



## Synthesis of Cationic Porphyrins as Potential Gene Transfection Carriers

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### ABSTRACT

Porphyrins are found having strong but reversible interaction with nucleic acid and thus can be used as complementary to other non-viral vectors in gene transfection delivery. Amphiphilic porphyrins with combination of hydrophobic and hydrophilic substituents along the peripheral of porphyrin macrocycle were synthesized with an attempt to facilitate the membrane penetration as well as better accumulation on the targeted cells. Besides, their fluorescent properties can be used as marker to identify their localization in the intracellular domain. Adler-Longo condensation method was employed to synthesize basic cationic porphyrins and novel asymmetrical amphiphilic cationic porphyrins. All compounds were characterized using <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, Ultraviolet (UV) and Infrared (IR) spectroscopy.

| Porphyrins | Condensation | Amphiphilic | Vectors | Gene Transfection |

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## 1. INTRODUCTION

*meso*-Substituted porphyrins have been extensively explored in laboratory synthesis due to their expanding array of applications in various fields, such as photodynamic therapy [1-3], artificial photosynthesis [4,5] and sensor. Recently, discovery of cationic porphyrins which have ability to form strong but reversible interaction with DNA [6,7], low toxicity towards cell and prolong survival [8] has sparked the interest of researchers to intensively investigate their use in gene therapy.

As low target specificity of non-viral vectors and severe immune response to viral vectors [9] always being the two main impediments to gene transfection efficiency, porphyrins with different number and distribution of hydrophilic and hydrophobic ligands are developed as an alternative to viral-mediated vectors. Besides, the fluorescence properties of porphyrin also can be employed as gene marker in the cellular domain [10].

*meso*-Substituted porphyrins are favoured over the  $\beta$ -substituted porphyrins in the laboratory synthesis due to the amendable substituents around the macrocycle peripheral. A well known method to synthesize the porphyrins and their derivatives is based on the condensation reaction between the pyrrole and the aldehydes derivatives [11,12]. Adler-Longo method was employed in this study to synthesize both symmetrical and asymmetrical porphyrins precursors which are then further functionalized to desired cationic porphyrins.

In the present work, basic cationic porphyrin as well as several novel asymmetrical and cationic amphiphilic porphyrins were successfully synthesized.

## 2. EXPERIMENTAL

### 2.1 Materials, Method and Instruments

<sup>1</sup>H NMR and <sup>13</sup>C NMR data were acquired on a Bruker Advance 400 MHz NMR spectrometer. UV-visible spectra were recorded using Perkin Elmer, Lambda 25 UV/VIS Spectrometer, while the infrared (IR) spectra were obtained using Shimadzu FTIR-8300 spectrometer. Both nujol mull and KBr were used in sample preparation. Melting point was recorded using Barnstead Electrothermal instrument. Thin layer chromatography used for monitor the reactions was performed on silica gel 60F<sub>254</sub> (Merck) pre-coated aluminium sheet. Column chromatography and flash chromatography were carried out using Scharlau Silica Gel 60 (70-230mesh) and Fluka Silica Gel 60 (230-400mesh).

### 2.2 Symmetrical Porphyrins

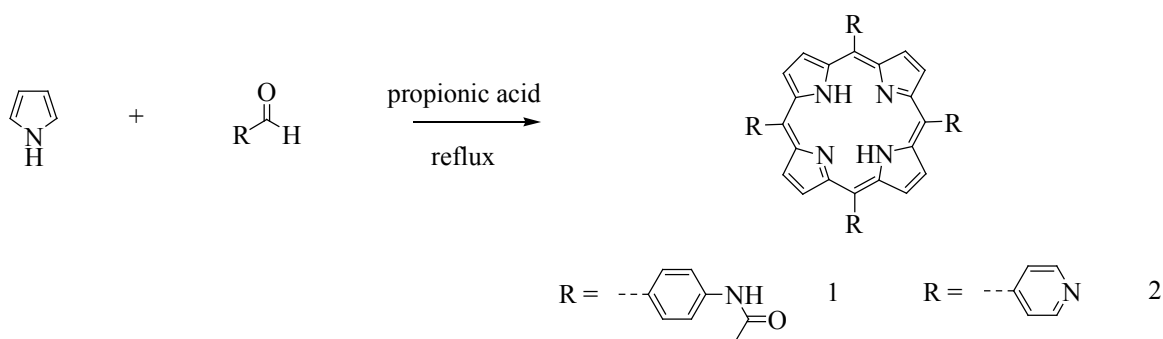
5,10,15,20-tetrakis(4-acetamidophenyl)porphyrin (1). Pyrrole (0.57mL, 8.15mmol) and 4-acetamidobenzaldehyde (1.33g, 8.15mmol) were refluxed in propionic acid (100mL) for 2h, controlled under TLC (methanol/*n*-hexane 3:2) (**Scheme 1**). 250mL distilled water was added into the cooled mixture and the resulting mixture was stirred in solid sodium acetate for 1h and then left

