Aminonaphthalimide-based Imidazolium Podands for Turn-On Fluorescence Sensing of Nucleoside Polyphosphates

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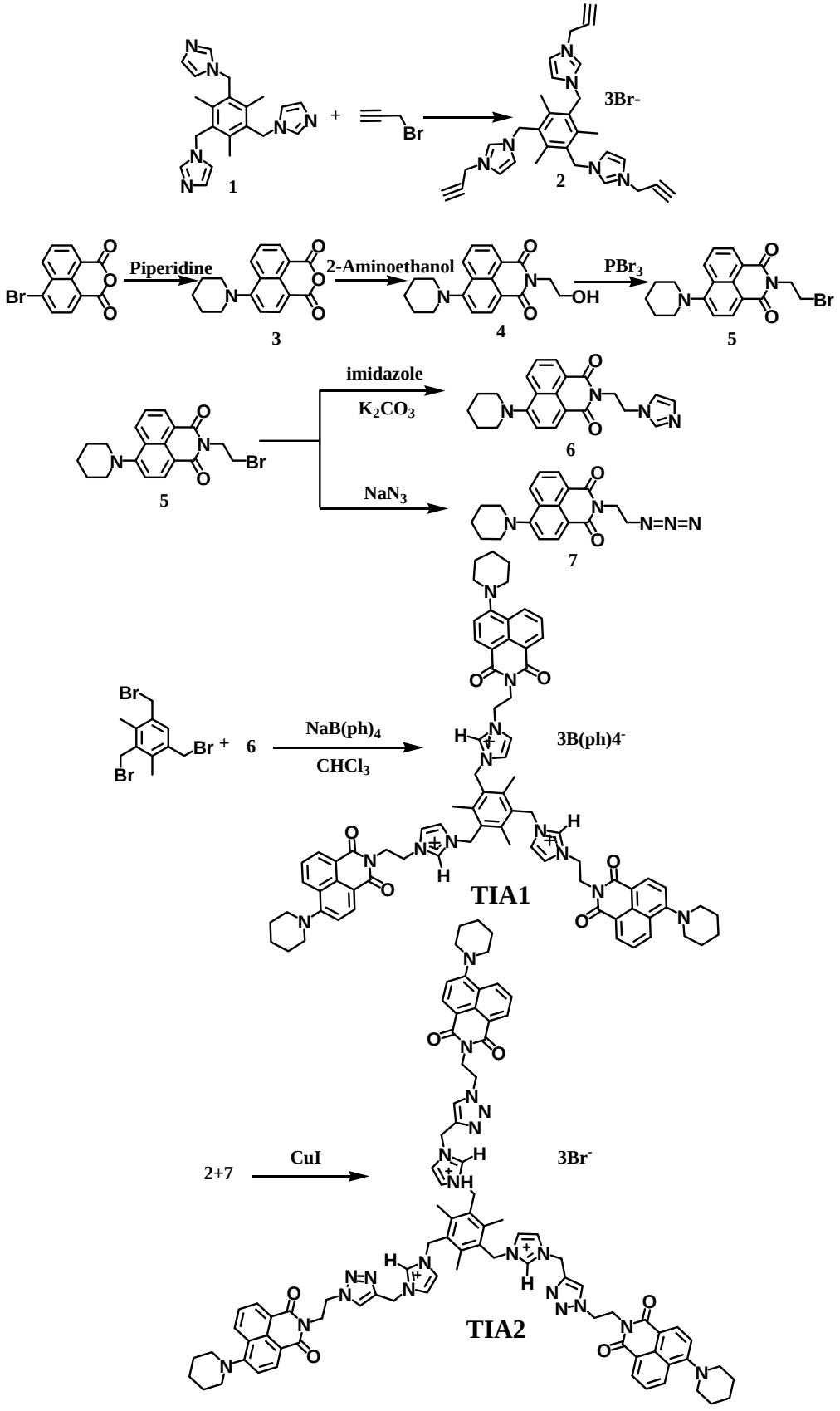
Contents

1. Experimental Section.



Scheme S1 Synthetic procedure of TIA1 and TIA2.

General

Materials unless otherwise stated, were obtained from commercial suppliers and used without further purification. Adenosine-5'-triphosphate disodium trihydrate (ATP), Adenosine-5'-diphosphate disodium (ADP), Adenosine-5'-monophosphate acid monodydrate (AMP), Cytidine-5'-triphosphate disodium dihydrate (CTP), Cytidine-5'-diphosphate disodium hydrate (CDP), Cytidine-5'-diphosphate acid (CMP), Guanosine-5'-triphosphate disodium hydrate (GTP), Guanosine-5'-diphosphate disodium (GDP), Guanosine-5'-monophosphate disodium (GMP), Uridine-5'-triphosphate trisodium (UTP), Uridine-5'-diphosphate disodium (UDP) and Uridine-5'-monophosphate disodium (UMP) were purchased from Bio Basic Inc.(BBI) company. 1,3,5-Tris(bromomethyl)-2,4,6-trimethylbenzene was synthesized according to the literature method.[1] ^{1}H NMR and ^{13}C NMR spectra were measured on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in d^6-DMSO, d^6-$CDCl_3$ or d^6-D_2O, TMS as internal standard). Mass spectrometric data were obtained on a API/MS mass spectrometry, GCT CA156 MS spectrometry and a LCQ-Tof MS spectrometry. The solution fluorescent spectra were measured on EDINBURGH FS920. Optical absorption spectra were measured on a TU-1900 Uv/Vis spectrophotometer at room temperature. Cell imaging were measured on Nikon eclipase TE2000-5 inverted fluorescence microscopy or Leica TCS-SP2 confocal microscope.

Synthesis of 1

1 was synthesized according the literature methord.[2]

Synthesis of 2

A mixture of 1,1',1''-(2,4,6-trimethylbenzene-1,3,5-triyl)tris(methylene)tris(1H-imidazole) (0.36 g, 1 mmol) and Propargyl bromide (0.476 g, 4 mmol) was heated to reflux in acetonitrile (20 mL) under nitrogen atmosphere for 12 h. The white precipitated formed was filtered, and dried in vacuo. Yield 0.66 g (76%). m.p. 255–256.6 ^{o}C; Anal calc. for $C_{30}H_{33}Br_3N_6$: C 50.23, H 4.64, Br 33.42, N 11.72%. Found: C 50.20, H 4.67, Br 33.32, N 11.81%; ^{1}H NMR (d^6-DMSO, ppm) δ: 9.29 (s, $1H_{triazole}$), 7.87 (s, 1H, $H_{triazole}$), 7.81 (s, $1H_{triazole}$), 5.60 (s, $2H_{CH2}$), 5.25 (d, $2H_{CH2}$, J = 2.8Hz), 3.82 (s, $1H_{allylene}$), 2.30 (s, $3H_{CH3}$); ^{13}C NMR (d^6-DMSO, ppm) δ: 141.63, 135.64, 129.23, 122.85, 122.27, 78.68, 78.65, 76.19, 47.91, 16.27; LCQ-Tof MS: 256.10 $[M+2H_2O]^{2+}$, 279.10

$[M+HBr]^{2+}$, 302.11 $[M+4CH_3OH]^{2+}$, 311.14 $[M+2DMF]^{2+}$.

Synthesis of 3

A mixture of 6-bromobenzoisochromene-1,3-dione (5.54 g, 20 mmol) and piperidine (2.23 g, 26.2 mmol) was heated to reflux in methoxylethanol (100 mL) under nitrogen atmosphere for 24 h. After removal of methoxylethanol, the residues was purified by silica gel column chromatography using dichloromethane as eluent to afford **3**. Compound **3** was used in the next reaction without any further characterization.

Synthesis of 4

A mixture of **3** (1.50 g, 5.33 mmol) and 2-Aminoethanol (0.4 mL, 6.66 mmol) was heated to reflux in EtOH (20 mL) under nitrogen atmosphere for 12 h. After cool to room temperature, yellow solid appeared, filter cake was washed by EtOH to afford compound **4**. The yield was 1.28 g (80%), yellow powder. Compound **4** was used in the next reaction without any further characterization.

Synthesis of 5

Phosphorus tribromide (2 mL) was added dropwise to **4** (1.46 g, 4.51 mmol) in dry chloroform at 0–5 ^{o}C and the mixture was allowed to reflux for 3 h. The reaction solution was washed by water and then extracted several times with dichloromethane. The extract was dried over sodium sulfate and was concentrated to give a yellow brown mixture, which was purified by silica gel column chromatography using petroleum ether/chloroform (1:1) as eluent to afford compound **5**. The yield was 0.8 g (56 %), yellow powder. m.p. 184.3–185.5 ^{o}C; Anal calc. for $C_{19}H_{19}BrN_2O_2$: C 58.93, H 4.95, Br 20.63, N 7.23, O 8.26%. Found: C 58.92, H 4.97, Br 20.62, N 7.20, O 8.29%; ^{1}H NMR (d^6-$CDCl_3$, ppm) δ: 8.6 (d, $1H_{Ar}$, J = 6Hz), 8.52 (d, 1 H_{Ar}, J = 8.4Hz), 8.49 (d, $1H_{Ar}$, J = 7.6Hz), 7.72 (t, $1H_{Ar}$, J = 7.6Hz), 7.24 (d, $1H_{Ar}$, J = 8.4Hz), 4.60 (t, $2H_{CH2}$, J = 7.2Hz), 3.67 (t, $2H_{CH2}$, J = 7Hz), 3.29 (m, $4H_{piperdine}$), 1.95 (m, $4H_{piperdine}$), 1.75 (m, $2H_{piperdine}$); ^{13}C NMR (d^6-$CDCl_3$, ppm) δ: 164.38, 163.82, 133.01, 131.39, 131.00, 130.08, 126.25, 125.45, 122.78, 115.00, 114.83, 54.61, 41.07, 27.98, 26.16, 24.29; GCT CA156 MS 386.0637.

Synthesis of 6

Imidazole (2 g, 29.4 mmol) was added to anhydrous potassium carbonate (K_2CO_3) (1.02 g, 7.34 mmol) and anhydrous tetrahydrofuran (THF) (50 mL). The mixture was stirred at room temperature for 10 min prior to the addition of **5** (5.69 g, 14.7 mmol). The mixture was then stirred under reflux for 24 h. After filtration, the THF was removed under vacuum to leave a yellow solid which was dissolved in dichloromethane (DCM) (40 mL) and washed with water (3×50 mL). The organic layer was then extracted using hydrochloric acid (HCl) (2 M, 3×30 mL) followed by water (2×50 mL). The combined acid layer was neutralised with solid saturated sodium bicarbonate ($NaHCO_3$) and then extracted into DCM (2×40 mL). The combined DCM layer was washed with water (3×50 mL), dried over anhydrous magnesium sulfate ($MgSO_4$) and filtered. Removal of DCM under vacuum gave a yellow solid, which was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH (20:1) as eluent to afford compound **6**. The yield was 0.66 g (12 %), yellow powder. m.p. 147–149 ^oC; Anal calc. for $C_{22}H_{22}N_4O_2$: C 70.57, H 5.92, N 14.96, O 8.55%. Found: C 70.62, H 5.97, N 14.91, O 8.50%; ^1H NMR (d^6-$CDCl_3$, ppm) δ: 8.55 (d, $1H_{Ar}$, J = 8Hz), 8.46 (d, $1H_{Ar}$, J = 8Hz), 8.40 (d, $1H_{Ar}$, J = 8Hz), 7.68 (t, $1H_{Ar}$, J = 8Hz), 7.54 (s, $1H_{imidazole}$), 7.17 (d, $1H_{Ar}$, J = 8Hz), 7.06 (s, $2H_{imidazole}$), 4.55 (t, $2H_{CH2}$, J = 6Hz), 4.34 (t, $2H_{CH2}$, J = 6Hz), 3.25 (m, $4H_{piperdine}$), 1.89 (m, $4H_{piperdine}$), 1.73 (m, $2H_{piperdine}$); ^{13}C NMR (d^6-$CDCl_3$, ppm) δ: 164.45, 163.87, 157.83, 137.37, 133.14, 131.41, 131.23, 130.10, 129.39, 126.27, 125.40, 122.44, 119.38, 115.00, 114.87, 54.54, 44.41, 40.10, 26.20, 24.32; API-MS m/z: 375.5 $[M+H]^+$;

