Electronic Supporting Information

First silicon (IV) phthalocyanine-nucleoside conjugates with high photodynamic activity

Xiao-Min Shen,[†] Bi-Yuan Zheng,[†] Xiu-Rong Huang, Lei Wang, Jian-Dong Huang*

(College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, China)

Contents

EXPERIMENTAL SECTION

1. General

Reactions were performed under an atmosphere of nitrogen. Acetone and toluene were distilled from sodium and anhydrous calcium sulfate, respectively. Chromatographic purifications were carried out on silica gel columns (100-200 mesh, Qingdao Haiyang Chemical Co., Ltd, China) with the indicated eluents. Size exclusion chromatography was performed on Bio-Rad Bio-Beads S-X3 beads with the indicated eluents. All other solvents and reagents were of reagent grade and used as received. Silicon phthalocyanine dichloride was prepared according to literature procedures.[1]

^{1}H NMR spectra were recorded on Bruker AVANCE III (500MHz or 400MHz) spectrometer. Chemical shifts were relative to internal TMS (δ =0 ppm). High resolution mass spectra were recorded on an Aligent ESI-Q-Tof 6520 mass spectrometer. Low resolution mass spectra were recorded on a Finnigan LCQ Deca xpMAX mass spectrometer. IR spectra were recorded on a Perkine-Elmer SP2000 FT-IR spectrometer, using KBr disks. Electronic absorption spectra were measured on a Shimadzu UV-2450 UV-vis spectrophotometer. Fluorescence spectra were taken on an Edinburgh FL900/FS900 spectrofluorometer.

The purity of all the new phthalocyanines was determined by HPLC and was found to be ≥ 98%. The analytical HPLC experiments were performed on a Kromasir KR100-10C18 column (5μm, 4.6 mm×250 mm) by using a Shimadzu LC-10AT controller with a SPD-M10A diode array detector. The conditions were set as follows: mobile phase from 5% B changed to 95% B over 30 min, then maintained 95% B for a further 20 min (solvent A = distilled water; solvent B = DMF). The column temperature was set at 30℃. The flow-rate was fixed at 0.5 mL min^{-1}.

Statistical analyses were performed by using the Student's *t*-test. *P* values of < 0.05 were considered as statistically significant.

2. Synthesis

2', 3'-O-isopropylideneuridine (9). A mixture of p-toluenesulfonic acid (1.72 g, 10 mmol) and dry acetone (20 ml) was titrated slowly into the mixture of uridine (245 mg, 1 mmol) and dry acetone (20 ml) in ice-water bath. The mixture was stirred at room temperature for 6 h. It was then neutralized with ice sodium bicarbonate solution (4% w.t.) until the bubble disappeared. The

solution was extracted with CH_2Cl_2 for three times. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to afford the product **9** as a faint yellow solid (242 mg, 85%). IR (KBr, cm^{-1}): 3245, 2986, 2935, 1703, 1671, 1468, 1372, 1274, 1121, 1078, 858, 803. ^1H NMR (DMSO-d6, 400MHz, ppm): δ 11.39 (s, 1H, pyrimidine-NH), 7.80 (d, J =8.0Hz, 1H, pyrimidine-NCH), 5.84 (s, 1H, 1′-H), 5.64 (d, J =8.0Hz, 1H, pyrimidine-COCH), 5.09 (s, 1H, OH), 4.90 (t, J =5.6Hz, 1H,2′-H), 4.75 (s, 1H, 3′-H）, 4.07 (s(br), 1H, 4′-H), 3.57-3.58 (m, 2H, 5′-H), 1.49 (s, 3H, Me), 1.29 (s, 3H, Me). MS (EI) : *m/z* calcd for $C_{12}H_{16}N_2NaO_6$ $(M+Na)^+$, 307.1; found, 307.3.

2', 3'-O-isopropyl-5-methyl-uridine (10). According to the above procedure, 5-methyl-uridine (258 mg, 1 mmol) was treated with p-toluenesulfonic acid (1.72 g, 10 mmol) in dry acetone (40 ml) for 16 h. The mixture was then neutralized with ice sodium bicarbonate solution (4% w.t.) to afford a clear solution. The solution was extracted with CH_2Cl_2 for three times. The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was further purified by silica gel column chromatography using ethyl acetate as the eluent to give a white solid **10** (253 mg, 85 %). IR (KBr, cm^{-1}): 3367, 3223, 1666, 1605, 1517, 1484, 1426, 1334, 1289, 1210, 1122, 1080, 909, 781, 758, 739. ^1H NMR ($CDCl_3$，400MHz，ppm): δ 8.92 (s(br), 1H, pyrimidine-NH), 7.14 (s, 1H, pyrimidine-NCH), 5.49 (d, J =3.6Hz, 1H,1′-H), 5.30 (s, 1H, OH), 5.07-5.10(m, 1H, 2′-H), 4.97-4.99 (m, 1H，3′-H), 4.25-4.28 (m, 1H, 3′-H), 3.90-3.93 (m, 1H, 5′-H), 3.78-3.82 (m, 1H, 5′-H), 1.92 (s, 3H, pyrimidine-Me), 1.57 (s, 3H, Me), 1.36 (s, 3H,Me). HRMS (ESI): *m/z* calcd for $C_{13}H_{19}N_2O_6$ $(M+H)^+$, 299.1243; found, 299.0491.

