Supporting Information

A More Natural Strategy: 5-Formyluracil as a Cornerstone for Aluminum Detection in Vitro and in Vivo

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Materials and Instruments and methods

Materials and Instruments.

Unless otherwise stated, all chemical reagents were purchased from Sigma-Aldrich and used without further purification. HRMS was performed on Thermo Scientific™ Dionex Ultimate 3000 Hybrid LTQ Orbitrap Elite Velos Pro (Thermo Scientific, USA). 1H nuclear magnetic resonance (NMR), 13C NMR spectra were recorded on Varian Mercury 400 spectrometers, respectively. DNA concentration was quantified by NanoDrop 2000c (Thermo Scientific, USA). Gel images were collected by the Pharos FX Molecular imager (Bio-Rad, USA). The UV absorption spectra were acquired with Shimadzu UV-2550. The fluorescent emission spectra were excited by PerkinElmer LS 55 (PerkinElmer, USA) or Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The thin layer chromatography plates were monitored by a portable UV-lamp (GL-9406, Jiangsu, China). The Confocal fluorescence microscopy imaging were obtained from PerkinElmer UltraVIEW VoX system (PerkinElmer, Boston, United States of America).

Synthesis of Compound frU and TPP-frU

Synthesis of Compound 1. Compound 1 was prepared according to the literature.[1]

Synthesis of Compound frU. 5-formyl-2'-deoxyuridine (52 mg, 0.21 mmol) and furan-2-carbohydrazide (30 mg, 0.24 mmol) were added in 10 mL methanol in a 25 mL round bottom flask. The reaction mixture was kept stirring and stayed at 37°C for 1 h. The solvent was removed on a rotary evaporator and the residue was purified by silica gel chromatography (DCM/MeOH = 30:1) to give frU (138 mg, yield 95%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 11.75 (d, J = 41.7 Hz, 2H), 8.36 (s, 2H), 7.95 (d, J = 11.1 Hz, 1H), 7.23 (d, J = 33.7 Hz, 1H), 6.68 (s, 1H), 6.31 – 5.98 (m, 1H), 5.31 (d, J = 4.1 Hz, 1H), 5.02 (d, J = 33.8 Hz, 1H), 4.25 (s, 1H), 3.86 (s, 1H), 3.52 (d, J = 48.4 Hz, 2H), 2.29 – 2.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6), δ (ppm): 162.47, 154.46, 150.24, 146.99, 146.36, 141.78, 138.05, 115.23, 112.53, 108.48, 88.27, 85.78, 70.93, 61.79, 40.62. HRMS (ESI+) $C_{15}H_{17}N_4O_7^+$ $[M+H]^+$ calculated 365.10918, found 365.11115.

Synthesis of Compound 2. Compound 1 (100 mg, 0.36 mmol) and furan-2-carbohydrazide (54 mg, 0.43 mmol) were added in 10 mL methanol in a 25 mL round bottom flask. The reaction mixture was kept stirring and stayed at 37°C for 1 h. The solvent was removed on a rotary evaporator and the residue was purified by silica gel chromatography (DCM/MeOH = 30:1) to give compound 2 (133 mg, yield 95%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 11.73 (d, J = 101.4 Hz, 2H), 8.36 (s, 1H), 8.24 (s, 1H), 7.92 (s, 1H), 7.30 (s, 1H), 6.68 (s, 1H), 6.18 (s, 1H), 5.55 (s, 1H), 4.24 (s, 1H), 3.91 (s, 1H), 3.73 – 3.54 (m, 2H), 2.44 – 2.14 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6), δ (ppm): 162.49, 154.67, 150.04, 146.94, 145.33, 141.68, 138.76, 115.30, 112.52, 108.68, 85.98, 85.22, 70.75, 51.89, 39.67. HRMS (ESI^+) $C_{15}H_{16}N_7O_6^+$ $[M+H]^+$ calculated 390.11566, found 390.11450.

Synthesis of Compound TPP-frU. Compound 2 (20 mg, 0.05 mmol), pent-4-yn-1-yltriphenylphosphonium iodide (25 mg, 0.05 mmol), sodium ascorbate (8 mg, 0.04 mmol) and CuSO4·5H2O (4 mg, 0.02 mmol) were suspended in a mixture of DMF and water (v/v=5 mL/5 mL) in a 25 mL round bottom flask. The reaction mixture was kept stirring and stayed at 60°C for 24 h under an Ar atmosphere. The solvent was removed on a rotary evaporator and the residue was purified by silica gel chromatography (DCM/MeOH = 30:1) to give TPP-frU (18 mg, yield 50%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 11.90 (s, 1H), 11.77 (s, 1H), 8.34 (s, 1H), 8.17 (s, 1H), 8.12 (s, 1H), 7.88 (s, 4H), 7.76 – 7.70 (m, 13H), 7.22 (d, J = 3.0 Hz, 1H), 6.68 (s, 1H), 6.12 (t, J = 6.6 Hz, 1H), 4.71 (dd, J = 14.2, 3.3 Hz, 1H), 4.59 (dd, J = 14.2, 8.8 Hz, 1H), 4.32 – 4.22 (m, 2H),

3.57 (d, J = 10.5 Hz, 2H), 2.82 (d, J = 6.7 Hz, 2H), 2.29 – 2.18 (m, 2H), 1.88 – 1.80 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6), δ (ppm): 162.42, 158.69, 154.69, 150.05, 146.75, 145.48, 141.57, 135.42, 133.97, 130.64, 124.27, 119.18, 118.33, 115.42, 112.75, 108.54, 86.61, 85.92, 71.52, 46.02, 25.79, 22.51, 20.71, 20.03. 31P NMR (162 MHz, DMSO-d6), δ (ppm): 23.85. HRMS (ESI+) $C_{38}H_{37}N_7O_6P^+$ $[M\text{-}I]^+$ calculated 718.25374, found 718.25163.

Cell Culture.

HeLa cells were from Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (Hyclone, China supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37 °C and incubated in an atmosphere of 5/95 (v/v) of CO_2/air. Before imaging, cells were placed at 25-Petri dishes and allowed to adhere for 12 hours.

MTT assays.

HeLa cells were used to test the cytotoxicity of probe TPP-frU according to the MTT assay. In general, cells were seeded in 96-well plates at a density of 10^3-10^4 cells per well and cultured for 12 h. Then the primary cell culture medium was replaced by 100 μL fresh medium with different concentration of TPP-frU and incubated for another 24 h. After that, 10 μL of MTT solution (5 mg/mL in PBS) was added into each well maintained at 37 ℃ for 4 hours. Then the medium containing MTT in each well was removed, followed by adding 100 μL of DMSO to dissolve the purple crystals. The optical density readings at 492 nm were recorded using a plate reader. Each experiment was performed with 5 wells parallel. The cell viability was expressed by the average values ± standard deviation (SD).

Confocal imaging of TPP-frU in living cells.

HeLa cells were passed and plated into a 35 mm confocal dish (Nest, China) for 12 h. For labelling, the cells were washed with DMEM without serum for three times. Then incubated with Al3+ for 2 h before adding 300 μM TPP-frU for 3 h at 37 °C. And the cells were washed with PBS buffer for three times before confocal imaging.

ODN-5fU labeling reaction conditions.

Generally, ODN-5fU labeling reaction can be divided into two steps. Firstly, ODNs (100 μM, 2 μL) containing 5fU were performed in NaOAc buffer (1 M, pH = 5.0, 10 μL) with 2-furoic hydrazine (10 mM in water, 2 μL) at 37 oC for 4 h in a 1.5 mL tube in a thermo-shaker (Ningbo Biocotek Scientific Instrument Co., Ltd., China, 1200 r.p.m.). Then the DNA without purification was analyzed with RP-HPLC chromatography (Shimadzu LC-6AD) at 260 nm. Column: ODS-SP column (5 μm, 250 mm ×4.6 mm) (GL Science Inc., Japan); Eluent: mobile phase A (100 mM TEAA buffer, pH 7.0) and B (acetonitrile); Concentration of B: 5%-30%/0-30 min; Flow rate: 1.0 mL·min–1; Column oven: 35 °C. Or after purification with the cold ethanol precipitation, characterized with 20% denaturing polyacrylamide gel electrophoresis (PAGE).

Enzymatic digestion of ODNs.

DNA (100 μM, 8μL), Degradase Plus (1 μL), 10× Degradase Plus reaction buffer (2.5 μL) (Zymo Research), ddH2O (13.5 μL). The mixture was incubation at 37 °C for 2 h to digest the DNA to constituent nucleosides. The nucleoside mixture was under high speed centrifugation (12000 g, 10 min.) to remove the enzymes.

