Supporting Information

Visualization and Quantification of Cellular RNA Production and Degradation Using a Fluorescence and Mass Spectrometry-Combined Characterization Assay

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Author Contributions
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1. Materials and Instrumentation

Materials. All the chemicals and reagents are commercially available and used without further purification. DCM, THF and toluene are distilled under nitrogen before use. 4,4'-dimethoxybenzophenone, 4-bromobenzophenone, trimethylborate, benzoyl chloride, bis(triphenylphosphine)palladium chloride, boron tribromide, 1,3-propanesultone and 1,3-propanediol di-p-tosylate are purchased from TCI. Sodium ascorbate (SA), Copper sulfate ($CuSO_4$), formaldehyde, dimethylsulfoxide (DMSO), tris(3-hydroxypropyltriazolylmethyl) amine (THPTA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich.

Alexa488-azide are purchased from Invitrogen. Actinomycin D (Dactinomycin) are purchased from Abcam. Dulbecco's Modified Essential Medium (DMEM), penicillin, streptomycin, Fetal bovine serum (FBS) and trypsin-EDTA solution are purchased from Gbico.

Instrumentation. ^{1}H and ^{13}C NMR spectra is measured on Bruker ARX 400 NMR spectrometer using DMSO as solvent, and tetramethylsilane (TMS; $\delta = 0$ ppm) was chosen as internal reference. Photoluminescence spectra are measured on PerkinElmer LS 55 spectrofluorometer and UV spectra are measured on Biochrom Libra S80PC double beam spectrometer. High-resolution mass spectra (HRMS) were recorded on an AB TripleTOF 5600 plus System (AB SCIEX, Framingham, USA) Mass Spectrometer System operating in a positive ion mode. The HPLC profiles were acquired using Waters e2695 equipment (flow rate 1mL/min) with Atlantis T3 Column (100Å, 5 μm, 4.6 mm X 250 mm, 1/pkg). Particle sizes are measured using a Brookhaven ZetaPlus potential analyzer (Brookhaven instruments corporation, USA). Confocal lasing scanning microscopic (CLSM) images and fluorescence spectra are obtained on confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM780) using ZEN 2009 software (Carl Zeiss).

2. Synthetic Procedures

Synthesis of p^{6}A (*N*^{6}-Propargyladenosine). To a solution of 6-chloropurine riboside (1.5 mmol, 429 mg) in absolute EtOH (10 mL), Et_3N, (4.5 mmol, 455 mg) and 2-propargylamine (4.5 mmol, 248 mg) were added. The mixture was stirred at 80℃ for 3 h, cooled to room temperature and the solvent was removed under vacuum to leave syrupy residue. The addition of dry Et_2O precipitated Et_3NHCl, which was filtered off. The crude residue after evaporation was crystallized from MeOH to afford the desired product (366 mg). Yield = 80%. ^{1}H NMR (400 MHz, DMSO) δ 8.39 (d, J = 14.5 Hz, 1H), 8.35 – 8.15 (m, 2H), 5.90 (d, J = 6.1 Hz, 1H), 5.47 (d, J = 6.2 Hz, 1H), 5.35 (dd, J = 7.0, 4.7 Hz, 1H), 5.21 (d, J = 4.7 Hz, 1H), 4.61 (dd, J = 11.2, 6.0 Hz, 1H), 4.25 (s, 2H), 4.14 (dt, J = 16.5, 8.2 Hz, 1H), 3.96 (q, J = 3.5 Hz, 1H), 3.68 (dt, J = 12.0, 4.2 Hz, 1H), 3.61 – 3.51 (m, 1H), 3.03 (d, J = 11.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 152.20, 140.16, 87.82, 85.81, 81.79, 73.51, 72.33, 70.56, 61.55,

45.38, 8.71.

Compound **4** was synthesized according to the procedures reported in literature.[1]

Synthesis of Compound 1. To a dry THF solution (250 mL) of zinc powder (200 mmol, 13 g), 4,4'-dimethoxybenzophenone (20 mmol, 4.8 g) and 4-bromobenzophenone (24 mmol, 6.2 g), titanium tetrachloride (120 mmol, 23 g) was add under nitrogen atmosphere at -78℃. After that, the reaction mixture was allowed to warm to room temperature slowly and then stirred to reflux for overnight. The resulting solution was quenched with 100mL HCl solution. The solvent was extracted with DCM for 3 times and then evaporated under reduced pressure. After that the crude product was purified by silica gel chromatography with petroleum ether/ dichloromethane (6:1) as eluent to afford the desired product (5 g). Yield = 53%. ^{1}H NMR (400 MHz, DMSO) δ 7.38 – 7.28 (m, 2H), 7.21 – 7.03 (m, 3H), 6.97 (dd, *J* = 11.1, 9.6 Hz, 2H), 6.91 – 6.79 (m, 6H), 6.71 (dd, *J* = 19.1, 8.8 Hz, 4H), 3.68 (d, *J* = 8.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 157.87, 157.79, 143.32, 143.11, 140.50, 137.41, 135.33, 135.25, 132.83, 132.01, 131.94, 130.79, 130.71, 127.94, 126.41, 119.34, 113.33, 113.15, 54.91, 54.88.

Synthesis of Compound 2. To a dry THF solution (150 mL) of compound 1 (4.2 mmol, 2 g) and trimethylborate (8.4 mmol, 0.87 g), n-butyllithium (1.6 M solution, 6.7 mmol) was add under nitrogen atmosphere at -78℃ and kept for 2 h. After that, the reaction mixture was allowed to warm to room temperature slowly and then stirred to for 3 h. The resulting solution was quenched with 45mL HCl solution (3 M). The solvent was extracted with DCM for 3 times and then evaporated under reduced pressure. After that the crude product was purified by silica gel chromatography with petroleum ether/ tetrahydrofuran (4:1) as eluent to afford the desired product (1.2 g). Yield = 65%.

Synthesis of Compound 3. To a dry toluene solution (100 mL) of compound 2 (1.4 mmol, 600 mg), K_3PO_4•$3H_2O$ (2.1 mmol, 440 mg) and $Pd(PPh_3)_2Cl_2$ (0.07 mmol, 48.3 mg), benzoyl chloride (1.7 mmol, 233 mg) was add under nitrogen atmosphere. After that, the reaction mixture was heated at 80℃ overnight. The resulting solution was extracted with DCM for 3 times and then evaporated under reduced pressure.

After that the crude product was purified by silica gel chromatography with petroleum ether/ tetrahydrofuran (4:1) as eluent to afford the desired product (300 mg). Yield = 43%.

Synthesis of Compound 4. To a solution of compound 3 (0.3 mmol, 150mg) in anhydrous DCM (100 mL), boron tribromide (2.4 mmol, 600 mg) was added at 0℃. The reaction was stirred at room temperature overnight. The resulting solution was quenched with 100mL water. The solvent was extracted with DCM for 3 times and then evaporated under reduced pressure. After that the crude product was purified by silica gel chromatography with petroleum ether/ tetrahydrofuran (1:1) as eluent to afford the desired product (130 mg). Yield = 93%.

