

**Supplementary Material to Accompany:** Tetrakis-acridinyl peptide: A novel fluorometric reagent for nucleic acid analysis based on the fluorescence dequenching upon DNA bind

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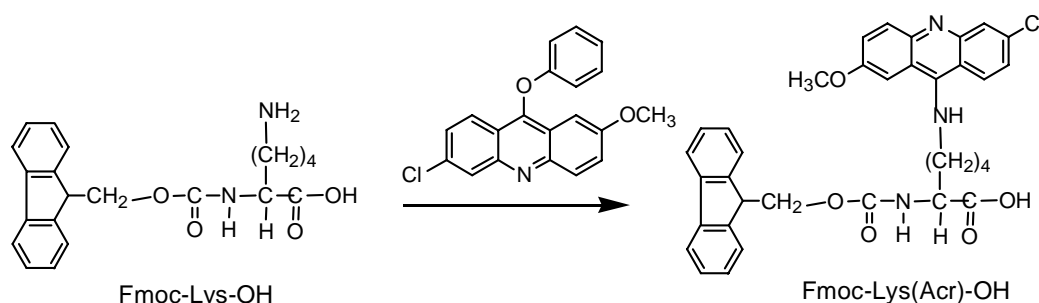
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## Synthesis

### 6-Chloro-2-methoxy-9-phenoxyacridine

6-Chloro-2-methoxy-9-phenoxyacridine was prepared from 6,9-dichloro-2-methoxyacridine by the procedure described previously (Takenaka, S.; Sato, H.; Ihara, T.; Takagi, M. *J. Heterocyclic Chem.* **1997**, *34*, 123-127).

### Fmoc-Lys(Acr)-OH

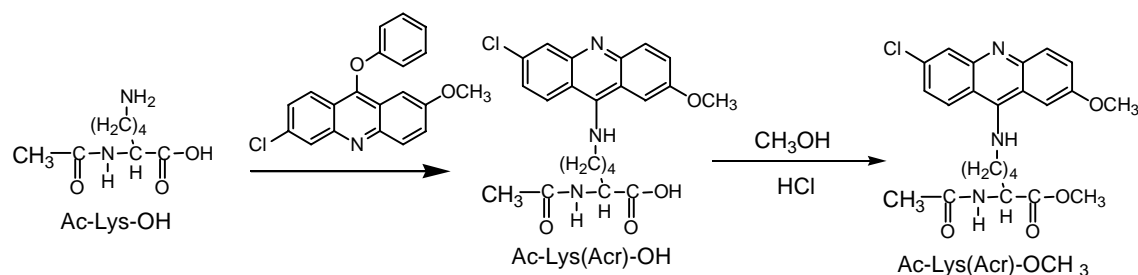


6-Chloro-2-methoxy-9-phenoxyacridine (4.54 g, 13.5 mmol) was dissolved at 80 °C in 30 g of phenol in a 50 ml beaker. While stirring with a magnetic bar at 55-65 °C, Fmoc-Lys-OH (4.42 g, 12.0 mmol) was added and the mixture was stirred for 2 h at 55-65 °C. After cooling to room temperature, 300 ml of ethyl ether was added while stirring vigorously. The yellow precipitate formed was filtered and washed with ethyl ether. The product was dried under vacuum to give 7.57 g (92% yield) of Fmoc-Lys(Acr)-OH as a yellow solid, mp. 142-143.5 °C. Time of flight mass spectroscopy gave 610.8 of  $[C_{21}H_{33}N_3O_5Cl]^+$  for Fmoc-Lys(Acr)-OH (theory 610.1).