2', 3'-O-isopropyl-cytidine (11). According to the above procedure, cytidine (243 mg, 1 mmol) was treated with p-toluenesulfonic acid (1.72 g, 10 mmol) in dry acetone (40 ml) for 6 h. The mixture was then centrifuged, and the residue was washed with acetone. The crude product was purified by recrystallisation with DMF/ethyl acetate (1:20, v/v) to afford **11** as a white solid (269 mg, 95 %). IR (KBr, cm^{-1}): 3068, 1727, 1693, 1650, 1540, 1383, 1204, 1123, 1033, 1010. ^1H NMR (DMSO-d6, 400MHz, ppm): δ 9.49 (s, 1H, pyrimidine-H), 8.41 (s, 1H, NH_2), 8.09 (d, J =7.6Hz, 1H, NH_2), 6.05 (d, J =7.6Hz, 1H, pyrimidine-H), 5.76 (d, J =1.2Hz, 1H, 1′-H), 4.86 (t, J =3.0Hz, 1H, 2′-H), 4.70-4.72 (m, 1H, 3′-H), 4.21 (d, J =2.8Hz, 1H, 4′-H), 3.51-3.61 (m, 2H, 5′-H), 1.45 (s, 3H, Me), 1.25(s, 3H, Me).

2', 3'-O-isopropyl-5-N-cytidine (12). According to the above procedure, 5-N-cytidine (244 mg, 1 mmol) was treated with p-toluenesulfonic acid (1.72 g, 10 mmol) in dry acetone (40 ml) for 24

h. The mixture was then added with ice sodium bicarbonate solution (4% w.t.) until the formation of white precipitate. The precipitate was collected by filtration and washed with acetone. It was then dried in vacuum to afford **12** as a white solid (256 mg, 90 %). IR(KBr, cm^{-1}): 3415, 3291, 1693, 1610, 1470, 1212, 1158, 1110. ^{1}H NMR (DMSO-d6, 400MHz, ppm)：δ 8.41(s, 1H, pyrimidine-H), 7.60-7.63 (m, 2H, NH_2), 5.72 (d, J =5.2Hz, 1H,1′-H), 5.08 (s(br), 1H, OH), 4.96-4.98 (m, 1H, 2′-H), 4.76-4.78 (m, 1H, 3′-H), 4.10-4.13 (m, 1H, 4′-H), 3.53-3.63 (m, 2H, 5′-H), 1.48 (s, 3H, Me), 1.29 (s, 3H, Me). HRMS (ESI): *m/z* calcd for $C_{11}H_{17}N_4O_5$ $(M+H)^+$, 285.1199; found, 285.1188.

Silicon phthalocyanine 1. A mixture of silicon(IV) phthalocyanine dichloride (40 mg, 0.065 mmol), 2',3'-O-isopropyl-uridine (**9**) (148 mg, 0.52 mmol) and NaH (0.42 mmol) in toluene (20 mL) were refluxed for 24 h. After evaporating the solvent in vacuo, the residue was washed thoroughly with water, followed by column chromatography on a silica gel using ethyl acetate as the eluent. The crude product was further purified by size-exclusion chromatography by using THF as the eluent to give a blue solid **1** (47 mg, 65.6 %). IR (KBr, cm^{-1}): 3444, 1718, 1695, 1522, 1429, 1374, 1336, 1291, 1081, 911, 760, 734. ^{1}HNMR ($CDCl_3$, 400MHz, ppm): δ 9.66-9.68 (m, 8H, Pc-H_α), 8.44-8.46 (m, 8H, Pc-H_β), 7.44 (s, 2H, pyrimidine-NH), 4.86 (d, J =8.0 Hz, 2H, pyrimidine-NCH), 4.43 (d, J =4.0 Hz, 2H, pyrimidine-COCH), 4.06 (d, J =8.0Hz, 2H, 1′-H), 1.90-1.92 (m, 2H, 2′-H), 1.36-1.39 (m, 2H, 3′-H), 0.88 (s, 6H, Me), 0.65 (s, 6H, Me), 0.41 (d, J =5.6Hz, 2H, 4′-H), -1.22 to -1.25 (m, 2H, 5′-H), -2.40 to -2.43 (m, 2H, 5′-H). HRMS (ESI): *m/z* calcd for $C_{56}H_{46}N_{12}NaO_{12}Si$ $(M+Na)^+$, 1129.3025; found, 1129.2948. Anal.cacld for $C_{56}H_{46}N_{12}O_{12}Si$: C 60.75, H 4.19, N 15.18; found: C 60.37, H 4.59, N 14.83. HPLC: Rt = 39.07 min.

Silicon phthalocyanine 2. According to the procedure described for SiPc **1**, silicon(IV) phthalocyanine dichloride (40 mg, 0.065 mmol), 2',3'-O-isopropyl-5-methyl-uridine **10** (155 mg, 0.52 mmol) and NaH (0.42 mmol) in toluene (20 mL) was refluxed for 24 h to give **2** as a blue solid (30 mg, 41%). IR (KBr，cm^{-1}): 3447, 2926, 739, 1519, 1428, 1334, 1291, 1123, 1081, 911, 760. ^{1}HNMR ($CDCl_3$, 400MHz, ppm): δ 9.62-9.64 (m, 8H, Pc-H_α), 8.40-8.42 (m, 8H, Pc-H_β), 7.43 (s, 2H, pyrimidine-NH), 4.73 (s, 2H, pyrimidine-NCH), 4.17 (d, J =3.6Hz, 2H, 1′-H), 2.17-1.90 (m, 2H, 2′-H), 1.82-1.84 (m, 2H, 3′-H), 1.52 (s, 6H, Me), 0.88 (s, 6H, Me), 0.61 (s, 6H, Me), 0.51 (d, J =5.6Hz, 2H, 4′-H), -1.29 to -1.32(m, 2H, 5′-H), -2.28 to -2.40 (m, 2H, 5′-H). HRMS (ESI): *m/z* calcd for $C_{58}H_{50}N_{12}NaO_{12}Si$ $(M+Na)^+$, 1157.3338; found, 1157.3219.

Anal.cacld for $C_{58}H_{52}N_{12}O_{13}Si$ (**2**·H_2O): C 60.41, H 4.55, N 14.58; found: C 60.85, H 4.17, N 14.89. HPLC: Rt = 39.58 min.