Synthesis of Compound 5. To a solution of sodium azide (10 mmol, 650 mg) in anhydrous DMF (100 mL), 1,3-propanediol di-p-tosylate (10 mmol, 3.84 g) was added. The reaction was stirred at room temperature overnight. The resulting solution was quenched with 100mL water. The solvent was extracted with DCM for 3 times and then evaporated under reduced pressure. After that the crude product was purified by silica gel chromatography with petroleum ether/ tetrahydrofuran (4:1) as eluent to afford the desired product (1.91 g). Yield = 75%. ^{1}H NMR (400 MHz, DMSO) δ 7.92 – 7.70 (m, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 4.19 – 3.96 (m, 2H), 3.37 (dd, *J* = 7.6, 5.6 Hz, 2H), 2.43 (s, 3H), 1.82 (p, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 144.99, 132.17, 130.16, 127.98, 127.58, 125.47, 67.95, 46.79, 27.69, 21.05.

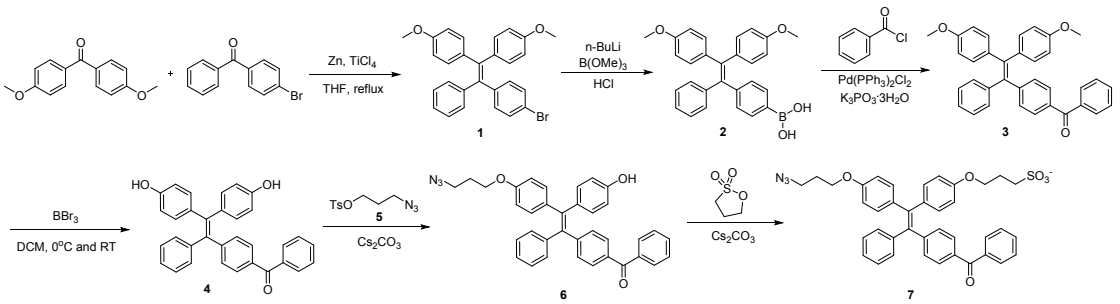
Synthesis of Compound 6. To a solution of compound 1 (0.42 mmol, 197 mg) and compound 2 (0.4 mmol, 102 mg) in anhydrous DMF (50 mL), cesium carbonate (1.2 mmol, 391 mg) was added. The reaction was stirred at 50℃ overnight. The resulting solution was quenched with 50 mL water. The solvent was extracted with DCM for 3 times and then evaporated under reduced pressure. After that the crude product was purified by silica gel chromatography with petroleum ether/tetrahydrofuran (4:1) as eluent to afford the desired product (139 mg) as yellow solid. Yield = 60%. ^{1}H NMR (400 MHz, DMSO) δ 9.46 (d, *J* = 14.4 Hz, 1H), 7.71 – 7.60 (m, 2H), 7.58 – 7.46 (m, 4H), 7.15 (dddd, *J* = 14.8, 10.0, 5.6, 1.7 Hz, 5H), 6.99 (dd, *J* = 6.7, 1.5 Hz, 2H), 6.93 – 6.84 (m, 3H), 6.81 – 6.64 (m, 4H), 6.59 – 6.47 (m, 2H), 3.95 (td, *J* = 6.0, 3.6 Hz,

2H), 3.55 – 3.43 (m, 2H), 2.04 – 1.81 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 195.18, 157.18, 157.05, 156.35, 156.20, 151.44, 148.84, 148.76, 143.43, 143.34, 141.85, 139.15, 137.22, 137.18, 135.44, 135.37, 134.24, 133.52, 133.44, 132.45, 132.18, 132.08, 130.87, 130.79, 129.36, 128.48, 128.00, 126.40, 124.88, 114.74, 114.62, 113.74, 113.60, 64.40, 64.36, 47.62, 34.35, 30.38, 28.08, 21.00. HRMS (ESI): calculated for C36H29N3O3 [M$^-$]: 550.2136; found: 550.2119.

Synthesis of Compound 7. To a solution of compound 3 (0.1 mmol, 55 mg) in anhydrous DMSO (20 mL), 1,3-propanesultone (0.2 mmol, 24 mg) and cesium carbonate (0.2 mmol, 65 mg) was added. The reaction was stirred at 50℃ for two days. After that the resulting solution was purified by HPLC to afford yellow solid. Yield = 50%. ^{1}H NMR (400 MHz, DMSO) δ 7.65 (dd, *J* = 7.3, 6.3 Hz, 2H), 7.54 (td, *J* = 8.2, 4.0 Hz, 4H), 7.27 – 7.08 (m, 5H), 7.04 – 6.95 (m, 2H), 6.93 – 6.81 (m, 5H), 6.78 – 6.63 (m, 4H), 3.96 (td, *J* = 10.7, 6.2 Hz, 4H), 3.57 – 3.39 (m, 2H), 2.54 (d, *J* = 5.1 Hz, 2H), 2.03 – 1.86 (m, 4H). HRMS (ESI): calculated for C39H34N3O6S- [M$^-$]: 672.2174; found: 672.2142.

RERERENCES

1. F. Hu, Y. Y. Huang, G. X. Zhang, R. Zhao, H. Yang, D. Q. Zhang, *Anal. Chem.* 2014, **86**, 7987.



Scheme S1. Synthetic Route to AIE-Active Fluorescent Probe SO_3-TPE-N_3.

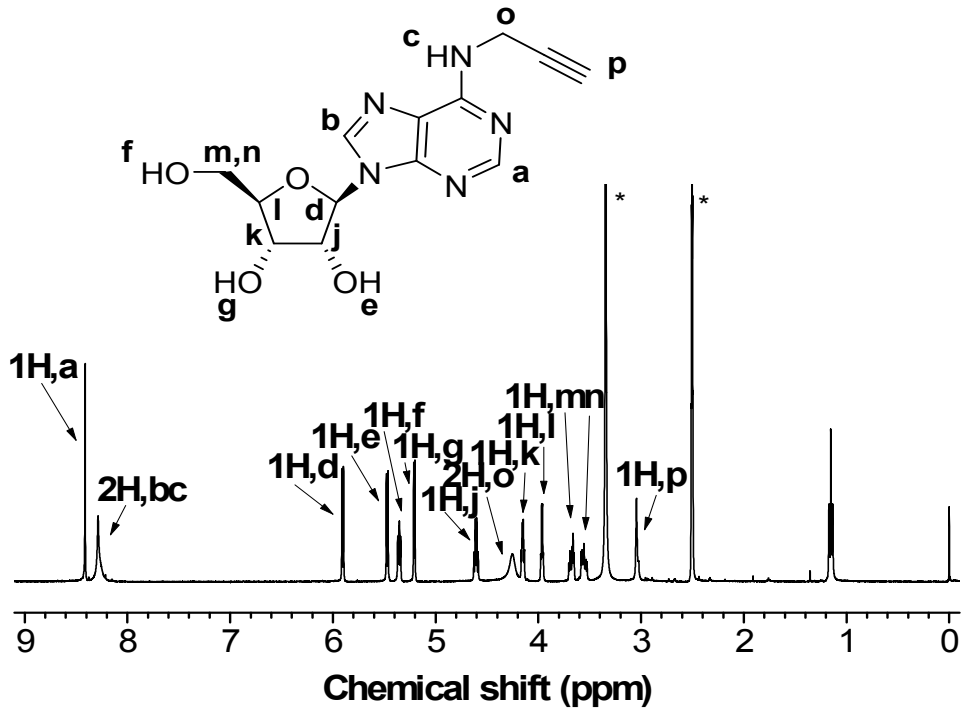


Fig. S1. ^{1}H NMR spectrum of p^{6}A in DMSO-d6.