Table S1. Models of oligonucleotides sequences.

Oligomer	**Sequence (from 5'to 3')**
ODN-T	GACTCAATAGCCGTA
ODN-5fU	GACTCAA5fUAGCCGTA
ODN-5fC	GACTCAA5fCAGCCGTA
ODN-AP	GACTCAAAPAGCCGTA

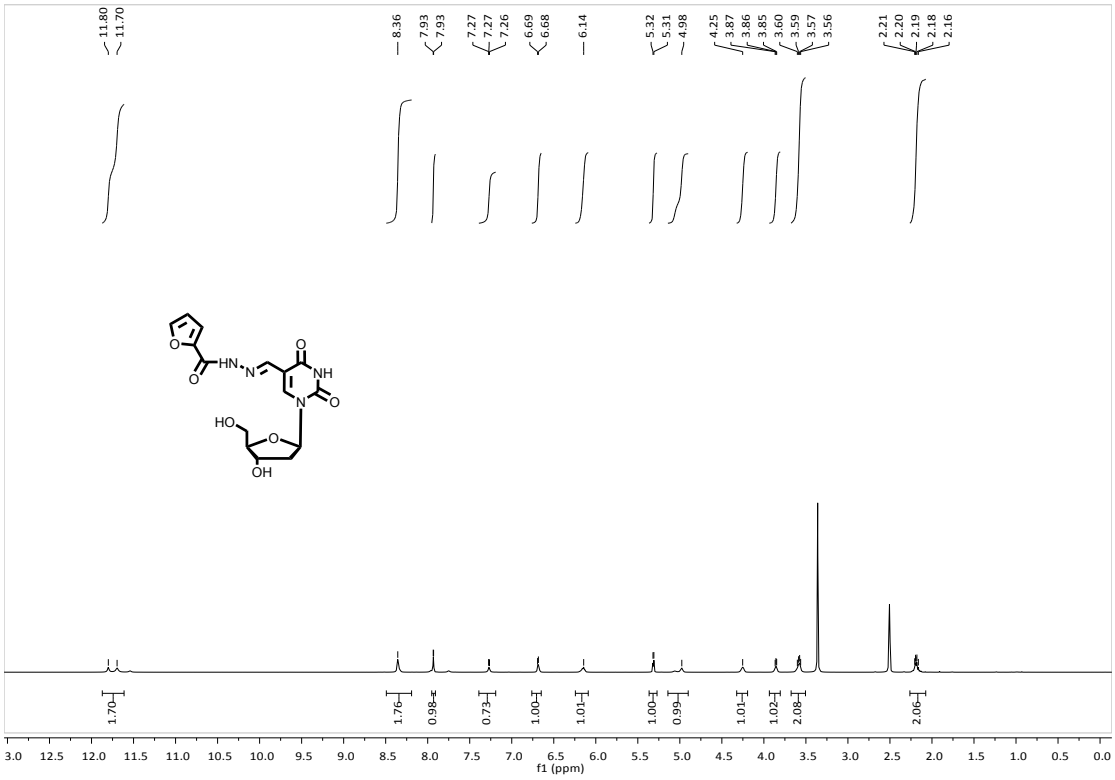


Figure S1. The ^{1}H NMR spectrum (400 MHz, in d6-DMSO) of frU.

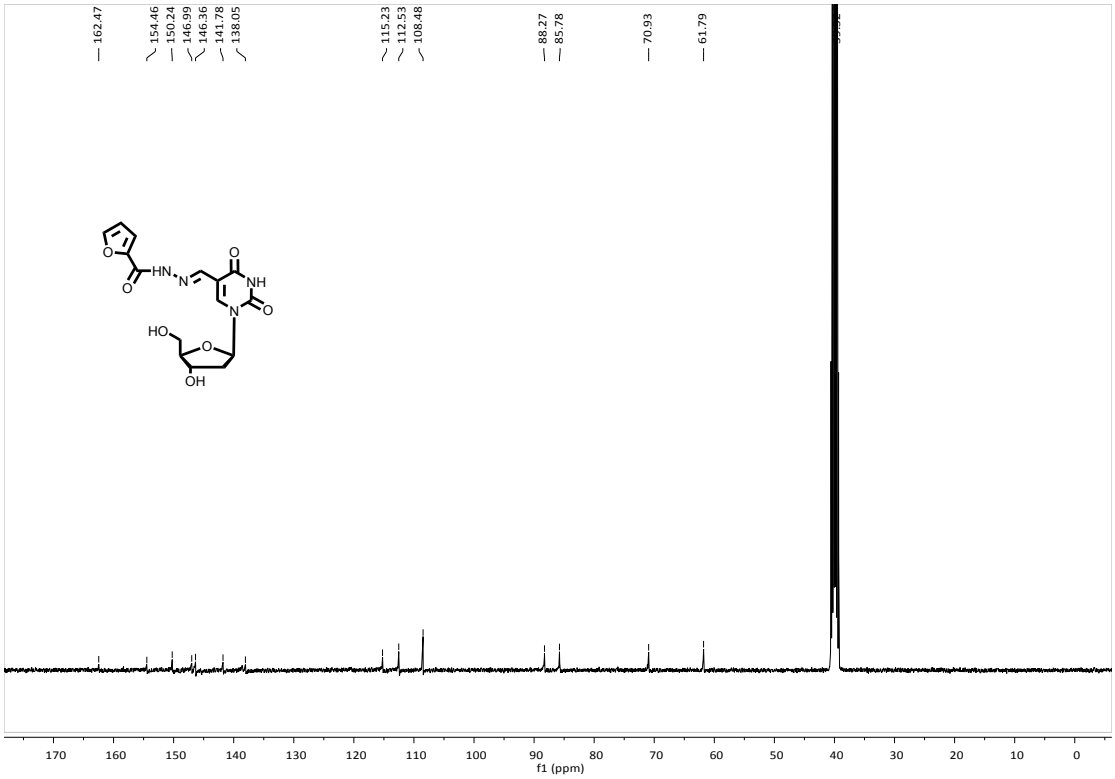


Figure S2. The ^{13}C NMR spectrum (400 MHz, in d6-DMSO) of frU.

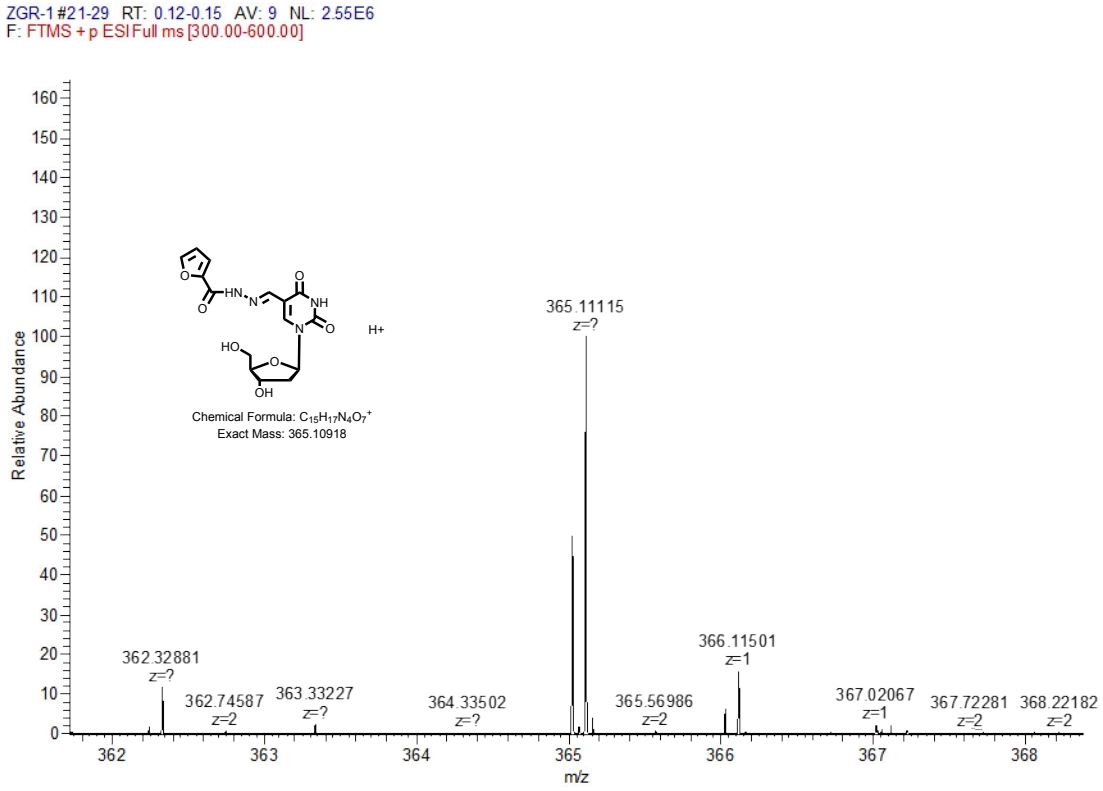


Figure S3. The HRMS spectrum of frU.