Silicon phthalocyanine 3. A mixture of silicon(IV) phthalocyanine dichloride (40 mg, 0.065 mmol), 2',3'-O-isopropyl-5- cytidine **11** (147 mg, 0.52 mmol) and NaH (0.42 mmol) in toluene (20 mL) was refluxed for 24 h. After evaporating the solvent under reduced pressure, the residue was washed thoroughly with water, followed by column chromatography on a silica gel using ethyl acetate/DMF (10:1, v/v) and then DMF as the eluent. The crude product was further purified by size-exclusion chromatography by using THF as the eluent to afford **3** as a blue solid **3** (37 mg, 52%). IR (KBr, cm^{-1}): 3370, 1519, 1428, 1335, 1291, 1123, 1081, 910, 760, 742. ^1H NMR (DMSO-d6, 400MHz, ppm): δ 9.70-9.76 (m, 8H, Pc-H_α), 8.53-8.58 (m, 8H, Pc-H_β), 7.25 (s(br), 2H, NH_2), 7.06 (s(br), 2H, NH_2), 5.38 (d, J =6.8 Hz, 2H, pyrimidine-H), 4.51 (d, J =3.6Hz, 2H, pyrimidine-H), 4.33 (d, J =7.6Hz, 2H, 1′-H), 1.85-1.88 (m, 2H, 2′-H), 1.24-1.31 (m, 2H, 3′-H), 0.76 (s, 6H, Me), 0.68 (d, J =6.0Hz, 2H, 4′-H), 0.57 (s, 6H, Me), -1.44 to -1.46 (m, 2H, 5′-H), -2.30 to -2.32 (m, 2H, 5′-H). HRMS (ESI): *m/z* calcd for $C_{56}H_{48}N_{14}NaO_{10}Si$ $(M+Na)^+$, 1127.3345; found, 1127.3377. Anal.cacld for $C_{56}H_{50}N_{14}O_{11}Si$ (**3**·H_2O): C 59.88, H 4.49, N 17.46; found: C 59.44, H 4.72, N 17.04. HPLC: Rt = 37.77min.

Silicon phthalocyanine 4. According to the procedure described for SiPc **3**, silicon(IV) phthalocyanine dichloride (40 mg, 0.065 mmol), 2',3'-O-isopropyl-5-N-cytidine **12** (148 mg, 0.52 mmol) and NaH (0.42 mmol) in toluene (20 mL) was refluxed for 24 h to give **4** as a blue solid (29 mg, 40%) . IR (KBr, cm^{-1}): 3373, 1633, 1519, 1472, 1429, 1335, 1291, 1164, 1124, 1082, 912,798, 760, 739. ^1H NMR ($CDCl_3$, 500MHz, ppm): δ 9.86-9.88 (m: 8H, Pc-H_α), 8.65-8.66 (m, 8H, Pc-H_β), 5.03 (s, 2H, pyrimidine-H), 4.25 (d, J =1.5Hz, 2H, 1′-H), 2.98 (s(br), 2H, NH_2), 2.91 (s(br), 2H, NH_2), 2.13-2.15 (m, 2H, 2′-H), 1.55 (d, J =3.5Hz, 2H, 3′-H), 0.99 (s, 6H, Me), 0.91 (s, 6H, Me), 0.51 (d, J =5.5Hz, 2H, 4′-H), -1.05 to -1.07 (m, 2H, 5′-H), -2.43 to -2.45 (m, 2H, 5′-H). HRMS (ESI): *m/z* calcd for $C_{54}H_{46}N_{16}NaO_{10}Si$ $(M+Na)^+$, 1129.3250; found,1129.3142. Anal.cacld for $C_{54}H_{48}N_{16}O_{11}Si$ (**4**·H_2O): C 57.64, H 4.30, N 19.92; found: C 58.01, H 3.92, N 19.78. HPLC: Rt = 38.33min.

3. Photophysical and Photochemical properties

Fluorescence quantum yields. The fluorescence quantum yields (Φ_F) were determined in the

same solvent (DMF) by the equation: $\Phi_{F(sample)}=(F_{sample}/F_{ref})\cdot(A_{ref}/A_{sample})\cdot\Phi_{F(ref)}$, where F, and A are the measured fluorescence (area under the fluorescence spectra) and the absorbance at the excitation position (610 nm), respectively. The unsubstituted zinc (II) phthalocyanine (ZnPc) in DMF was used as the reference [$\Phi_{F(ref)} = 0.28$].[2]

Singlet oxygen yields. The singlet oxygen quantum yields (Φ_{Δ}) were determined by a steady-state method using DPBF (1,3-diphenylisobenzofuran) as the scavenger in DMF.[3] The DMF solution of phthalocyanine (ca. 4 μM) containing DPBF (35 μM) were prepared in the dark and irradiated with red light, then DPBF degradation at 413 nm was monitored along with irradiated time. The singlet oxygen quantum yields (Φ_{Δ}) is calculated by the equation: $\Phi_{\Delta(sample)}=(k_{sample}/k_{ref})\cdot(A_{ref}/A_{sample})\cdot\Phi_{\Delta(ref)}$, where $\Phi_{\Delta(ref)}$ is the singlet oxygen quantum yield for the reference (un-substituted ZnPc) in DMF ($\Phi_{\Delta(ref)}=0.56$)[3], k_{sample} and k_{ref} are the DPBF photobleaching rates in the presence of the samples and reference, respectively; A_{ref} and A_{sample} are the absorbance at Q band (area under the absorption spectra in 610-750 nm) of the samples and reference, respectively. The light source consisted of a 150 W halogen lamp, a water tank for cooling and a color glass filter cut-on 610 nm. The fluence rate (λ > 610 nm) was 1.0 mW·cm^{-2}.

4. In vitro photodynamic activity assay

The HepG2 human hepatocarcinoma cells (from ATCC) were maintained in RPMI 1640 medium (Hyclone) supplemented with 10% fetal bovine serum, penicillin (50 units mL^{-1}) and streptomycin (50 μg mL^{-1}).

