Supporting information

A ratiometric ESIPT probe based on 2-aza-cope rearrangement for rapid and selective detection of formaldehyde in living cells

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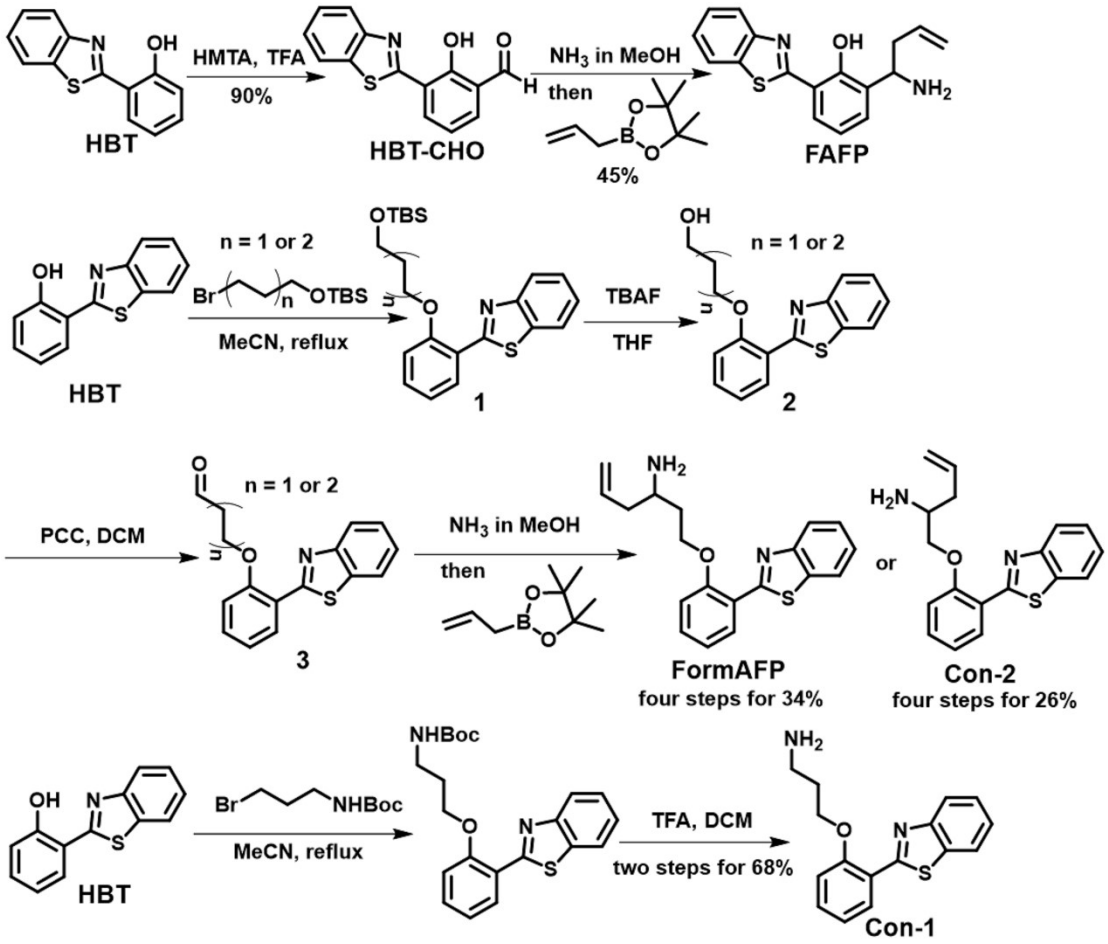
1. Synthesis and characterization



Scheme S1. Synthetic routes for **FAFP**, **FormAFP**, **Con-1** and **Con-2.**

Synthesis of FAFP. The compound **HBT-CHO** was prepared according to previous studies.[1-2] Briefly, to 10 mL MeOH, 5 mL of NH_3 solution (7 N in MeOH) and **HBT-CHO** (257 mg, 1.0 mmol) were added. The mixture was stirred for 0.5 h at 0 °C and allylboronic acid pinacol ester (360 mg, 2.0 mmol) was added. Then the solution was moved to ambient room condition and reacted for 0.5 h. After evaporating the solvent, the obtained residue was subjected to silica gel chromatography purification (petroleum ether/DCM = 1:1) to yield **FAFP** (white solid, 148 mg, 45%).^{1}H NMR (500 MHz, Chloroform-d): δ 8.01 (d, J = 7.3 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 7.9 Hz, 1H), 7.45-7.40 (m, 2H), 7.31 (t, J = 7.0 Hz, 1H), 6.94 (d, J = 2.2 Hz, 1H), 5.80-5.68 (m, 1H), 5.16–5.04 (m, 2H), 4.17 (t, J = 6.9 Hz, 1H), 2.62–2.50 (m, 2H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, Chloroform-d) δ 165.08, 160.97, 152.06, 135.77, 135.08, 131.73,

127.07, 126.92, 126.11, 124.13, 122.03, 121.83, 119.70, 118.60, 54.67, 20.75. MALDI-TOF-MS: m/z calcd. for C17H16N2OS, $[M+H]^+$ 297.1062, found 297.11.

