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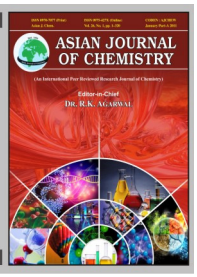

Synthesis, Photophysical Properties and DNA-Binding Studies of *p*-Hydroxy and *m*-Hydroxy Amphiphilic Porphyrins

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Three *p*-hydroxy and *m*-hydroxy amphiphilic porphyrins had been synthesized and fully characterized by ^{1}H NMR, IR and FAB-MS. Their photophysical properties were also determined. Furthermore, the UV-visible absorption and fluorescence emission titration experiments indicate that porphyrins and DNA existed an outside binding mode. In addition, the DNA binding constant of 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP were determined.

Keywords: Porphyrins, Amphiphilic, Photophysical, DNA-binding.

INTRODUCTION

During the past two decades, a great interest has been drawn to porphyrin-based materials. The porphyrins are known for their multiple biological functions and excellent metal complexing ability. And, porphyrin-based materials were widely used in many areas including photodynamic therapy[1], antiviral[2], anticancer therapies[3], NLO materials[4], OLED and solar cell[5]. Porphyrins and its derivatives are also one of themost studied DNA binding agents[6]. According to some previous studies, there are several binding modes to native and synthetic double stranded DNA. The binding modes were marked affected by periphery groups, positive charges and the nature of DNA of the porphyrin derivatvies. It has been shown that the grooving binding, outside random binding and stacking along the DNA template are the common bonding modes[7].

It was reported that hydroxylporphyrins derivatives showed good water solubility and bioactivities[8]. Moreover, these porphyrins could be easily synthesized and separated. However, to the best of our knowledge, there are few reported about the DNA bonding properties of the hydroxylporphyrins. For such reasons, we prepared a series of three *p*-hydroxy and *m*-hydroxy amphiphilic porphyrins, in which a hydroxy is attached to the *para* and *meta* position. The main purpose of this paper is to reveal the DNA binding mode of the hydroxyl amphiphilic porphyrins and CT-DNA. At the same time, the novel porphyrins were fully characterized by IR, MS and ^{1}H NMR.

EXPERIMENTAL

The 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 on a BrukerAvance III 400MHz NMR spectrometer. Chemical shifts of ^{1}H NMR spectra were referenced to internal deuteriated solvents and then recalculated to $SiMe_4$ (δ0.00). FAB mass spectra were obtained with a Quattro micro API mass spectrometer. Electronic absorption spectra in the UV-Visible region were recorded with a Shimadzu UV-2401 PC spectrophotometer instrument. The IR spectra (KBr pellets) were recorded with a Nicolet 5700 FTIR spectrometer. Steady-state visible fluorescence and PL excitation spectra were recorded on a Cary Eclipse spectrofluorimeter.

General procedure

3*p*-OH-TPP: A solution of benzaldehyde (0.53 g, 5 mmol) and *p*-hydroxybenzaldehyde (1.83 g, 15 mmol) in propionic acid (200 mL) was heated to 130 °C. Then freshly distilled pyrrole (1.34 g, 20 mmol) in propionic acid (50 mL) was added slowly to the solution over a period of 0.5 h. The reaction mixture was heated to reflux for another 0.5 h and then cooled to room temperature. Then the reaction mixture was reduced to dry under reduced pressure. The resultant was chromatographed on a silica gel column with $CHCl_3$ as eluent. The fourth band gave the desired 3*p*-OH-TPP. Yield: 106 mg (3.2 %). ^{1}H NMR ($CDCl_3$): δ = 8.86-8.90 (m, 8H), 8.23-8.25 (m, 4H), 8.09-8.11 (m, 4H), 7.76-7.81 (m, 5H), 7.23-7.25 (m,4H) and

-2.75 (s, 2H). FAB MS (positive mode): *m/z* = 647 [M-OH + H]$^+$. IR (KBr, ν_{max}, cm^{-1}): λ = 3508, 1608, 1510, 1350, 1267, 1171, 966, 799 and 723.

4*p*-OH-TPP: benzaldehyde (0.53 g, 5 mmol), *p*-hydroxybenzaldehyde (1.83 g, 15 mmol) and propionic acid (200 mL) were used. The fifth band gave the desired 4*p*-OH-TPP. Yield: 200 mg (5.9 %). ^{1}H NMR ($CDCl_3$): δ = 8.86-8.90 (m, 8H), 8.22-8.25 (m, 4H), 8.09-9.11 (m, 4H), 7.77-7.79 (m, 4H), 7.24-7.26 (m, 4H) and -2.75 (s, 2H). FAB MS (positivemode): *m/z* = 647 [M-2OH + H]$^+$. IR (KBr, ν_{max}, cm^{-1}): λ = 3945, 1599, 1506, 1469, 1340, 1261, 1171, 970, 800 and 711.

