Supporting information to
Water-soluble and fluorescent dendritic perylene bisimides for live-cell imaging

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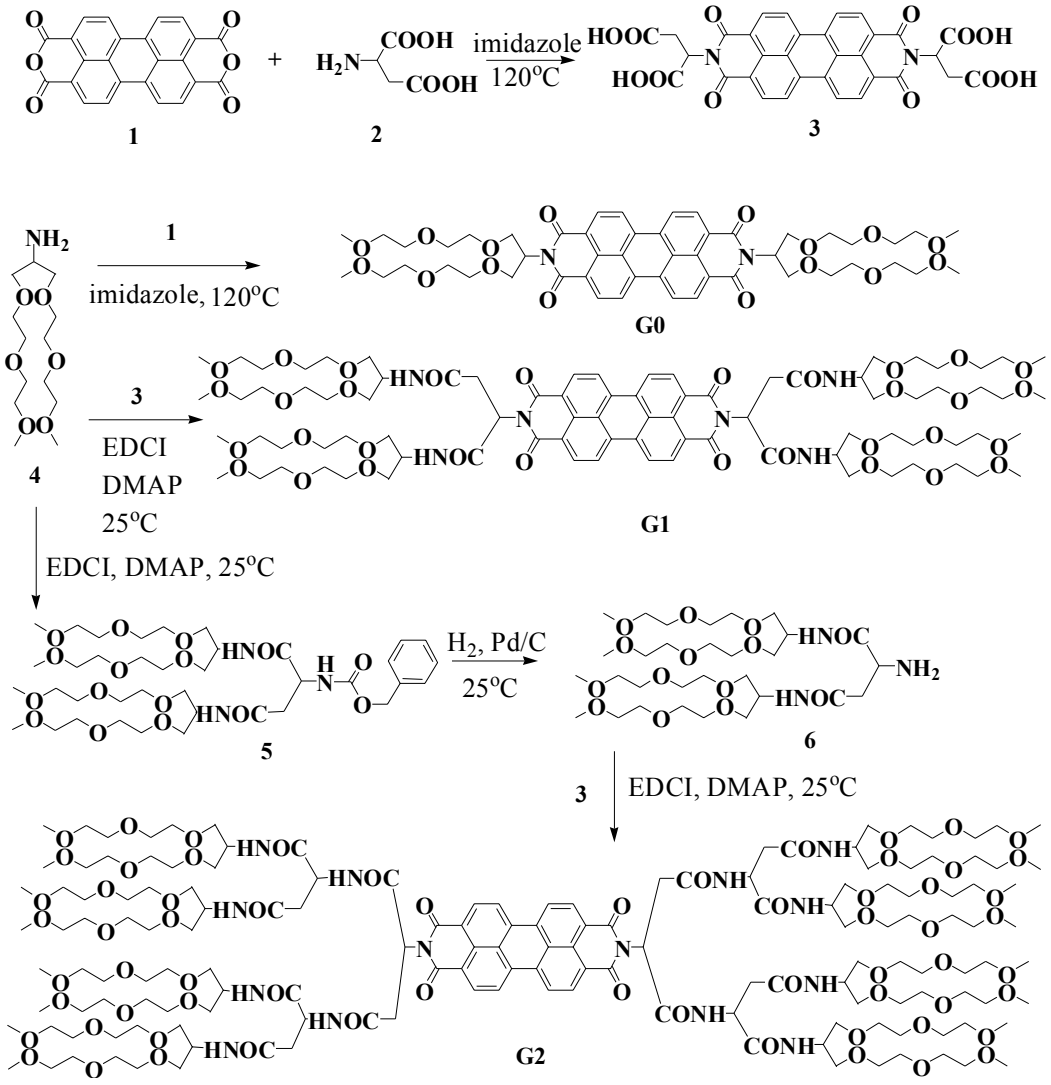
1. General Information

Perylenetetracarboxylic acid dianhydride PTCDA was purchased from Liaoning Liangang Pigment and Dyestuff Chmeicals Co. Ltd. L-Aspartic acid and N-carbobenzyloxy-L-Aspartic acids were purchased from Yangzhou Baosheng Bio-Chemical Co. Ltd. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), 4-Dimethylaminopyridine and 1-Hydroxybenzotrizole were purchased from Shanghai Medpep Co. Ltd. Imidazole, 2-(2-Methoxyethoxy)ethanol and solvents were purchased from Sinopharm Chemical Reagent Co.Ltd, and used without any further purification. Solvents used for precipitation and column chromatography were distilled under normal atmosphere. The ^{1}HNMR spectra were recorded at 20 °C on 400 or 600 MHz NMR spectrometer (Bruker).Chemical shifts are reported in ppm at room temperature using CDCl3 as solvent and tetramethylsilane as internal standard unless indicated otherwise. Abbreviations used for splitting patterns are s = singlet, d = dublett, t = triplet, qui = quintet, m = multiplet. Mass spectra were carried out using MALDI-TOF/TOF matrix assisted laser desorption ionization mass spectrometry with autoflexIII smartbeam (Bruker Daltonics Inc). UV/Vis spectra were recorded with a Shimadzu WV-2550 spectrophotometer. Size distribution of the chromophores in aqueous solution was measured by dynamic light scattering (DLS) with a vertically polarized He-Ne laser Wyatt technology). The scattering angle was fixed at 90. and the measurement was carried out with constant temperature at 25^{o}C. The samples were freshly prepared, and then put in dark for 24 h. Before the measurement, the samples were filtered using 0.45 μm PTFE membrane filters to eliminate any dust particles. The concentration of the solution for the calculation of quantum yields was10^{-5}mol/L. The excitation wavelength is 492nm. The reference and the samples are equal in optical density at the excitation wavelength. *N*,*N*'-Dicyclohexyl-perylene-3,4:9,10-tetracarboxylic acid bisimide in chloroform was used as reference (Φr = 1.00).

