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Synthesis of β-Substituted Cationic Porphyrins and Their Interactions with DNA

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Abstract—The β-substituted cationic porphyrins (**7**, **8** and **10**) have been synthesized and their interactions with plasmid DNA investigated. We found that substituents at the β-position of porphyrins (**7** and **8**) have apparently affected their interactions with DNA compared with non-β-substituted porphyrins (**10**).

Substituents at the β-position of porphyrins are found to exert much larger steric and electronic effects on the porphyrin ring than those at the *meso*-aryl position. The β-substituents often induce the porphyrin into a non-planar conformation which may control the biological properties in tetrapyrrole systems like the photosynthetic centers, vitamin B_{12} and P-450.[1] Therefore, synthetic methodology studies of β-substituted porphyrins have been widely investigated by many groups using coupling chemistry.[2] It is known that the photoeffects of porphyrins have been previously studied by Fiel and co-workers on DNA–porphyrin interaction.[3] Then, a lot of publications on DNA–porphyrin interaction have been published to study their interaction modes, structure effects, charges effects and so on.[4] However, to our knowledge, the β-substituting effects of cationic porphyrins on DNA–porphyrin interaction have not been reported. Since the natural porphyrin derivatives are β-substituted porphyrin families, obviously, the β-substituting effect of porphyrins on DNA–porphyrin interaction will be more significant for their biological studies. Herein, we first report the synthesis of β-substituted cationic porphyrins and then their interactions with plasmid DNA.

The synthetic strategy to obtain cationic porphyrin β-tetramethyl-mono (4-*N*-trimethylanilinium) triphenyl porphyrin **7** and β-tetraphenyl-mono (4-*N*-trimethyl anilinium)triphenyl porphyrin **8** was involved in Suzuki coupling chemistry starting from β-tetrabromoporphyrin (Scheme 1). Mono aryl-nitro porphyrin **2** was prepared by mixing fuming HNO_3 with β-tetrabromo porphyrin **1**[2c] in $CHCl_3$ at 5 °C[5] in 24.3% yield. Then, porphyrin **3** or **4** was obtained by mixing compound **2** with methyl or phenyl boronic acids in toluene or DMF catalyzed by

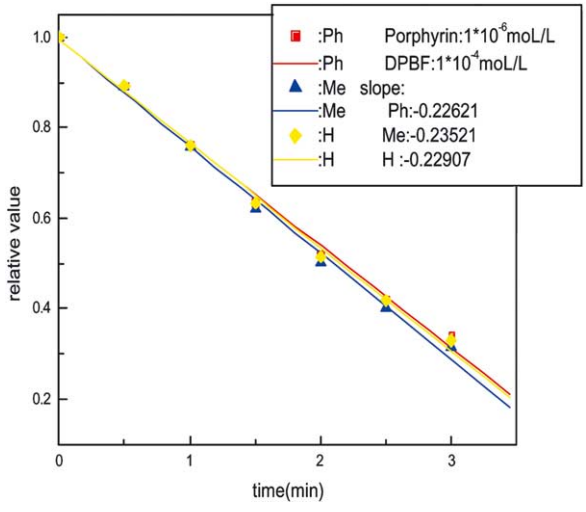

Figure 1. Decomposition of DPBF by compounds **7**, **8** and **10**. Porphyrin (1.0×10^{-6} M) and DPBF (1.0×10^{-4} M) were irradiated in pyridine.