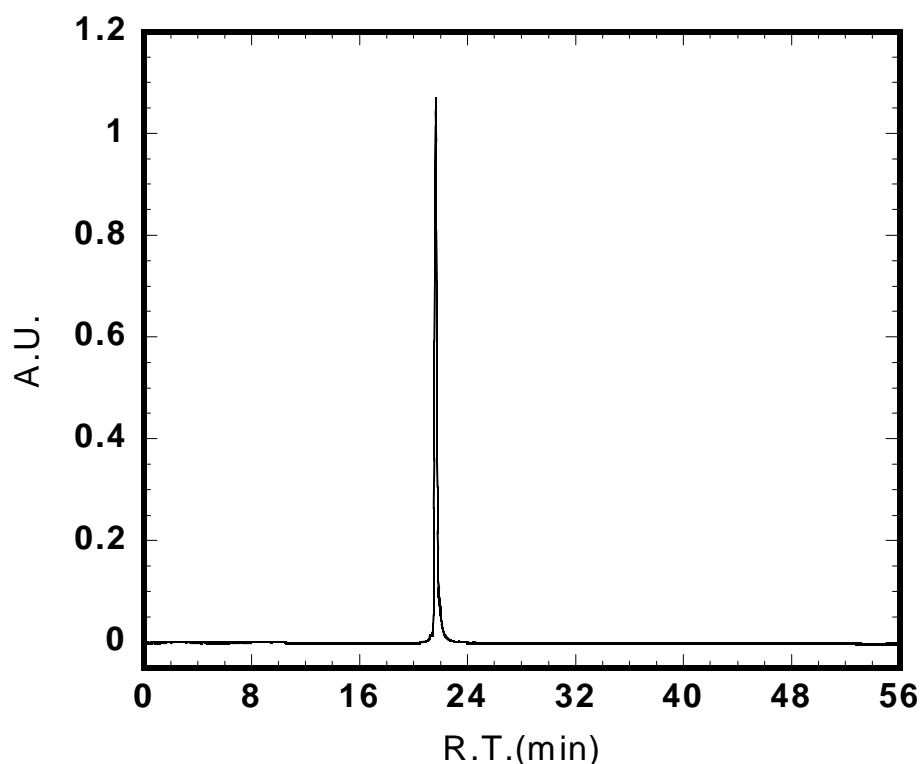
### Teterakis-acridinyl peptide (**1**)

Teterakis-acridinyl peptide **1** was assembled on a peptide synthesizer (PE Applied Biosystems, model 431A) by using Fmoc chemistry. Starting with 200 mg of Fmoc-NH SAL resin, 500 mg of a **1**-immobilized resin were obtained. This resin (500 mg) was suspended in a mixture of m-cresol (0.1 ml), thioanisole (0.6 ml), and trifluoroacetic acid (TFA, 4.3 ml) and stirred at room temperature for 1 h. After removing TFA under reduced pressure, ethyl ether (20 ml) was added on ice, the mixture was sonicated and incubated for five minutes. The supernatant was removed and ethyl acetate (20 ml) was added to this precipitate, the mixture was sonicated and incubated for five minutes. The yellow solid collected by filtration was washed with ethyl ether and then dried under vacuum to give 318 mg of the product. Peptide **1** was purified by reversed phased HPLC (Figure S1). The eluent, consisting of 0.1% TFA (A) and 0.1% TFA in 70% acetonitrile (B), was run in a linear gradient of 0-100% of (B) over 40 min at a flow rate of 1.0 ml/min. Time of flight mass spectroscopy gave 2309.03 of  $[C_{118}H_{157}N_{25}O_{15}C_{14}]^+$  for **1**+H<sup>+</sup> (theory 2307.51)(Figure. S2).