2. Synthesis and characterization of Perylene Bisimides

Scheme S1 Summary of synthetic route



Compound **5**

1.04g N-carbobenzyloxyl-aspartic acid (3.9mmol) was dissolved in 50ml dry DCM. 1.8g EDCI (9.3mmol) was added, and the mixture was stirred at 0℃for 30min. Subsequently, 2.8g of 4 (9.3mmol) and 0.12g DMAP (1.0mmol) were added and the reaction stirred at room temperature for 24 hours. Solvent was removed by rotary evaporation. The residue was purified by column chromatography on silica gel to afford 2.4g of compound 5 (76%) as a white solid. ^{1}H NMR (600 MHz, CDCl): δ2.59 (m, 1H), 2.85 (m, 1H), 3.38(m, 12H),3.49~ 3.63(m, 40H), 4.17 (m, 2H), 4.53 (m, 1H), 5.12 (m,2H), 6.42 (d, J=7.8 Hz, 1H), 6.55 (d, J=8.4 Hz, 1H), 7.09 (d, J=8.4 Hz, 2H),7.32(m,5H); MALDI-TOF MS m/z: 822.4[M+H]+, 844.4 [M+Na]+, Calcd for C38H67N3O16: 821.5

Compound **6**

1.47g Compound **5** (1.8mmol) was dissolved in 70ml MeOH, and 0.412g of 10% Pd/C catalyst was added. The mixture was hydrogenated at 2 bar H_2 for overnight. The reaction mixture was then filtered and the solvent was removed under reduced pressure to give of yellow oil (1.20g, 96%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ: δ2.17 (s, 2H), 2.39 (m, 1H),2.75 (m, 1H), 3.38(m, 12H), 3.49~ 3.63(m, 40H), 3.78 (m, 1H), 4.20 (m, 2H), 6.83 (d, J=8.4 Hz,1H), 7.63 (d, J=9.1 Hz, 1H); MALDI-TOF MS m/z: 688.4 [M+H]+, Calcd for C30H61N3O14: 687.4

Compound **G0**

0.45g 2,5,8,12,15,18-hexaoxa-10-nonadecanamine (1.5mmol), 0.30g 3,4,9,10-perylenetetracarboxylic dianhydride (0.8mmol), 5.76g imidazole and 67mg $Zn(OAc)_2$ (0.3mmol) were heated at 165□ overnight. The reaction mixture solidified upon cooling and was dissolved in 2M HCl. DCM was added and the phases were separated. The water phase was extracted thoroughly with DCM and then the combined organic layers were dried over Na_2SO_4 and concentrated. Lastly, imidazole-free material was obtained by column chromatography on silica gel to give of deep-red solid (470mg, 65%).^{1}H NMR (600 MHz, $CDCl_3$): δ 3.28 (s, 12H), 3.42 (m, 8H), 3.57(m, 8H), 3.64 (m, 12H),3.73 (m, 4H), 3.99 (m, 4H), 4.21 (m, 4H), 5.72 (m, 2H), 8.45 (m, 4H), 8.56 (m,4H); ^{13}CNMR(150MHz, $CDCl_3$):|52.2, 59.1, 69.4, 70.5, 70.62, 70.63, 72.0, 123.6, 126.4, 129.7, 131.7, 134.6, 164.0. MALDI-TOF MS m/z: 969.4 [M+Na]+, Calcd for C50H62N2O16: 946.4.

Compound **G1**

Compound 3 (100mg, 0.16mmol) and EDCI (148mg, 0.77mmol), HOBt (117mg, 0.77mmol) were dissolved in 40ml dry DMF. The mixture were cooled to 0□ and stirred for 0.5h before removal of the ice bath. Subsequently, compound 4 (283mg, 0.96mmol) was added. The reaction was stirred for 2d at room temperature. After that, the solvent DMF was evaporated under reduced pressure. The solid mixture was dried under vacuum at 60□, and then purified by column chromatography on silica gel to yield pure red product (192mg, 69%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 2.61 (m, 2H), 2.91 (m, 2H), 3.33 (m, 24H), 3.44~ 3.64 (m, 80H), 4.16 (m, 2H),4.29 (m, 2H), 6.08 (m,2H), 6.72 (d, J=8.4 Hz, 2H), 7.42 (d, J=8.4 Hz, 2H), 8.51 (m, 4H), 8.61(m,4H); ^{13}CNMR(100MHz ,CDCl): 36.8, 48.9, 51.8, 59.0, 69.3, 70.5, 77.0, 123.2, 126.1, 129.3, 131.6, 134.5, 163.1, 168.3, 170.6; MALDI-TOF MS m/z: 1753.7 [M+Na]+, Calcd for C84H126N6O32: 1730.8.