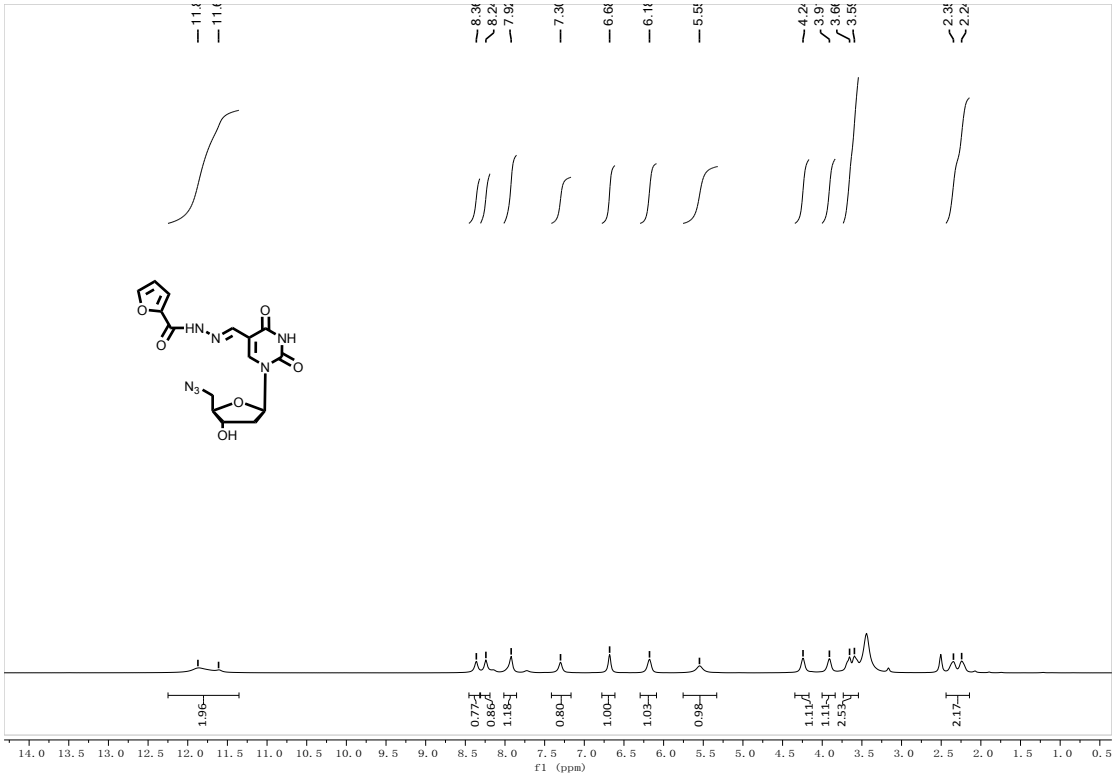


Figure S4. The ^{1}H NMR spectrum (400 MHz, in d6-DMSO) of compound 2.

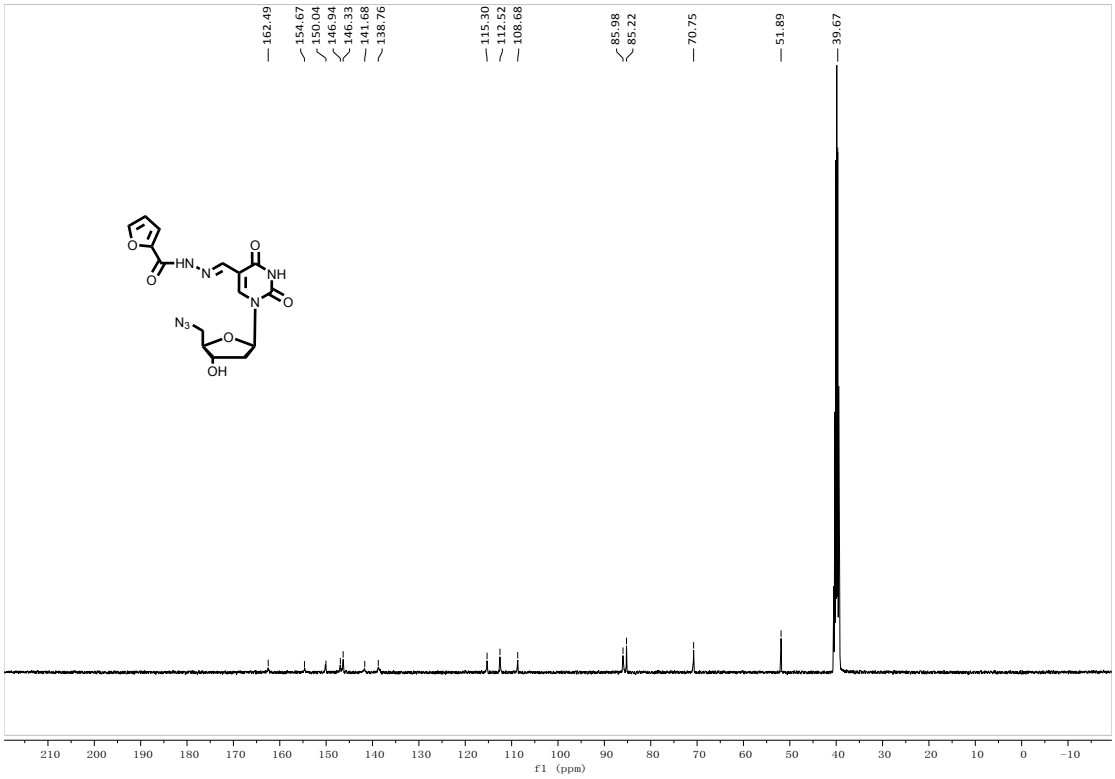


Figure S5. The ^{13}C NMR spectrum (400 MHz, in d6-DMSO) of compound 2.

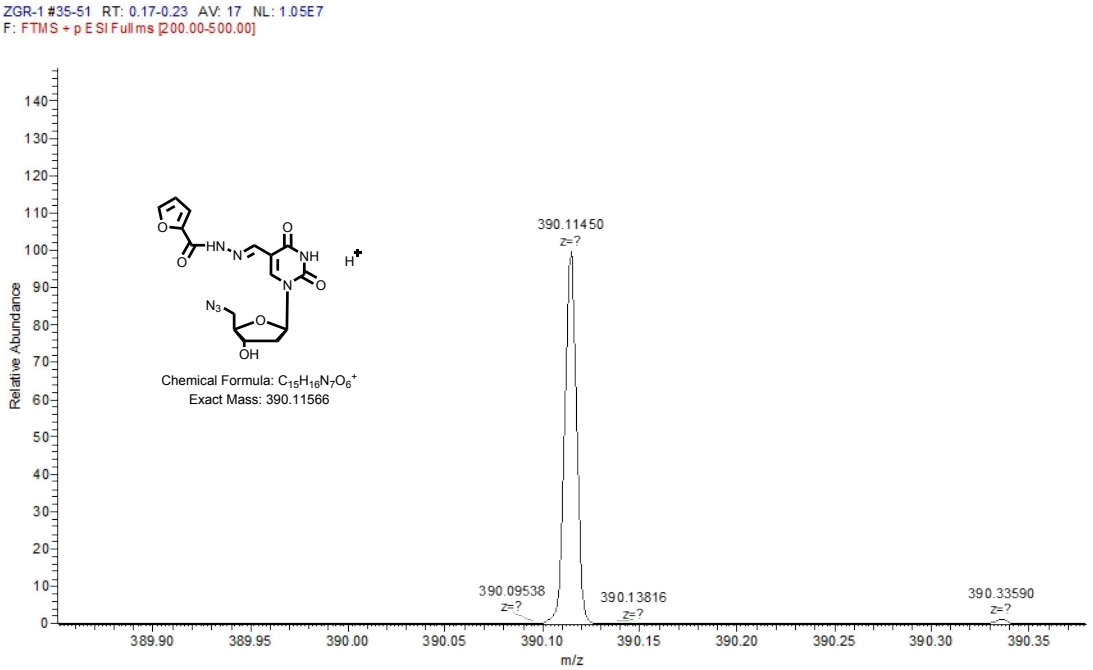


Figure S6. The HRMS spectrum of compound 2.

