

Supporting Information for

“A Bispyrene Derivative as a Selective Fluorescent Probe for RNA”

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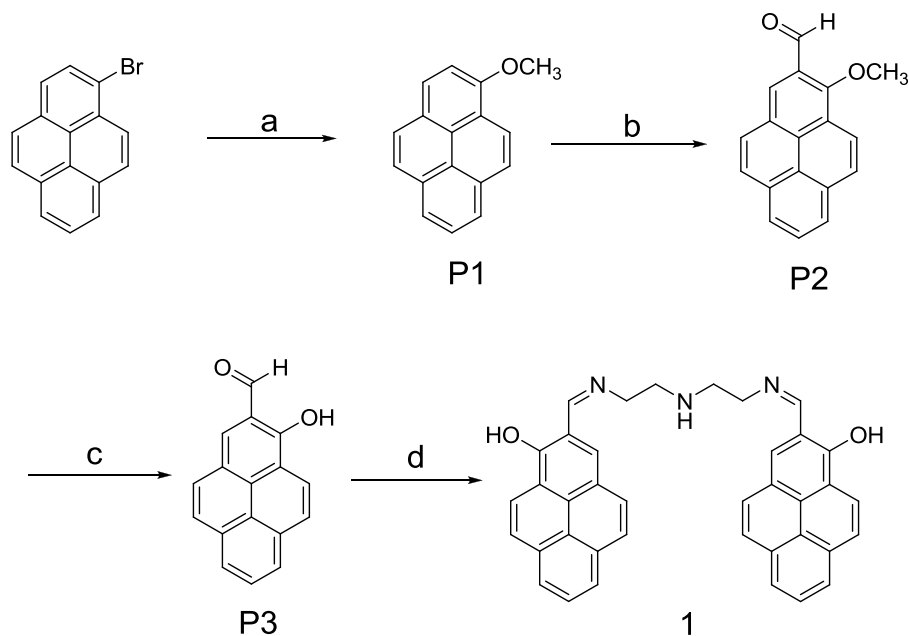
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1. Experiment Section

General Consideration: All materials used for synthesis were obtained from Aldrich-Sigma or TCI. All organic solvents for synthesis were of analytical grade and were obtained commercially. They were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) with 0.25-mm Merck silica gel plates (60F-254) under irradiation by UV-lamp (254 nm). Merck Millipore silica gel (230-400 mesh) was used in the column-chromatography purification. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra were measured on a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). Absorption spectra were recorded in a 10 × 10-mm quartz cuvettes (HELLMA; type number 100-QS) on a Thermo Fisher Scientific Evolution 201 UV/Visible spectrophotometer under the control of a PC system (Windows 8) with the professional software supplied by the

manufacturer. Fluorescence spectra were measured in a 10 × 10 mm disposable cuvette (HELLMA; type number 101.650-QG) on a RF-5301/PC spectrofluorophotometer (Shimadzu) controlled by a PC (Windows XP) running software provided by the manufacturer.



Scheme S1. Reagents and conditions: (a) Na, CuI, MeOH, reflux, 36h, 60%; (b) n-butyllithium, 0°C; tetramethylethylenediamine, 4h; DMF, 22h, 30%; (c) AlCl₃, CH₂Cl₂, room temperature, 4h, 72%; (d) diethylenetriamine, CH₂Cl₂, room temperature, 24h, 80%.

Synthesis of P1

1-bromo-pyrene (5 g, 18 mmol) and CuI (0.38 g, 2 mmol) were mixed in the fresh sodium methylate solution and were refluxed for 36 hours under N₂. The above mixture was quenched by 300 ml cool water and then acidified to pH=1 by 2 M HCl. The light brown precipitate was filtered and washed with water. The product was purified by silica column chromatography using a hexane/dichloromethane mixture (20:1) as eluent. Then recrystallized in ethanol to get **P1** as light yellow solid. Yield: 2.5 g (60 %) ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 4.18 (s, 1H), 7.54-7.57 (d, 1H), 7.86-8.22 (m, 7H), 8.43-8.47 (d, 1H).