Compound **G2**

Compound 3 (150mg, 0.24mmol) and EDCI (444mg, 2.3mmol), DMAP (12mg, 0.1mmol), HOBt (177mg, 1.2mmol) were dissolved in25ml dry DMF. The mixture were cooled to 0□ and stirred for 0.5h before removal of the ice bath. Subsequently, 4

(1125mg, 1.639mmol) was added. The reaction was stirred for 4d at room temperature. After that, the solvent DMF was evaporated under reduced pressure. The solid mixture was dried under vacuum at 60□, and then purified by column chromatography on silica gel to yield the red product (597mg, 75%).^{1}H-NMR (600 MHz, $CDCl_3$): δ 2.59 (m, 6H), 2.81 (m, 6H), 3.36 (m, 48H), 3.42~ 3.66 (m, 160H), 4.14 (m, 8H), 4.77(m, 4H), 6.19 (m,2H), 6.75 (d, J=8.4 Hz, 4H), 7.20 (d, J=8.4 Hz, 2H), 7.45 (d, J=8.4 Hz, 4H), 7.90 (d, J=8.4 Hz, 2H), 8.71 (m, 8H); ^{13}CNMR(100MHz ,CDCl): 33.0, 36.4, 47.9, 49.7, 52.0, 53.9, 70.6, 70.9, 72.6, 73.1, 124.5, 129.2, 130.3, 133.8, 134.5, 165.9, 175.1, 175.4; MALDI-TOF MS m/z: 3299.7 [M+Na]+, Calcd for C152H254N14O64: 3322.5

3 Fluorescence quantum yield measurements

Fluorescence quantum yields were calculated from the integrated intensity under the emission band (*I*) using the following equation:

$$\Phi = \Phi_r \frac{I}{I_r} \frac{OD_r}{OD} \frac{n^2}{n_r^2}$$

where *OD* is the optical density of the solution at the excitation wavelength and *n* is the refractive index. The excitation wavelength is 492nm. The reference and the samples are equal in UV-vis absorption density at the excitation wavelength. *N*,*N*'-Dicyclohexyl-perylene-3,4:9,10-tetracarboxylic acid bisimide in chloroform was used as reference (Φr = 1.00).

4 Photo-stability measurements

The photostability of PEPBIs was determined by the UV-vis absorption and the fluorescence quantum yields method. The aqueous solution of PEPBIs was irradiated under 254 nm UV light (100W, 5cm distance between solution and UV lamp). The concentration of the solution for the calculation of quantum yields was 10^{-5}mol/L.

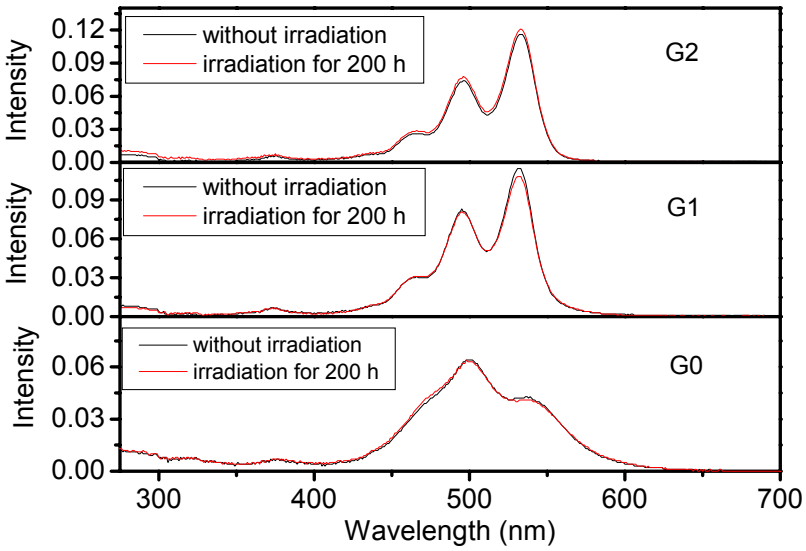


Figure S1 UV-vis spectra of PEPBIs in water with 10^{-5} M

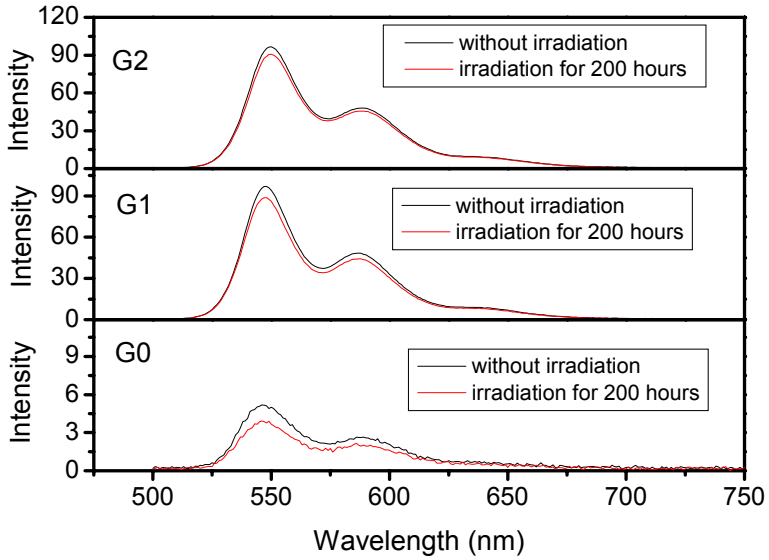


Figure S2 Photoluminescence spectra of PEPBIs in water with 10^{-5} M

Table S1 Fluorescence quantum yields of PEPBIs in water before and after irradiation

Compound	G0	G1	G2
Aqueous solution without irradiation	**4%**	**65%**	**93%**
Aqueous solution with irradiation for 200 h	**3%**	**62%**	**91%**

5 pH sensitivity measurements

The steady-state absorption spectra of PEPBIs at various pH in PBS buffer were obtained with a Shimadzu WV-2550 spectrophotometer. The emission spectra of PEPBIs at various pH in PBS buffer were measured on the Shimadzu RF-5301pc spectrophotometer. As can be seen in Figure S1 and S2, the PEPBIs are very stable with no observable changes in either the absorption or emission spectra.