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overnight. Precipitate formed was collected and the impurities were removed by chromatography. Organic solvent was evaporated and the residue was dried under vacuum to afford a dark purple solid porphyrin (320mg, 18.60%). mp > 400°C.  $R_f$ =0.51 (methanol/*n*-hexane 3:2).  $^1\text{H NMR}$  (400MHz, DMSO)  $\delta_{\text{H}}$  ppm -2.92 (s, 2H, pyrrole N-H), 2.22 (s, 12H, COCH<sub>3</sub>), 8.04 (d, 8H,  $J$ =8.4Hz, *m*-phenyl), 8.12 (d, 8H,  $J$ =8.4Hz, *o*-phenyl), 8.85 (s, 8H,  $\beta$ -pyrrole), 10.51 (s, 4H, NHCO). UV-Vis (MeOH)  $\lambda_{\text{max}}$ /nm (log  $\epsilon$ ) 417.81 (5.14), 515.02 (4.76), 551.18 (3.60), 591.12 (3.30), 648.76 (3.33). IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>)(nujol): 3340.65 (N-H), 3164.83 (=C-H aromatic), 2923.59 and 2853.72 (C-H), 1664.09 (C=O amide), 1459.32 (C=C aromatic), 1377.01 and 1304.15 (C-N), 1154.74 (N-H bend).

5,10,15,20-tetrakis(4-pyridyl)porphyrin (2). Pyrrole (20.82mL, 0.3mol) and 4-pyridinecarboxaldehyde (28.2mL, 0.3mol) were refluxed in propionic acid (200mL) for 2h,



**Scheme 1** Synthesis of Symmetrical Porphyrins

### 2.3 Asymmetrical Porphyrins

5-propyl-10,15,20-tris(4-pyridyl)porphyrin (3). Pyrrole (13.88mL, 0.2mol), 4-pyridinecarboxaldehyde (14.12mL, 0.15mol) and butanal (4.45mL, 0.05mol) were refluxed in propionic acid (100mL) for 2h, controlled under TLC (CH<sub>2</sub>Cl<sub>2</sub>/methanol 100:3) (**Scheme 2a**). The cooled mixture was added with distilled water and stirred in solid sodium acetate for 1h. Precipitation was allowed to take place overnight and precipitate was collected after 24h. The product mixtures were then dissolved in dichloromethane and the desired porphyrin was obtained as the fourth fraction using column chromatography (silica gel, dichloromethane/ethyl acetate 100:15). Recrystallization of the target fraction afforded a purple solid (227mg, 0.79%). mp > 400°C. TLC analysis (CH<sub>2</sub>Cl<sub>2</sub>/methanol 100:3)  $R_f$  0.26.  $^1\text{H NMR}$  (400MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  ppm -2.82 (s, 2H, pyrrole N-H), 1.36 (t, 3H,  $J$ =7.2Hz, CH<sub>3</sub>), 2.62 (m, 2H, CH<sub>2</sub>), 5.05 (t, 2H,  $J$ =8Hz, CH<sub>2</sub>), 8.16 (dd, 6H,  $J$ =5.6Hz,  $J$ =10.8Hz, *m*-py), 8.82 (s, 4H,  $\beta$ -pyrrole), 8.91 (d, 2H,  $J$ =4.8Hz,  $\beta$ -pyrrole), 9.04 (s, 2H,  $\beta$ -pyrrole), 9.07 (d, 4H,  $J$ =5.6Hz, *o*-py), 9.59 (d, 2H,  $J$ =5.2Hz, *o*-py).

5-hexyl-10,15,20-tris(4-pyridyl)porphyrin (4a). The synthesis process is similar to the procedure employed in

controlled under TLC (methanol/*n*-hexane 3:2) (**Scheme 1**). 250mL distilled water was added into the cooled mixture. The resulting mixture was stirred in solid sodium acetate for 1h and left overnight. Precipitate formed was filtered and washed with methanol to afford a purple solid porphyrin (6.39g, 13.77%). mp > 400°C.  $R_f$ =0.43 (methanol/*n*-hexane 3:2).  $^1\text{H NMR}$  (400MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  ppm -2.92 (s, 2H, pyrrole N-H), 8.18 (d, 8H,  $J$ =6Hz, *m*-py), 8.89 (s, 8H,  $\beta$ -pyrrole), 9.08 (d, 8H,  $J$ =5.6Hz, *o*-py).  $^{13}\text{C NMR}$  (400MHz, CDCl<sub>3</sub>)  $\delta$  ppm 117.79, 129.30, 148.39, 149.79. UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\text{max}}$ /nm (log  $\epsilon$ ) 415.93 (5.59), 512.00 (4.28), 544.78 (3.71), 586.98(3.75), 642.73(3.32). IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>)(nujol): 3390.10 (N-H), 3181.31 (=C-H aromatic), 1459.18 (C=C aromatic).