Synthesis of compound 1. Add (3-bromopropoxy) (*tert*-butyl) dimethylsilane (17.6 mmol, 4.45g) to 2-(2-hydroxyphenyl) benzothiazoline (8.8 mmol, 2 g) and K_2CO_3 (26.4 mmol, 3.64g) were dissolved in a solution of MeCN (50 mL). The resulting mixture was stirred at 80°C for 1 h. Then, the reaction was cooled to room temperature. The reaction was quenched with NaCl solution, diluted with EtOAc, washed twice with water and once with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained crude product was purified by flash chromatography (20:1 hexane/ethyl acetate) to obtain the compound **1** (6.06g, 90%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.35 (dtq, J = 13.7, 7.9, 7.9, 1.9, 1.7, 1.7 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.63-7.56 (m, 1H), 7.43 (td, J = 7.6, 1.6 Hz, 1H), 7.36 (td, J = 7.6, 1.3 Hz, 1H), 7.31-7.22 (m, 2H), 7.09-7.01 (m, 1H), 4.10 (t, J = 6.0 Hz, 2H), 3.65 (t, J = 6.1 Hz, 2H), 1.91 (p, J = 6.2 Hz, 2H), 0.98 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (125 MHz, Chloroform-d) δ 163.27, 155.72, 152.05, 135.65, 130.07, 127.97, 126.64, 125.37, 125.01, 124.62, 122.15, 121.97, 113.73, 66.98, 61.18, 31.02, 25.90, 18.31, -5.20. MALDI-TOF-MS: m/z calcd. for C22H29NO2SSi, $[M+H]^+$ 400.1767, found 400.18.

Synthesis of compound 2. Compound **1** (3.5g, 8.8 mmol) and tetrabutylammonium fluoride trihydrate (3.3g, 10.6mmol) were added into THF (40 mL), and the resulting mixture stirred for 3h at room temperature. The THF was removed under reduced pressure, and the resulting solution was extracted with EtOAc (30 mL×3), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification via column chromatography (2:1 hexanes/ethyl acetate) provided compound **2** as a white solid (2.5g, 98% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.38 (dtq, J = 13.7, 7.9, 7.9, 1.9, 1.7, 1.7 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.54-7.47 (m, 1H), 7.46-7.35 (m, 2H), 7.15-7.08 (m, 1H), 7.02 (dt, J = 8.4, 1.6 Hz, 1H), 4.32 (td, J = 5.9, 1.7 Hz, 2H), 4.02 (q, J = 5.5 Hz, 2H), 2.24 (p, J = 5.8 Hz, 2H). ^{13}C NMR (125 MHz, Chloroform-d) δ 163.27, 155.95, 152.11, 135.61, 130.25, 127.99, 126.63, 125.26,

124.90, 124.28, 122.08, 122.00, 113.83, 67.35, 59.78, 32.1. MALDI-TOF-MS: m/z calcd. for C16H15NO2S, $[M+H]^+$ 286.0902, found 286.08.

Synthesis of FormAFP. Compound **2** (50mg, 0.177mmol) was added to DCM (4 mL). To this solution was added a solution of pyridinium chlorochromate (0.28 mmol, 60 mg) in DCM (40 mL). The resulting solution was stirred at room temperature for 4 hours. Then, the solution was filtered through a silica gel column and purified by flash chromatography (10:1 hexane/ethyl acetate) to remove the chromium salt to obtain a crude product and proceed directly to the next reaction. The crude product was dissolved in NH_3 solution (1.0 mL, 7.0 N in MeOH), and the reaction mixture was stirred at room temperature for 30 min. Then the mixture was added allylboronic acid pinacol ester (35.7 mg, 0.21 mmol) in NH_3 solution (1.0 mL, 7.0 N in MeOH), and the mixture was stirred for 2 h. After evaporating the solvent, the obtained residue was subjected to silica gel chromatography purification. (DCM/MeOH = 10:1) to obtain compound **FormAFP** as a yellow solid. (21 mg, 40%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.54 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.46 (ddd, J = 29.0, 14.9, 7.5 Hz, 3H), 7.21-7.07 (m, 2H), 5.98-5.77 (m, 1H), 5.22-5.16 (m, 2H), 4.42-4.37 (m, 2H), 3.34-3.23 (m, 1H), 2.40-2.23 (m, 2H), 2.20-1.92 (m, 2H). ^{13}C NMR (125 MHz, Chloroform-d) δ 163.18, 156.54, 152.16, 135.96, 135.18, 131.80, 130.02, 129.75, 125.95, 124.62, 122.80, 122.27, 121.20, 118.01, 112.37, 66.75, 48.07, 43.06, 36.62. MALDI-TOF-MS: m/z calcd. for C19H20N2OS, $[M+H]^+$ 325.1375, found 325.14.

Synthesis of Con-1. Add *tert*-butyl (3-bromopropyl) carbamate (8.8 mmol, 2.08g) to 2-(2-hydroxyphenyl) benzothiazoline (4.4 mmol, 1 g) and K_2CO_3 (13.2 mmol, 1.82g) were dissolved in a solution of MeCN (50 mL). The resulting mixture was stirred at 80°C for 3 h. Then, the reaction was cooled to room temperature. After evaporating the solvent, the crude product was dissolved in TFA/DCM (V/V, 20 mL/40mL), and the reaction mixture was stirred at room temperature for 4 h. The THF was removed under reduced pressure, and the resulting solution was extracted with DCM (30 mL×3), then

dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification via column chromatography (DCM/MeOH = 10:1) provided compound **Con-1** as a yellow solid (1.2g, 68% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.30 (d, J = 13.7 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 7.63-7.56 (m, 1H), 7.41 (td, J = 7.6, 1.6 Hz, 1H), 7.38 (td, J = 7.6, 1.3 Hz, 1H), 7.31-7.22 (m, 2H), 7.09-7.01 (m, 1H), 4.16 (t, J = 6.1 Hz, 2H), 3.13 (p, J = 6.2 Hz, 2H), 1.92 (p, J = 6.2 Hz, 2H). ^{13}C NMR (125 MHz, Chloroform-d) δ 163.27, 155.95, 152.11, 135.61, 130.25, 127.99, 126.63, 125.26, 124.90, 124.28, 122.08, 122.00, 113.94, 67.90, 36.58, 31.3. MALDI-TOF-MS: m/z calcd. for C16H16N2OS, $[M+H]^+$ 285.1062, found 285.11.