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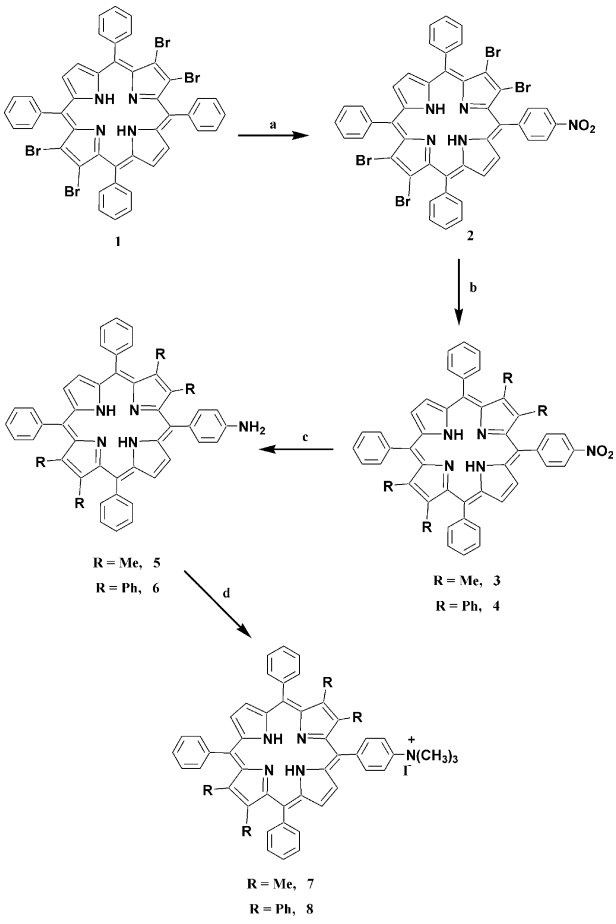
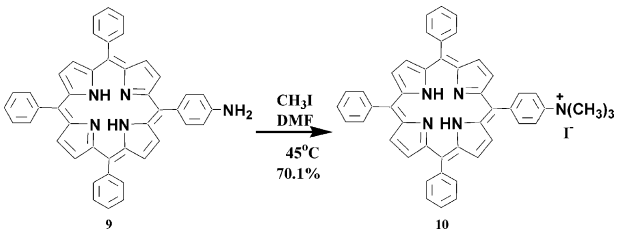


Scheme 1. Synthesis of quaternary ammonium porphyrins **7**, **8**: (a) fuming HNO_3, 5 °C, 3 h, 24.3%; (b) $MeB(OH)_2$, K_2CO_3, $Pd(Ph_3P)_4$, toluene, 95–105 °C, 3 days, 87.2%; $PhB(OH)_2$, Na_2CO_3, $Pd(Ph_3P)_4$, DMF, 12 h, 75.9%; (c) $SnCl_2{\cdot}2H_2O$, cHCl, 70 °C, 3 h (for Me, 87.7%), 12 h (for Ph, 82.6%); (d) CH_3I, DMF, 40 °C, 5 h, 61.1% (Me), 42.3% (Ph).

$Pd(Ph_3P)_4$ at 95–105 °C for 3 days or 12 h[2c] in 87.2 or 75.9% yields. Reduction of nitro porphyrin **3** or **4** was finished by $SnCl_2{\cdot}2H_2O$ in the concentrated HCl. Porphyrins **5** and **6** were obtained in the yields of 87.7 and 82.6%, respectively. Finally, methylation of amino groups of porphyrins **5** and **6** was performed by mixing with methyl iodide in DMF, respectively. Porphyrins **7** and **8** were obtained in the yields of 42.3 and 61.1%. Cationic porphyrin **10** without β-substituents was prepared by mixing porphyrin **9**[5] with methyl iodide in DMF and the yield was 70.1% (Scheme 2). New compounds were fully characterized by ^1H NMR, HRMS and UV.[6]

It is known that photo-induced DNA damage by porphyrins was involved in the production of singlet oxygen.[7] Further to our initial study of porphyrins **7**, **8** and **10** interacting with DNA, measurement of singlet oxygen production by measuring the decomposition of 1,3-diphenyliso benzofuran (DPBF)[8] for these porphyrins has been carried out and the results are shown in Figure 1.

The slope of the plot of bleached absorption versus illumination time is proportional to the rate of production of singlet oxygen.[8] Therefore, the rate of singlet



Scheme 2. Synthesis of quaternary ammonium porphyrin **10**.

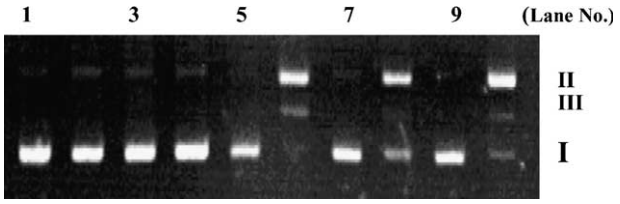


Figure 2. Cleavage of supercoiled pBR322 DNA by compounds **7**, **8** and **10**. 10-μL reaction mixtures contained 63 ng of plasmid DNA. Lane 1: DNA alone; lane 2: DNA + hν; lane 3: DNA + DMF (4%); lane 4: DNA + DMF (4%) + hν; lane 5: DNA + 7 (10 μM); lane 6: DNA + 7 (10 μM) + hν; lane 7: DNA + 10 (10 μM); lane 8: DNA + 10 (10 μM) + hν; lane 9: DNA + 8 (10 μM); lane 10: DNA + 8 (10 μM) + hν.

