Supporting Information

Fluorescent Wittig reagent as a novel ratiometric probe for quantification of 5-formyluracil and its application in cell imaging

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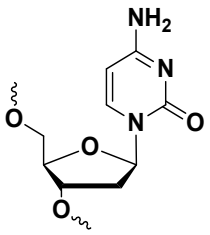
Materials and instruments

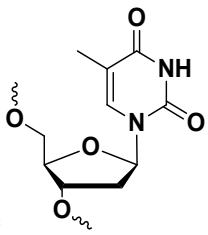
Unless otherwise noted, all chemical reagents were purchased from commercial suppliers and used without further purification. All solvents were dried according to the standard methods prior to use. In the optical spectroscopic studies, all of the solvents were either HPLC or spectroscopic grade. The canonical oligodeoxynucleotides (ODN-C, ODN-T) and the modified oligodeoxynucleotides (ODN-5fU, ODN-5fC, ODN-AP) were bought from Sangon Biotech (Shanghai, China) and Takara (Dalian, China), respectively. The nucleic acid stains SuperRed (NO.: BS354B) was purchased from biosharp (Hefei, China).

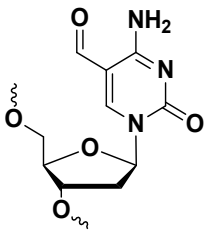
Thin layer chromatography (TLC) was performed on silica gel plates, and spots were visualized under UV light. Column chromatography was carried out using 200-300 mesh silica gel (Qingdao Ocean Chemicals). NMR spectra were recorded on a Bruker AMX-400 spectrometer at 25℃ (^{1}H NMR: 400 MHz, ^{13}C NMR: 101 MHz) and chemical shifts (λ) are expressed in parts per million (ppm) using the internal standard tertramethylsilane or the deuterated solvent ($CDCl_3$, DMSO-d_6, Methanol-d_4) as reference. Spin multiplicities in ^{1}H NMR are reported as singlet (s), doublet (d), double doublet (dd), double double doublet (ddd), triplet (t), triplet of triplet (tt), multiplet/overlapping peaks (m) or broad (br). The High-resolution mass spectra (HRMS) were obtained on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer. pH values were determined by a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. UV absorption spectra were recorded on a Persee TU-1901 UV-visible spectrophotometer. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer. Gel imaging was monitored with Azure Biosystems C600. Cell imaging was performed on a Zeiss LSM 780 confocal laser scanning microscope.

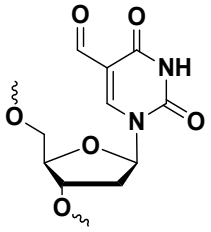
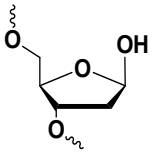
Sequences of oligodeoxynucleotides

5’-GACTCAAXAGCCGTA-3’

ODN-C: X= 

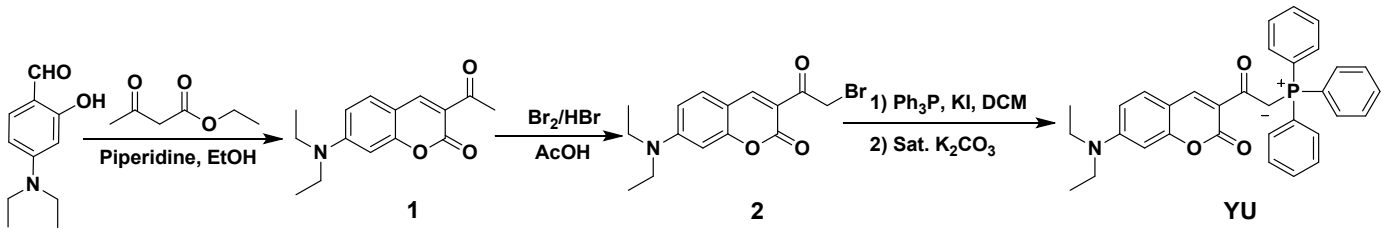
ODN-T: X= 

ODN-5fC: X= 

ODN-5fU: X=  ODN-AP: X= 

Synthesis

Synthesis of YU



Scheme S1. The synthetic routes of **YU**.

Synthesis of compound 1

Compound **1** was synthesized according to a literature method.[1] Briefly, 4-(Diethylamino)salicylaldehyde (1.93 g, 10 mmol), ethyl acetoacetate (1.85 g, 15 mmol) and 0.1 mL piperidine were dissolved in 50 mL absolute ethanol. Then, the mixture was refluxed for 5 h. After cooling to room temperature, the yellow precipitate was filtered and recrystallized from ethanol to afford compound **1** (2.15 g, 8.3 mmol). Yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.39 (d, J = 9.0 Hz, 1H), 6.61 (dd, J = 9.0, 2.4 Hz, 1H), 6.46 (d, J = 2.3 Hz, 1H), 3.45 (q, J = 7.1 Hz, 4H), 2.67 (s, 3H), 1.24 (t, J = 7.1 Hz, 6H).

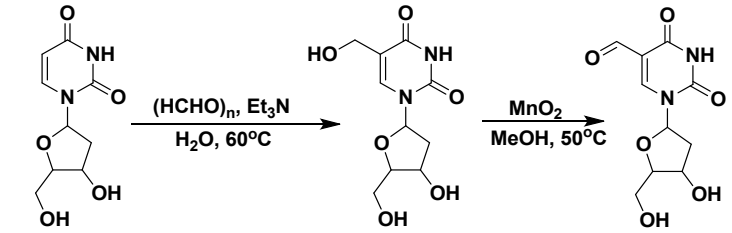
Synthesis of compound 2

Compound **2** was synthesized following a previously reported method.[2] Bromine (1.48 g, 9.25 mmol) was added dropwise to a solution of compound **1** (1.5 g, 5.78 mmol) dissolved in 150 mL acetic acid containing 50% HBr aq. (2.8 g, 17.3 mmol). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and 100 mL water was added, followed by sodium bicarbonate until pH = 8-9. The mixture was then extracted with CH_2Cl_2 (100 mL×3), and organic extracts were combined, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield the crude product. Further purification by silica gel column chromatography eluting with CH_2Cl_2 gave compound **2** (1.29 g, 3.81 mmol). Yield: 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.43 (d, J = 9.0 Hz, 1H), 6.64 (dd, J = 9.0, 2.5 Hz, 1H), 6.48 (d, J = 2.4 Hz, 1H), 4.77 (s, 2H), 3.48 (q, J = 7.2 Hz, 4H), 1.25 (t, J = 7.1 Hz, 6H).

Synthesis of YU

To a solution of Compound **2** (1.29 g, 3.81 mmol) and triphenylphosphine (1.12 g, 4.57 mmol) in CH_2Cl_2, catalytic amount of potassium iodide was added and the reaction mixture was heated at reflux for 8 h. Then, the solvent was evaporated and the residue was purified by flash column chromatography (CH_2Cl_2: Methanol = 30:1, v/v) to afford the intermediate as a red solid. Subsequently, the red solid was dissolved in 50 mL CH_2Cl_2 mixed with 50 mL saturated K_2CO_3 aq. and stirred vigorously for additional 3 h at room temperature. After that, the organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Finally, the crude solid was purified by silica gel column chromatography (CH_2Cl_2: Methanol = 50:1, v/v) to provide **YU** as an orange solid (1.57 g, 3.03 mmol). Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.73 (dd, *J* = 12.5, 7.4 Hz, 6H), 7.58-7.45 m, 9H), 7.31 (d, *J* = 8.7 Hz, 1H), 6.54 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.48 (d, *J* = 2.1 Hz, 1H), 5.55 (d, *J* = 28.6 Hz, 1H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ177.9, 162.1, 157.3, 151.2, 144.1, 133.4, 133.3, 132.1, 130.4, 123.0, 128.9, 127.5, 126.5, 109.0, 96.7, 77.2, 56.5, 55.4, 44.9, 12.6; HRMS (ESI) *m/z* calcd for $C_{33}H_{30}NO_3P$ $[M+H]^+$: 520.2042, found: 520.2040.

Synthesis of 5fU



Scheme S2. The synthetic routes of **5fU**.

Synthesis of 5-hydroxymethyluracil (5hmU)

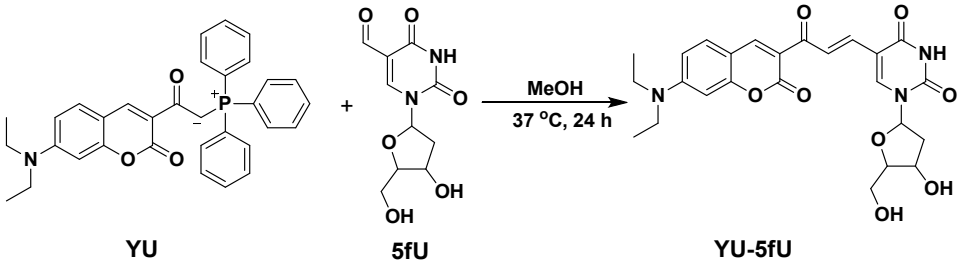
The intermediate 5hmU was synthesized according to literature.[3,4] Briefly, a suspension of 2'-deoxyuridine (5.25 g, 23.0 mmol) and paraformalclehyde (3.11 g, 103.5 mmol) in 80 mL H_2O was treated with 5.6 mL triethylamine and heated at 60°C for 4 days. During the reaction, paraformaldehyde (4.49 g, 149.5 mmol) was added once daily, and triethylamine (1 mL) and water (10 mL) were supplied on the second day. After that, the solvent was evaporated under reduced pressure and the residue was recrystallized in MeOH to afford 5hmU as a white solid (4.11g, 15.9 mmol). Yield = 70%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H), 6.26 (t, *J* = 6.7 Hz, 1H), 4.36 (q, *J* = 3.3 Hz, 1H), 4.28 (s, 2H), 3.88 (q, *J* = 3.3 Hz, 1H), 3.77-3.67 (m, 2H), 2.27-2.15 (m, 2H).

Synthesis of 5-formyl-2'-deoxyuridine (5fU)[3,4]

The obtained 5hmU (0.70 g, 2.7mmol) was added into a 25 mL round-bottom flask and dissolved with 10 mL MeOH followed by activated manganese dioxide (0.94 g, 10.9 mmol). The suspension

was heated with stirring at 50°C for 12 h. After cooling to room temperature, the mixture was filtered through celatom. The filtrate was collected and concentrated *in vacuo*. Further purification by silica gel column chromatography (CH_2Cl_2: Methanol = 6:1) afforded 5fU as a white solid (0.60 g, 2.35 mmol). Yield: 79%. ^{1}H NMR (400 MHz, D_2O) δ 9.66 (s, 1H), 8.80 (s, 1H), 6.25 (t, *J* = 6.1 Hz, 1H), 4.49 (q, *J* = 4.7 Hz, 1H), 4.13 (q, *J* = 4.3 Hz, 1H), 3.93-3.78 (m, 2H), 2.58-2.41 (m, 2H).

Synthesis of YU-5fU

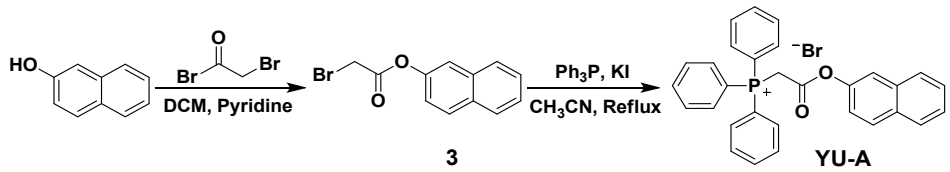


Scheme S3. The synthetic route of **YU-5fU**.