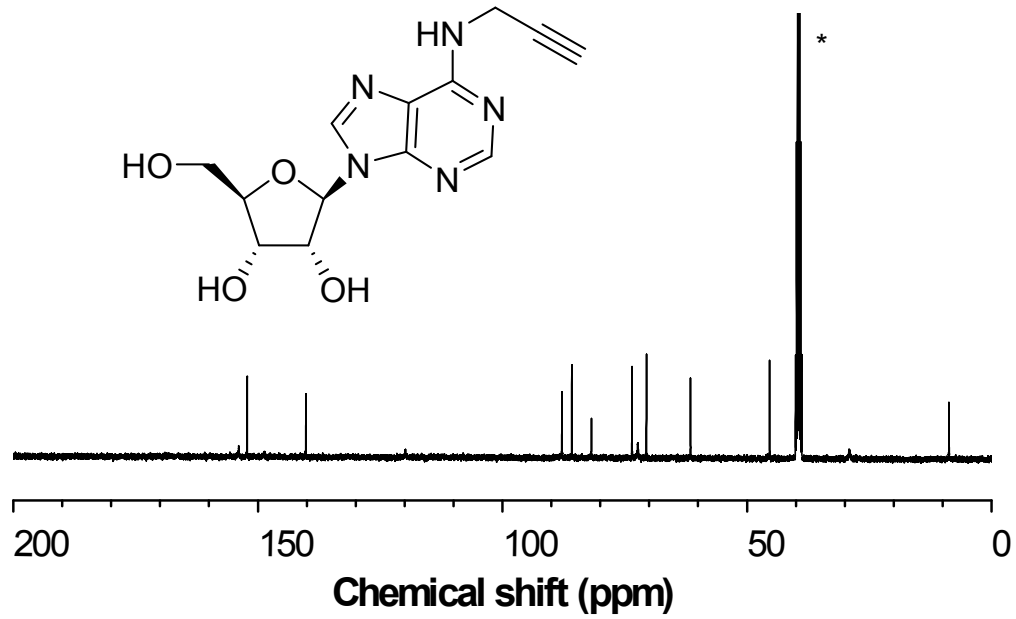


Fig. S2. ^{13}C NMR spectrum of p^6A in DMSO-d6.

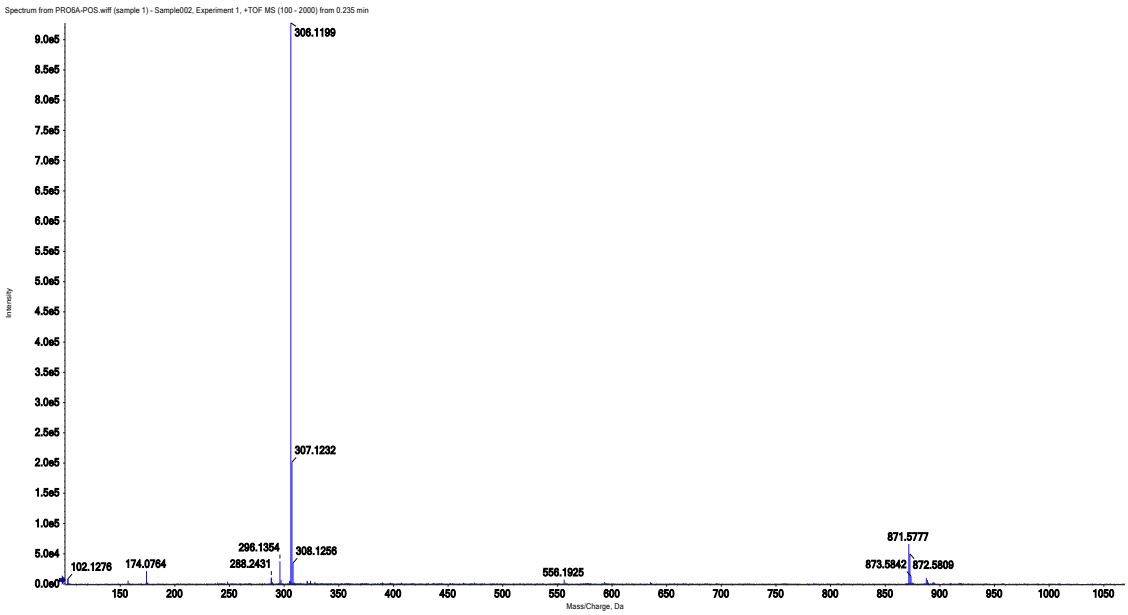


Fig. S3. High resolution mass spectrum of p^6A.

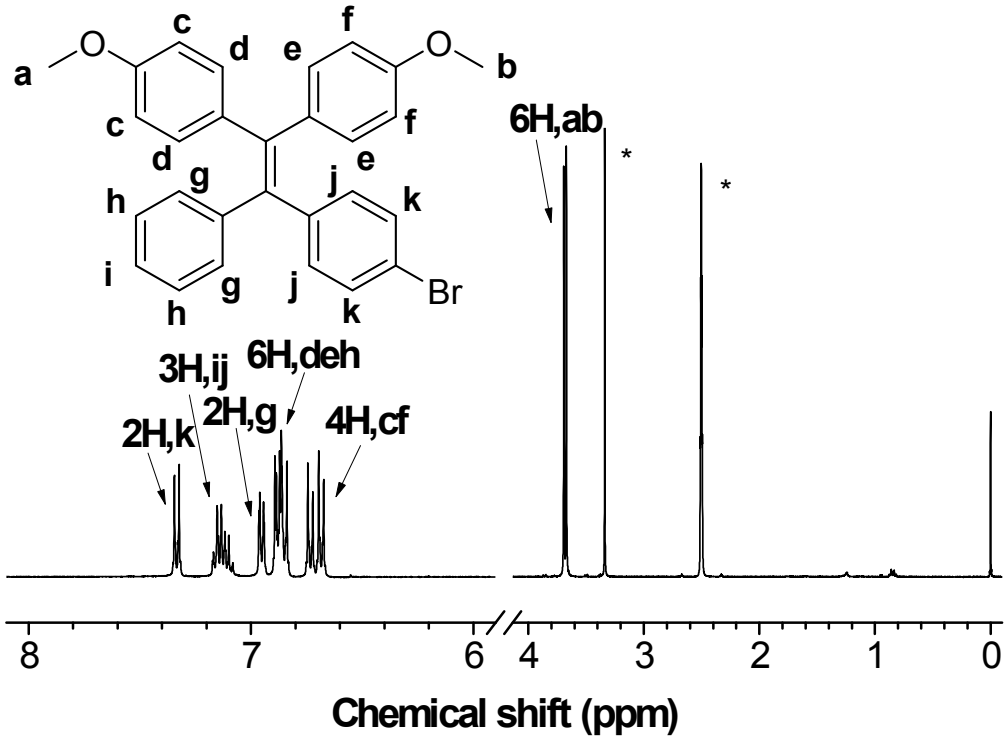


Fig. S4. ^{1}H NMR spectrum of compound 1 in DMSO-d6.