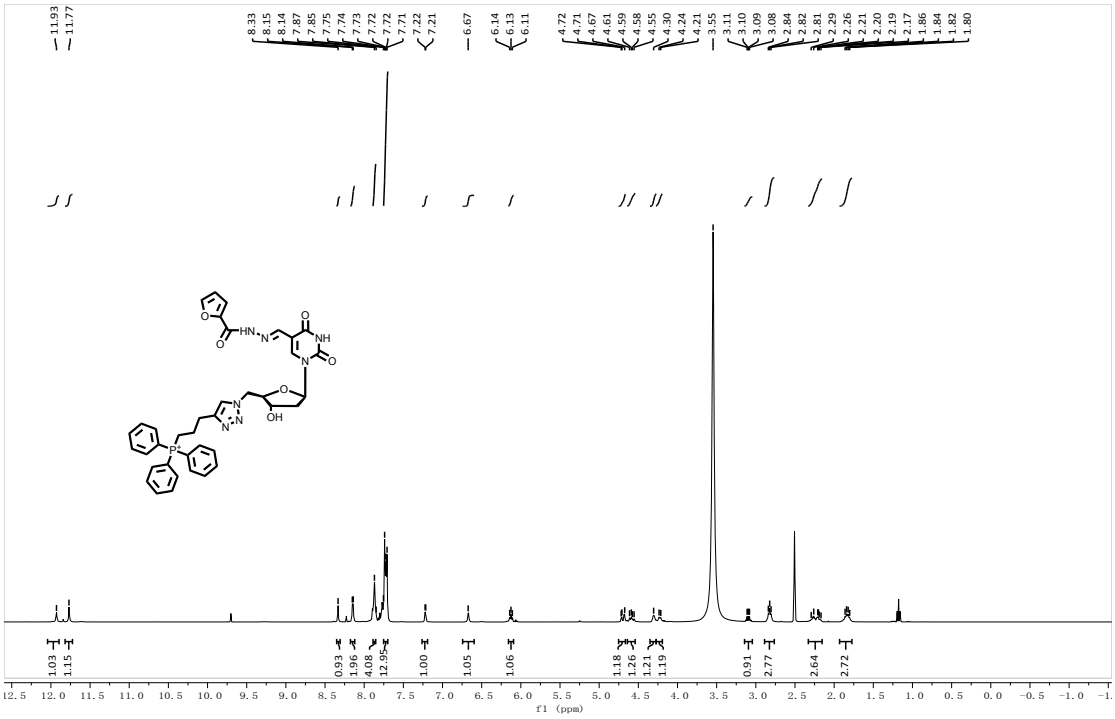


Figure S7. The ^{1}H NMR spectrum (400 MHz, in d6-DMSO) of TPP-frU.

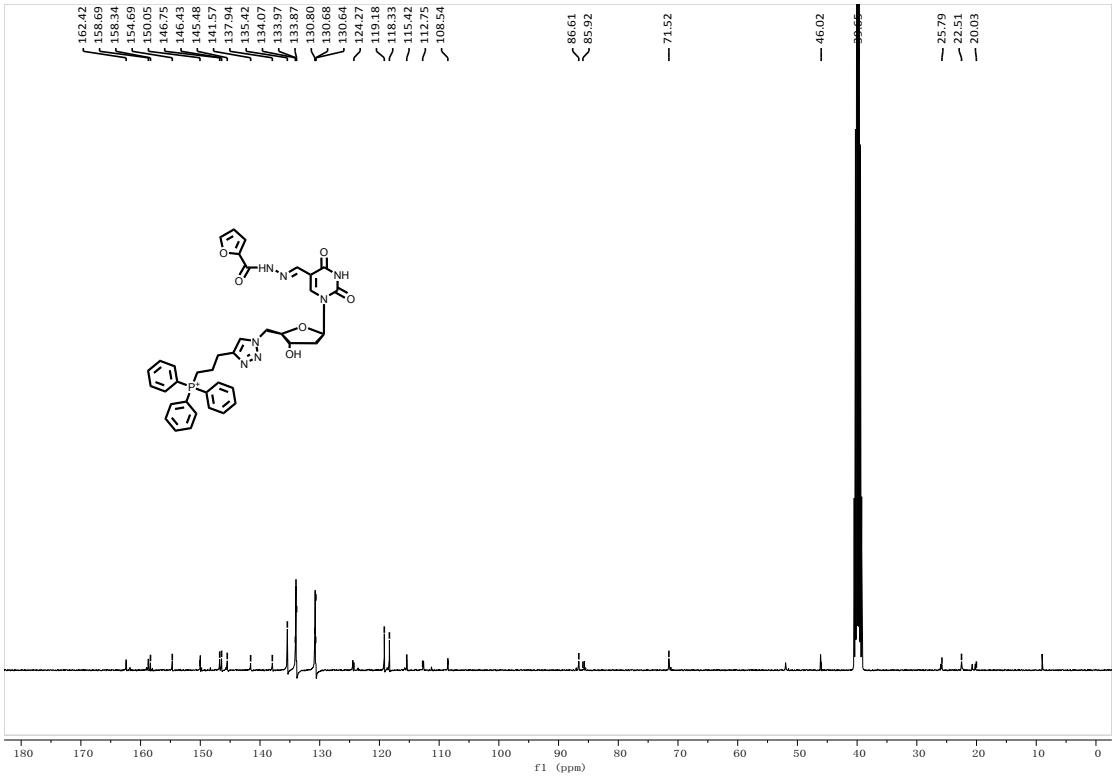


Figure S8. The ^{13}C NMR spectrum (400 MHz, in d6-DMSO) of TPP-frU.

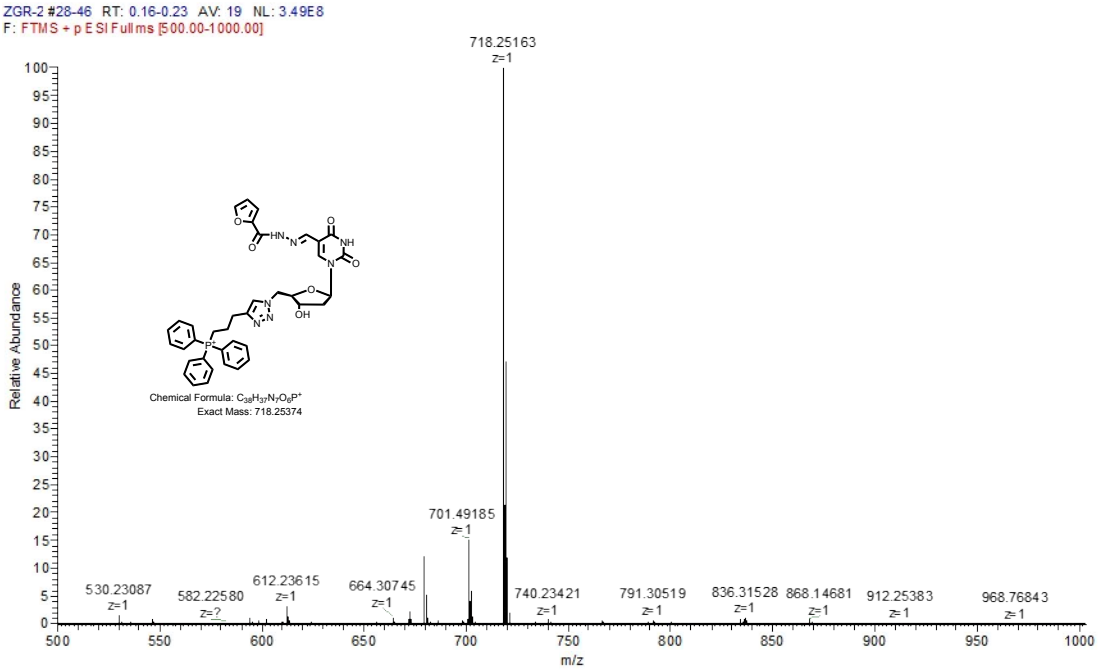


Figure S9. The HRMS spectrum of TPP-frU.

