Supporting information to

Guanidinium-dendronized Perylene bisimides as stable, water-soluble fluorophores for live-cell imaging

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1. Materials and Characterization

All chemicals and reagents were used as received from commercial sources without purification. Anhydrous diethyl ether and tetrahydrofuran were distilled from sodium/ benzophenone ketyl prior to use. The monomers **1**, **2**, and **5** were synthesized according to the literature.[1-3]

The NMR spectra were collected on a Varian Mercury Plus 500 spectrometer with tetramethylsilane as the internal standard. Matrix-assisted laser desorption/ionization (MALDI) experiments were carried out using a Shimadzu AXIMA-CFR *plus* time-of-flight mass spectrometer (Kratos Analytical, Manchester, UK.). UV-Vis spectra were recorded on a Shimadzu 3150 PC spectrophotometer. Fluorescence measurement was carried out on a Shimadzu RF-5301 PC spectrofluorophotometer with a xenon lamp as a light source. Dynamic laser light scattering measurement was performed using photon correlation spectroscopy (Nano ZS90 zetasizer, Malvern Instruments Corp, U.K.) at 25 °C under a fixed angle of 90° in disposable polystyrene cuvettes. The measurements were obtained using a He–Ne laser of 633 nm. And the measurement time was about 5 min and each run underwent ~ 20 subruns. Each value reported is the average of three measurements.

Fluorescence quantum yields (Φ_f) of the **PBI-G2** in aqueous solution was measured by using Cresyl Violet (Φ_f = 0.54 in methanol) as standards. Fluorescence quantum yield was calculated from the integrated intensity under the emission band using the following equation:

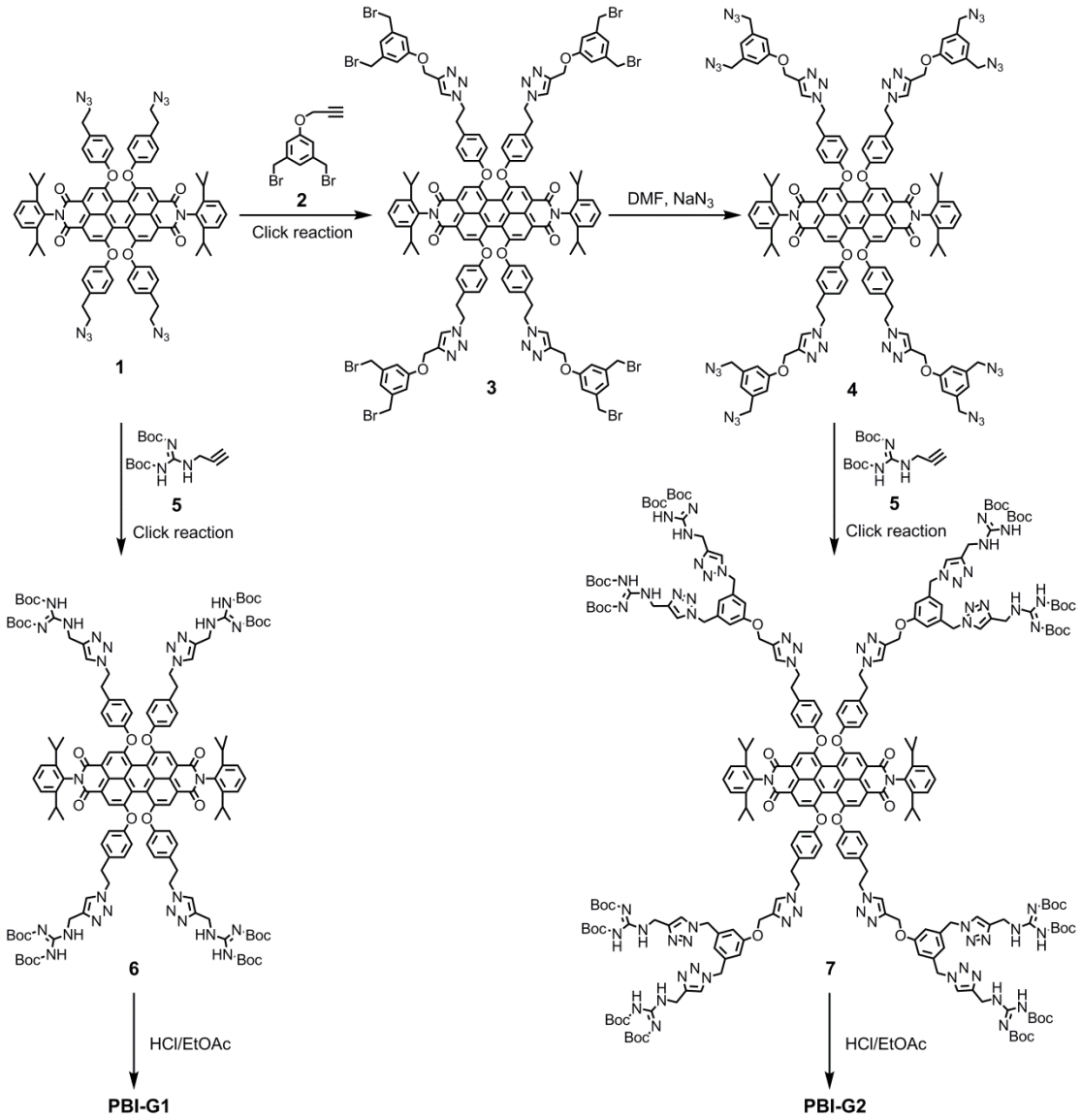
$$\Phi_S = \Phi_R \frac{I_S A_R n_S^2}{I_R A_S n_R^2}$$