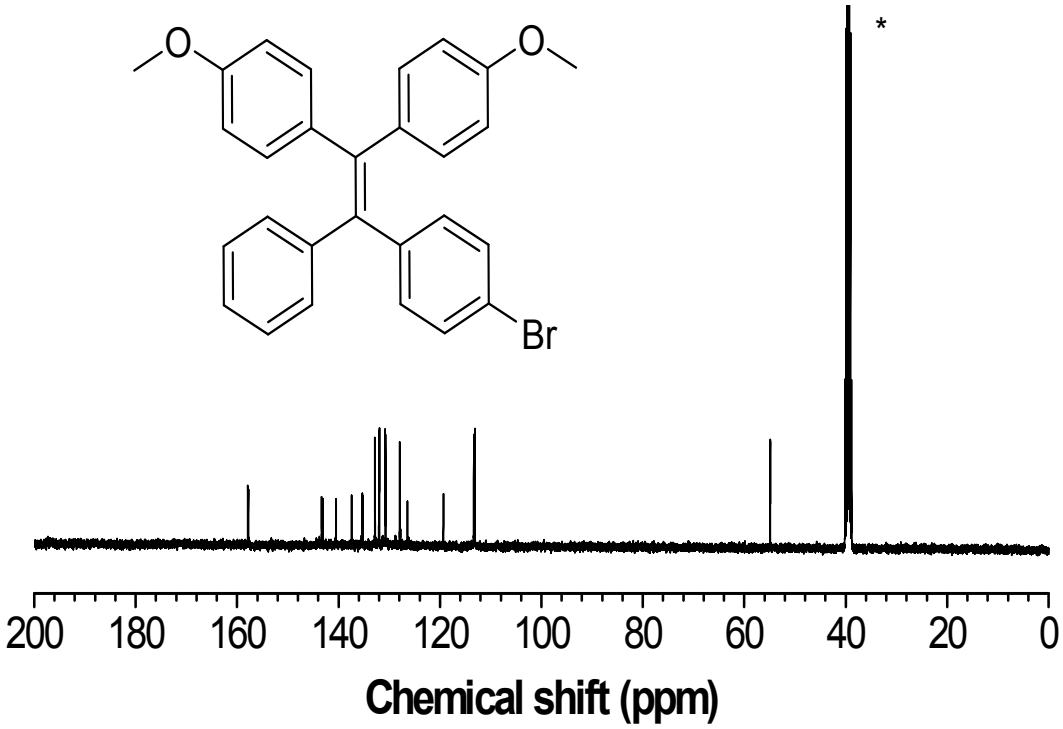


Fig. S5. ^{13}C NMR spectrum of compound 1 in DMSO-d6.

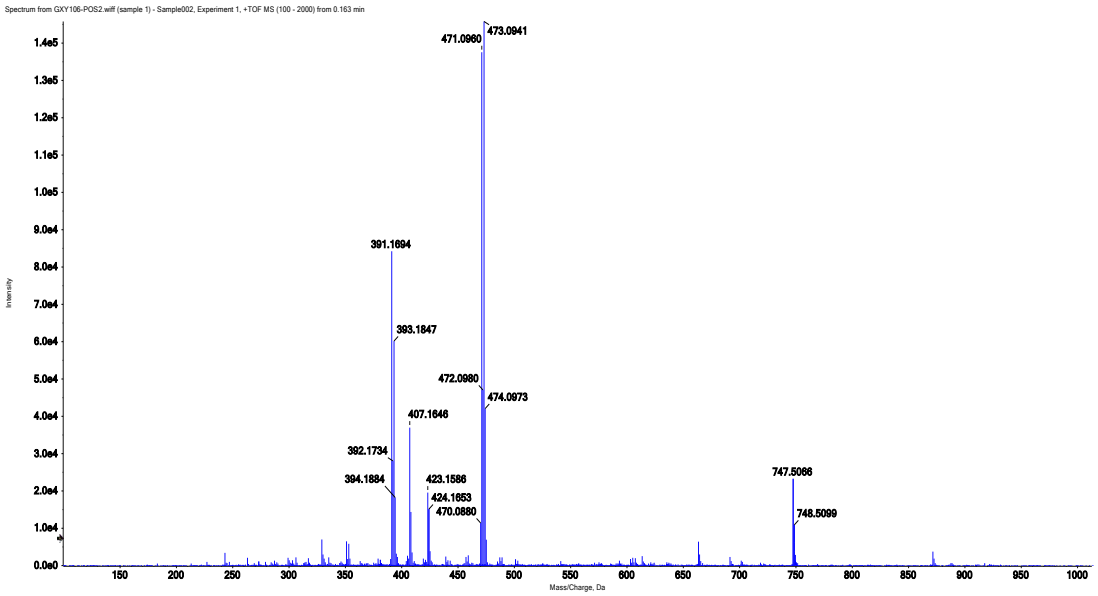


Fig. S6. High resolution mass spectrum of compound 1.

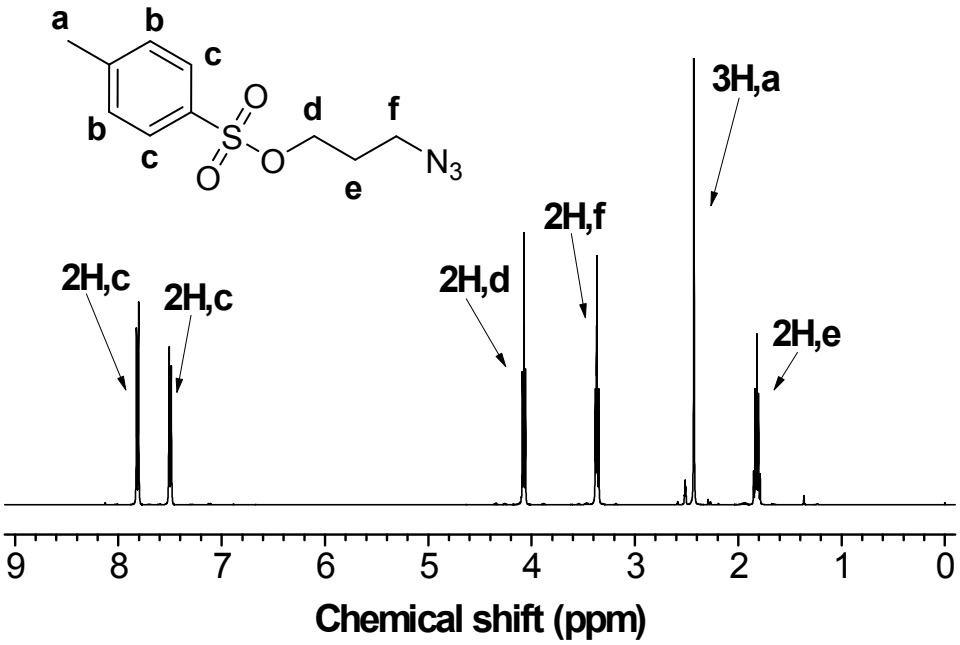


Fig. S7. ^{1}H NMR spectrum of compound 5 in DMSO-d6.