4*m*-OH-TPP: A solution of *m*-hydroxybenzaldehyde (2.44 g, 20 mmol) in propionic acid (200 mL) was heated to 130 °C. Then freshly distilled pyrrole (1.34 g, 20 mmol) in propionic acid (50 mL) was added slowly to the solution over a period of 0.5 h. The reaction mixture was heated to reflux for another 0.5 h and then cooled to room temperature. Then the reaction mixture was reduced to dry under reduced pressure. The resultant was chromatographed on a silica gel column with $CHCl_3$ as eluent. Yield: 149 mg (4.4 %). ^{1}H NMR ($CDCl_3$): δ = 9.90 (s, 4H), 8.90 (m, 6H), 7.61-7.63 (m, *J* = 8.4Hz, 10H), 7.32-7.39 (m, 2H), 6.85-7.08 (m, 2H)and -2.96 (s, 2H). FAB MS (positive mode): *m/z* = 679 [M]$^+$. IR (KBr, ν_{max}, cm^{-1}): λ = 3387, 1701, 1582, 1440, 1288, 1223, 1163, 1080, 922 and 802.

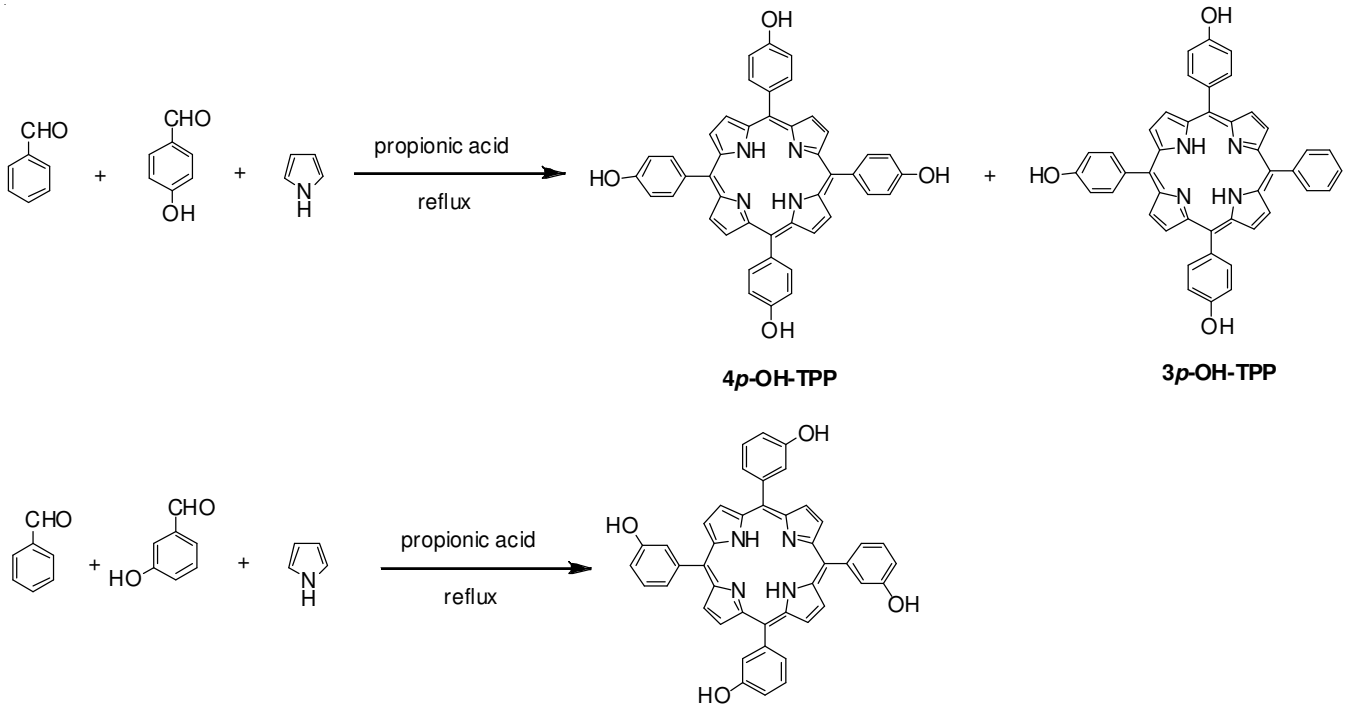
RESULTS AND DISCUSSION

Synthesis of hydroxy amphiphilic porphyrins: The synthetic routes for the preparation of hydroxy amphiphilic-porphyrins are shown in **Scheme-I**. *p*-hydroxybenzaldehyde, benzaldehyde and pyrrole was refluxed in propionic acid to give the products 3*p*-OHTPP (3.2 %) and 4*p*-OHTPP (5.9 %). *m*-Hydroxybenzaldehyde and pyrrole was refluxed in propionic acid to give the product 4*m*-OH-TPP (4.4 %). The amphiphilic porphyrins showed good solubility in common organic solvents like $CHCl_3$, CH_2Cl_2, CH_3OH, DMF and DMSO *etc*. The complexes were fully characterized by ^{1}H NMR, IR, FAB-MS, UV-visible spectrum and fluorescence analysis. The ^{1}H NMR spectra of hydroxyl amphiphilic porphyrins show singlets at δ = -2.75 to -2.96 ppm for the inner NH protons of the porphyrin rings (experimental section).