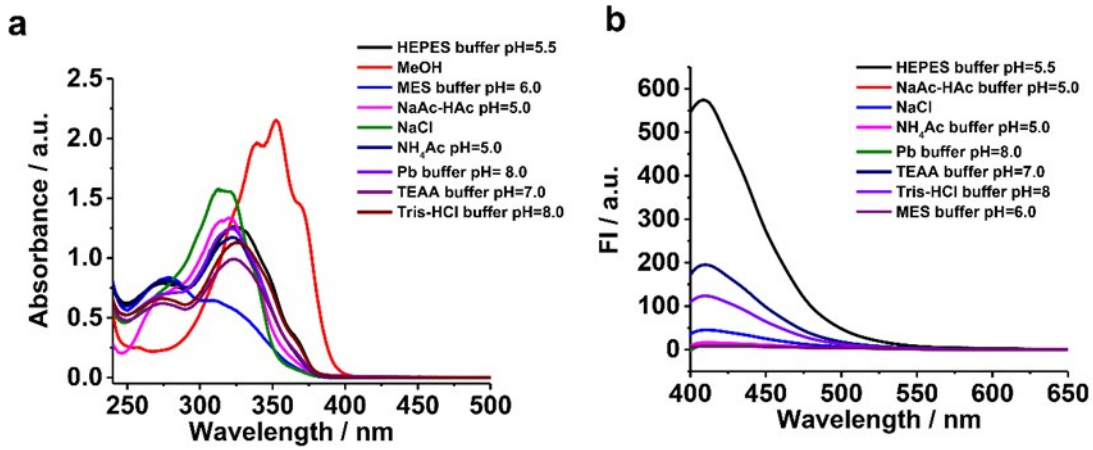


Figure S10. (a) UV absorption spectra and (b) Fluorescent emission spectra of the compound frU (10 mM, 1 µL) with Al^{3+} (100 mM, 1 µL) which was added into 398 µL various solvents.

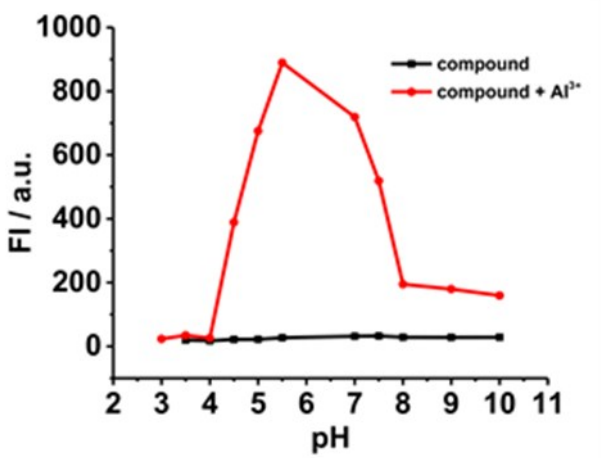


Figure S11. Effect of pH on the emission intensity TPP- frU and its Al^{3+} complex at 410 nm.

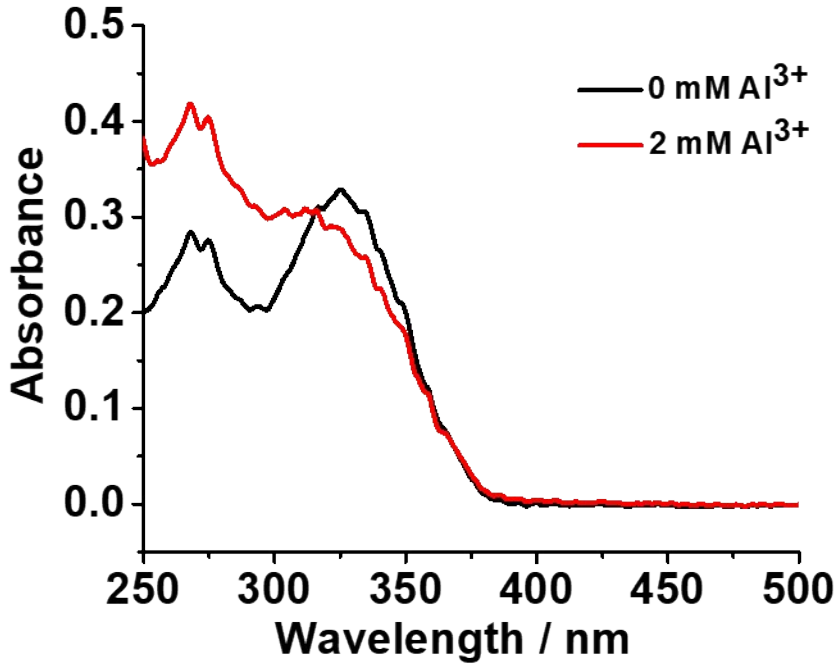


Figure S12. The absorbance spectrum of frU with or without addition of Al^{3+}.

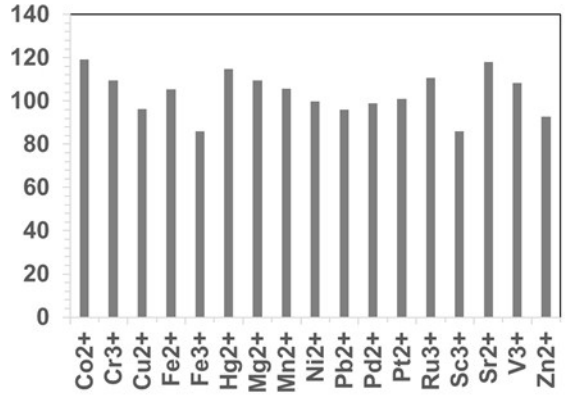


Figure s13. The selectivity of TPP-frU toward Al^{3+} over various biological relevant metal ions was investigated. Other potential competitive metal ions can't affect the response of TPP-frU to Al^{3+} via the fluorescence spectra. The fluorescence spectra of TPP-frU and Al^{3+} complex after addition of potential competitive metal ions.

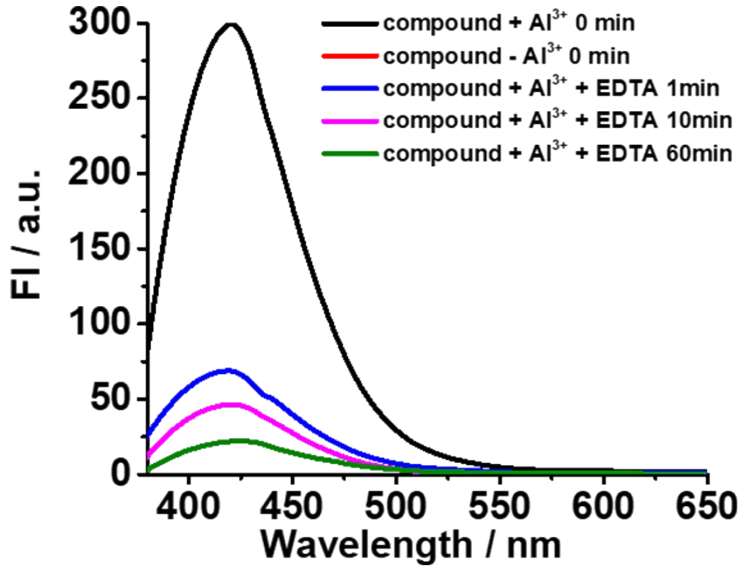


Figure 14. The fluorescence turn-on signal of the TPP-frU- Al^{3+} complex was reversible upon addition of 10 equiv. of EDTA.