Synthesis of YU-5fU

5fU (8.0 mg, 0.03 mmol) was added to a solution of **YU** (12.0 mg, 0.02 mmol) in 3 mL methanol. The reaction mixture was kept stirring at 37 ℃ for 24 h. A color change from yellow to orange was observed. Then, the solvent was evaporated in vacuo and the crude product was purified by silica gel column chromatography (CH_2Cl_2: Methanol = 10:1, v/v) to afford **YU-5fU** as an orange powder in more than 85% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.47 (d, J = 25.5 Hz, 2H), 8.24 (d, J = 15.6 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.39 (d, J = 15.6 Hz, 1H), 6.79 (d, J = 8.8 Hz, 1H), 6.59 (s, 1H), 6.15 (t, J = 6.5 Hz, 1H), 5.29 (s, 1H), 5.17 (s, 1H), 4.30-4.24 (m, 1H), 3.84-3.80 (m, 1H), 3.63 (dd, J = 30.9, 12.3 Hz, 2H), 3.49 (q, J = 6.8 Hz, 4H), 2.25-2.13 (m, 2H), 1.15 (t, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d6) δ 186.6, 160.1, 158.6, 153.2, 149.8, 148.5, 144.9, 132.6, 124.5, 116.4, 110.5, 109.7, 108.3, 96.3, 88.1, 85.3, 70.3, 61.3, 44.9, 12.8.; HRMS (ESI) *m/z* calcd for $[M+H]^+$: 498.1876, found: 498.1870.

Synthesis of YU-A



Scheme S4. The synthetic route of **YU-A**.

Synthesis of compound 3

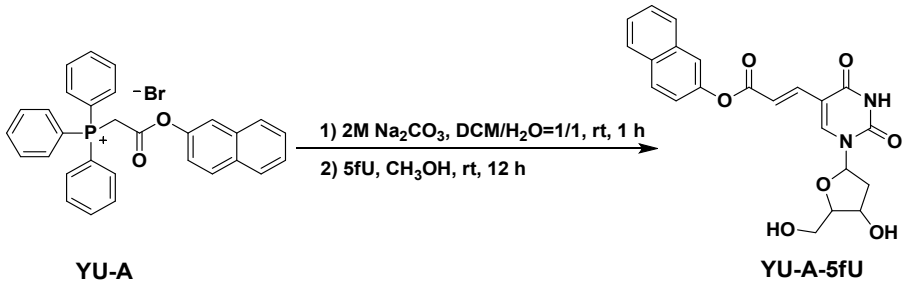
Compound **3** was synthesized according to literature.[5] 2-Naphthol (2 g, 13.87 mmol) was added to a 100 mL round-bottom flask, evacuated, and backfilled with nitrogen three times. Anhydrous

CH_2Cl_2 was added to dissolve the solid, and the reaction flask was cooled to 0 ℃. Pyridine (2.2 mL, 27.69 mmol) was added, and the resulting mixture was stirred for 5 min to equilibrate the temperature. Bromoacetyl bromide (1.8 mL, 20.81 mmol) was added slowly at 0 ℃ and stirred at that temperature for 15 min before removing the flask from the cooling bath and allowed the mixture to warm to room temperature for an additional 12 h. The reaction was diluted with H_2O (50 mL), and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield a light brown solid. The crude product was purified by silica gel column chromatography (DCM) to afford compound **3** as a white solid (1.5270 g, 5.76 mmol). Yield: 41.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89-7.81 (m, 3H), 7.62 (d, J = 2.2 Hz, 1H), 7.52-7.49 (m, 2H), 7.28-7.25 (m, 1H), 4.11 (s, 2H).

Synthesis of YU-A

To a solution of Compound **3** (1.5270 g, 5.76 mmol) and triphenylphosphine (1.5108 g, 5.76 mmol) in acetonitrile, catalytic amount of potassium iodide was added and the reaction mixture was heated at reflux for 6 h. Then, the solvent was evaporated and the residue was purified by flash column chromatography (CH_2Cl_2: Methanol = 100:1, v/v) to provide **YU-A** as a white solid (2.278 g, 4.32 mmol). Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J = 13.4, 7.9 Hz, 6H), 7.78-7.69 (m, 4H), 7.69-7.59 (m, 8H), 7.44-7.36 (m, 2H), 7.24 (d, J = 1.8 Hz, 1H), 6.91 (dd, J = 8.9, 2.2 Hz, 1H), 5.96 (d, J = 13.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 163.7, 147.5, 135.3, 135.2, 134.1, 134.0, 133.3, 131.6, 130.4, 130.3, 129.5, 127.6, 126.7, 126.0, 120.3, 118.4, 118.2, 117.3, 33.8, 33.2; HRMS (ESI) *m/z* calcd for $[M]^+$: 447.1508, found: 447.1500.

Synthesis of YU-A-5fU



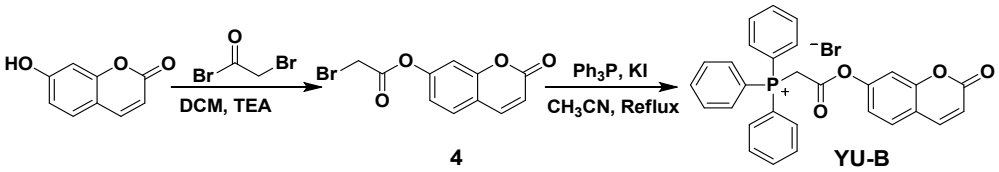
Scheme S5. The synthetic route of **YU-A-5fU**.

Synthesis of YU-A-5fU

YU-A (528 mg, 1 mmol) was dissolved in 10 mL CH_2Cl_2 mixed with 10 mL 2 mol/L Na_2CO_3 aq. and stirred vigorously for 1 h at room temperature. After that, the organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was redissolved in 5 mL methanol, into which 5fU (180 mg, 0.7 mmol) was added and the reaction mixture was kept stirring at room

temperature for 12 h. Then the solvent was evaporated and the crude product was purified by silica gel column chromatography (CH_2Cl_2: Methanol = 10:1, v/v) to afford **YU-A-5fU** as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ 8.60 (s, 1H), 7.91-7.83 (m, 3H), 7.65 (s, 1H), 7.61 (d, *J* = 2.6 Hz, 1H), 7.53-7.45 (m, 2H), 7.28 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.17 (d, *J* = 15.8 Hz, 1H), 6.28 (t, *J* = 6.4 Hz, 1H), 4.46-4.42 (q, *J* = 3.9 Hz, 1H), 3.97 (dd, *J* = 6.6, 3.2 Hz, 1H), 3.90-3.75 (m, 2H), 2.41-2.25 (m, 2H); HRMS (ESI) *m/z* calcd for $[M+Na]^+$: 447.1168, found: 447.1184.

Synthesis of YU-B



Scheme S6. The synthetic route of **YU-B**.

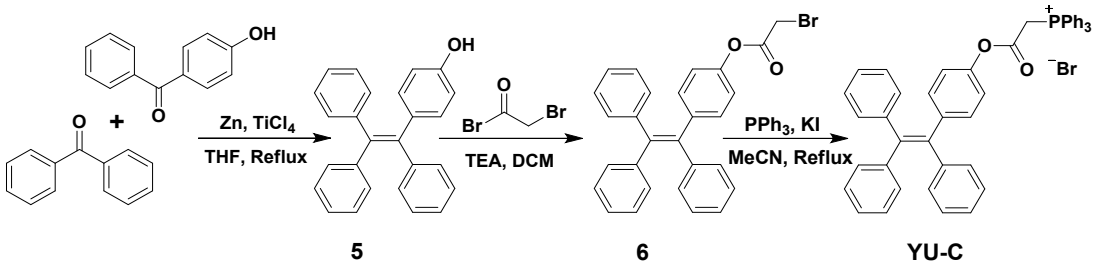
Synthesis of compound 4

7-Hydroxycoumarin (0.81 g, 5 mmol) was added to a 50 mL round-bottom flask, evacuated, and backfilled with nitrogen three times. Anhydrous CH_2Cl_2 was added to dissolve the solid, and the reaction flask was cooled to 0 ℃. Triethylamine (1.38 mL, 10 mmol) was added, and the resulting mixture was stirred for 5 min to equilibrate the temperature. Bromoacetyl bromide (0.65 mL, 7.5 mmol) was added slowly at 0 ℃ and stirred at that temperature for 15 min before removing the flask from the cooling bath and allowed the mixture to warm to room temperature for an additional 5 h. The reaction was diluted with H_2O (50 mL), and the organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was further purified by silica gel column chromatography (DCM) to yield compound **4** as a white solid (1.2578 g, 4.4 mmol). Yield: 88.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 9.6, 4.0 Hz, 1H), 7.52 (dd, *J* = 8.5, 4.3 Hz, 1H), 7.25 (d, *J* = 4.5 Hz, 1H), 7.20-7.13 (m, 1H), 7.13-7.05 (m, 1H), 6.42 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.08 (d, *J* = 4.5 Hz, 2H).

Synthesis of YU-B

To a solution of compound **4** (1.2578 g, 4.44 mmol) and triphenylphosphine (11.736 g, 4.47 mmol) in acetonitrile, catalytic amount of potassium iodide was added and the reaction mixture was heated at 80 ℃ for 7 h. Then, the solvent was evaporated and the residue was purified by flash column chromatography (CH_2Cl_2: Methanol = 100:1, v/v). Unfortunately, we failed to obtain **YU-B** due to the instability of its aromatic carboxylate structure.

Synthesis of YU-C



Scheme S7. The synthetic route of **YU-C**.

Synthesis of compound 5

Compound **5** was synthesized according to a literature method.[6] 4-Hydroxybenzophenone (1.9 g, 10 mmol), benzophenone (2.2 g, 12 mmol), and Zn powder (2.9 g, 44 mmol) were added to a 250 mL two-necked round-bottom flask. The flask was evacuated under vacuum and flushed with nitrogen three times. Then 80 mL newly dried THF was added. The solution was cooled to 0 ℃, into which $TiCl_4$ (2.4 mL, 22 mmol) was added. The reaction mixture was refluxed overnight. After cooling to room temperature, 80 mL dilute hydrochloric acid (1 mol/L) was added, followed by DCM extraction. The collected organic layer was dried over anhydrous $MgSO_4$. After solvent evaporation, the crude product was purified by silica gel column chromatography (PE: EA = 10:1, v/v) and compound **5** was obtained as a white solid (1.74 g, 5 mmol). Yield: 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 7.18-7.05 (m, 9H), 6.97-6.91 (m, 6H), 6.74 (d, J = 7.4 Hz, 2H), 6.50 (d, J = 7.3 Hz, 2H).

Synthesis of compound 6

Compound **5** (0.2 g, 0.574 mmol) was added to a 25 mL round-bottom flask, evacuated, and backfilled with nitrogen three times. Anhydrous CH_2Cl_2 was added to dissolve the solid, and the reaction flask was cooled to 0 ℃. Triethylamine (0.119 mL, 0.86 mmol) was added, and the resulting mixture was stirred for 5 min to equilibrate the temperature. Bromoacetyl bromide (0.075 mL, 0.86 mmol) was added slowly at 0 ℃ and stirred at that temperature for 15 min before removing the flask from the cooling bath and allowed the mixture to warm to room temperature for an additional 4 h. After that, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (PE: EA = 20:1, v/v) to yield compound **6** as a white solid (0.2037 g, 0.45 mmol). Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15-7.08 (m, 9H), 7.07-7.00 (m, 8H), 6.86-6.90 (m, 2H), 4.01 (s, 2H).

Synthesis of YU-C

To a solution of compound **6** (0.721 g, 1.54 mmol) and triphenylphosphine (0.404 g, 1.54 mmol) in acetonitrile, catalytic amount of potassium iodide was added and the reaction mixture was heated at reflux for 10 h. Then, the solvent was evaporated and the residue was purified by flash column

chromatography (CH_2Cl_2: Methanol = 50:1, v/v). Due to its instability, no pure **YU-C** was finally obtained, but the characteristic peak (-CH_2-) linking TPE and triphenylphosphine was found in the ^1H NMR. HRMS (ESI) *m/z* calcd for $[M]^+$: 651.2447, found: 651.2443.