Synthesis of P2

1.6M butyl-lithium (10.9 mL, 18 mmol) was added slowly to the solution of compound **P1** (4.1 g, 17.6 mmol) in 70 mL dry hexane under N₂ at 0 °C. Then tetramethyl-ethylerediamine (2.66 mL) was injected to the above solution, then the solution color changed to red. After 4 hours, DMF (1.63 mL, 56 mmol) was added by syringe, and the mixture was continue stirred

for 22 hours at room temperature. Finally the reaction was stopped by adding 0.5 M HCl. Then the hexane was removed by evaporation to get yellow solid. And the solid was washed with water and extracted with dichloromethane. The organic phase was dried over Na₂SO₄. The solvent was evaporated out and the residue was purified by silica column chromatography using dichloromethane as eluent to obtain **P2** as yellow powder. Yield: 1.4 g (30 %) ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 4.19 (s, 1H), 8.06-8.11 (m, 6H), 8.39-8.43 (m, H), 8.61 (s, 1H), 10.83 (s, 1H).

Synthesis of **P3**

compound **P2** (3 g, 11.4 mmol) dissolved in 30 mL dry CH₂Cl₂ was added to the solution of AlCl₃ (6 g, 45 mmol) in 40 mL dry CH₂Cl₂ at 0°C. Then the solution was stirred at room temperature. 4 hours later, 0.1 M HCl was added to the red mixture. The mixture extracted with dichloromethane, and the organic phase was dried over Na₂SO₄. The solvent was evaporated and the residue was purified by silica column chromatography using dichloromethane as eluent to obtain **P3** as yellow powder. Yield: 2 g (72 %) ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 7.79-8.12 (m, 7H), 8.44-8.47 (d, H), 10.27 (s, 1H), 11.88 (s, 1H).

Synthesis of **1**

The crude **P3** (100mg, 0.41mmol) was dissolved in CH₂Cl₂ and diethylenetriamine (0.02mL, 0.20mmol) was added to the reaction mixture. The reaction mixture was stirred at RT for 12h and a precipitate was formed. The precipitate was filtered and washed with cold CH₂Cl₂ to give **1** as a red solid. Yield: 181mg (80%). m.p. = 203°C ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 14.37 (s, OH), 8.70 (s, 2H), 8.23 (d, *J* = 9.34 Hz, 2H), 7.92-7.85 (m, 6H), 7.73 (d, *J* = 7.73 Hz, 4H), 7.56 (s, 4H), 3.89 (s, 4H), 3.16 (s, 4H), 1.39 (s, NH). ¹³C NMR (CDCl₃, 62.5 MHz) : δ 132.78, 132.49, 127.26, 127.00, 126.30, 124.74, 124.27, 124.18, 119.87, 115.36, 113.41, 15.76, 12.48, 11.99. HRMS (FAB) calcd for C₃₈H₃₀N₃O₂ [M+H]⁺ 560.2260; found 560.2335

