Supporting Information

A water-soluble two-photon ratiometric triarylboron probe with nucleolar targeting by preferential RNA binding

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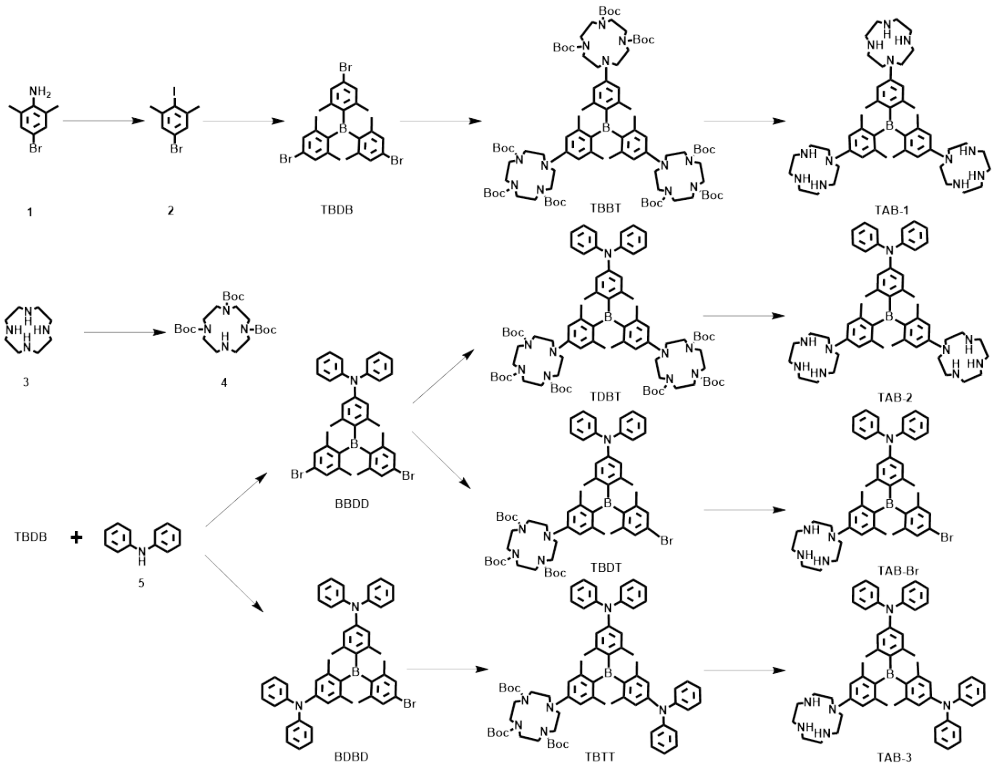
Experimental Details

1. General information

All chemical reagents were purchased from J&K (Beijing, China) and used without further purification. RNA and DNA were purchased from sigma. RNA and DNA were directly dissolved into water for using. Absorption spectra were recorded on Hitachi UV-3010. The fluorescence spectra were obtained on Hitachi F-7000. Cells were analyzed using a confocal microscope (OLYMPUS FV 1000-IX81 for single-photon excited fluorescence, Leica TCS SP8 for two-photon excited fluorescence). ^{1}H NMR spectra were obtained on BrukerAvance III 400 H (400 MHz) spectrometers.

2. Synthsis of TAB-1, TAB-2, TAB-Br and TAB-3.



Scheme S1. The synthetic route to compounds TAB-1, TAB-2, TAB-Br and TAB-3.

Compound 2 and Compound 4 was synthesized according to the previous reported procedure.

Synthesis of Compound TBDB

Under a N_2 atmosphere at room temperature, a hexane solution of n-BuLi (1.6 M, 20mL, 32 mmol) at -78 °C to a solution of Compound 2 (10.0 g, 32 mmol) in dry Et_2O (60 mL) was added dropwise. The reaction mixture was allowed to warm to 0 °C and stirred for 20 min. Then, $BF_3{\cdot}Et_2O$ (1.25 mL, 10mmol) was dropped into the mixture at -78 °C. The reaction mixture was warmed up to room temperature and stirred overnight. The solvent was removed under vacuum to give crude product, which was purified by column chromatography on silica gel to afford the pure product as a white solid (4.0 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 1.97 (s, 18H), 7.11 (s, 6H). MALDI-TOF (m/z): Calcd. For $C_{24}H_{24}BBr_3$ 561.9, found 562.9.

