Supporting Information to

Assembly-Disassembly Driven Off-On Fluorescent Perylene Bisimides Probes for Detecting and Tracking of Targeted Protein in Live Cell

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1. General Information

Materials

Perylenetetracarboxylic acid dianhydride was purchased from Liaoning Liangang Pigment and Dyestuff Chmeicals Co. Ltd. 3-Butyn-1-ol was purchased from J&K Company N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), 4-Dimethylaminopyridine and 1-Hydroxybenzotrizole were purchased from Shanghai Medpep Co. Ltd. D-Biotin, acetic acid, N-methyl-2- pyrrolidone, Bromine, 2-(2-Methoxyethoxy)ethanol, CuI and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd, and used without any further purification. $Pd(PPh_3)_4$ was purchased from Shanghai Chiral Chemistry Co., Ltd. Solvents used for precipitation and column chromatography were distilled under normal atmosphere. Avidin, bovine serum albumin, and ovalbumin were purchased from Sigma-Aldrich.

The ^{1}H-NMR spectra were recorded at 20 °C on 600 MHz NMR spectrometer (Bruker). The ^{13}C-NMR spectra were recorded at 20 °C on 150 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 = doublet, 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. Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer. AFM images were obtained with a tapping-mode on an Agilent Technologies 5500 scanning probe microscope. TEM was performed on a JEOL JEM-1011 transmission electron microscope operated at an acceleration voltage of 100 kV.

Preparation of Cell Cultures

HeLa cells were cultured in RPMI-1640 medium (Sigma-Aldrich, Inc.) supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 ug/ml). All cells were maintained in a humidified atmosphere of 5/95 (v/v) of CO2/air at 37 °C. The cells were passed and

plated on 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom.

Live cell imaging

HeLa cells were incubated with the probes solution and Avidin solution (1.0 X 10^{-5}mol/L) for 3 h, and then washed three times with PBS buffer. The sequential incubation experiments: HeLa cells were incubated with avidin for 3h, washed twice with PBS buffer to remove the free avidin in solution and on the cell surface, and then incubated with **APBI-1** for 3h. The fluorescence images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000) that was equipped with a 488 nm laser and a band-pass (500−600 nm) emission filter.

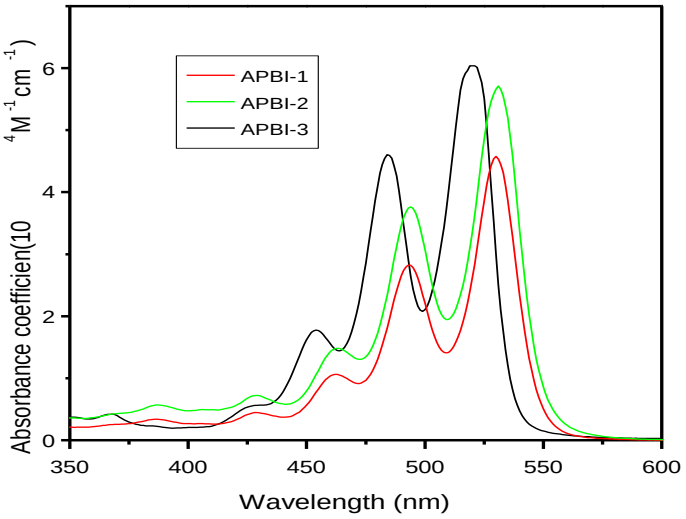
Determination of the detection limit

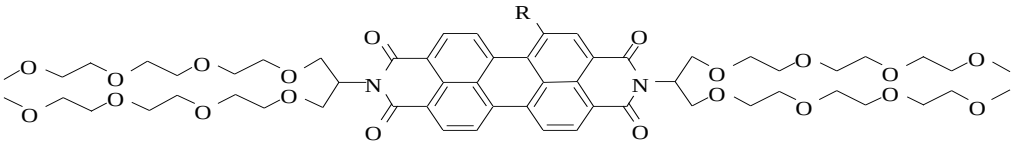
The detection limit DL of **APBI-1** for avidin was determined from the following equation:

$$DL = K \times Sb/S$$

Where K = 2; Sb is the standard deviation of the blank solution; S is the slope of the calibration curve.

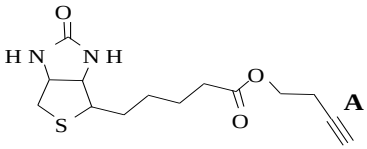
2 **Supplementary Figures**



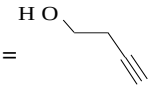


A P B I

A P B I-1 : R =



A P B I-2 : R =



A P B I-3 : R = H

Figure S1 Ultraviolet-visible absorption of **APBI** in THF and the chemical structure of **APBI**

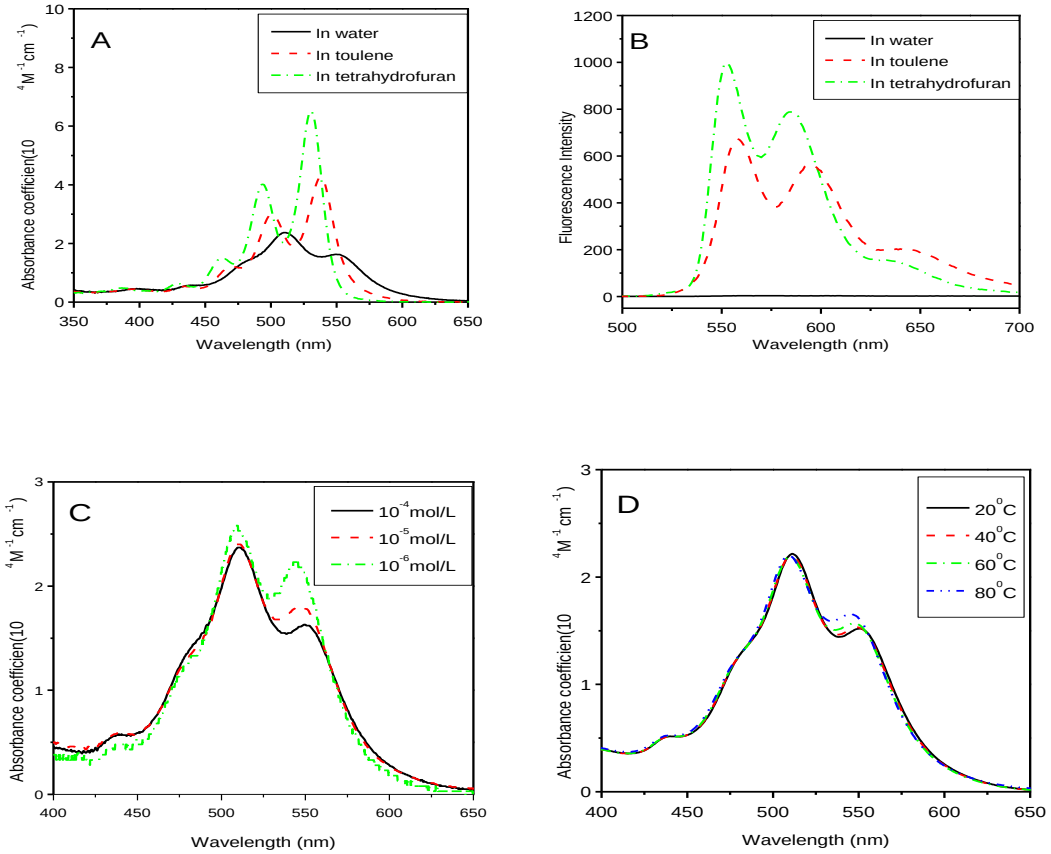


Figure S2.Ultraviolet-visible absorption (A) and Fluorescence spectroscopy (B) of APBI-1 in different solution; the concentration-dependent (C) and temperature-dependent (D) ultraviolet-visible absorption spectroscopy of APBI-1 in aqueous solution.