Compounds **1-4** were first dissolved in DMF (1.0 mM) and the solutions were diluted to 80 μM with 1 % (wt) aqueous solution of Cremophor EL (Sigma, 1 g in 100 mL of water). The solutions were clarified with 0.45 μm filter, and then diluted with the cellular culture medium to appropriate concentrations.

About 2 x 10^4 cells per well in this medium were incubated in 96-well plate and incubated overnight at 37 ^{o}C in a humidified 5% CO_2 atmosphere. The cells were then incubated with 200 μl of the above phthalocyanine solutions for 2 h under the same conditions. After that, the cells were rinsed with PBS and re-fed with 100 μl of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a color glass filter cut-on 610 nm. The fluence rate (λ > 610 nm) was 15 mW·cm^{-2}.

An illumination of 30 min led to a total fluence of 27 J cm^{-2}.

Cell viability was determined by the colorimetric 3-(4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[4] After illumination, the cells were incubated at 37 °C under 5 % CO_2 overnight. An MTT (Sigma) solution in PBS (25 μl, 5 mg ml^{-1}) was added to each well followed by incubation for 4 h under the same environment. A 200 μl of DMSO was then added to each well. The 96-well plate was agitated on a Thermo-Labsystems microplate reader (Multishan MK3) at ambient temperature for 20 seconds before the absorbance at 490 nm at each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the equation: Cell Viability (%) = $[\Sigma(A_i/\bar{A}_{control} \times 100)] / n$, where A_i is the absorbance of the ith data (i = 1, 2, …, n), $\bar{A}_{control}$ is the average absorbance of the control wells, in which the phthalocyanine was absent, and n (≥3) is the number of the data points.

5. Intracellular Fluorescence studies

About 6×10^4 HepG2 cells in the culture medium (0.5 mL) were seeded on a coverslip and incubated overnight at 37°C under 5% CO_2. The medium was then removed. The cells were incubated with the solutions of phthalocyanies **1-4** in the medium (2 mL, 2 μM) for 2 h under the same conditions. The cells were then rinsed with PBS and viewed with a Nikon C2 confocal microscope. Upon excited at 637 nm under the same light intensity, fluorescence signals of **1-4** were monitored at 640-700 nm, respectively. The images were then digitized and analyzed by using the Nikon C2 ROI Fluorescence Statistics. The intracellular fluorescence intensities (a total of 20 cells for each sample) were also determined.

6. Subcellular Localization Studies

HepG2 cells were cultivated on a coverslip as described above, and incubated with the solutions of phthalocyanies **1-4** in the medium (2 mL, 2 μM) for 2 h. And then the cells were incubated with MitoTracker Green (Molecular Probes, 0.2 μM) in PBS for a further 30 min. The cells were then rinsed with PBS and viewed with a Nikon C2 confocal microscope. Mito Tracker Green was excited at 488 nm and monitored at 499-529 nm, while compounds **1-4** were excited at 637 nm and monitored at 640-700 nm. The subcellular localization of **1-4** was revealed by comparing the intracellular fluorescence images caused by MitoTracker and these phthalocyanines.

7. Apoptosis analysis.

We studied the cell damage mechanism induced by compound **1** by examining the dual fluorescence of Annexin V-FITC (fluorescein isothiocyanate) and propidium iodide (PI) (Apoptosis Detection Kit, Beyotime, China).[5]

About 6×10^4 HepG2 cells in the culture medium (0.5 mL) were seeded on a coverslip and incubated overnight at 37°C under 5% CO_2. The medium was then removed. The cells were incubated with the solution of **1** in the medium (2 mL, 2 μM) for 2 h under the same conditions. After being rinsed with PBS and refilled with 100 μl of the culture medium, the cells were illuminated using a red light (λ>610 nm, 15 $mW\cdot cm^{-2}$, 27 $J\cdot cm^{-2}$) or kept in the dark. After 24 h of incubation, the cells were then suspended in 500μL of binding buffer containing 5 μL Annexin V-FITC and 5 μL PI, and were incubated at 37℃ for 10 min in the dark. Cells were washed twice and then evaluated using a Nikon C2 fluorescence confocal microscope. Annexin V-FITC was excited at 488 nm and monitored at 499-529 nm, while PI was excited at 543 nm and monitored at 552-617 nm. The cell populations at different phase of cell death, namely viable (annexin V-FITC $^-$ / PI^-), early apoptotic (annexin V-$FITC^+$ / PI^-), and necrotic or late-stage apoptotic (annexin V-$FITC^+$ / PI^+), were determined by digitally merging fluorescence images and phase contrast images.