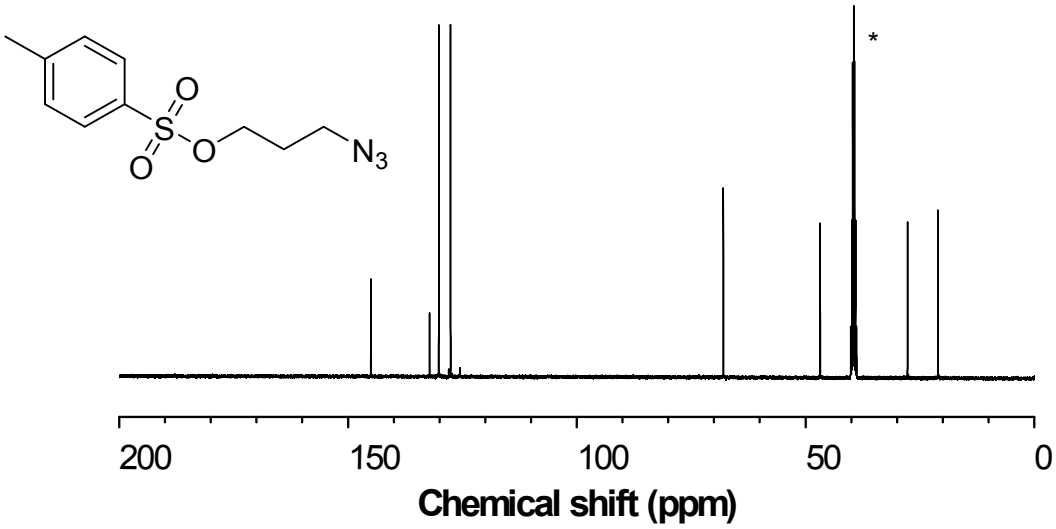


Fig. S8. ^{13}C NMR spectrum of compound 5 in DMSO-d6.

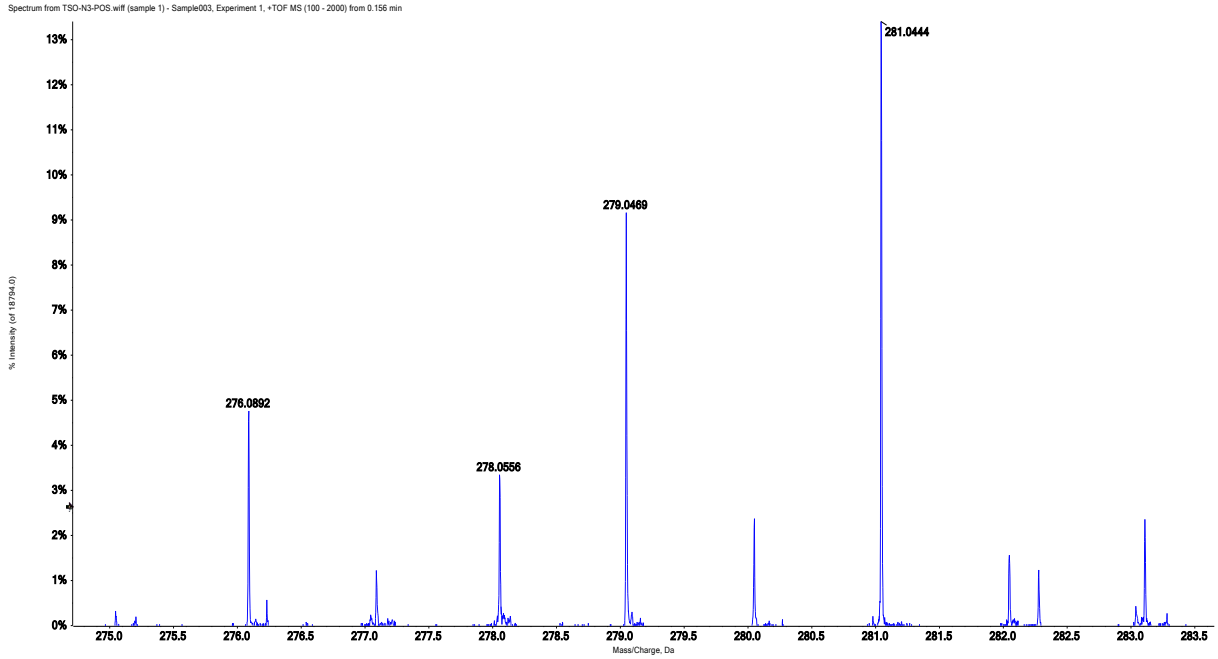


Fig. S9. High resolution mass spectrum of compound 5.

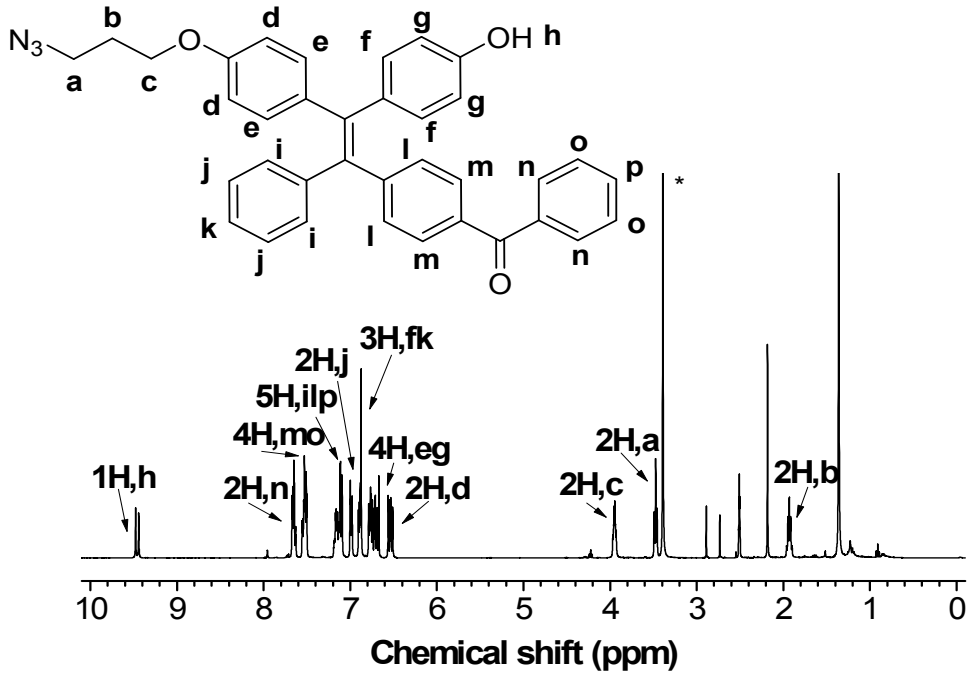


Fig. S10. ^{1}H NMR spectrum of compound 6 in DMSO-d6.