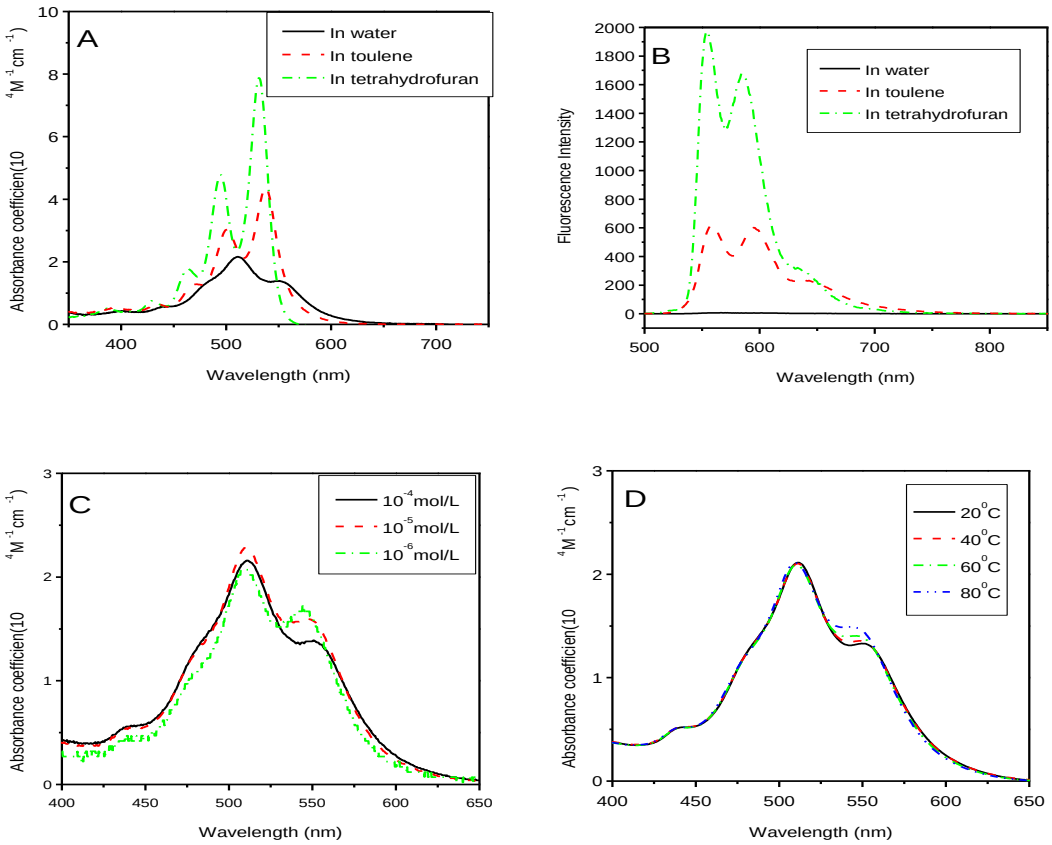


Figure S3. Ultraviolet-visible absorption (A) and Fluorescence spectroscopy (B) of **APBI-2** in different solution; the concentration-dependent (C) and temperature-dependent (D) ultraviolet-visible absorption spectroscopy of **APBI-2** in aqueous solution.

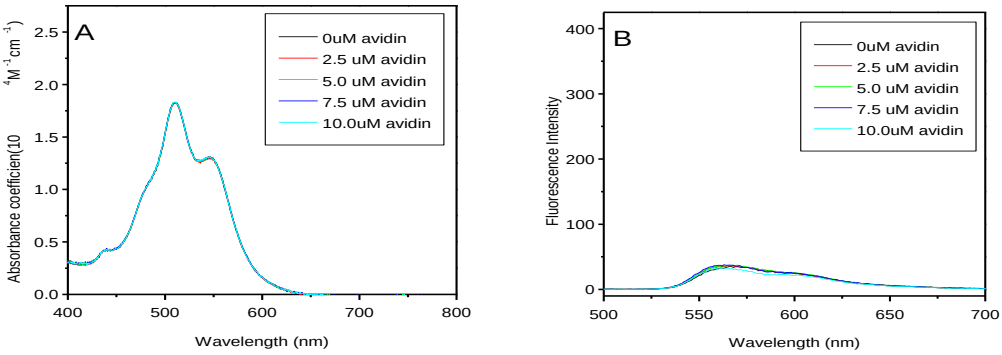


Figure S4 Spectroscopic analyses of -specific **APBI-2** probe. (A) UV-vis absorption spectral changes of probe 1 (10 μ M) upon addition of avidin (0-10 μ M). (B) Fluorescence spectral changes of **APBI-2** (10 μ M) upon the addition of avidin (0-10 μ M)

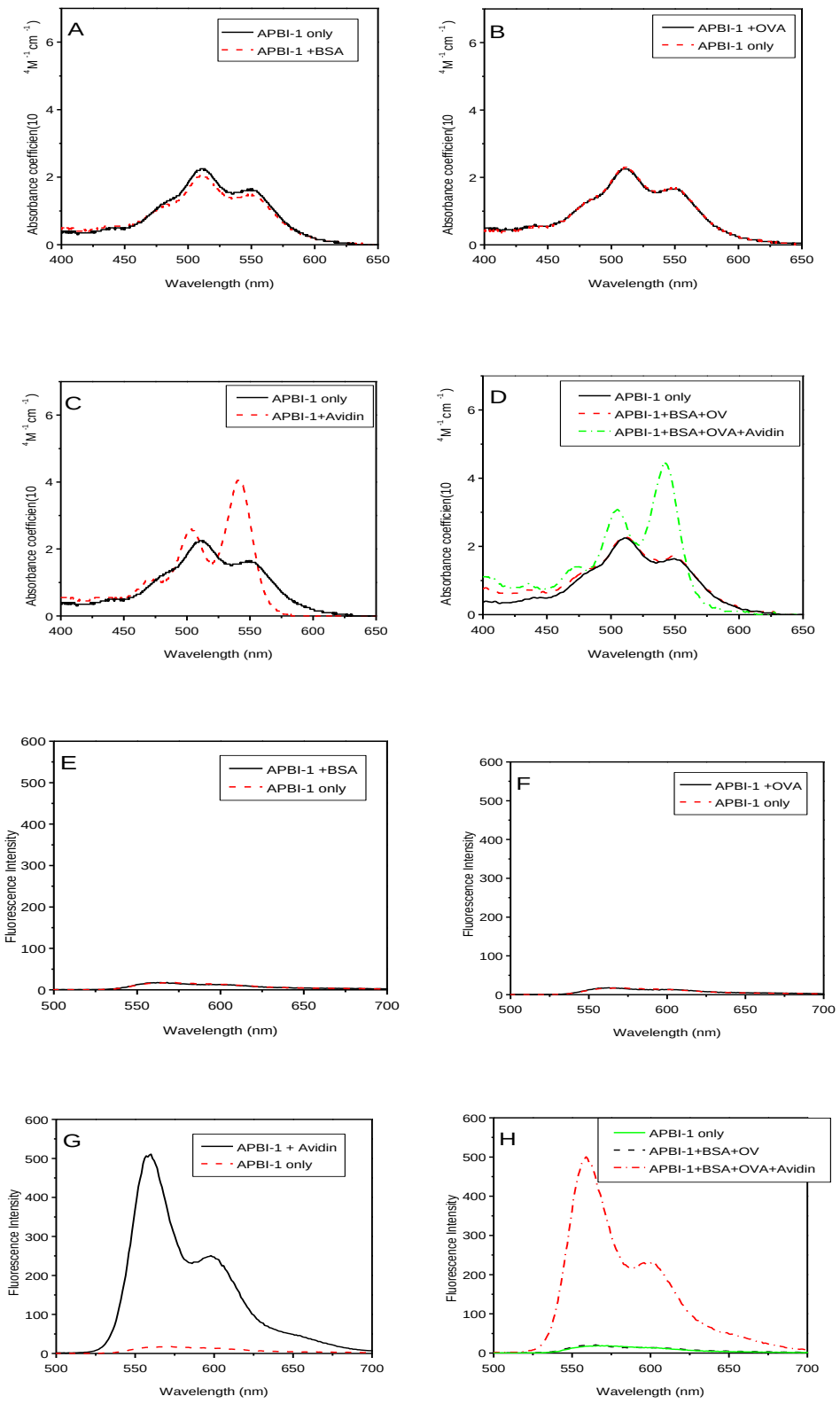


Figure S5 Ultraviolet-visible absorption (A-D) and Fluorescence spectroscopy (E-H) of **APBI-1**without or with avidin, or bovine serum albumin, and ovalbumin. **APBI-1** (10uM) in PBS (pH = 7.4).