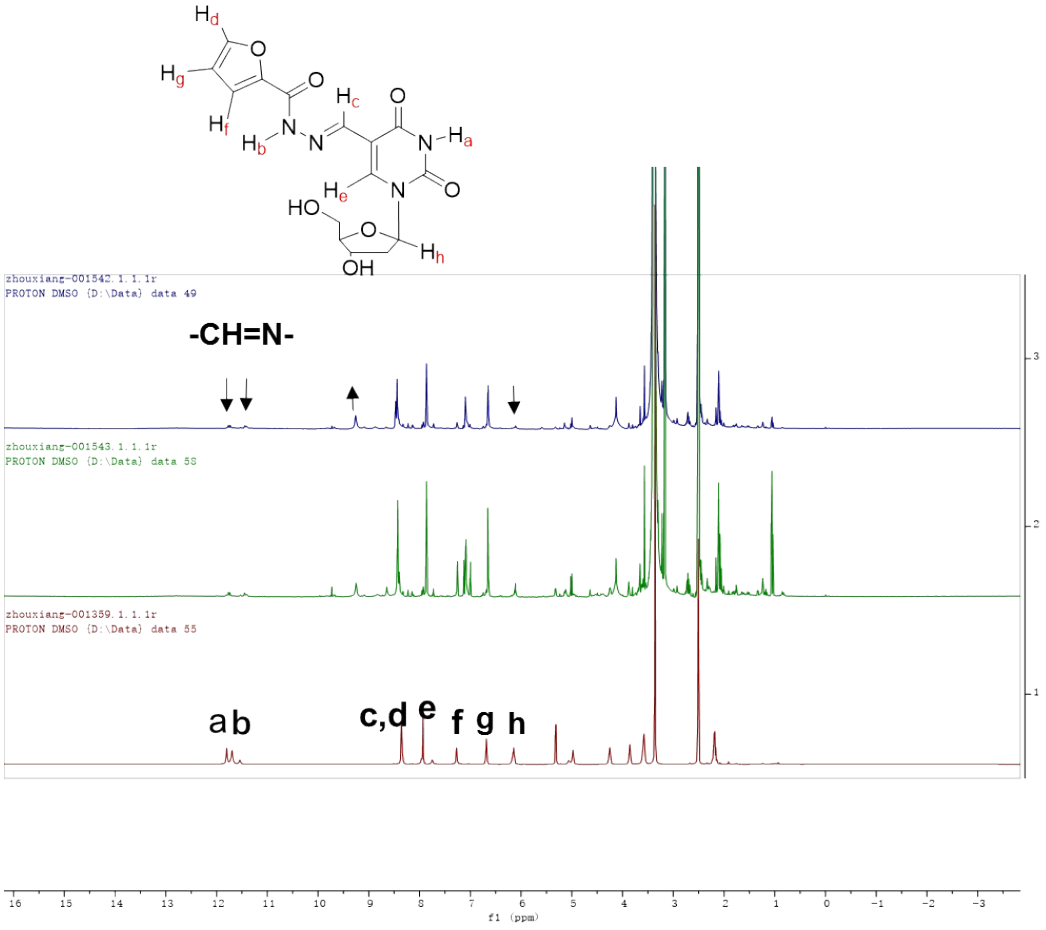


Figure s15. ^{1}H NMR of probe in d6-DMSO upon addition of various equiv. of Al^{3+}.

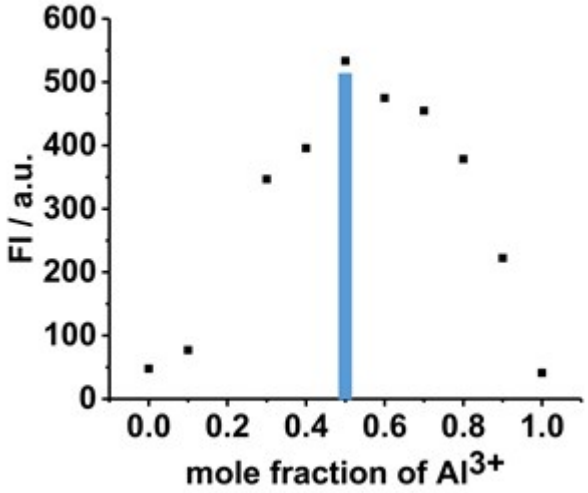
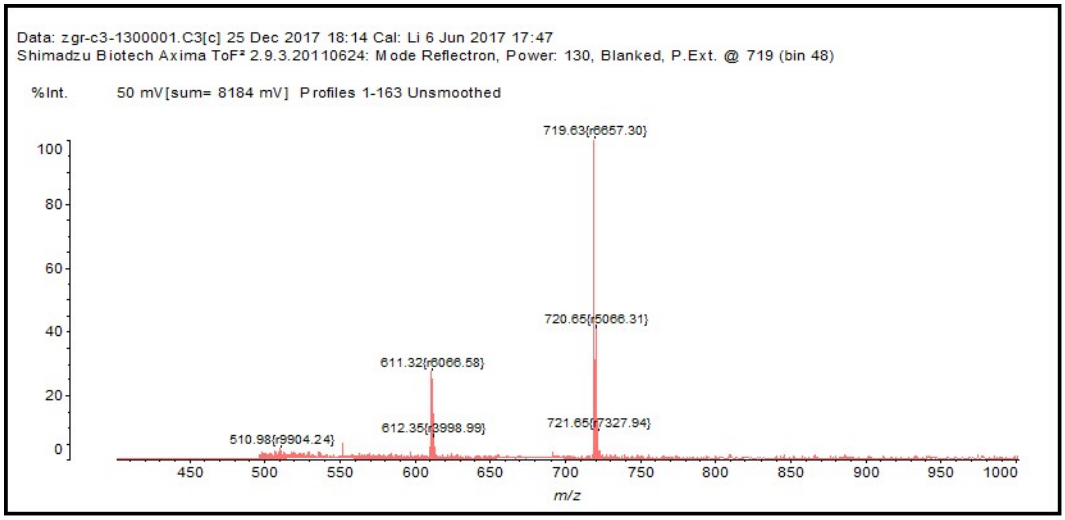


Figure S16. The Job's plot examined between TPP- frU and Al^{3+} by fluorescence.

(a) TPP-frU calculated 718.25, found 719.63.



(b) TPP-frU + Al^{3+} + $2H_2O$ complex, calculated 781.25, found 780.33.

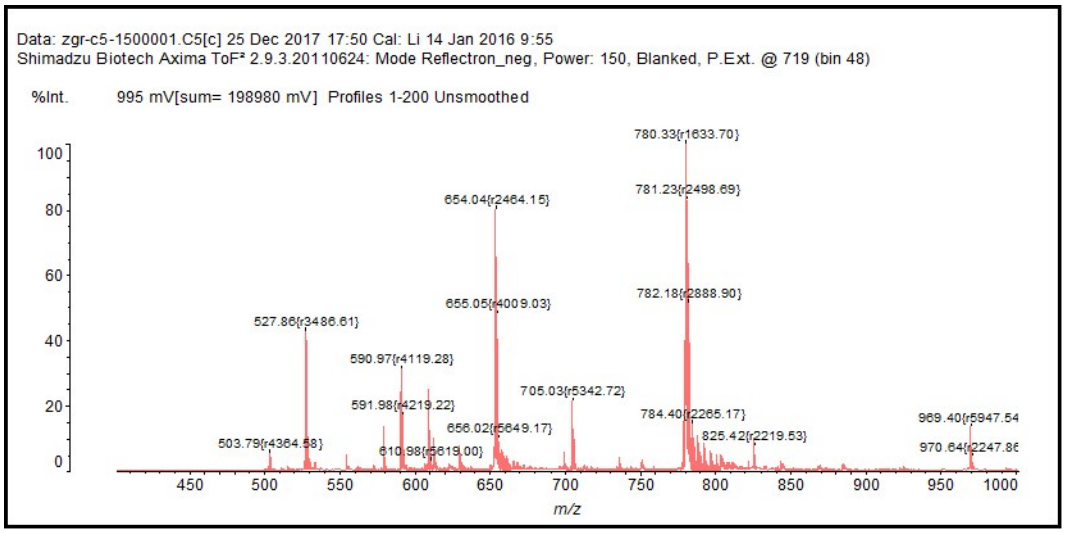


Figure S17. MALDI-TOF Mass Spectra.

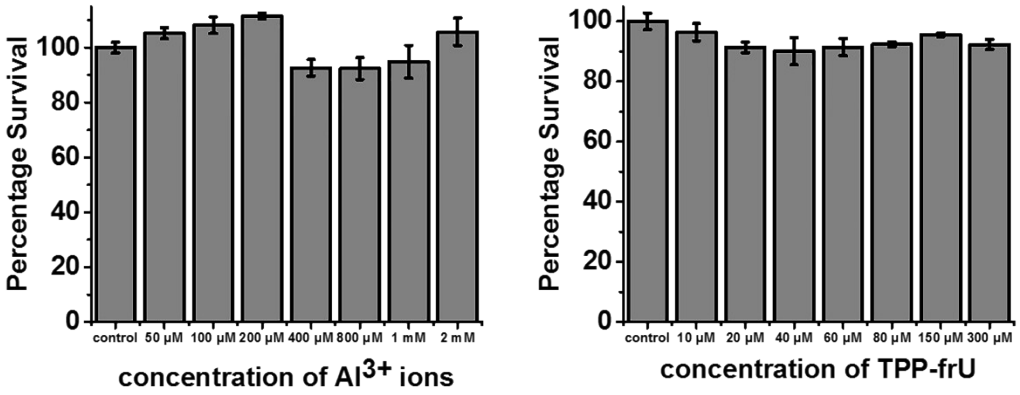


Figure s18. Cell viability of HeLa cells after incubation with different concentrations of Al^{3+} or TPP-frU for 24 h.