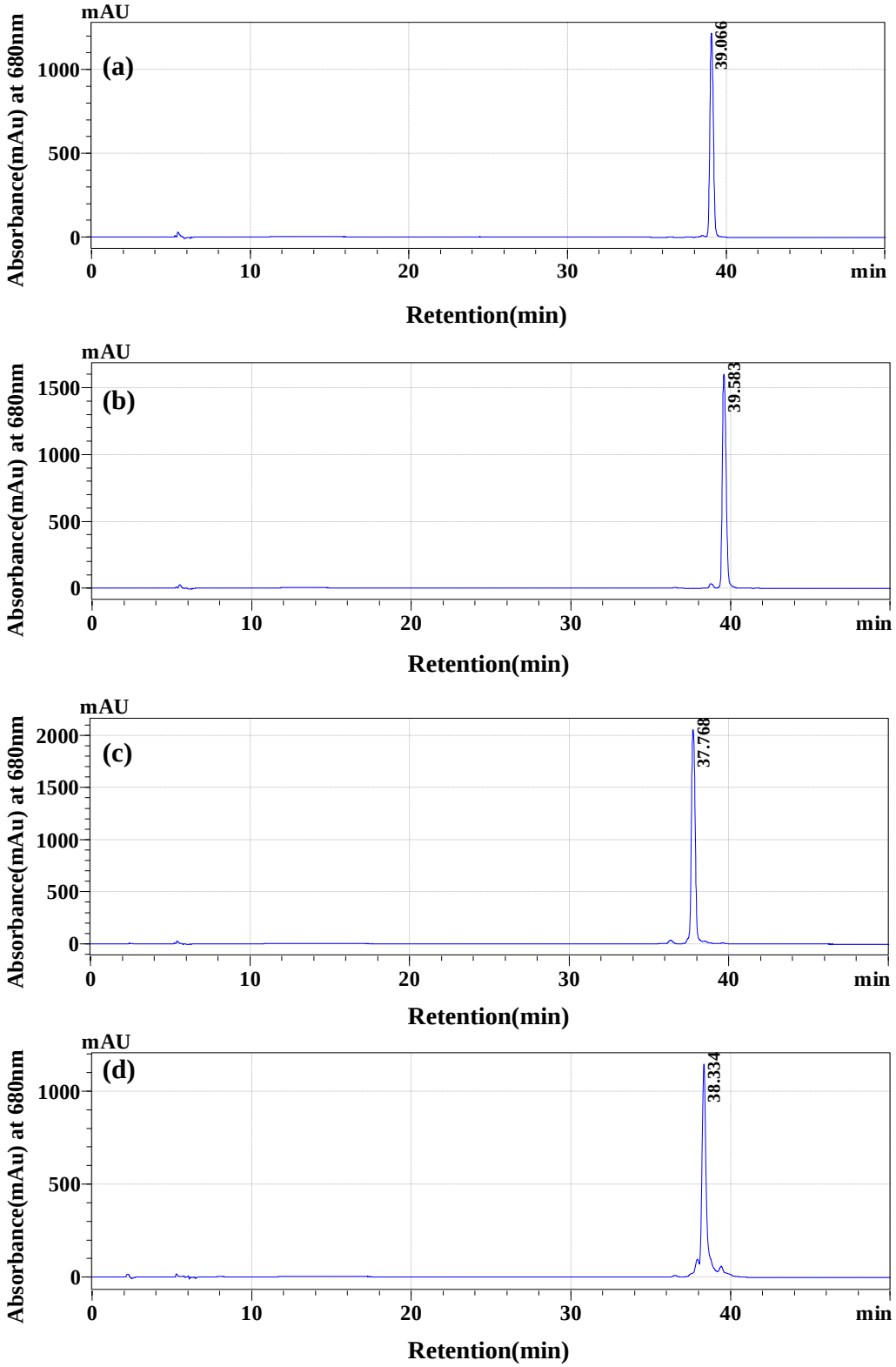
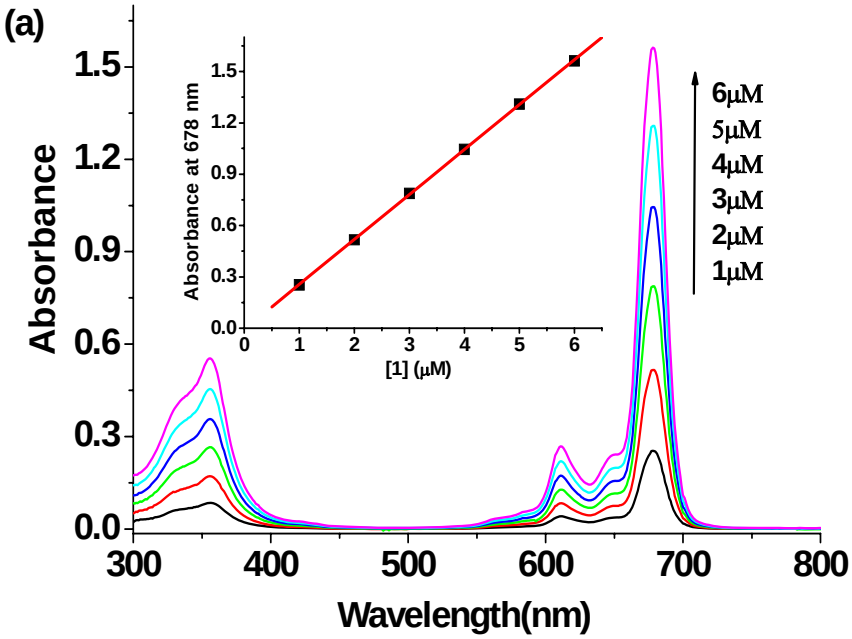
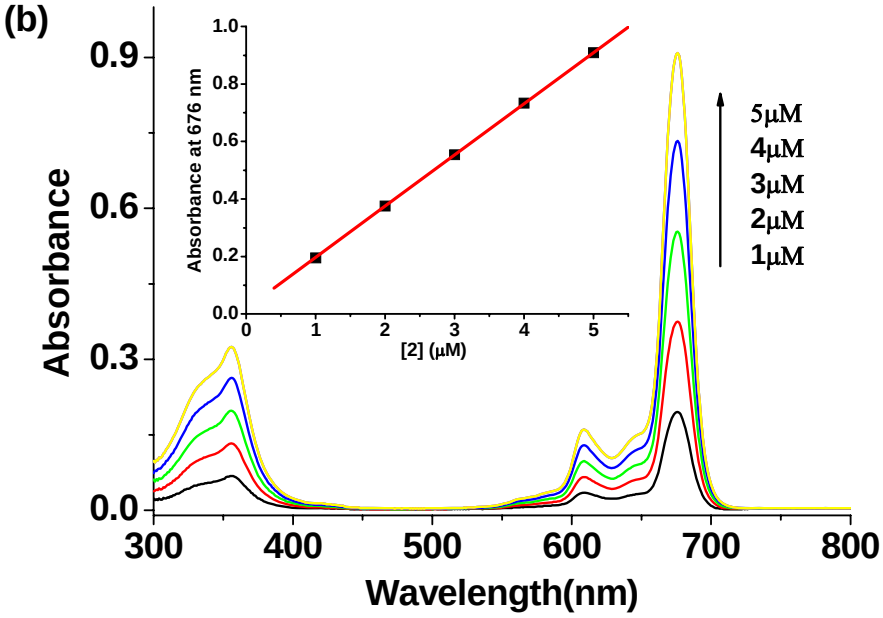


Figure S1. HPLC profiles of SiPcs **1**(a), **2**(b), **3**(c), and **4**(d).