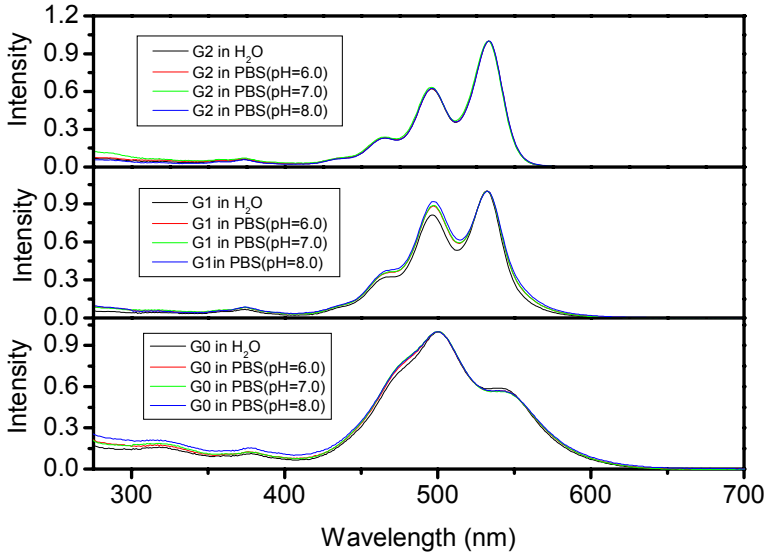


Figure S3 UV-vis spectra of PEPBIs in PBS buffers with 10^{-5} M

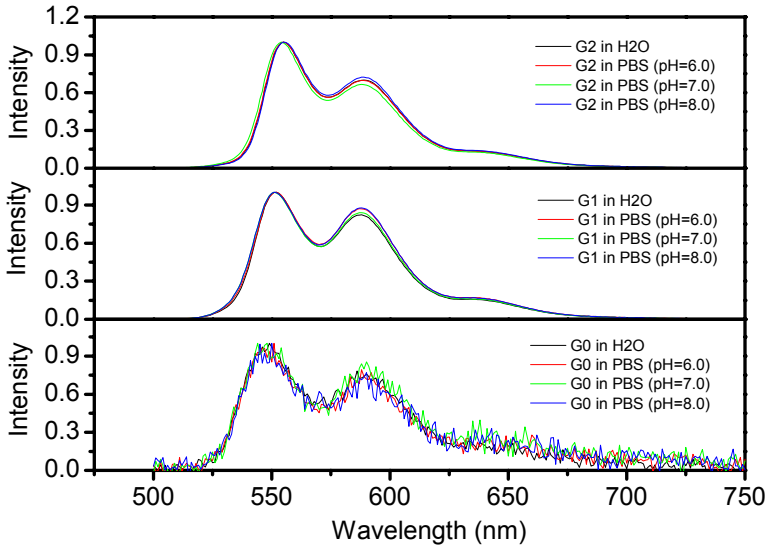


Figure S4 Photoluminescence spectra of PEPBIs in PBS buffers with 10^{-5}

6 Cytotoxicity assay

Cytotoxicity Test : MTT assays were performed to assess the metabolic activity of Hela cells. Hela cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 2×10^4 cells/mL. After 36 h incubation, the medium was replaced by PEPBIs solution at the concentration of 1.0 X 10^{-5}mol/L, and the cells were then incubated for 24, 48, and 72 h, respectively. After the designated time intervals, the wells were washed twice with 1×PBS buffer and freshly prepared MTT (10μL, 5mg/mL) solution in culture medium was added into each well. The MTT medium solution was

carefully removed after 3 h incubation in the incubator. DMSO (100μL) was then added into each well and gently shaken for 10 min at room temperature to dissolve all the precipitate formed. The absorbance of MTT at 490 nm was monitored by the ELX-800 microplate reader (ELISA Reader). Cell viability was expressed by the ratio of the absorbance of the cells incubated with PEPBIs solution. Each result is an average of data from six wells; 100% viability was determined using untreated cells.

7 Live cell imaging

HeLa cells were grown in Minimum Essential Medium Eagle (MEM medium, Sigma) in a 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom. The MEM medium was then replaced by the **G2** solution (1.0×10^{-5}mol/L) for 1 h, and then washed three times with PBS buffer. Conventional fluorescence images were obtained using inverted microscope (Olympus IX71) equipped with a DP72 CCD.