Denaturing PAGE analysis of ODNs after incubation with YU

ODNs (0.1 mM in ddH_2O, 50 μL), **YU** (10 mM in EtOH, 25 μL) and 25 μL EtOH were added into 1.5 mL microcentrifuge tube, respectively. The mixture was vortexed for 1 min and incubated at 37 ℃ for 48 h. Then, 3 μL reaction product, 6 μL deionized formamide and 1 μL loading buffer were mixed well and immediately transferred onto a 20% denaturing PAGE prepared by using 1xTBE (89 mM Boric acid, 2 mM EDTA, 89 mM Tris base, pH = 8.3) containing 7 M urea. The PAGE was performed in 1xTBE buffer at a constant voltage of 150 V for about 1 h at room temperature. We scanned the final polyacrylamide gel electrophoresis products with Azure Biosystems C600 operated in the fluorescence mode (Custom-RGB-G). Finally, the gel was stained with 3×SuperRed for 45 min to visualize other DNA bands.

Fluorescence analysis of ODNs after incubation with YU

ODNs (0.1 mM in ddH_2O, 50 μL), **YU** (10 mM in EtOH, 25 μL) and 25 μL EtOH were added into 1.5 mL microcentrifuge tube, respectively. The mixture was vortexed for 1 min and incubated at 37 ℃ for 48 h. After vacuum freeze-drying, the residue was dissolved in 500 μL water. Excess **YU** was removed by extraction with CH_2Cl_2 (400 μL × 3). The aqueous layer was then collected to scan fluorescence spectrum upon excitation at 495 nm.

Fluorescence imaging of intracellular 5fU mutations with YU

Fluorescence imaging of intracellular 5fU generated by γ-irradiation.

Specifically, Hela cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic at 37 ℃ in a 5% CO_2/95% air incubator. For fluorescence imaging, Hela cells were seeded in a 1 ml cuvette and incubated for 24 h. Then, the cells were exposed to a ^{60}Co γ-source at a dose rate of 18 Gy/min for 60 min (room temperature). Immediately after irradiation, the cells were fixed with 80% EtOH for 20 min and further incubated with **YU** (10 μM) at 37 ℃ for another 16 h. After being washed with PBS three times, the cell imaging was

operated on a confocal laser scanning microscope (Zeiss LSM 780, Germany) upon an excitation at 488 nm.

Fluorescence imaging of exogenous 5fU or intracellular 5fU generated by Fenton's reagent.

Similarly, Hela cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic at 37 ℃ in a 5% CO_2/95% air incubator. For fluorescence imaging, Hela cells were seeded in a 1 ml cuvette and incubated for 24 h. Then, the cells were treated with Fenton's reagent ($FeSO_4$: H_2O_2, 1:5) or 5fU for 20/60 min at 37 ℃. Next, the culture medium was replaced with a fresh one containing 30% glycerol, followed by the addition of 10 μM **YU** and incubated for another period. After being washed with PBS three times, the confocal fluorescent images were captured with an excitation light at 488 nm (Zeiss LSM 780, Germany).

Additional spectra, tables and images

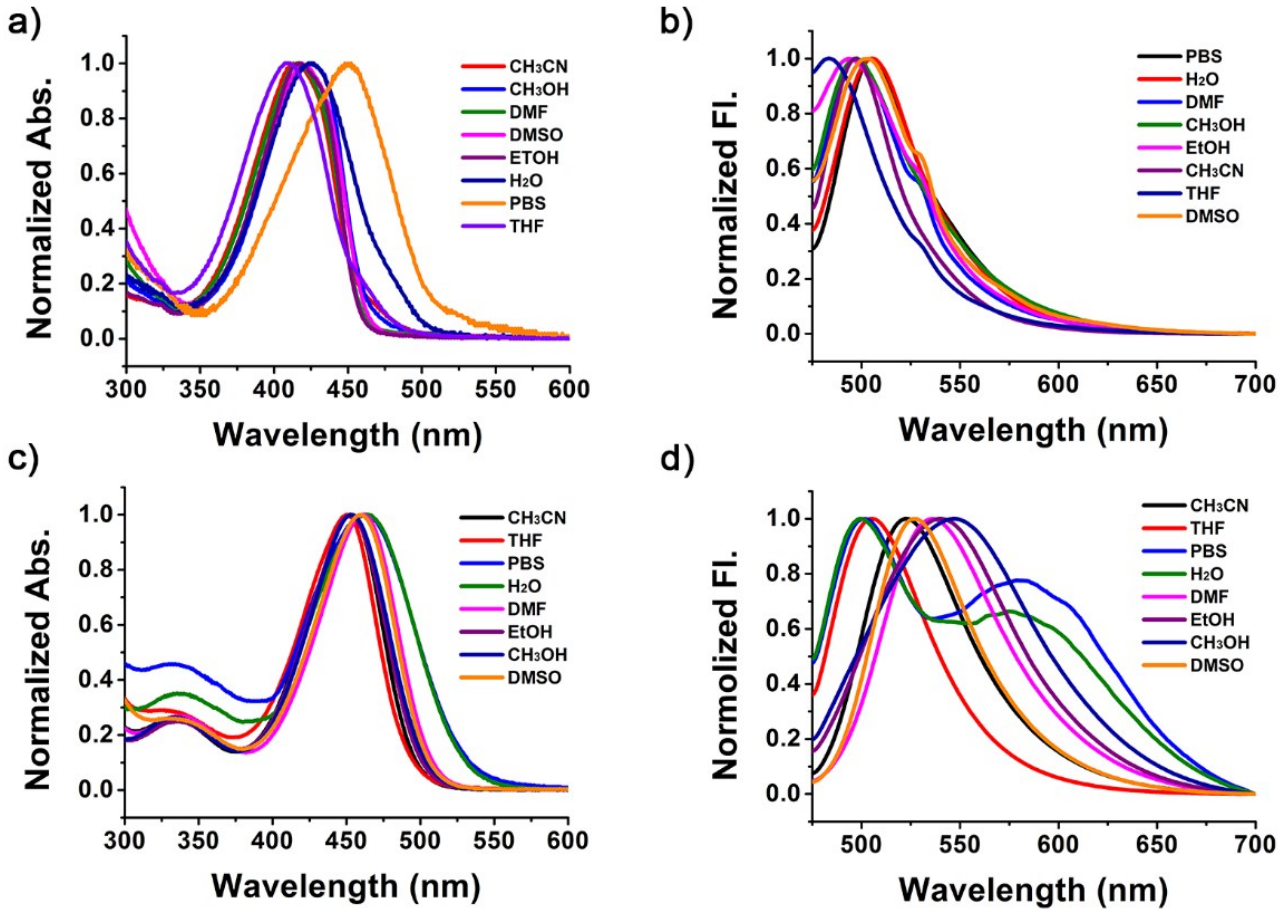


Fig. S1 Normalized UV absorption spectra and fluorescence emission spectra (λ_{em}: 460 nm) of **YU** (a, b) and **YU-5fU** (c, d) in different solvents, including CH_3CN, CH_3OH, DMF, DMSO, EtOH, H_2O, PBS and THF.

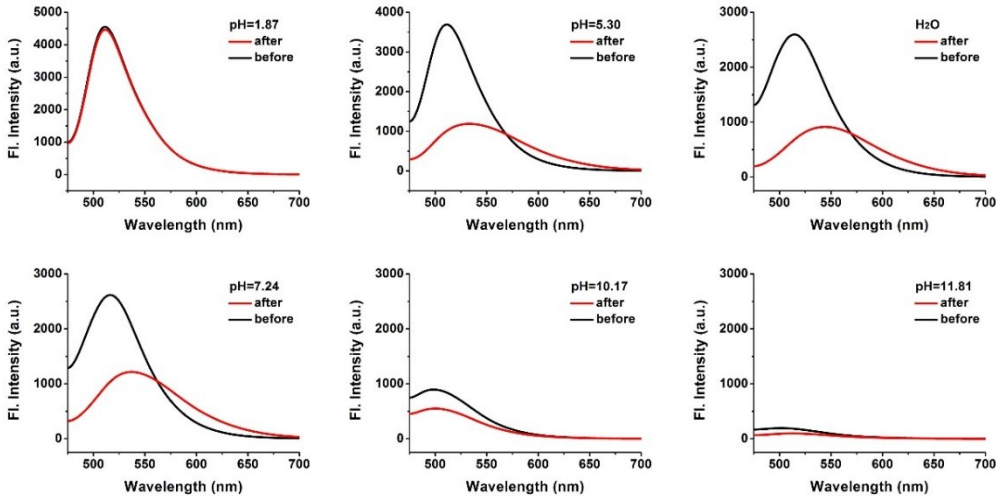


Fig. S2 Fluorescence spectra of **YU** (10 μM) before (black) and after (red) incubation with 5fU (10 eq.) in different pH solutions containing 50% EtOH at 37 ℃ for 48 h.

Table S1 Maximum fluorescence emission wavelength of YU and YU-5fU in different solvents.

	CH_3CN	THF	PBS	H_2O	DMF	EtOH	CH_3OH	DMSO	EtOH/ H_2O=1/1
YU	497	483.6	505.2	505.6	497.2	493.8	496.8	503.8	497.8
YU-5fU	523	505.4	579.4	575.4	536.2	540.2	547.8	526.4	555
Red shift	26	21.8	74.2	69.8	39	46.4	51	22.6	57.2

Table S2 Maximum UV absorption wavelength of YU and YU-5fU in different solvents.

	CH_3CN	THF	PBS	H_2O	DMF	EtOH	CH_3OH	DMSO	EtOH/ H_2O=1/1
YU	415	411	452	422	417	418	422	419	411
YU-5fU	453	450	464	465	462	452	454	460	468
Red shift	38	39	12	43	45	34	32	41	57

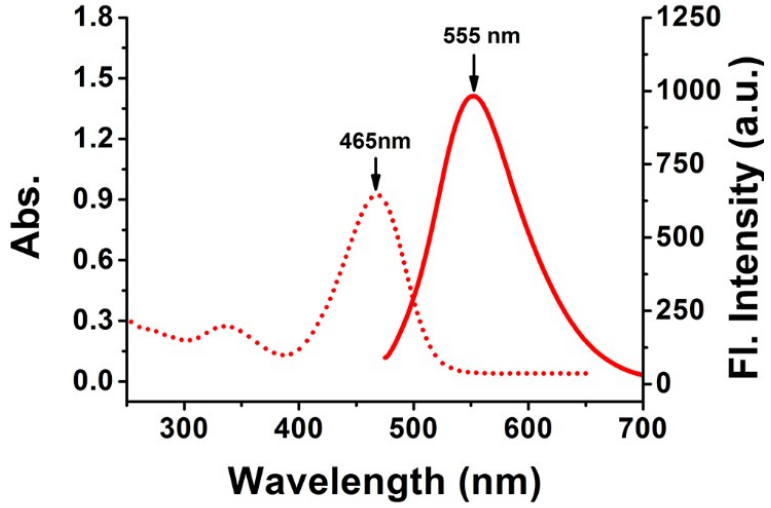


Fig. S3 UV absorption spectra and fluorescence emission spectra (λ_{em}: 460 nm) of **YU-5fU** in EtOH/H_2O = 1/1.

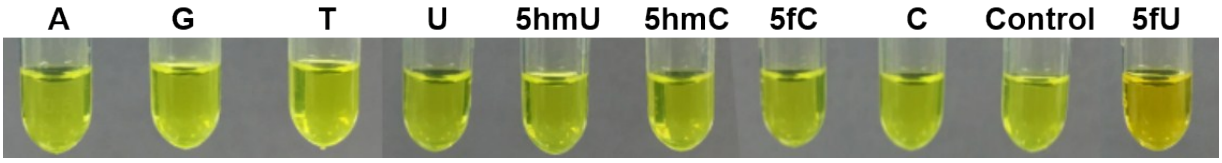


Fig. S4 The images of 10 μM **YU** after incubation with 30 eq. 5fU and other interfering species, including A, G, T, U, 5hmU, 5hmC, 5fC and C. 10 μM **YU** without any nucleoside served as the control.