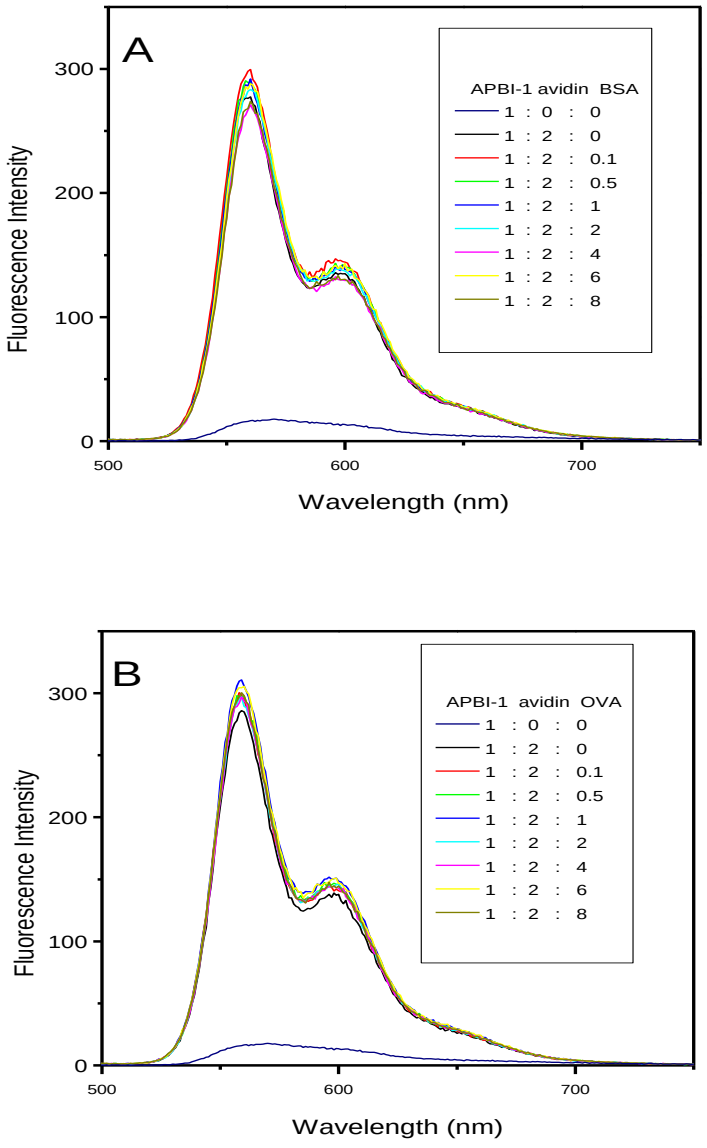


Figure S6 Fluorescence spectroscopy (A-B) of **APBI-1** with avidin, bovine serum albumin, and ovalbumin. **APBI-1** (10uM) in PBS (pH = 7.4).

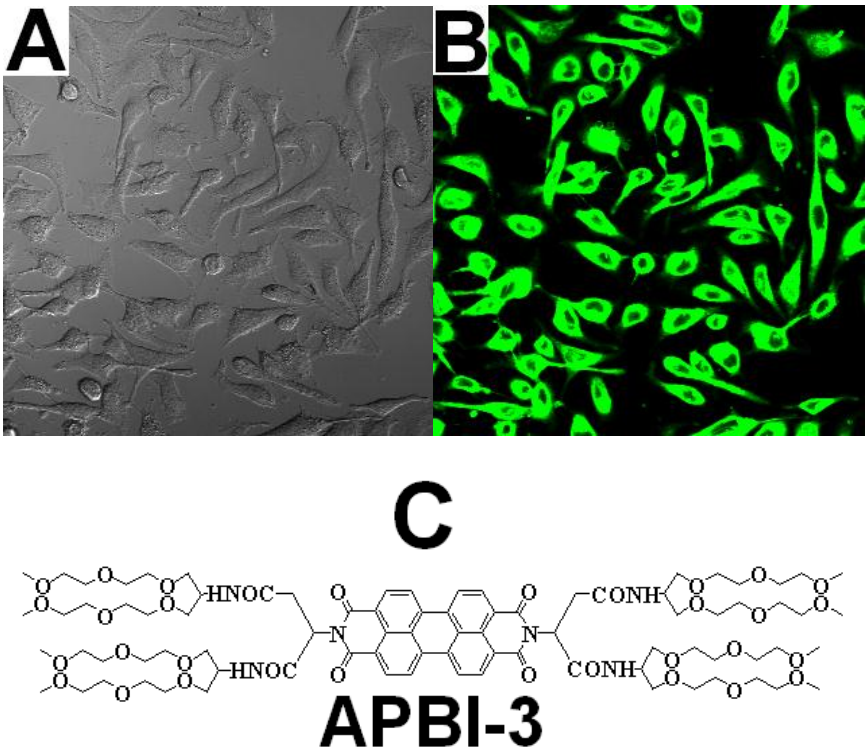
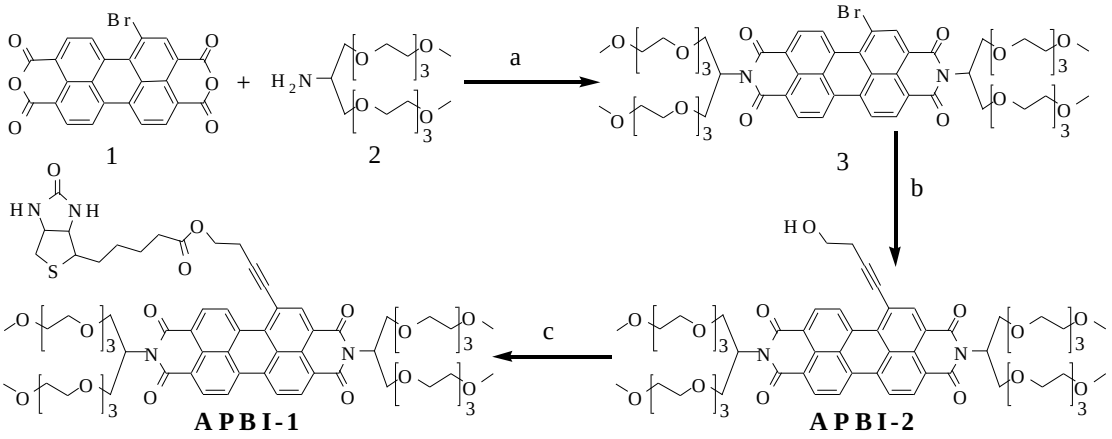


Figure S7 Confocal microscopy images of living Hela cells treated with **APBI-3** probe and protein: the bright-field (A) and confocal fluorescence (B) images of Hela cells incubated with **APBI-3;** (C) the chemical structure of **APBI-3**.

3. Synthesis and Characterization of Perylene Bisimides

Scheme S1 Summary of synthetic route



Reagents and conditions: (a) acetic acid, N-methyl-2- pyrrolidone, 80°C, 1h; (b) CuI, $Pd(PPh_3)_4$, TEA, toluene, 55°C,1h; (d) EDCI, DMAP, DMF/CH_2Cl_2, 25°C, 25h.