preparing (3) using pyrrole (2.78mL, 0.04mol), 4-pyridinecarboxaldehyde (2.82mL, 0.03mol) and heptanal (1.39mL, 0.01mol) (**Scheme 2a**). Recrystallization of the fifth fraction from column chromatography (silica gel, dichloromethane/ethyl acetate 100:15) afforded a purple compound (250mg, 4.00%). mp > 400°C.  $R_f$  =0.14 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3).  $^1\text{H NMR}$  (400MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  ppm -2.82 (s, 2H, pyrrole N-H), 0.94 (t, 3H,  $J$ =7.2Hz, CH<sub>3</sub>), 1.42 (m, 2H, CH<sub>2</sub>), 1.51 (m, 2H, CH<sub>2</sub>), 1.84 (m, 2H, CH<sub>2</sub>), 2.57 (m, 2H, CH<sub>2</sub>), 5.06 (t, 2H,  $J$ =7.6Hz, CH<sub>2</sub>), 8.16 (m, 6H, *m*-py), 8.82 (s, 4H,  $\beta$ -pyrrole), 8.91 (d, 2H,  $J$ =4.8Hz,  $\beta$ -pyrrole), 9.06 (m, 6H, *o*-py), 9.58 (d, 2H,  $J$ =4.8Hz,  $\beta$ -pyrrole). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\text{max}}$ /nm (log  $\epsilon$ ) 416.68(5.55), 513.89 (3.98), 548.17 (3.94), 589.99(3.84), 647.26(3.72).

5,15-dihexyl-10,20-bis(4-pyridyl)porphyrin (4b). Obtained as the third fraction during the synthesis of (4a) as purple solid (60mg, 0.95%). mp > 400°C.  $R_f$  =0.43 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3).  $^1\text{H NMR}$  (400MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  ppm -2.75 (s, 2H, pyrrole N-H), 0.93 (t, 6H,  $J$ =7.2Hz, CH<sub>3</sub>), 1.40 (m, 4H, CH<sub>2</sub>), 1.51 (m, 4H, CH<sub>2</sub>), 1.80 (m, 4H, CH<sub>2</sub>), 2.52 (m, 4H, CH<sub>2</sub>), 4.98 (t, 4H,  $J$ =8Hz, CH<sub>2</sub>), 8.16 (m, 4H, *m*-py), 8.83 (d, 4H,  $J$ =4.4Hz,  $\beta$ -pyrrole), 9.06 (d, 4H,  $J$ =5.6Hz, *o*-py), 9.49 (d, 4H,  $J$ =4.8Hz,  $\beta$ -pyrrole). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)

$\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 417.06 (5.71), 516.15 (4.61), 550.43 (4.28), 592.63 (4.02), 651.02 (4.17).

5,10-dihexyl-15,20-bis(4-pyridyl)porphyrin (4c).

Obtained as the fourth fraction during the synthesis of (4a) as purple solid (240mg, 3.79%). mp > 400°C.  $R_f$  = 0.20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm -2.74 (s, 2H, pyrrole N-H), 0.96 (t, 6H,  $J$ =7.2Hz, CH<sub>3</sub>), 1.42 (m, 4H, CH<sub>2</sub>), 1.55 (m, 4H, CH<sub>2</sub>), 1.85 (m, 4H, CH<sub>2</sub>), 2.57 (m, 4H, CH<sub>2</sub>), 5.03 (t, 4H,  $J$ =7.6Hz CH<sub>2</sub>), 8.14 (d, 4H,  $J$ =5.6Hz,  $m$ -py), 8.74 (s, 2H,  $\beta$ -pyrrole), 8.83 (d, 2H,  $J$ =4.4Hz,  $\beta$ -pyrrole), 9.04 (d, 6H,  $J$ =5.6Hz,  $o$ -py), 9.52 (d, 2H,  $J$ =4.8Hz,  $\beta$ -pyrrole), 9.62 (s, 2H,  $\beta$ -pyrrole). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 417.06 (5.57), 516.15 (4.13), 550.81 (3.78), 593.00 (3.55), 649.89 (3.58).

5-(methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)porphyrin (5a). The synthesis process is similar to the procedure employed in preparing (3) using pyrrole (13.88mL, 0.2mol), 4-pyridinecarboxaldehyde (14.12mL, 0.15mol) and methyl-4-formylbenzoate (5.21g, 0.05mol) (Scheme 2b). The desired porphyrin was obtained as the fifth fraction using column chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/ethyl acetate 100:15). Recrystallization of the target fraction afforded a purple crystal compound (681mg, 2.02%). mp > 300°C  $R_f$  = 0.23 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm -2.80 (s, 2H, pyrrole N-H), 4.14 (s, 3H, OCH<sub>3</sub>), 8.18 (dd, 6H,  $J$ =1.6Hz,  $J$ =4.4Hz,  $m$ -py), 8.31 (d, 2H,  $J$ =8.4Hz, phenyl-H), 8.48 (d, 2H,  $J$ =8.4Hz, phenyl-H), 8.86 (d, 2H,  $J$ =4.4Hz,  $\beta$ -pyrrole), 8.88 (s, 6H,  $\beta$ -pyrrole), 9.08 (m, 6H,  $o$ -py). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 417.06 (5.64), 513.13 (4.57), 547.04 (4.13285), 588.11 (4.13276), 643.86 (3.99).