## Ac-Lys(Acr)-OMe (2)



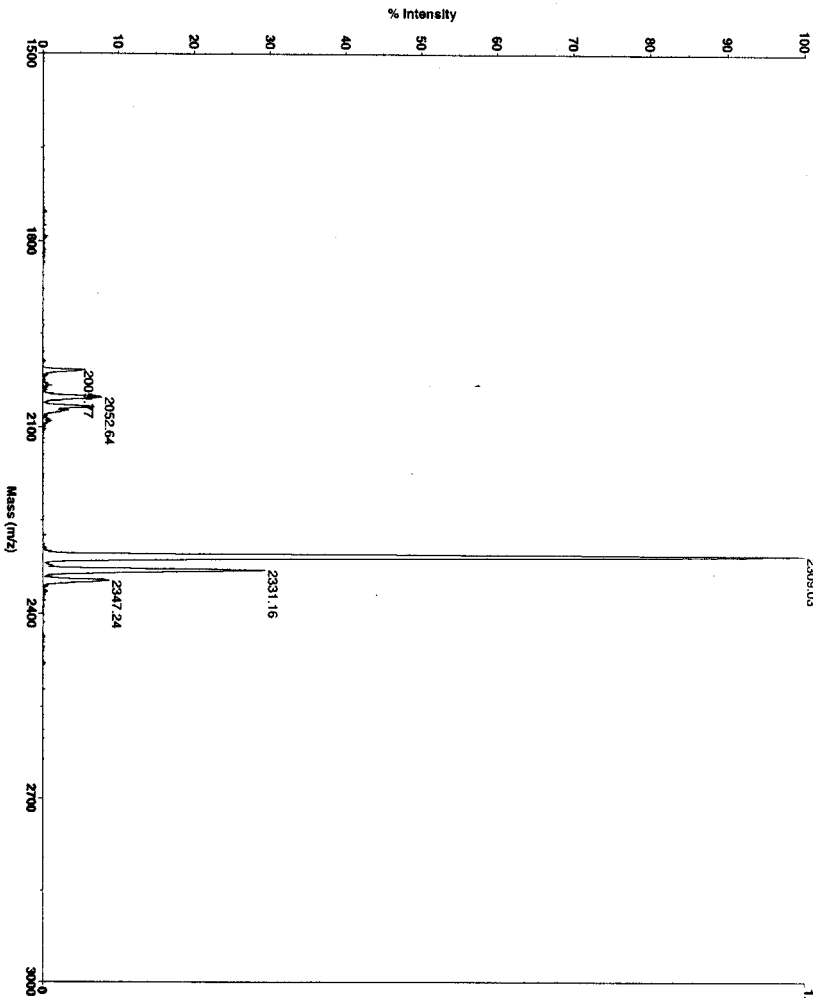
6-Chloro-2-methoxy-9-phenoxycridine (757 mg, 2.25 mmol) was dissolved at 80 °C in 5.0 g of phenol in a 50 ml beaker. While stirring with a magnetic bar at 60 °C, Ac-Lys-OH (377 mg, 2.0 mmol) was dissolved over 40 min and the mixture was stirred for 1 h at 55-60 °C. After cooling to room temperature, 50 ml of ethyl ether was added while stirring vigorously. The yellow precipitate formed was filtered and washed with ethyl ether. The solid was dried under vacuum to give 876 mg of Ac-Lys(Acr)-OH. To a suspension of crude Ac-Lys(Acr)-OH (876 mg) in dry methanol was introduced dry hydrogen chloride and the mixture was stirred for 4 h. Upon removal of the solvent under vacuum, a solid was obtained. The product was purified by recrystallization from dry methanol and dry ethyl ether to yield 633 mg (74 %) of **2** as a yellow solid, mp. 135-138 °C. <sup>1</sup>H NMR (250 MHz, DMSO-d<sub>6</sub>): δ 1.4 (q, 2H), 1.7 (quin, 2H), 1.8 (s, 3H), 1.9 (br, 2H), 3.6 (s, 3H), 4.0 (s, 3H), 4.1 (q, 2H), 4.2 (q, 1H), 7.5 (d, 8.3 Hz, 1H), 7.7 (d, 9.4 Hz, 1H), 7.9 (d, 9.4 Hz, 1H), 8.0 (s, 2H), 8.3 (d, 8.3 Hz, 1H), 8.5 (d, 8.6 Hz, 1H), 9.9 ppm (br, 1H) (Figure S3).



**Figure 1S.** Reversed phase HPLC of **1**. The eluent, consisting of 0.1% TFA (A) and 0.1% TFA in 70% acetonitrile (B), was run in a linear gradient of 0-100% of (B) over 40 min at a flow rate of 1.0 ml/min.