Compound **3**

A suspension of brominated perylene bisanhydride 1 (2.00 g, 4.2mmol) prepared according to literature procedures, compound 2 (3.33g 8.7mmol), and acetic acid (1.22g, 20.4 mmol) in 50 mL of N-methyl-2-pyrrolidinone was stirred at 85 °C under N_2 for 1 hour. The solvent was removed under reduced pressure. After the mixture was cooled to room temperature, the mixture was dried in a vacuum. The crude product was purified by silica gel column chromatography on silica gel with CH_2Cl_2/CH_3CH_2OH (30:1) as eluent. The third band was collected and after the solvent was removed by rotary evaporation, compound 3 was obtained as a red powder (2197mg, 43%). ^{1}H-NMR (600MHz, $CDCl_3$): δ 9.80 (d, J=8.4 Hz ,1H), 8.91 (s, 1H), 8.70 (s, 3H), 8.64 (d, J=2.4 Hz ,1H), 8.63 (d, J=3.0 Hz ,1H), 5.72 (m, 2H), 4.21-4.19 (m, 4H), 4.00-3.95 (m, 4H), 3.74-3.72 (m, 4H), 3.61-3.51 (m, 44H) 3.35 (s,12H);^{13}C-NMR(150MHz,$CDCl_3$):δ163.9, 163.6, 163.5, 162.7, 133.8, 133.5, 133.4, 133.3, 128.9, 128.5, 128.0, 127.9, 126.8, 123.8, 123.1, 120.8, 71.9, 70.5, 70.4, 70.3, 69.3, 69.1, 59.0, 52.4, 52.2; MALDI-TOF MS m/z Calcd for C58H77BrN2O20: 1202.4, found: 1225.4 [M+Na]+.

Compound **APBI-2**

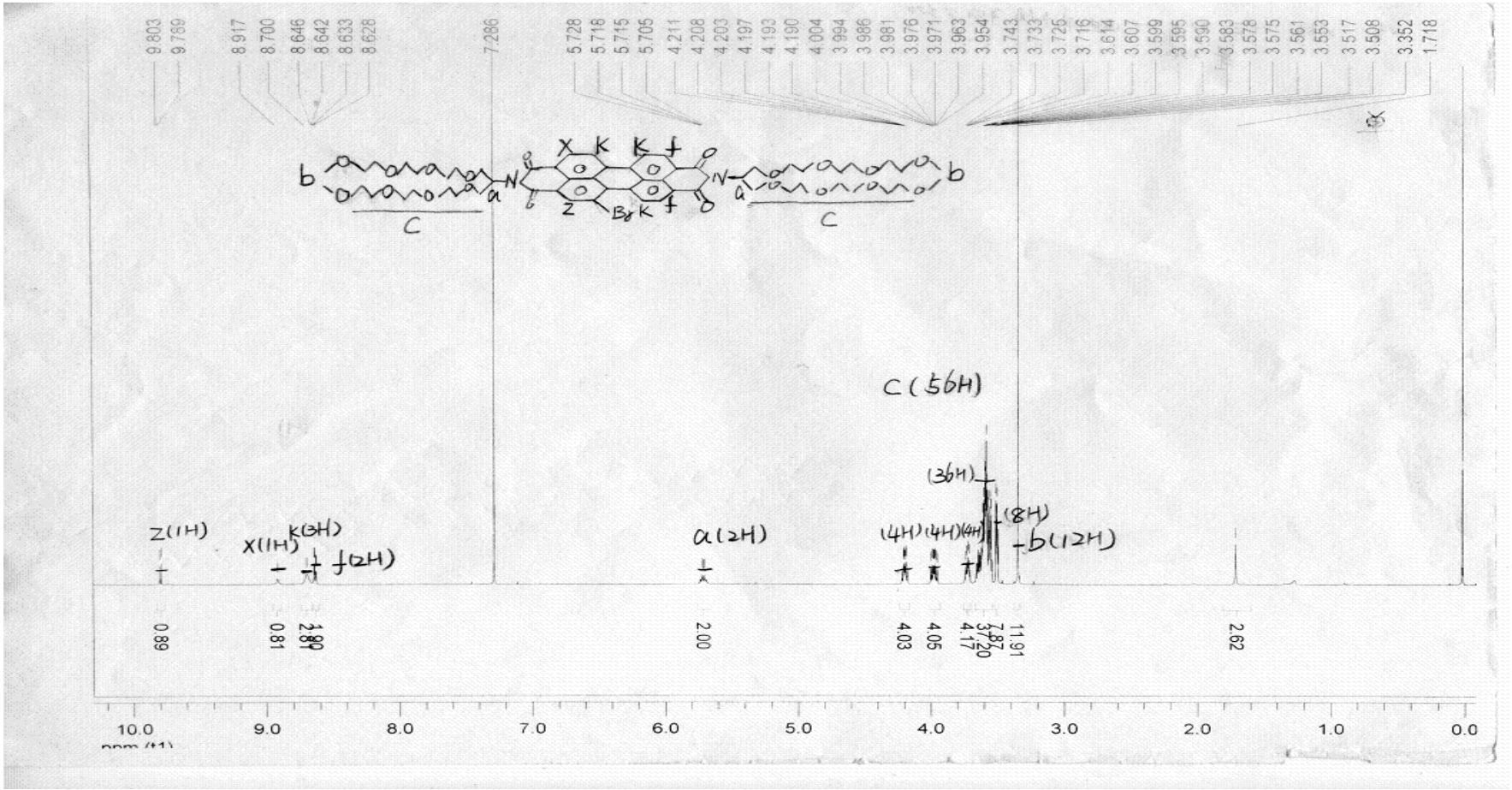
In a glove box filled with dry nitrogen, compound 3 (300 mg, 0.25 mmol), 3-Butyn-1-ol (35mg, 0.500mmol), $Pd(PPh_3)_4$ (58 mg, 0.050 mmol) CuI (14 mg, 0.075mmol), 57 mL toluene and 14 mL TEA were mixed. The mixture was stirred under nitrogen for 1 hour at 55 °C. The reaction mixture was cooled to room temperature and 2M HCl was added dropwise to acidic pH,extracted with Methylene dichloride and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by silica gel column chromatography on silica gel with CH_2Cl_2/CH_3CH_2OH (20:1) as eluent. After the solvent was removed by rotary

evaporation, compound **APBI-2** was obtained as a red powder 248mg (83%). ^{1}H-NMR (600MHz, $CDCl_3$): δ 9.95 (t, J=2.1 Hz,1H), 8.51 (br, 3H), 8.29 (s,3H), 5.77-573 (m, 2H), 4.29-3.50 (m, 56H), 4.02 (m, 2H), 3.35 (s, 12H), 2.92 (t, J=6.0Hz, 2H);^{13}C-NMR(150MHz,$CDCl_3$):δ 163.8, 163.5, 163.2, 163.1, 133.6, 133.5, 133.2, 133.1, 128.4, 127.7, 126.3, 125.6, 123.1, 122.5, 120.4, 101.9, 83.3, 71.9, 70.5, 70.4, 70.3, 69.4, 69.3, 60.4, 59.0, 52.2, 24.4; MALDI-TOF MS m/z Calcd for C62H82N2O21: 1190.5, found: 1213.5 [M+Na]+.

