Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Synthesis and Optical Properties of Water-Soluble Biperylene-based Dendrimers

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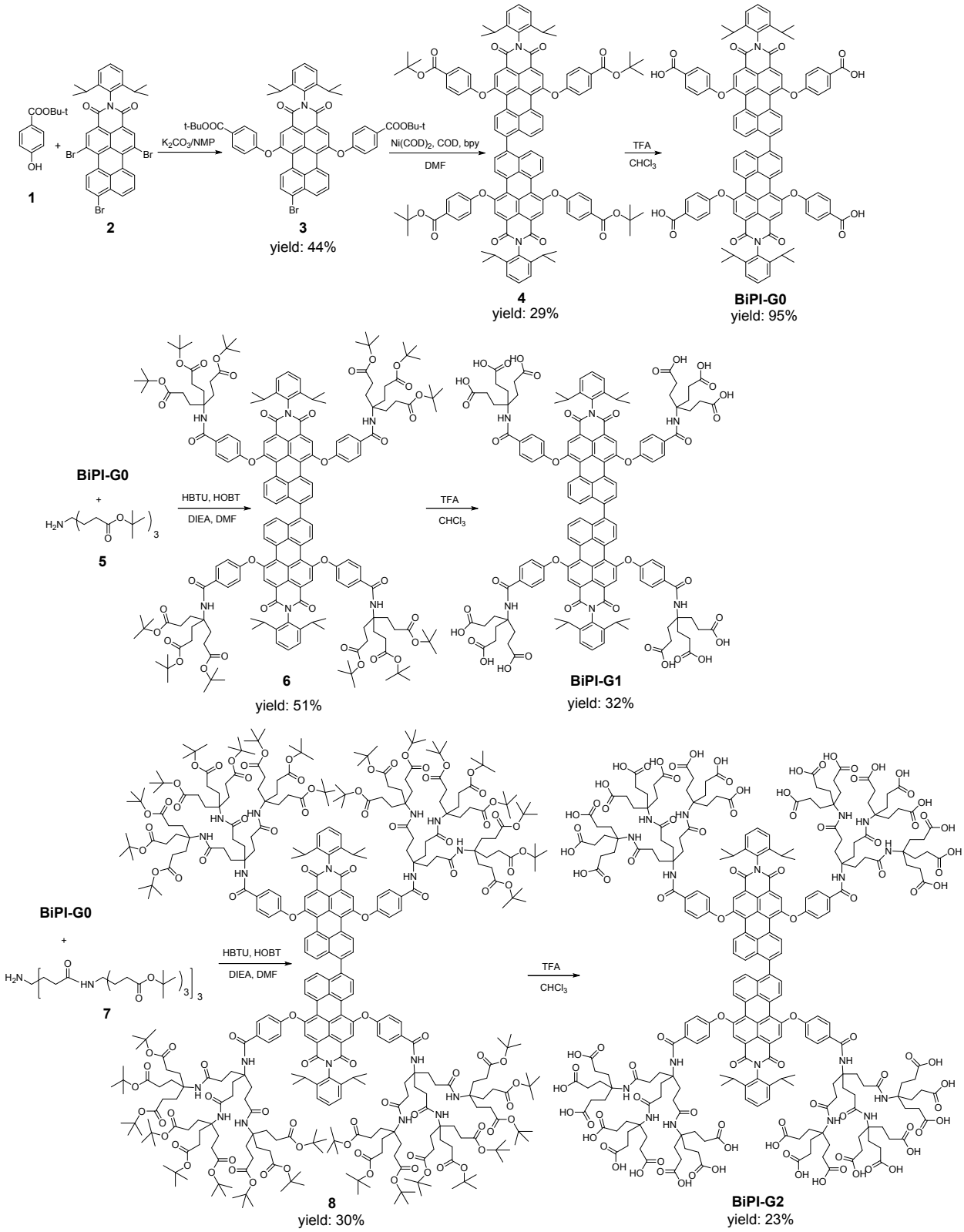
Supporting Information

General: The solvents used are ACS or HPLC grade. Compound **5** was purchased from Frontier Scientific (catalog# NTN1693). Compounds **1**, **2** and **7** were synthesized by following the procedures reported.[1-3] Column chromatography was performed on silica gel (standard grade, 60A, Sorbtech). ^{1}H and ^{13}C NMR spectra were recorded on the Brucker DRX 300 MHz and Brucker Avance III 400 MHz instruments. MALDI-TOF mass spectra were recorded on a PerSeptive Voyager STR MS spectrometer. UV/Vis spectra were recorded on a Cary 100 Bio UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer.

Compound 3:
Compound **1** (1.03 g, 5.31 mmol), **2** (1.8 g, 2.5 mmol), and potassium carbonate (0.7 g, 5.1 mmol) were stirred in N-methyl-2-pyrrolidone (100 mL) at 80 ˚C under argon for 6 h. After being cooled to room temperature, the mixture was poured into brine (800 mL). The resulting red precipitate was collected by filtration and washed with water (100 mL × 3). The crude product was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/50) as the eluent resulting in **3** (1.24 g, 44 %) as a red solid. ^{1}H NMR ($CDCl_3$): δ = 9.22 (dd, 1H, *J* = 0.9 & 6.9 Hz), 9.00 (d, 1 H, *J* = 8.4 Hz), 8.33-8.36 (m, 3 H), 7.98-8.02 (m, 4 H), 7.86 (d, 1 H, *J* = 8.4 Hz), 7.67 (t, 1 H, *J* = 8.1 Hz), 7.46 (t, 1 H, *J* = 8.1 Hz), 7.29-7.34 (m, 2 H), 7.07-7.11 (m, 4 H), 2.71 (sep, 2 H, *J* = 6.9 Hz), 1.58 (s, 18 H), 1.15 (d, 12 H, *J* = 6.9 Hz).

Compound 4:
A mixture of bis(1,5-cyclooctadiene)nickel (0) ($Ni(COD)_2$) (1 g, 3.6 mmol), 2,2'-bipyridine (bpy) (0.567 g, 3.6 mmol) and 1,5-cyclooctadiene (COD) (0.33 g, 3.06 mmol) in anhydrous DMF (20 mL) was heated at 70 ˚C under argon for 0.5 h. Next, **3** (1.24 g, 1.31 mmol) in anhydrous DMF (20 mL) was added dropwise and the resulting mixture was heated at 80 ˚C for 5 h. After being cooled to room temperature, the mixture was poured into H_2O (150 mL) and extracted with ethyl acetate (100 mL × 3). The organic layer was dried over anhydrous sodium sulfate. The crude product was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/100 to 1/50) as the eluent resulting in **4** (0.33 g, 29 %) as a red solid. ^{1}H NMR ($CDCl_3$): δ = 9.27 (d, 2H, *J* = 8.1 Hz), 9.19 (d, 2H, *J* = 7.8 Hz), 8.39 (s, 2 H), 8.38 (s, 2H), 7.99-8.03 (m, 8 H), 7.59 (d, 2 H, *J* = 8.1 Hz), 7.53 (d, 2 H, *J* = 8.4 Hz), 7.47 (t, 2 H, *J* = 7.8 Hz), 7.41 (t, 2 H, *J* = 7.8 Hz), 7.32 (d, 4 H, *J* = 7.8 Hz), 7.09-7.16 (m, 8 H), 2.71-2.77 (m, 4 H), 1.57 (s, 36 H), 1.17 (d, 12 H, *J* = 6.9 Hz), 1.16 (d, 12 H, *J* = 6.9 Hz). ^{13}C NMR ($CDCl_3$): δ = 165.11, 162.99, 159.49, 152.20, 152.14, 145.79, 145.77, 140.51, 132.89, 132.13, 132.10, 132.05, 131.77, 130.59, 129.76, 129.52, 129.25, 128.72, 128.49, 128.43, 127.91, 127.84, 127.40, 127.37, 127.29, 126.41, 126.29, 124.66, 124.19, 122.27, 117.54, 117.44, 81.31, 81.29, 29.30, 28.34, 24.19, 24.18. MS (MALDI-TOF): calcd. for $C_{112}H_{100}N_2O_{16}$ [M] *m/z* 1729.71, found *m/z* 1729.86.

BiPI-G0:
Compound **4** (0.1 g, 57.8 μmol) was dissolved in chloroform (15 mL) and then trifluoroacetic acid (5 mL) was added. The resulting mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation and the residue was washed by dichloromethane (10 mL × 3). **BiPI-G0** (82 mg, 95 %) was obtained as a red solid. MS (MALDI-TOF): calcd. for $C_{96}H_{68}N_2O_{16}$ *m/z* 1505.46, found *m/z* 1505.73. Due to aggregation of the dye molecule at high concentration needed, ^{1}H and ^{13}C NMR spectra could not be obtained.



Scheme S1. Synthesis of the **BiPI** dendrimers.

Compound 6:

A mixture of BiPI-G0 (35 mg, 23 μmol), HBTU (53 mg, 140 μmol), and HOBt (19 mg, 140 μmol) in anhydrous DMF (4 mL) was stirred at room temperature for 5 min. DIEA (35 μL, 201 μmol) was then added and the mixture was stirred for another 10 min. Next, aminotriester **5** (58 mg, 140 μmol) was added to the acid solution and the resulting mixture was stirred at room temperature under argon for 48 h. The solvent was removed by rotary evaporation and the resulting solid was purified by thin layer chromatography using large silica gel plates with dichloromethane/ethyl acetate (25/1) as the eluent resulting in compound **6** (36 mg, 51 %) as a red solid. ^{1}H NMR ($CDCl_3$): δ = 9.33 (d, 2H, *J* = 8 Hz), 9.21 (dd, 2H, *J* = 0.8&8 Hz), 8.36 (s, 2 H), 8.35 (s, 2H), 7.87 (d, 4H, *J* = 7.6 Hz), 7.85 (d, 4H, *J* = 8 Hz), 7.64 (d, 2 H, *J* = 8 Hz), 7.60 (dd, 2 H, *J* = 0.8&8.4 Hz), 7.42-7.48 (m, 4 H), 7.31 (d, 4 H, *J* = 7.6 Hz), 7.19 (d, 4 H, *J* = 8.8 Hz), 7.15 (d, 4 H, *J* = 8.8 Hz), 6.93 (s, 2H), 6.92 (s, 2H), 2.73 (hex, 4 H, *J* = 6.8 Hz), 2.30 (t, 24 H, *J* = 7.2 Hz), 2.12 (t, 24 H, *J* = 7.2 Hz), 1.41 (s, 108 H), 1.16 (d, 12 H, *J* = 6.8 Hz), 1.15 (d, 12 H, *J* = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ = 173.30, 165.84, 163.01, 158.58, 158.26, 152.60, 152.45, 145.80, 145.76, 140.57, 132.95, 132.06, 131.11, 131.00, 130.65, 129.64, 129.61, 129.26, 128.48, 128.15, 127.53, 127.51, 127.31, 125.89, 124.38, 124.13, 122.18, 118.18, 118.02, 80.94, 58.04, 58.02, 30.40, 30.09, 29.27, 28.21, 24.19, 24.17. MS (MALDI-TOF): calcd. for $C_{184}H_{225}N_6O_{36}$ [M+H] *m/z* 3096.60, found *m/z* 3096.82.