Synthesis of Con-2. (2-bromoethoxy) (tert-butyl) dimethylsilane being used instead of (3-bromopropoxy) (*tert*-butyl) dimethylsilane, the synthesis of **Con-2** was simulated to that of **FormAFP** (30 mg, overall yield 26%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.46 (d, J = 7.9 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.99 – 7.92 (m, 1H), 7.58 – 7.37 (m, 3H), 7.15 (ddd, J = 8.2, 7.4, 1.1 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 5.90 (ddt, J = 17.2, 10.1, 7.2 Hz, 1H), 5.27 – 5.16 (m, 2H), 4.28 – 4.22 (m, 1H), 4.10 (dd, J = 9.0, 7.2 Hz, 1H), 3.65 – 3.56 (m, 1H), 2.53 (dt, J = 13.3, 6.5 Hz, 1H), 2.43 (dt, J = 14.1, 7.3 Hz, 1H). ^{13}C NMR (125 MHz, Chloroform-d) δ 164.69, 156.30, 152.25, 136.56, 135.01, 130.21, 127.87, 126.62, 125.31, 124.91, 122.08, 121.98, 121.91, 117.73, 114.06, 71.63, 51.95, 38.5. MALDI-TOF-MS: m/z calcd. for C18H18N2OS, $[M+H]^+$ 311.1218, found 311.12.

References

1 Zijun Huang, Shuangshuang Ding, Dehuan Yu, Feihu Huang, Guoqiang Feng. *Chem. Commun.*, **2014**, 50, 9185—9187.

2 Yalong Zheng, Hanchen Zhang, Dihua Tian, Dechen Duan, Fang Dai, Bo Zhou. *Spectrochim. Acta A*, **2020**, 238, 118429.

2. Selected spectra and data referred in the paper

Table S1. Photophysical properties of **FAFP** and **FormAFP** in EtOH/PBS buffer solution (1:50 v/v, 10 mM, pH=7.4)

Compounds	Abs / nm	Em / nm	Quantum yield	Response time	Selectivity
FAFP	365	460 With FA: 535	0.35 With FA: 0.38	70 min, The pseudo first order kinetics rate constant: 0.006/s with 0.2 mM FA	75-fold over other RCS
FormAFP	340	380 With FA: 520	0.27 With FA: 0.24	10 min, The pseudo first order kinetics rate constant: 0.052/s with 0.2 mM FA	70-fold over other RCS

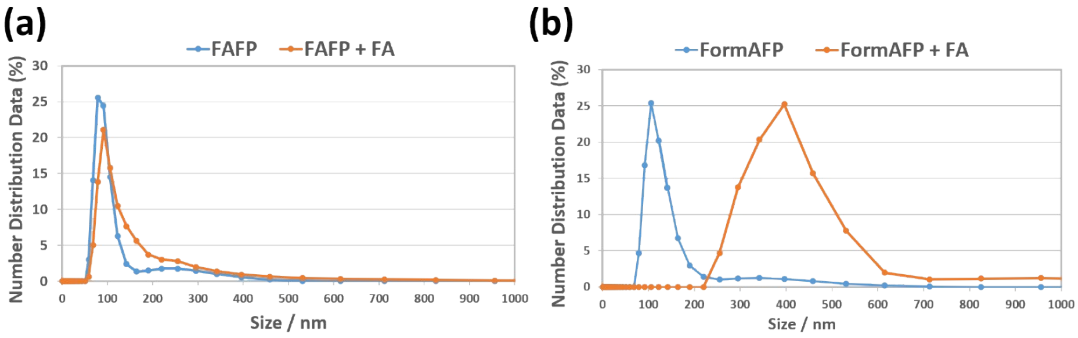


Figure S1. Particle size distribution of (a) **FAFP** (10 μM), (b) **FormAFP** (10 μM) obtained by dynamic light scattering (DLS) without or with 100 μM FA in EtOH/PBS (1:50 v/v, 10 Mm, pH=7.4).

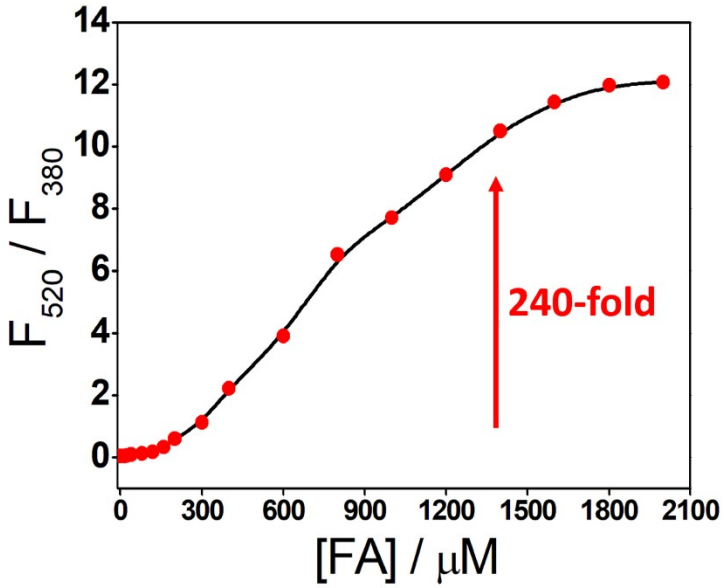


Figure S2. Fluorescence response of **FormAFP** (10 μM) at ratio of $F_{520\ nm}$ / $F_{380\ nm}$ to concentrations of FA (0–2mM) in buffer solution at 37℃. Conditions: experiments were test in EtOH/PBS buffer solution (1:50 v/v, 10 mM, pH=7.4), excitation was set at 340 nm.

