasmid DNA. Plasmid DNA (pBluescript, 0.5 μg), enriched with the double helix covalently-closed circular, 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 porphyrins (test sample) were added into the Eppendorf tubes to give a final volume of 20 μL. 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 mL of the 6x sample dye solution (which contained 20% 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 x 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 mg/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).[12]

Singlet Oxygen Production

1,3-diphenylisobenzofuran (DPBF) was used as a selective singlet oxygen acceptor with a wavelength > 500 nm. The samples were illuminated by light of 50 watt. DPBF and porphyrins were prepared in dark, with a concentration of 50 mM and 1 mM respectively. DPBF only, and DPBF with H_2TMPyP were used as control group.[13] The absorbance at 418 nm under 20 °C were collected.

RESULTS AND DISCUSSION

DNA Photocleavage

The ability of DNA photocleavage was measured by monitoring the conversion of supercoiled conformer (Form I) to the open-circular conformer (Form II) upon nicking by a DNA-cleavage with the singlet oxygen generation.[9] The results were illustrated in *Fig.* 3.

In the DNA photocleavage activities, H_2TMPyP had a higher photocleavage ability, which reached 97% at 3 μM, than those of two bisporphyrins due to its stronger electronic

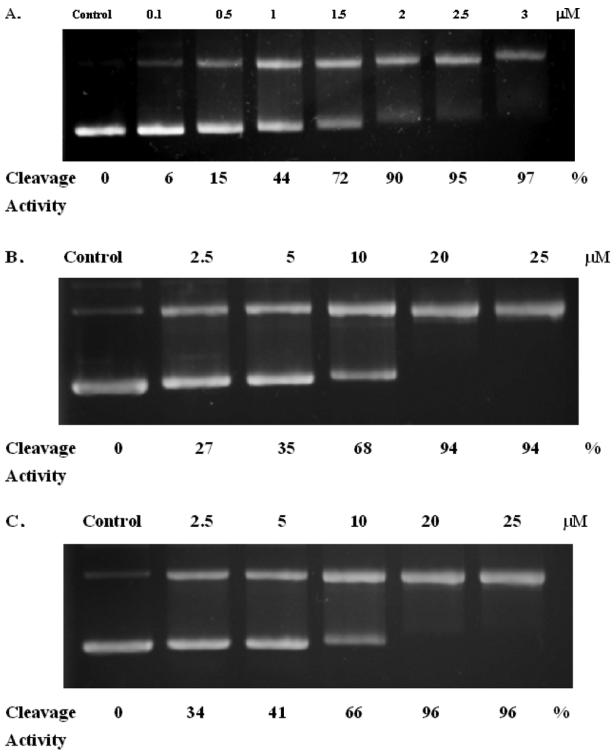


***Fig.* 3.** Agarose gel electrophoresis images of DNA photocleavageassays of H_2TMPyP (A), **4a** (B), and **4b** (C) as a function of their concentrations.

effect than **4a** and **4b**. It suggests that H_2TMPyP could provided a better binding to the anionic area of DNA. Meanwhile, at 10 μM, **4a** and **4b** had photocleavage activity of 68% and 66% respectively and showed the promising photocleavage activity at higher concentration. The DNA photocleavage activity of cationic bisporphyrins **4a** and **4b** was ca. 3-fold higher than that of bisporphyrins with amidinophenyl group (19% at 10 μM),[13] similar to cationic bisporphyrin substituted with CN group by $-O(CH_2)_3O-$ as a linker (73% at 11 μM)[12] in our previous paper. Seen from the data and their structure, the hydrophilicity increasing from the tetra-charge introduction in the photosensitizers, such as H_2TMPyP, could increase the yield of $^1O_2$, but the hydrophobicity increasing from the porphyrin ring tail led to lower the $^1O_2$ generation in virtue of the occurrence of the self-assembly of bisporphyrins with amidinophenyl group, **4a** and **4b**.[14] These results also underscored the importance of a close-range interaction with DNA and an existence of cationic group in the molecule structure.