where Φ_S is the fluorescence quantum yield of the sample, Φ_R is the fluorescence quantum yield of the standard, I_S and I_R are the integrated emission intensities of the sample and the standard, respectively, A_S and A_R are the absorbance of the sample and the standard at the excitation wavelength (540 nm), respectively, and n_S and n_R are the refractive indexes of the corresponding solutions (pure solvents were assumed).

The aqueous solution of **PBI-G2** (10^{-5} mol L^{-1}) was exposure to natural light for two weeks. And the photostability of **PBI-G2** was determined by the UV-Vis absorption and the fluorescence spectra.

2. Synthetic Procedures

The guanidinium encapsulations of PBIs were synthesized by copper-catalyzed azide-alkyne cycloaddition (CuAAC) "click" reaction with azide-terminated PBIs and *N*-propargylguanidine. The key intermediate azide-terminated PBI **1** was obtained according to literature procedures.[4] A standard click chemistry conditions ($CuSO_4 \cdot 5H_2O$ and sodium ascorbate) between compound **1** and 1,3-dibromomethyl-5-propargyloxy-benzene **2** was used to produce the bromo-substituted PBI **3**, which in turn was treated with sodium azide to produce azide-substituted PBI **4**. Compound **5** reacted with **1** and **4** (CuAAC "click" reaction) yielded the Boc-protected, fully guanidinylated derivatives **6** and **7**, respectively. Subsequent deprotection of the Boc groups by HCl-EtOAc afforded the target dendrimers **PBI-G1** and **PBI-G2**, which process four and eight guanidinium groups, respectively. The target compounds were purified by dialysis and fully characterized by ^{1}H NMR, ^{13}C NMR and MALDI-TOF mass spectroscopy.



Scheme S1. The synthetic routes of **PBI-G1** and **PBI-G2**. Click reaction: $CuSO_4.5H_2O$ (5 mol %), Sodium ascorbate (10 mol %), THF/H_2O (1:1), rt, 12 h.

Synthesis of compound 3:

Compound **1** (50 mg, 0.037 mmol) and compound **2** (70.1 mg, 0.222 mmol) were dissolved in THF (4 mL), and an aqueous solution of $CuSO_4 \cdot 5H_2O$ (9.2 mg, 0.037 mmol) and sodium ascorbate (14.7 mg, 0.074 mmol) were added. The mixture was stirred for 12 h at room temperature and diluted with 10 ml of water (a pale precipitate occurred). The suspension was extracted with EtOAc. The organic layers were combined and dried with Na_2SO_4. The solvent was evaporated under vacuum. The residue was purified by silica-gel column chromatography using EtOAc-CH_2Cl_2 (v/v = 1/4) as the eluent to give the product as a dark red solid (81.4 mg) with a yield of 84%. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.20 (s, 4H), 7.51 (s, 4H), 7.43 (t, J = 7.85 Hz, 2H), 7.29 (d, J = 7.80 Hz, 4H), 7.02 (d, J = 8.27 Hz, 8H), 6.97 (s, 4H), 6.90 (s, 8H), 6.88 (d, J = 8.47 Hz, 8H), 5.18 (s, 8H), 4.56 (t, J = 6.85 Hz, 8H), 4.34 (s, 16H), 3.19 (t,

J = 6.74 Hz, 8H), 2.74-2.69 (m, 4H), 1.13 (d, *J* = 5.78 Hz, 24H). ^{13}C NMR (500 MHz, $CDCl_3$, *δ*): 163.18, 158.48, 155.94, 154.15, 145.59, 143.39, 139.70, 133.50, 133.16, 130.51, 130.39, 129.57, 123.97, 123.35, 122.93, 122.32, 120.57, 120.43, 120.33, 120.04, 115.55, 61.95, 51.60, 35.94, 32.83, 29.11, 24.06.