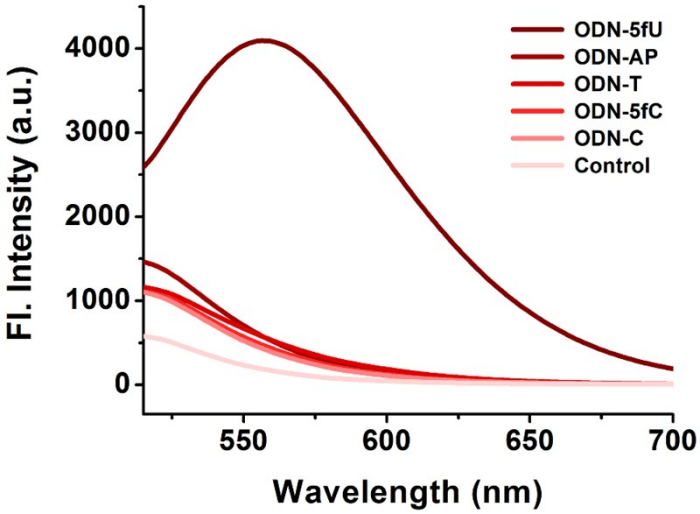


Fig. S5 Fluorescence spectra of **YU** after reaction with ODN-5fU, ODN-5fC, ODN-AP, ODN-T and ODN-C. λ_{ex}: 495 nm.

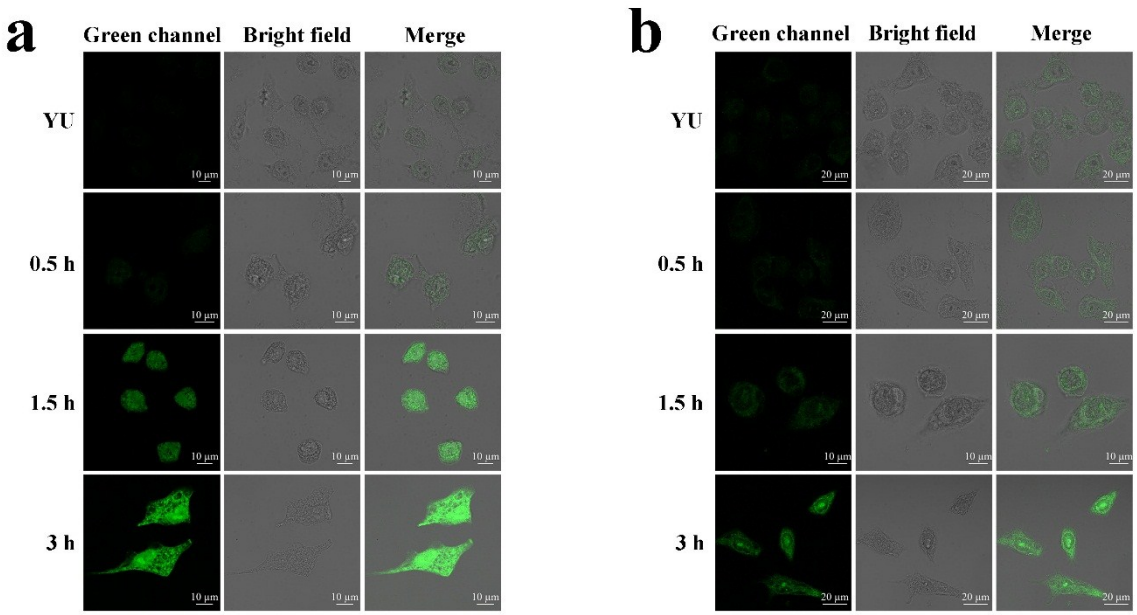


Fig. S6 Confocal fluorescence imags of Hela cells pretreated with 25 μM Fenton's reagent (the left group) or 10 μM 5fU (the right group) for 20 min and then incubated with 10 μM **YU** in DMEM containing 30% glycerol at 37 ℃ for 0.5 h (the second line), 1.5 h (the third line), 3 h (the fouth line), respectively. λ_{ex}=488 nm, λ_{em}=500-700 nm.

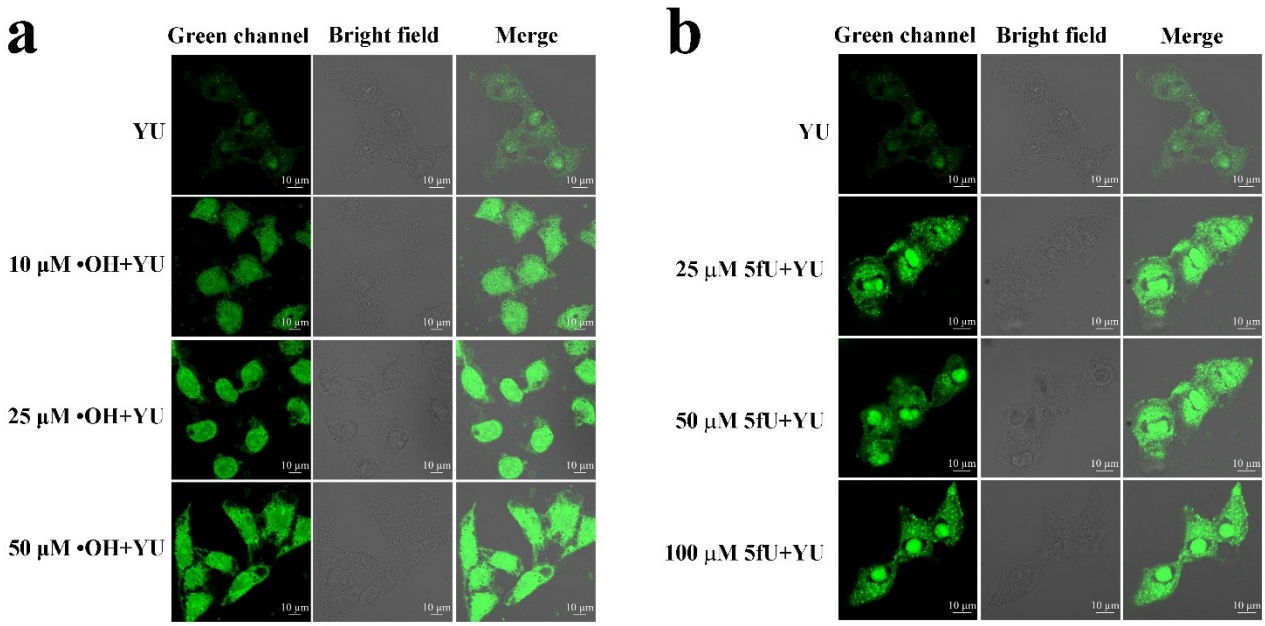
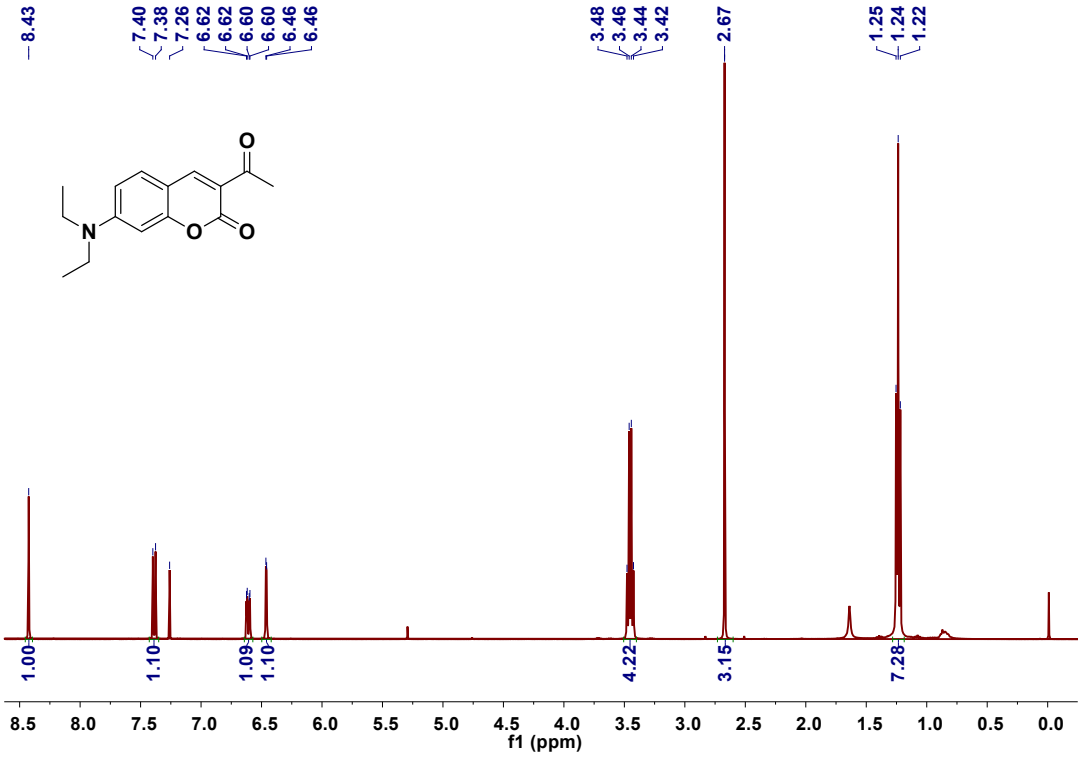
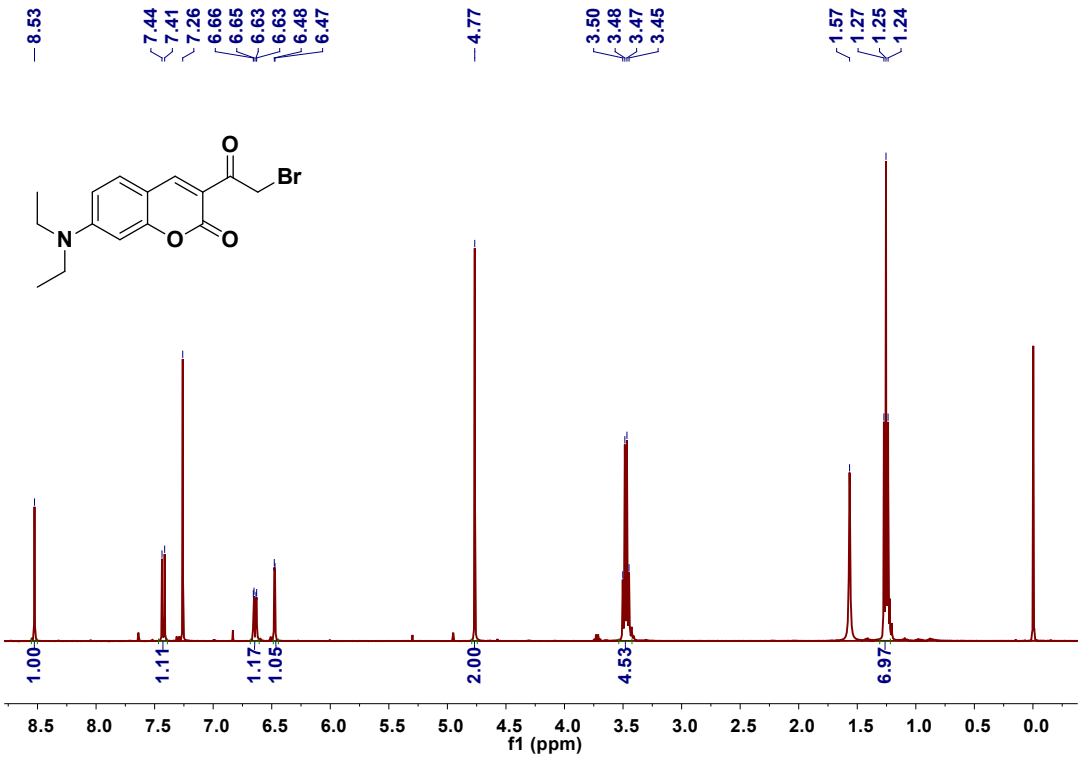


Fig. S7 Confocal fluorescence imags of Hela cells pretreated with differenet concentrations of Fenton's reagent (the left group) or 5fU (the right group) for 60 min and then incubated with 10 μM **YU** in DMEM containing 30% glycerol at 37 ℃ for 16 h. λ_{ex}=488 nm, λ_{em}=500-700 nm.