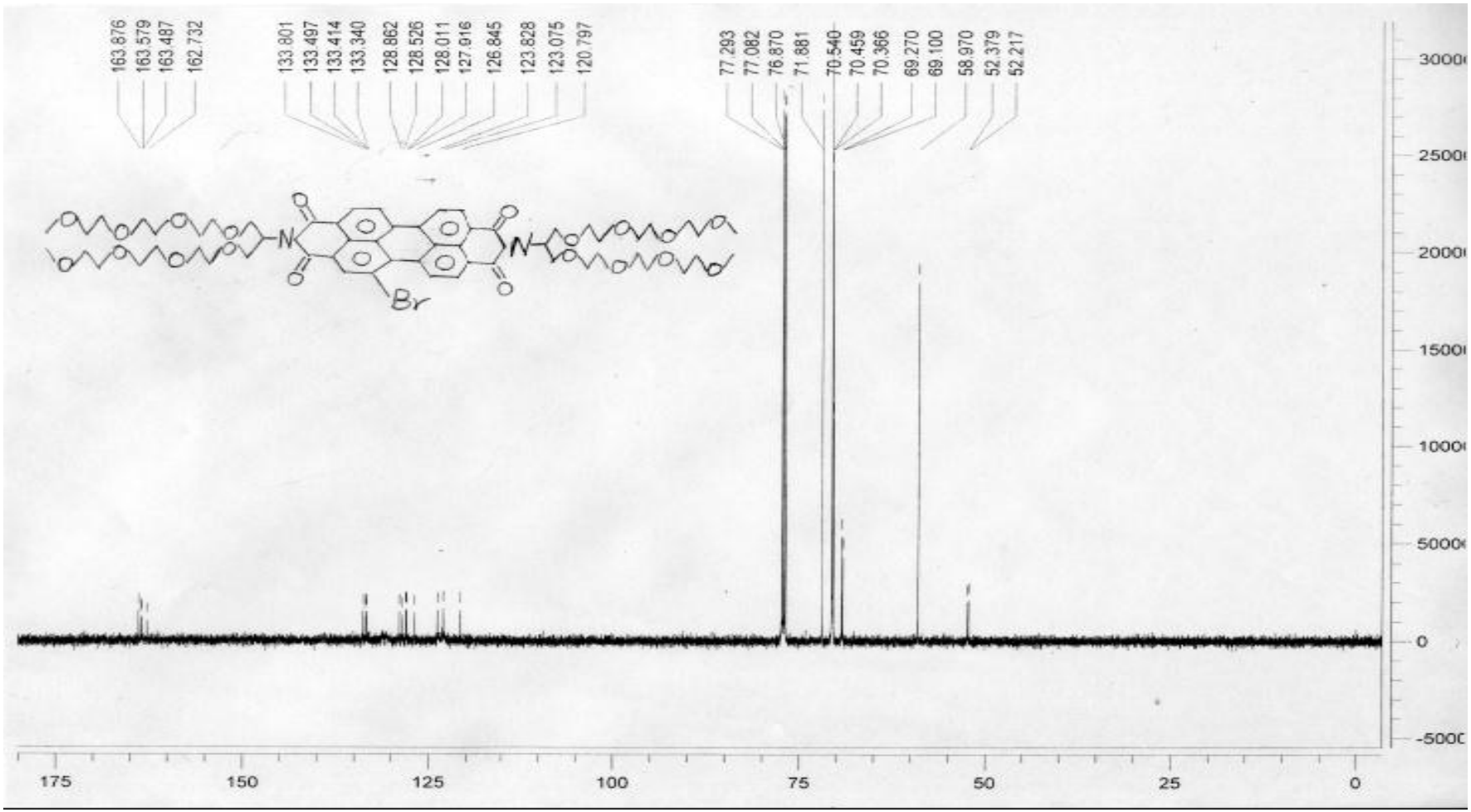
Compound **APBI-1**

D-(+)-biotion (41.046mg,0.168mmol) was dissolved in 0.8mL of DMF, EDCI (32.2mg,0.17mmol) was added, and the mixture was stirred at 0℃ for 30 min. Compound APBI-2 (100.0mg, 0.084mmol), DMAP (2.05mg, 0.017mmol) and 3.2mL of DCM were added and the mixture stirred at room temperature for 25 hours. Solvent was removed by rotary evaporation. The mixture was dried in a vacuum. The crude product was purified by silica gel column chromatography on silica gel with CH_2Cl_2/CH_3CH_2OH(15:1) as eluent. After the solvent was removed by rotary evaporation, compound **APBI-1** was obtained as red solid 66mg (56%).^{1}H-NMR (600MHz, $CDCl_3$): δ 10.24 (d, J=8.4 Hz,1H), 8.70-8.63 (m, 6H), 5.74(m, 2H),4.92(s,1H) ,4.85(s,1H),4.51 (d, J=12.6 Hz,3H),4.31(s,1H),4.22-3.49 (m, 56H), 3.35(s,12H),3.14 (m, 1H), 3.09 (t, J=6.3 Hz, 2H), 2.90 (d, J=18 Hz, 1H) , 2.72 (d, J=12.6 Hz, 1H), 2.50 (t, J=7.2 Hz, 2H), 1.73(m,2H), 1.48(m,2H), 1.29(m,2H); ^{13}C-NMR(150MHz,$CDCl_3$):δ173.4,163.9, 163.7, 163.3, 163.2, 134.2, 134.1, 133.9, 133.8, 128.9, 128.2, 126.9, 126.4, 126.3, 123.5, 123.0, 120.2, 98.5, 83.6, 71.9, 70.5, 70.5, 70.4, 70.3, 69.4, 69.2, 61.8, 61.7, 60.1, 59.0, 55.3, 52.3, 52.2, 40.5, 33.9, 28.3, 28.2, 24.8, 20.9; MALDI-TOF MS m/z Calcd for C72H96N4O23S: 1416.6, found: 1439.6 [M+Na]+.

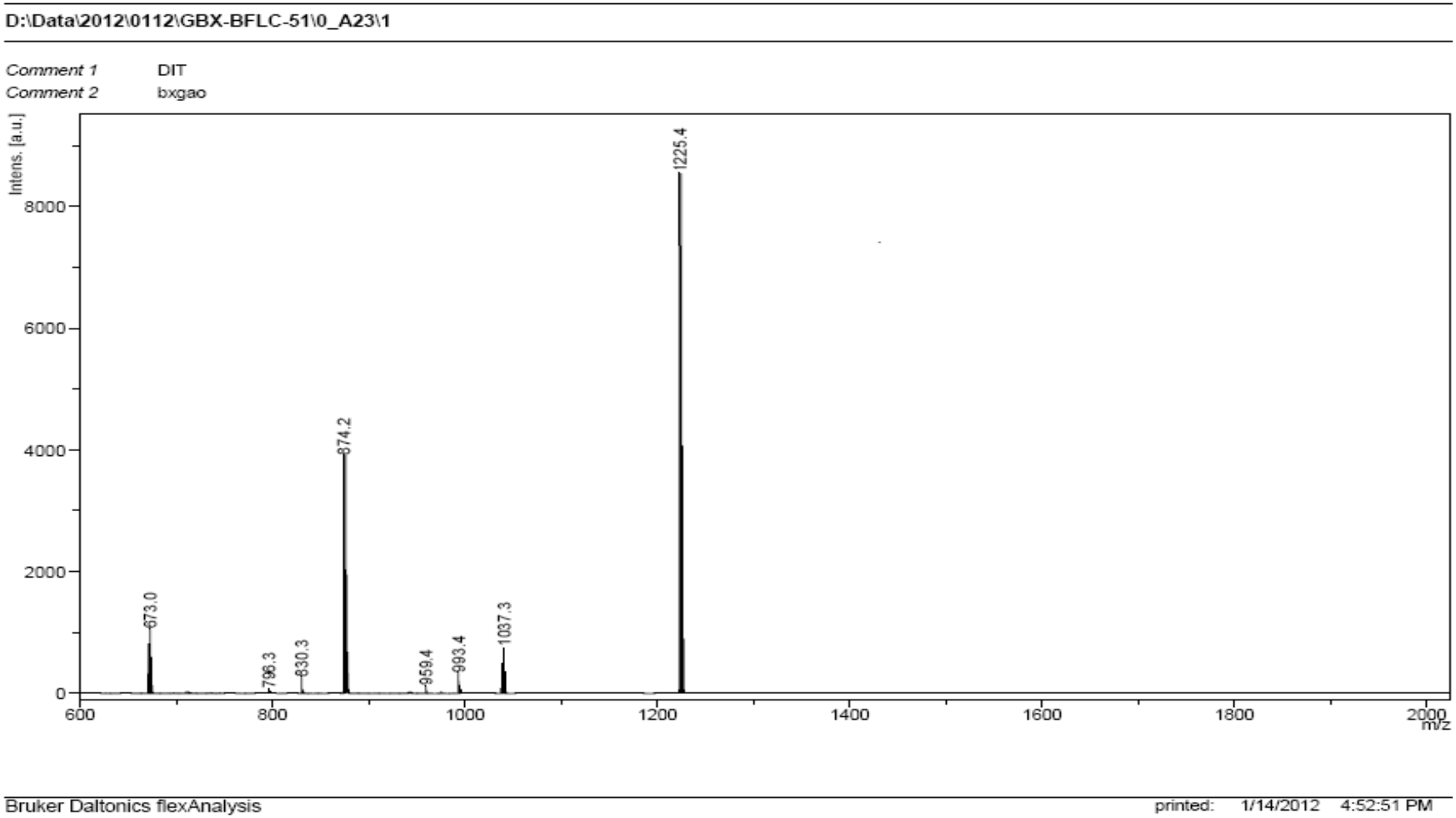
Part B: ^{1}H-NMR, ^{13}C-NMR spectrum and MALDI-TOF spectrum of APBI