Synthesis of 7

N-bromobutyl-4-piperidine-1.8-naphthalimide (1.1 g, 2.6 mmol), NaN_3 (0.8 g, 12 mmol) were added into 20 mL ethanol. Followed by warmed at 110 ^oC for 6 hr, the solvent was removed by rotate evaporator to afford product. m.p. 138–139.6 ^oC; Anal calc. for $C_{19}H_{19}N_5O_2$: C 65.32, H 5.48, N 20.04, O 9.16%. Found: C 65.20, H 5.60, N 20.10, O 9.22%; ^1H NMR ($CDCl_3$, ppm) δ: 8.58 (d, $1H_{Ar}$, J = 8Hz), 8.50 (d, $1H_{Ar}$, J = 8Hz), 8.40 (d, $1H_{Ar}$, J = 8Hz), 7.68 (t, $1H_{Ar}$, J = 16Hz), 7.18 (d, $1H_{Ar}$, J = 8Hz), 4.43 (t, $2H_{CH2}$, J = 12Hz), 3.65 (t, $2H_{CH2}$, J = 16Hz), 3.24 (m, $4H_{piperdine}$), 1.89 (m, $4H_{piperdine}$), 1.73 (m, $2H_{piperdine}$); ^{13}C NMR ($CDCl_3$, ppm) δ: 164.62, 164.05, 157.62, 133.03, 131.33, 130.98, 130.08, 126.26, 125.38, 122.73, 115.39, 114.75, 54.54, 48.95, 38.64, 26.21, 24.34; API-MS m/z: 350.3 $[M+H]^+$, 372.3 $[M+Na]^+$, 721.5 $[2M+Na]^+$;

Synthesis of TIA1

1,3,5-Tris(bromomethyl)-2,4,6-trimethylbenzene (0.16 g, 0.41 mmol) and **6** (0.46 g, 1.23 mmol) were dissolved in $CHCl_3$ (20 mL) and stirred at reflux for 15 h. During this time, a white precipitate formed. The product was filtered off and washed with $CHCl_3$ to give the desired tribromo anions product as a yellow powder. A solution of the mixture of $3Br^-$ product (0.6 g, 0.39 mmol) and $NaB(C_6H_5)_4$ (0.88 g, 2.6 mmol) was stirred at room temperature in CH_3OH (30 mL) for 1 hr. The yellow precipitated formed was filtered, washed with methanol and diethyl ether, and dried in vacuo. Yield 0.66 g (76%). m.p. 190–192 ^oC; Anal calc. for $C_{150}H_{141}B_3N_{12}O_6$: C 80.42, H 6.34, B 1.45, N 7.5, O 4.29%. Found: C 80.40, H 6.36, B 1.42, N 7.52, O 4.30%; ^1H NMR (d^6-DMSO, ppm) δ: 9.02 (s, $1H_{imidazole}$), 8.27 (d, $1H_{Ar}$, J = 4Hz), 8.25 (d, $1H_{Ar}$, J = 4Hz), 8.17 (d, $1H_{Ar}$, J = 8Hz), 7.77 (s, $1H_{imidazole}$), 7.70 (t, $1H_{Ar}$, J = 8Hz), 7.57 (s, $1H_{imidazole}$), 7.17 (m, $1H_{Ar}$), 5.49 (s, $2H_{CH2}$), 4.46 (s, $2H_{CH2}$), 4.38 (s, $2H_{CH2}$), 3.13 (M, $4H_{piperdine}$), 2.24 (s, $3H_{CH3}$), 3.13(m, $4H_{piperdine}$), 1.79 (m, $4H_{piperdine}$), 1.64 (m, $2H_{piperdine}$); ^{13}C NMR (d^6-DMSO, ppm) δ: 164.03, 163.53, 163.04, 162.55, 156.78, 141.10, 136.00, 135.44, 132.22, 130.68, 130.48, 129.16, 125.58, 125.21, 125.19, 125.15, 123.50, 121.97, 121.92, 121.39, 114.64, 114.23, 53.82, 47.69, 25.59, 23.70, 15.86; LCQ-Tof MS: 427.29 $[M]^{3+}$.

Synthesis of TIA2

A mixture of **2** (0.33 g 0.416 mmol), **7** (0.53 g 1.5 mmol), CuI (20 mg 0.1 mmol) was heated to reflux in THF (30 mL) for 48 hr. The solvent was removed by rotate evaporator to afford crude product. The crude product was recrystallizated in CH_3OH and ether. m.p. 203–205 ^oC; Anal calc. for $C_{87}H_{90}Br_3N_{21}O_6$: C 59.19, H 5.14, Br 13.58, N 16.66, O 5.44%. Found: C 59.17, H 5.16, Br 13.55, N 16.68, O 5.45%; ^1H NMR (d^6-DMSO, ppm) δ: 9.30 (s, $1H_{imidazole}$), 8.39 (s, $1H_{triazole}$), 8.34 (d, $1H_{Ar}$, J = 8Hz), 8.31 (d, $1H_{Ar}$, J = 8Hz), 8.24 (d, $1H_{Ar}$, J = 8Hz), 7.79 (s, $1H_{imidazole}$), 7.74 (t, $1H_{Ar}$, J = 8Hz), 7.62 (s, $1H_{imidazole}$), 7.23 (d, $1H_{Ar}$, J = 8Hz), 5.59 (s, $2H_{CH2}$), 5.54 (s, $2H_{CH2}$), 4.71 (m, $2H_{CH2}$), 4.40 (m, $2H_{CH2}$), 3.15 (m, $4H_{piperdine}$), 2.29 (m, $3H_{CH3}$), 1.79 (m, $4H_{piperdine}$), 1.65 (m, $2H_{piperdine}$); ^{13}C NMR (d^6-DMSO, ppm) δ: 163.50, 162.88, 156.86, 141.26, 140.14, 135.67, 132.37, 130.79, 130.66, 129.36, 129.22, 125.78, 125.52, 125.34, 122.77, 122.31, 122.06, 114.86, 114.43, 53.93, 47.95, 47.57, 43.55, 25.69, 23.82, 16.32; LCQ-Tof MS: 508.42 $[M]^{3+}$, 802.57 $[M+HBr]^{2+}$.

Preparation of fluorometric nucleotides titration solutions.

Stock solutions (10 mM) of the sodium salts of nucleotides of ADP, ATP, AMP, CDP, CTP, CMP, GDP, GTP, GMP, UDP, UTP, UMP in 1:1 DMF/H_2O (v:v) solvents were prepared. Stock solution of **TIA1** and **TIA2** (1 mM) were also prepared in distilled CH_3CN solution. Test solutions were prepared by placing 40 uL of host stock solution into a quartz cell of 1 cm optical path length including 2 mL distilled CH_3CN or aqueous solution, and then adding an appropriate aliquot of each nucleotides stock by using a micro-syringe. In competition experiments, 4 equiv. amounts of sodium salts of nucleotides stock was added to the solution of **TIA1** with 4 equiv. amounts of ADP in CH_3CN.

NMR Titration Method

All NMR spectra were measured on a VARIAN INOVA-400 spectrometer at 298 K. (a) A solution (1 mM) of host **TIA1** in d^6-DMSO was titrated with 3 eq ADP in d^6-DMSO. (b) A solution (1 mM) of host **TIA2** in d^6-DMSO:D_2O = 8:2 (v:v) was titrated with appropriate aliquot of ATP, GTP and UTP stock in D_2O by using a micro-syringe. The chemical shift changes of the proton of imidazolium and 1, 8-naphthalimides units were monitored.

Association Constants Calculation:

The binding constant was calculated from the fluorescent titration curve according to the equation.

$$\text{Log } ((F-F_{min})/(F_{max}-F))=\log k + n \log [c]$$

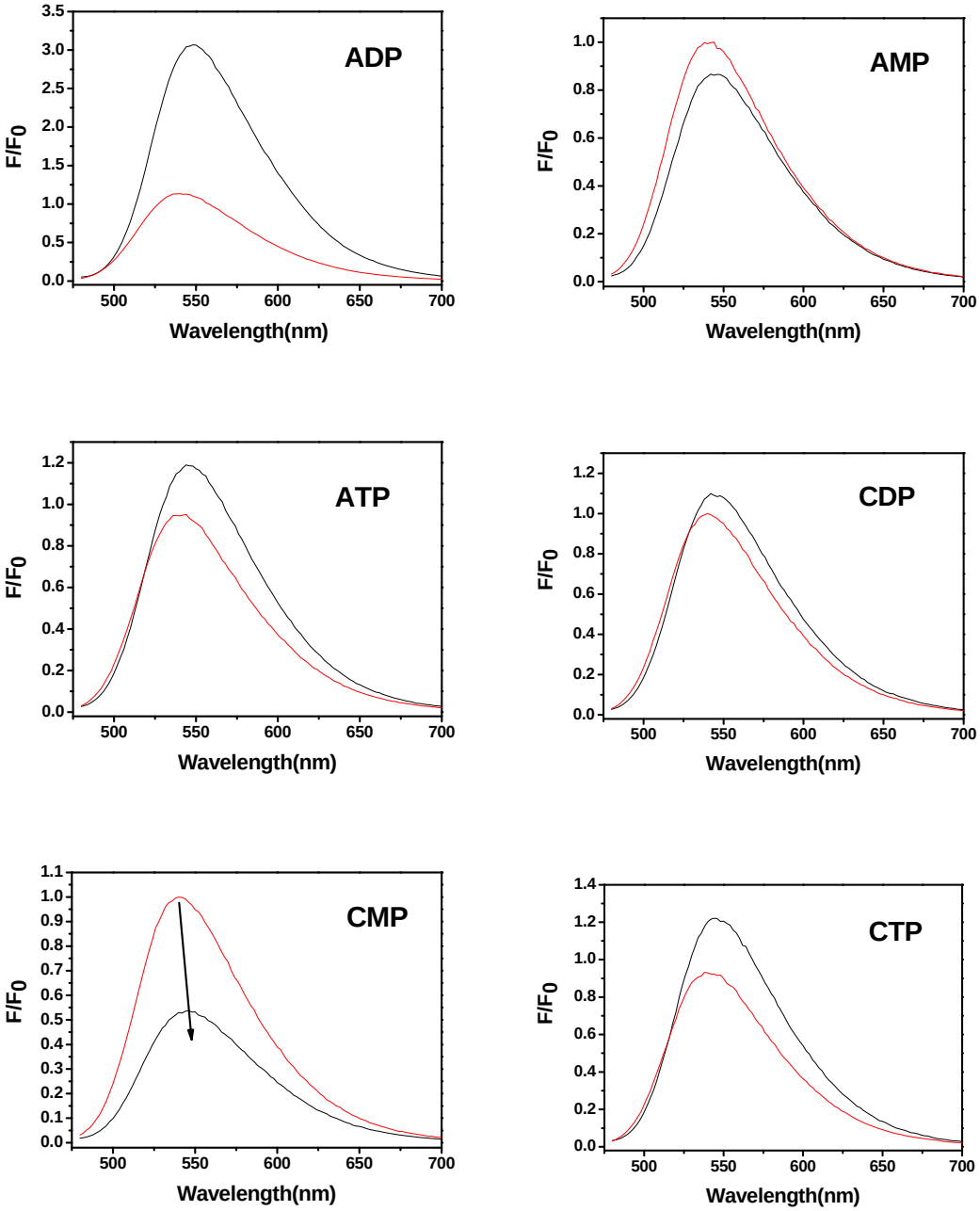
where A is fluorescent of **TIA1** (**TIA2**) at 548 nm (557 nm, 560 nm and 558 nm) upon addition of different amount of nucleoside polyphosphates. [c] stands for the concentration of nucleoside polyphosphates.

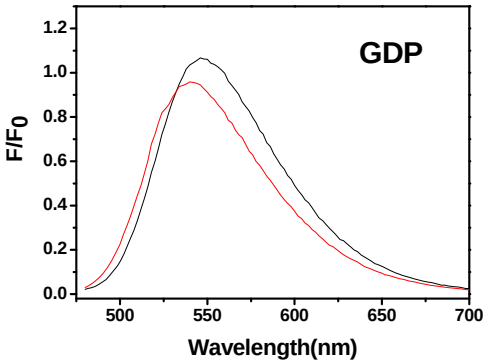
Cell incubation and imaging

HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded on 18 mm glass coverslips for confocal fluorescence imaging and in 24-well flat-bottomed plates for Nikon eclipase TE2000-5 inverted fluorescence microscopy. After 12 h, HeLa cells were

incubated with 10 μM compound **TIA1** or **TIA2** (in the culture medium containing 0.5% DMSO) for 30 min at 37^{o}C under 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times before incubating with 40 eq ATP and ADP for another 30 min, and cells were rinsed with PBS three times again. The fluorescence imaging of intracellular ADP in HeLa cells was observed under Nikon eclipase TE2000-5 inverted fluorescence microscopy with a 20×objective lens (excited with blue light). For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular ADP fluorescence. Confocal fluorescence imaging of intracellular ATP in HeLa cells was observed under a Leica TCS-SP2 confocal microscope. Excitation wavelength of laser was 458 nm. Emissions were centered at 510±15nm and 590±15nm (double channel). MetaFluor (Universal Imaging Corp.) was used as imaging analysis software.