PE Biosystems Voyager System 1180

Spec #1[BP = 2308.8, 15990]



Mode of operation: Linear  
 Extraction mode: Delayed  
 Polarity: Positive  
 Acquisition control: Manual  
 Accelerating voltage: 20000 V  
 Grid voltage: 94%  
 Guide wire D: 0.05%  
 Extraction delay time: 200 nsec  
 Acquisition mass range: 1500 -- 3000 Da  
 Number of laser shots: 50/Spectrum  
 Laser intensity: 1103  
 Calibration type: Default  
 Calibration matrix: a-Cyano-4-hydroxycinnamic acid  
 Low mass gate: 500 Da  
 Digitizer start time: 24.4887  
 Bin size: 2 nsec  
 Number of data points: 50000  
 Vertical scale: 1000 mV  
 Vertical offset: 0%  
 Input bandwidth: 100 MHz

Sample well: 89  
 Plate ID: PLATE1  
 Serial number: 1180  
 Instrument name: Voyager-DE  
 Plate type filename: C:\VOYAGER\100 well plate.plt  
 Lab name: PE Biosystems

Absolute x-position: 36399.8  
 Absolute y-position: 17584.3  
 Relative x-position: -747.885  
 Relative y-position: 756.84  
 Shots in spectrum: 50  
 Source pressure: 7.207e-007  
 Mirror pressure: 0  
 TC2 pressure: 0.02283  
 TIS gate width: 30  
 TIS flight length: 940

Acquired: 13:58, December 20, 2001  
 D:\user\d01\12.200013.dat

Printed: 14:00, December 20, 2001

Figure S2. Time of flight mass spectrum of 1. The m/z value of 2309.03 is consistent with that of  $[C_{118}H_{157}N_{25}O_{15}Cl_4]^+$  (theory 2307.51).