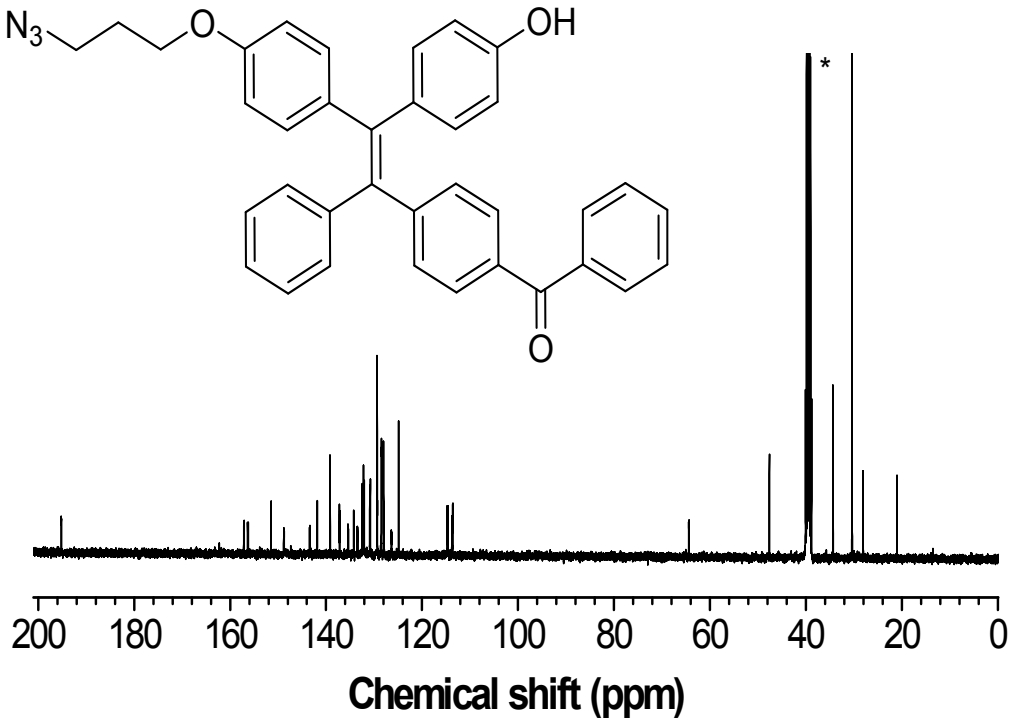


Fig. S11. ^{13}C NMR spectrum of compound 6 in DMSO-d6.

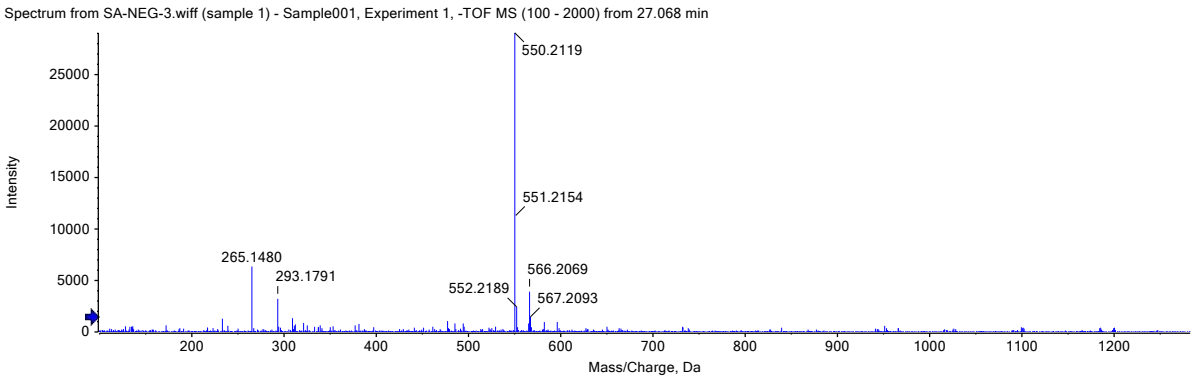


Fig. S12. High resolution mass spectrum of compound 6.

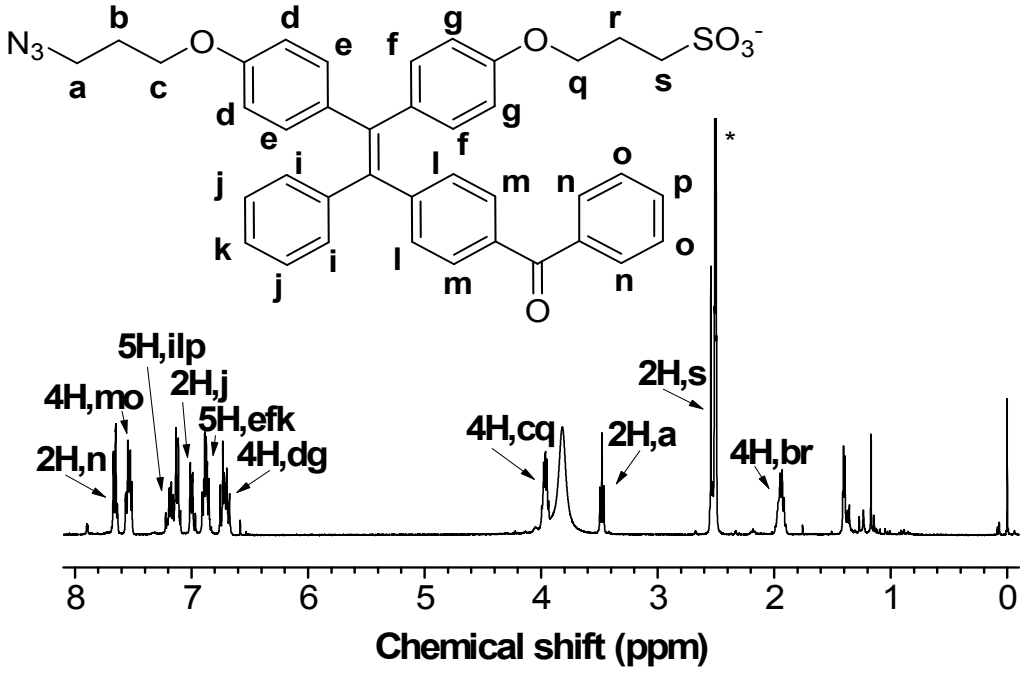


Fig. S13. ^{1}H NMR spectrum of compound 7 in DMSO-d6.

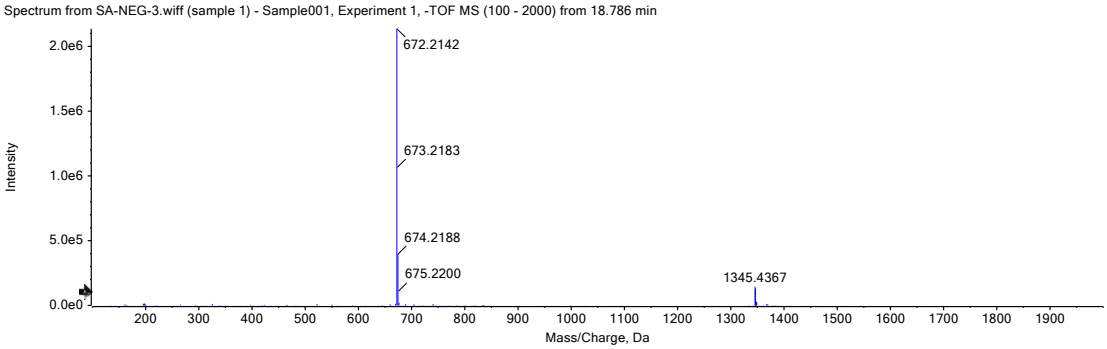


Fig. S14. High resolution mass spectrum of compound 7.