Part B: ^{1}H-NMR, ^{13}C-NMR spectrum and MALDI-TOF spectrum of **PEPBIs**

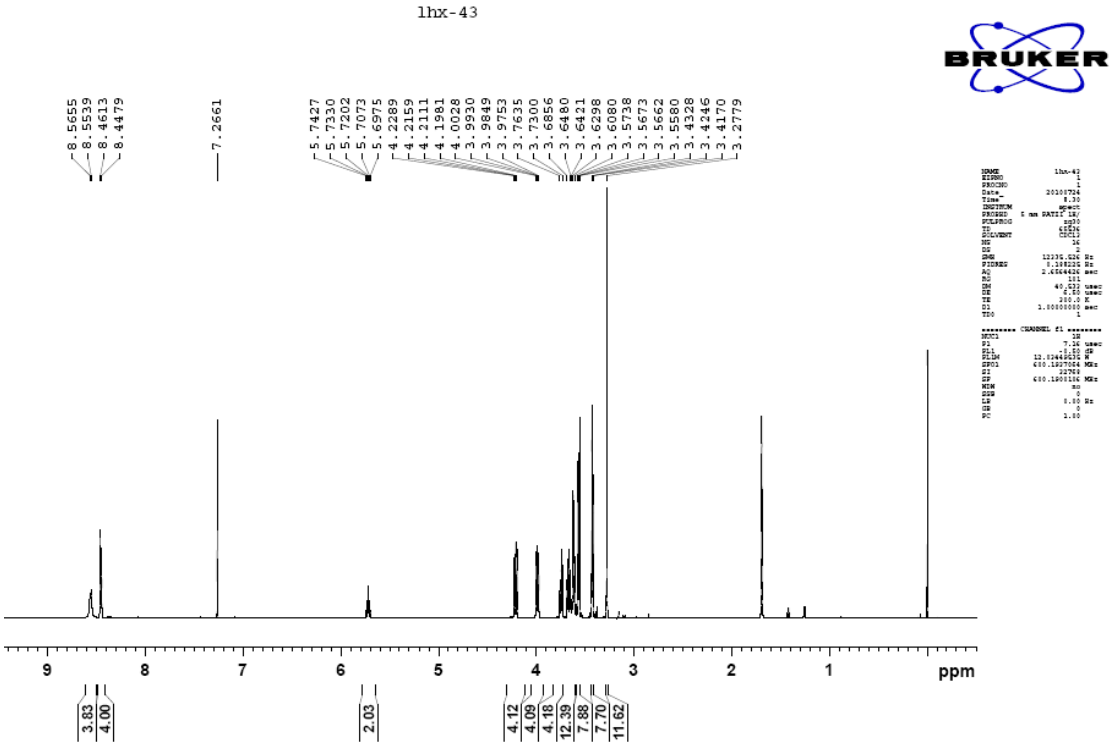


Figure S5 ^{1}H-NMR spectrum of compound **G0** in $CDCl_3$

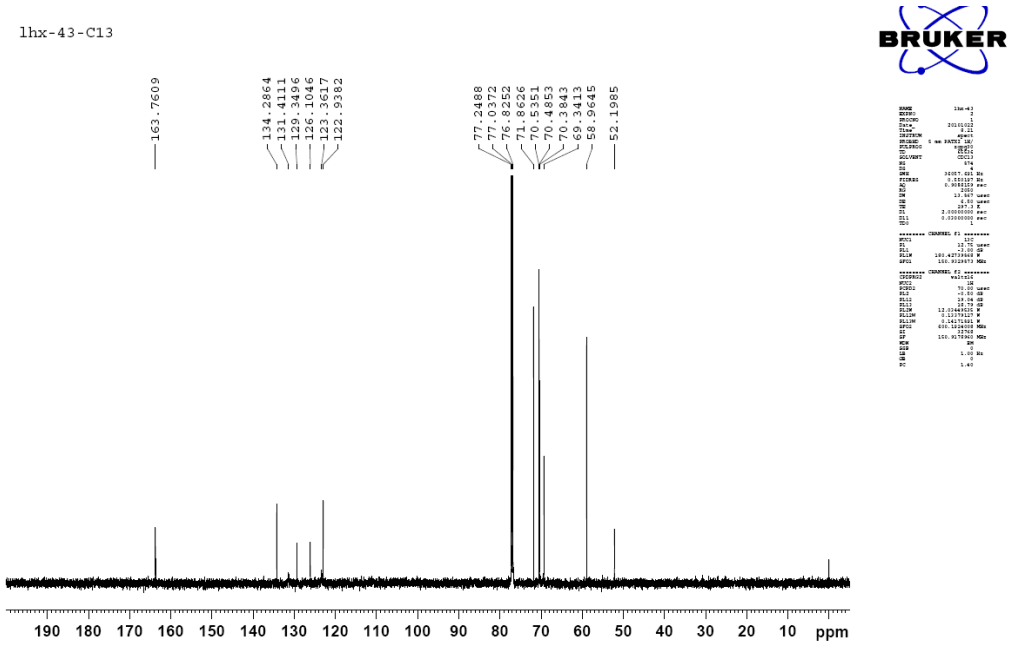