Synthesis of Compound TBBT

Compound 4 (1.5g, 3.2mmol), TBDB (560mg, 1mmol), sodium tert-butoxide (864mg, 9mmol), palladium acetate (27 mg, 0.12mmol) and P (t-Bu)$_3$ (25mg, 0.12mmol)were placed into a three-necked flask under a nitrogen atmosphere and was stirred at 90 °C for 24 h. The reaction mixture was then cooled to 20 °C, evaporated in vacuum. The crude product was purified by column chromatography (SiO_2, PE–EtOAc 5:1–1:1) to give compound TBBT(860

mg, 50%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 1.46 (s, 81H), 1.97 (s, 18H), 3.10-3.70(m, 48H), 6.29 (s, 6H). MALDI-TOF (m/z): Calcd. For $C_{93}H_{153}BN_{12}O_{18}$ 1738.2, found 1737.2.

Synthesis of Compound BBDD and BDBD

A solution of diphenylamine (1g, 6mmol) in dry toluene (20mL) was added dropwise over 2h to a mixture of TBDB, $Pd(dba)_2$ (69 mg, 0.12mmol), BINAP(150mg, 0.24mmol), sodium tert-butoxide (1.15 g, 12mmol) in dry toluene was placed in a three-necked flask under a nitrogen atmosphere and was stirred at 90 °C for 24 h. After cooling, the reaction was quenched by adding water and then was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and evaporated under vacuum. The products were purified by column chromatography (SiO_2, PE–EtOAc 5:1–1:1) to give compound BBDD (650mg, 25%) and BDBD (880mg, 30%) as yellow solid. BBDD: ^{1}H NMR (400 MHz, CD_2Cl_2) , δ (TMS, ppm): 1.86 (s, 6H), 1.97 (s, 6H), 2.20(s, 6H), 6.60 (s, 2H), 6.90-7.14 (m, 8H), 7.19-7.30 (m, 6H). MALDI-TOF (m/z): Calcd. For $C_{36}H_{34}BBr_2N$ 651.1, found 651.2.

BDBD: ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 1.78 (s, 6H), 1.90 (s, 6H), 1.99(s, 6H), 6.52 (d, 4H, J=11.1), 6.95 (m, 4H), 7.01 (m, 10H), 7.18 (m, 8H). MALDI-TOF (m/z): Calcd. For $C_{48}H_{44}BBrN_2$ 740.3, found 739.4.

Synthesis of Compound TDBT and TBDT

Compound TDBT and TBDT were synthesized according to the same procedure as that of TBBT. Yellow solid of TDBT and TBDT was obtained in 45% and 28%, respectively. Compound 4 (1g, 2mmol), BBDD (650mg, 1mmol), sodium tert-butoxide (576mg, 6mmol), $P(t\text{-}Bu)_3$ (25mg, 0.12mmol) and palladium acetate (27 mg, 0.12mmol). TDBT: ^{1}H NMR (400 MHz, CD_2Cl_2) , δ (TMS, ppm): 1.30(s, 54H), 1.75-1.93(m, 18H), 3.18-3.35(m, 32H), 6.20(d, 4H, J=9.3), 6.47(s, 2H), 6.85(m, 2H), 6.89(d, 4H, J=7.8), 7.12(m, 4H). MALDI-TOF (m/z): Calcd. For $C_{82}H_{120}BN_9O_{12}$ 1434.7, found 1434.0. TBDT: ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 1.32(s, 27H), 1.72-1.94(m, 18H), 3.19-3.38(m, 16H), 6.20(d, 2H, J=12.0), 6.42(d, 2H, J=7.6), 6.48(s, 2H), 6.78(m, 2H), 6.87(d, 4H, J=10.0), 7.11(m, 4H). MALDI-TOF (m/z): Calcd. For $C_{59}H_{770}BBrN_5O_6$ 1043.5, found $[M - Br]^+$ = 963.8.

Synthesis of Compound TBTT

Compound TBTT was synthesized according to the same procedure as that of TBBT. Yellow solid of TDBT and TBDT was obtained in 82%. Compound 4 (566mg, 1.2mmol), BDBD (740mg, 1mmol), sodium tert-butoxide (278 mg, 3mmol). $P(t\text{-}Bu)_3$ (25mg, 0.12mmol) and palladium acetate (27 mg,0.12mmol). ^{1}H NMR (400 MHz, CD_2Cl_2) , δ (TMS, ppm): 1.43-1.45 (m, 27H), 1.91 (s, 6H), 1.96 (s, 6H), 2.05 (s, 6H) , 3.31-3.51 (m, 16H), 6.33 (s, 2H), 6.62 (d, 4H, J=6.8) , 7.01 (m, 4H) ,7.07 (m, 8H) , 7.25 (m, 8H). MALDI-TOF (m/z): Calcd. For $C_{71}H_{87}BN_6O_6$ 1130.7, found 1129.0.