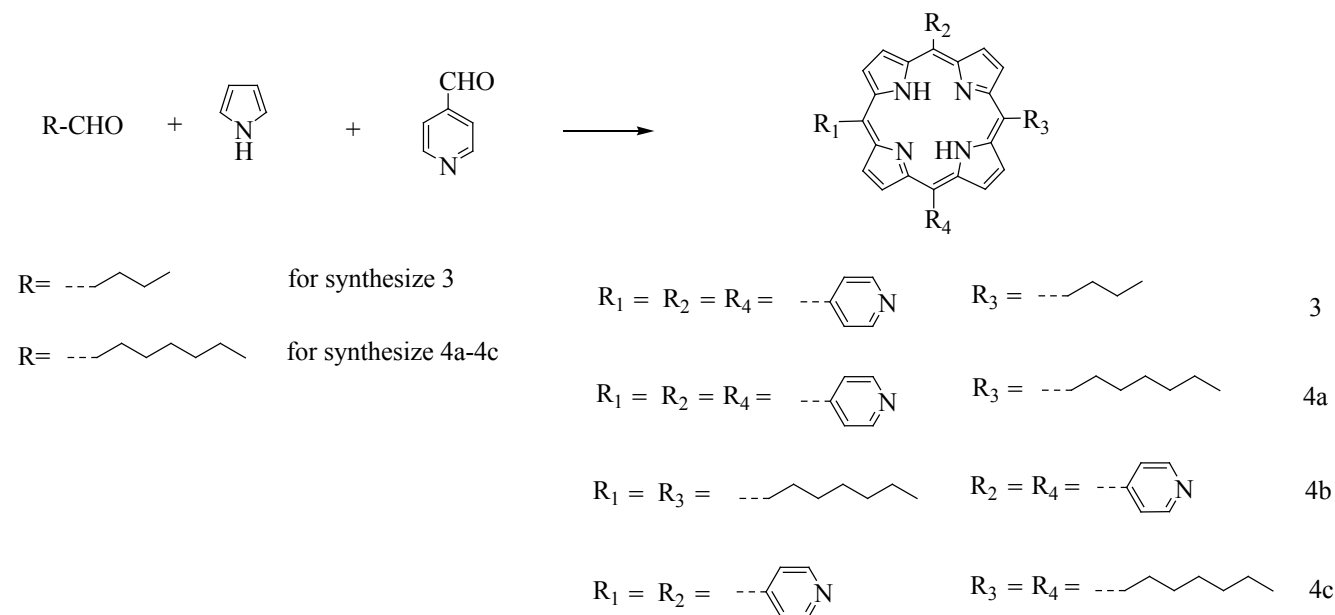
5,10,15,20-tetra(methoxycarbonylphenyl)porphyrin (5b). Obtained as the first fraction during the synthesis of (5a) as purple solid (4.9mg, 0.01%). mp > 300°C.  $R_f$  = 1.00 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm

-2.80 (s, 2H, pyrrole N-H), 4.13 (s, 12H, OCH<sub>3</sub>), 8.31 (d, 8H,  $J$ =8Hz, phenyl-H), 8.46 (d, 8H,  $J$ =8Hz, phenyl-H), 8.83 (s, 8H,  $\beta$ -pyrrole).

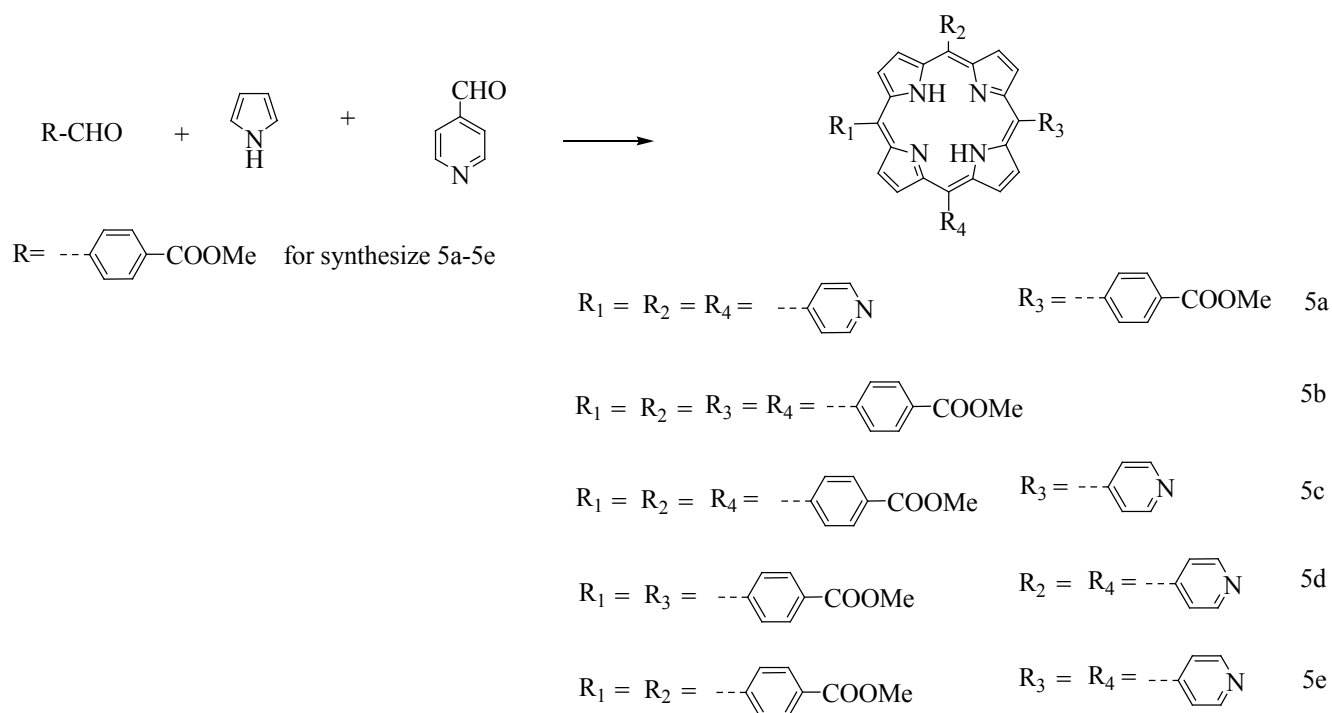
5,10,15-tri(methoxycarbonylphenyl)-20-(4-pyridyl)porphyrin (5c). Obtained as the second fraction during the synthesis of (5a) as purple solid (94.4mg, 0.24%). mp > 300°C.  $R_f$  = 0.63 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm -2.83 (s, 2H, pyrrole N-H), 4.13 (s, 9H, OCH<sub>3</sub>), 8.18 (dd, 2H,  $J$ =1.6Hz,  $J$ =4.4Hz,  $m$ -py), 8.31 (d, 6H,  $J$ =8Hz, phenyl-H), 8.47 (dd, 6H,  $J$ =1.2Hz,  $J$ =8Hz, phenyl-H), 8.84 (s, 6H,  $\beta$ -pyrrole), 8.86 (d, 2H,  $J$ =4.8Hz,  $\beta$ -pyrrole), 9.06 (d, 2H,  $J$ =5.6Hz,  $o$ -py). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 418.95 (5.38), 515.02 (4.50), 549.30 (4.41), 589.61 (4.40), 645.37 (4.39).