BiPI-G1:

Trifluoroacetic acid (1 mL) as added to a solution of compound **6** (36 mg, 11.6 μmol) in chloroform (4 mL) and the resulting mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation. The residue was dissolved in methanol (10 mL) and precipitated out after diethyl ether (100 mL) was added. BiPI-G1 (9 mg, 32 %) was obtained as a red solid after filtration. ^{1}H NMR (*d*4-MeOD): δ = 9.17 (br.s, 2H), 8.35 (br.s, 2H), 8.11 (br.s, 2 H), 7.85 (br.s, 2H), 7.74 (d, 4H, *J* = 8 Hz), 7.43 (t, 4H, *J* = 8 Hz), 7.29 (d, 8 H, *J* = 8 Hz), 7.03 (br.s, 10 H), 2.69-2.72 (m, 4 H), 2.28-2.32 (m, 24 H), 2.12-2.14 (m, 24 H), 1.04-1.09 (m, 24 H). MS (MALDI-TOF): calcd. for $C_{136}H_{128}N_6O_{36}$ *m/z* 2421.84, found *m/z* 2421.72. Due to aggregation of the dye molecule at high concentration needed, ^{13}C NMR spectrum could not be obtained.

Compound 8:

A mixture of BiPI-G0 (30 mg, 20 μmol), HBTU (33 mg, 87 μmol), and HOBt (12 mg, 89 μmol) in anhydrous DMF (4 mL) was stirred at room temperature for 5 min. DIEA (22 μL, 126 μmol) was then added and the mixture was stirred for another 10 min. After that, **7** (172 mg, 120 μmol) was added to the acid solution. The resulting mixture was stirred at room temperature under argon for 48 h. The solvent was removed by rotary evaporation and the residue was purified by thin layer chromatography using large silica gel plates with dichloromethane/ethyl acetate (40/3) as the eluent resulting in compound **8** (43 mg, 30 %) as a red solid. ^{1}H NMR ($CDCl_3$): δ = 9.41 (d, 2H, *J* = 8.4 Hz), 9.28 (d, 2H, *J* = 8 Hz), 8.69 (s, 2 H), 8.68 (s, 2 H), 8.27 (s, 2H), 8.26 (s, 2 H), 8.05 (t, 8H, *J* = 8.8 Hz), 7.66 (d, 4H, *J* = 8.4 Hz), 7.64 (d, 2 H, *J* = 8 Hz), 7.46 (t, 4 H, *J* = 8 Hz), 7.40 (t, 4 H, *J* = 7.6 Hz), 7.30 (d, 4 H, *J* = 8.8 Hz), 7.24-7.25 (m, 4 H), 6.01 (s, 12H), 2.67-2.76 (m, 4 H), 2.26-2.27 (m, 24 H), 2.16-2.20 (m, 96 H), 1.92-1.96 (m, 72 H), 1.39 (s, 324 H), 1.11-1.14 (m, 24 H). ^{13}C NMR ($CDCl_3$): δ = 173.09, 172.68, 166.1, 162.90, 158.14, 153.18, 145.76, 145.70, 140.30, 132.88, 132.05, 130.69, 130.02, 129.66, 129.38, 129.03, 128.29, 127.75, 127.09, 123.88, 121.95, 118.92, 80.61, 58.02, 57.57, 34.67, 32.01, 31.84, 29.90, 29.82, 29.08, 28.26, 28.08, 24.10, 24.08. MS (MALDI-TOF): calcd. for $C_{400}H_{596}N_{18}O_{96}$ *m/z* 7192.24, found *m/z* 7191.80.

BiPI-G2:

Compound **8** (43 mg, 6 μmol) was dissolved in chloroform (4 mL) and then trifluoroacetic acid (1 mL) was added. The resulting mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation. The residue was dissolved in methanol (4 mL) and precipitate by adding diethyl ether (50 mL). BiPI-G2 (7 mg, 23 %) was obtained as a red solid after filtration. ^{1}H NMR (*d*6-DMSO): δ = 9.43 (d, 2H, *J* = 8 Hz), 9.27 (d, 2H, *J* = 7.2 Hz), 8.09 (s, 2 H), 8.08 (s, 2 H), 7.95-8.04 (m, 8H), 7.87 (d, 2H, *J* = 8 Hz), 7.63 (d, 2H, *J* = 7.6 Hz), 7.55 (d, 2 H, *J* = 7.6 Hz), 7.42 (d, 6 H, *J* = 8 Hz), 7.36 (d, 4 H, *J* = 8.4 Hz), 7.31 (d, 4 H, *J* = 6.8 Hz), 7.25 (br.s, 12 H), 2.65-2.67 (m, 4 H), 2.10 (br.s, 96 H), 1.82-1.92 (m, 96 H), 1.04 (br.s, 24 H). ^{13}C NMR (*d*6-DMSO): δ = 174.94, 172.90, 165.74, 163.07, 157.64, 157.57, 153.77, 153.85, 145.90, 140.30, 132.91, 132.83, 132.64, 131.94, 130.88, 130.64, 129.35, 127.62, 127.07, 126.62, 124.26, 123.28, 121.85, 119.61, 119.08, 58.05, 56.81, 31.06, 30.74, 29.53, 28.86, 28.55, 28.15, 24.19. MS (MALDI-TOF): calcd. for $C_{256}H_{308}N_{18}O_{96}$ *m/z* 5171.98, found *m/z* 5172.40.

Photophysical Study of BiPI-G0, BiPI-G1 and BiPI-G2

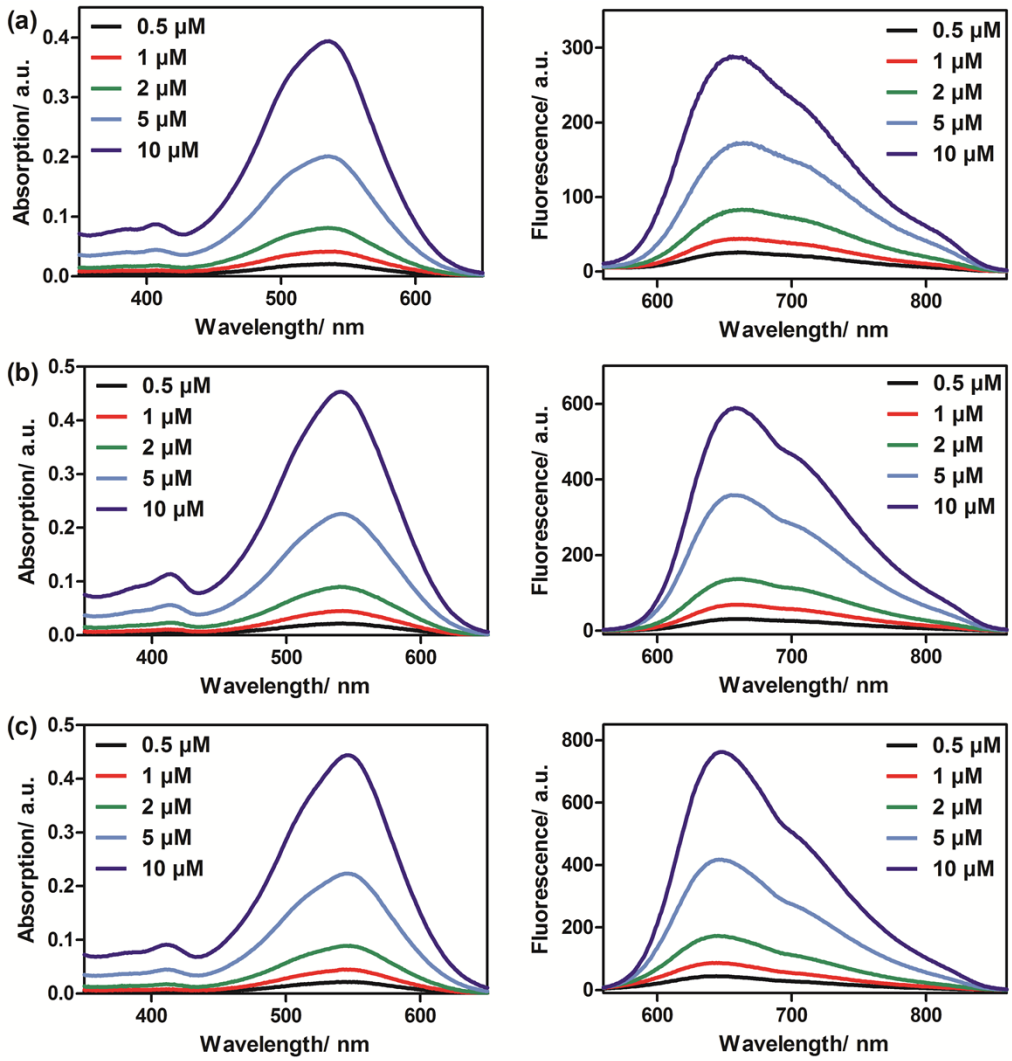


Figure S1. The concentration-dependent absorption and emission (λ_{ex}= 530 nm) spectra of (a) **BiPI-G0**, (b) **BiPI-G1** and (c) **BiPI-G2** in water.