2. Figure S1 Family of Fluorescence spectra of TIA1 (red line) in CH_3CN solution (20 μM) upon the addition of 20 eq various ribonucleotide polyphosphates(black line).

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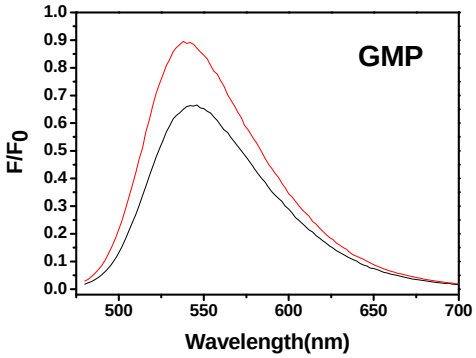
Wavelength(nm)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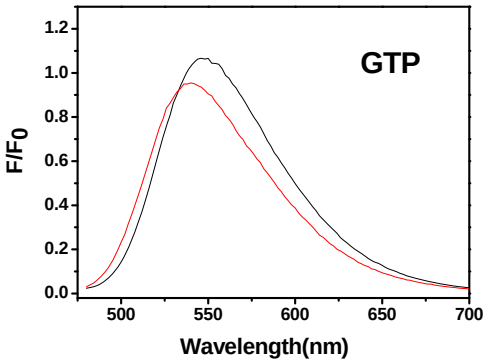
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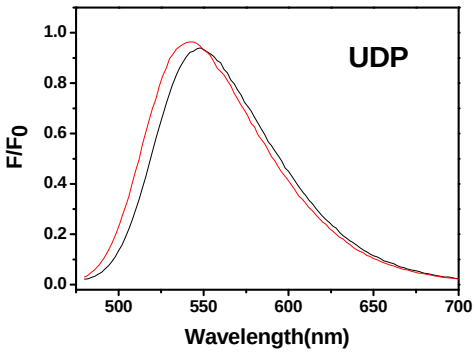
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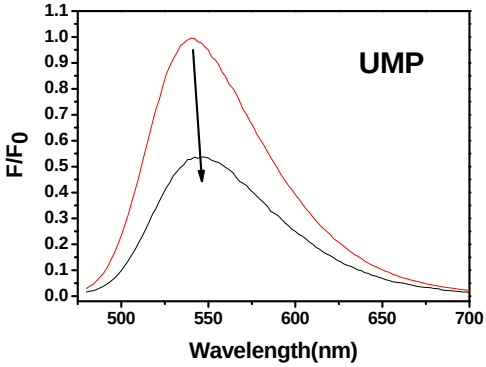
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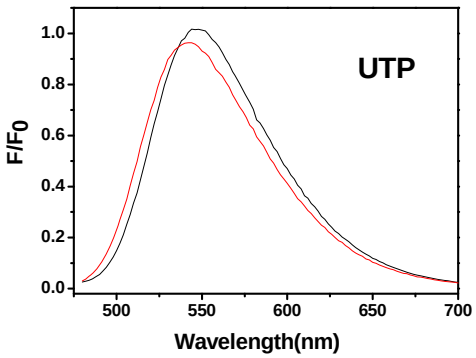
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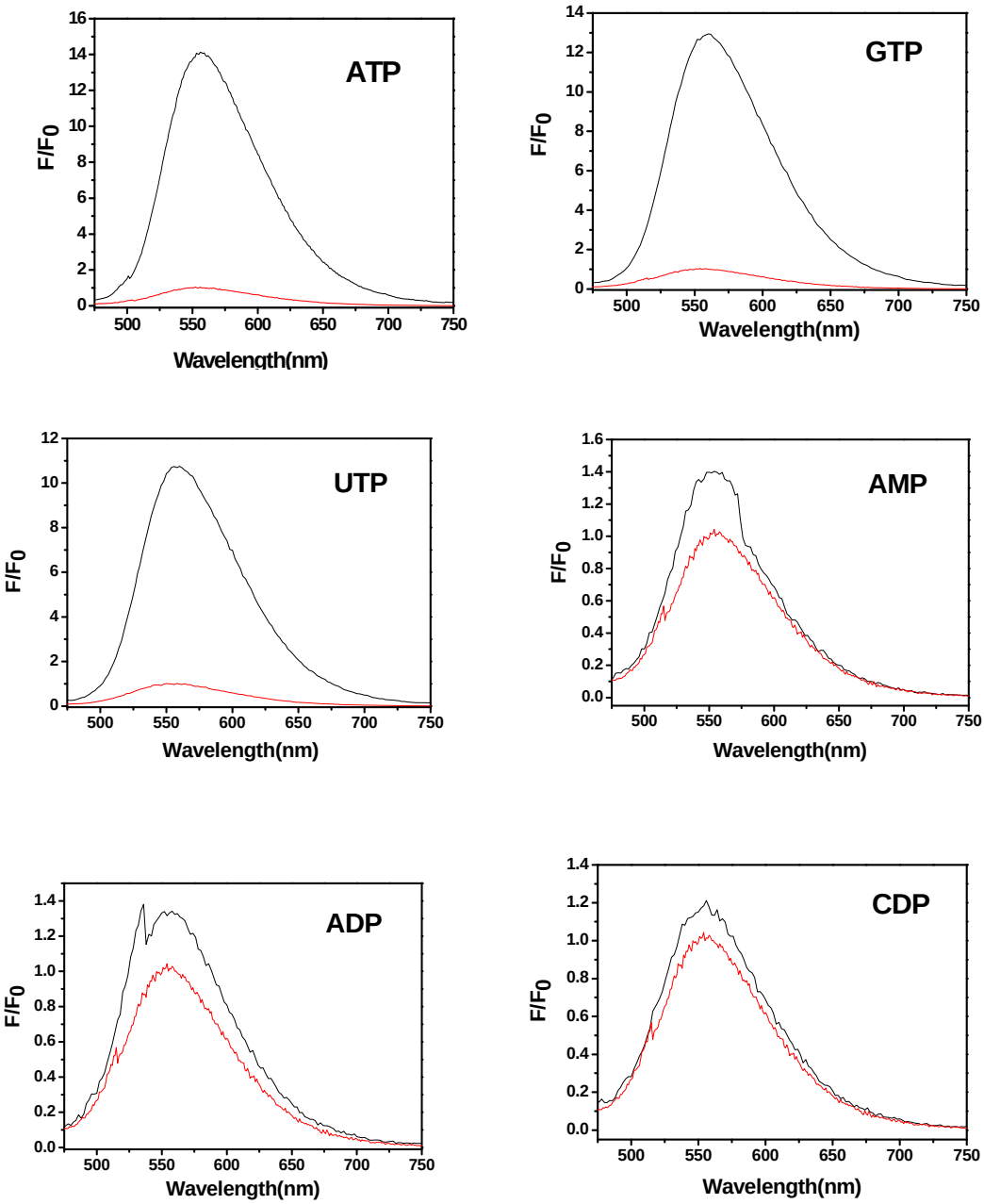
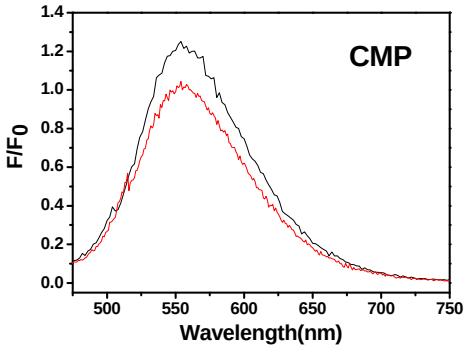
F/F0
Wavelength(nm)

3. Figure S2 Family of Fluorescence spectra of TIA2 (red line) in aqueous solution (20 μM) upon the addition of 20 eq various ribonucleotide polyphosphates(black line).

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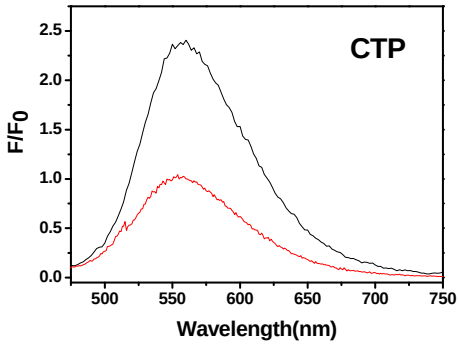
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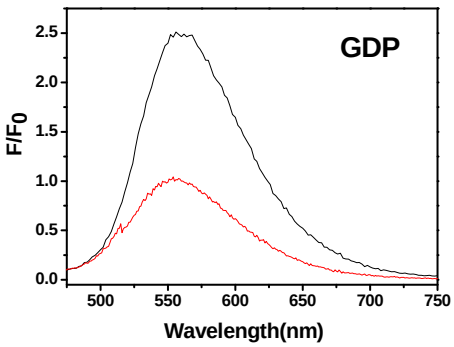
Wavelength(nm)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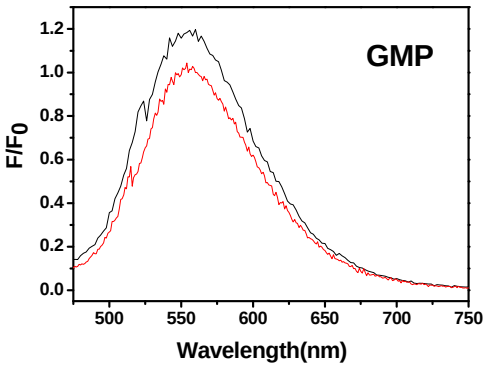
Wavelength(nm)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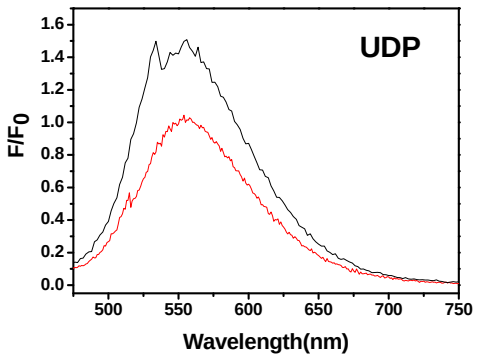
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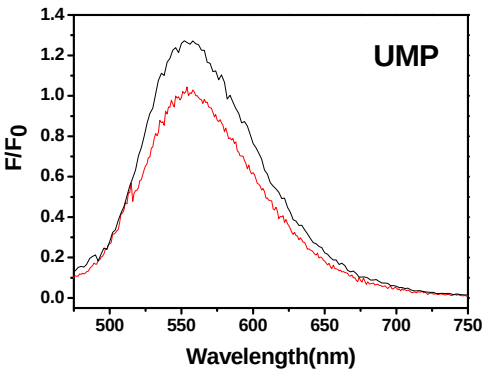
F/F0
Wavelength(nm)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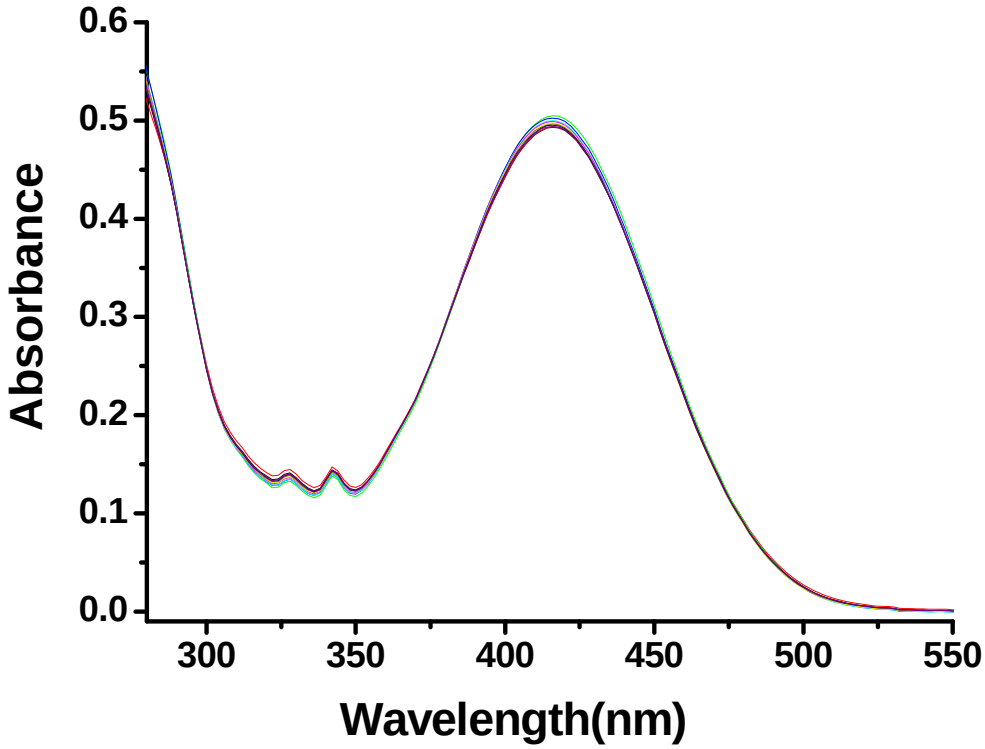
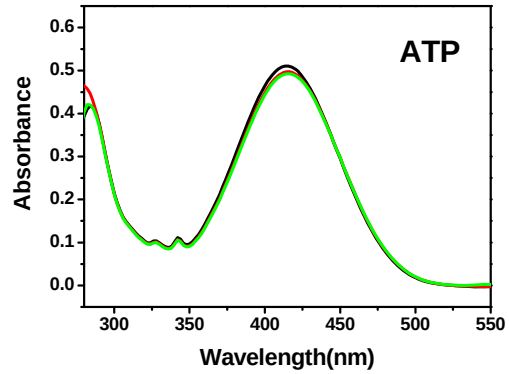
Wavelength(nm)