Figure S6 ^{13}C NMR spectrum of compound **G0** in $CDCl_3$

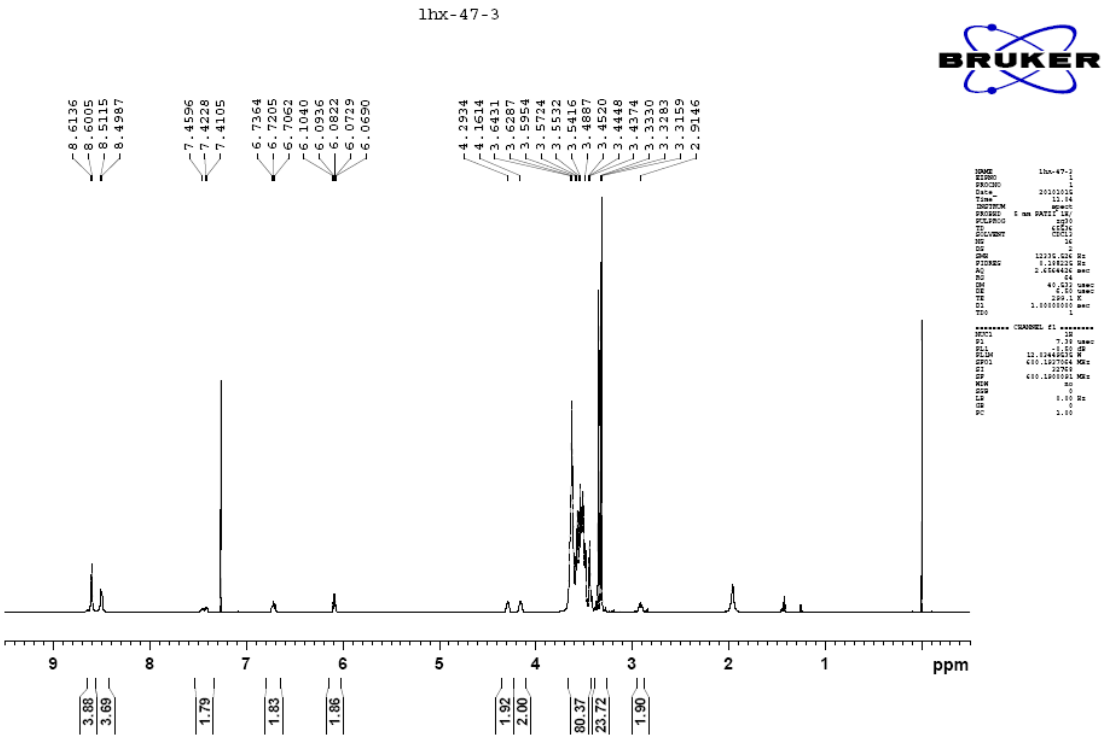


Figure S7 ^{1}H-NMR spectrum of compound **G1** in $CDCl_3$

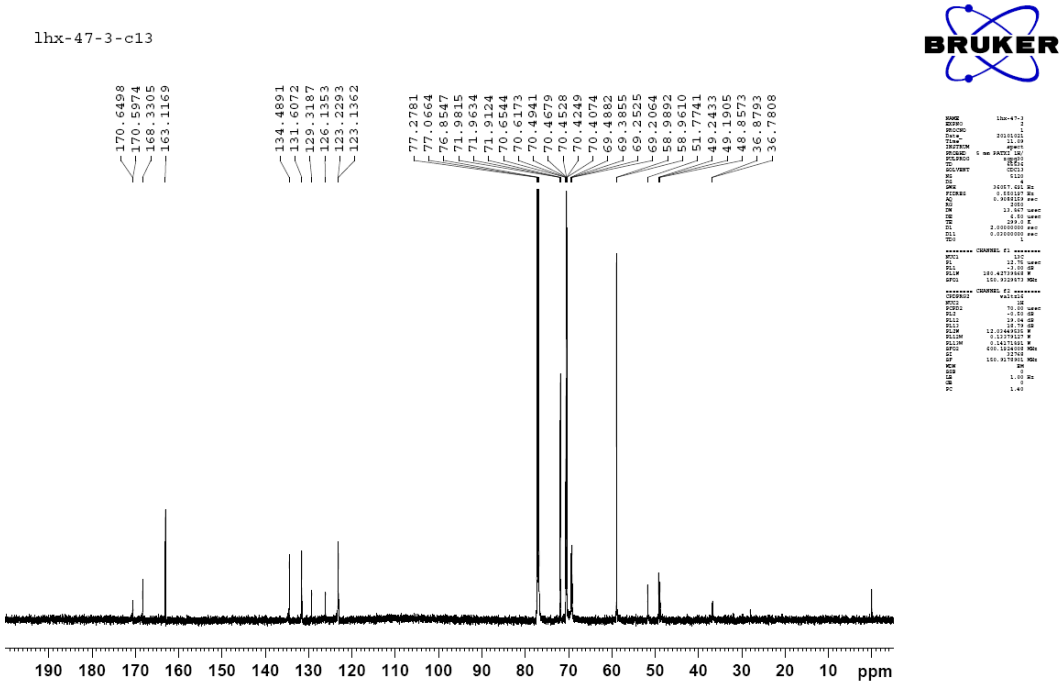


Figure S8 ^{13}C NMR spectrum of compound **G1** in $CDCl_3$.