5,15-di(methoxycarbonylphenyl)-10,20-bis(4-pyridyl)porphyrin (5d). Obtained as the third fraction during the synthesis of (5a) as purple solid (169.9mg, 0.46%). mp > 300°C.  $R_f$  = 0.44 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm -2.86 (s, 2H, pyrrole N-H), 4.14 (s, 6H, OCH<sub>3</sub>), 8.18 (dd, 4H,  $J$ =1.6Hz,  $J$ =4.4Hz,  $m$ -py), 8.31 (d, 4H,  $J$ =8Hz, phenyl-H), 8.48 (d, 4H,  $J$ =8Hz, phenyl-H), 8.86 (q, 8H,  $J$ =4.8Hz,  $\beta$ -pyrrole), 9.06 (dd, 4H,  $J$ =4.4Hz,  $J$ =1.6Hz,  $o$ -py). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 417.81 (5.52), 513.51 (4.30), 548.17 (3.96), 589.99 (3.94), 644.99 (3.93).

5,10-di(methoxycarbonylphenyl)-15,20-bis(4-pyridyl)porphyrin (5e). Obtained as the fourth fraction during the synthesis of (5a) as purple solid (695.8mg, 1.9%). mp > 300°C.  $R_f$  = 0.43 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta_H$  ppm -2.85 (s, 2H, pyrrole N-H), 4.14 (s, 6H, OCH<sub>3</sub>), 8.18 (d, 4H,  $J$ =5.6Hz,  $m$ -py), 8.31 (d, 4H,  $J$ =8Hz, phenyl-H), 8.48 (d, 4H,  $J$ =8Hz, phenyl-H), 8.86 (d, 8H,  $J$ =8Hz,  $\beta$ -pyrrole), 9.07 (d, 4H,  $J$ =5.6Hz,  $o$ -py). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 418.95 (5.68), 513.89 (4.57), 548.17 (4.19), 589.24 (4.14), 644.99 (4.05).



Scheme 2a Synthesis of Asymmetrical Porphyrins.



Scheme 2b Synthesis of Asymmetrical Porphyrins.

## 2.4 Basic Cationic Porphyrins

5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrin (6). Porphyrin (2) (0.1g, 0.16mmol) was dissolved in 20mL dimethylformamide. The solution was warmed to 130°C and methyl-*p*-toluenesulfonate (1mL) was added. The mixture was refluxed for 4h. Precipitate formed was filtered and washed with chloroform to afford a purple solid porphyrin (80mg, 72.91%). mp > 400°C.  $R_f=0.03$  (CH<sub>2</sub>Cl<sub>2</sub>/ACN 1:2). <sup>1</sup>H NMR (400MHz, DMSO)  $\delta_H$  ppm -3.13 (s, 2H, pyrrole N-H), 4.71 (s, 12H, N-CH<sub>3</sub>), 8.97 (d, 8H,  $J=6.8$ Hz, *m*-py), 9.17 (s, 8H,  $\beta$ -pyrrole), 9.46 (d, 8H,  $J=6.8$ Hz, *o*-py). UV-Vis (MeOH)  $\lambda_{max}/nm$  (log  $\epsilon$ ) 423.84 (4.40), 515.02 (3.33), 548.92 (2.98), 591.87 (2.94), 646.12 (2.59).