Absorption titrations: The UV-visible absorption spectra of amphiphilic porphyrinsin the absence and presence of increasing amounts of CT DNA are given in Fig. 1. The UV-visible absorptions of these amphiphilic porphyrins retain the characteristics features of H_2TPP. They show a strong Soret band in the blue region and weak Q-bands in the visible region. The titration of DNA induces large spectral perturbations in the Soret bands of all the porphyrins. The hypochromism reach 58.0, 61.4 and 42.8 % for 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP, respectively (Table-1). These porphyrins exhibited a remarkable decrease in intensity, but the absorption bands do not have any red shift with the titration of DNA.

The UV-visible spectra of amphiphilic porphyrins show no red shift and hypochromism of Soret bands from 42.8 to 61.4 %, which indicated the porphyrins adopt an outside binding mode[9]. All physical data about UV-titration experiments are listed inTable-1.

Emission titrations: The fluorescence spectra of these amphiphilic porphyrins also retain the characteristics features of H_2TPP. The products emitted at about 650 and 720 nm. The fluorescence spectra and data of amphiphilic porphyrins are shown in Fig. 2. We also carried out the emission titration experiments for 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP with DNA (Fig. 2). In the presence of CT DNA, a decrease in intensity of fluorescence emission is depicted for all porphyrins (21.4-



Scheme-I: Synthesis of 4*p*-OHTPP, 3*p*-OHTPP and 4*m*-OHTPP

TABLE-1
CHANGES IN ELECTRONIC SPECTRA OF AMPHIPHILIC PORPHYRINS IN THE ABSENCE OR PRESENCE OF CT-DNA IN *tris*-HCl BUFFER (pH = 7.2, 0.05 M NaCl)

Compound	UV–visible on the Soret band				Fluorescence emission	$K_{app}(M^{-1})$
	λ_{max} (free) nm	λ_{max} (bound) nm	$\Delta\lambda$ (nm)	H (%)	Decrease of intensity (%)	
4*p*OH-TPP	430	430	0	58.0	34.7	$1.54 \times 10^7\ M^{-1}$
3*p*OH-TPP	433	433	0	61.4	50.1	$1.59 \times 10^7\ M^{-1}$
4*m*OH-TPP	426	426	0	42.8	21.4	$1.47 \times 10^7\ M^{-1}$

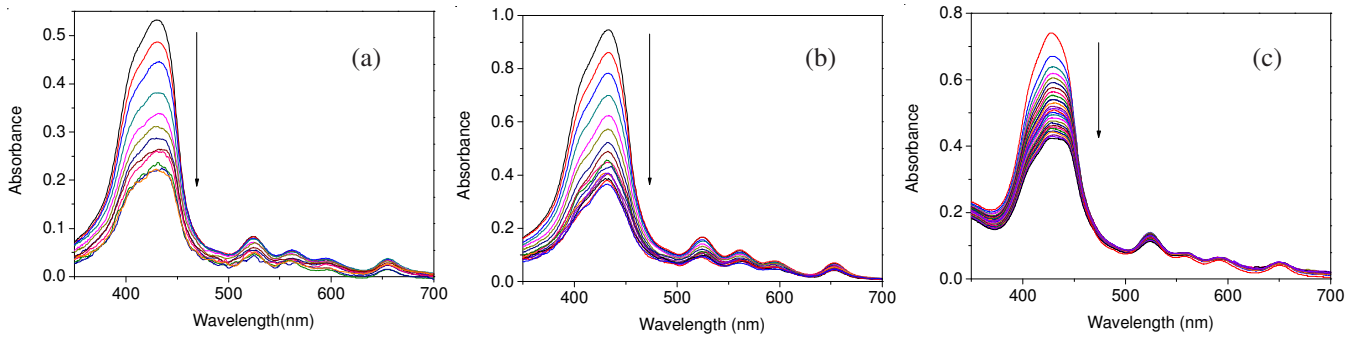


Fig. 1. Absorption spectra of 4*p*-OHTPP (a), 3*p*-OHTPP (b) and 4*m*-OHTPP (c) in *tris* buffer (pH = 7.2, 0.05 M NaCl) at 25 °C in the presence of increasing amounts of CT DNA. [Por] = 10 μM. Arrows indicate the change in absorbance upon increasing the DNA concentration