Synthesis of Compound TAB-1

Compound TBBT (174 mg, 0.1mmol) was dissolved in CH_2Cl_2 (5ml) and trifluoroacetic acid (0.2 ml, 2.8mmol) was added. The reaction mixture was stirred for 24 h (until no starting compound was indicated by TLC (SiO_2, MeOH), evaporated, and dried under vacuum to yield a viscous solid. Then the solid was dissolved in MeOH (10ml) and poured onto the column of strongly basic anion exchanger (Ion exchanger III; Merck). The column was washed with 100 ml MeOH. The solution was dried under vacuum to give 83mg (99%) of TAB-1 as yellow solid. ^{1}H NMR (400 MHz, CD_3OD), δ (TMS, ppm): 1.86 (s, 18H), 1.91 (s, 6H), 2.53-2.74 (m, 36H), 3.26 (m, 12H) , 6.50 (s, 6H). ^{13}C NMR (75 MHz, CD_3OD) δ = 150.7, 141.9, 138.4, 131.0, 128.5, 122.3, 116.5, 116.0,65.3, 60.1, 48.2, 45.1, 30.3, 22.4, 20.2, 19.5, 18.7 ppm. MALDI-TOF (m/z): Calcd. For $C_{48}H_{81}BN_{12}$ 836.7, found 837.9. Melting point：155.6 °C.

Synthesis of Compound TAB-2

Compound TDBT (144 mg, 0.1mmol) was dissolved in CH_2Cl_2:CH_3OH (5:2) (7ml) and hydrochloric acid (2 ml) was added. The reaction mixture was stirred for 48 h (until no starting compound was indicated by TLC (SiO_2, MeOH), evaporated, and dried under vacuum to yield yellow solid. Then the solid was dissolved in MeOH (10ml) and poured onto the column of strongly basic anion exchanger (Ion exchanger III; Merck). The column was washed

with 100 ml MeOH. The solution was dried under vacuum to give 82mg (99%) of TAB-1 as yellow solid.^{1}H NMR (400 MHz, CD_3OD) , δ (TMS, ppm): 1.84 (s, 6H), 1.94 (s, 6H), 2.01 (s, 6H), 2.62-2.82 (m, 24H), 3.34 (m, 8H), 6.56 (m, 6H), 6.98 (m, 6H), 7.22 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ = 151.3, 148.6, 147.6, 142.1, 141.8, 128.9, 124.4, 122.7, 121.9, 116.6, 51.1, 22.4, 22.0 ppm. MALDI-TOF (m/z): Calcd. For $C_{52}H_{72}BN_9$ 833.6, found 834.8. ；Melting point：144.6 °C.

Synthesis of Compound TAB-Br

Compound TAB-Br was synthesized according to the same procedure as that of TAB-2. Compound TBDT (104 mg, 0.1mmol), TAB-Br (73mg, 99%) was obtained as Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) , δ (TMS, ppm): 1.77-2.18 (m, 18H), 2.56-3.04 (m, 12H), 3.18-3.34 (m, 4H), 6.33 (d, 1H, J=12.7), 6.50-6.60 (m, 3H), 6.85(m, 3H), 6.97(m, 5H), 7.19(m, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ = 147.7, 128.9, 124.5, 122.7, 51.1, 45.1, 21.8 ppm. MALDI-TOF (m/z): Calcd. For $C_{44}H_{53}BBrN_5$ 743.4, found [M-Br] = 664.6. Melting point：130.2 °C.

Synthesis of Compound TAB-3

Compound TAB-3 was synthesized according to the same procedure as that of TAB-2. Compound TBDT (115 mg, 0.1mmol), TAB-3 (87mg, 99%) was obtained as Yellow solid. ^{1}H NMR (400 MHz, CD_3OD), δ (TMS, ppm): 1.89 (s, 6H), 1.97 (s, 6H), 2.07 (s, 6H), 2.68-2.92 (m, 12H), 3.48 (m, 4H), 6.56-6.61 (m, 6H), 6.97-7.06(m, 12H), 7.24-7.28(m, 8H). ^{13}C NMR (75 MHz, CD_3OD) δ = 149.4, 143.7, 131.0, 125.4, 123.8, 119.0, 116.5, 53.0, 47.3, 46.9, 24.0 ppm. MALDI-TOF (m/z): Calcd. For $C_{56}H_{63}BN_6$ 830.5, found 831.7. Melting point：140.8 °C.

3. Cell culture and viability assay

Mouse fibroblast cells (NIH/3T3) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with glucose (4.5 g/L), L-glutamine, sodiumpyruvate,and 10% fetal bovine serum (FBS). The cells were plated on glass bottomed dishes at 37 °C under 5% CO_2 atmosphere before imaging. Cell images were obtained using a confocal microscope FV1000-IX81 and were analyzed with FV10-ASW software.