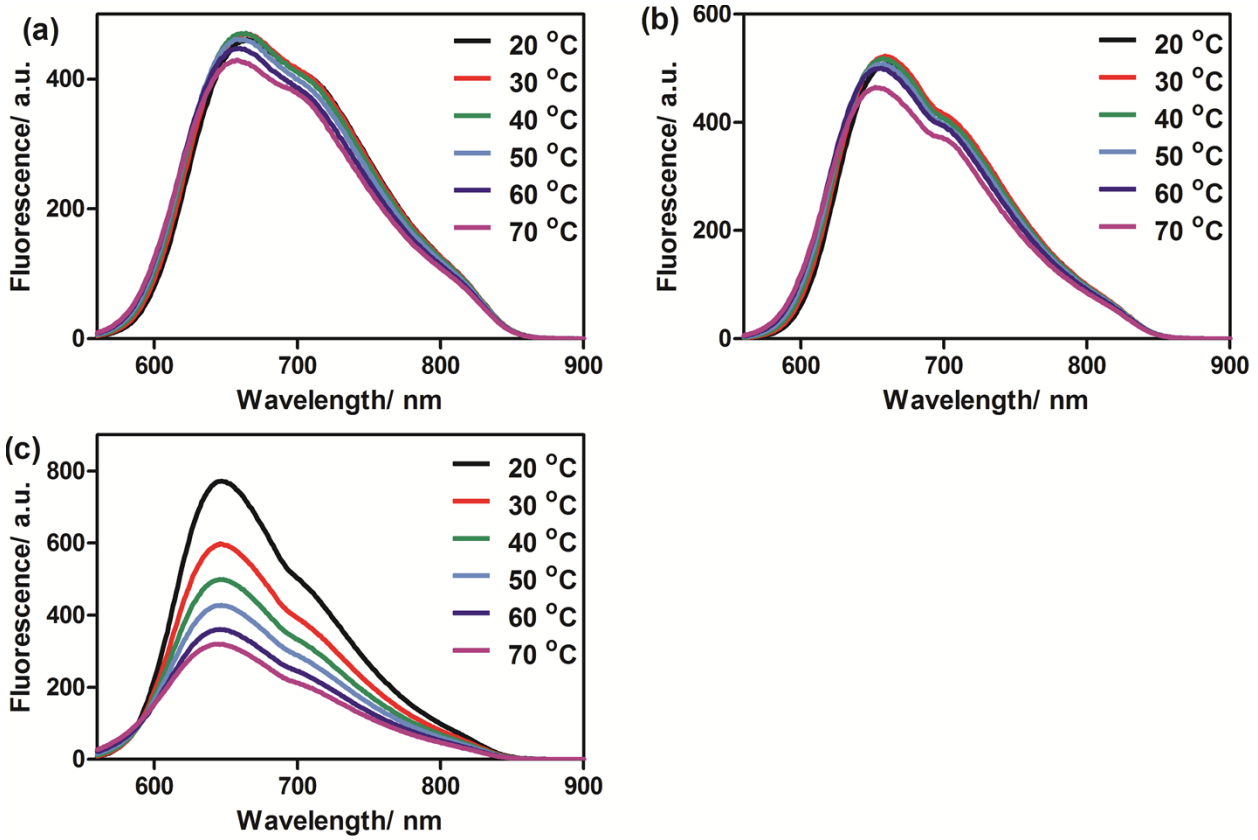


Figure S2. The temperature-dependent emission spectra (λ_{ex}= 530 nm) of (a) BiPI-G0, (b) BiPI-G1 and (c) BiPI-G2 in water at a concentration of 10 μM.

Cytotoxicity

The cell viability assay was performed by using a mouse malignant astrocytoma cell line, wild type delayed brain tumor (WT-DBT). WT-DBT cells were cultured in DMEM containing 10% fetal bovine serum, 4 mM glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. WT-DBT cells were seeded in 24-well plates at density of ~7×10^4 cells/mL for 24 h in a water-jacketed incubator (37 °C, 5% CO_2). Cells were then treated with indicated concentrations of BiPI-G0, BiPI-G1 and BiPI-G2 for 24 h (0 μM, 1 μM, 5 μM to 10 μM). After 24 h incubation, the cells from each group were harvested by trypsinization using a 0.05% trypsin–EDTA solution. Cell toxicity was assessed by a Hemocytometer-based trypan blue dye exclusion method[4]. Indocyanine green (ICG) (Sigma-Aldrich) was used as the negative control and Doxorubicin Hydrochloride (Fisher Scientific) was used as the positive control.

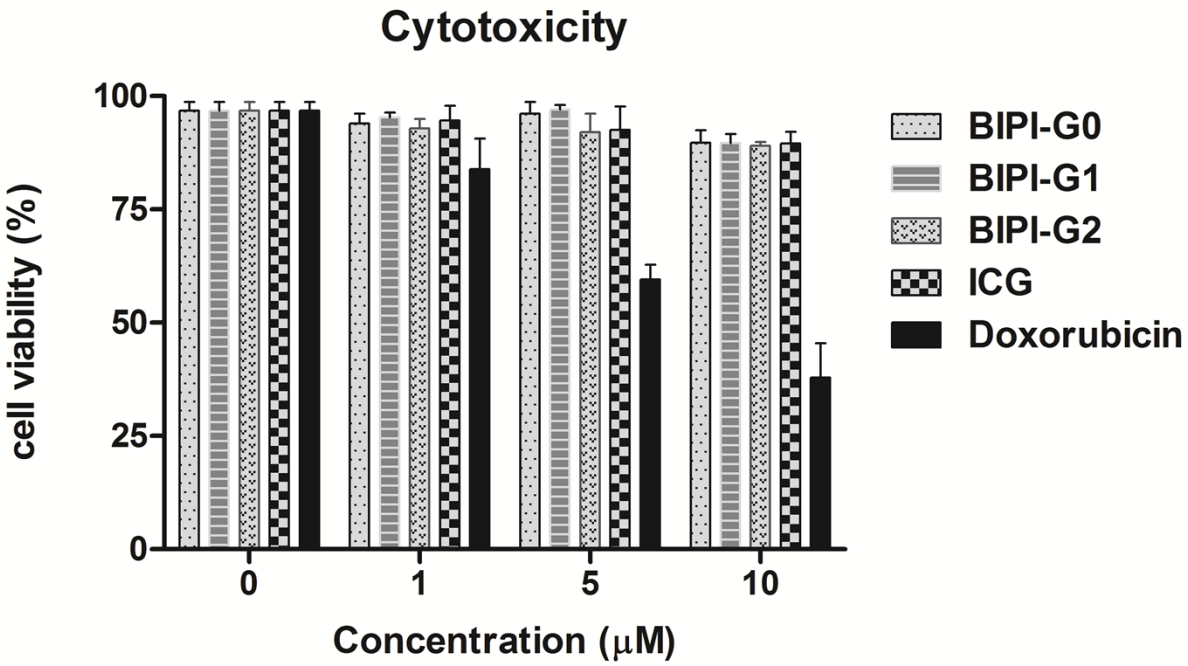


Figure S3. Cytotoxicity study of BiPI-G0, BiPI-G1 and BiPI-G2. To evaluate the cytotoxicity of BiPI-G0, BiPI-G1 and BiPI-G2, WT-DBT cells were incubated with 0, 1, 5, 10 μM of these dendrimers for 24 h. Cell toxicity was measured using the Hemocytometer-based trypan blue dye exclusion method. Indocyanine gree (ICG), the only clinically approved near infrared fluorescent dye, was used as the negative control. Doxorubicin, a commonly used chemotherapy drug, served as the positive control. Each data point represents the mean ± SD based on triplicate samples.

Cell fluorescent imaging of BiPI-G0, BiPI-G1 and BiPI-G2

WT-DBT cells were seeded into 35 mm MatTek dishes (MatTek Corporation) and cultured for 24 h. Cells were treated with 5 μM BiPI-G0, BiPI-G1 and BiPI-G2 at 37 °C for 3 h in a water-jacketed incubator (37 °C, 5% CO_2). After being washed with PBS twice to remove residues of dendrimers, the cells were imaged using the Zeiss Axio Observer fluorescent microscope equipped with the ApoTome 2 imaging system. Rhodamine filter was used to image fluorescent probes (Excitation/Emission: 538-562/570-640 nm). Differential interference contrast (DIC) images were obtained through Trans light DIC.

Quantification of BiPI dendrimer uptake in cells

WT-DBT cells were incubated with 5 μM of BiPI-G0, BiPI-G1 or BiPI-G2 for 3 h. Cells were washed with PBS once. Fluorescence intensity of the dendrimers uptake in cells and those left in cell medium and PBS was measured respectively, using a SynergyTM H4 Hybrid Multi-Mode Microplate Reader. Specific excitation/emission wavelength that corresponds to the maximum absorption/emission of each BiPI dendrimer was used to measure fluorescence intensity as follows: 535/663 nm (BiPI-G0), 540/661 nm (BiPI-G1), and 545/646 nm (BiPI-G2).

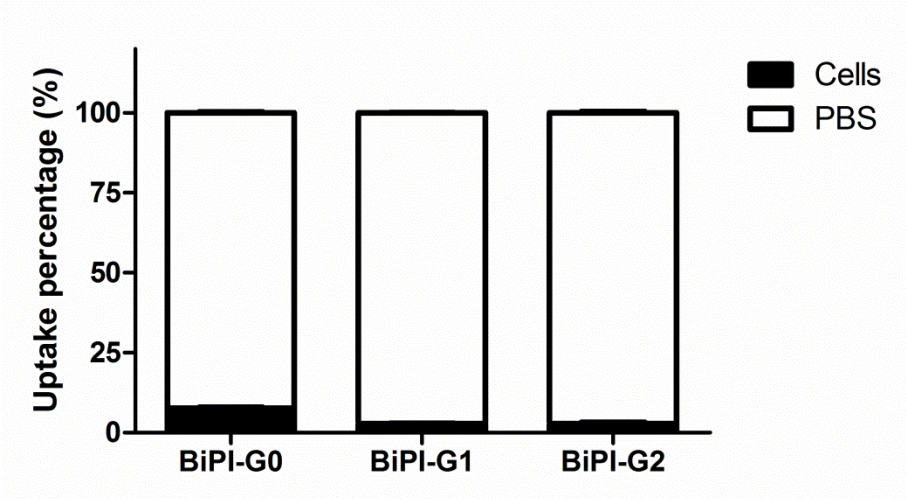


Figure S4. Cellular uptake of dendrimers. Cells were incubated with 5 μM of BiPI-G0, BiPI-G1 and BiPI-G2 for 3 h. Fluorescence intensity of the uptaken (in cells) and unbound (in cell medium) molecules was measured respectively. Each data point represents the mean ± standard error of the mean (SEM) based on triplicate samples.