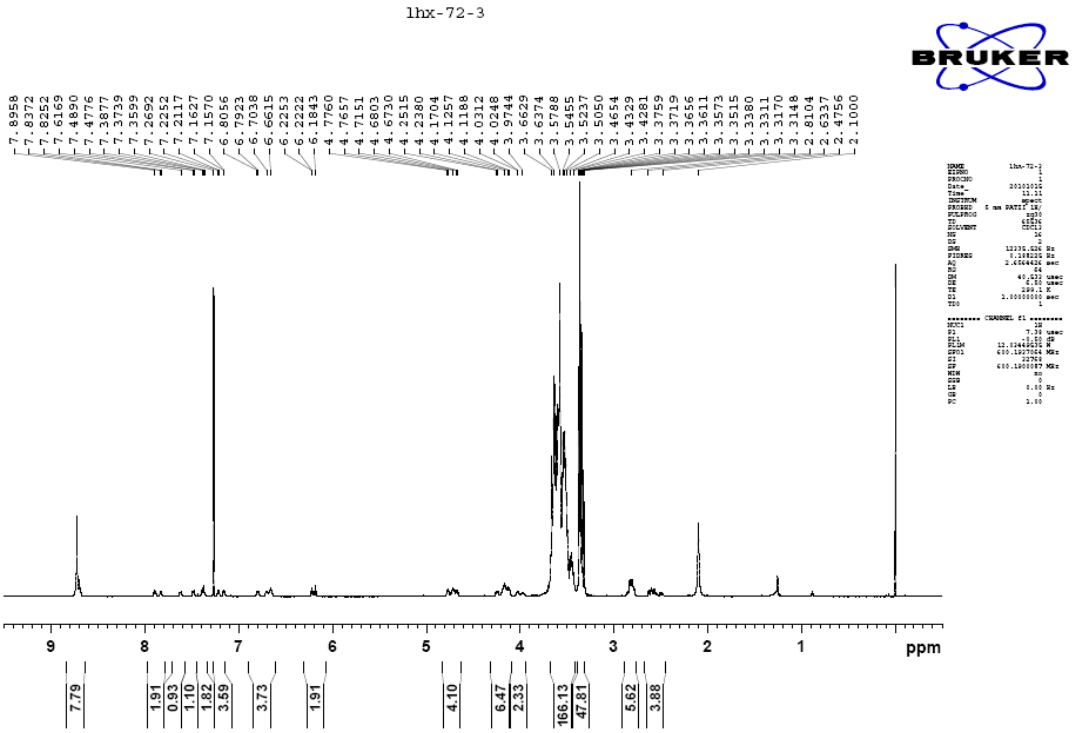


Figure S9 ^{1}H-NMR spectrum of compound **G2** in $CDCl_3$

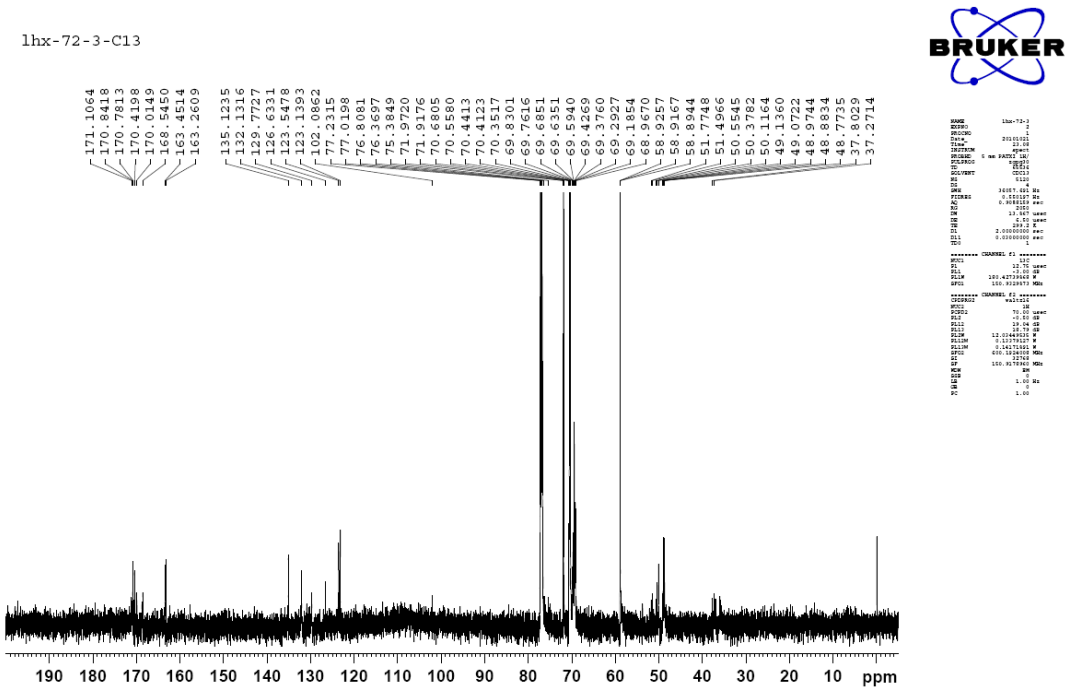


Figure S10 ^{13}C NMR spectrum of compound **G2** in $CDCl_3$

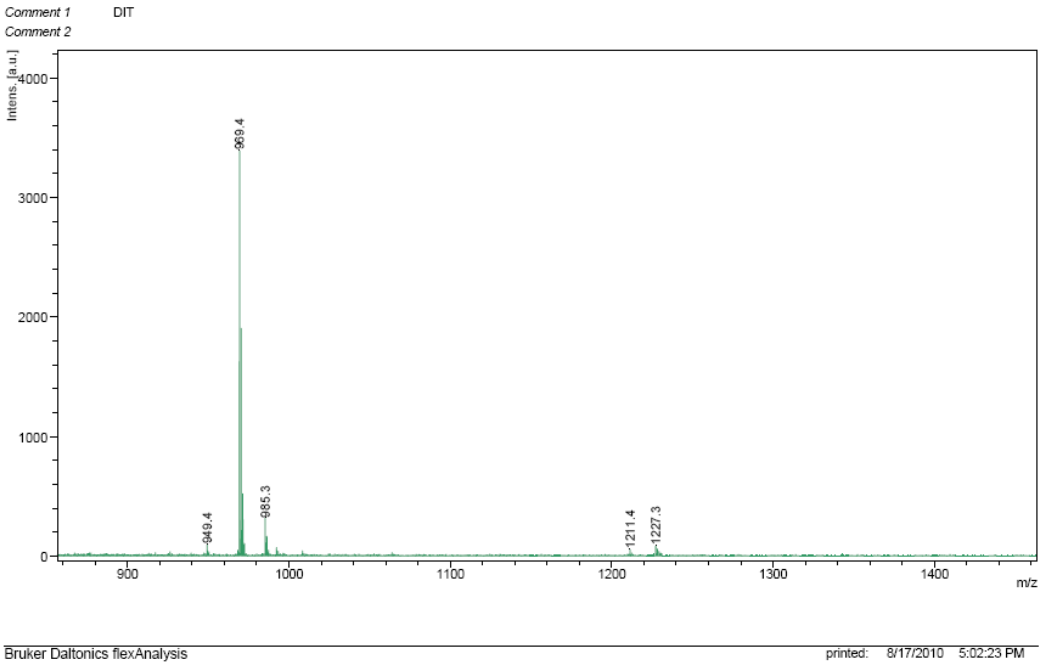