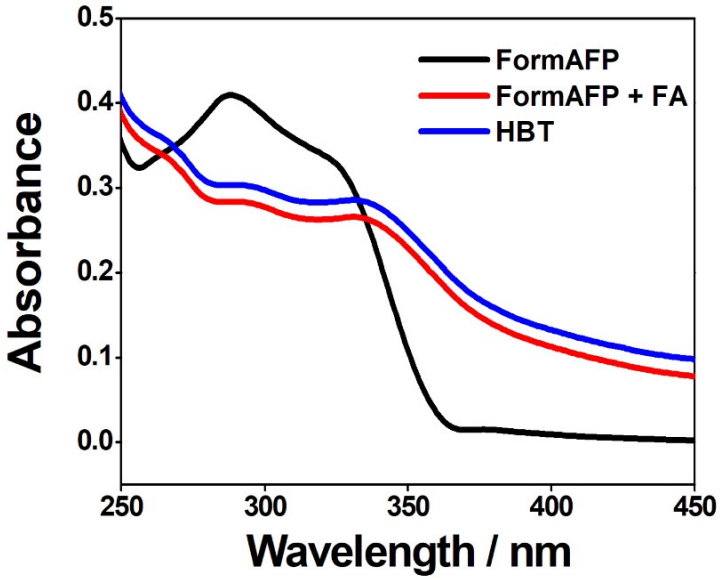


Figure S3. The absorption spectrum of 10 μM **FormAFP**, 10 μM **HBT** and 10 μM **FormAFP** adding with 0.2 mM FA at 37℃. Conditions: experiments were test in EtOH/PBS buffer solution (1:50 v/v, 10 mM, pH=7.4), excitation was set at 340 nm.

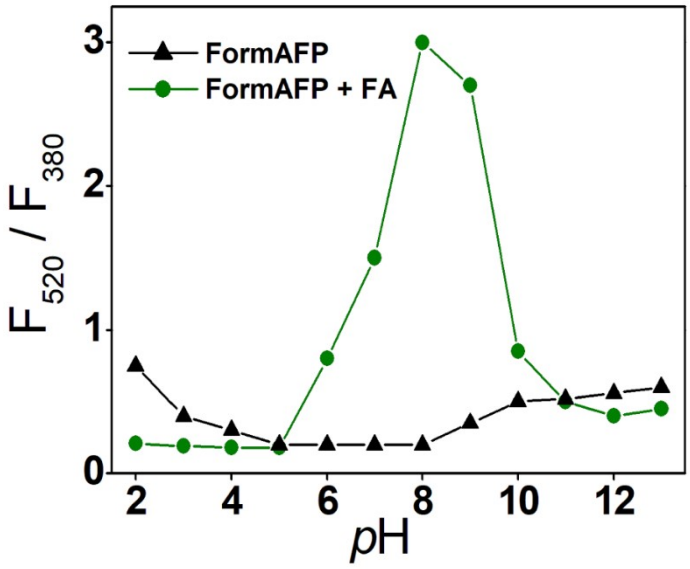


Figure S4. The ratio of $F_{520\ nm}/F_{380\ nm}$ of **FormAFP** (10 μM, black line) and 10 mM **FormAFP** upon addition of 100 μM FA (green line) with different pH values from pH 2.0 to pH 13.0 at 37°C. Conditions: experiments were test in EtOH/PBS buffer solution (1:50 v/v, 10 mM, pH=7.4), excitation was set at 340 nm.

Table S2. Components of artificial urine (in ddH_2O, mmol / L) (pH = 6.8)

$CaCl_2$	$MgCl_2$	Na_2SO_4	$Na_3Citrate$	KH_2PO_4	KCl	NH_4Cl	Urea	NaCl	Creatinine
3.8	3.0	14.5	2.2	21.8	16.4	17.2	41.6	72.1	8.8

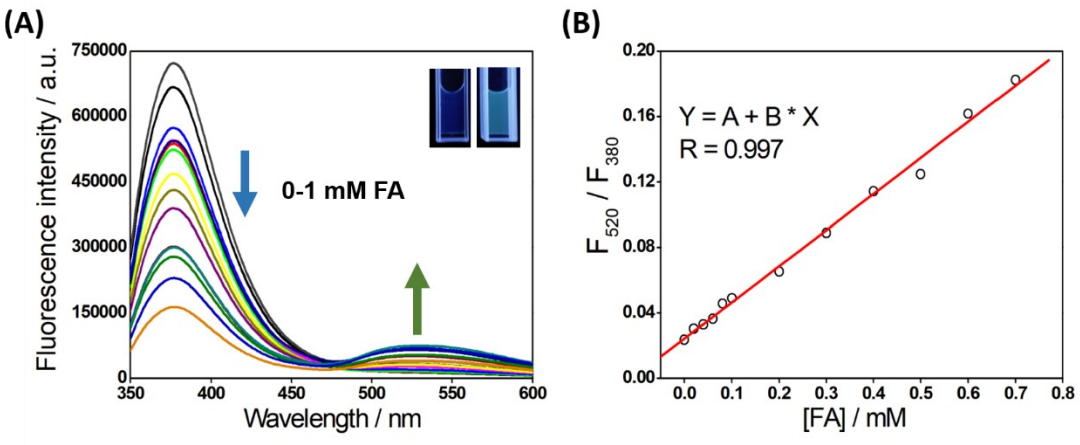


Figure S5. (A) The fluorescence emission spectra of **FormAFP** (10 μM) with different concentrations of FA (1 mM) at 37℃ in artificial urine, (Inset) The corresponding photographs of **FormAFP** (10 μM) without or with 1 mM FA under a UV lamp at 365 nm; (B) The ratio of $F_{520\ nm}$ / $F_{380\ nm}$ versus the concentrations of FA (0-1 mM). The linear relationship between the ratio of $F_{520\ nm}$ / $F_{380\ nm}$ and the concentration of FA (0–1.2mM). Conditions: experiments were test artificial urine, excitation was set at 340 nm.