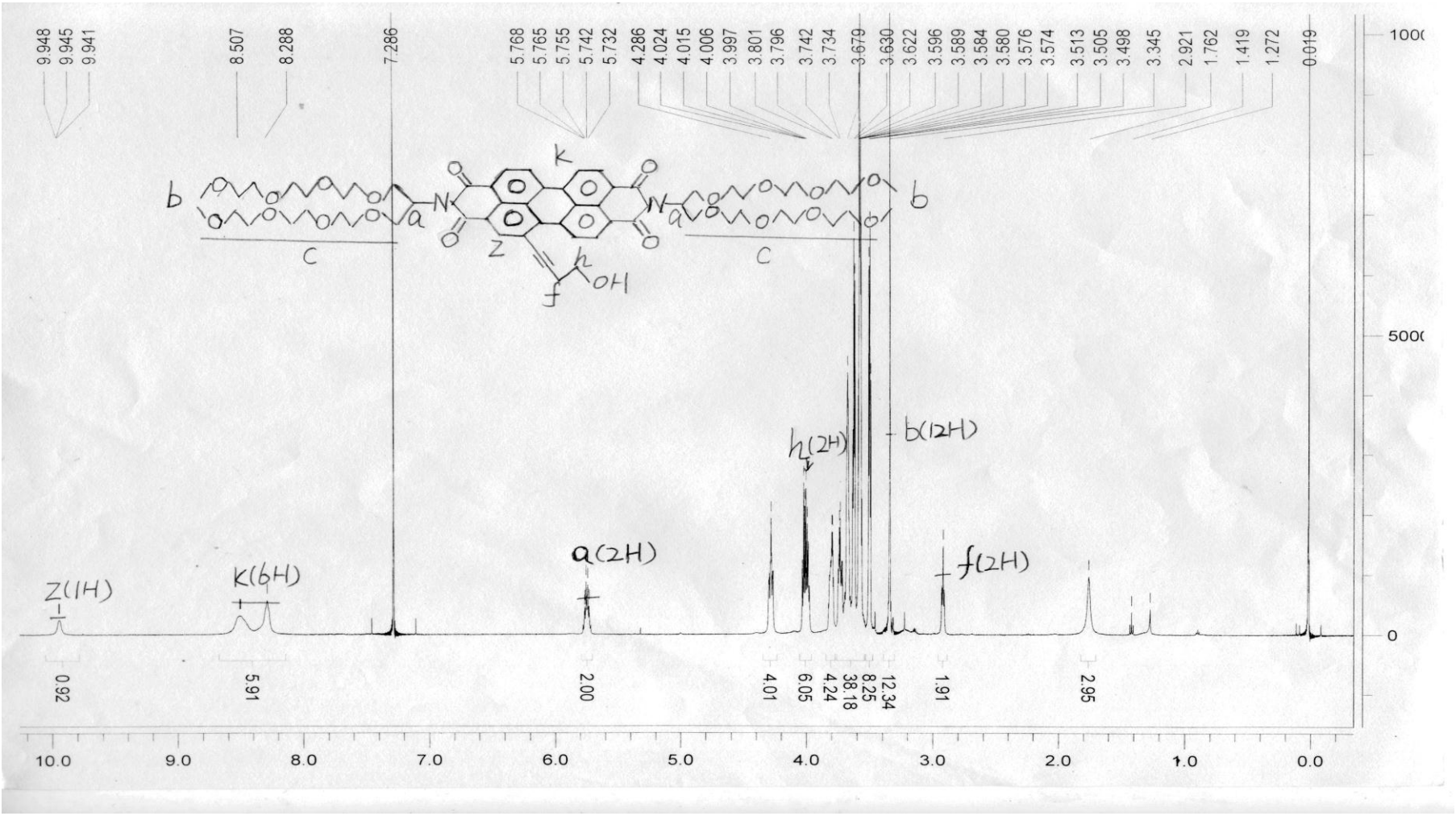
^{1}H-NMR spectrum of compound 3 in $CDCl_3$



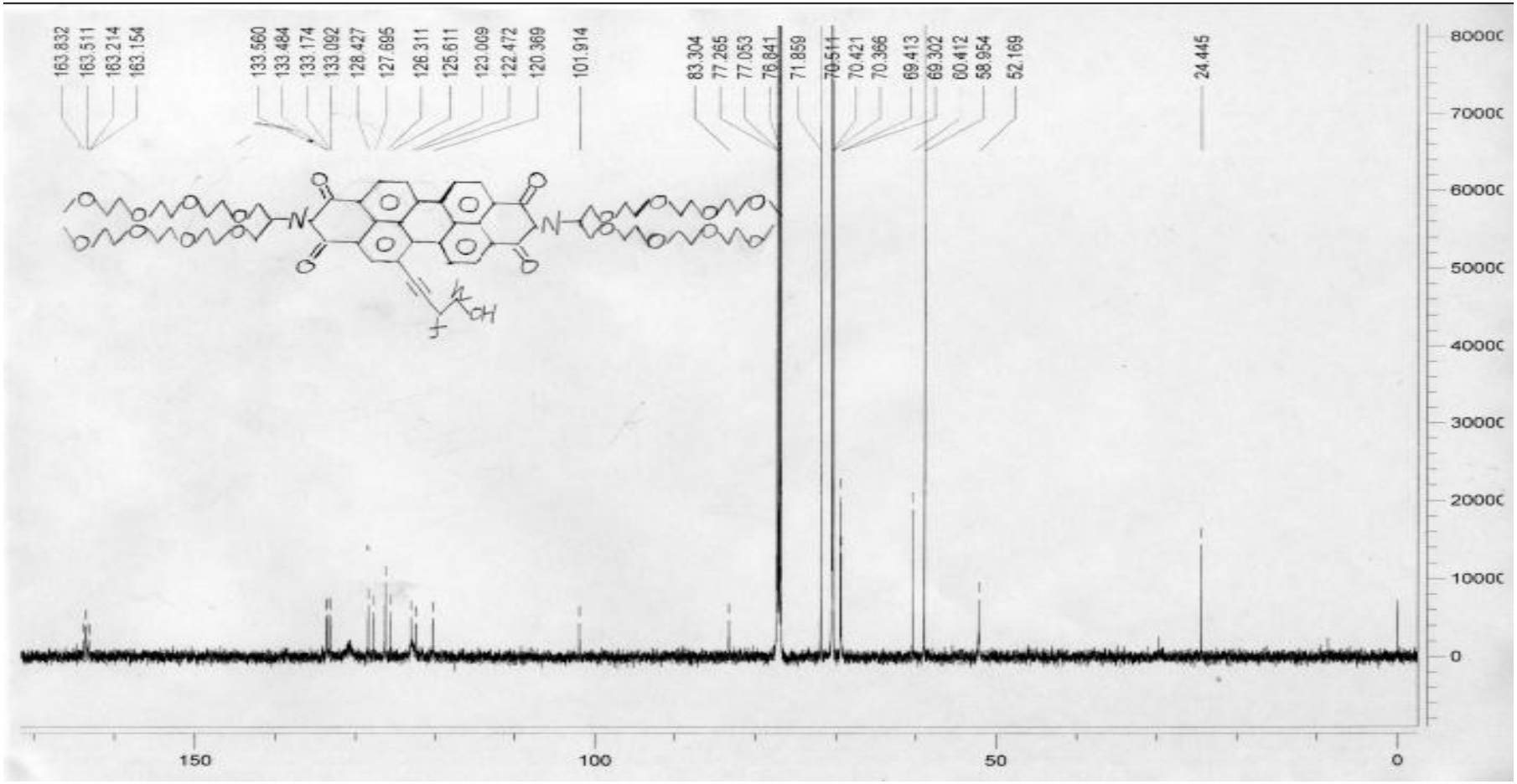
^{13}C-NMR spectrum of compound 3 in $CDCl_3$