Concentration-dependent and incubation time-dependent cell uptake.

To measure concentration-dependent uptake, WT-DBT cells were treated with 0, 0.625, 1.25, 2.5, 5, or 10 μM of each BiPI dendrimer for 3 h. To measure incubation time-dependent uptake, cells were treated with 5 μM of each BiPI dendrimer for indicated time (0 min, 5 min, 30 min, 1 h, 3 h, 6 h, 24 h). Fluorescence intensity was measured after cells were washed with PBS once. Specific excitation/emission wavelength that corresponds to the maximum absorption/emission of each BiPI dendrimer was used to measure fluorescence intensity as follows: 535/663 nm (BiPI-G0), 540/661 nm (BiPI-G1), and 545/646 nm (BiPI-G2).

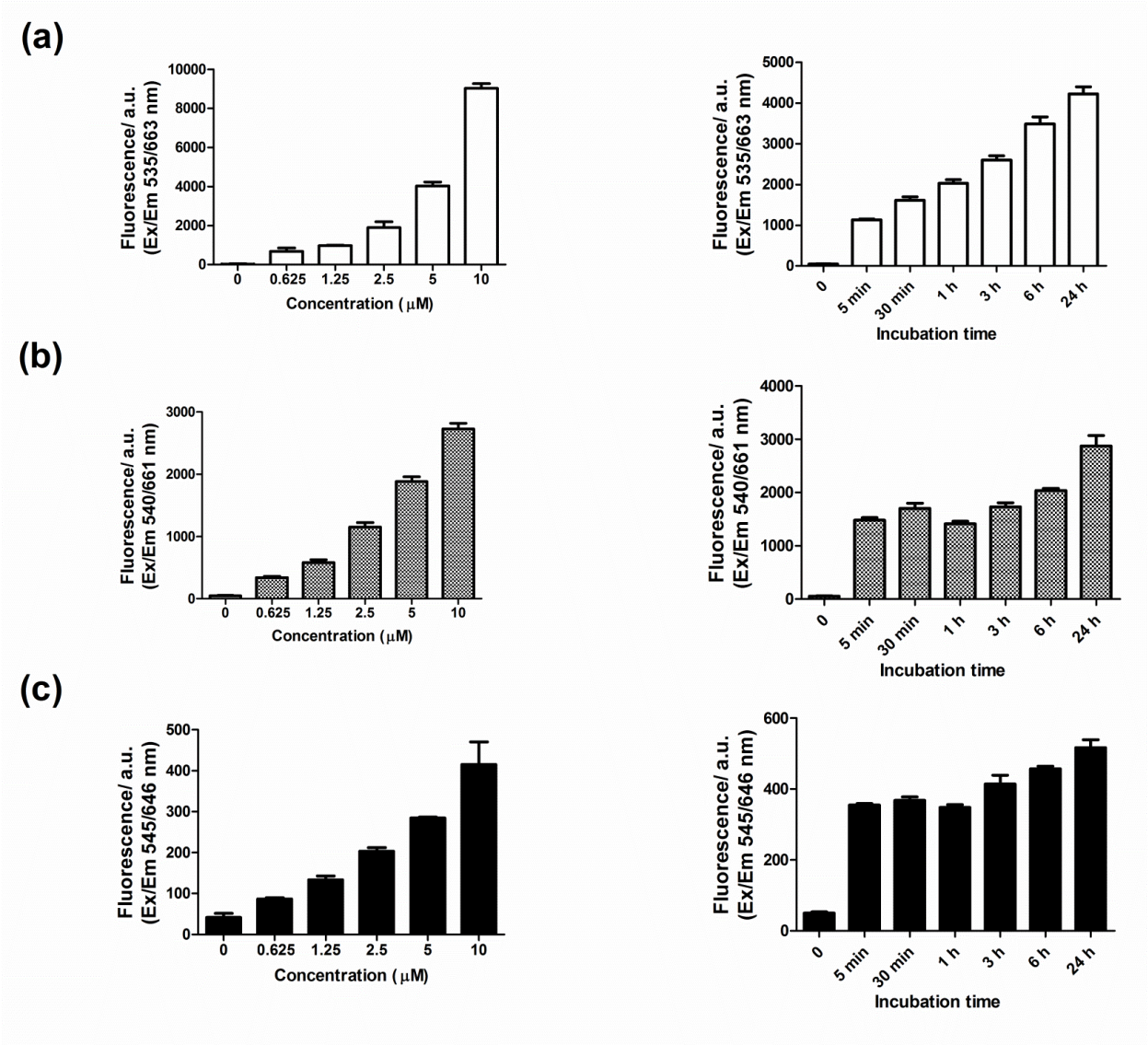


Figure S5. Concentration-dependent and incubation time-dependent cell uptake of dendrimers. For the measurement of concentration-dependent uptake, cells were treated with 0, 0.625, 1.25, 2.5, 5, or 10 μM of each BiPI dendrimer for 3 h. For the measurement of incubation time-dependent uptake, cells were treated with 5 μM of each BiPI dendrimer for indicated time (0 min, 5 min, 30 min, 1 h, 3 h, 6 h, 24 h). Left: concentration-dependent cellular uptake. Right: incubation time-dependent cellular uptake. (a) BiPI-G0. (b) BiPI-G1. (c) BiPI-G2. Each data point represents the mean ± standard error of the mean (SEM) based on triplicate samples.

Reference

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2. Y. Geerts, H. Quante, H. Platz, R. Mahrt, M. Hopmeier, A. Bohm, K. Mullen, *J Mater Chem* 1998, **8**, 2357.
3. C. Ornelas, R. Pennell, L. F. Liebes, M. Weck, *Org Lett,* 2011, **13**, 976.
4. T. Umemura, M. Naoi, T. Takahashi, Y. Fukui, T. Yasue, M. Ohashi, T. Nagatsu, *Biochem Med Metab Biol,* 1990, **44**, 51.

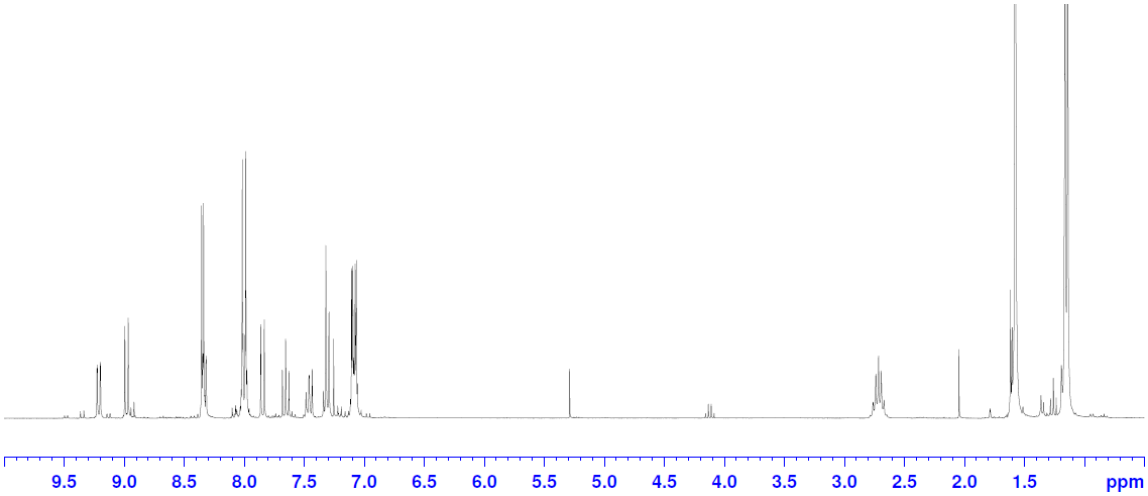

Figure S6 ^{1}H NMR spectrum of compound 3.

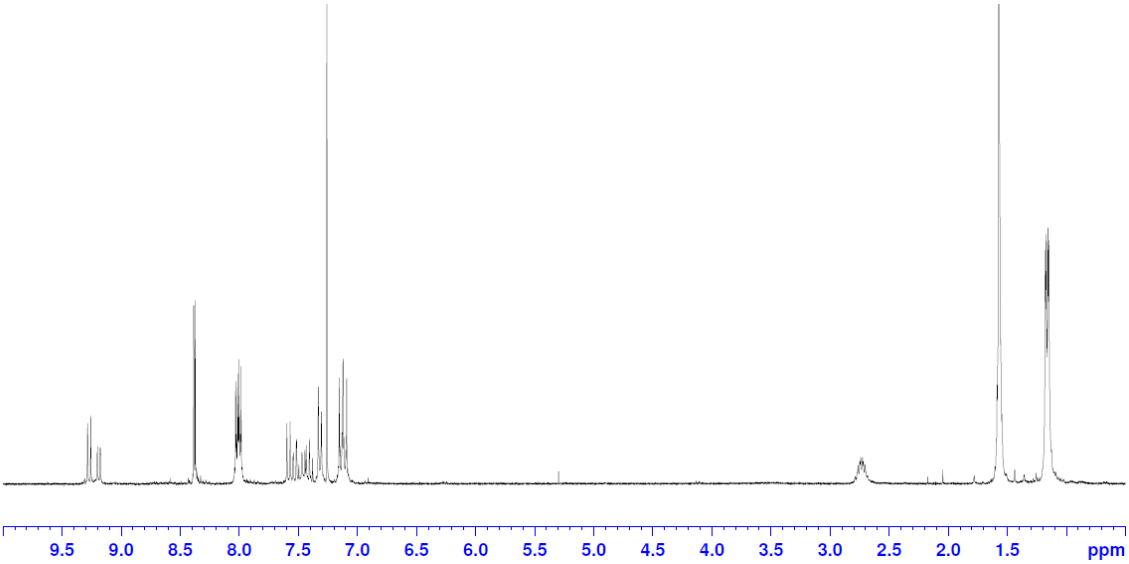

Figure S7 ^{1}H NMR spectrum of compound 4.