4. Figure S3 Absorption spectra of TIA1 (20 mM) upon addition of increasing amounts of ADP in DMF/H_2O (95:5) solution.



5. Figure S4 Absorption spectra of TIA2 (20 mM) upon addition of increasing amounts of ATP (top picture), GTP (middle picture) and UTP (bottom picture) in DMF/H_2O (95:5) solution.



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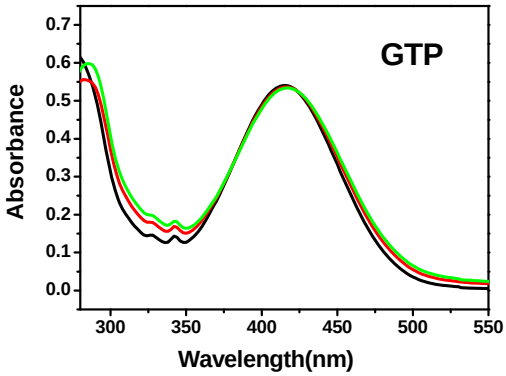
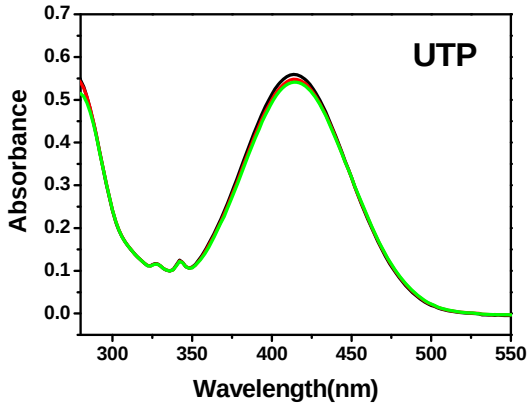
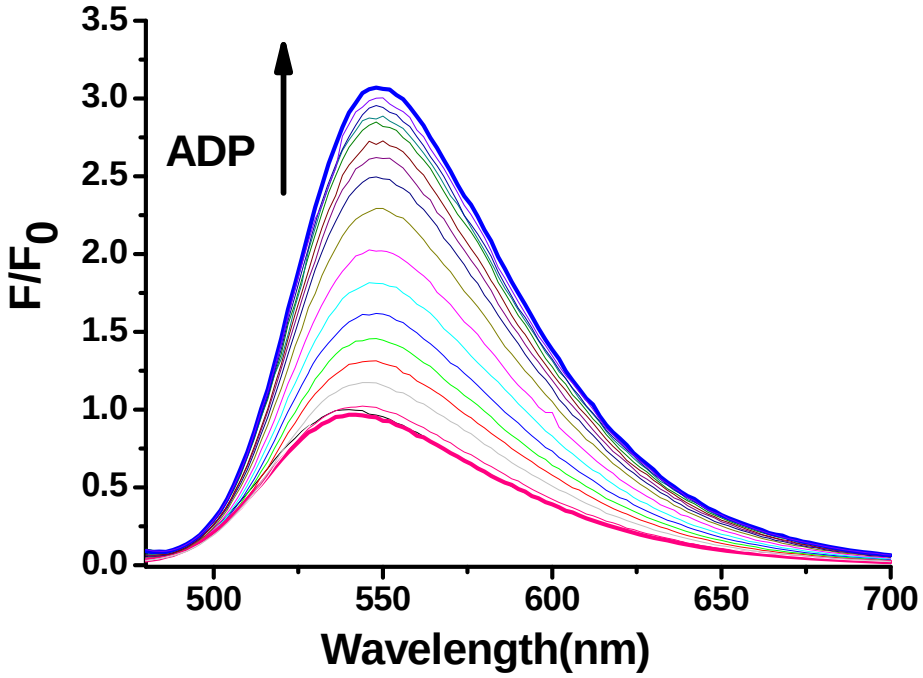
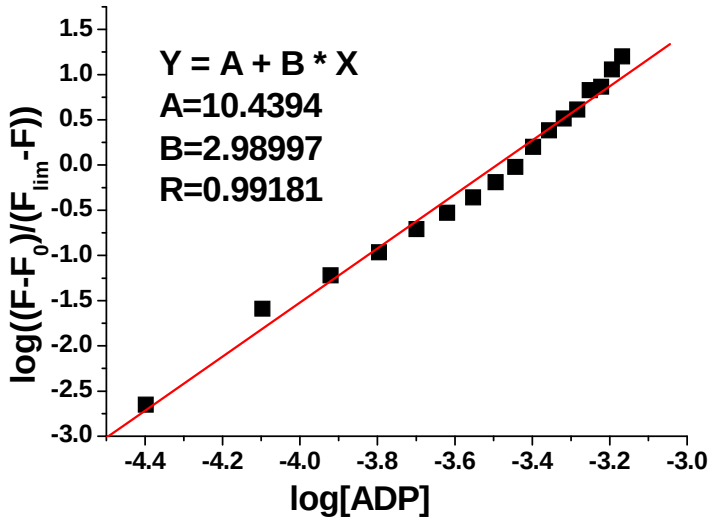


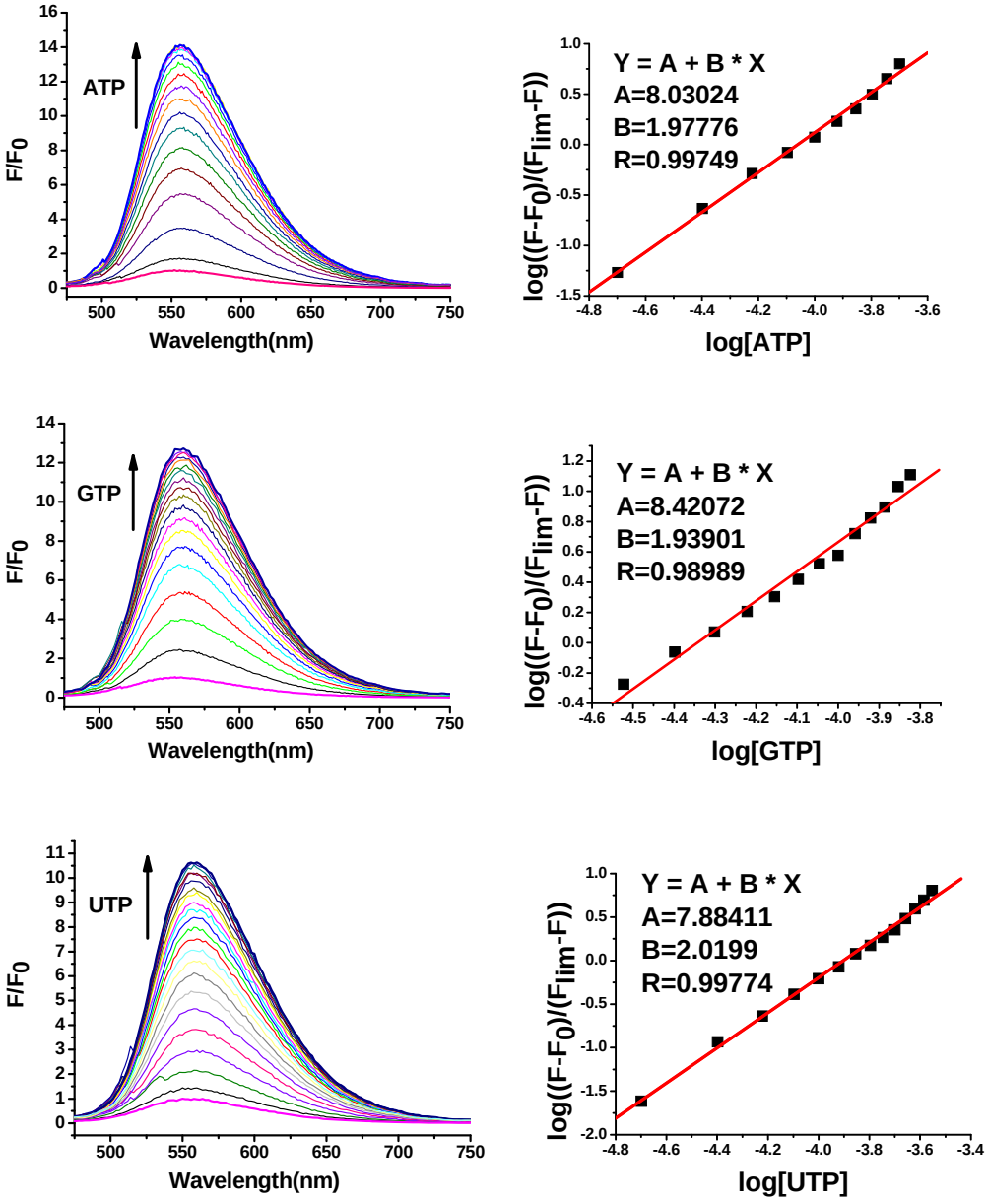


6. Figure S5 Fluorescence spectra of TIA1 (20 μM) in CH_3CN solution upon addition of increasing concentrations of ADP. Scan slit:2 nm. liner of log ((F-F_0)/(F_{lim}-F) vs. log [ADP]. (A present fluorescence of TIA1 at 548 nm).