5,10-bis(*N*-methyl-4-pyridyl)-15,20-bis(4-pyridyl)porphyrin or 5,15-bis(*N*-methyl-4-pyridyl)-10,20-bis(4-pyridyl) porphyrin (7). Porphyrin (2) (0.1g, 0.16mmol) was dissolved in 40mL chloroform. Excess methyl iodide (10mL) was added and the mixture was stirred under nitrogen atmosphere at 40°C for 24 h. Precipitate formed was filtered and washed with chloroform. Drying of precipitate under vacuum afforded a purple solid porphyrin (110mg, 100%). mp > 400°C.  $R_f=0.94$  (ACN/water 7:3). <sup>1</sup>H NMR (400MHz, DMSO)  $\delta_H$  ppm -3.07 (d, 2H,  $J=5.2$ Hz, pyrrole N-H), 4.69 (s, 6H, N-CH<sub>3</sub>), 8.26 (d, 4H,  $J=4.4$ Hz, *m*-py), 8.98 (s, 6H, *m*-py,  $\beta$ -pyrrole and *o*-py), 9.06 (d, 8H,  $J=5.6$ Hz, *m*-py,  $\beta$ -pyrrole and *o*-py), 9.43 (s, 4H, *o*-py). UV-Vis (MeOH)  $\lambda_{max}/nm$  (log  $\epsilon$ ) 418.19 (5.26), 512.76 (4.13), 548.92 (3.73), 588.86 (3.67), 648.76 (1.55). IR  $\nu_{max}$  (cm<sup>-1</sup>) (nujol): 3362.63 (N-H), 3170.32 (=C-H aromatic), 2924.85

and 2853.94 (C-H, from methanol solvent), 1637.88 (N-H bend), 1459.49 (C=C aromatic).

5,10,15-tris(*N*-methyl-4-pyridyl)-20-(4-pyridyl)porphyrin (8). Porphyrin (2) (0.1g, 0.16mmol) was dissolved in 40mL chloroform. Excess methyl iodide (10mL) was added and the mixture was stirred under nitrogen atmosphere at 40°C for 24h. Precipitate formed was filtered and washed with chloroform. Drying of precipitate under vacuum afforded a purple solid porphyrin (90mg, 82.04%). mp > 400°C.  $R_f=0.03$  (CH<sub>2</sub>Cl<sub>2</sub>/ACN 1:5). <sup>1</sup>H NMR (400MHz, DMSO)  $\delta_H$  ppm -3.07 (d, 2H,  $J=4.8$ Hz, pyrrole N-H), 4.70 (d, 9H,  $J=8$ Hz, N-CH<sub>3</sub>), 8.26 (d, 4H,  $J=4.4$ Hz, *m*-py), 8.98 (m, 8H, *o*-py, *m*-py and  $\beta$ -pyrrole), 9.07 (m, 8H, *o*-py, *m*-py and  $\beta$ -pyrrole), 9.43 (d, 4H,  $J=5.2$ Hz, *o*-py, *m*-py and  $\beta$ -pyrrole).

## 2.5 Amphiphilic Cationic Porphyrins

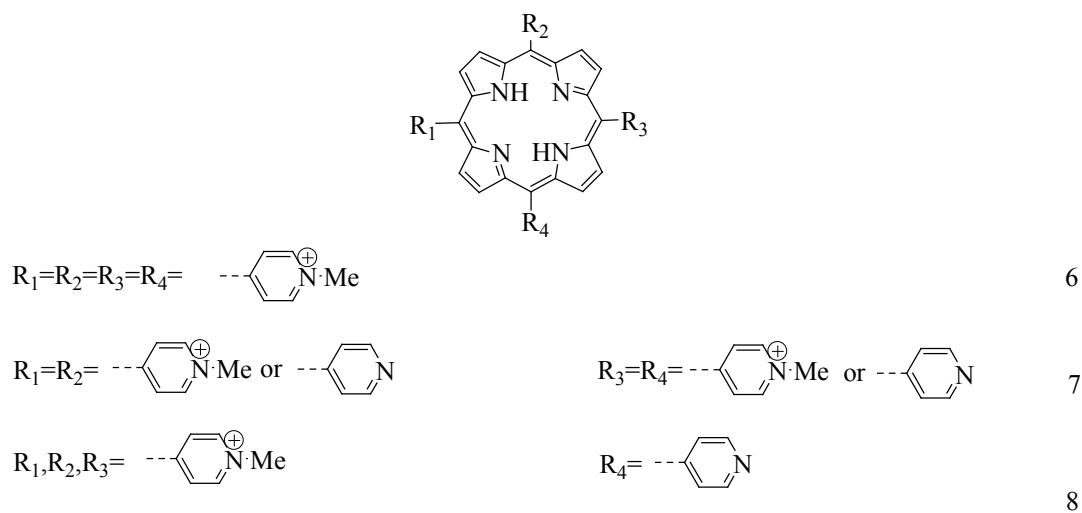
5-hexyl-10,15,20-tris(*N*-methyl-4-pyridyl)porphyrin (9). Porphyrin (4) (140mg, 0.22mmol) was dissolved in 40mL chloroform. Excess methyl iodide (7.1mL) was added and the mixture was stirred under nitrogen atmosphere at 40°C for 24 h. Precipitate formed was filtered and washed with chloroform to afford a purple solid porphyrin (150mg, 100%). mp > 400°C.  $R_f=0$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3). <sup>1</sup>H NMR (400MHz, DMSO)  $\delta_H$  ppm -3.00 (s, 2H, pyrrole N-H), 0.87 (t, 3H,  $J=7.2$ Hz, CH<sub>3</sub>), 1.32 (m, 2H, CH<sub>2</sub>), 1.44 (m, 2H, CH<sub>2</sub>), 1.80 (m, 2H, CH<sub>2</sub>), 2.43 (m, 2H, CH<sub>2</sub>), 4.71 (m, 9H, N-CH<sub>3</sub>), 5.16 (t, 2H,  $J=7.6$ Hz, CH<sub>2</sub>), 8.94 (d, 2H,  $J=6.4$ Hz, *m*-py), 8.98 (d, 4H,  $J=6$ Hz, *m*-py), 9.08 (s, 6H,  $\beta$ -pyrrole),