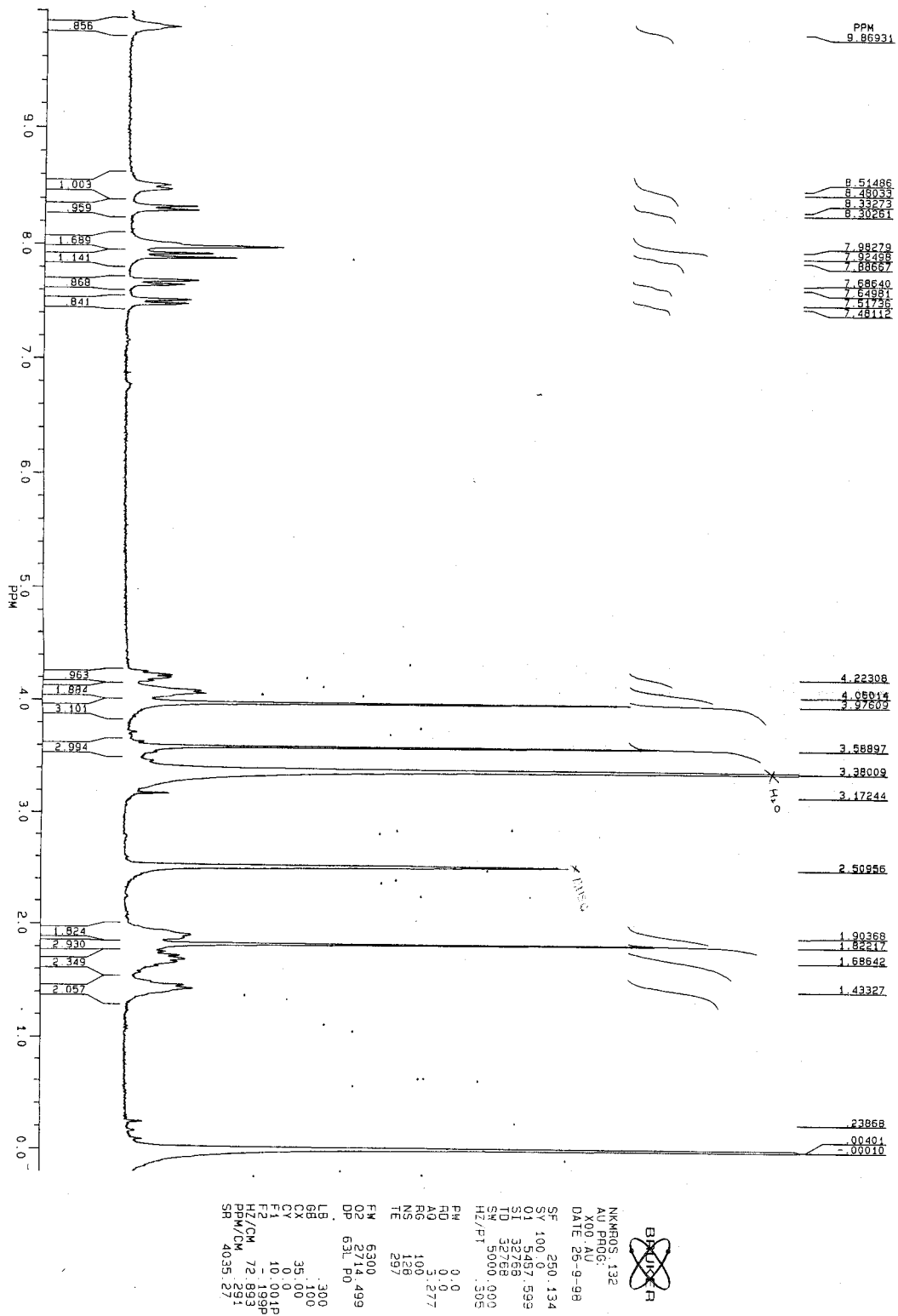
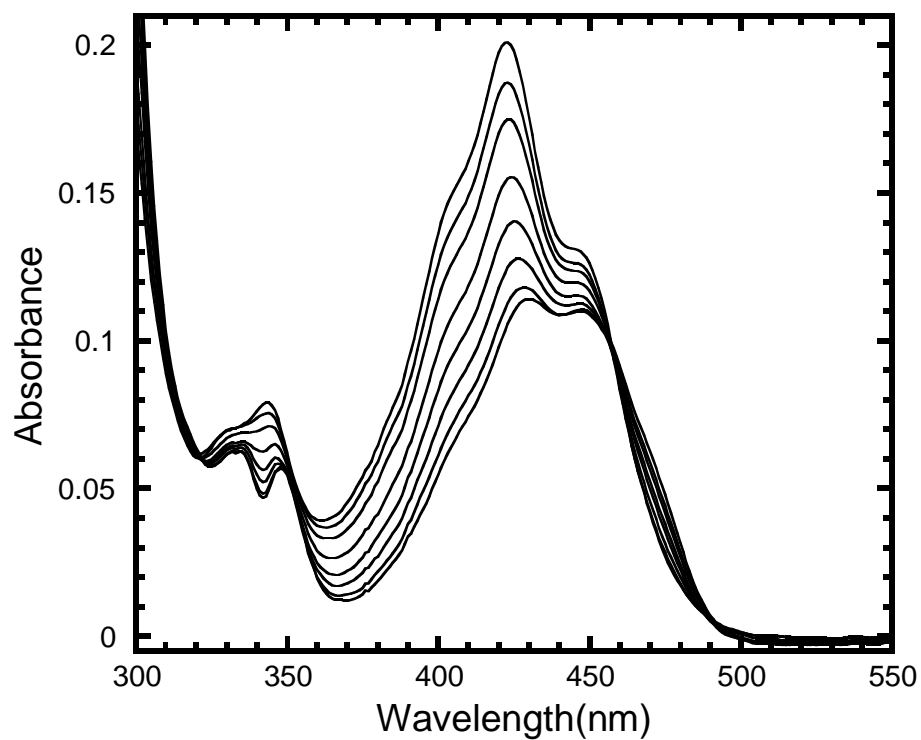
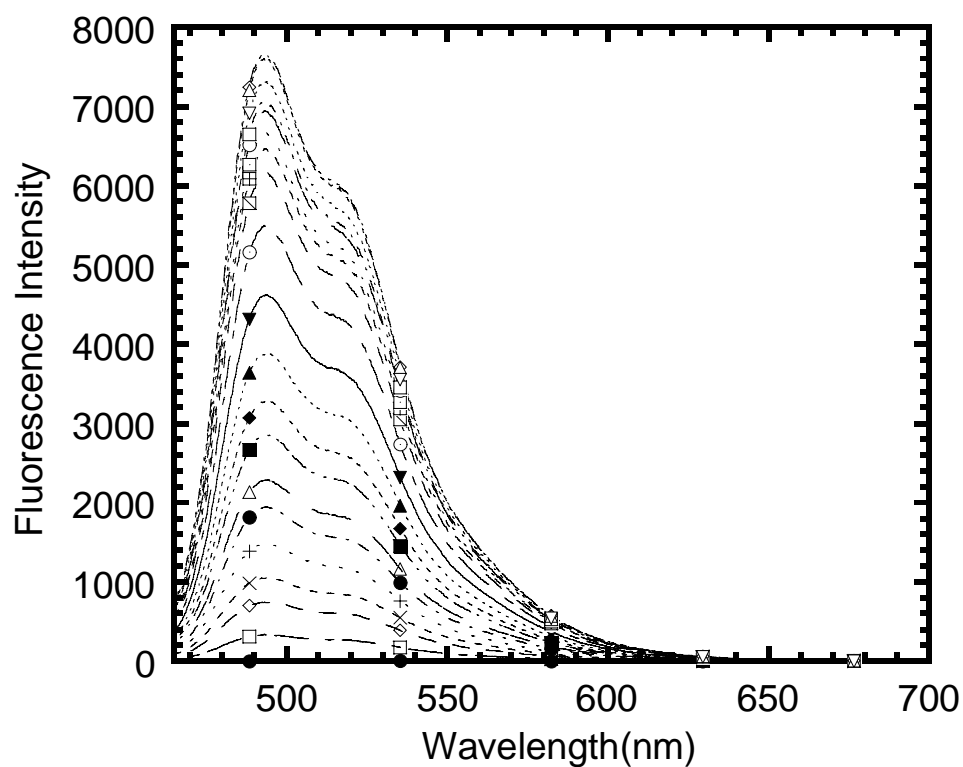