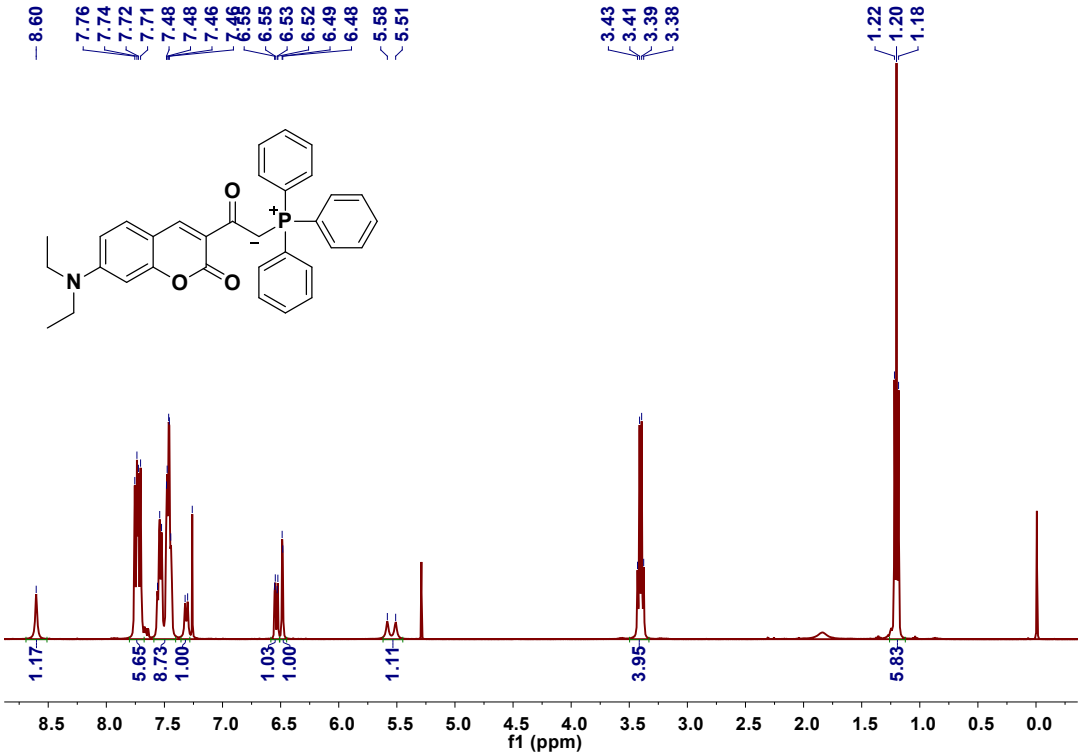
^{1}NMR, ^{13}NMR and ESI-MS spectra of synthesized compounds



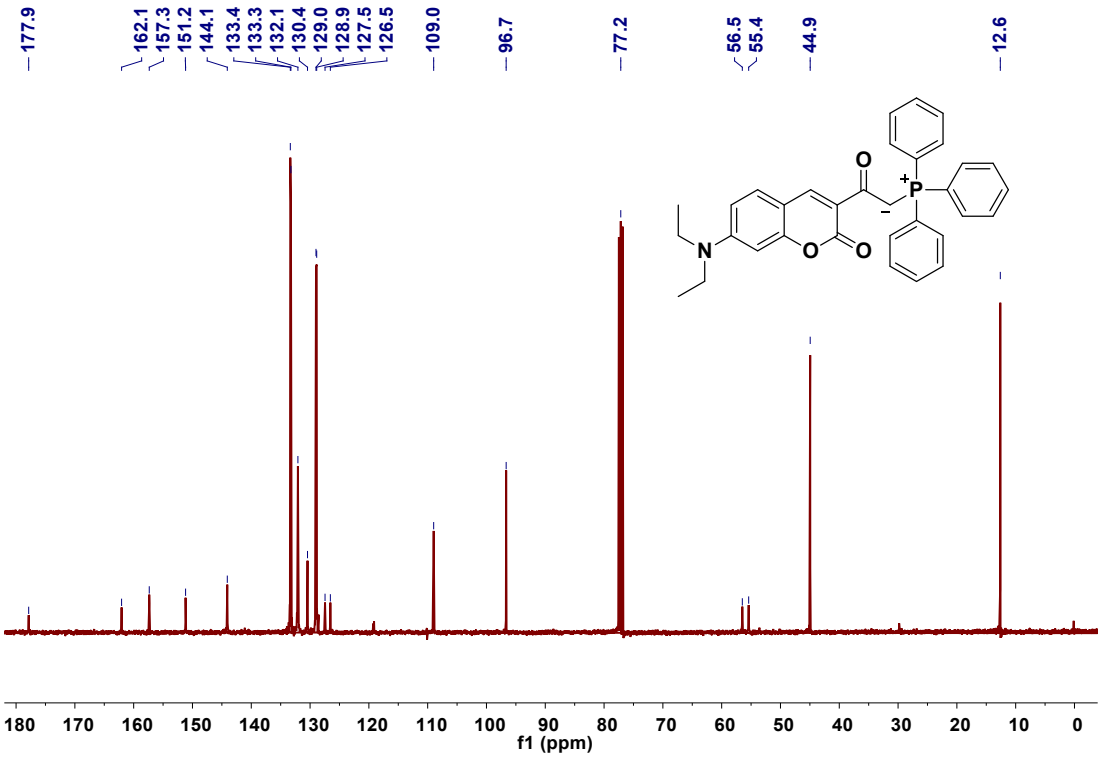
The ^{1}H NMR spectra of compound **1** in $CDCl_3$.



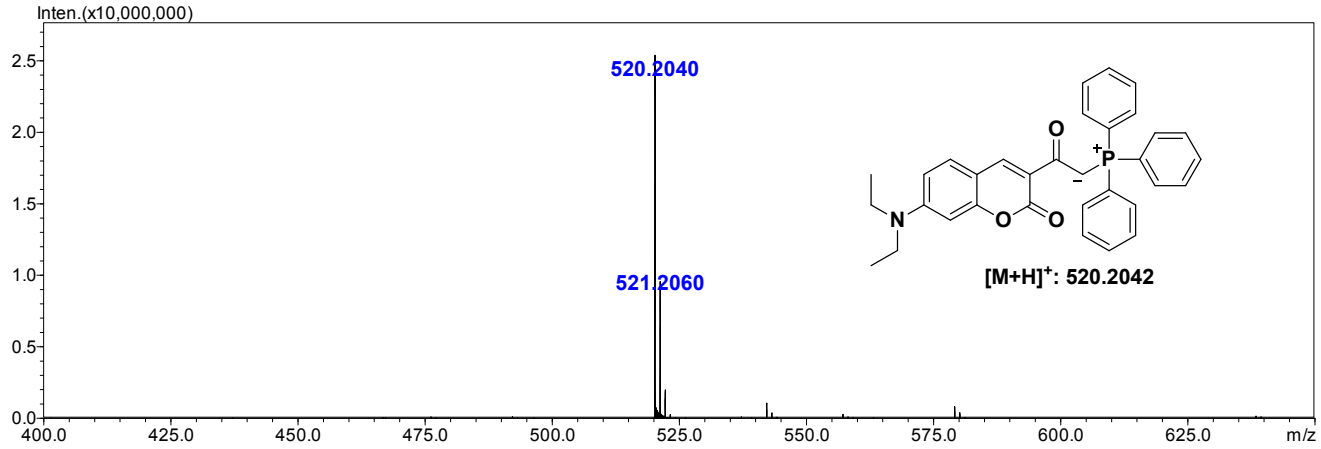
The ^{1}H NMR spectra of compound **2** in $CDCl_3$.



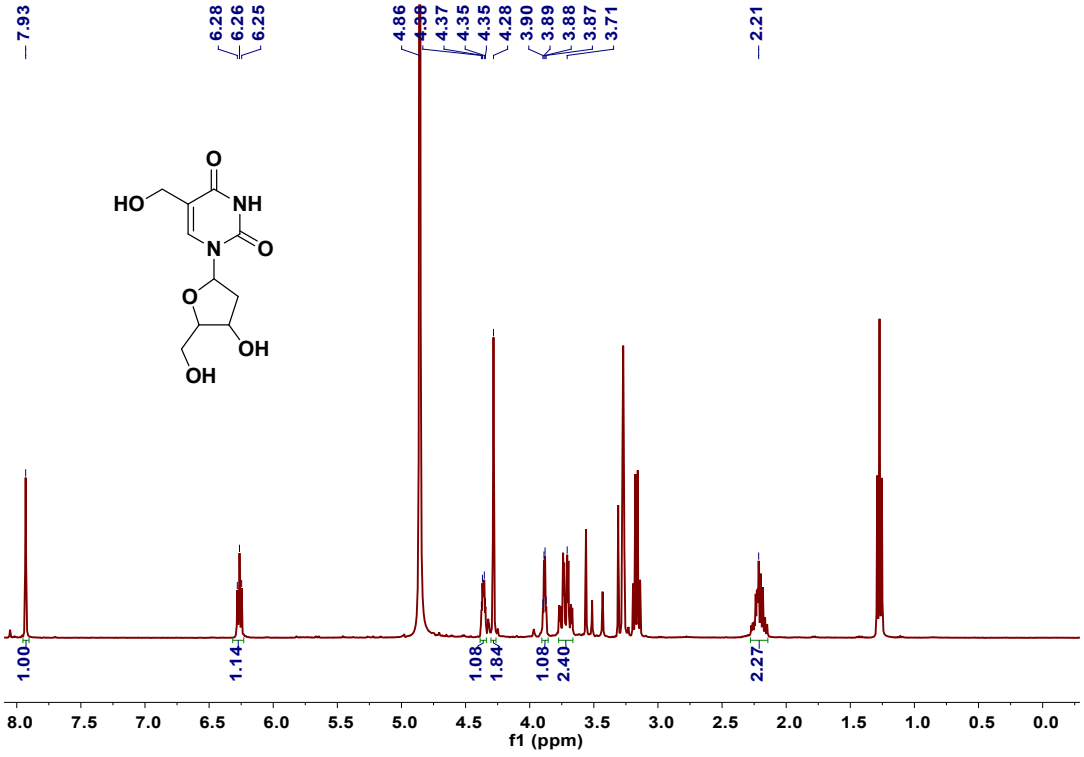
The ^{1}H NMR spectra of **YU** in $CDCl_3$.