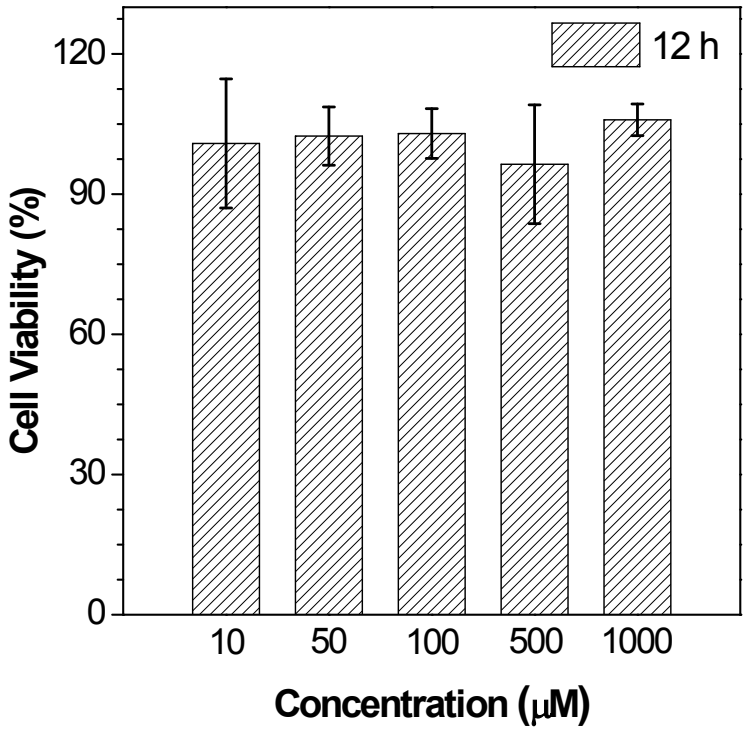


Fig. S15. MTT assay of p^6A on HeLa cells.

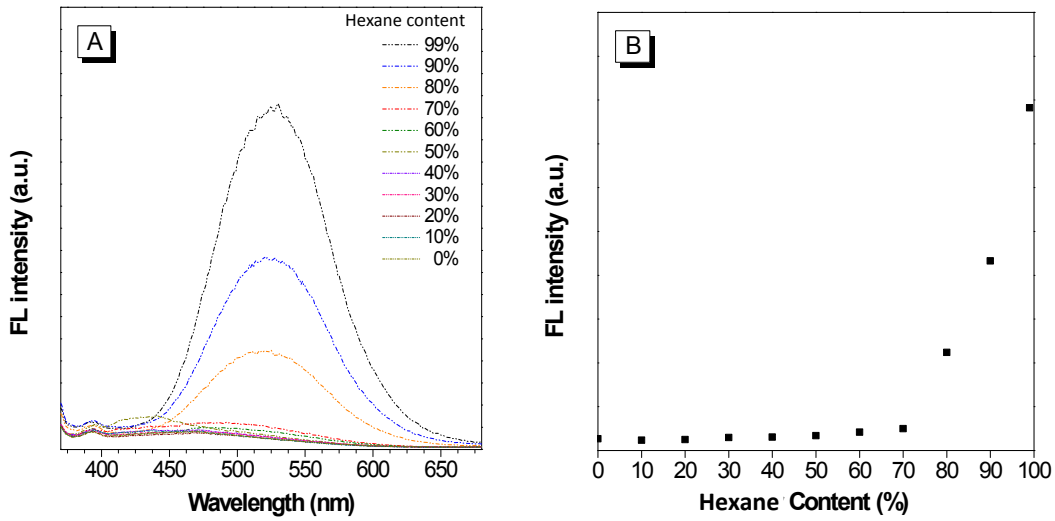


Fig. S16. (A) Fluorescence (FL) spectra of probe in THF/hexane mixtures with different hexane fractions. (B) Plot of relative peak intensity (I/I_0) versus different compositions of the solvent mixtures of SO_3-TPE-N_3. Concentration of SO_3-TPE-N_3: 10 μM; Excitation wavelength: 350 nm.

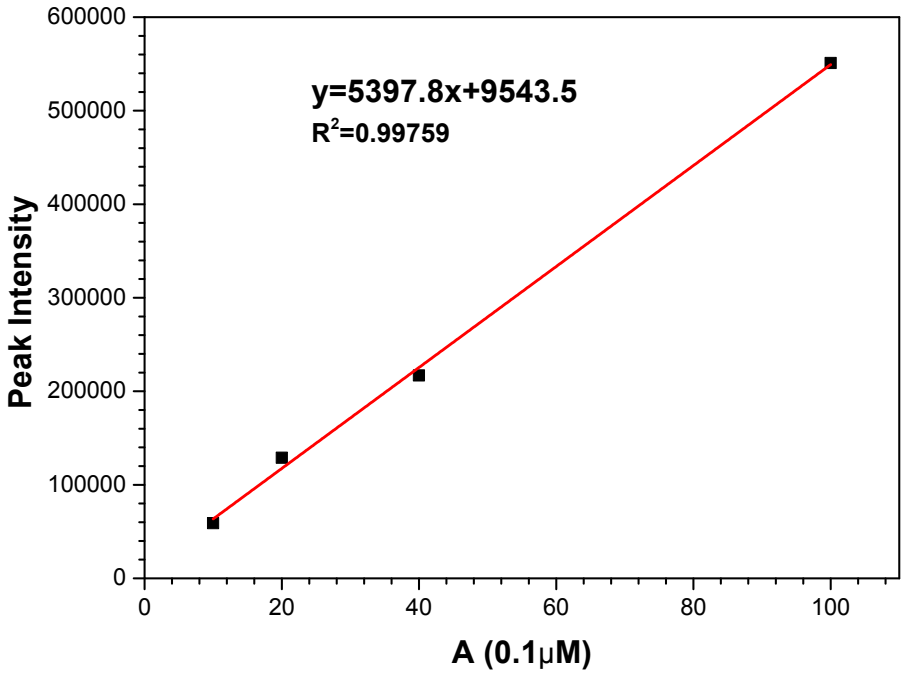


Fig. S17. Standard curve for p^{6}A 年 RNA synthesis.

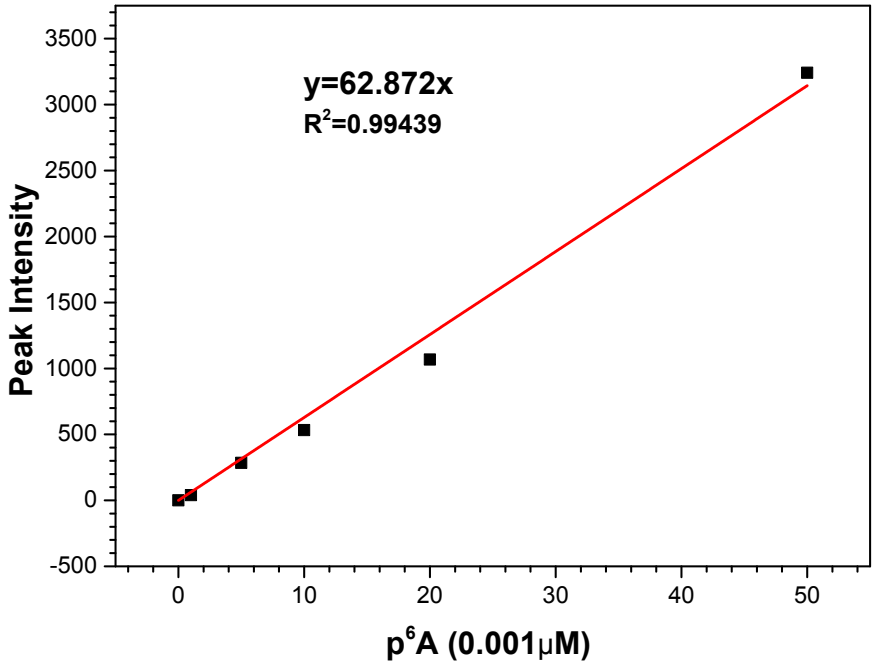


Fig. S18. Standard curve for adenosine in RNA synthesis.