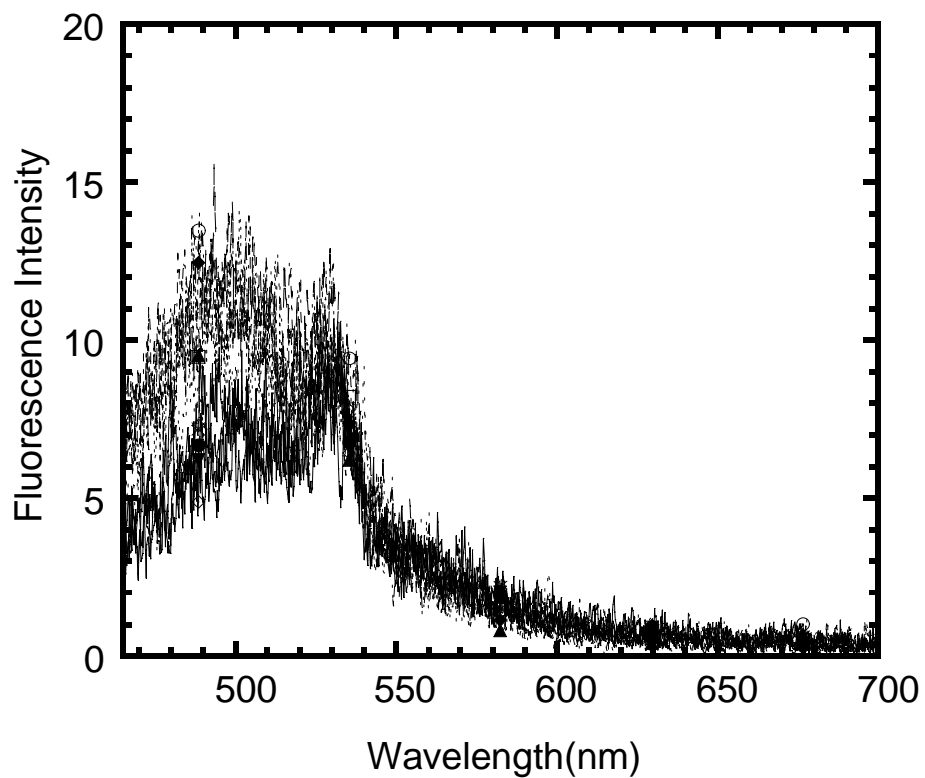
Figure S3.  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO-d}_6$ ) of Ac-Lys(Acr)-OMe, 2.