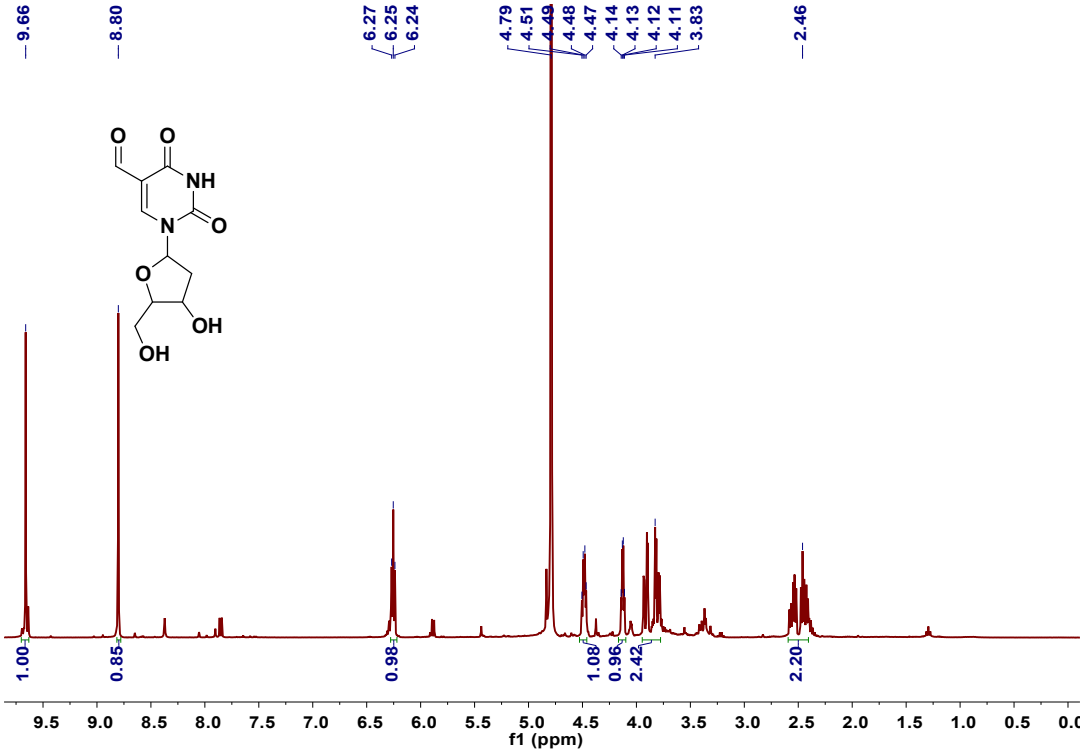
The ^{13}C NMR spectra of **YU** in $CDCl_3$.



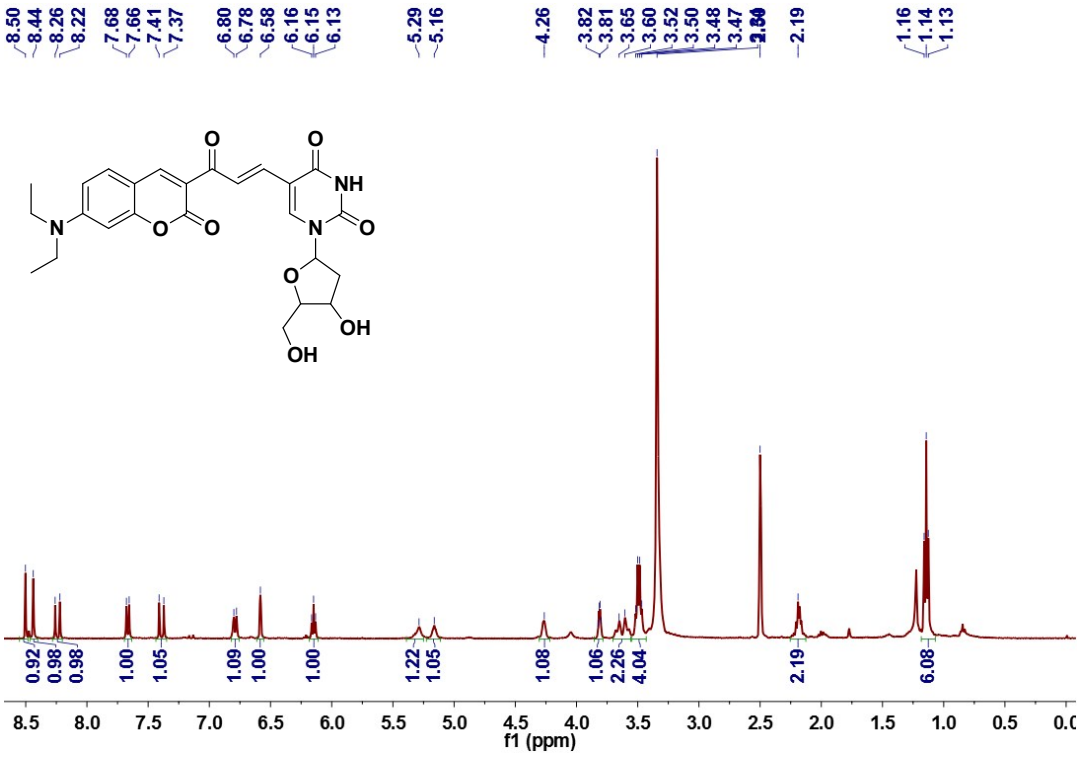
The ESI-MS spectra of **YU**.



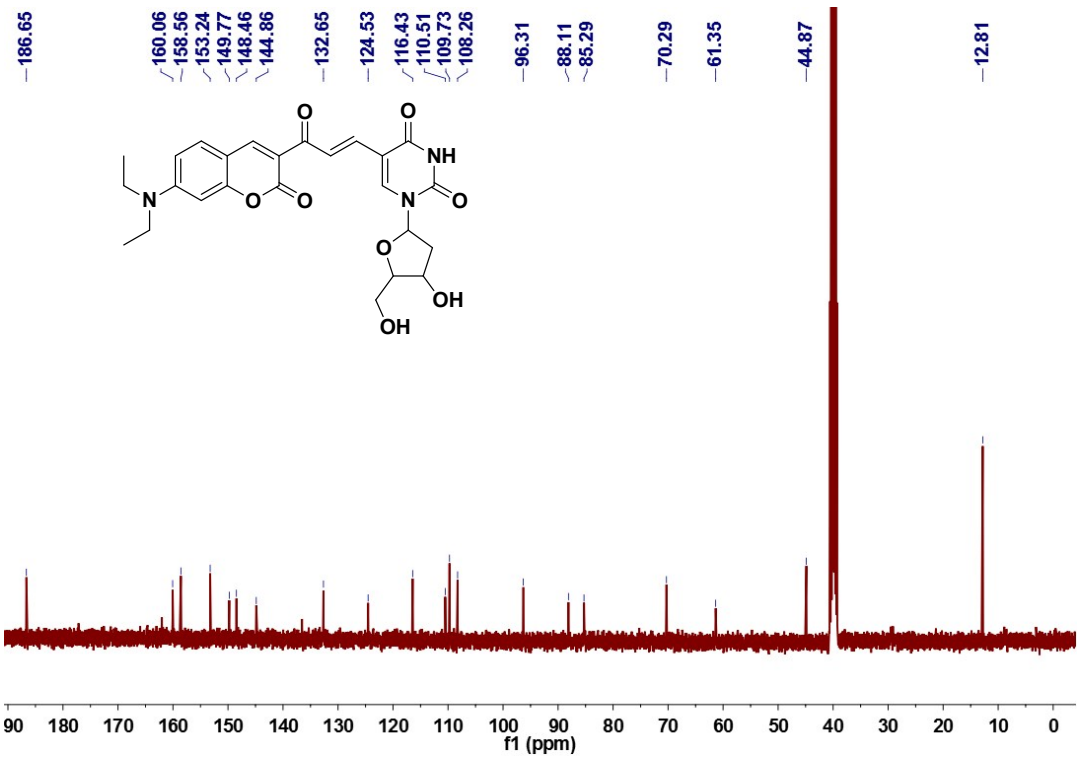
The ^{1}H NMR spectra of **5hmU** in CD_3OD.



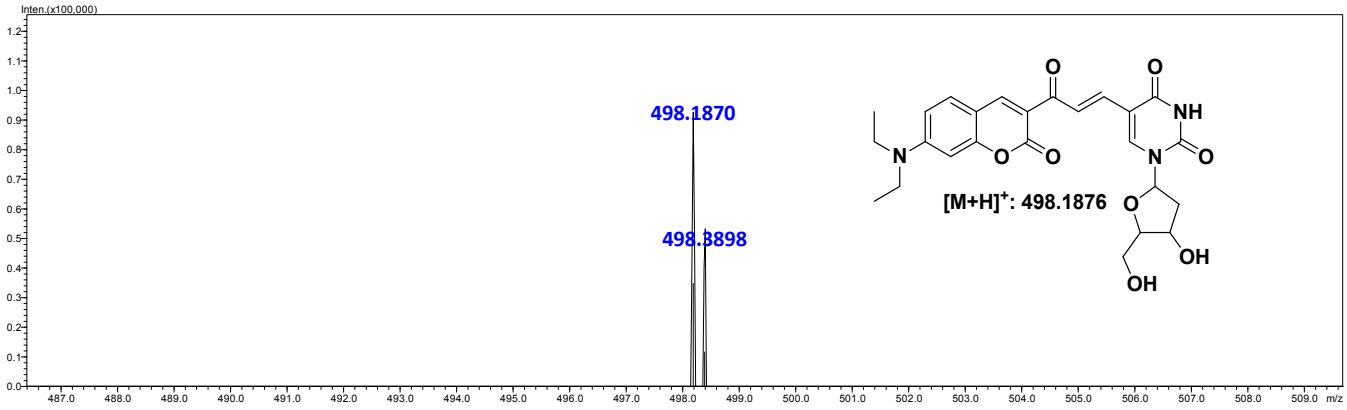
The ^{1}H NMR spectra of **5fU** in D_2O.



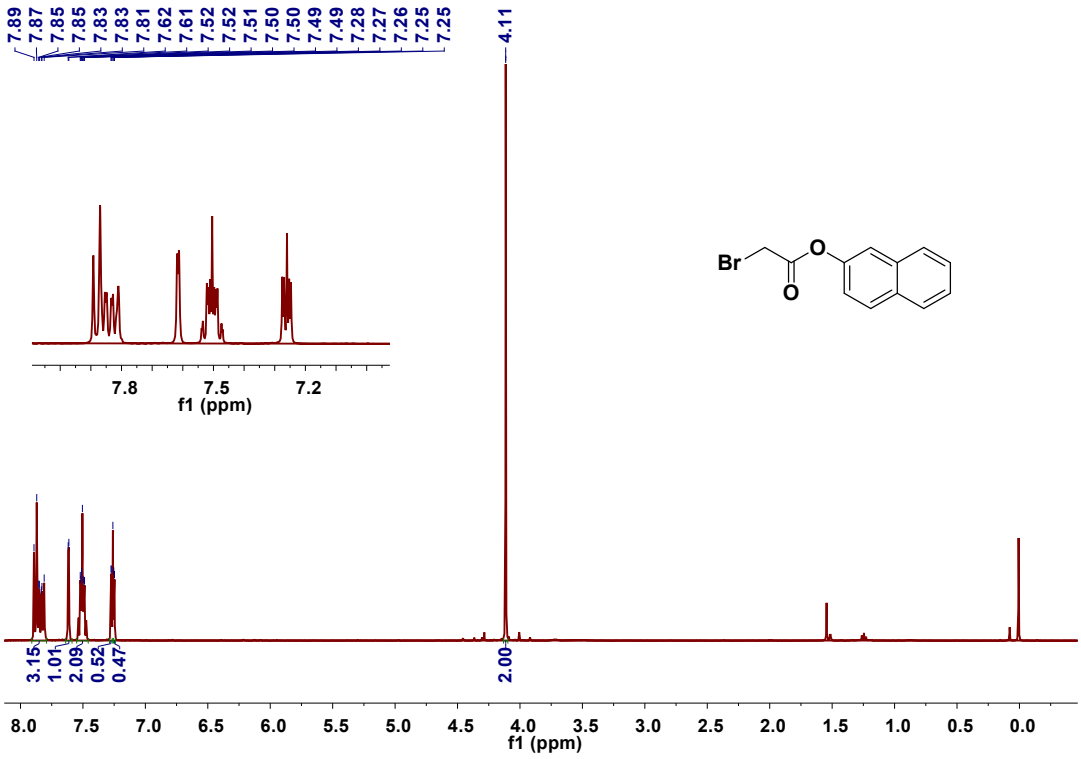
The ^{1}H NMR spectra of **YU-5fU** in DMSO-d6.