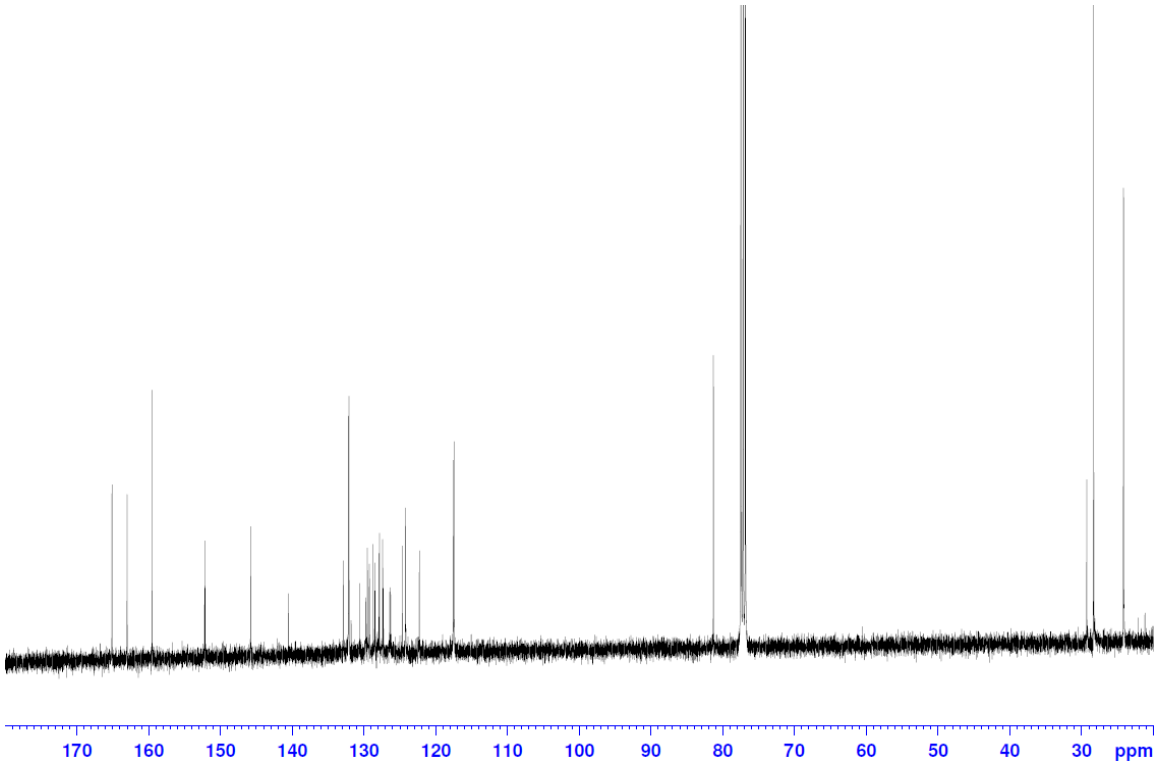

Figure S8 ^{13}C NMR spectrum of compound 4.

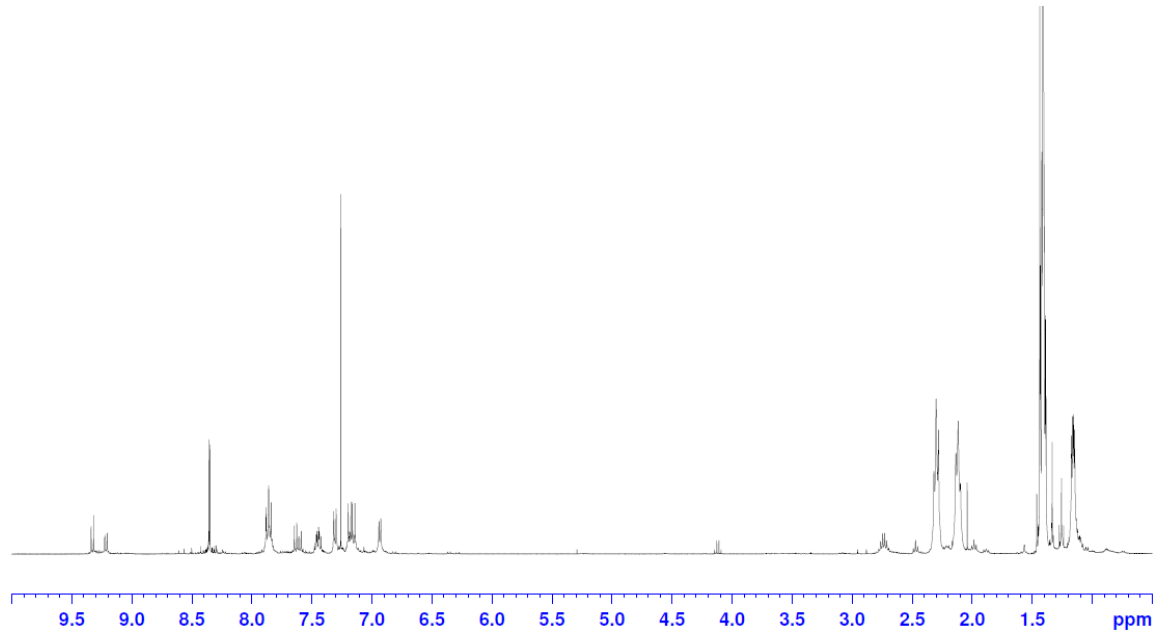

Figure S9 ^{1}H NMR spectrum of compound 6.

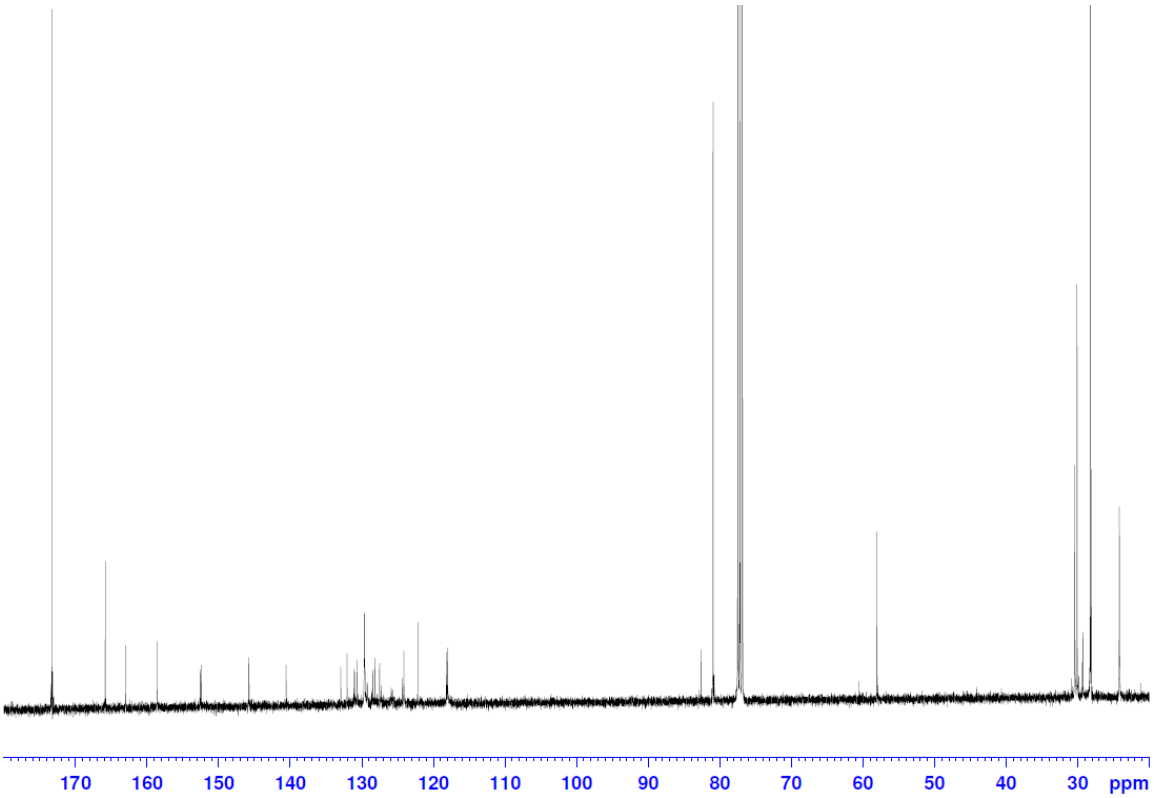

Figure S10 ^{13}C NMR spectrum of compound 6.