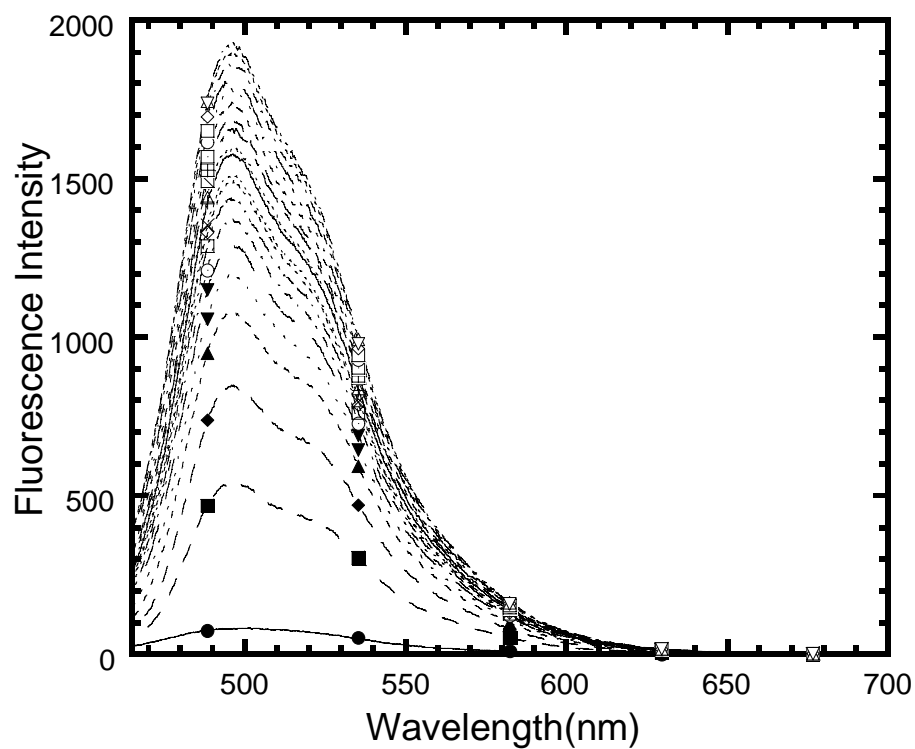
**Figure S4.** Spectral shifts of 0.7  $\mu\text{M}$  **1** in 10 mM MES buffer and 1 mM EDTA containing 0.2 M NaCl (pH 6.25) on titration with [poly(dA-dT)]<sub>2</sub>. The DNA base pair concentrations were 0, 0.30, 0.75, 1.50, 2.10, 2.70, 3.23, and 3.68  $\mu\text{M}$  from top to bottom.



**Figure S5.** Fluorometric titration of 1.6  $\mu\text{M}$  **1** with [poly(dA-dT)]<sub>2</sub> in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25 °C. The DNA base pair concentrations were 0, 1.13, 2.26, 3.39, 4.52, 5.65, 6.78, 7.91, 9.04, 10.17, 11.30, 12.43, 13.56, 14.69, 15.82, 18.08, 20.34, 24.86, 33.90, and 42.94  $\mu\text{M}$  from bottom to top. The excitation wavelength was 457 nm.



**Figure S6.** Fluorometric titration of 1.6  $\mu\text{M}$  **1** with  $[\text{poly}(\text{dG-dC})]_2$  in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25  $^\circ\text{C}$ . The DNA base pair concentrations were 0, 1.08, 2.16, 3.24, 4.32, 9.72, 15.12, 20.52, 25.92, and 31.32  $\mu\text{M}$  from bottom to top. The excitation wavelength was 451 nm.



**Figure S7.** Fluorometric titration of 1.6  $\mu\text{M}$  A666 with  $[\text{poly}(\text{dA-dT})_2]$  in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25  $^\circ\text{C}$ . The DNA base pair concentrations were 0, 1.22, 2.44, 3.66, 4.88, 6.10, 7.32, 8.54, 9.76, 10.98, 12.20, 13.42, 15.86, 18.30, 23.18, 32.94, 43.92, 73.20, 106.14, and 117.12  $\mu\text{M}$  from bottom to top. The excitation wavelength was 453 nm.