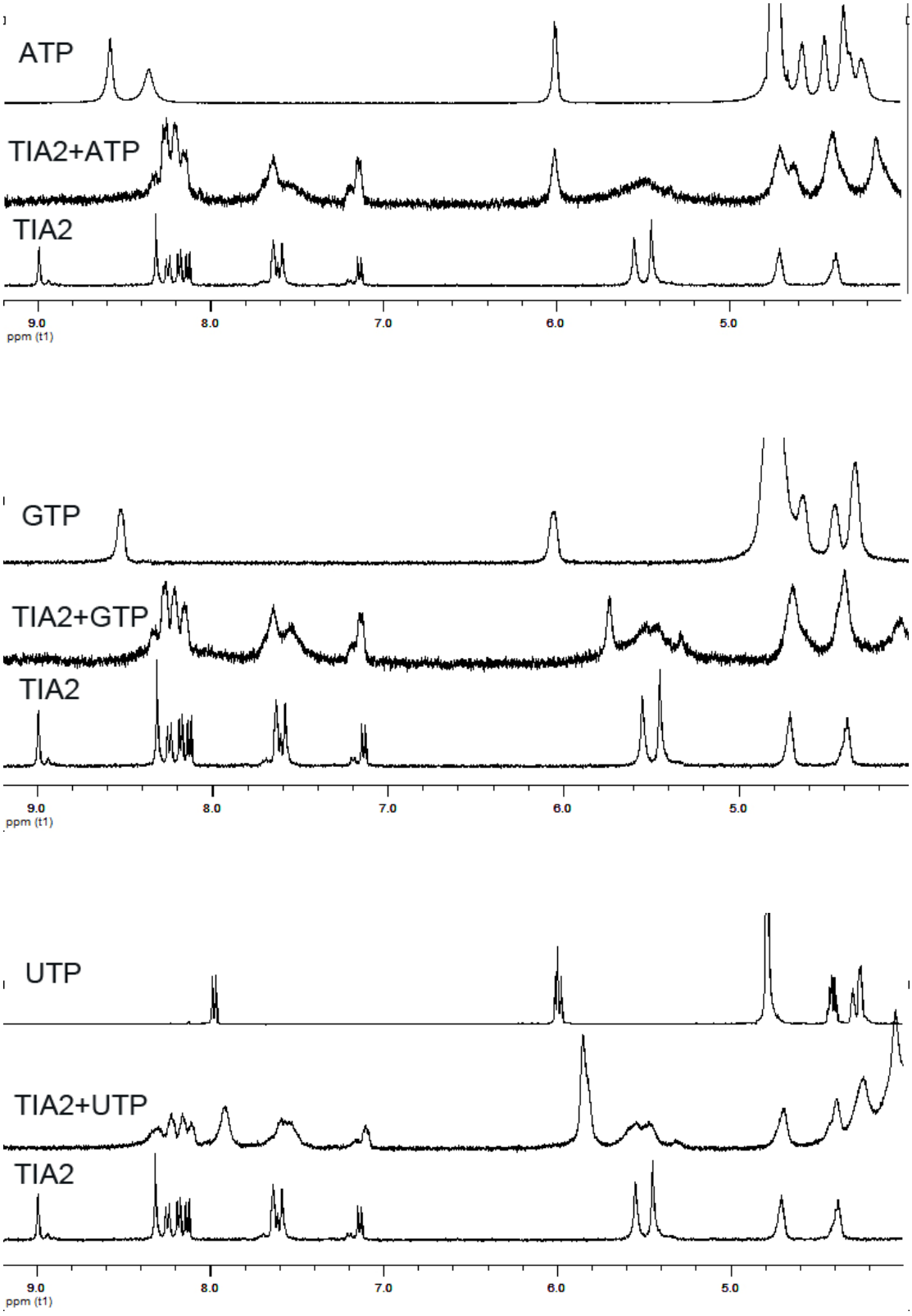




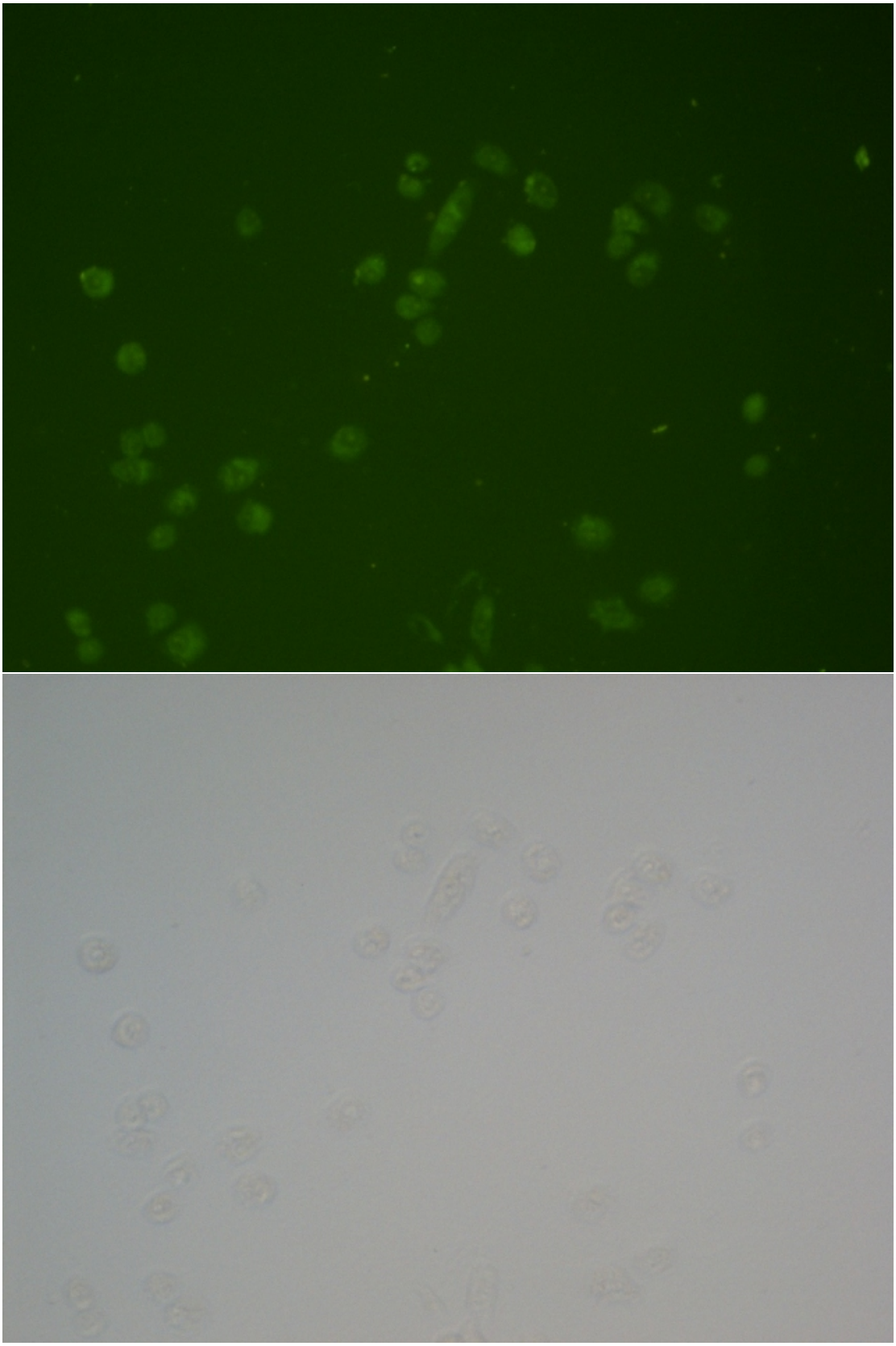
7. Figure S6 Fluorescence spectra of TIA2 (20 μM) in aqueous solution upon addition of increasing concentrations of ATP, GTP and UTP with an excitation wavelength at 396 nm. Scan slit:2 nm. liner of log ((F-F_0)/(F_{lim} -F) vs. log [XTP]. (A present fluorescence of TIA2 at 557 nm, 560 nm and 558 nm, respectily).



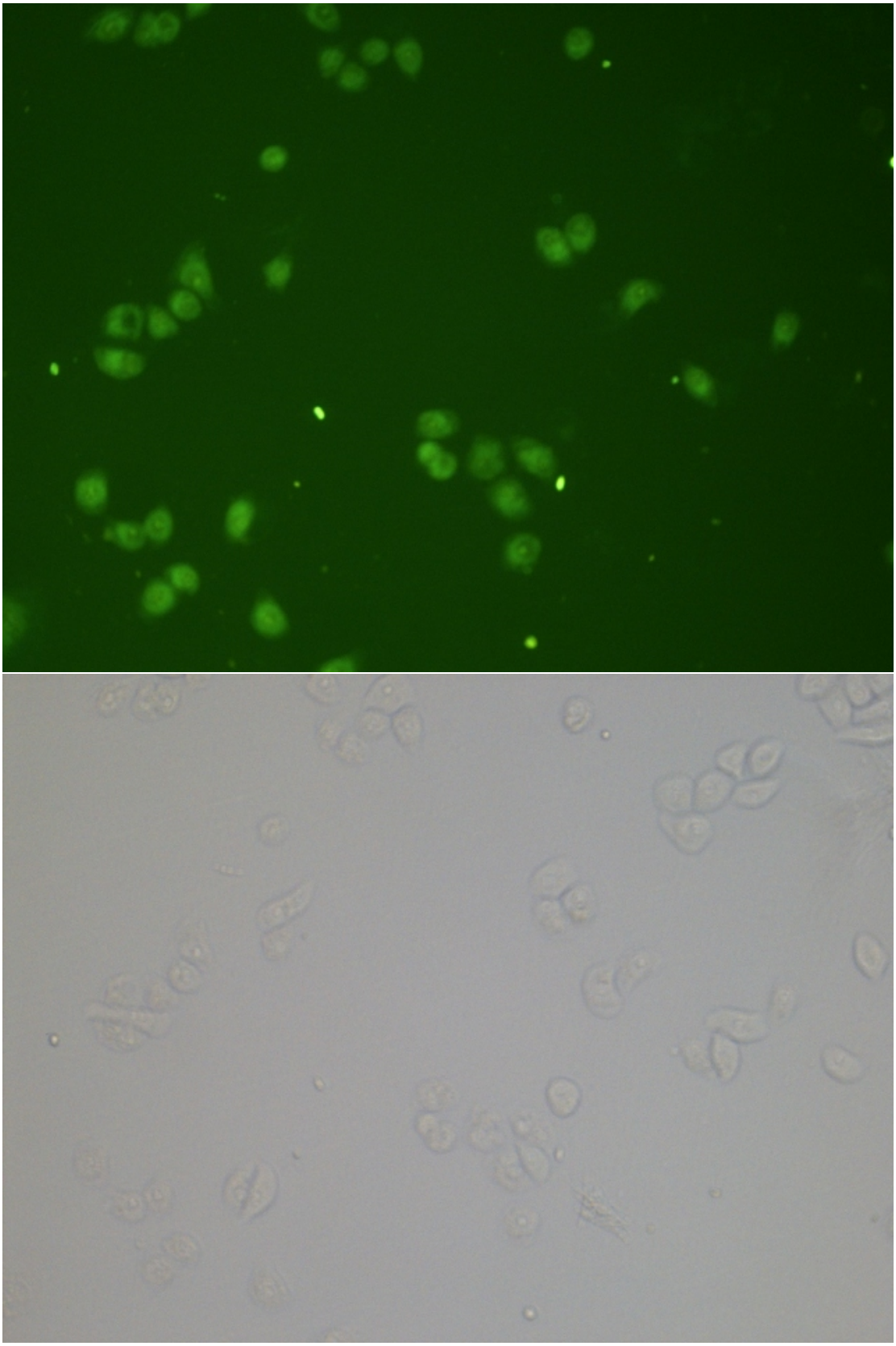
8. Figure S7 Partial ^{1}H-NMR spectra for **TIA2**+ATP (top picture), **TIA2**+GTP (middle picture) and **TIA2**+UTP (bottom picture).



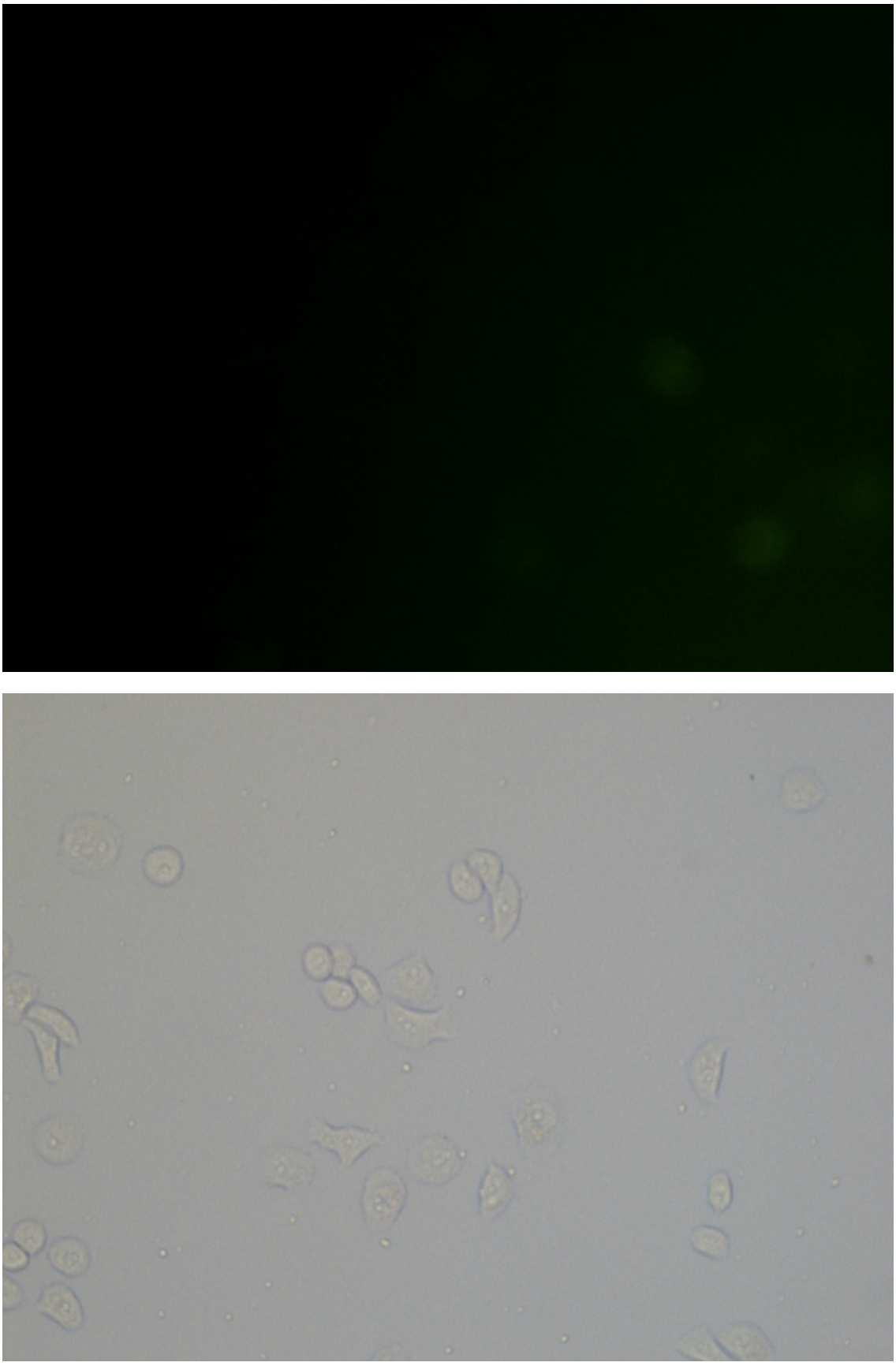
9. Figure S8 HeLa cell imaging of **TIA1**.