2. Characterization of Probe 1:

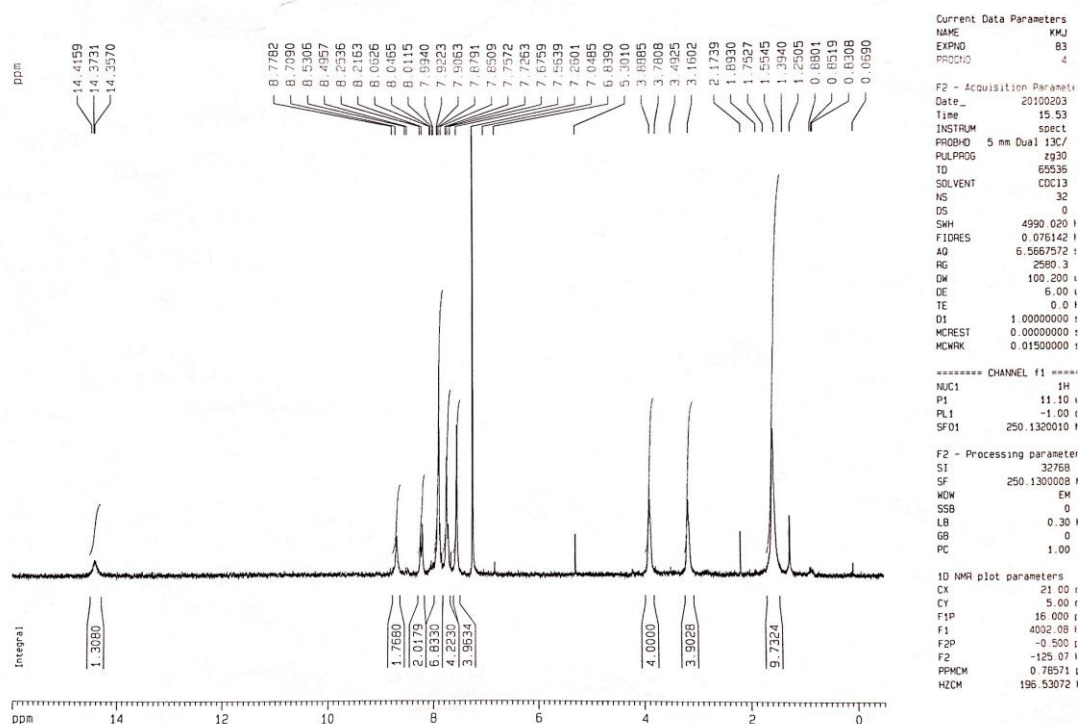


Figure S1. ^1H NMR spectra of **1** in CDCl_3 .

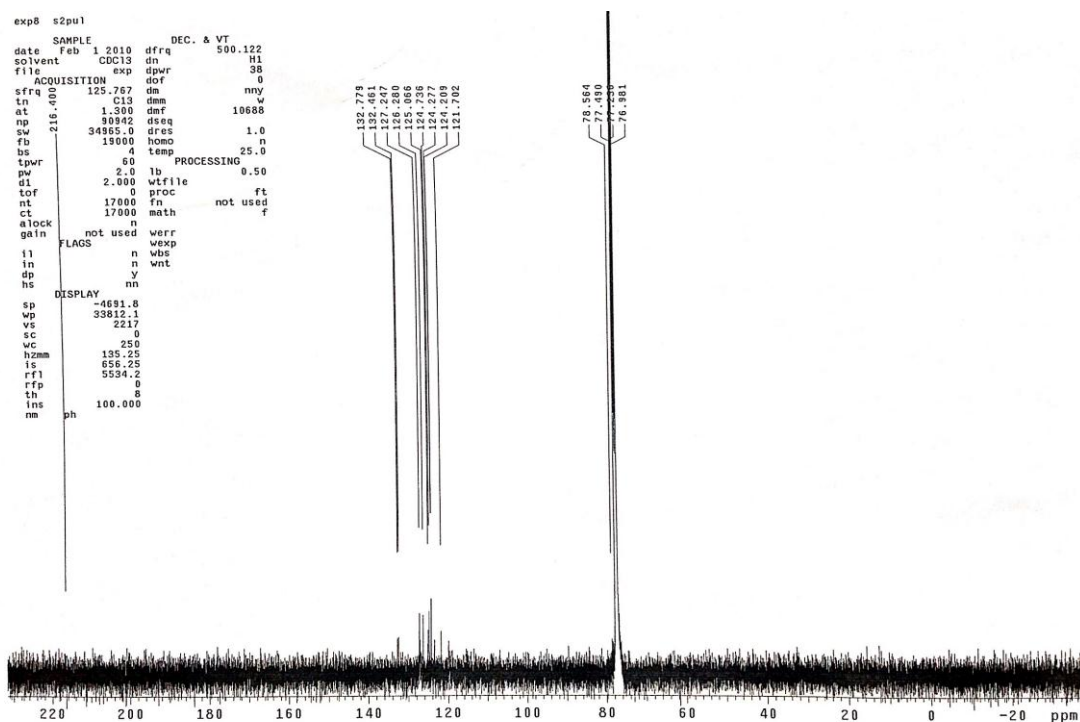


Figure S2. ^{13}C NMR spectra of **1** in CDCl_3 .