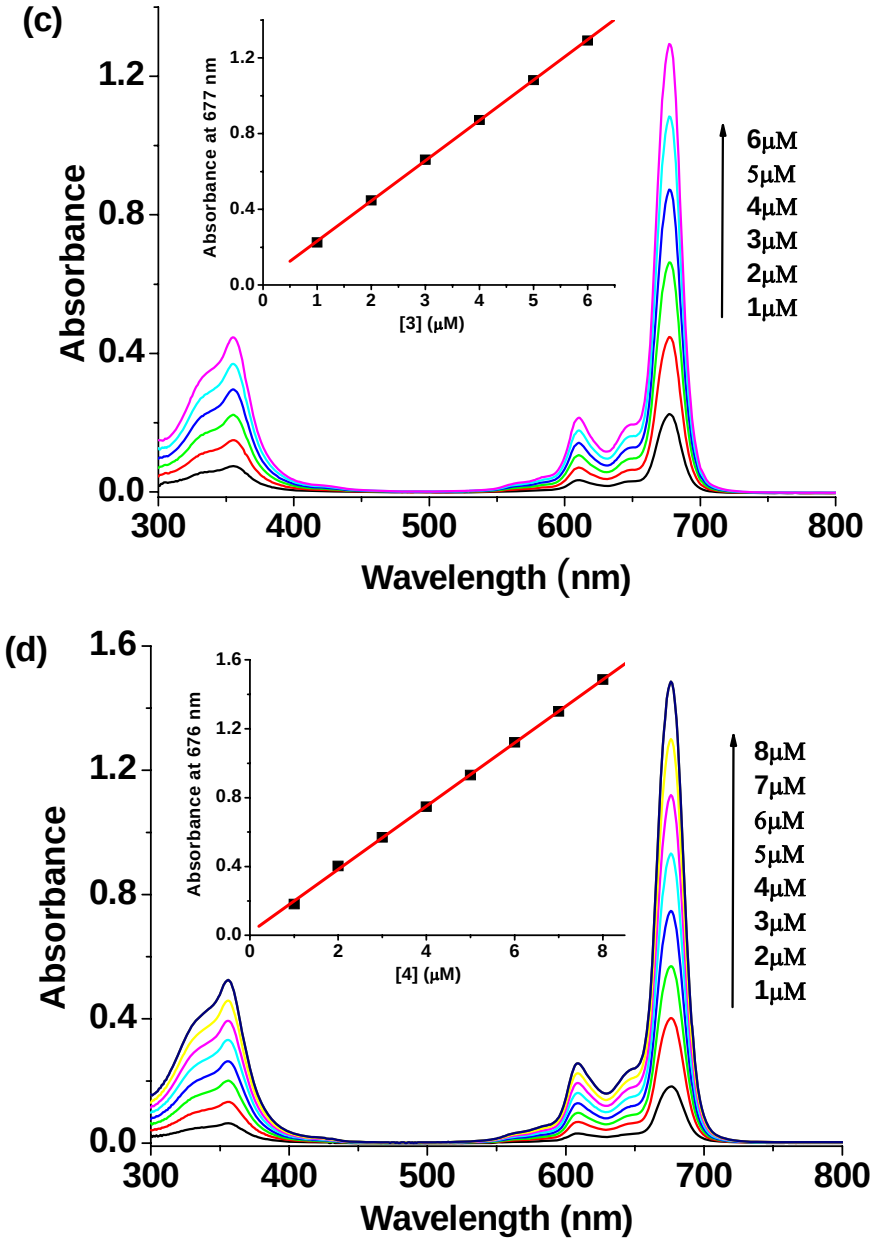
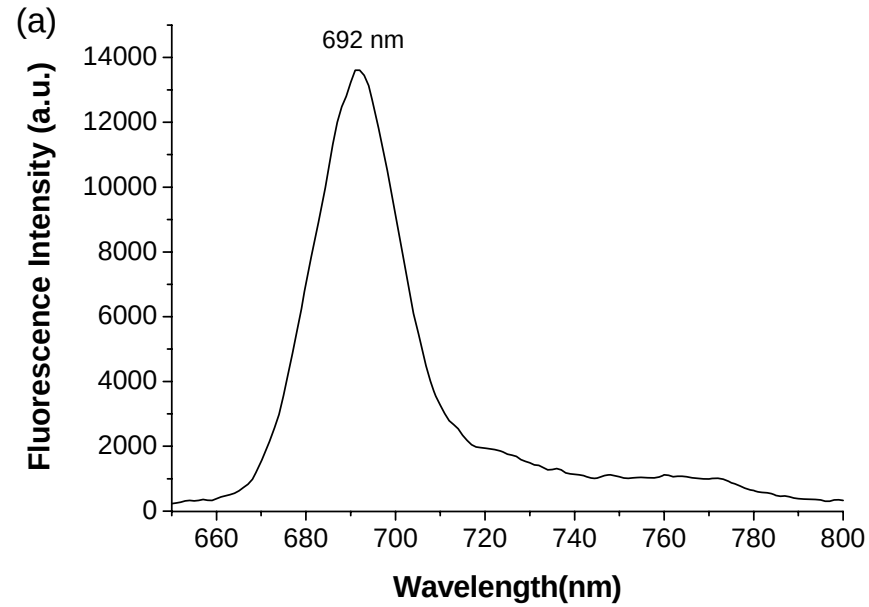
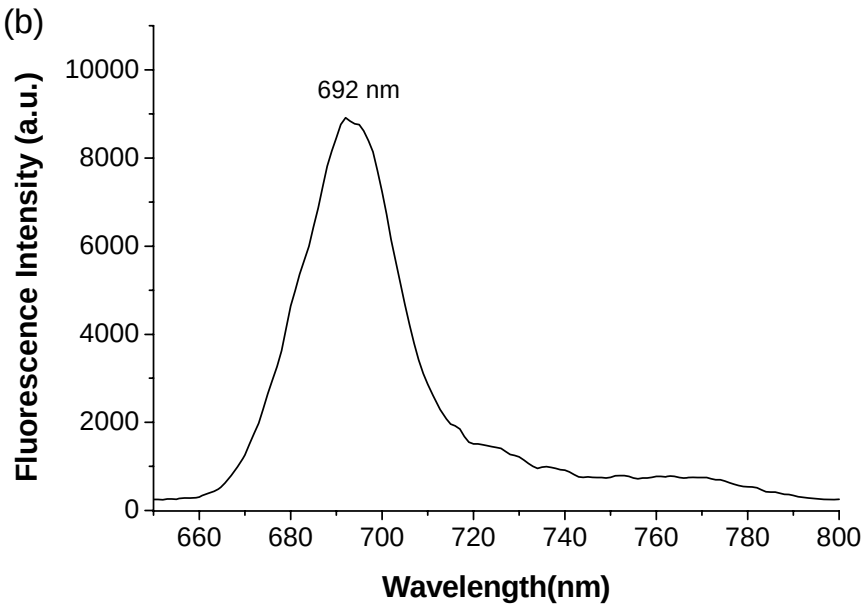