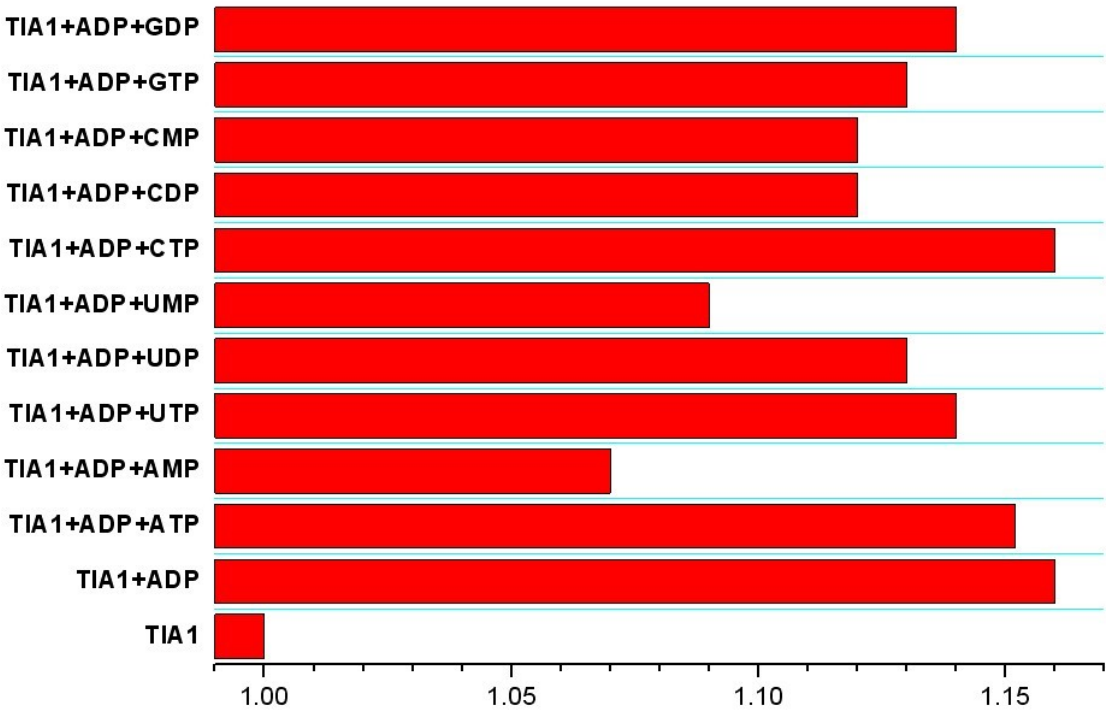
10. Figure S9 HeLa cell imaging of TIA1+ADP.



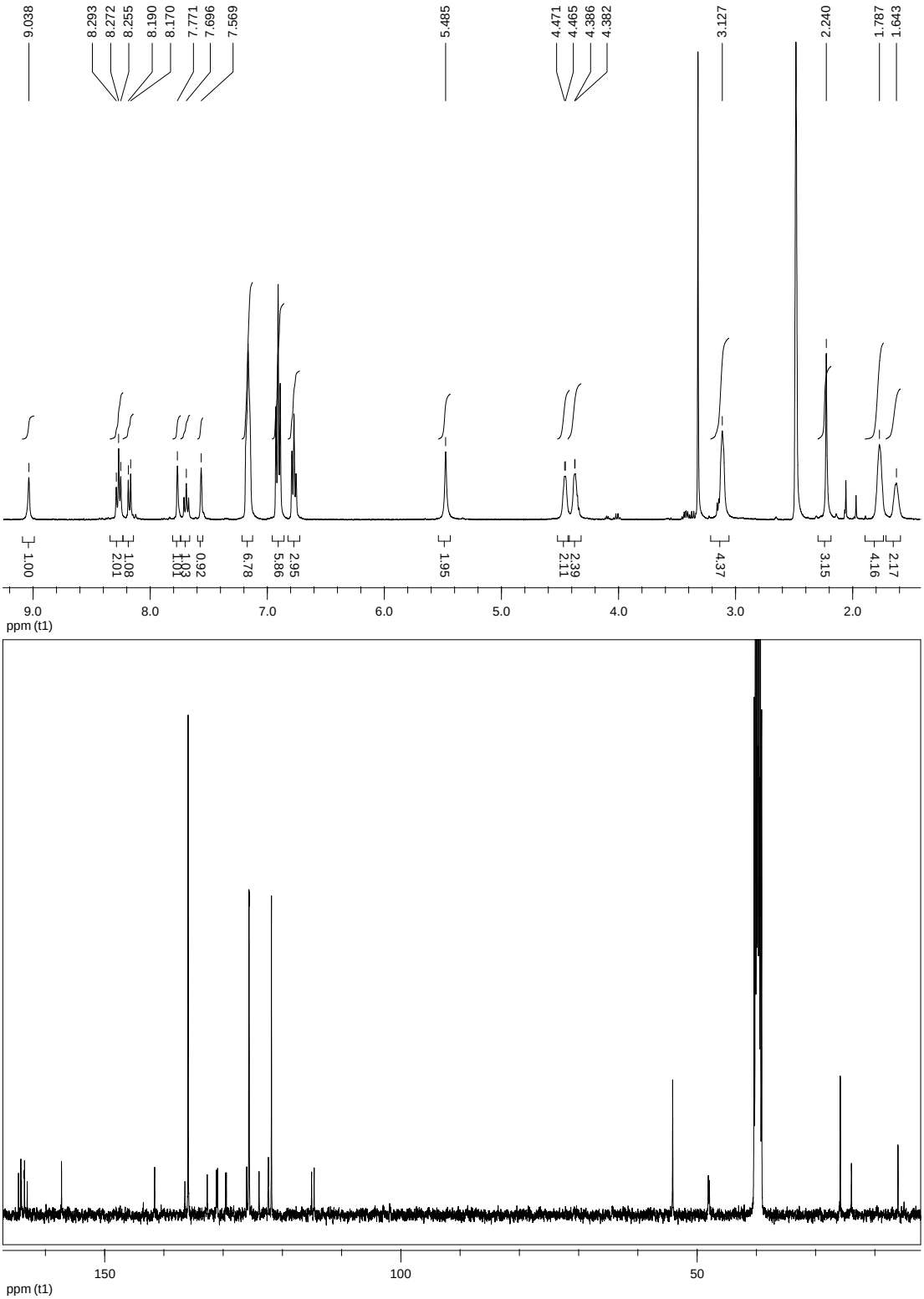
11. Figure S10 HeLa cell imaging of **TIA1+**ATP.



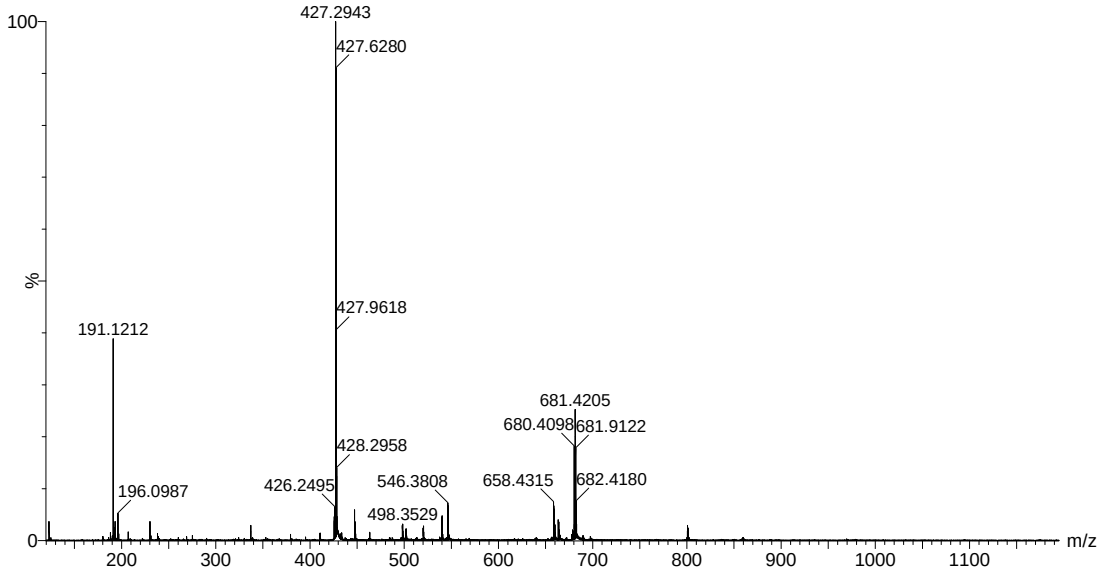
12. Figure S11. Competitive experiments of ADP (80μM) with TIA1（20 μM）in CH_3CN solution in the presence of various nucleosides (80μM).



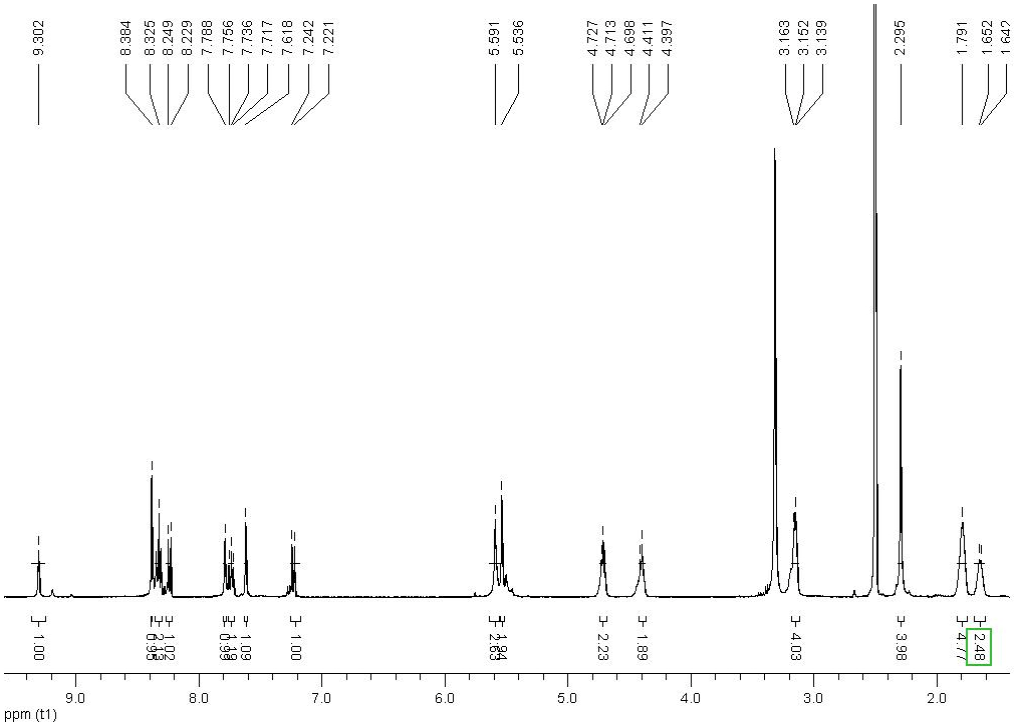
13. Figure S12. **^{1}H-NMR (top picture) and ^{13}C-NMR (bottom picture) spectra of the TIA1 in d^{6}-DMSO**