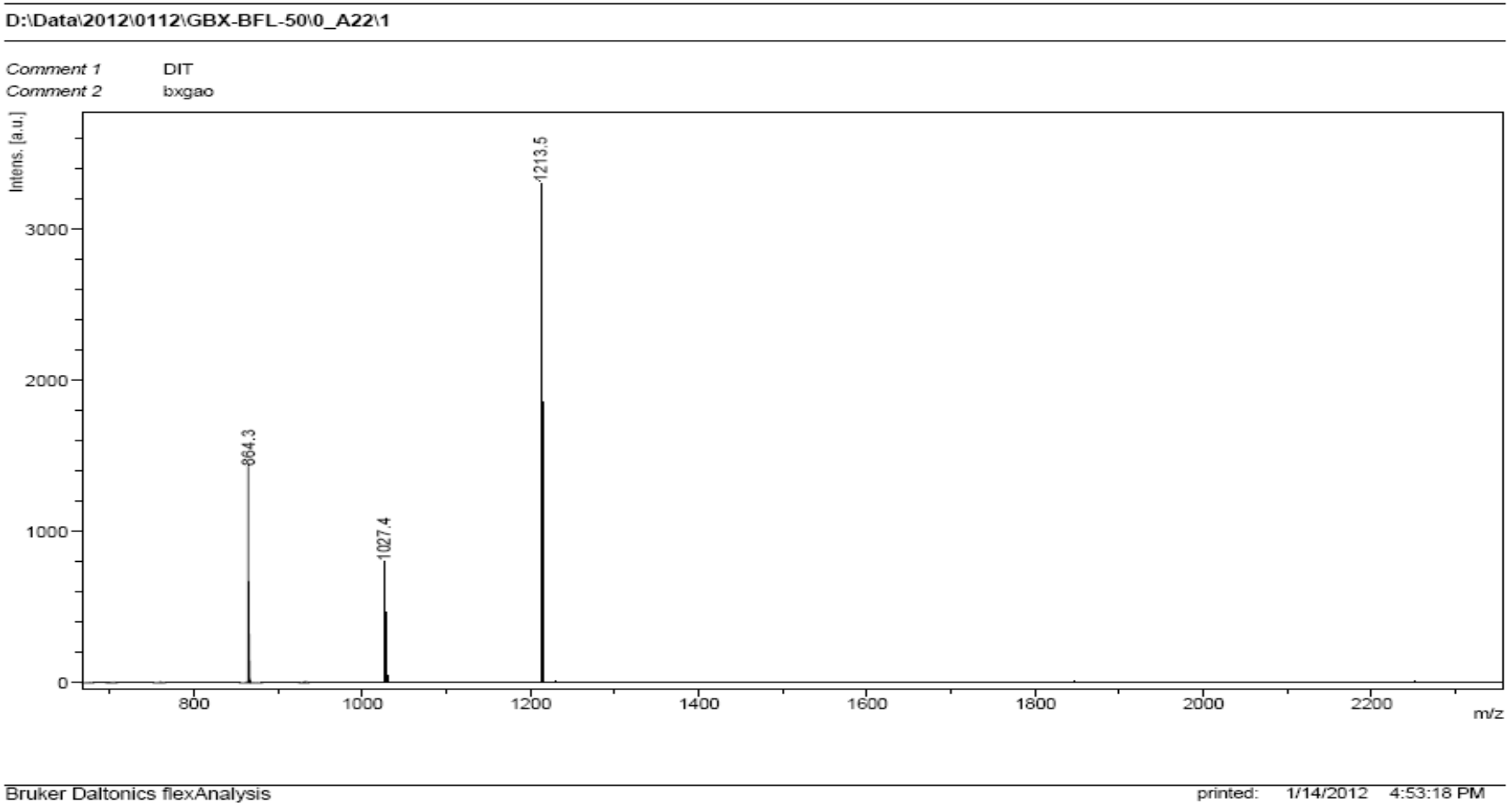
MALDI-TOF spectrum of compound 3



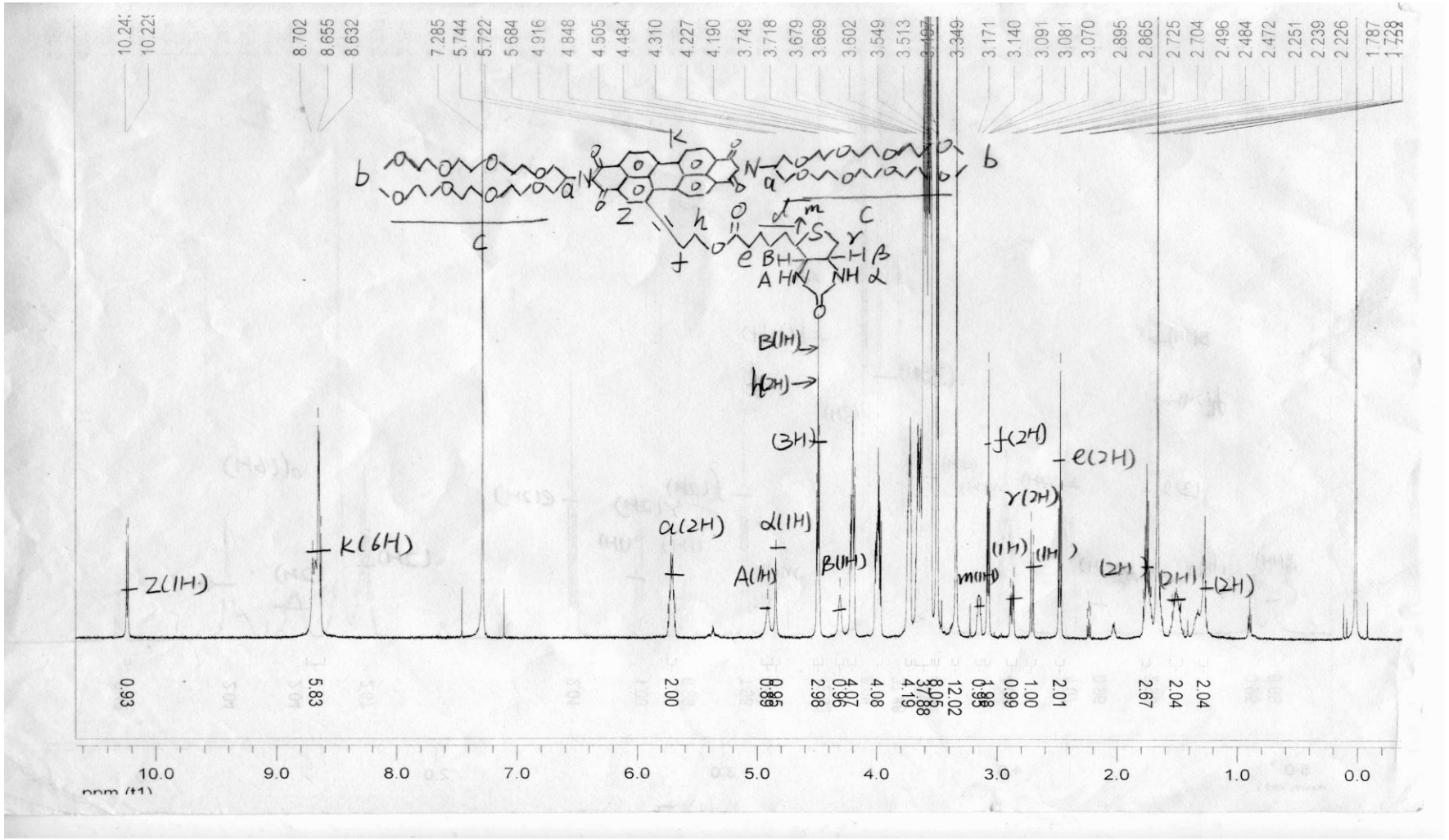
^{1}H-NMR spectrum of **APBI-2** in $CDCl_3$