Figure S2 Electronic absorption spectra of SiPcs **1**(a), **2**(b), **3**(c), and **4**(d) in DMF at different concentrations. The inset plots the Q-band absorbance versus the concentration of **1-4.**





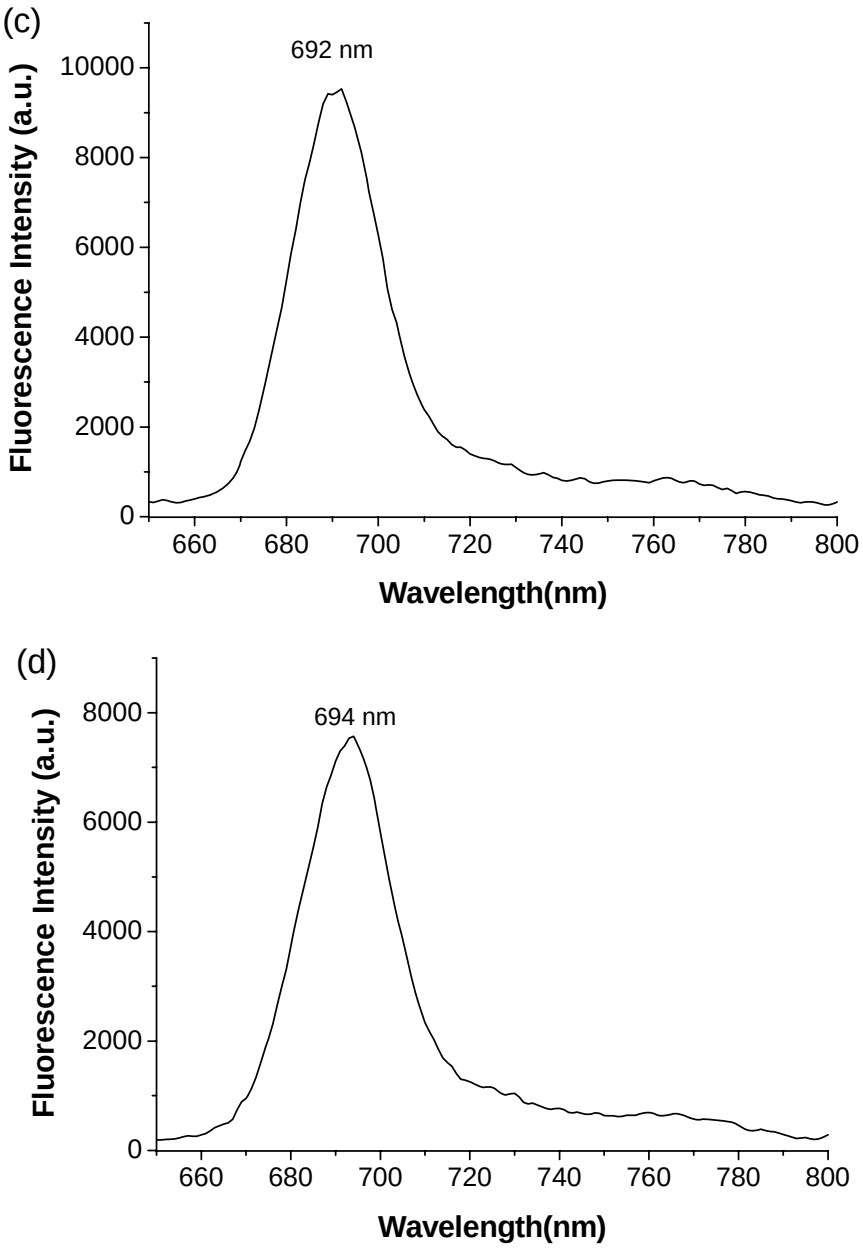


Figure S3. Fluorescence spectra of SiPcs **1**(a), **2**(b), **3**(c), and **4**(d) in the RPMI1640 culture medium (all at 2 μM).