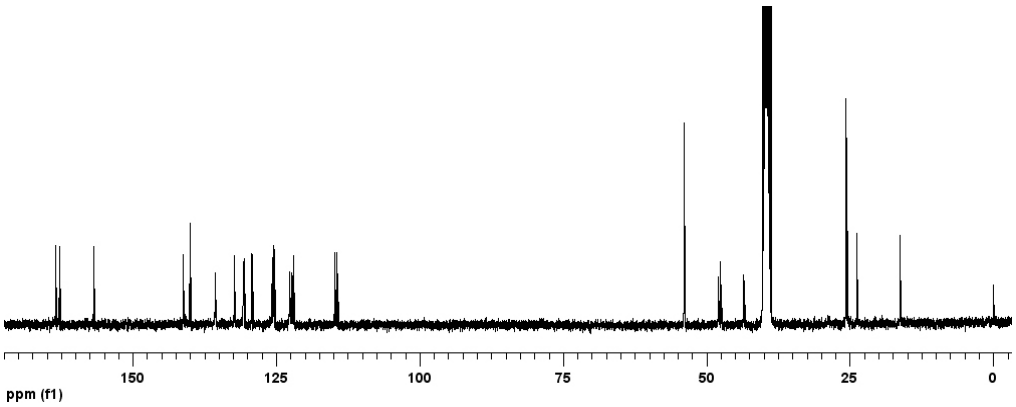


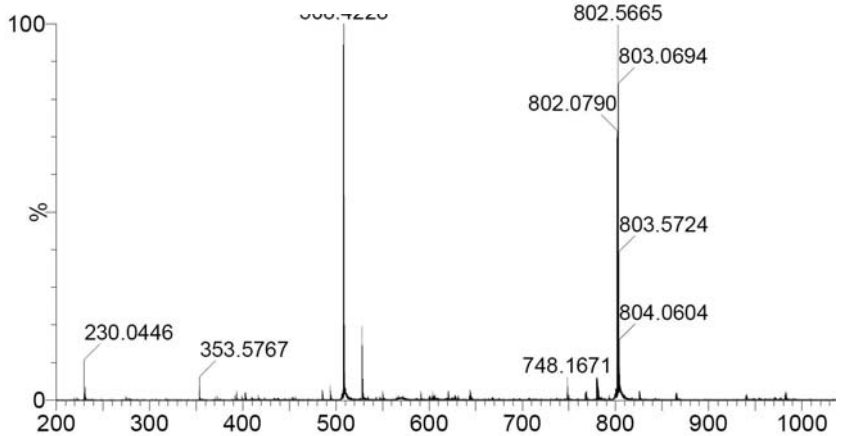
14 Figure S13. Mass spectrum of compound of TIA1.



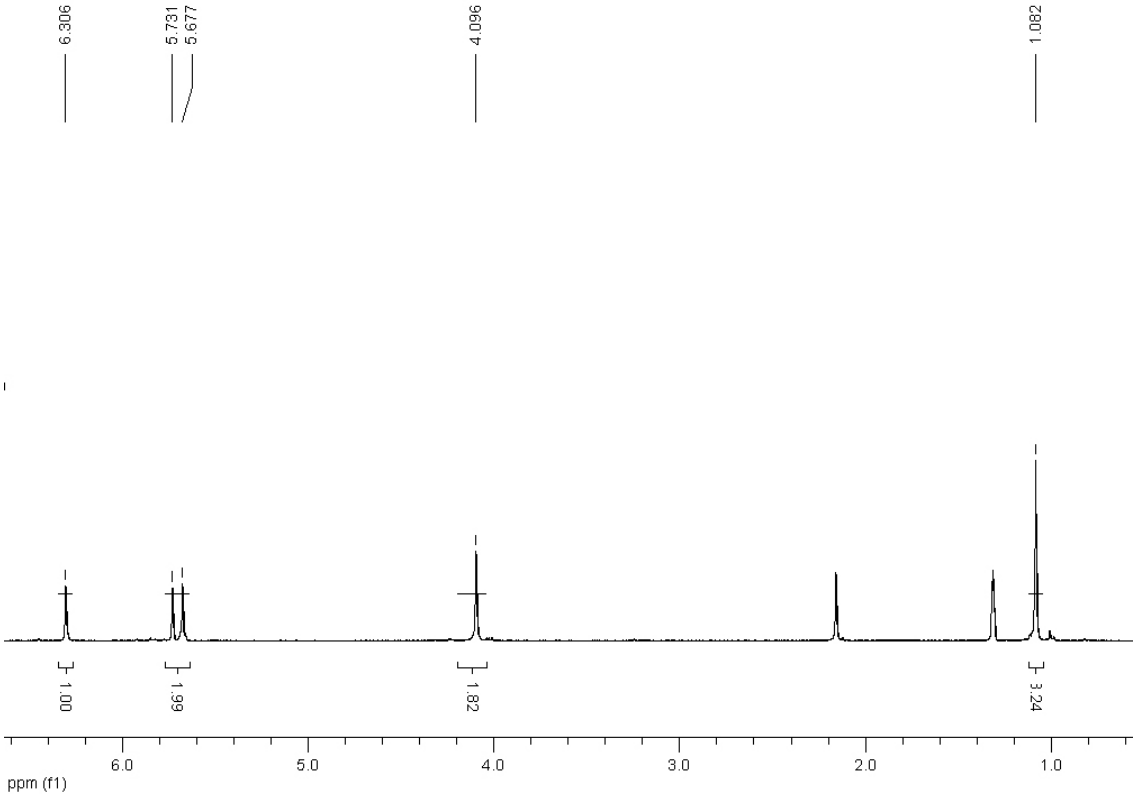
15. Figure S14. **^{1}H-NMR (top picture), ^{13}C-NMR (middle picture) and Mass (bottom picture) spectra of the TIA2 in d^{6}-DMSO**



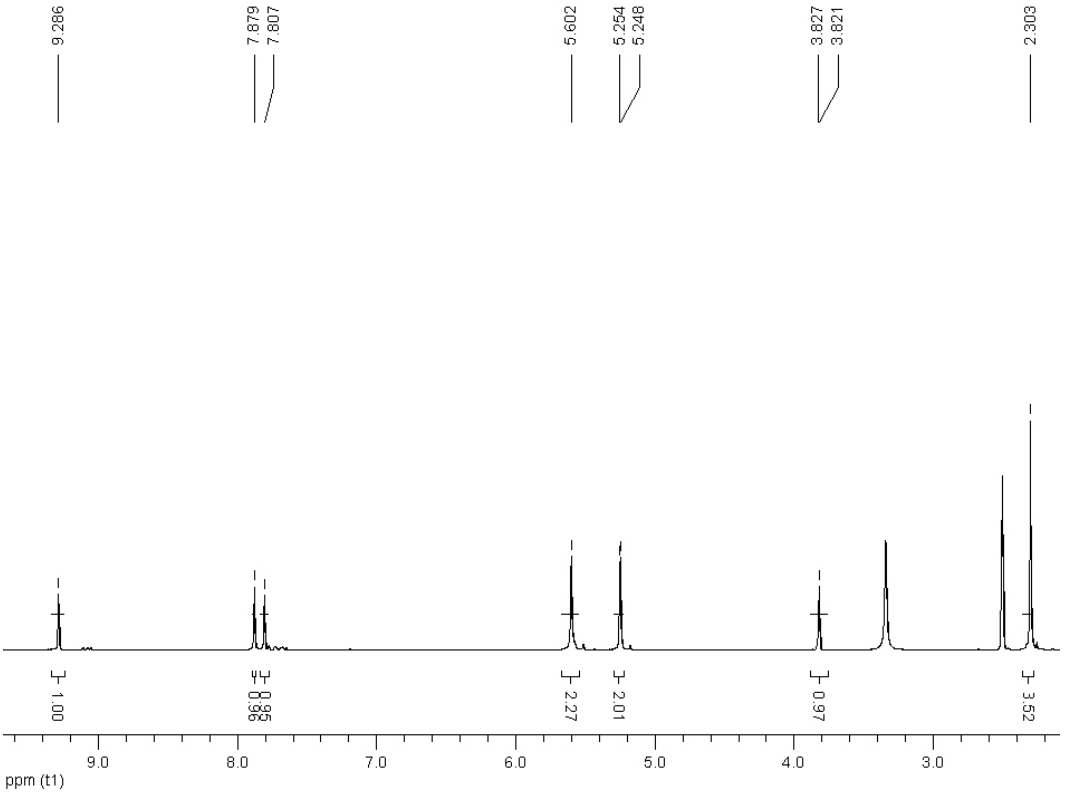


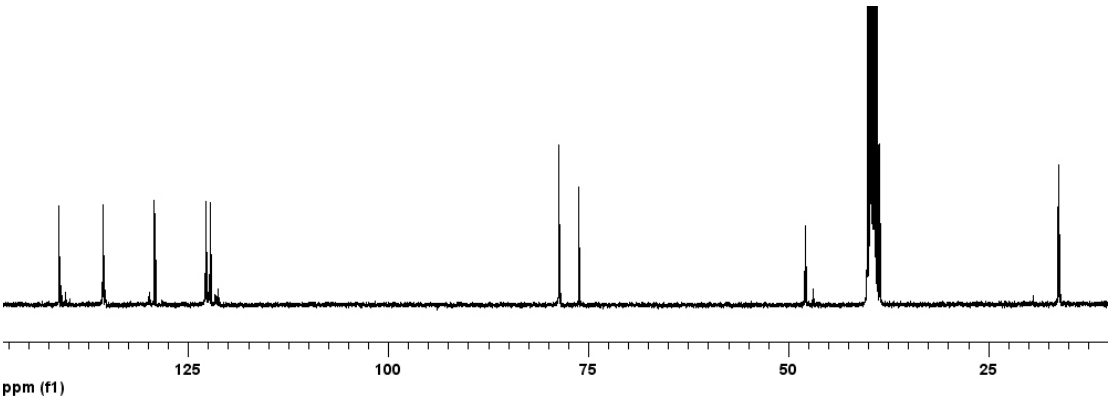


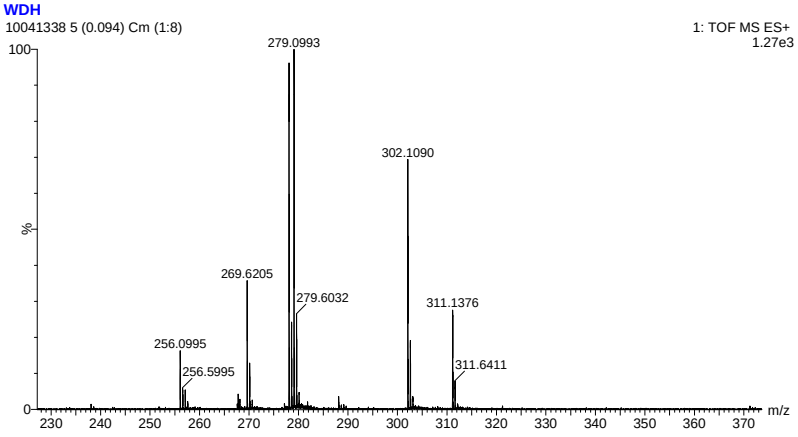
16. Figure S15. H^1 NMR spectrum of compound of 1.