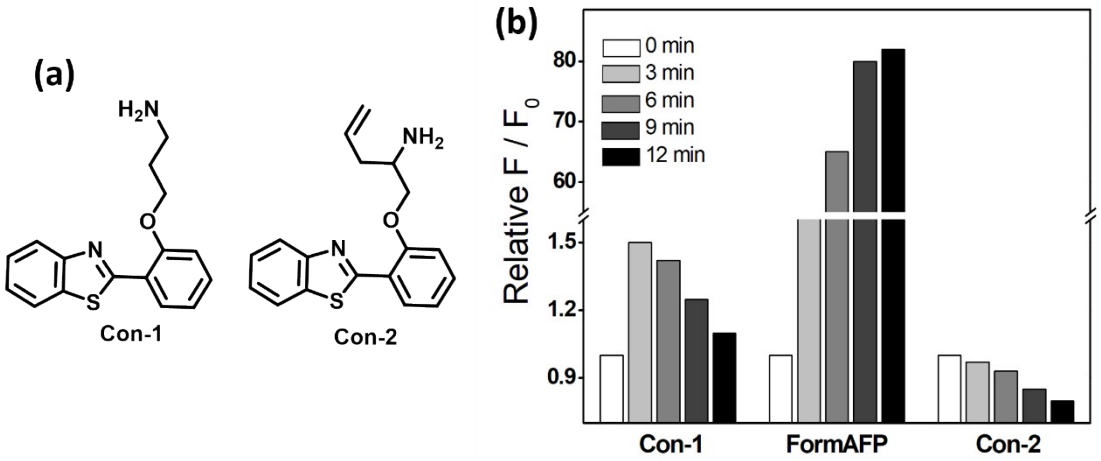


Figure S6. (a) Chemical structures of **Con-1** and **Con-2**; (b) The changes of fluorescence emission intensity of **Con-1**, **Con-2** and **FormAFP** (10 μM) with 10 equiv. FA at 37℃ in EtOH/PBS buffer solution (1:50 v/v, 10 mM, pH=7.4) over a period of time (12 min).

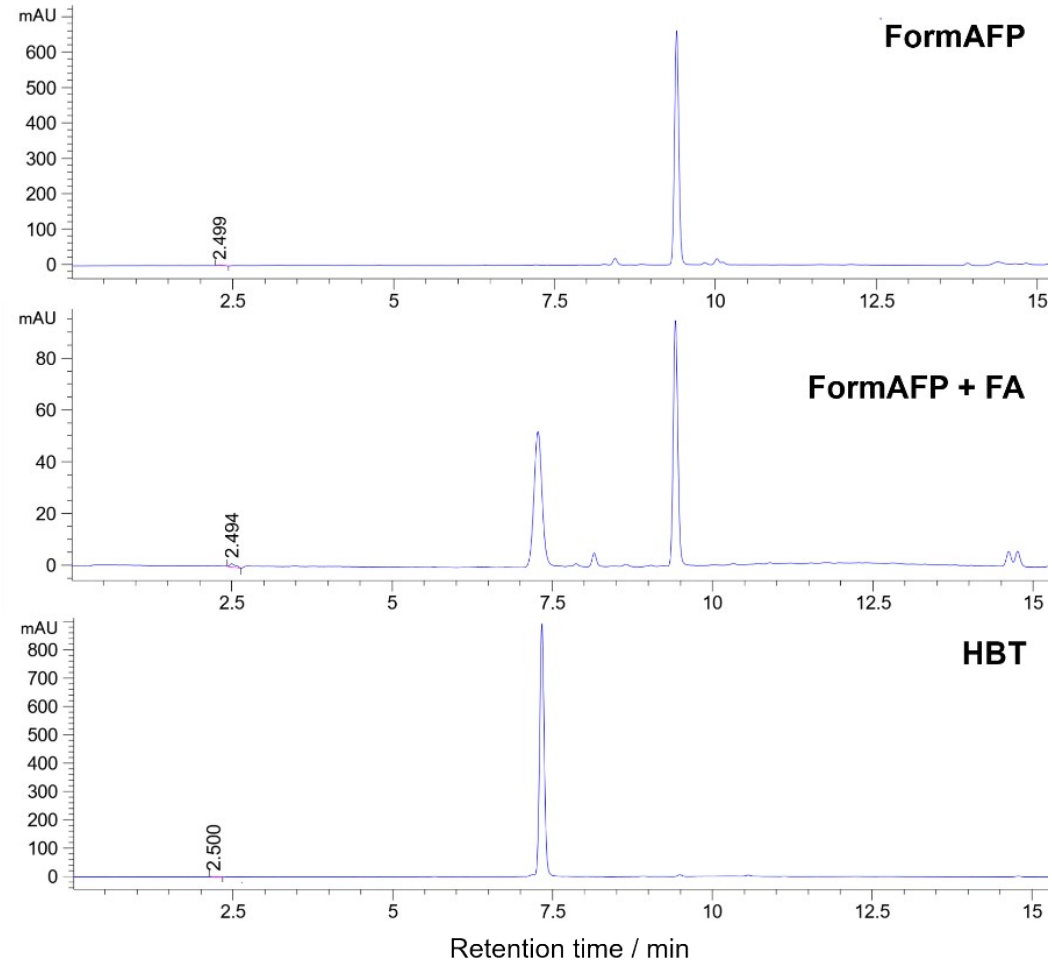


Figure S7. HPLC chromatogram analysis. Upper curve: **FormAFP** (10 μM), Middle curve: **FormAFP** (10 μM) + formaldehyde (100 μM), Lower curve: compound HBT (10 μM). HPLC analysis condition: The mobile-phase eluents were water/methanol (from 1/2 to 1/1 v/v) during 20 min, detection wavelength was set at 245 nm.