Synthesis of compound 4:

To an anhydrous DMF (5 mL) solution of compound **3** (170 mg, 0.065 mmol), NaN_3 (42.3 mg, 0.65 mmol) was added, and the mixture was stirred for 21 h at 85 °C. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 and washed with brine. The organic layers were combined and dried with Na_2SO_4. The solvent was evaporated under vacuum. The residue was purified by silica-gel column chromatography using EtOAc-CH_2Cl_2 (v/v = 1/4) as the eluent to give the product as a dark red solid (143.4 mg) with a yield of 95%. ^{1}H NMR (500 MHz, $CDCl_3$, *δ*): 8.21 (s, 4H), 7.53 (s, 4H), 7.42 (t, *J* = 7.75 Hz, 2H), 7.29 (d, *J* = 7.56 Hz, 4H), 7.03 (d, *J* = 8.48 Hz, 8H), 6.90 (d, *J* = 8.80 Hz, 8H), 6.88 (s, 8H), 6.84 (s, 4H), 5.20 (s, 8H), 4.55 (t, *J* = 6.85 Hz, 8H), 4.25 (s, 16H), 3.19 (t, *J* = 6.87 Hz, 8H), 2.75-2.69 (m, 4H), 1.13 (d, *J* = 5.59 Hz, 24H). ^{13}C NMR (500 MHz, $CDCl_3$, *δ*): 163.21, 158.82, 155.94, 154.18, 145.63, 143.45, 137.69, 133.51, 133.15, 130.55, 130.35, 129.56, 123.96, 123.31, 122.89, 120.63, 120.42, 120.40, 120.33, 120.07, 114.33, 61.95, 54.33, 51.55, 35.89, 29.10, 24.02.

Synthesis of compound 6:

Compound **6** was synthesized according to the same procedure described for compound **3**. Compound **6** was purified by silica-gel column chromatography using EtOAc-CH_2Cl_2 (v/v = 1/1) as the eluent to give the product as a dark red solid with a yield of 85%. ^{1}H NMR (500 MHz, $CDCl_3$, *δ*): 11.44 (s, 4H), 8.78 (s, 4H), 8.21 (s, 4H), 7.55 (s, 4H), 7.43 (t, *J* = 7.64 Hz, 2H), 7.29 (d, *J* = 7.85 Hz, 4H), 7.09 (d, *J* = 8.35 Hz, 8H), 6.92 (d, *J* = 8.46 Hz, 8H), 4.70 (d, *J* = 4.65 Hz, 8H), 4.56 (t, *J* = 6.81 Hz, 8H), 3.21 (t, *J* = 6.80 Hz, 8H), 2.72-2.66 (m, 4H), 1.50 (s, 36 H), 1.45 (s, 36 H), 1.13 (d, *J* = 6.07 Hz, 24 H). ^{13}C NMR (500 MHz, $CDCl_3$, *δ*): 163.36, 163.16, 155.95, 155.84, 154.28, 152.96, 145.58, 143.81, 133.40, 133.12, 130.46, 130.33, 123.92, 122.91, 122.38, 120.66, 120.36, 120.31, 120.24, 83.25, 79.41, 51.46, 36.45, 35.97, 29.70, 28.31, 28.02, 24.02.

Synthesis of compound 7:

Compound **7** was synthesized according to the same procedure described for compound **3**. Compound **7** was purified by silica-gel column chromatography using MeOH-EtOAc (v/v = 2/100) as the eluent to give the product as a dark red solid with a yield of 80%. ^{1}H NMR (500 MHz, $CDCl_3$, *δ*): 11.42 (s, 8H), 8.76 (s, 8H), 8.17 (s, 4H), 7.56 (s, 12H), 7.38 (t, *J* = 6.29 Hz, 2H), 7.23 (d, *J* = 7.07 Hz, 4H), 7.05 (d, *J* = 5.52 Hz, 8H), 6.87 (d, *J* = 7.18 Hz, 8H), 6.80 (s, 8H), 6.74 (s, 8H), 5.37 (s, 16H), 5.08 (s, 8H), 4.65 (s, 16H), 4.55 (t, *J* = 6.85 Hz, 8H), 3.17 (t, *J* = 6.87 Hz, 8H), 2.66-2.65

(m, 4H), 1.45 (d, J = 10.07 Hz, 144H), 1.06 (s, 24H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 163.28, 163.17, 159.00, 155.94, 155.82, 154.16, 152.93, 145.58, 144.29, 142.90, 137.15, 133.41, 133.13, 130.47, 130.33, 123.90, 123.49, 122.86, 122.60, 120.68, 120.27, 120.12, 119.92, 114.62, 83.28, 79.37, 61.67, 53.56, 51.45, 36.40, 35.83, 29.04, 28.26, 28.00, 23.98.