9.46 (d, 6H,  $J=6.4\text{Hz}$ , *o*-py), 10.00 (d, 2H,  $J=2.8\text{Hz}$ ,  $\beta$ -pyrrole). UV-Vis (MeOH)  $\lambda_{\text{max}}/\text{nm}$  (log  $\epsilon$ ) 424.97 (5.71), 518.41 (4.61), 554.95 (4.34), 593.00 (4.18), 651.78 (4.00).

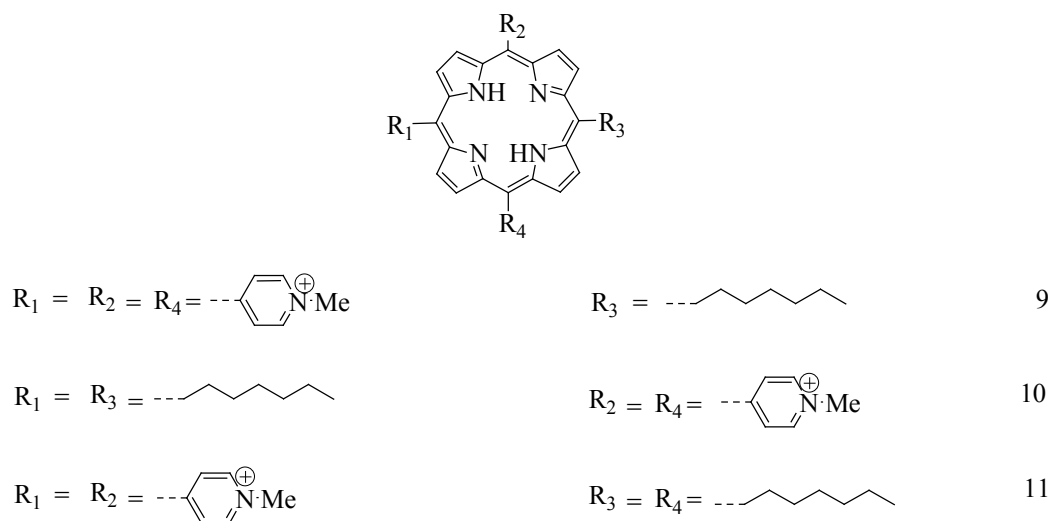
5,15-dihexyl-10,20-bis(*N*-methyl-4-pyridyl)porphyrin (10). Porphyrin (4d) (20mg, 0.03 mmol) was dissolved in 25mL chloroform. Excess methyl iodide (2mL) was added and the mixture was stirred under nitrogen atmosphere at 40°C for 24 h. Precipitate formed was filtered and washed with chloroform to afford a purple solid porphyrin (21mg, 100%). mp > 400°C.  $R_f = 0$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  100:3).  $^1\text{H NMR}$  (400MHz, DMSO)  $\delta_{\text{H}}$  ppm -2.97 (s, 2H, pyrrole N-H), 0.84 (t, 6H,  $J=7.2\text{Hz}$ ,  $\text{CH}_3$ ), 1.29 (m, 4H,  $\text{CH}_2$ ), 1.40 (m, 4H,  $\text{CH}_2$ ), 1.72 (m, 4H,  $\text{CH}_2$ ), 2.43 (m, 4H,  $\text{CH}_2$ ), 4.70 (s, 6H, N- $\text{CH}_3$ ), 5.05 (t, 4H,  $J=7.6\text{Hz}$ ,  $\text{CH}_2$ ), 8.95 (d, 4H,  $J=6.4\text{Hz}$ , *m*-py), 8.99 (d, 4H,  $J=4.8\text{Hz}$ ,  $\beta$ -pyrrole), 9.40 (d, 4H,  $J=6.4\text{Hz}$ , *o*-py), 9.85 (d, 4H,  $J=4.8\text{Hz}$ ,  $\beta$ -pyrrole). UV-Vis (MeOH)  $\lambda_{\text{max}}/\text{nm}$  (log  $\epsilon$ )

421.96 (5.61), 518.03 (4.60), 558.72 (4.53), 597.15 (4.28), 655.92 (4.48).