NIH/3T3 cells, pre-washed twice, were incubated with 10 μ M in cultured medium without FBS at 37°C under 5% CO_2 for certain time. Then the cells were washed with PBS to remove unbounded probes for six times before *in situ* imaging by Olympus FV1000-IX81 confocal laser scanning microscopy using oil objective, with excitation by 405nm laser or 760nm. For RNase digest test, the cells was first incubated with 30 mg/mL DNase-Free RNase (GE) for 2 h. After rinsing with PBS twice, the cells were furhter incubated with TAB-2 for 35min

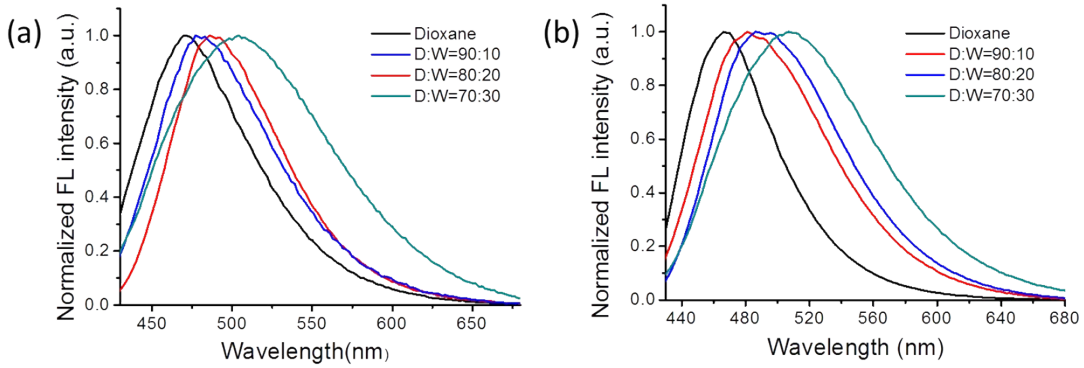


Figure S1. Fluorescence spectra of TAB-Br (a) and TAB-3 (b) in different ratios of water and 1,4-dioxane. Concentration: 1 μM; Excitation wavelength: 405 nm.

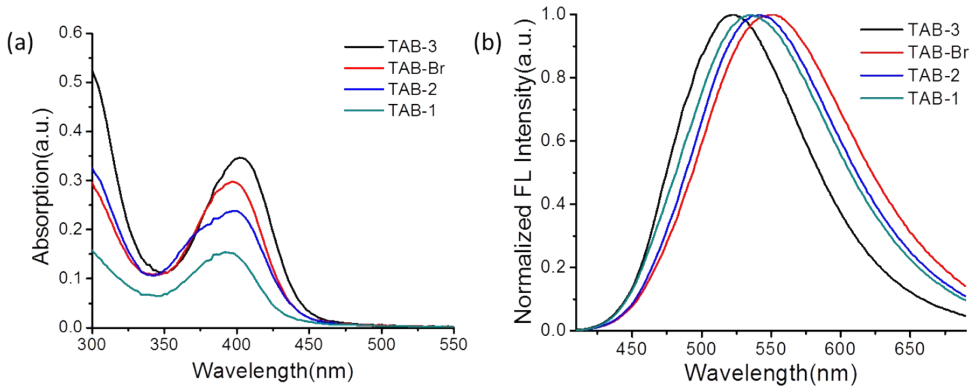


Figure S2. (a) UV absorption and (b) fluorescence spectra of TAB-1, TAB-2, TAB-Br and TAB-3 in DMSO (10 μM).

Table S1. Quantum yield (Φ) of TAB-1, TAB-2, TAB-Br and TAB-3 in DMSO determined by a Hamamatsu absolute PL quantum yield spectrometer C11347.

Compound	TAB-1	TAB-2	TAB-Br	TAB-3
Φ	0.501	0.500	0.519	0.565

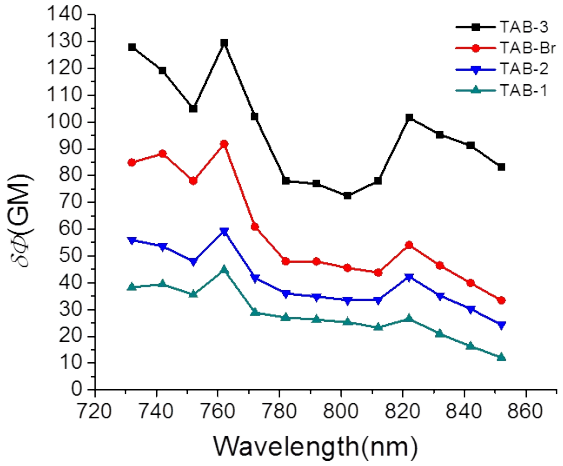


Figure S3. Two-photon action spectra of TAB-1, TAB-2, TAB-Br and TAB-3 in DMSO.