General procedure - Cleavage of Boc protecting groups with 3 M HCl-EtOAc:

The Boc-protected compound was dissolved in 3 M HCl-EtOAc. The mixture was stirred at 50 ^{o}C for 24 h. The solvent was removed at reduced pressure, the dark-red solid was dialyzed (cutoff 2000) against water for 2 days, and lyophilized to give the desired compound. Due to the bad solubility in water, **PBI-G1** was not further characterized. **PBI-G2**: dark red solid, yield: 91%. ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.27 (s, 4H), 8.18-8.09 (s, 8H), 7.97 (s, 4H), 7.42 (t, J = 6.30 Hz, 2H), 7.33-7.21 (broad, 12H), 7.01-6.91 (broad, 12H), 6.87 (broad, 4H), 5.54 (s, 16H), 5.11 (s, 8H), 4.61 (s,8H), 4.41 (s, 16H), 3.21-3.11 (m, 8H), 2.69-2.66 (m, 4H), 1.01 (s, 24H). ^{13}C NMR (500 MHz, DMSO-d_6, δ): 163.20, 158.82, 157.65, 155.87, 153.90, 145.90, 143.70, 142.59, 138.42, 134.71, 133.08, 131.03, 130.09, 125.04, 124.27, 123.81, 122.98, 120.44, 120.33, 119.37, 114.66, 61.63, 52.98, 50.86, 36.64, 35.56, 25.57, 24.19. MS (MALDI-TOF): calcd for [$C_{156}H_{174}Cl_8N_{62}O_{12}$ – 8Cl^-], 3107.48; found, 3104.50.

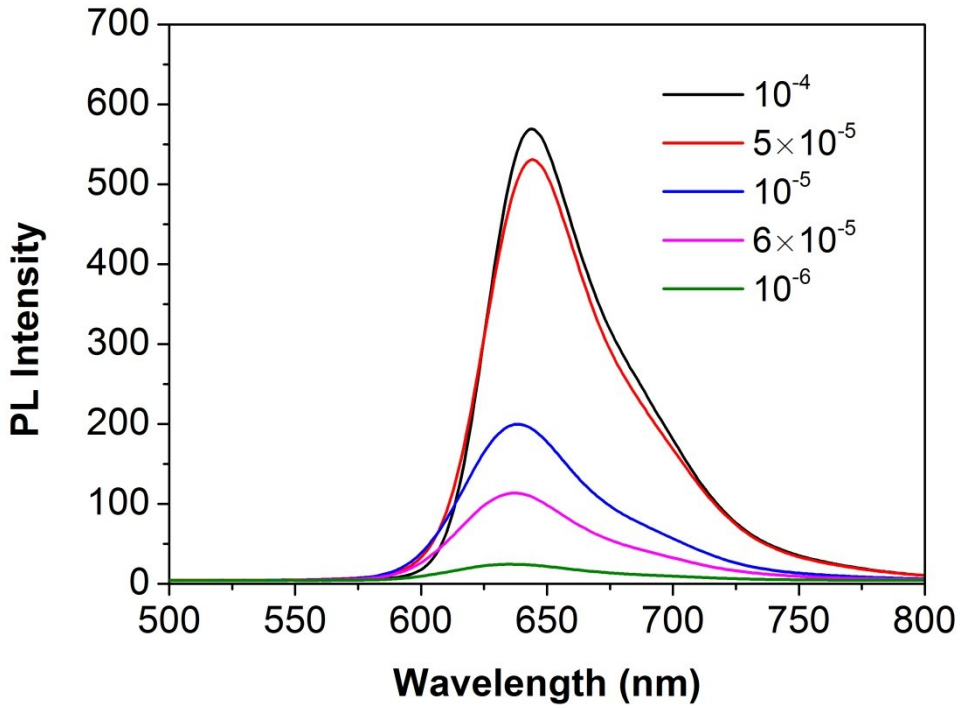


Figure S1. The fluorescence spectra of **PBI-G2** aqueous solutions at concentrations from 10^{-6} to 10^{-4} mol L^{-1}.