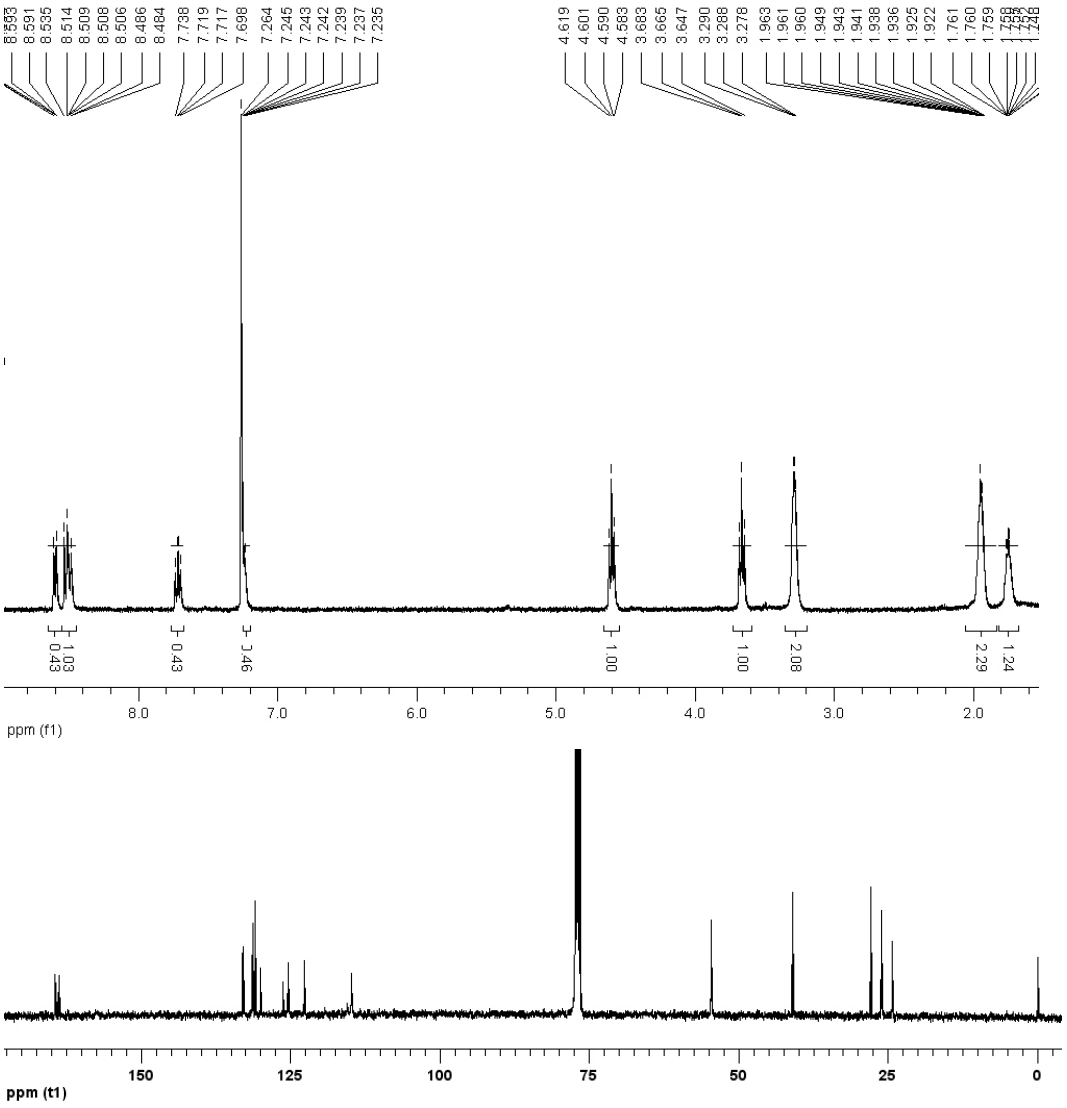
17. Figure S16. **^{1}H-NMR (top picture), ^{13}C-NMR (middle picture) and Mass (bottom picture) spectra of the 2.**

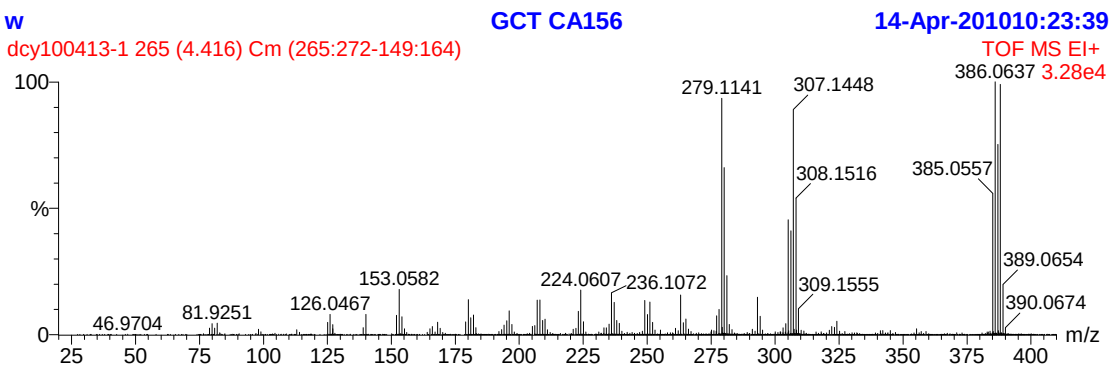




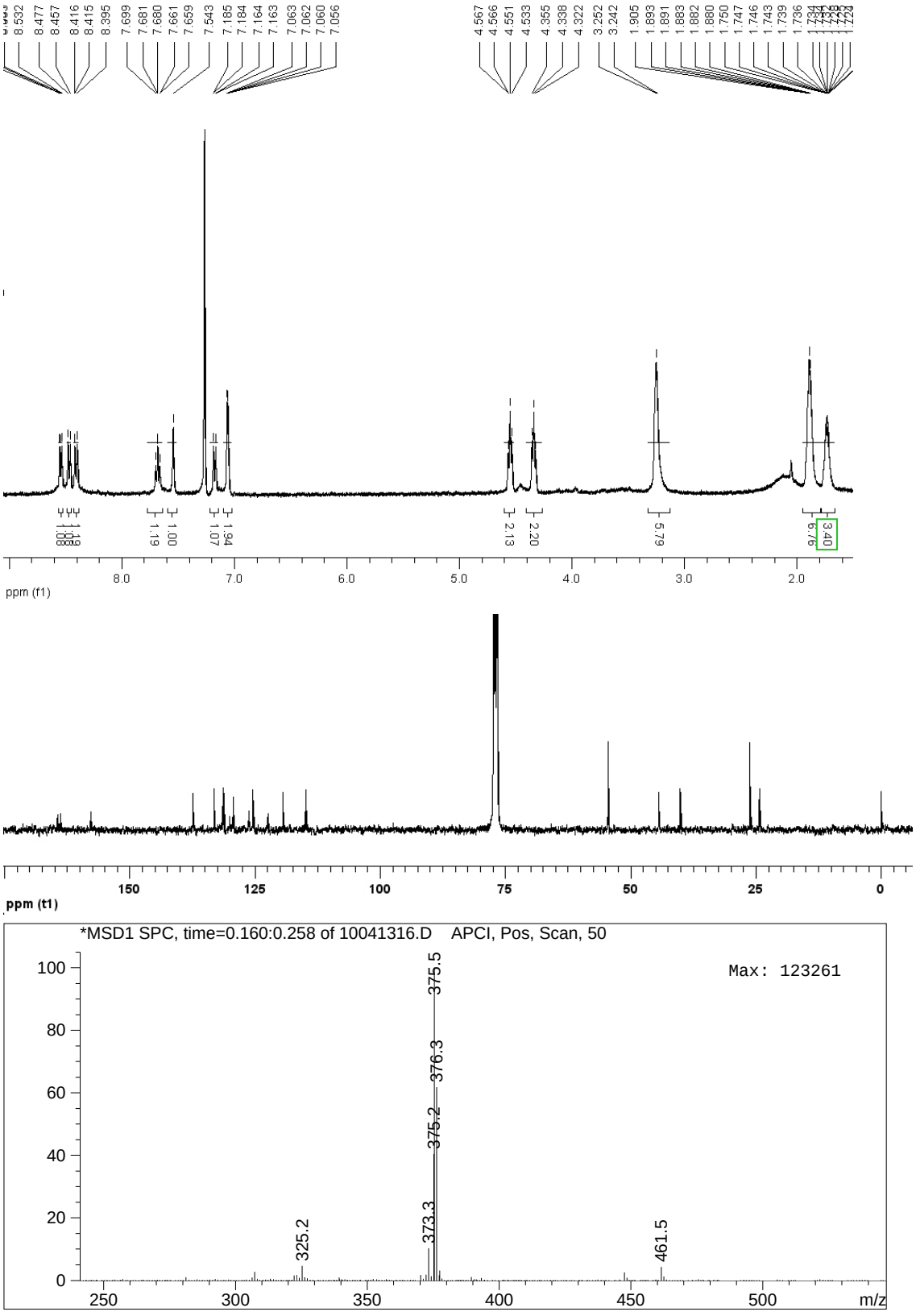


18. Figure S17. ^{1}H-NMR (top picture), ^{13}C-NMR (middle picture) and Mass (bottom picture) spectra of the 5.

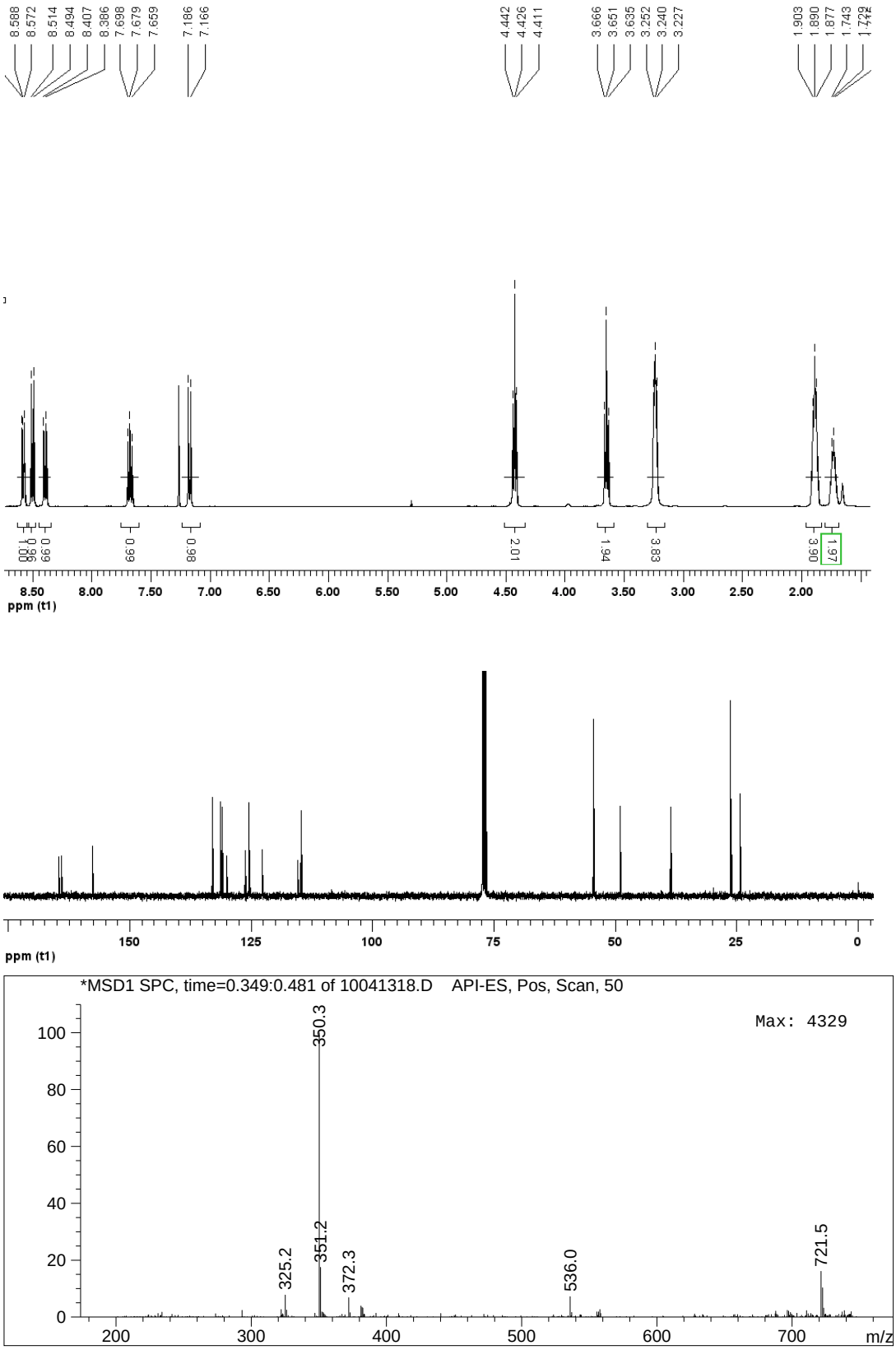




19. Figure S18. **^{1}H-NMR (top picture), ^{13}C-NMR (middle picture) and Mass (bottom picture) spectra of the 6.**



20. Figure S19. **^{1}H-NMR (top picture), ^{13}C-NMR (middle picture) and Mass (bottom picture) spectra of the 7.**



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(2) H. K. Liu, W. Y. Sun, H. L. Zhu, K. B. Yu and W. X. Tang, *Inorganica Chimica Acta*. 1999, 129-135.