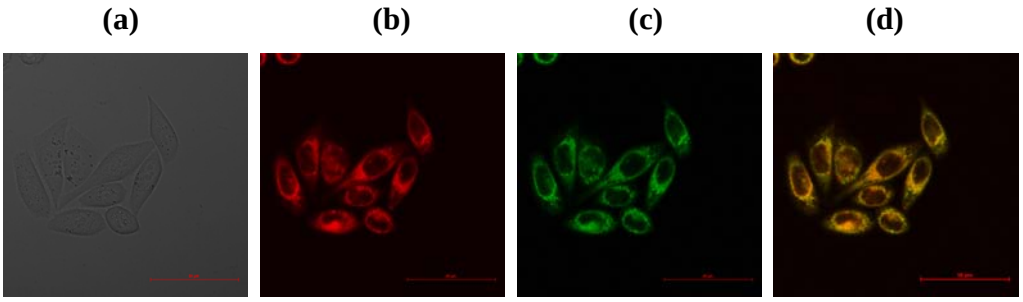


Figure S4. Subcellular fluorescence and localization of SiPc **1** in HepG2 cells (at 2 μM for 2 h). (a) the bright field image, (b) SiPc **1** fluorescence (in red) , (c) Mito Tracker Green fluorescence (in green), and (d) overlays of SiPc **1** and Mito Tracker Green fluorescences. Scale bar: 50 μm.

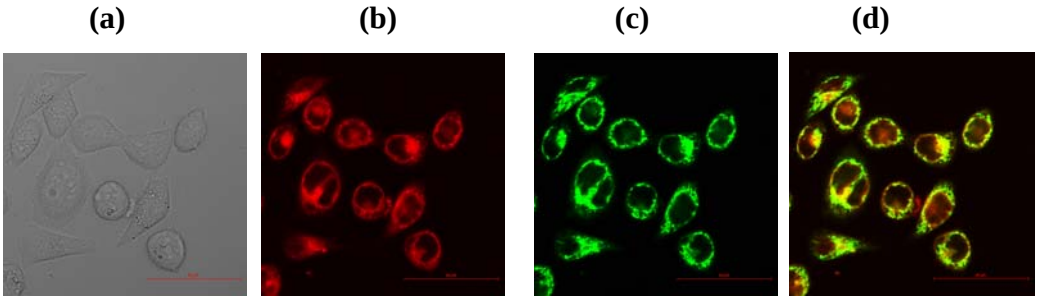


Figure S5. Subcellular fluorescence and localization of SiPc **2** in HepG2 cells (at 2 μM for 2 h). (a) the bright field image, (b) SiPc **2** fluorescence (in red) , (c) Mito Tracker Green fluorescence (in green) and (d) overlays of SiPc **2** and Mito Tracker Green fluorescences. Scale bar: 50 μm.

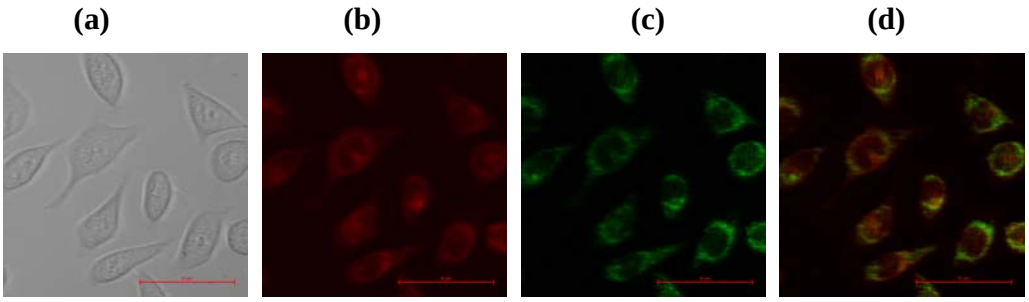


Figure S6. Subcellular fluorescence and localization of SiPc **3** in HepG2 cells (at 2 μM for 2 h). (a) the bright field image, (b) SiPc **3** fluorescence (in red), (c) Mito Tracker Green fluorescence (in green), and (d) overlays of SiPc **3** and Mito Tracker Green fluorescences. Scale bar: 50 μm.

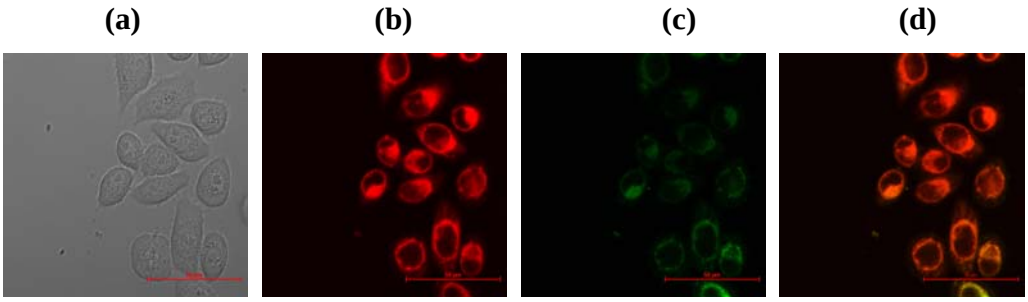


Figure S7. Subcellular fluorescence and localization of SiPc **4** in HepG2 cells (at 2 μM for 2 h). (a) the bright field image, (b) SiPc **4** fluorescence (in red), (c) Mito Tracker Green fluorescence (in green) and (d) overlays of SiPc **4** and Mito Tracker Green fluorescences. Scale bar: 50 μm.

References

1 C. W. Dirk, T. Inabe, K. F. J. Schoch and T. J. J. Marks, *J. Am. Chem. Soc.* 1983, **105**, 1539-1550.

2 I. Scalise and E. N. Durantini, *Bioorg. Med. Chem*., 2005, **13**, 3037-3045.

3 (a) M. D. Maree, N. Kuznetsova and T. Nyokong, *J. Photochem. Photobiol. A*, 2001, **140**, 117–125; (b) W. Spiller, H. Kliesch, D. Wohrle, S. Hackbarth, B. Roder and G. Schnurpfeil, *J. Porphyrins Phthalocyanines*, 1998, **2**, 145-148

4 H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto, *J. Immunol. Methods*, 1986, **93**, 157-165.

5 I. Vermes, C. Haanena, H. Steffens-Nakkena and C. Reutellingsperger, *J. Immunol. Methods*, 1995, **184**, 39-51.