

Supplementary Information

Materials

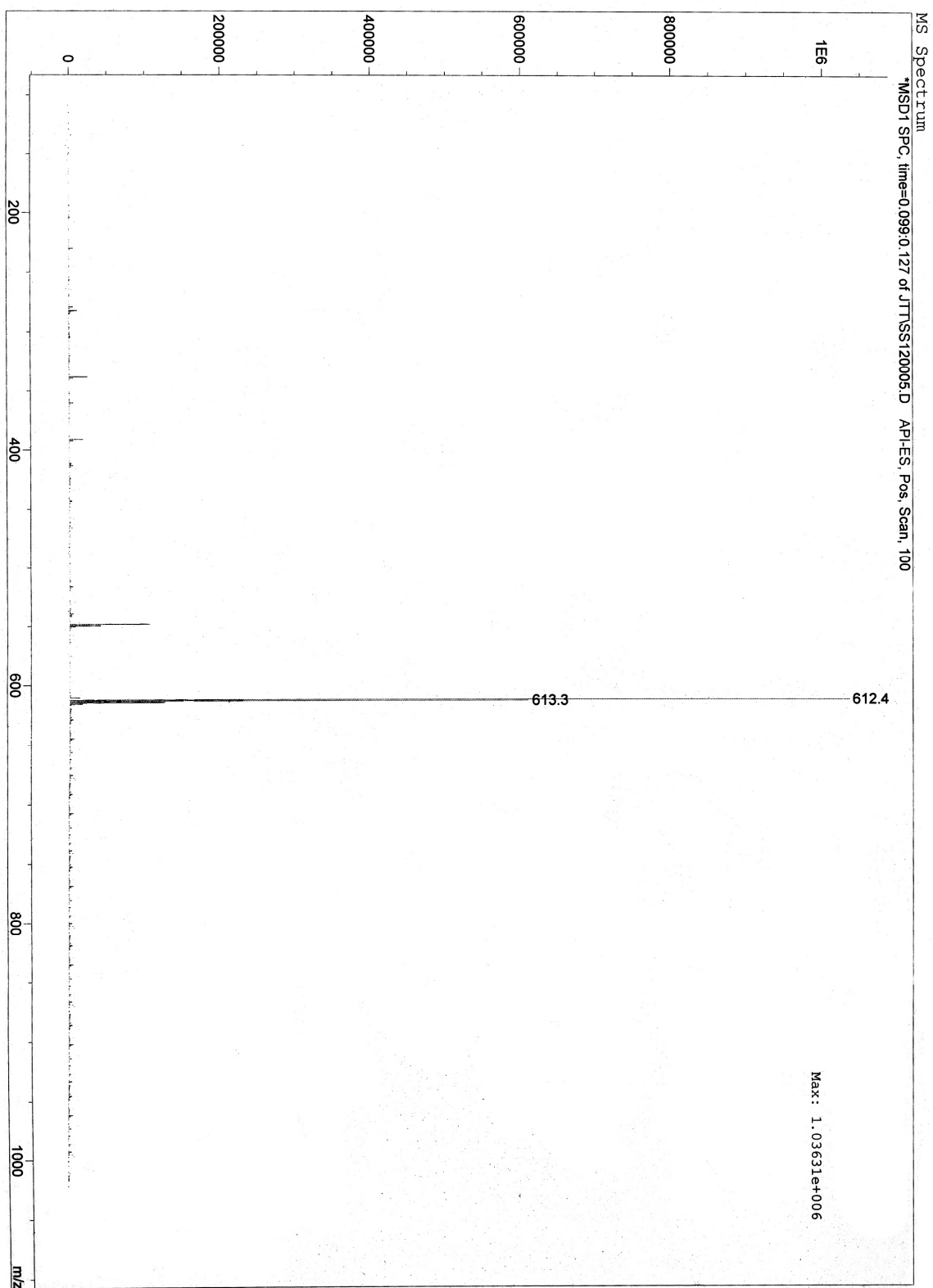
3-aminophenol was purchased from Sigma-Aldrich Co. All other reagents and solvents were purchased from commercial sources and of the highest grade. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. Analytical thin-layer chromatography was performed using GF254 silica gel (precoated sheets, 0.77 mm thick, Taizhou Si-Jia Biochemical Plastic Company). HepG2 cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences.