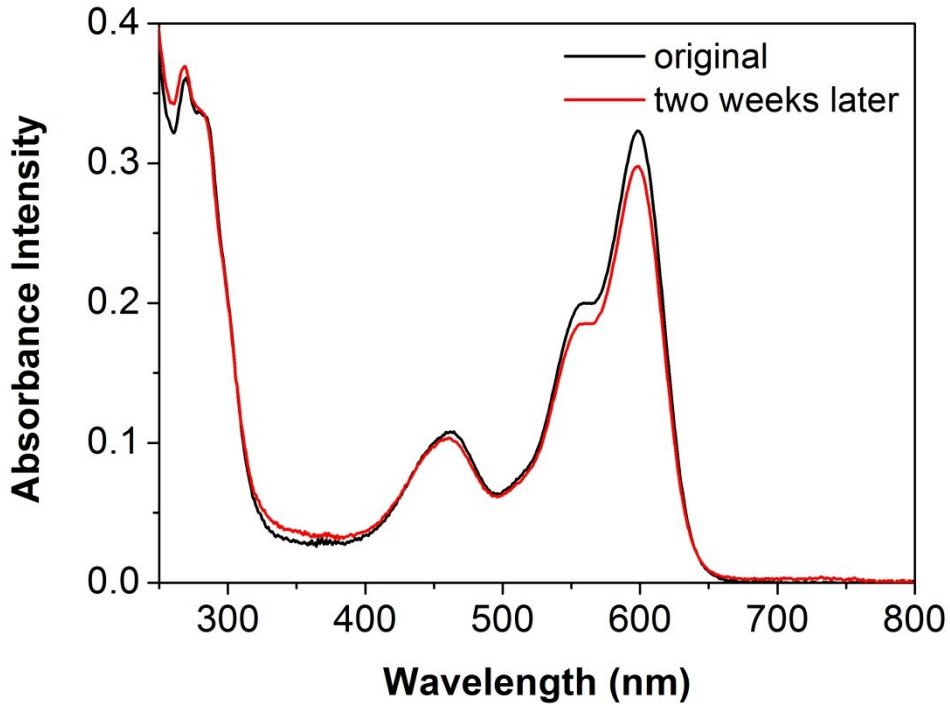


Figure S2. UV-Vis spectra of **PBI-G2** in water (10^{-5} mol L^{-1}) before and after exposure to natural light for two weeks.

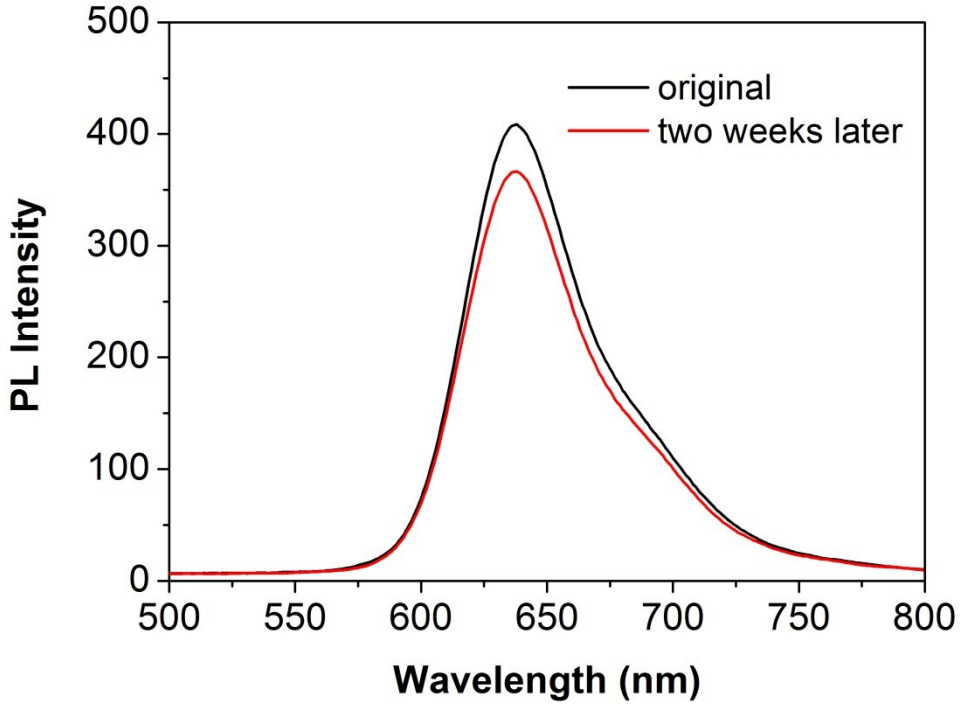


Figure S3. Fluorescence spectra of **PBI-G2** in water (10^{-5} mol L^{-1}) before and after exposure to natural light for two weeks.

3. Cell labeling

Prior to labeling, hela cell line was seeded into the 6 wells at a density of 10^4 cells/well and incubated for 48 h at 37 ^oC under a humidified 5% CO_2 atmosphere in 2 mL Dulbecco's modified eagle media (DMEM) supplemented with 10% fetal bovine serum, 100 units mL^{-1} penicillin and 0.1 mg mL^{-1} of streptomycin. The culture media were replaced with fresh culture media containing **PBI-G2** at a concentration of 2.5 μM, and the cells were dyed for 24 and 48 h. The cells were then washed with phosphate buffered saline (PBS, pH = 7.4) thoroughly to remove unattached **PBI-G2** in the medium. The cells were then fixed in 4% formaldehyde in PBS for 10 min at room temperature. After rising with PBS, the cells were stained with 4', 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for visualization of the nuclei. The stained cells were examined by OLYMPUS IX7O fluorescence microscopy.