Generation of Singlet Oxygen

The production rate of singlet oxygen had to be considered due to its significance to DNA photocleavage.[10] DPBF can absorb the singlet oxygen generated by those porphyrins to cause the reduction of their photochemical ability. The relative singlet oxygen production rate of H_2TMPyP, **4a**, and **4b** were measured based on the $^1O_2$-induced bleaching of DPBF. According to the relationship between A/A_0 absorbed by DPBF and illumination time, the $^1O_2$ production rate was evaluated. A steeper variation trend indicated a faster production rate: **4a**~**4b** > H_2TMPyP.

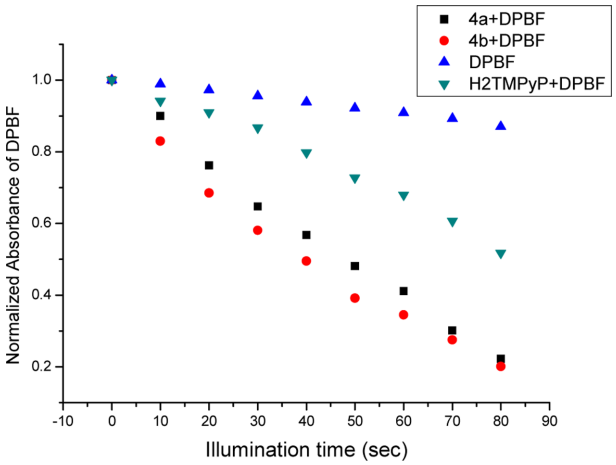


***Fig.* 4.** Normalized absorbance of DPBF (50 mM in DMSO) at 418 nm as a function of photo-irradiation time in the absence (control) and presence of 1 mM of H_2TMPyP, **4a** and **4b**.

The non β-substituted bisporphyrin had a similar $^1O_2$ quantum yield compared with the β-substituted bisporphyrin. The reason was that the molecular conjugated system and charge characteristics was not affected evidently in virtue of connecting two porphyrin rings with flexible chains of β-substitution or not. Cationic bisporphyrins **4a** and **4b** generated singlet oxygen much faster than H_2TMPyP owing to the interaction of the chromophoric group of molecules leading more energy to be excited.[11] But H_2TMPyP possesses four positive charge so as to increase the intermolecular repulsion and lower $^1O_2$ production rate. It seemed inconsistent that **4a** and **4b** had a faster singlet oxygen generation rate, but a lower photocleavage ability than H_2TMPyP through the results of DNA photocleavage and $^1O_2$ generation. Moreover, different solvent system could also affect these results. DMSO was selected in singlet oxygen production test, but buffer solution using in DNA photocleavage. The generation of $^1O_2$ depended on both the solvent and the solubility of porphyrins, which had been verified by Ishikawa who found that the synthesized bisporphyrins performed complete generation of singlet oxygen in DMSO, but weaker, even none, than that generation in buffer solution. So the porphyrins' dependency on solvents could be also verified by their difference in photodynamic properties in solvents.[15]

CONCLUSION

β-substituted and non β-substituted cationic bisporphyrin, **4a** and **4b**, were prepared and their structures were confirmed by ^{1}H NMR, IR, UV-vis, MS and elemental analysis. By contrast with H_2TMPyP, the DNA photocleavage activity and singlet oxygen production rate were evaluated. At the concentration of 10 μM, the activities of DNA photo cleavage of porphyrin **4a** and **4b** were 68% and 66% respectively, and both cationic bisporphyrins generated the singlet oxygen more efficiently than H_2TMPyP. These results suggest that cationic bisporphyrins could be developed as potential photosensitizers in PDT. Additionally, the β-substituted modification or not with a linker had insignificant effect on these measurements. Cellular uptake and cytotoxicity tests are currently under way.

Acknowledgments. The authors are grateful for the financial support of the National Natural Science Foundation of China (grant No.20902071) and Scientific Research Foundation of Wuhan Institute of Technology (grant No. 10122021).

And the publication cost of this paper was supported by the Korean Chemical Society.

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