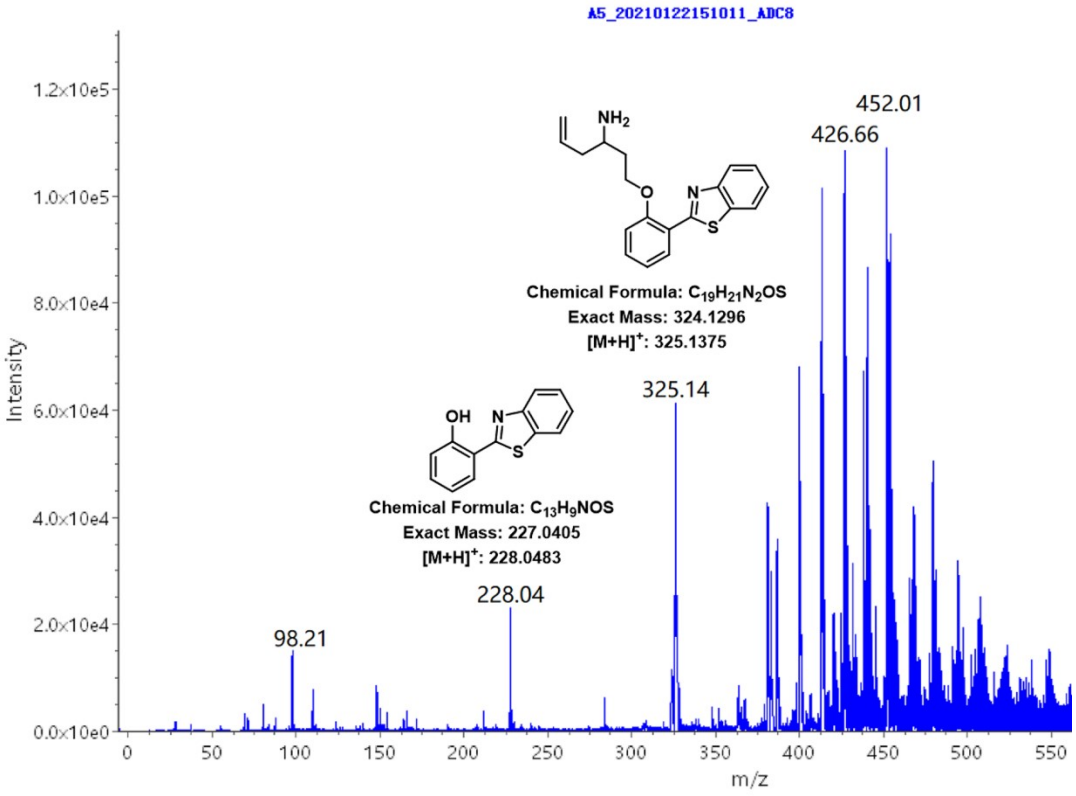


Figure S8. MALDI-TOF-MS spectrum of the reaction product of 50 mM FA and 10 mM **FormAFP**.

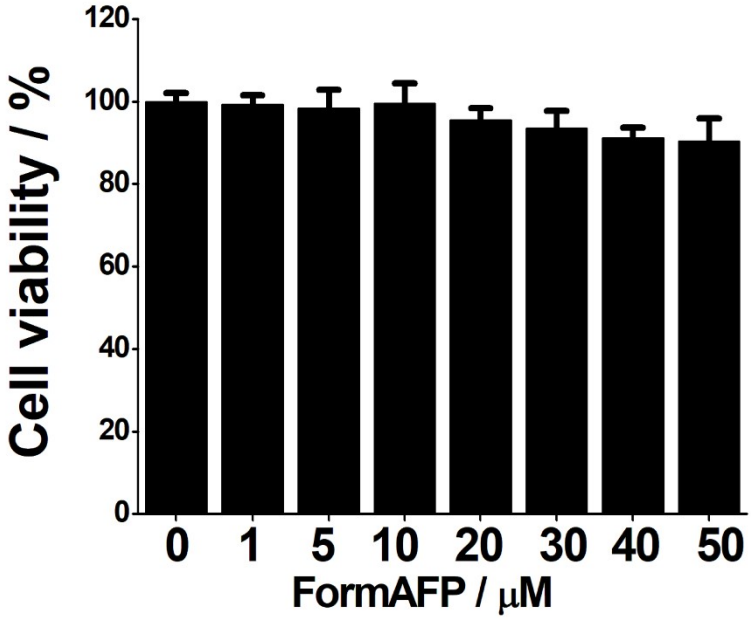


Figure S9. Cell viability of MCF7 treated with different concentrations of **FormAFP** (0-30 mM) for 24 h in fresh medium. The results are the mean standard deviation of five separate measurement.