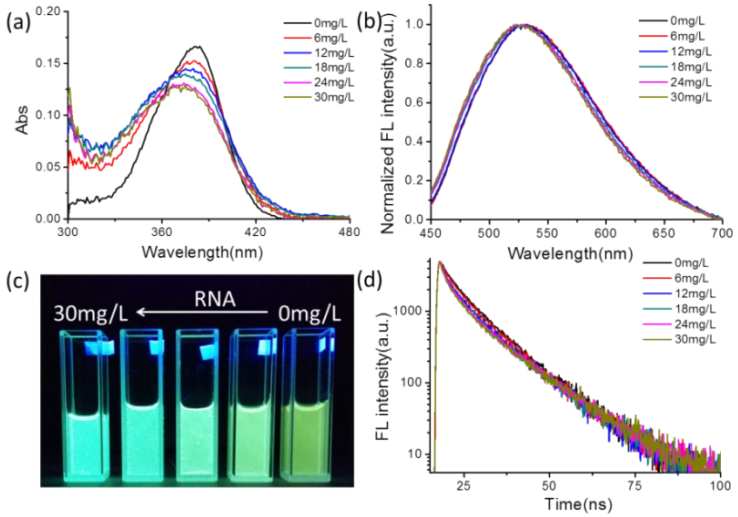


Figure S4. (a) UV absorption, (b) fluorescence spectra, (c) visual color, and (d) fluorescence decay changes for

TAB-1 with the addition of different amounts of RNA from *Torula* yeast. Concentration of TAB-1: 10 μM; Excitation wavelength: 405 nm.

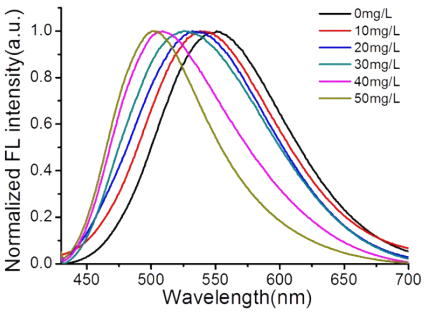


Figure S5. Fluorescence spectra changes for TAB-2 with the addition of different amounts of single-stranded DNA. Concentration of TAB-2: 10 μM; Excitation wavelength: 405 nm.

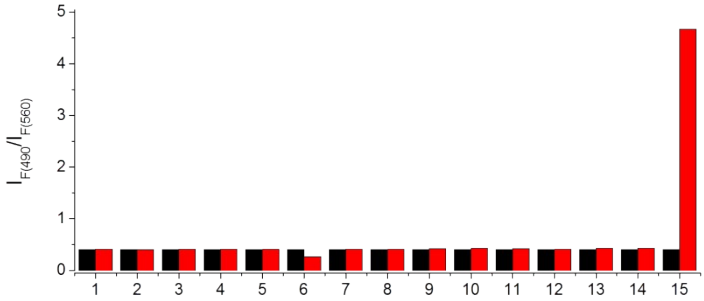


Figure S6. Fluorescence responses of TAB-2 to various substances. 1) A (100 μM), 2) C (100 μM), 3) G (100 μM), 4) T (100 μM), 5) U (100 μM), 6)TMP (100 μM), 7) AMP (100 μM), 8) CMP(100 μM), 9) GMP(100 μM), 10) UMP(100 μM), 11)Cys (100 μM), 12) Hcy (100 μM), 13) GSH(100 μM), 14) Glucose (100 μM), 15)RNA (30 mg/L).

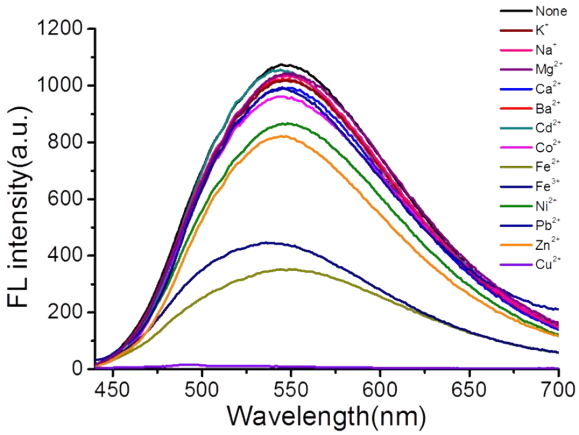


Figure S7. Fluorescence response of TAB-2 in HEPES(5mM, pH=7.4) for various metal ions.