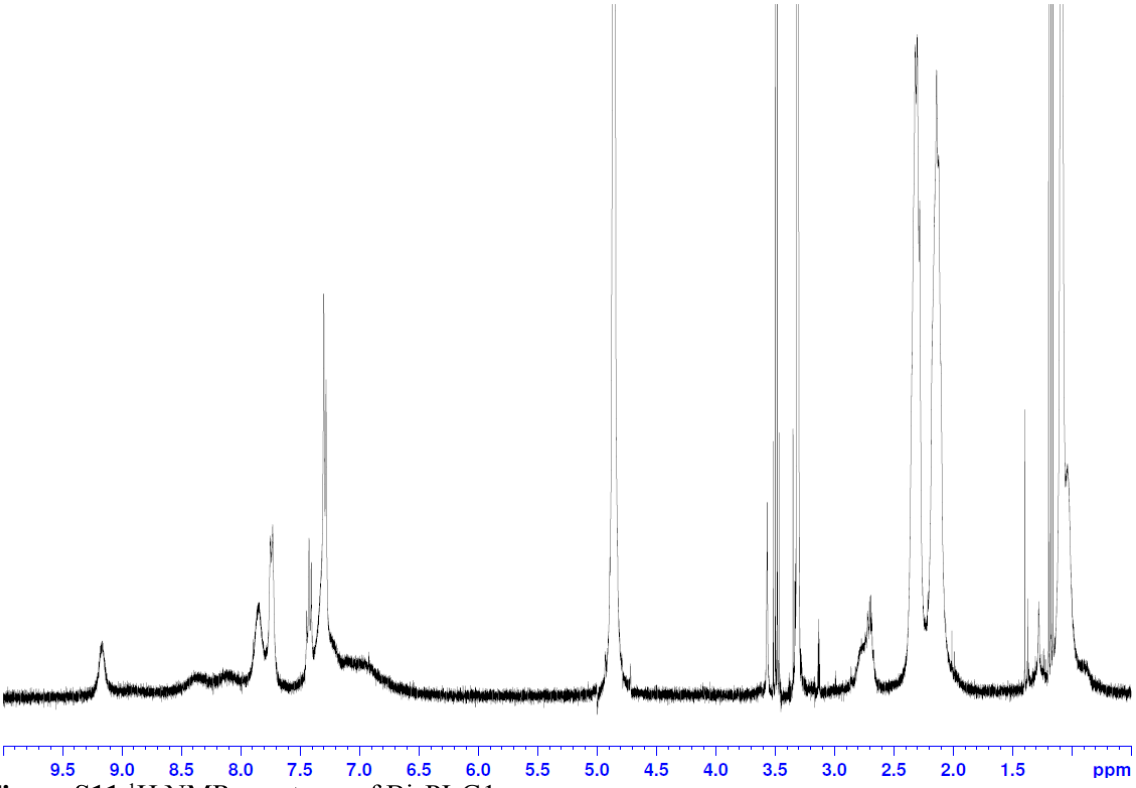

Figure S11 ^{1}H NMR spectrum of BisPI-G1.

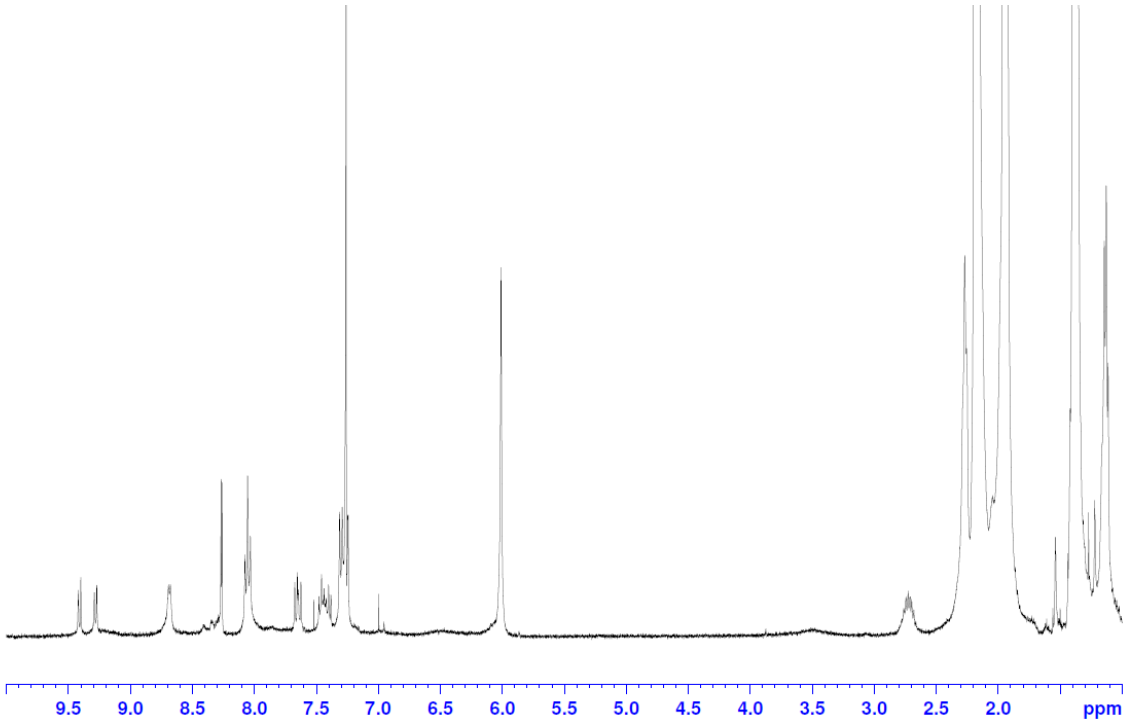

Figure S12 ^{1}H NMR spectrum of compound 8.

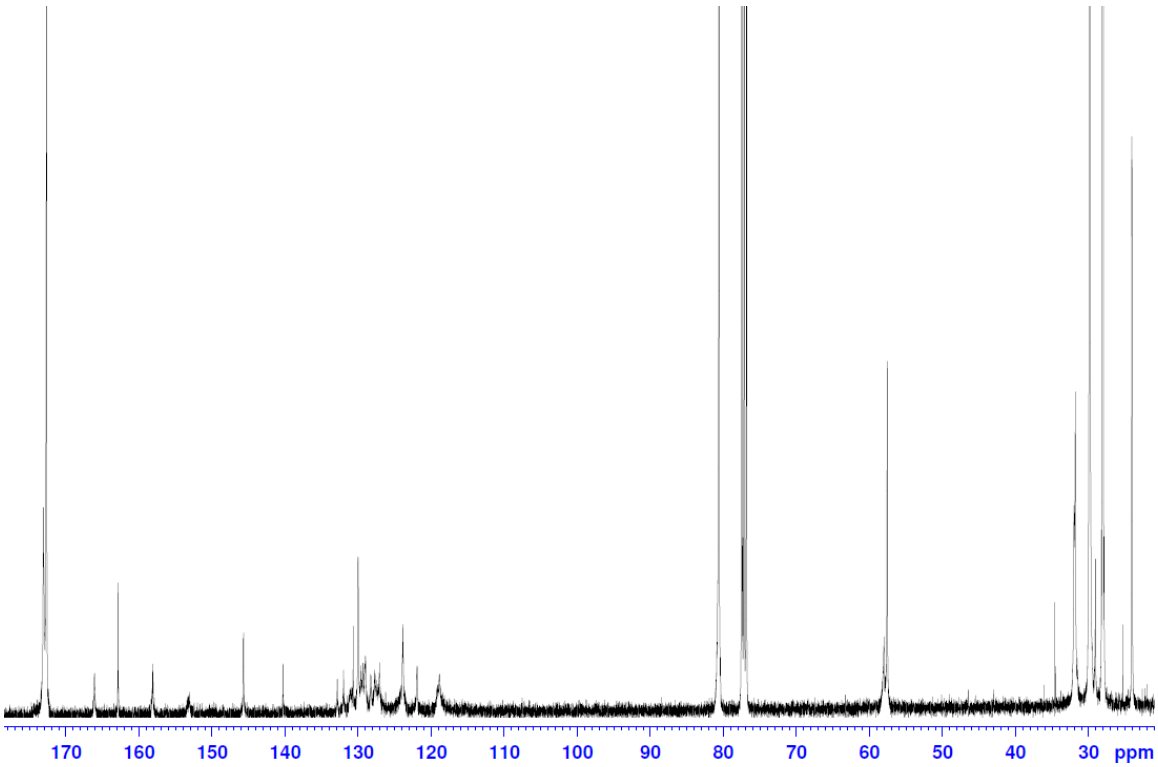

Figure S13 ^{13}C NMR spectrum of compound 8.

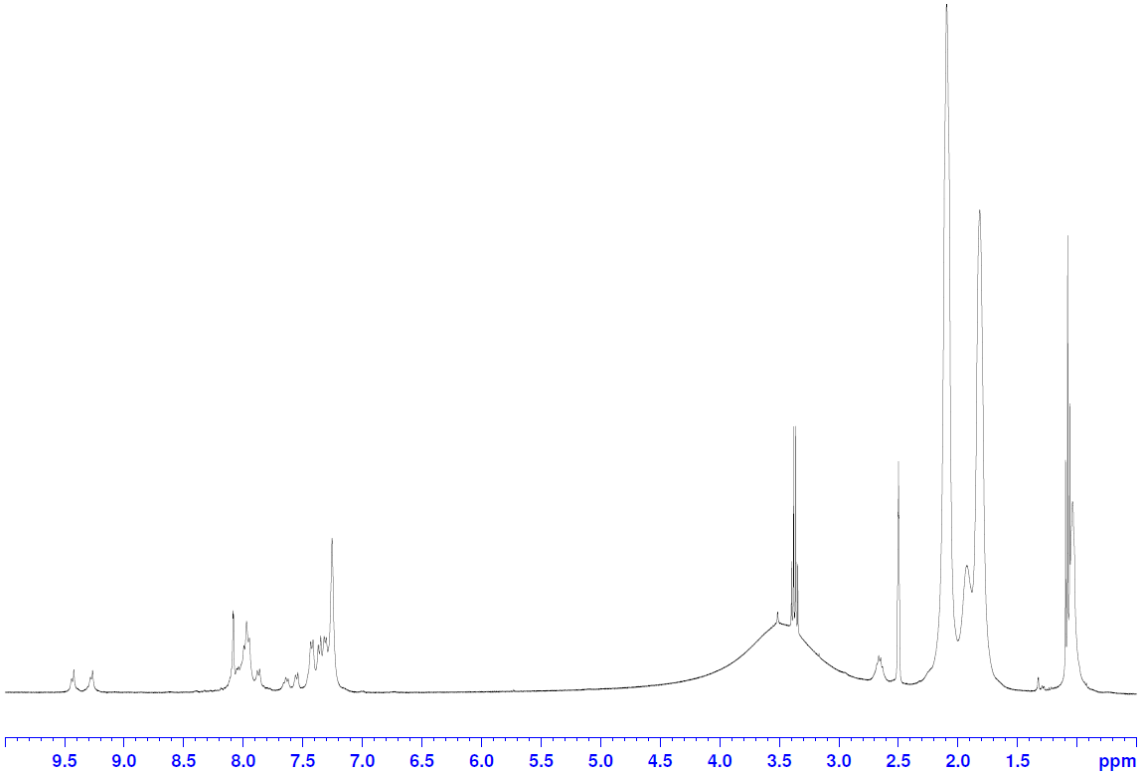

Figure S14 ^{1}H NMR spectrum of BisPI-G2.