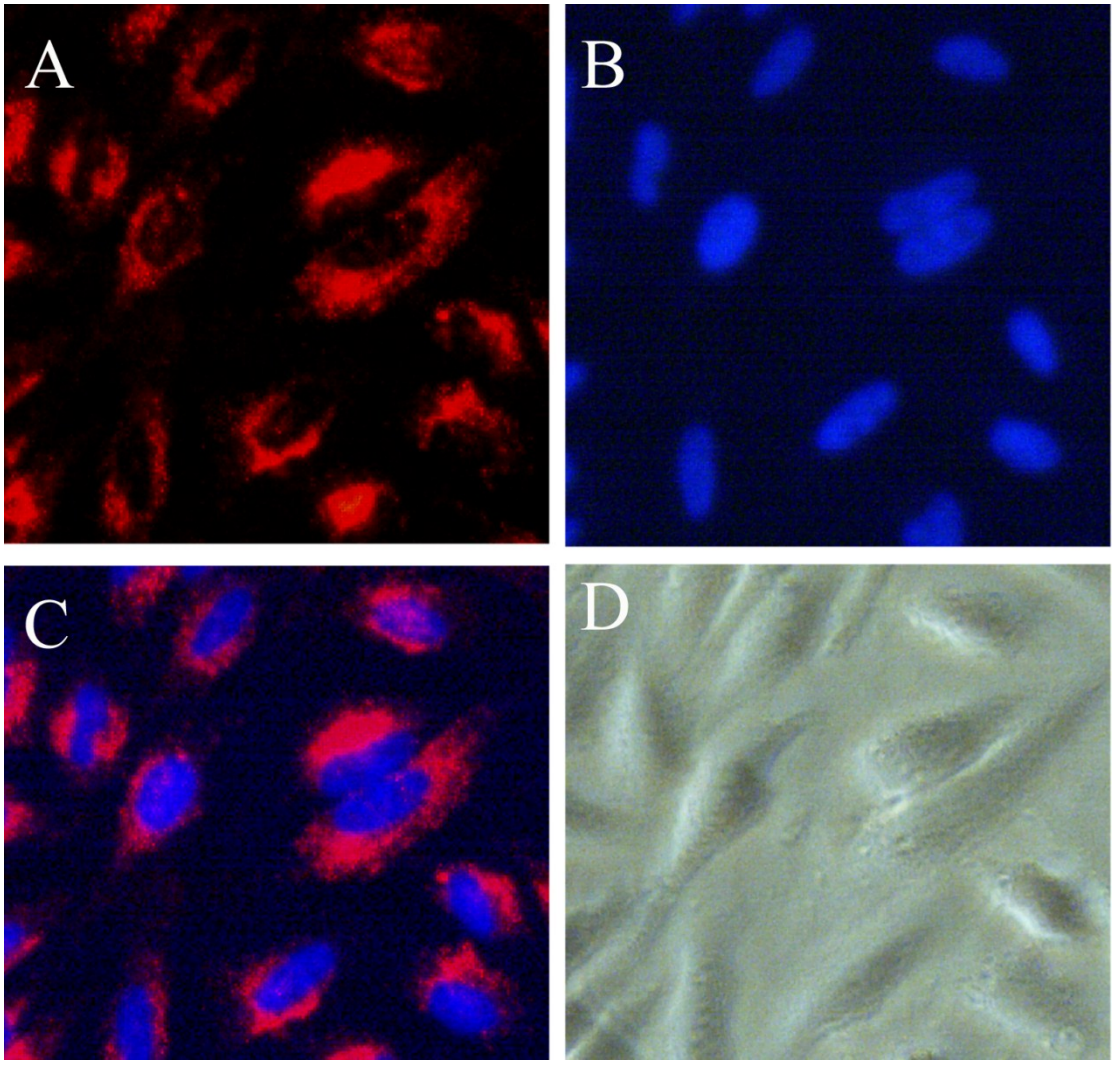


Figure S4. Fluorescent microscopy images of hela cells after 48 h incubation (Magnified 100 times). (A) Stained by **PBI-G2** showing the red fluorescence distributed in cytoplasm. (B) The cells' nuclei (blue) were labeled by DAPI. (C)

Merged A and B. (D) Brightfield.

4. Cytotoxicity test

Cell Counting Kit-8 (CCK-8, Dojindo) were performed to assess the cell viability activity of hela cell. Hela cells were seeded in 96-well plates at an intensity of 1000 cells/well in 100 μL culture media. After 48 h incubation, the medium was replaced with fresh culture media containing **PBI-G2** at a concentration of 2.5 μM. After incubated for 24 and 48 h, the cells were washed twice with PBS buffer and then incubated in freshly prepared CCK-8 solution in a culture medium for 2 h. The absorbance of CCK-8 was monitored at 450 nm on a microplate reader (Thermo) .Cell viability was expressed by the ratio of the absorbance of the cells incubated with **PBI-G2** solution. Each results is an average of data from seven wells, 100% viability was determined using untreated cell.