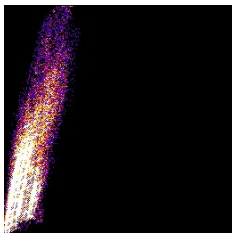


Figure S19. Colocalization scatter plot for TPP-frU in mitochondria of HeLa cells.

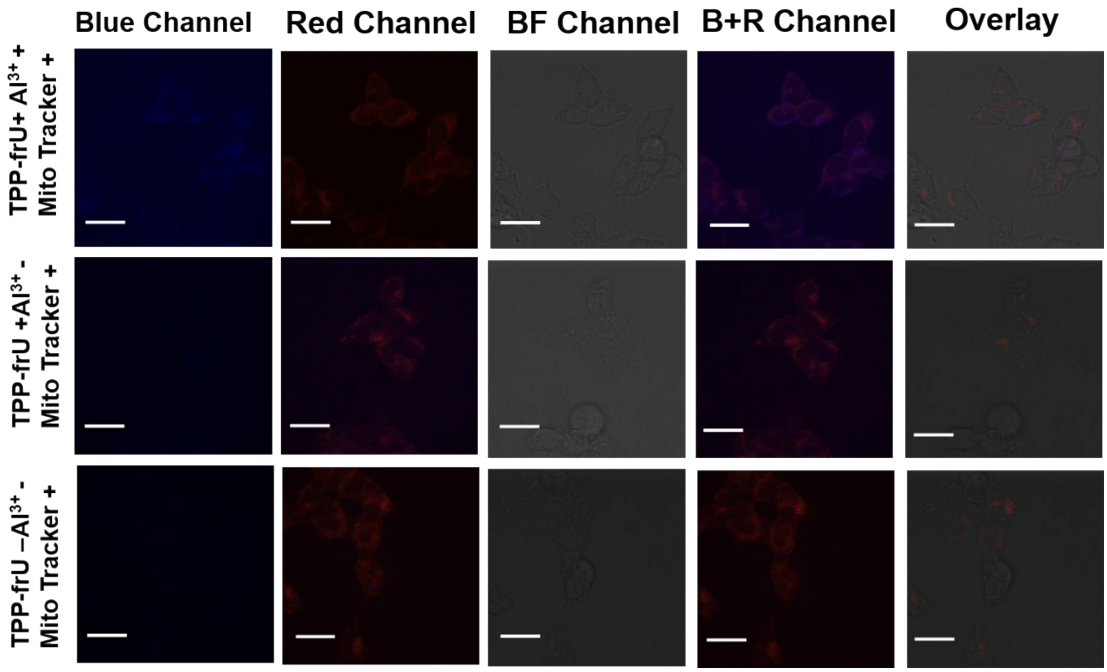


Figure S20. Fluorescent images of 293T cells stained with TPP-frU (blue light) and overlapped with Mito-Tracker (red light). All images share the same scale bar of 24 μm.

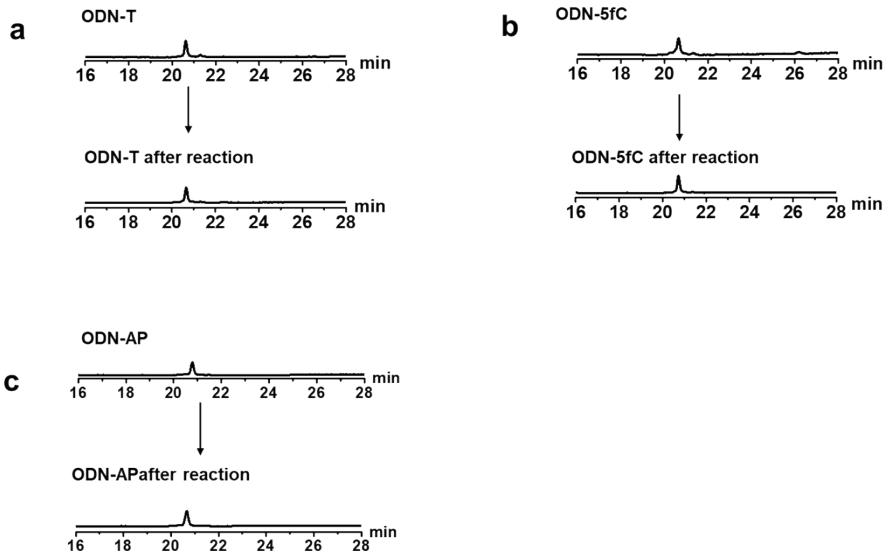


Figure S21. RP-HPLC trace at λ=260 nm of ODN-T, ODN–5fC, ODN-AP which was generated by the reaction fr under optimized conditions.

5’-GACTCAA5fUAGCCGTA-3’ 5’- GACTCAAfrUAGCCGTA-3’

calculated 4680.8, found 4680.4. calculated $[M+K^+]^+$ 4719.7, found 4718.1

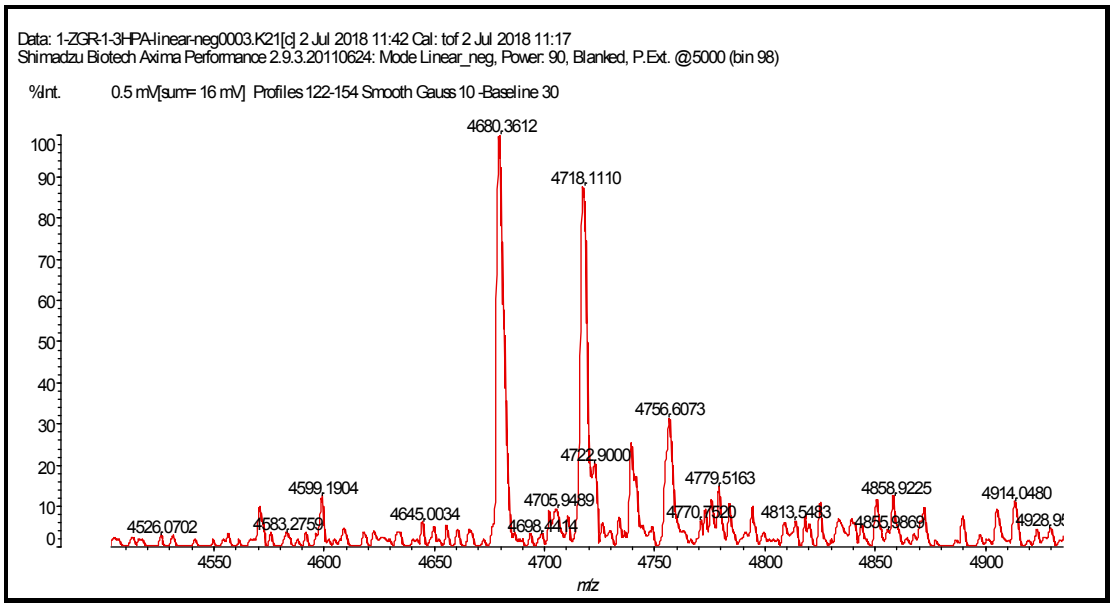
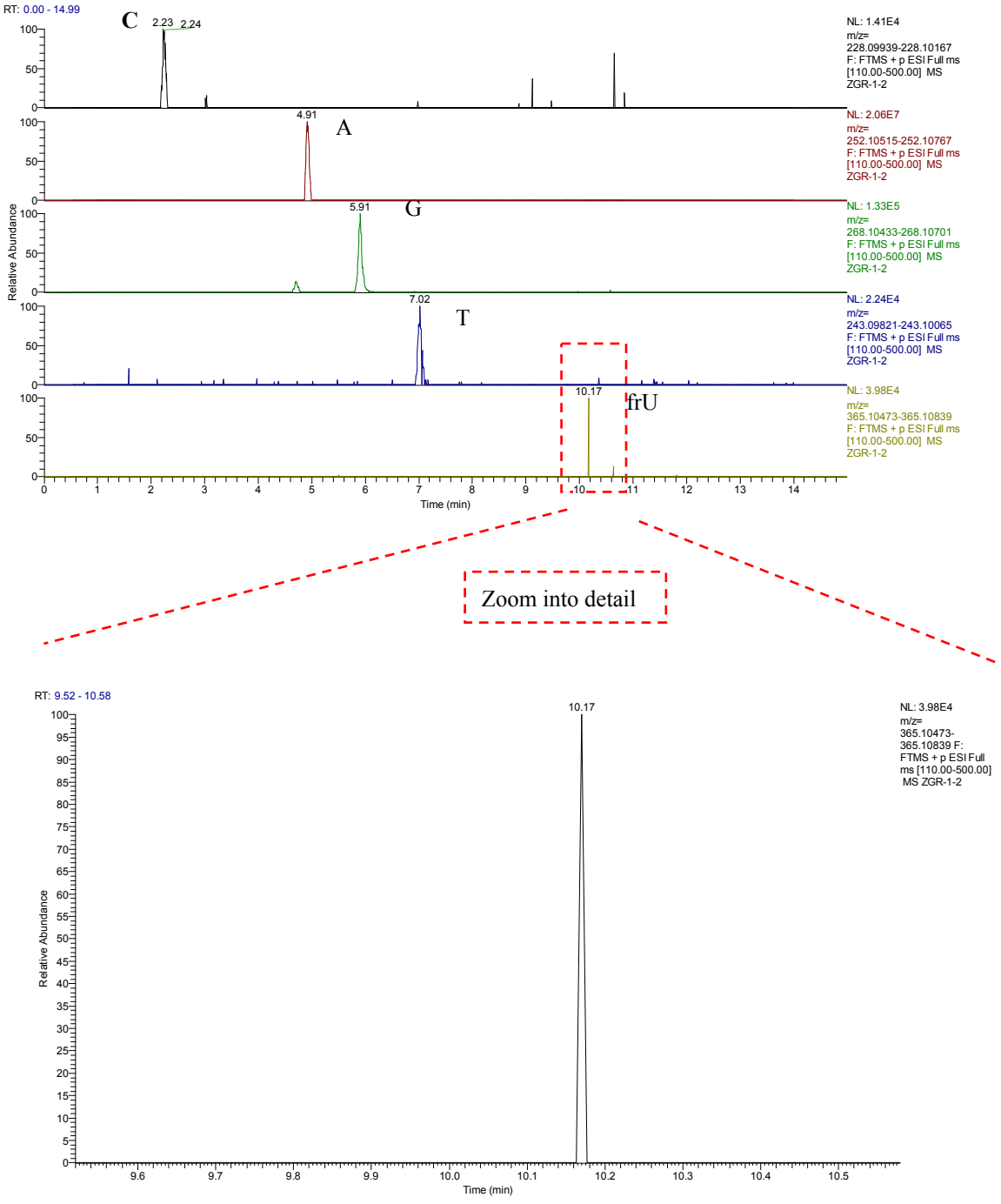