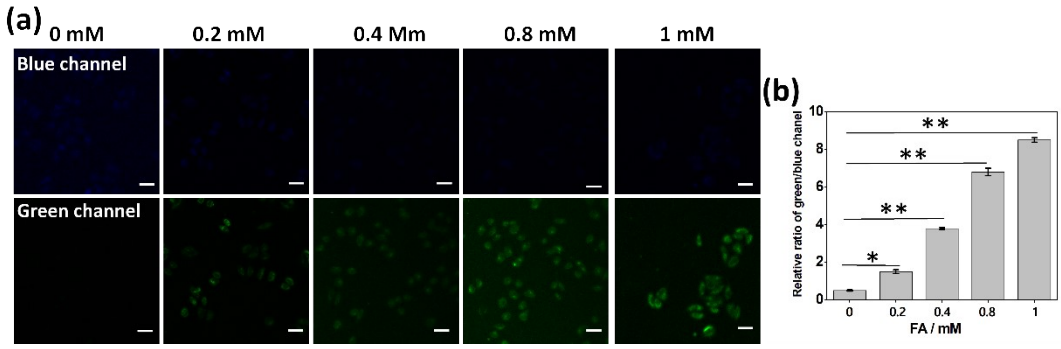


Figure S10. (a) Confocal fluorescence images of MCF7 cells with the addition of **FormAFP** with different concentrations at 0, 0.2, 0.4, 0.8 and 1 mM for 30 min; (b) Mean fluorescence intensities ratio of green/blue channel of MCF-7 cells treated in (a). Error bars denote SEM, n = 6; n.s.=not significance, * P <0.005, ** P <0.0005. Excitation is set at 350 nm, blue channel: 380–460 nm, green channel: 500–600 nm. The scale bar is 40 μm.

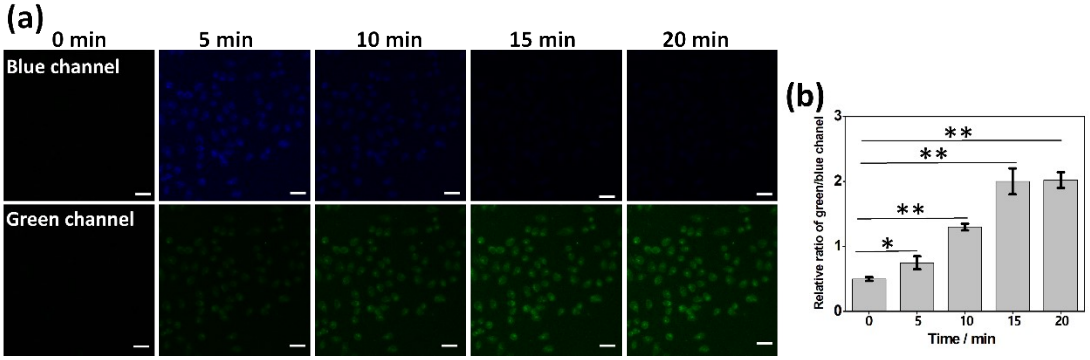


Figure S11. (a) Confocal fluorescence images of MCF7 cells with the addition of **FormAFP** recorded at 0, 5, 10, 15 and 20 min; (b) Mean fluorescence intensities ratio of green/blue channel of MCF-7 cells treated in (a). Error bars denote SEM, n = 6; n.s.=not significance, * P <0.005, ** P <0.0005. Excitation is set at 350 nm, blue channel: 380–460 nm, green channel: 500–600 nm. The scale bar is 40 μm.

3. NMR spectra and mass spectra

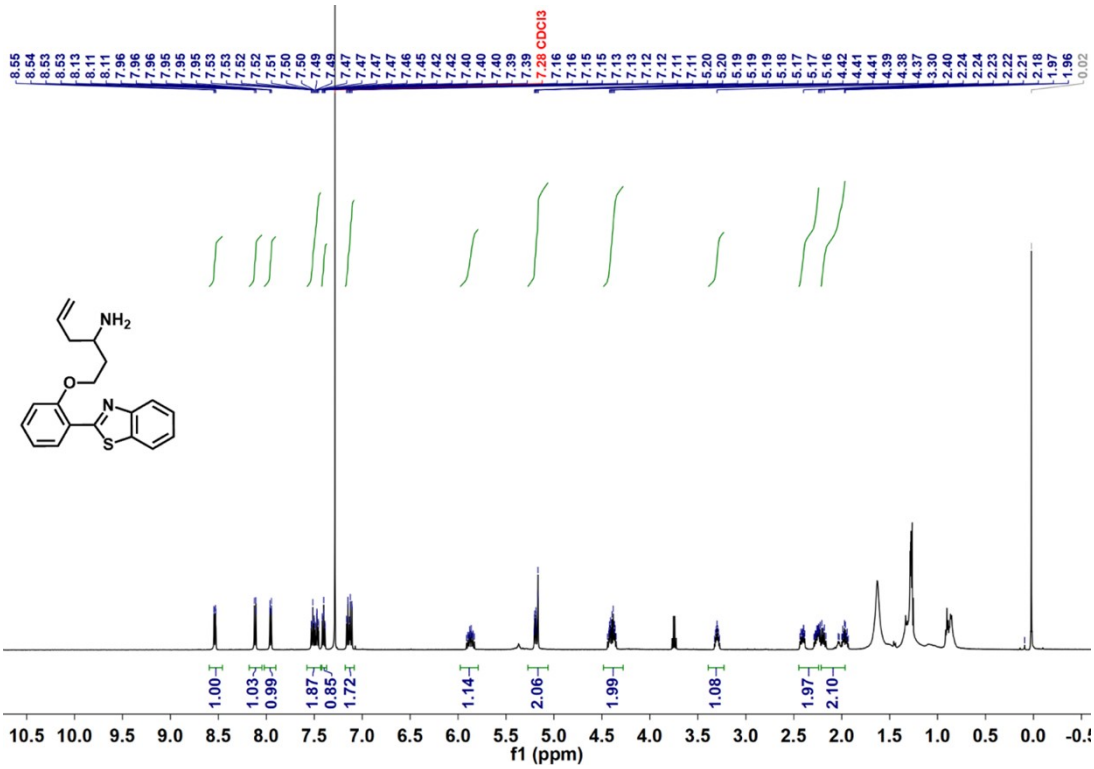


Figure S12. The ^{1}H NMR spectrum of **FormAFP** in $CDCl_3$ (500 MHz).