Figure S11 MALDI-TOF spectrum of compound **G0**

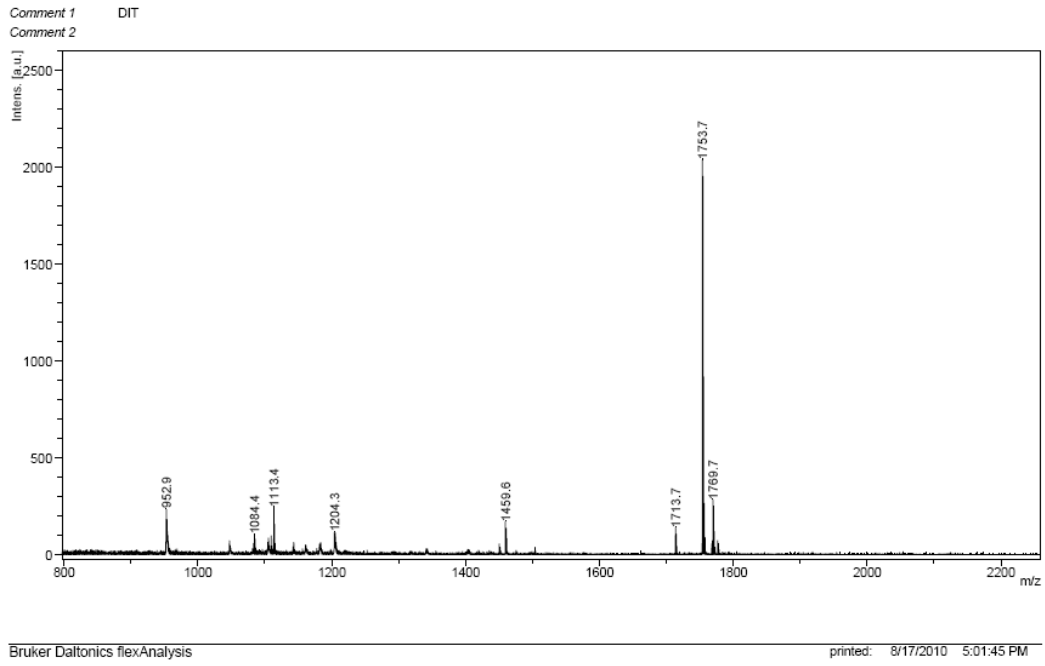


Figure S12 MALDI-TOF spectrum of compound **G1**

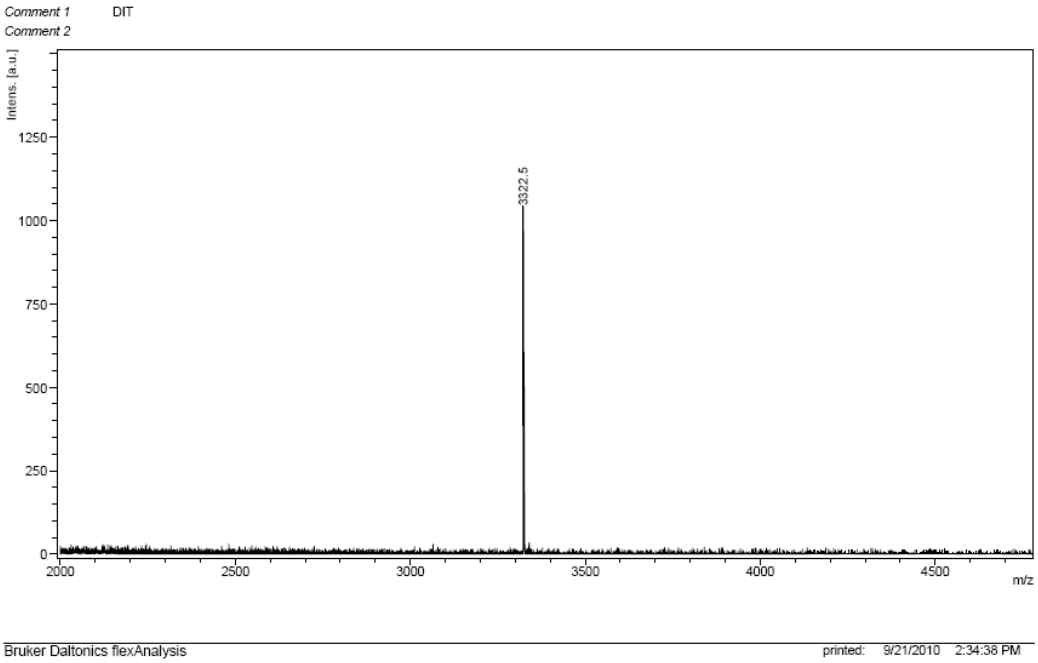


Figure S13 MALDI-TOF spectrum of compound **G2**