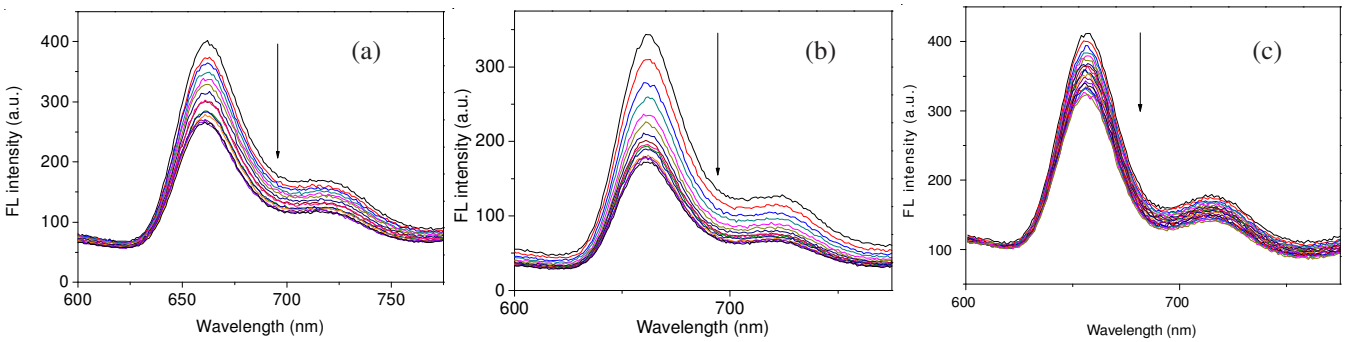


Fig. 2. Emission spectra of 4*p*-OHTPP (a), 3*p*-OHTPP (b) and 4*m*-OHTPP (c) in *tris*buffer (pH = 7.2, 0.05 MNaCl) at 25 °C in the presence of increasing amounts of CT DNA. [Por] = 10 μM. Arrows indicate the change in emission upon increasing the DNA concentration

50.1 %, Table-1). This phenomenon indicated that self stacking of the neighbouring porphyrin molecules to each other along the DNA surface.

DNA binding constant experiments: We carried out the EB competitive binding experiments and the florescence quenching plot are given in Fig. 3. The I_0/I *vs.* [Por]/[DNA] plot is good agreement with the linear Stern-Volmer equation ($I/I_0 = 1 + KQ$) with a slope of 11.72, 12.02 and 10.65 for 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP, respectively. We can also learn Fig. 3 that 50 % of EB molecules were replaced from DNA-bound EB at a concentration ratio of [Por]/[EB] = 0.65, 0.63 and 0.68 for 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP, respectively. The K_{app} of EB in the experimental condition is $1 \times 10^7\ M^{-1}$, therefore, the K_{app} of 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP were $1.54 \times 10^7\ M^{-1}$ and $1.59 \times 10^7\ M^{-1}$ and $1.47 \times 10^7\ M^{-1}$, respectively (Table-1)[10].

Conclusion

Three hydroxy amphiphilic porphyrins were prepared and fully characterized by ^1H NMR, IR, FAB-MS and UV-visible spectroscopy. The UV-visible spectra of amphiphilic porphyrins show no red shift and hypochromism of Soret bands from 42.8

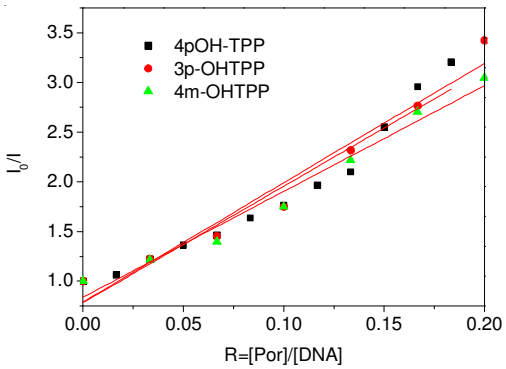


Fig. 3. Florescence quenching curves of DNA-bound EB by amphiphilic porphyrins in *tris* buffer (pH = 7.2, 0.05 M NaCl). ([DNA] = 100 μM, [EB] = 16 μM, λ_{exc} = 537 nm)

to 61.4 %, which indicated the porphyrins adopt an outside binding mode. The emission titration experiments indicate that self stacking of the neighbouring porphyrin molecules to each other along the DNA surface.The DNA binding constant experiments revealed the K_{app} of 4*p*OH-TPP, 3*p*OH-TPP and 4*m*OH-TPP were $1.54 \times 10^7\ M^{-1}$ and $1.59 \times 10^7\ M^{-1}$ and $1.47 \times 10^7\ M^{-1}$, respectively.

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