5,10-dihexyl-15,20-bis(*N*-methyl-4-pyridyl)porphyrin (11). Porphyrin (4e) (90mg, 0.14mmol) was dissolved in 40mL chloroform. Excess methyl iodide (9mL) was added and the mixture was stirred under nitrogen atmosphere at 40°C for 24 h. Precipitate formed was filtered and washed with chloroform to afford a purple solid porphyrin (50mg, 55.56%). mp > 400°C.  $R_f = 0$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  100:3).  $^1\text{H NMR}$  (400MHz, DMSO)  $\delta_{\text{H}}$  ppm -2.89 (s, 2H, pyrrole N-H), 0.88 (t, 6H,  $J=7.2\text{Hz}$ ,  $\text{CH}_3$ ), 1.33 (m, 4H,  $\text{CH}_2$ ), 1.46 (m, 4H,  $\text{CH}_2$ ), 1.80 (m, 4H,  $\text{CH}_2$ ), 2.44 (m, 4H,  $\text{CH}_2$ ), 4.70 (s, 6H, N- $\text{CH}_3$ ), 5.10 (t, 4H,  $J=7.4\text{Hz}$ ,  $\text{CH}_2$ ), 8.93 (d, 4H,  $J=6\text{Hz}$ , *m*-py), 8.97 (s, 4H,  $\beta$ -pyrrole), 9.42 (d, 4H,  $J=6.4\text{Hz}$ , *o*-py), 9.90 (s, 4H,  $\beta$ -pyrrole). UV-Vis (MeOH)  $\lambda_{\text{max}}/\text{nm}$  (log  $\epsilon$ ) 424.97 (5.38), 521.80 (4.36), 559.10 (4.17), 594.89 (4.01), 651.02 (2.86).



**Scheme 3:** Synthesis of Basic Cationic Porphyrins



**Scheme 4** Synthesis of Amphiphilic Cationic Porphyrins

### 3. RESULTS & DISCUSSION

Adler-Longo method was employed in this study to produce various of *meso*-substituted porphyrins by refluxing pyrrole and aldehyde derivatives in propionic acid. This method allows wider selection of substituted aldehydes and represents a facile and straightforward approach to obtain the desired products.

The asymmetrical porphyrins (3-5) were synthesized by carrying out mixed condensation reaction of pyrrole and aldehyde derivatives. This results in very large number of potential products which can be further functionalized into their corresponding cationic porphyrins. For instance, 5-hexyl-10,15,20-tris(*N*-methyl-4-pyridyl) porphyrin (9) is a product acquired from methylation of its porphyrin precursor (4a), while cationic porphyrin (11) was derived from porphyrin (4c).

Column chromatography using dichloromethane/ethyl acetate (100:15) as eluent, is efficient enough to separate all compounds from the product mixture. In fact, *cis*- and *trans*- isomers were successfully separated in this study and obtained in pure form by column chromatography separation.

Previous study showed that *trans*-isomer of *meso*-(bis)-4'-pyridyl-(bis)-4'-carboxymethylphenylporphyrins is eluted before the *cis*-isomers since the pyridyl groups have a higher affinity towards silica gel compared to methyl-ester group [13]. In accordance to this finding, we hypothesize that *cis*- isomer possesses asymmetrical polarity axis and thus makes it becomes more polar than the *trans*- isomer. Therefore, it was postulated that 5,10-dihexyl-15,20-bis(4-pyridyl)porphyrin (4c) with both higher polarity pyridyl groups located at one side was eluted just after 5,15-dihexyl-10,20-bis(4-pyridyl)porphyrin (4b).

Excess methyl iodide was utilized as methylation agent for most porphyrin precursors. However, the same result could not be obtained in synthesizing water soluble 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrin (6). Interpretation from the NMR spectrum shows only dimethylation occurred to form postulated dimethylated porphyrin (7). Increase amount of methyl iodide only result in trimethylated porphyrin (8). Therefore, different method was employed using methyl-*p*-toluenesulfonate as the alkylating agent and finally the desired tetramethylated porphyrin (6) was obtained.

UV spectra of all products are very similar, with their Soret bands lie in the range between 416nm to 422nm, while four Q bands are distributed along the wavelength ranging from 512nm to 655nm. The different intensity of Q bands in each spectrum is used to classify each compound into different type of porphyrin such as etio, rhodo, oxorhodo and phyllo type. Most porphyrins synthesized in this study are found as etio-type porphyrin. IR spectra cannot clearly elucidate the full structure of the synthetic compounds in this study but only identify presence or absence of simple functional group within a compound.

On the other hand, all compounds display a singlet peak in the range of -2.7ppm to -3ppm in proton NMR. This unique peak is assigned to the two inner pyrrole N-H protons which are highly shielded by the ring current induced by the external field and therefore shifted upfield, beyond tetramethylsilane (TMS). The existence of singlet peak instead of doublet is due to rapid exchange of chemically equivalent protons at the core of macrocycle. All alkyl groups resonate in the range of 0.8ppm to 5ppm while the *m*-pyridyl protons,  $\beta$ -pyrrole protons and *o*-pyridyl protons shows chemical shift at down field, ranging from 8ppm to 10ppm. The N-CH<sub>3</sub> and OCH<sub>3</sub> are resonating at 4.70ppm and 4.13ppm respectively.

### 4. CONCLUSION

All symmetrical and several novel asymmetrical porphyrins were successfully synthesized using the Adler-Longo method. Methylation of porphyrins was performed using excess methyl iodide except 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrin (6) which requires methyl-*p*-toluenesulfonate as methylation agent to prevent incomplete reaction. Second stage of the study will focus on evaluating the porphyrins as alternative gene carrier for gene therapy.

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