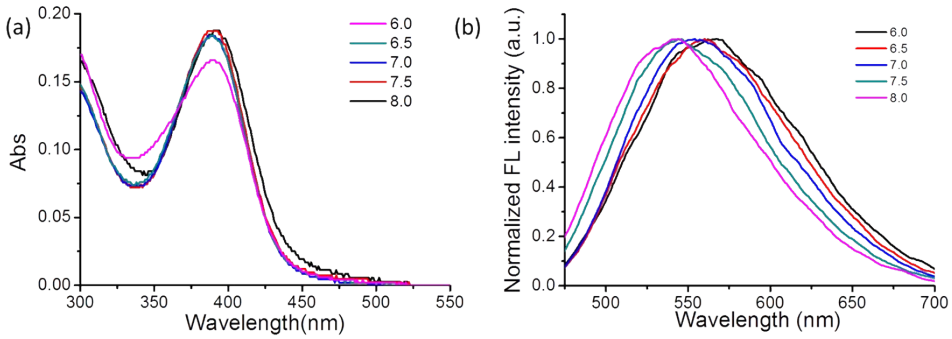


Figure S8. (a) UV absorption spectra and (b) Fluorescence spectra of TAB-2 in water at different pH values.aa

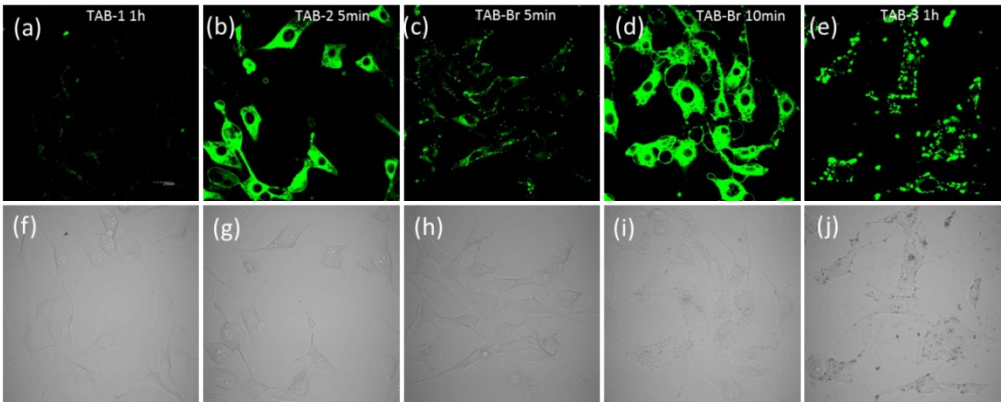


Figure S9. (a) - (e) Confocal fluorescence images of NIH/3T3 cells stained with TAB-1, TAB-2, TAB-Br and TAB-3 for different time interval at λexc = 405 nm by collecting from 500 to 550 nm; (f)-(j) are their corresponding bright-field images.

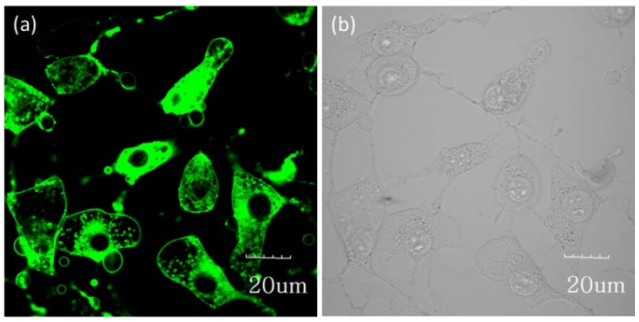


Figure S10. Confocal fluorescence images of NIH/3T3 cells stained with TAB-2 for 5 min for observing the process of endocytosis (the presence of many staining vesicles).

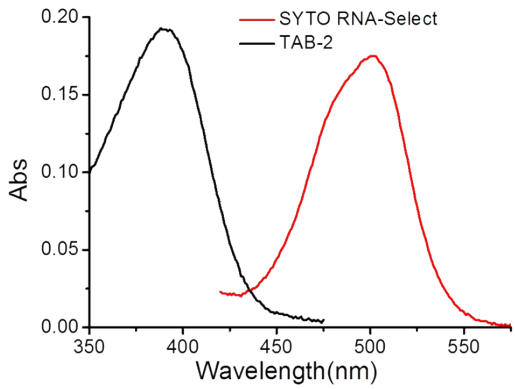


Figure S11. absorption spectra of TAB-2 and "SYTO RNA-Select" in DMSO (10 uM).