Figure S22. DNA MALDI-TOF Mass Spectrum. MALDI-TOF-spectrum of ODN-5fU after incubation with 2-furoic hydrazine.

(a)


RT: 0.00 - 14.99
C
2.23
2.24
NL: 1.41E4
m/z=
228.09939-228.10167
F: FTMS + p ESI Full ms
[110.00-500.00] MS
ZGR-1-2
4.91
A
NL: 2.06E7
m/z=
252.10515-252.10767
F: FTMS + p ESI Full ms
[110.00-500.00] MS
ZGR-1-2
5.91
G
NL: 1.33E5
m/z=
268.10433-268.10701
F: FTMS + p ESI Full ms
[110.00-500.00] MS
ZGR-1-2
7.02
T
NL: 2.24E4
m/z=
243.09821-243.10065
F: FTMS + p ESI Full ms
[110.00-500.00] MS
ZGR-1-2
10.17
frU
NL: 3.98E4
m/z=
365.10473-365.10839
F: FTMS + p ESI Full ms
[110.00-500.00] MS
ZGR-1-2
Relative Abundance
Time (min)
Zoom into detail
RT: 9.52 - 10.58
10.17
NL: 3.98E4
m/z=
365.10473-
365.10839 F:
FTMS + p ESI Full
ms [110.00-500.00]
MS ZGR-1-2
Relative Abundance
Time (min)

(b)

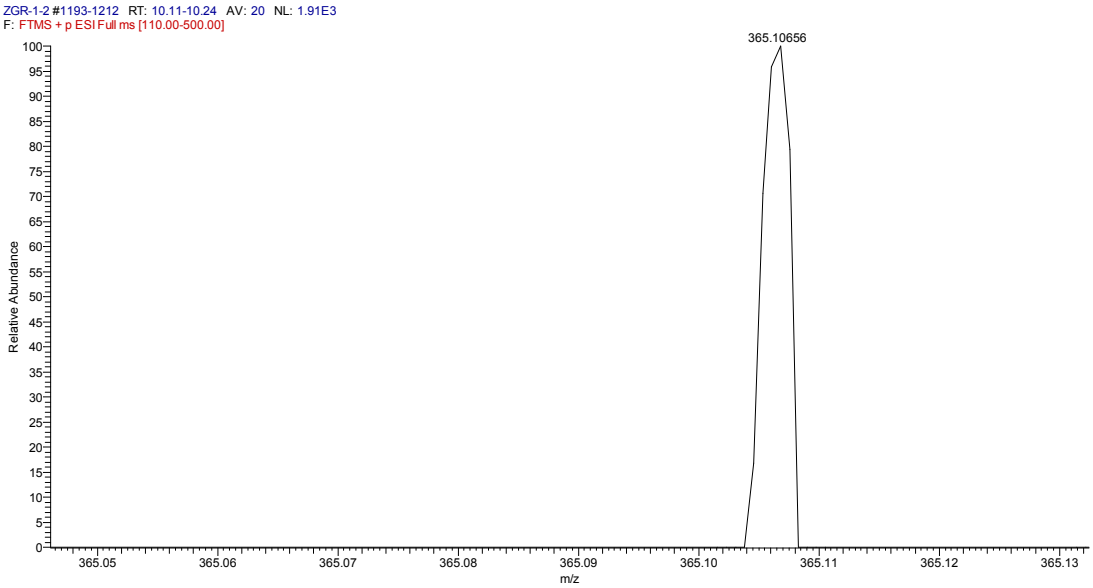


Figure S23. a) HPLC-MS extracted $[M+H]^+$ ion count for A, T, C, G, frU deoxynucleosides after digestion of DNA after labeled by fr. b) HRMS (ESI^+) of frU in HPLC-MS after digestion, HRMS $C_{15}H_{17}N_4O_7^+$ $[M+H]^+$ calculated 365.32145, found 365.10656.

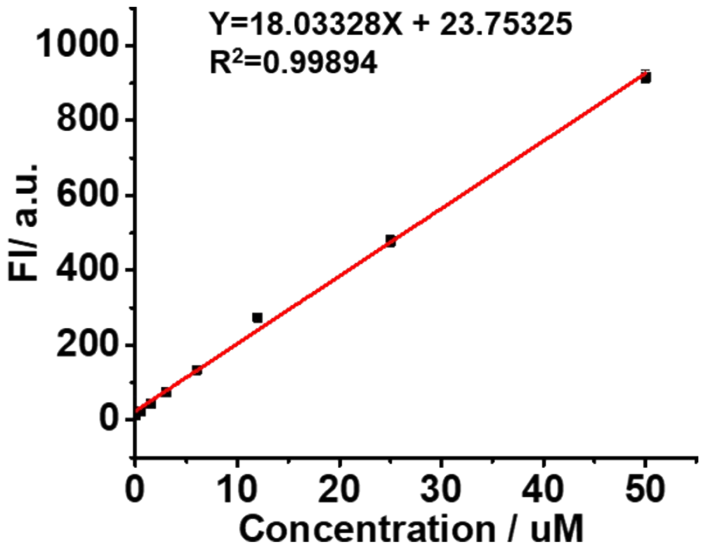


Figure S24. Correlation of the fluorescence intensity (at 430 nm) of 5fU after treatment with (furan-2-carbohydrazide with various DNA concentration.

R^2 = 0.98894

Equations: y = mx + C, where m= slope

Thus, y = 18.03328x + 23.75325

Here, slope (µM) = 18.03328

Standard deviation (SD) of the free ligand (measured by making repetition of 10 times) =0.60707

Calculation of detection limit:

DL = 3 X SD/Slope

= 3 X 0.60707/18.03328

= 0.1 uM

The limit of detection is calculated as 0.1 uM.

Notes and reference

1. C. X. Liu, G. R. Zou, S. Peng, Y. F. Wang, W. Yang, F. Wu, Z. R. Jiang, X. Zhang and X. Zhou, *Angew. Chem.-Int. Edit.*, 2018, **57**, 9689-9693.