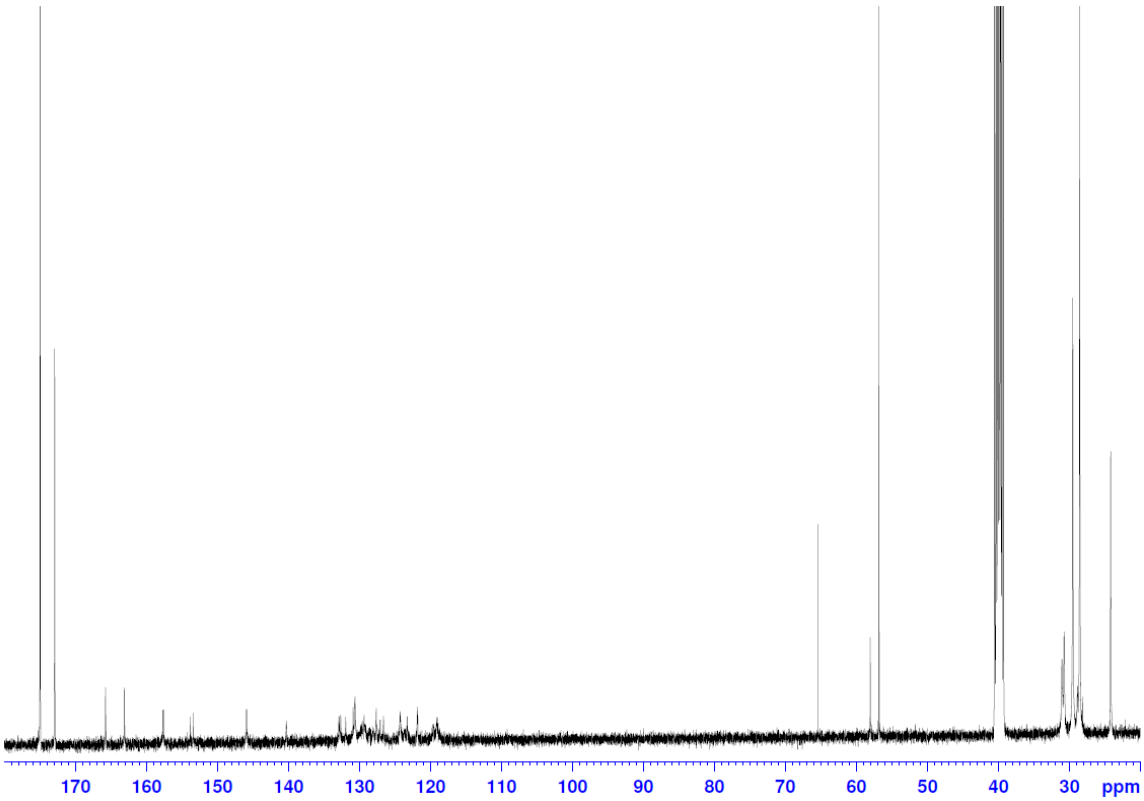

Figure S15 ^{13}C NMR spectrum of BisPI-G2.

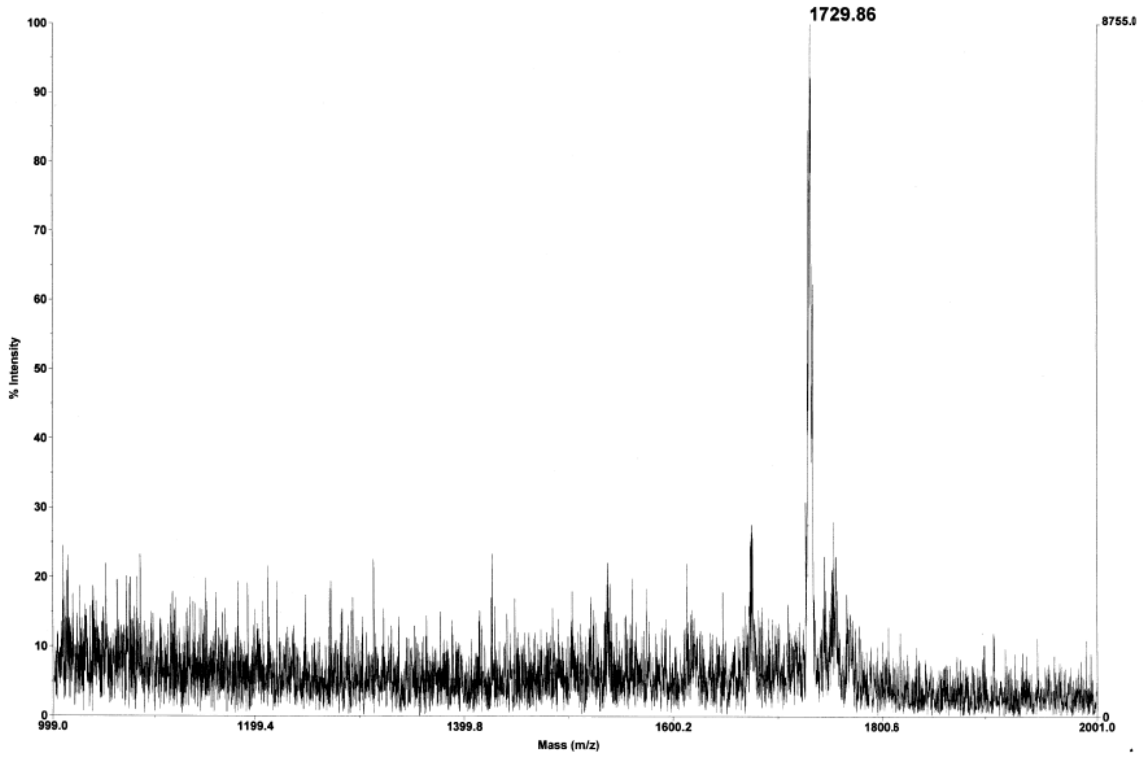

Figure S16 MALDI spectrum of compound 4.

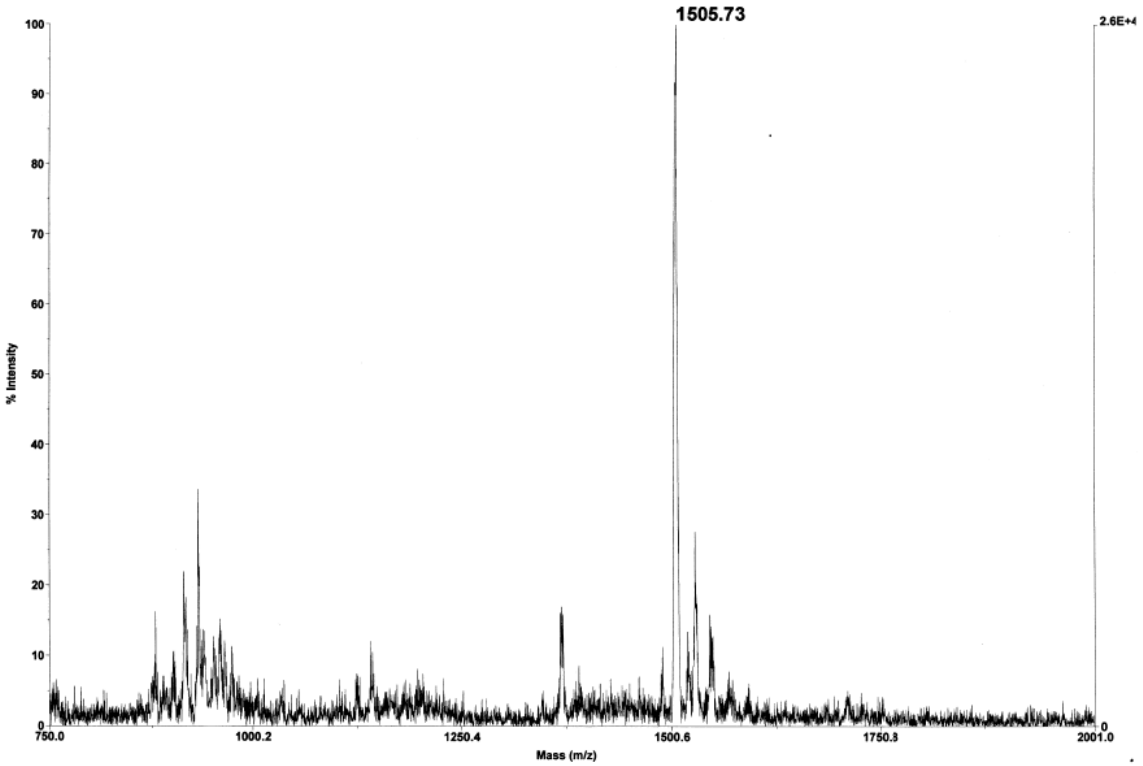

Figure S17 MALDI spectrum of BisPI-G0.