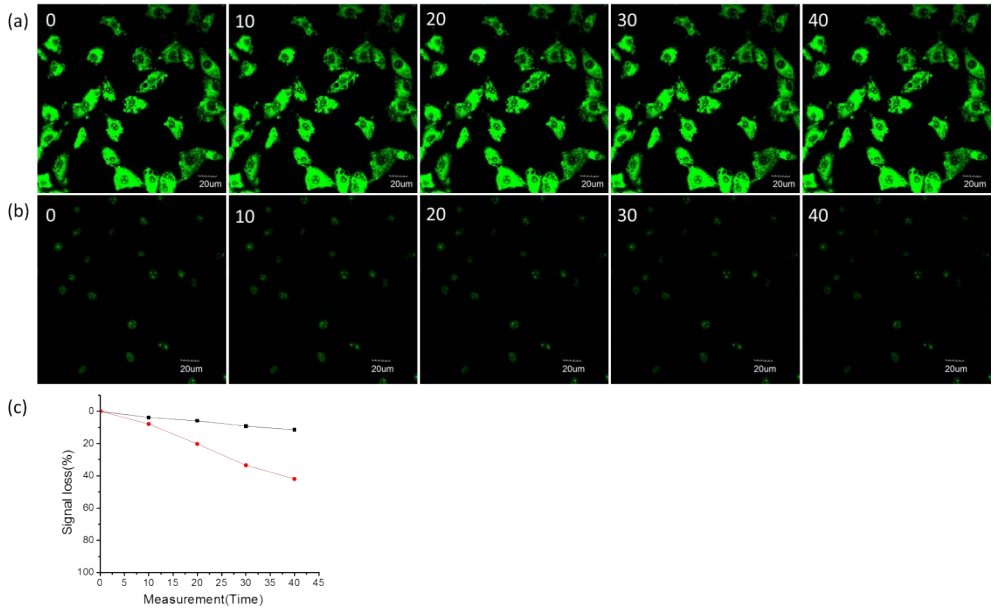


Figure S12. Fluorescent images of living NIH/3T3 cells stained with TAB-2 and SYTO RNA-Select with increasing number of scans (1, 10, 20, 30, 40 times) by exciting with (a) 405 nm and (b) 488 nm (laser power at the sample plane: 100 μ W); Collecting range: 500-550 nm; irradiation time: 15 s/scan. (c) Signal loss (%) of fluorescence emission of TAB-2 (black) and SYTO RNA-Select (red) with increasing number of scans.

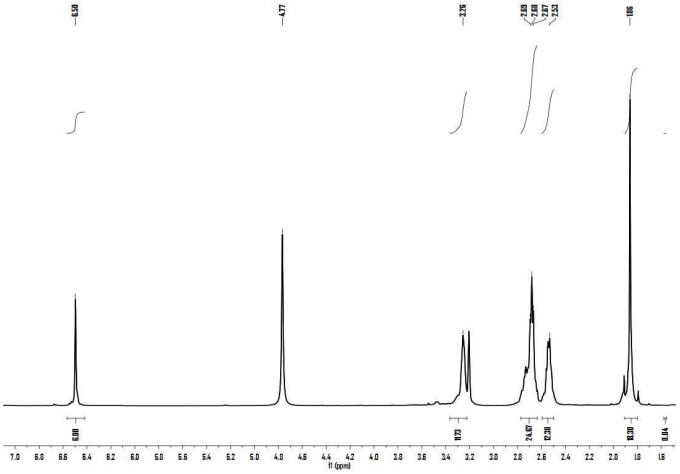


Figure S13 ^{1}HNMR spectra of TAB-1 in CD_3OD.

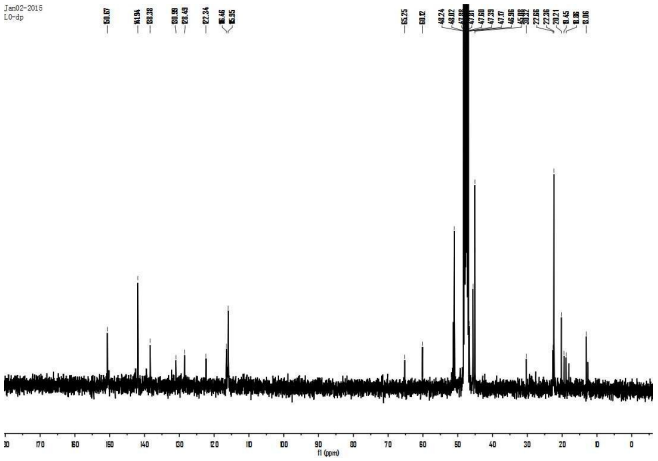


Figure S14 ^{13}CNMR spectra of TAB-1 in CD_3OD.

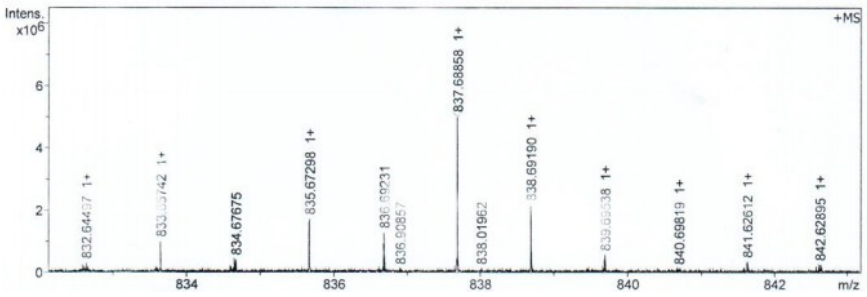


Figure S15 Mass spectra of TAB-1.