Apparatus

^1H NMR spectra were recorded on a Bruker Avance 300 MHz. Mass spectral determination was made on an Agilent HP 1100 LC-MSD (U.S). Fluorimetric spectra and traces were obtained with an Edinburgh FLS920 spectrofluorimeter (Edinburgh Instruments Ltd, England) equipped with a xenon lamp and 1.0 cm quartz cells. Absorption spectra were measured on a pharmaspect UV-1700 UV-Visible spectrophotometer (SHIMADZU). The pH measurements were performed on a PHS-3C digital pH-meter (LeiCi, Shanghai, China) with a combined glass-calomel electrode. Double-distilled water was used throughout. The images were taken using a LTE confocal laser scanning microscope (Germany Leica Co., Ltd) with an objective lens ($\times 40$). The excitation wavelength was 532 nm.

Synthesis

Preparation of AP-Cy. 1.333 g (2 mmol) of tricyanocyanine and 2.183 g (20 mmol) of 3-aminophenol were dissolved in 20 mL anhydrous DMF in a 50 mL round bottom flask. The mixture was stirred at 80-85°C under N_2 for four hours with progress monitored by TLC. Then it was cooled and added to 300 mL of ether with violent stirring. The obtained blue solid was filtered and dried under vacuum, then purified on silica gel chromatography eluted with ethyl acetate/methanol 20:1. ^1H NMR (300 MHz, CDCl_3): δ 1.04 (t, 6H, $J=7.5\text{Hz}$), 1.27 (s, 12H), 1.83 (m, 2H), 2.06 (m, 4H), 2.58 (m, 4H), 3.89 (t, 4H, $J=6.8\text{Hz}$), 5.84 (d, 2H, $J=14.0\text{Hz}$), 6.45 (m, 4H), 6.92-7.23 (m, 8H), 8.08 (d, 2H, $J=14.0\text{Hz}$). ESI-MS cal for $[\text{M}]^+ = 612.4$, found 612.4 (M-I).



Spectral analysis: AP-Cy was dissolved in acetonitrile to obtain 10 mM stock solutions, and then aliquots were diluted to 10 μ M with 40 mM buffer solution containing 40 mM acetic acid, phosphoric acid, boric acid. All the measurements were performed in the presence of 0.1 M NaCl to maintain a constant ionic strength. Slight variations in the pH of the solutions were achieved by adding the minimum volumes of NaOH or HCl. All the measurements were carried out at ambient temperature and in air-equilibrated solutions. Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} were added as the sulfates (200 μ M).

Figure S1. Picture of AP-Cy in buffer solution with different pH values. 10.50, 7.07, 3.58 from left to right, respectively.