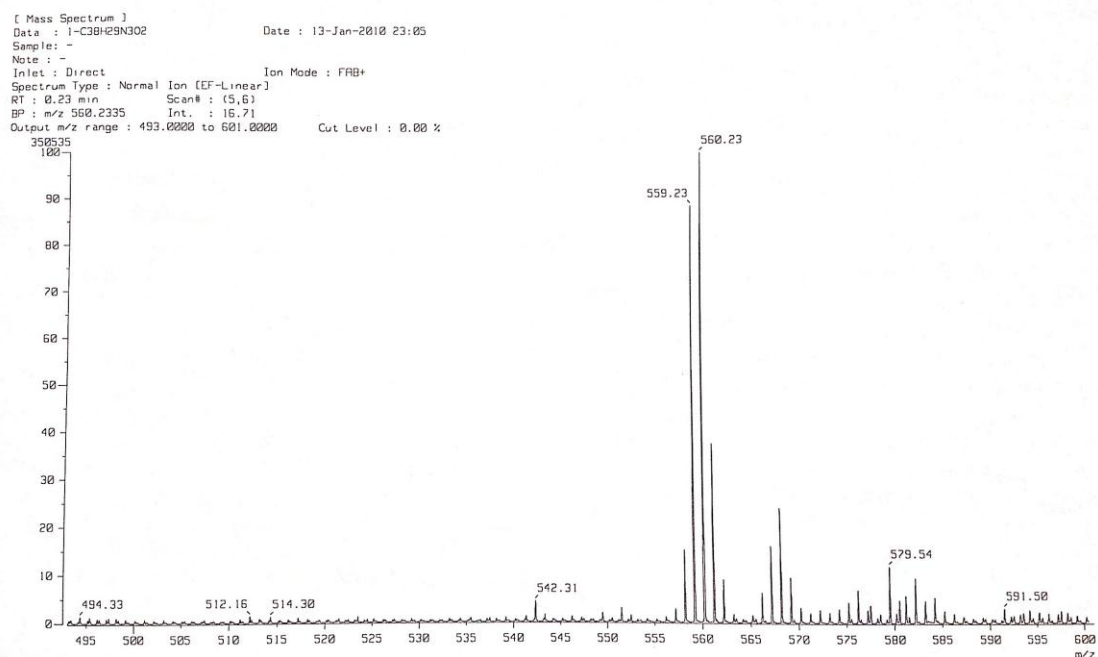


Figure S3. HRMS (FAB) spectra of **1**.