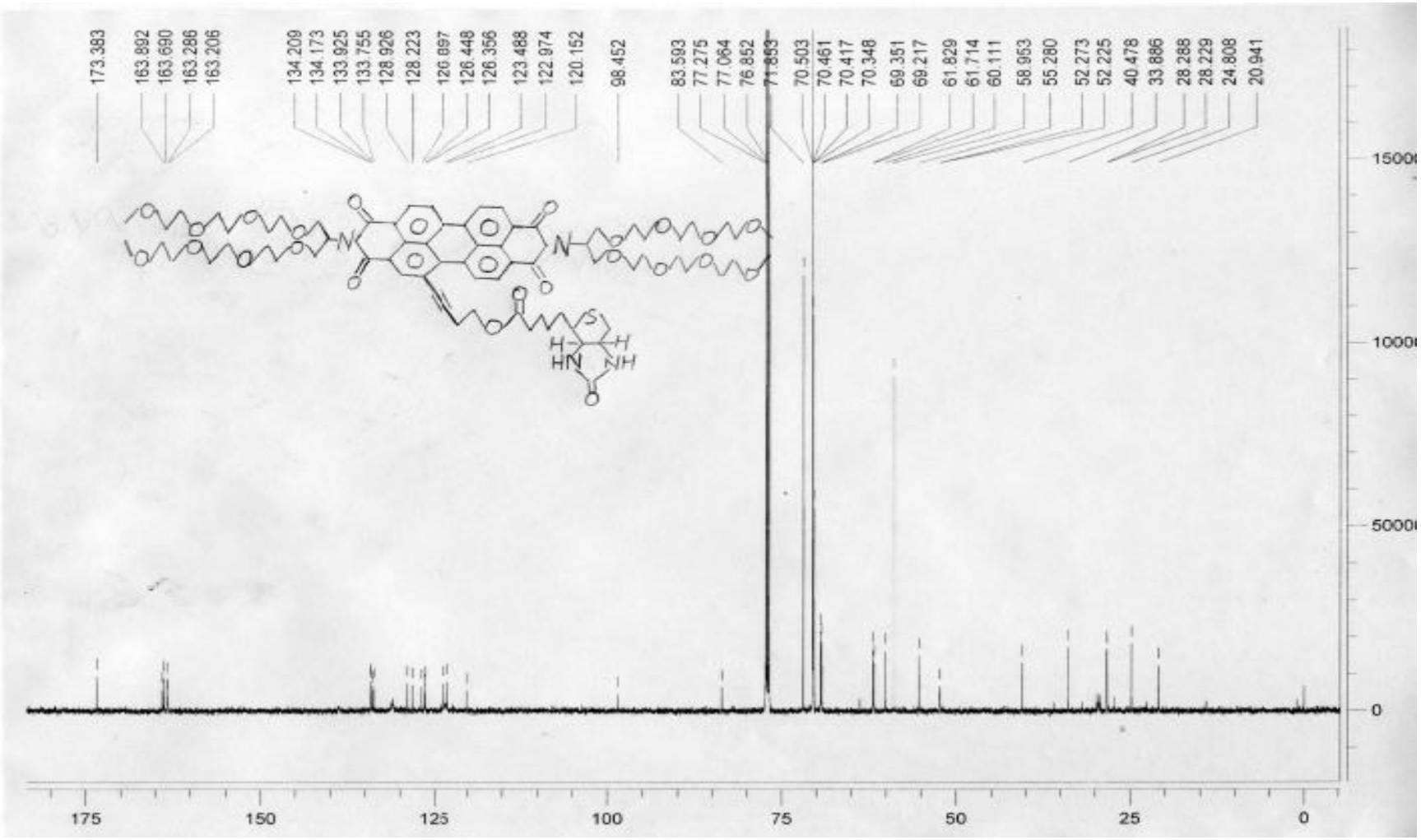
^{13}C-NMR spectrum of **APBI-2** in $CDCl_3$



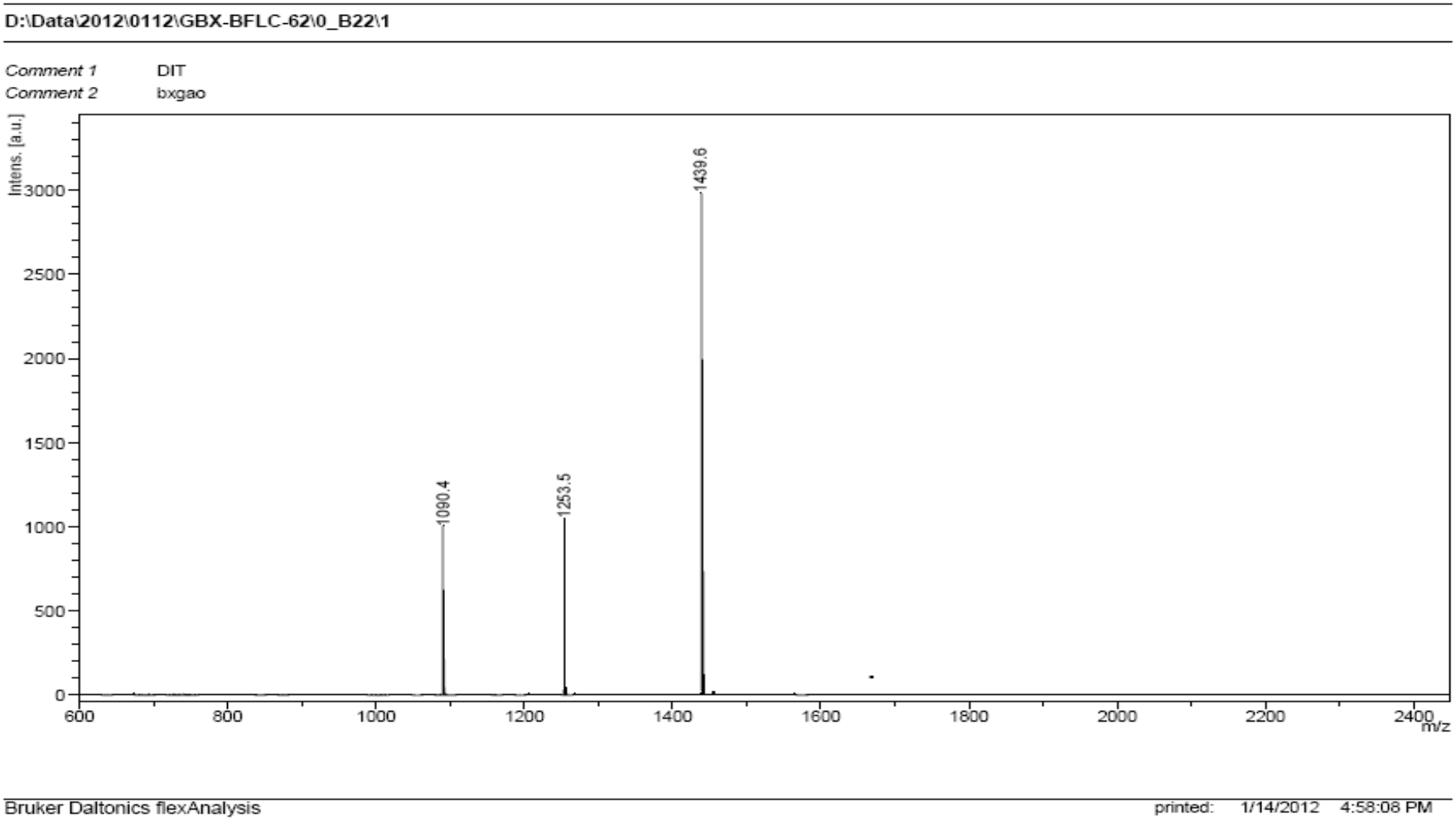
MALDI-TOF spectrum of APBI-2



^{1}H-NMR spectrum of **APBI-1** in $CDCl_3$



^{13}C-NMR spectrum of **APBI-1** in $CDCl_3$



MALDI-TOF spectrum of APBI-1