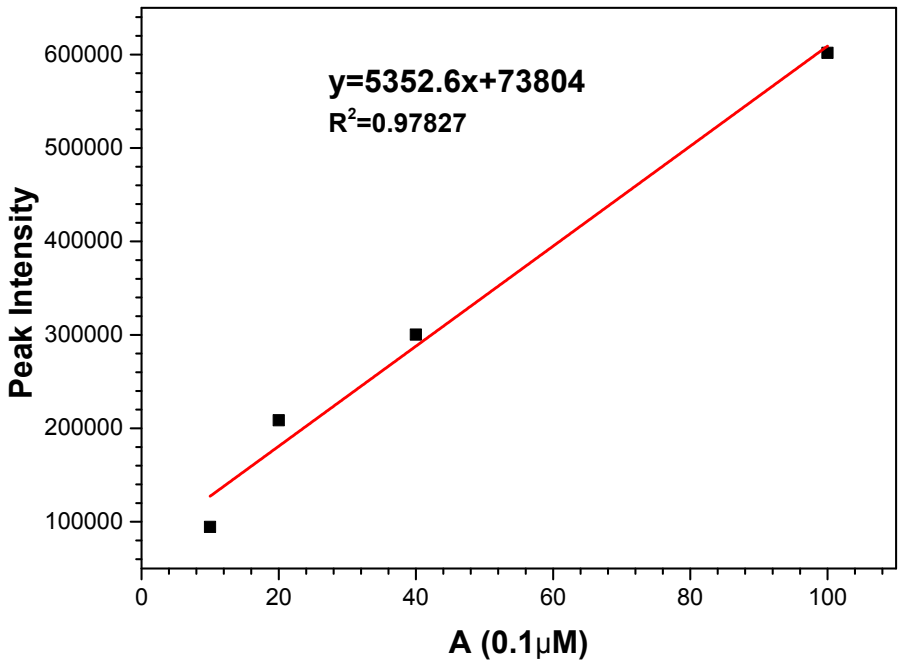


Fig. S19. Standard curve for p^6A in RNA decay.

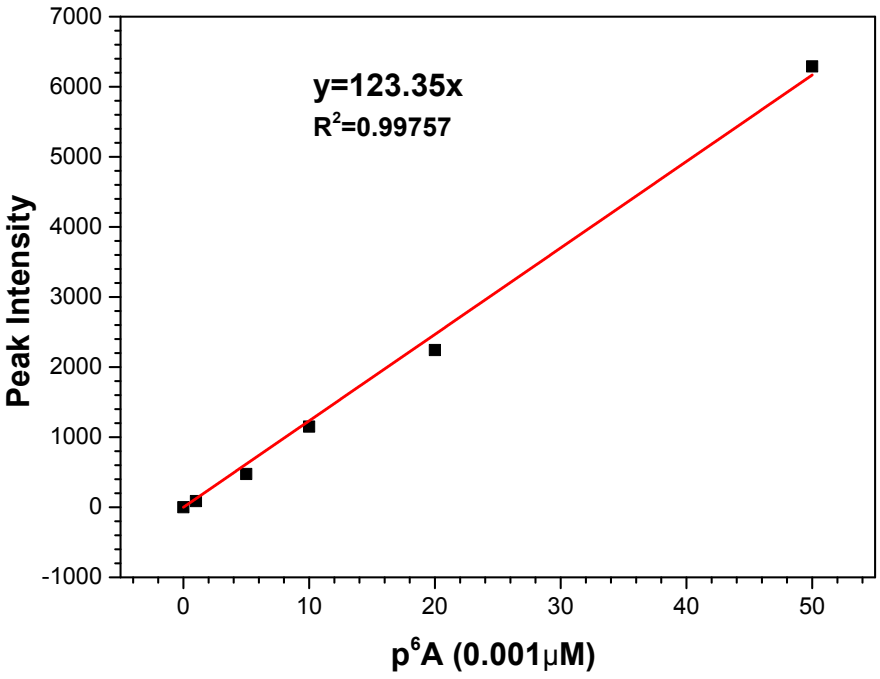


Fig. S20. Standard curve for adenosine in RNA decay.

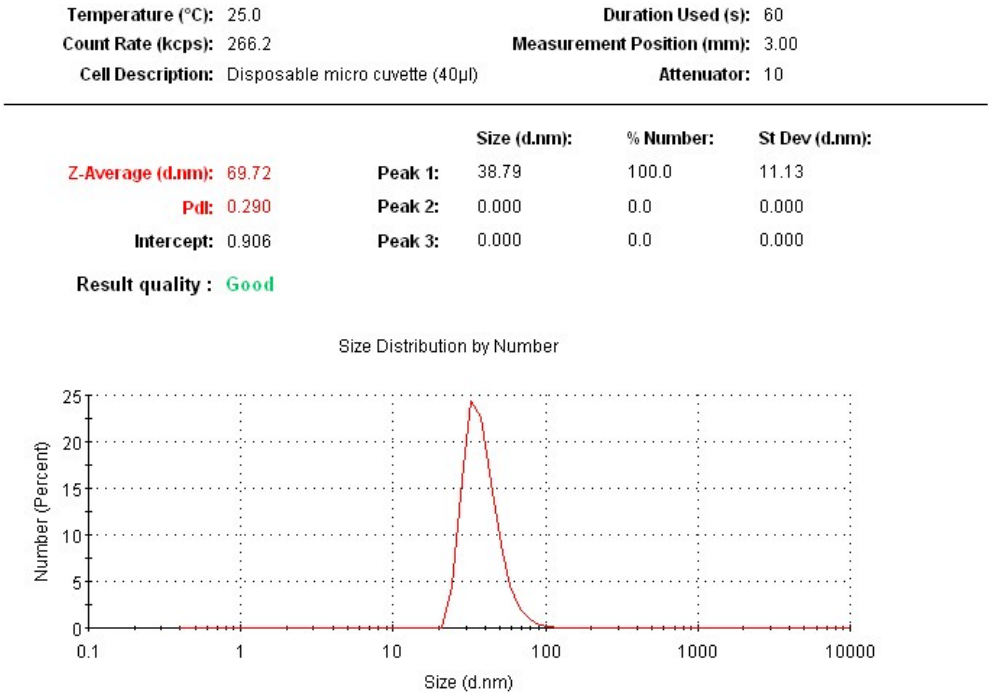


Fig. S21. Size distribution of probe in PBS buffer containing 1% DMSO at 25 ℃. Concentration: 10 μM