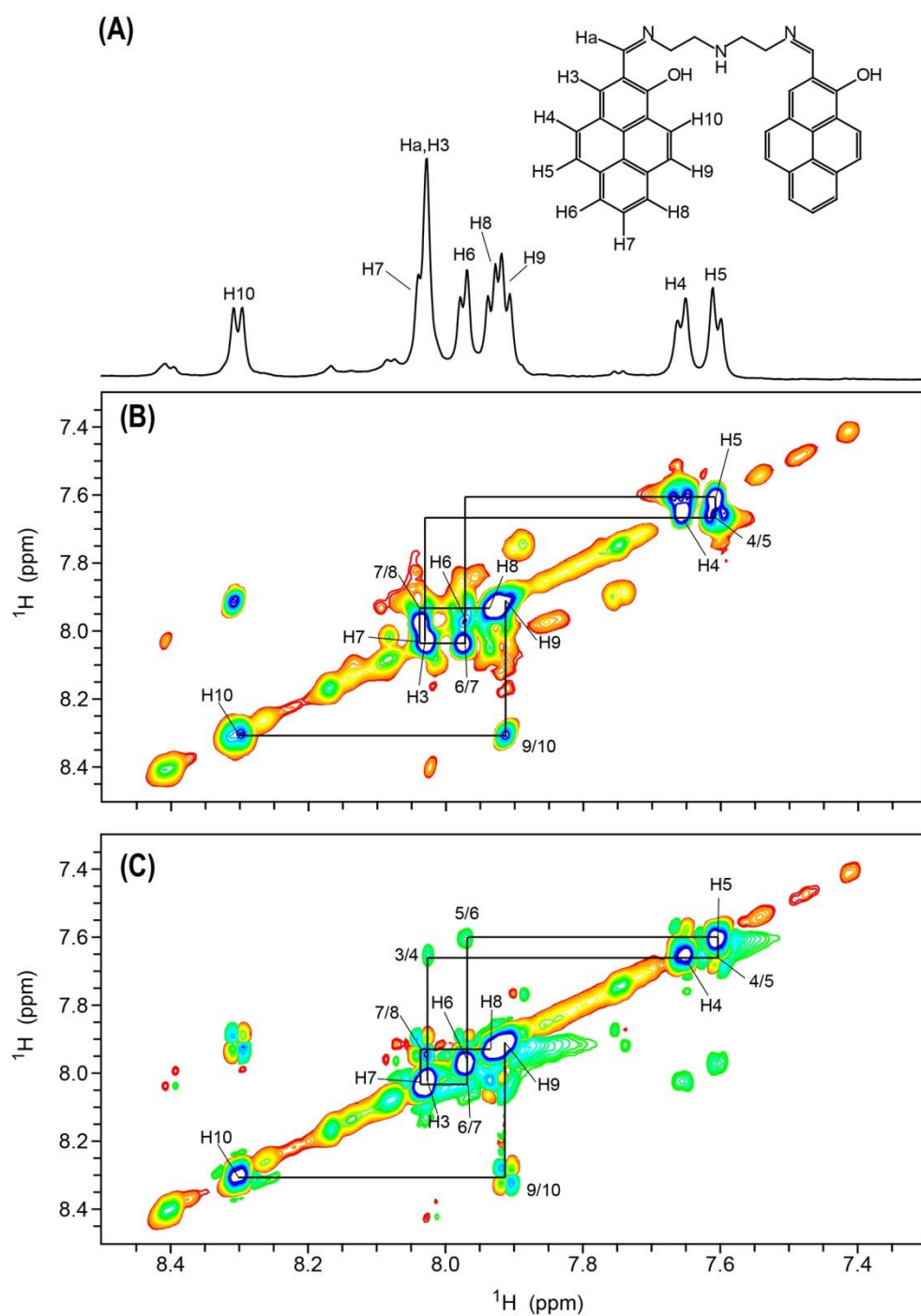


Figure S4. (A) Expanded 1D NMR spectrum of the **1** dissolved in DMSO-*d*₆ at 25 °C. Chemical structure of **1** is shown to the upper-right of spectrum. (B) Expanded TOCSY (mixing time: 80 ms) and (C) ROESY (mixing time: 250 ms) spectra of the **1** dissolved in DMSO-*d*₆ at 25 °C.

3. Spectrum analysis:

Stock solution of **1** (2 mM) was prepared in DMSO, which was then diluted to 20 μM in Tris-HCl buffer at pH 7.4. DNA and RNA were dissolved in deionized water and the concentration were determined from extinction coefficient at 260 nm using a UV-vis spectrophotometer.¹