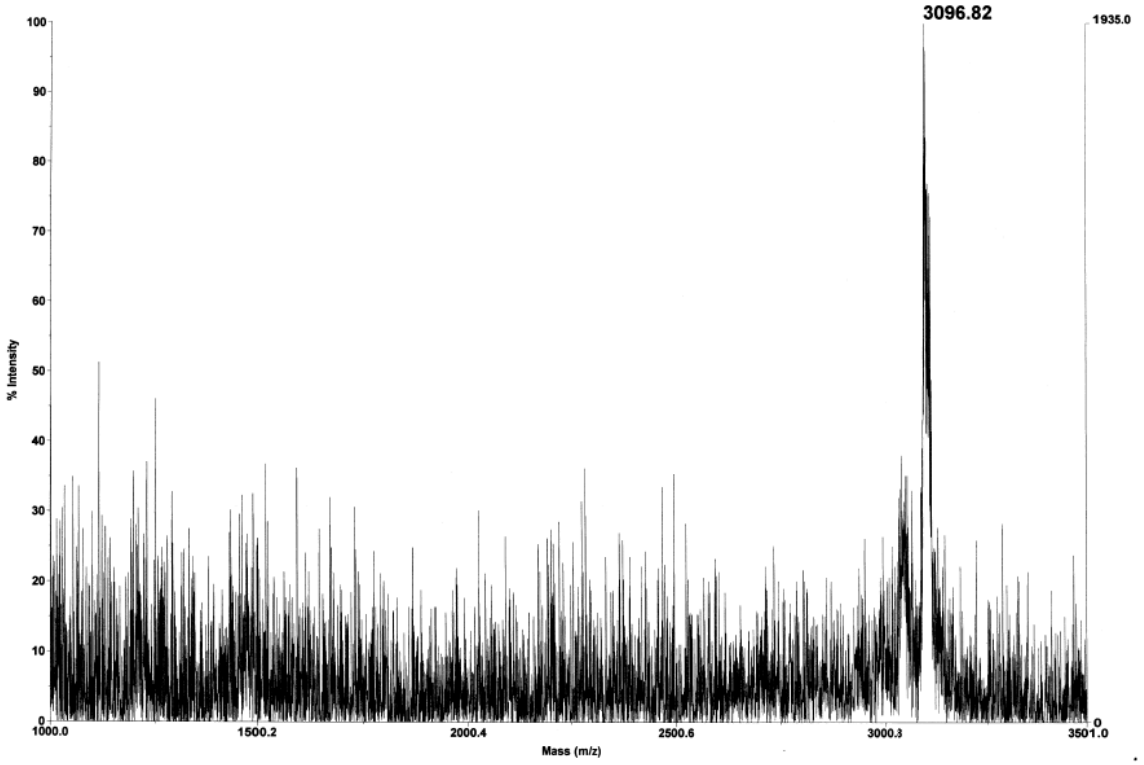

Figure S18 MALDI spectrum of compound 6.

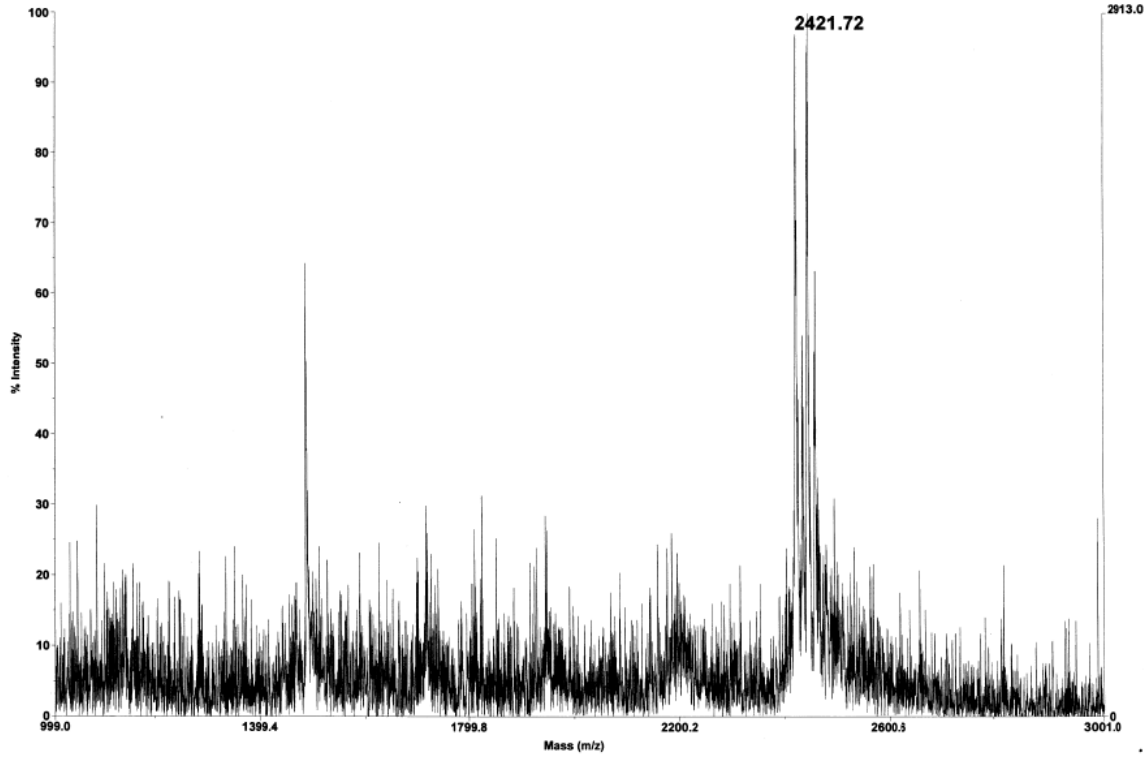

Figure S19 MALDI spectrum of BisPI-G1.

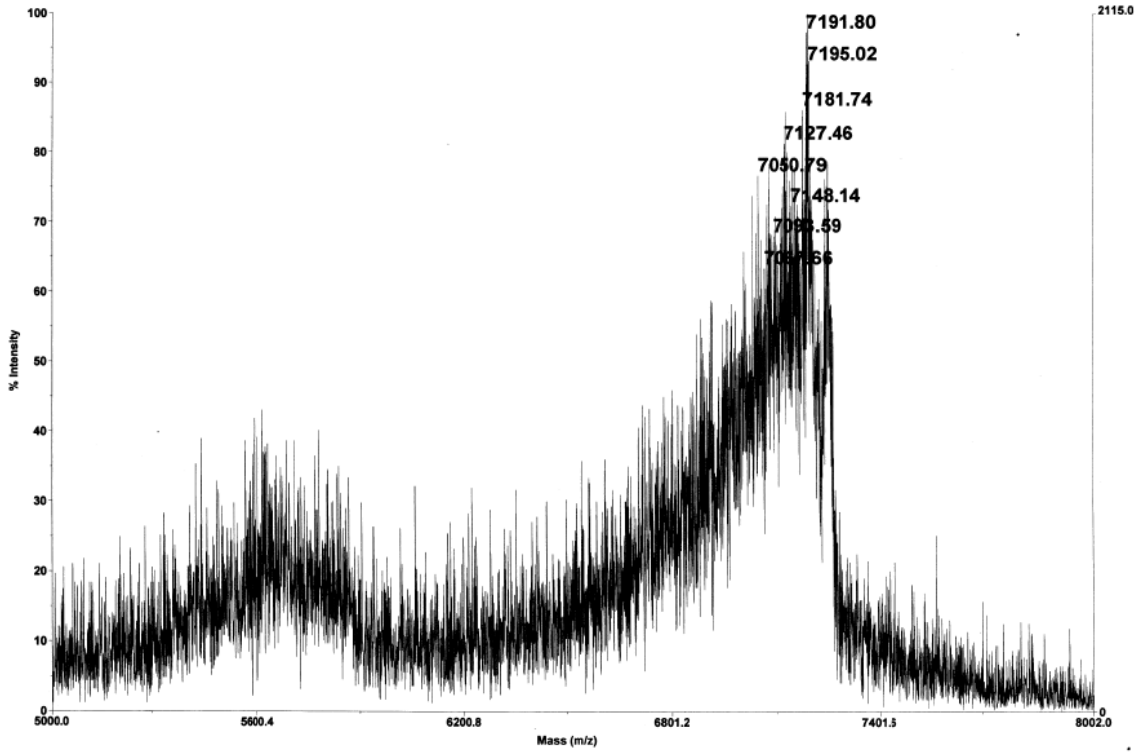

Figure S20 MALDI spectrum of compound 8.

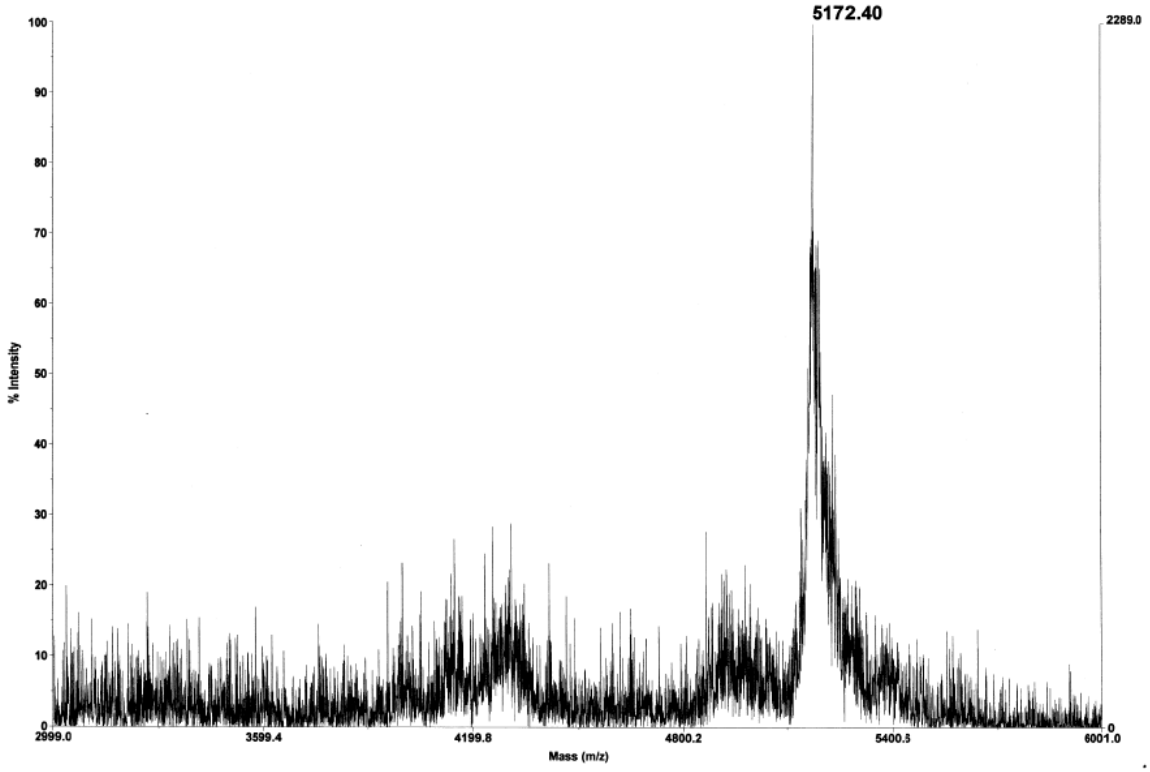

Figure S21 MALDI spectrum of BisPI-G2.