Table 1. The slopes (S) of the plots of bleached absorption of DPBF by photosensition of **7**, **8** and **10**

	7	**8**	**10**
S	−0.24	−0.23	−0.23

oxygen production for these porphyrins was not significantly different (Table 1). It implied that electronic effects at the β-position of these porphyrins did not change the yields of singlet oxygen production.

According to this result, if their interactions with DNA in the same binding modes, after photo-inducing, compounds **7**, **8** and **10** should have the same damage to DNA. However, tested results by agarose gel electrophoresis experiments gave us different data.

The cleaving ability of porphyrins **7**, **8** and **10** to plasmid DNA (pBR322) by illumination was investigated using agarose gel electrophoresis. All experiments were performed in buffer (pH = 8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 4% DMF)[9] and the samples were irradiated by high-pressure mercury lamp for 90 min at room temperature and the distance from the sample to the filament of the mercury lamp was 20 cm. Results of DNA cleavage for porphyrins **7**, **8** and **10** were illustrated in Figure 2.

Control experiments indicated that no cleavage of DNA happened in the presence of DMF. No cleavage of DNA was also observed if compounds **7**, **8** and **10** were mixed with DNA without illumination (lanes 5, 7 and 9). Apparently, compound **7** had the strongest ability to cleave DNA (lane 6), compound **10** was the weakest ability to cleave DNA (lane 8) among three compounds.

Both of compounds **7** and **8** could affect the conversion of supercoiled (Form I) DNA to nicked circular (Form II) and linear duplex (Form III) DNA at a concentration of 10 μM, whereas no Form III DNA was observed for compound **10** at a concentration of 10 μM. These results were not consistent with the yields of production of singlet oxygen for compounds **7**, **8** and **10**. It is reported that non-planar porphyrins occurred when the β-position of porphyrins was introduced by functional groups (methyl, aryl or Br).[10] Therefore, presumably, when compounds **7**, **8** and **10** interacted with DNA, as the steric effect of β-substituted porphyrins **7** and **8**, compounds **7** and **8** might have more better binding modes with DNA. Then, after illumination, both of them have a stronger ability to cleave DNA than that of compound **10**. Comparing with the abilities of DNA cleavage for compounds **7** and **8** themselves, methyl groups had less steric hindance than that of phenyl groups. Therefore, it is possible that β-methyl substituted porphyrin might have the better binding mode to plasmid DNA than β-phenyl substituted porphyrin.

In conclusion, we firstly synthesized β-substituted cationic porphyrins by coupling chemistry and studied their interactions with plasmid DNA. Our preliminary findings showed that the interaction modes between porphyrins and DNA might be affected after introducing substituents at β-positions of porphyrins. Further investigation about their binding modes, structure–activity relationship for β-substituted cationic porphyrins–DNA is undergoing.

Acknowledgements

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6. Selected data of porphyrin **8**: ^{1}H NMR δ_H ([$^2H_6$]-DMSO, 300 MHz): 8.29–7.98 (m, 7H), 7.73 (m, 6H), 7.53 (m, 3H), 7.28–7.20 (m, 8H), 6.97–6.84 (m, 19H), 3.73 (s, 2H), 3.66 (s, 7H), −2.12 (s, 2H), δ_C ([$^2H_6$]-DMSO, 75 MHz): 145.5, 141.7, 139.8, 136.1, 135.9, 134.8, 131.5, 131.1, 129.5, 128.5, 127.2, 126.6, 126.3, 125.9, 125.4, 121. 1, 120.7, 117.8, 117.6, 56.4, UV–vis λ_{max} (CH_3OH, nm, log ε): 428.0 (5.12), 496.0 (3.52), 525.0 (3.94), 637.0 (3.00), 670.5 (3.23), HRMS (ESI, *m*/*z*): calcd for $C_{71}H_{54}N_5$ [$M^+ - I$] 976.4374, found 976.4368.

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