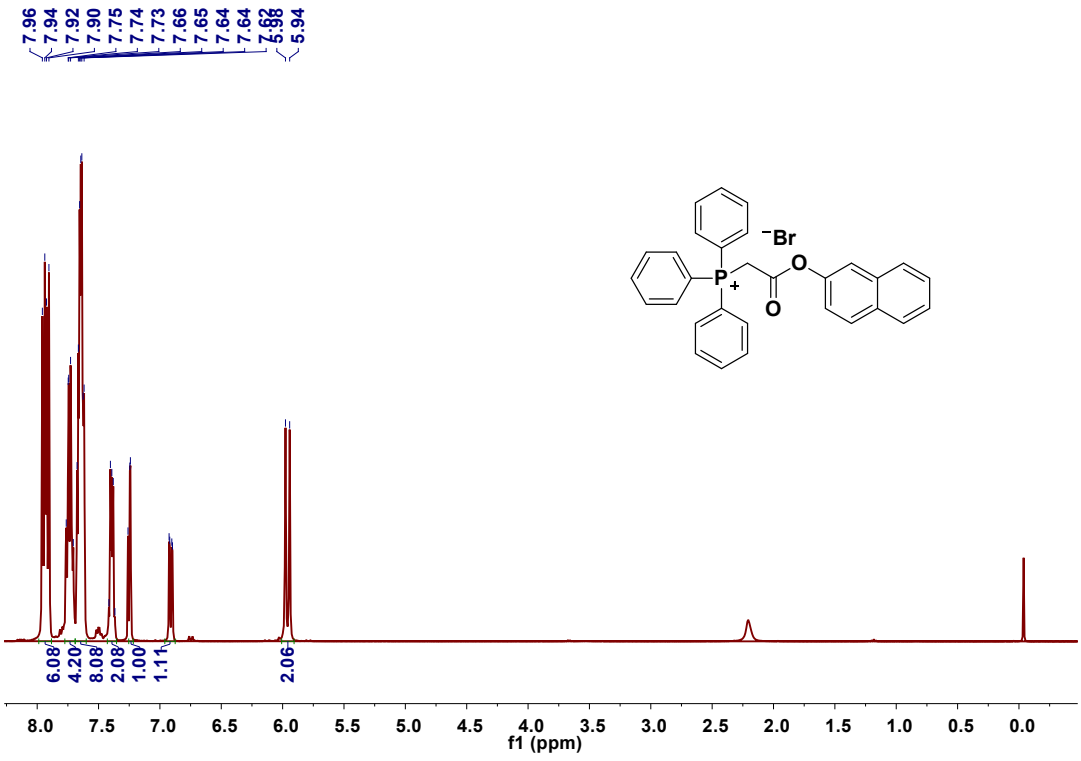
The ^{13}C NMR spectra of **YU-5fU** in DMSO-d6.



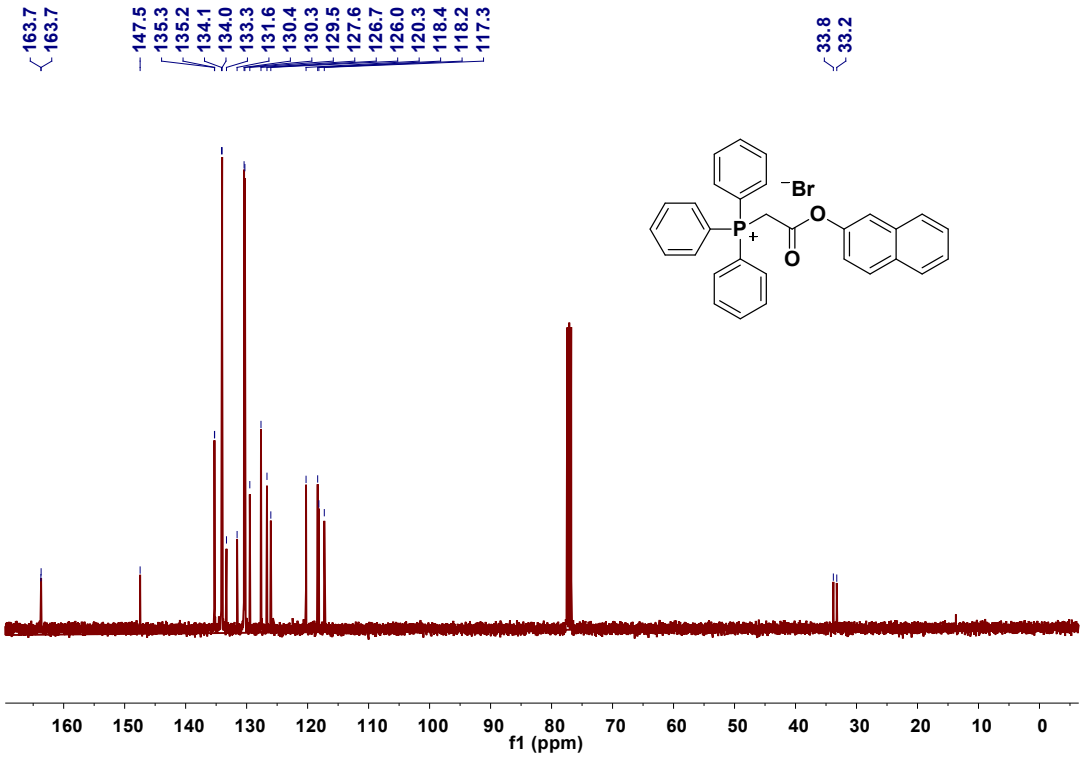
The ESI-MS spectra of **YU-5fU**.



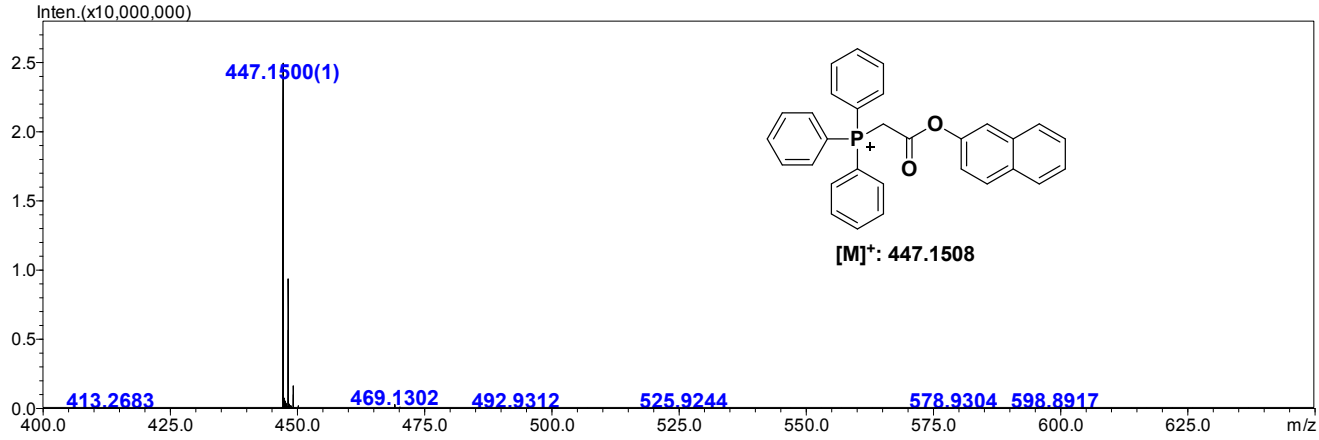
The ^{1}H NMR spectra of compound **3** in $CDCl_3$.



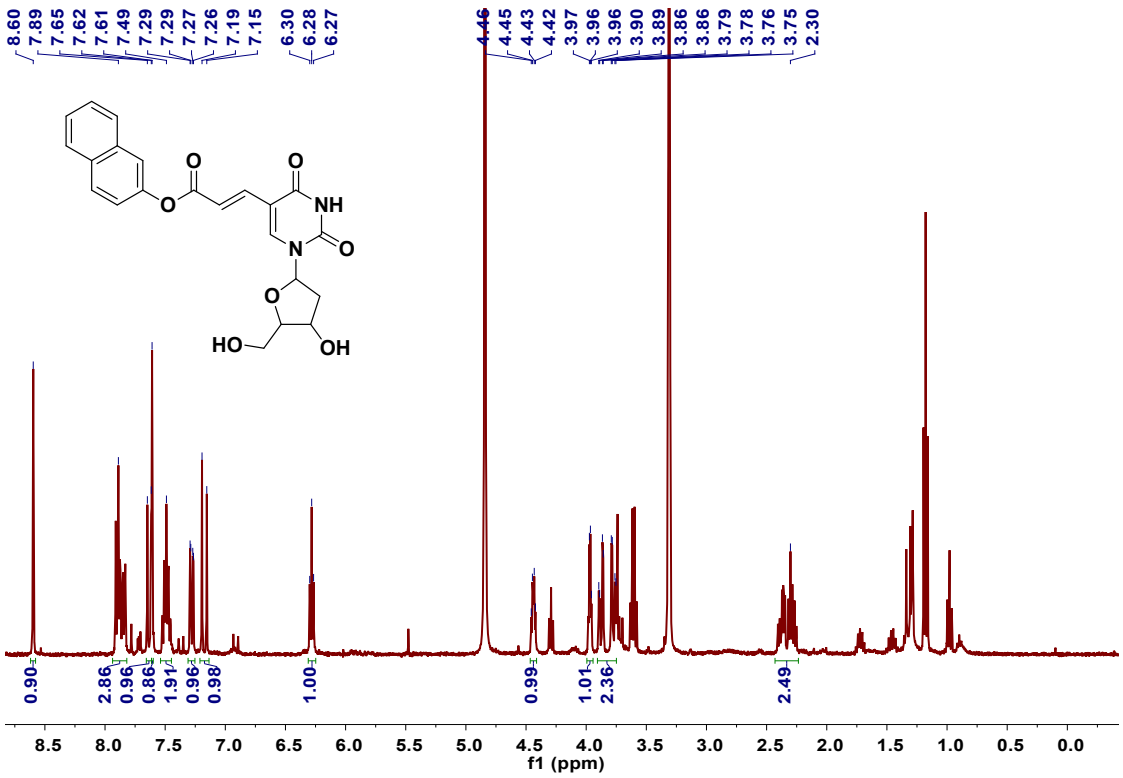
The ^{1}H NMR spectra of **YU-A** in $CDCl_3$.



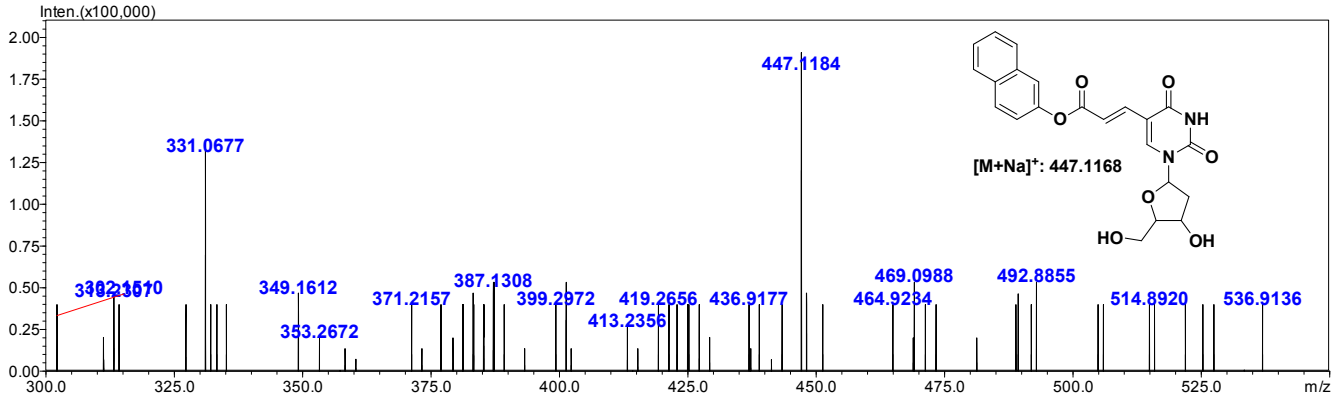
The ^{13}C NMR spectra of **YU-A** in $CDCl_3$.