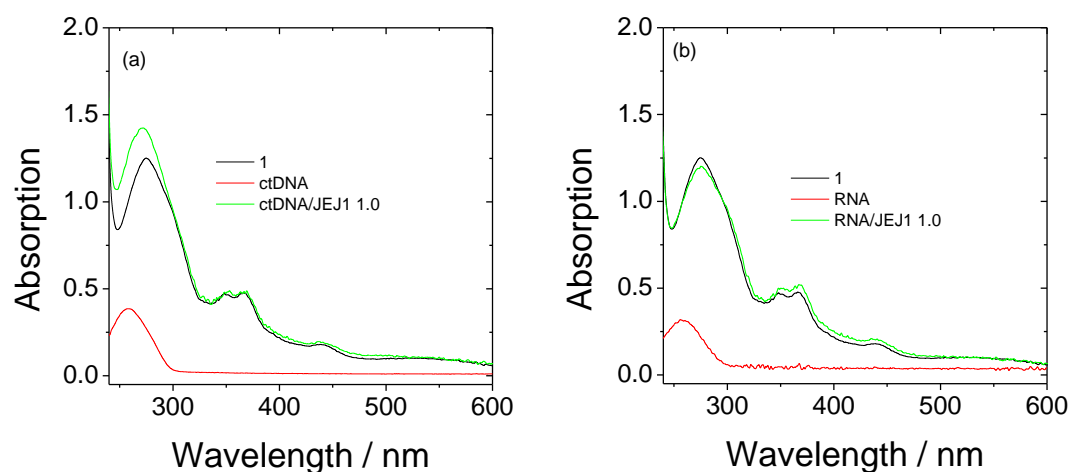


Figure S5. Absorption spectra of 20 μM **1**, ctDNA, RNA and the interaction of **1** with nucleic acid (mole ratios_{[base pairs]/[1]} = 1.0) are shown in (a), (b), in 0.05 M Tris-HCl (pH 7.4).

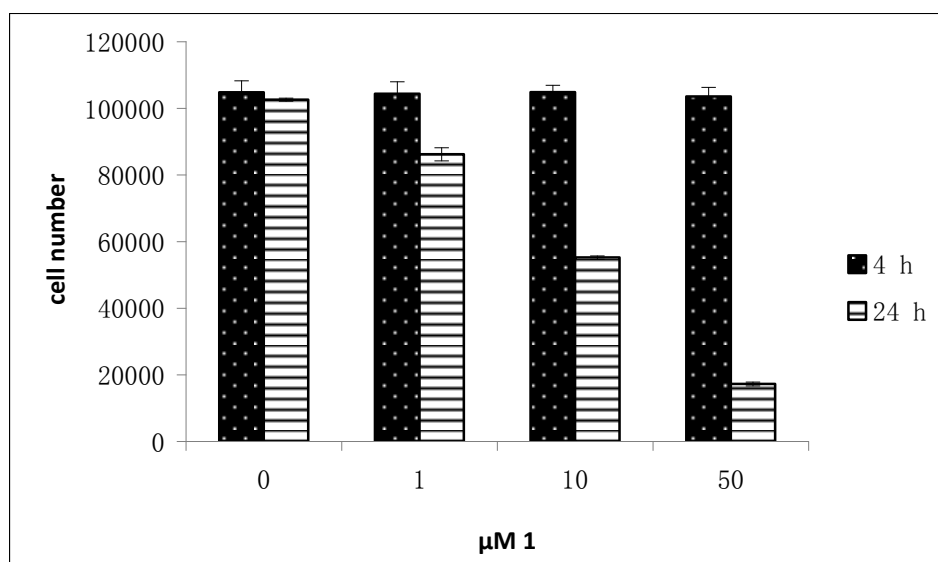


Figure S6. Cytotoxic effects of **1**. HeLa cells treated with 0, 1, 10, 50 μM **1** for 4, 24 h and counted the cell number. Results are expressed as mean \pm standard deviation of three independent experiments.

4. Reference:

- (1) Balapanuru ,J.; Yang, J.X.; Xiao, S.; Bao, Q.; Jahan, M.; Polavarapu, L.; Wei, J.; Xu ,Q.H.; Loh, K. P.
Angew. Chem. Int. Ed. **2010**, *49*, 6549 –6553.