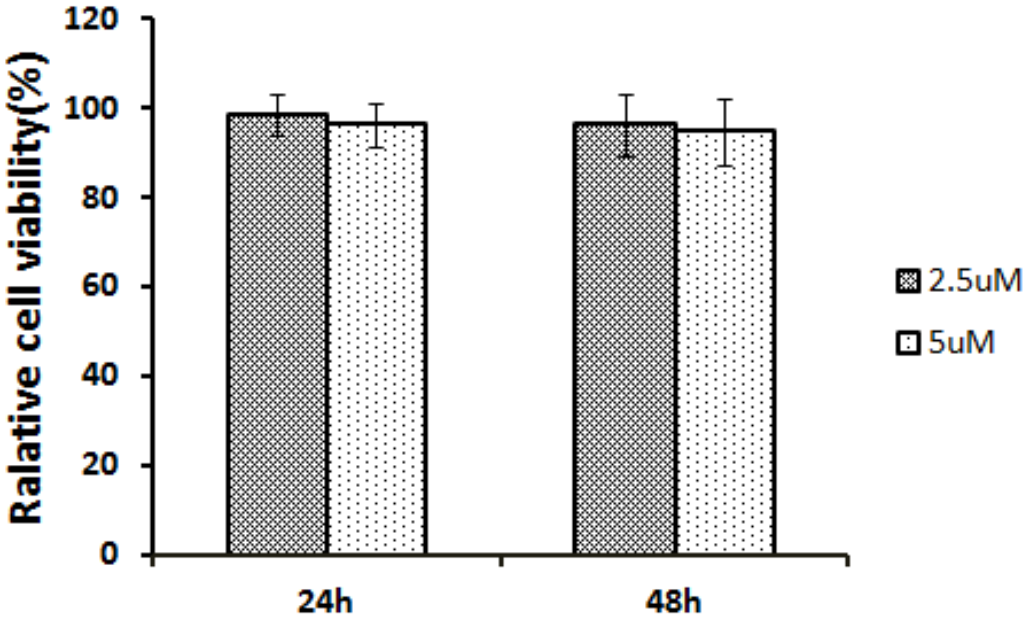


Figure S5. Hela Cell viability after incubation in cell culture medium containing 2.5 and 5 μM **PBI-G2** for 24 and 48 h, respectively.

5. ^{1}H NMR spectrum, ^{13}C NMR spectrum and MALDI-TOF spectrum of compound 7

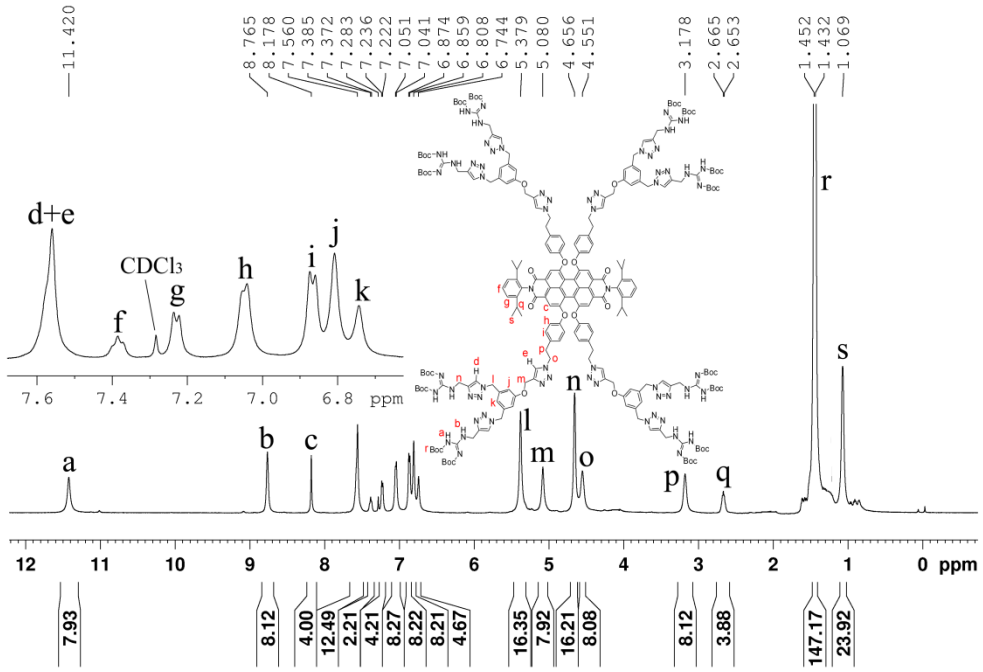


Figure S6. ^{1}H NMR spectrum of **7** in $CDCl_3$.