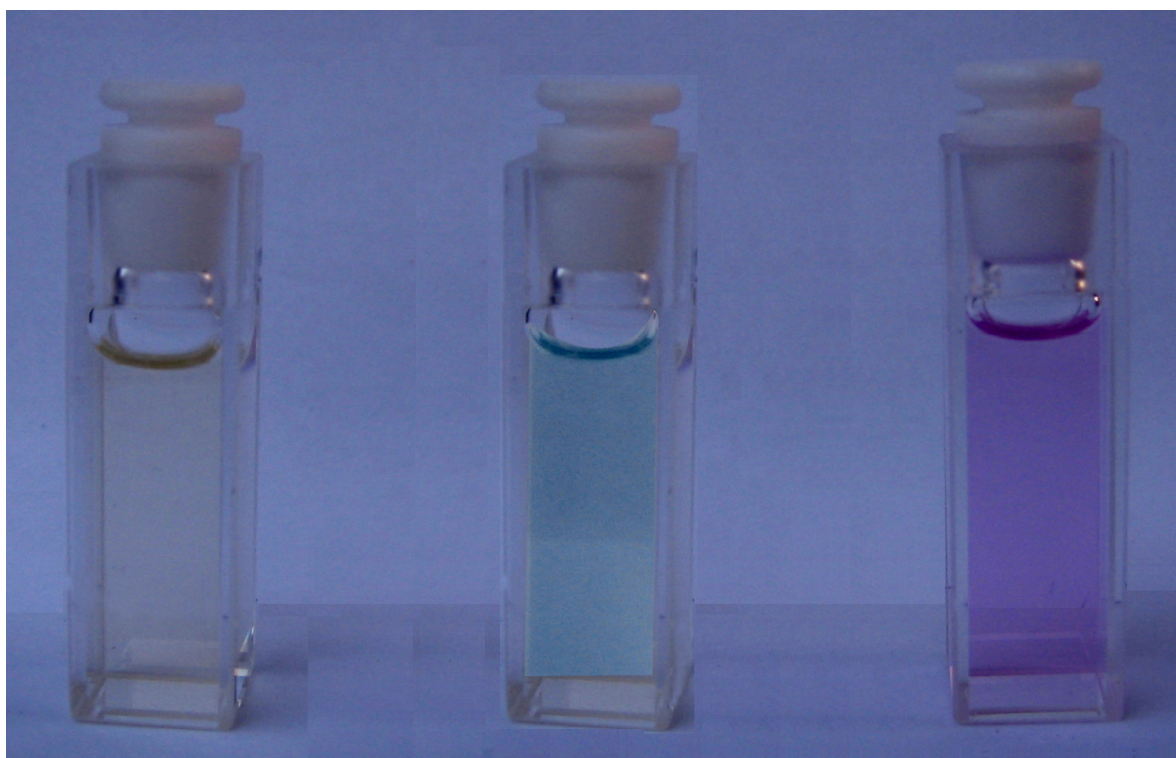


Figure S2. Relative fluorescence intensity at 615 nm versus pH titration curve for 10 μ M AP-Cy under acidic condition. ($\lambda_{ex} = 558$ nm)

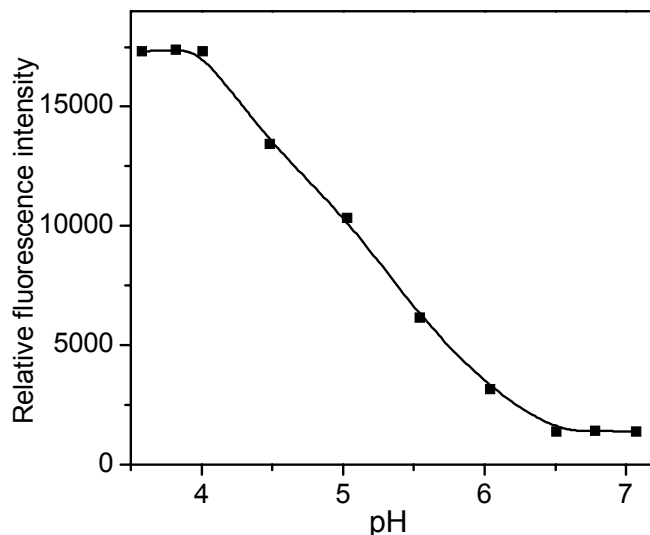
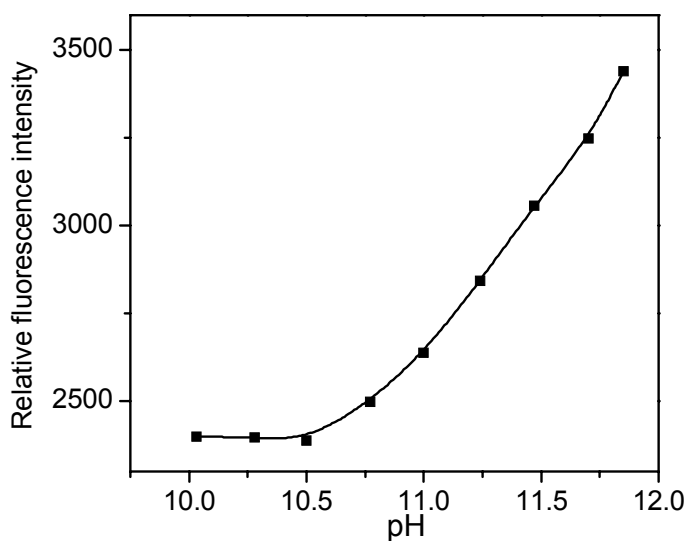


Figure S3. Relative fluorescence intensity at 517 nm versus pH titration curve for 10 μ M AP-Cy under basic condition. ($\lambda_{ex} = 468$ nm)



Confocal fluorescence imaging: HepG2 cells (human hepatocellular liver carcinoma cell line) were cultured in RPMI 1640 medium. One day before experiment cell suspensions were plated at a density of 5×10^4 cells/mL on 35-mm-diameter round glass coverslips. Then the cells were incubated with AP-Cy (10 μ M) for 1h at 37°C in 5% CO₂-95% air and washed three times with PBS buffer (0.1 M, pH 7.4) before imaging.