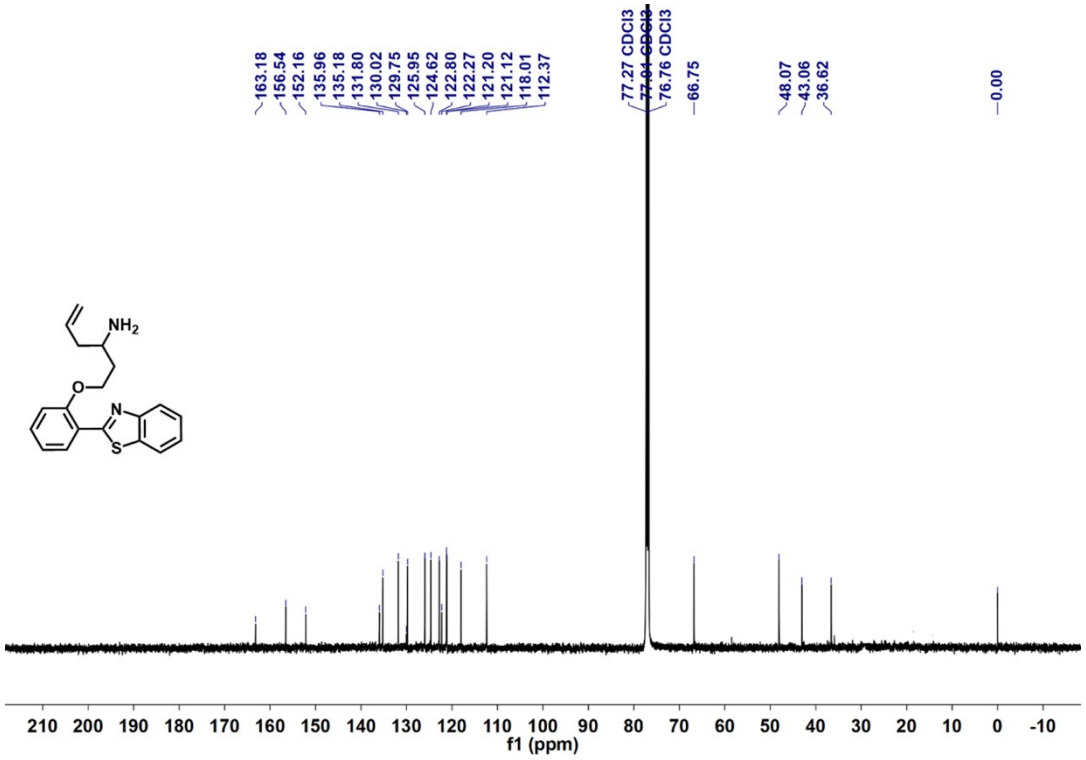


Figure S13. The ^{13}C NMR spectrum of **FormAFP** in $CDCl_3$ (125 MHz).

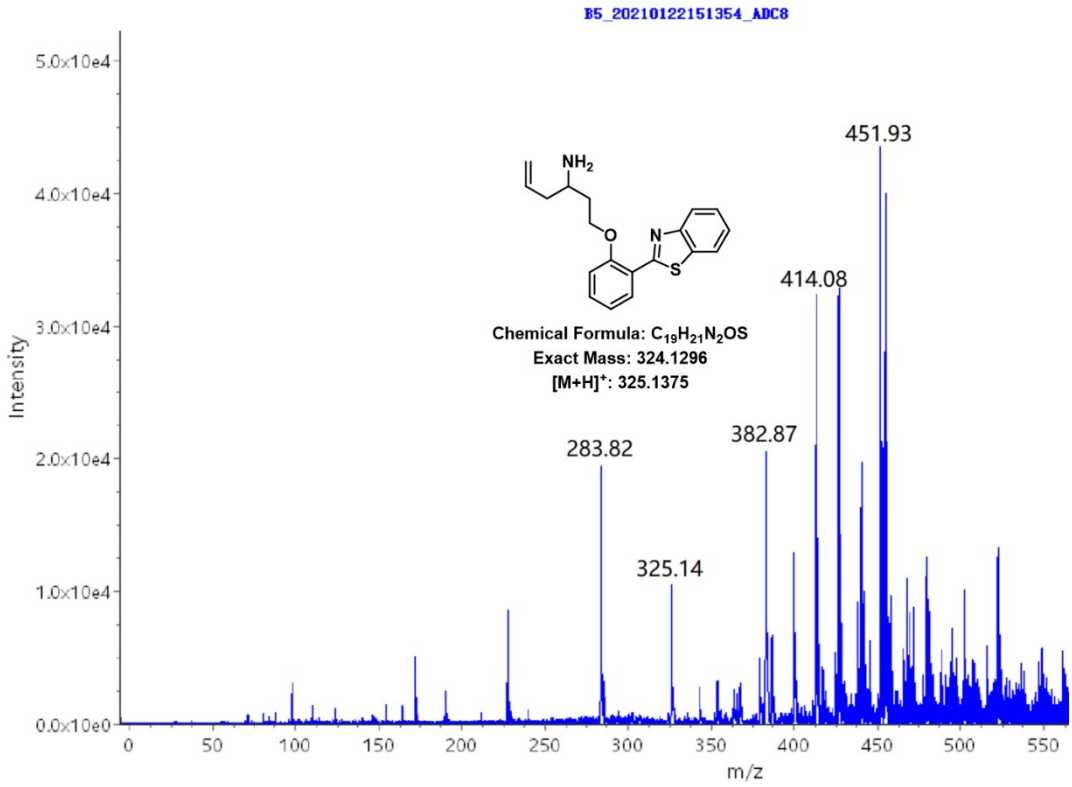


Figure S14. MALDI-TOF mass spectra of **FormAFP**.