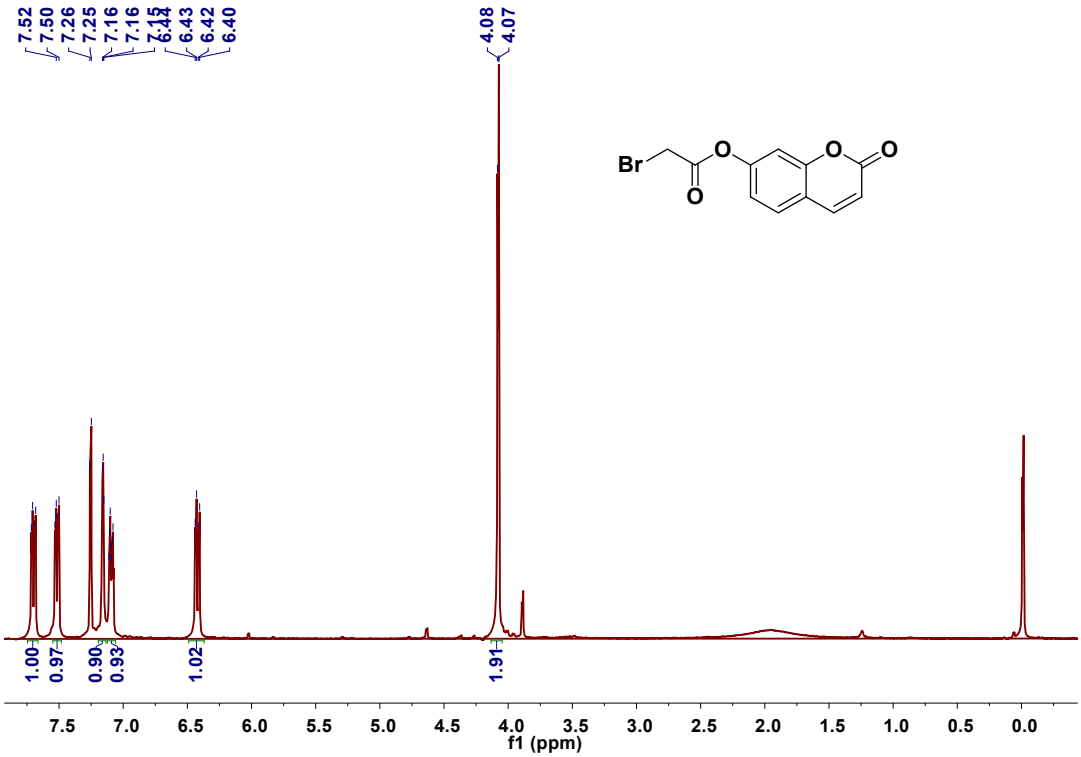
The ESI-MS spectra of **YU-A**.



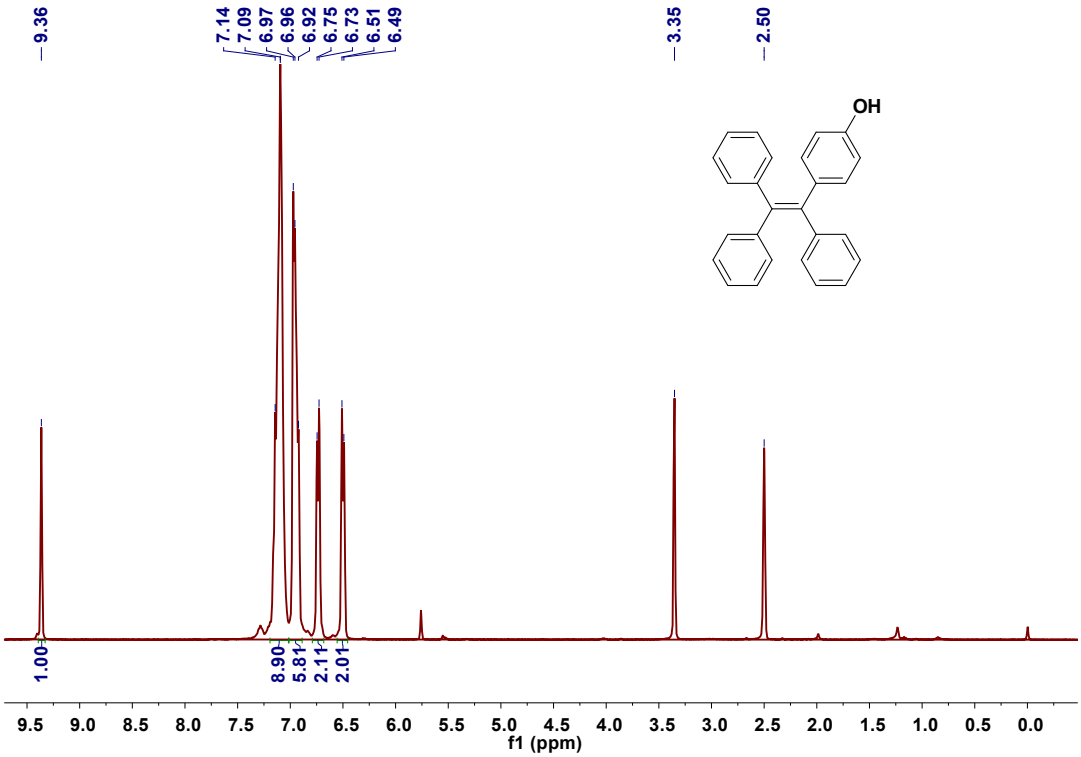
The ^{1}H NMR spectra of **YU-A-5fU** in CD_3OD.



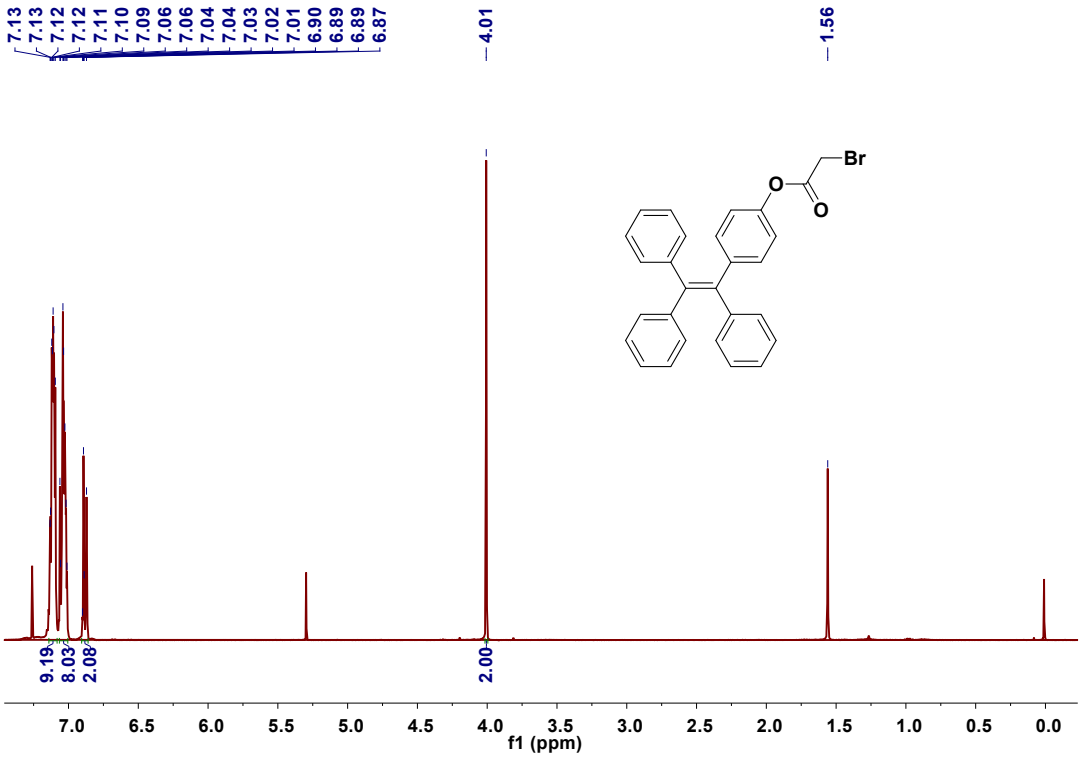
The ESI-MS spectra of **YU-A-5fU**.



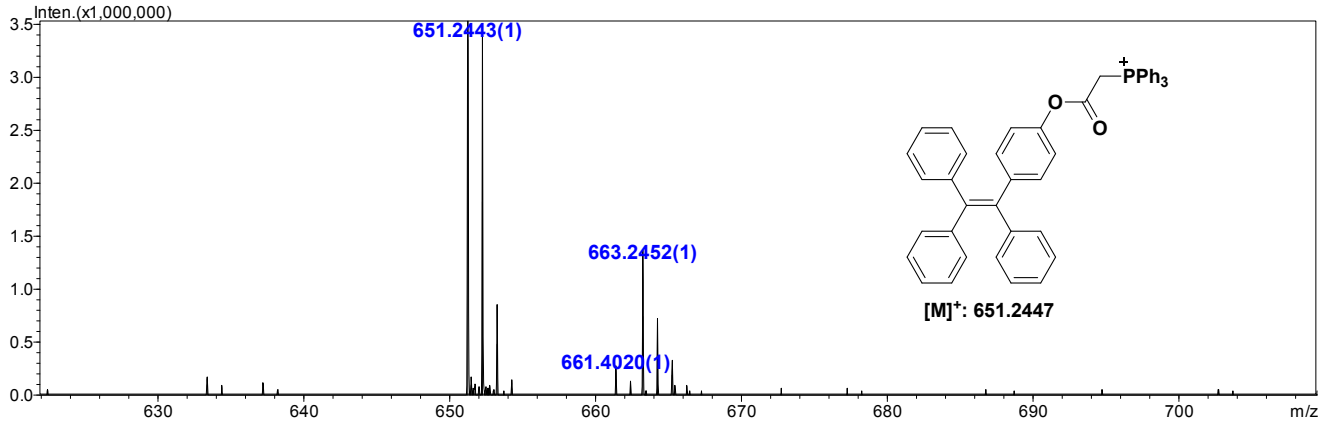
The ^{1}H NMR spectra of **compound 4** in $CDCl_3$.



The ^{1}H NMR spectra of **compound 5** in DMSO-d_6.



The ^{1}H NMR spectra of **compound 6** in $CDCl_3$.



The ESI-MS spectra of **YU-C**.

Supporting reference

S1 Wu, J. S.; Sheng, R. L.; Liu, W. M.; Wang, P. F.; Zhang, H. Y.; Ma, J. J. *Tetrahedron*, **2012**, *68*, 5458.

S2 Evans, V.; Duncanson, P.; Motevalli, M.; Watkinson, M. *Dyes Pigments*, **2016**, *135*, 36.

S3 Wang, C. J.; Xie, F.; Zhang, W. B. *Chinese J. Org. Chem.*, **2008**, *28*, 503.

S4 Guo, P.; Xu, X.; Qiu, X.; Zhou, Y.; Yan, S.; Wang, C.; Lu, C.; Ma, W.; Weng, X.; Zhang, X.; Zhou, X. *Org. Biomol. Chem.* **2013**, *11*, 1610.

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S6 Zhao, Q. L.; Li, K.; Chen, S. J.; Qin, A. J.; Ding, D.; Zhang, S. Z.; Liu, Y.; Liu, B.; Sun, J. Z.; Tang, B. Z. *J. Mater. Chem.* **2012**, *22*, 15128.