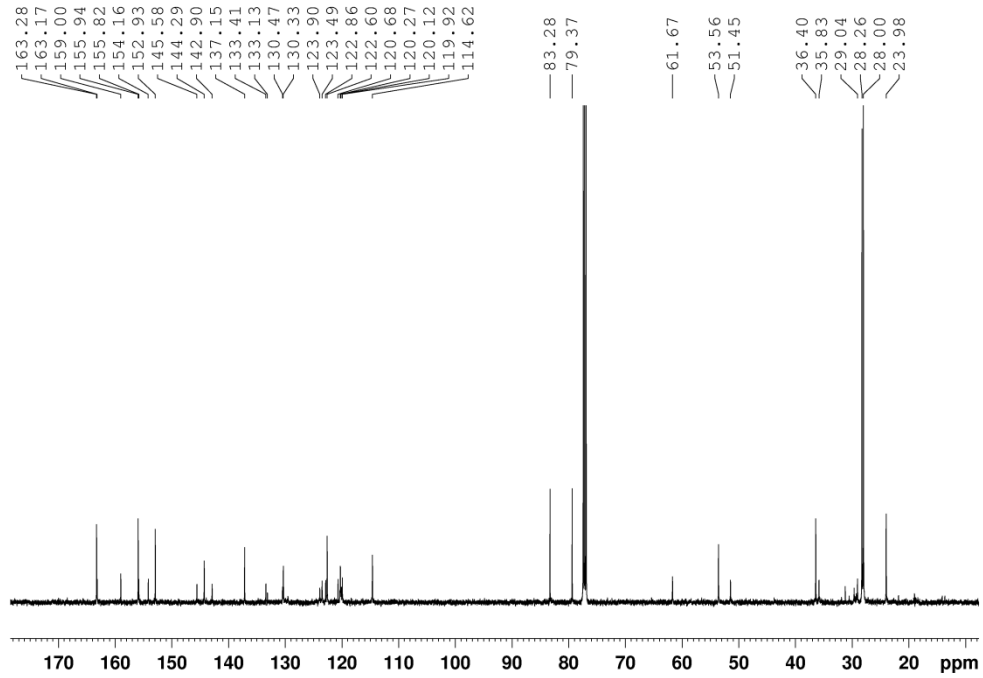


Figure S7. ^{13}C NMR spectrum of **7** in $CDCl_3$.

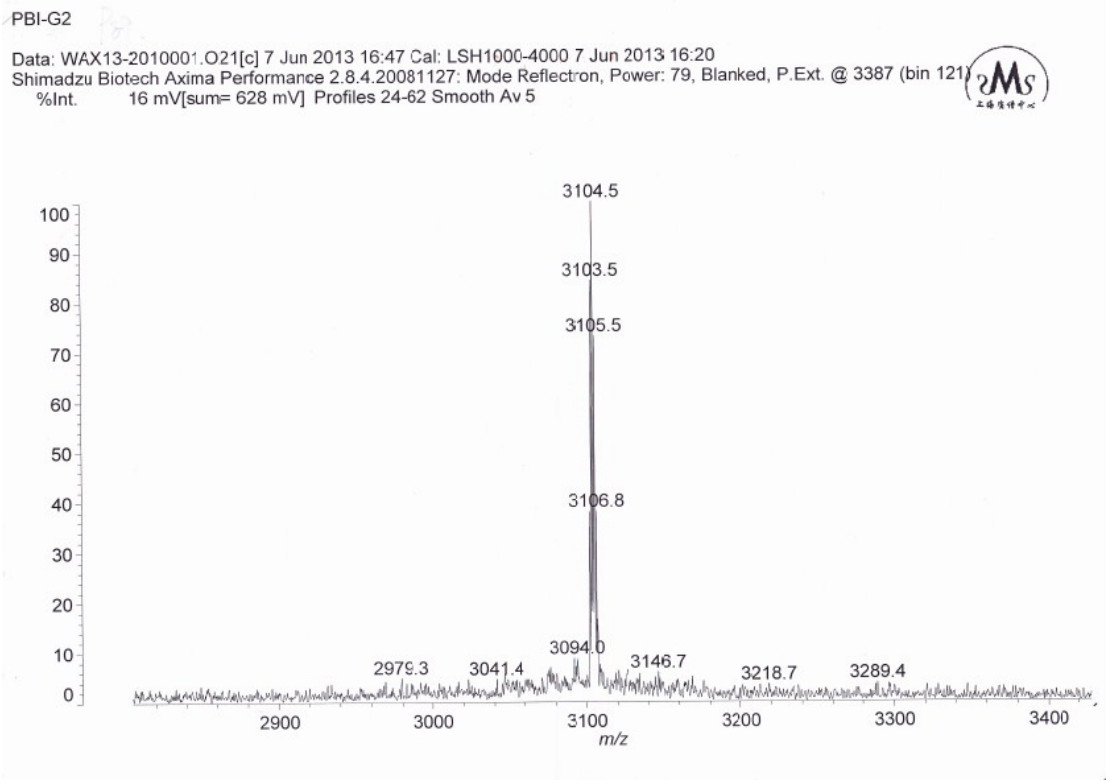


Figure S8. MS (MALDI-TOF) spectrum of **PBI-G2**.

References

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