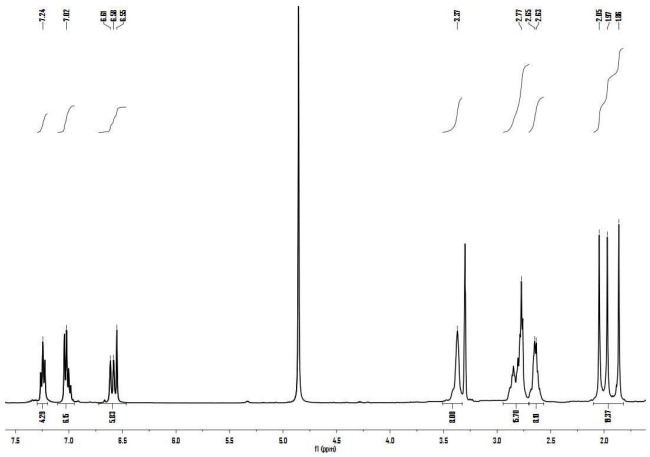


Figure S16 ^{1}HNMR spectra of TAB-2 in CD_3OD.

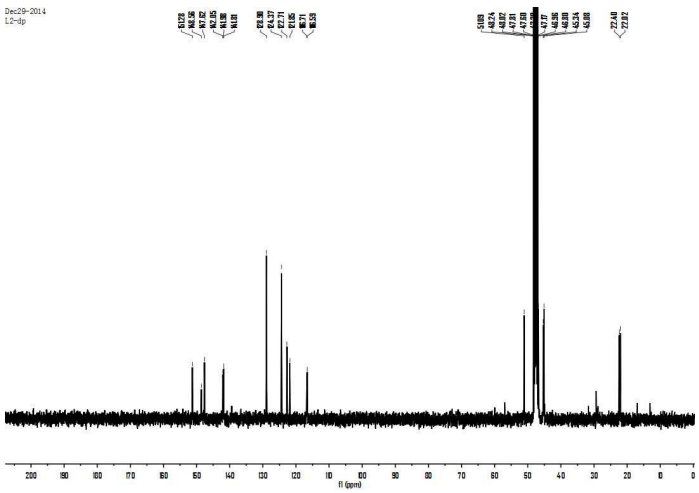


Figure S17 ^{13}CNMR spectra of TAB-2 in CD_3OD.

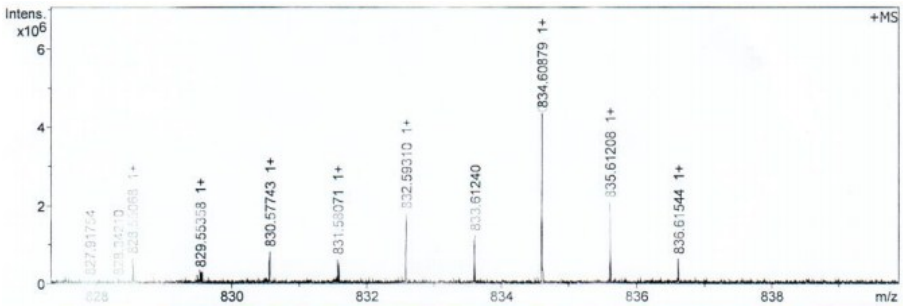


Figure S18 Mass spectra of TAB-2.

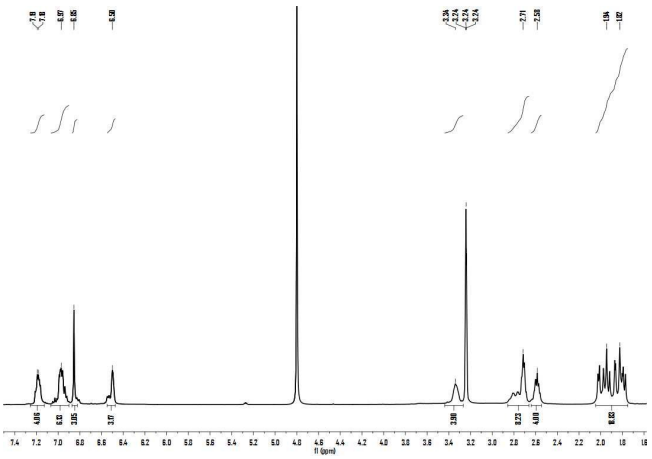


Figure S19 ^{1}HNMR spectra of TAB-Br in CD_3OD.

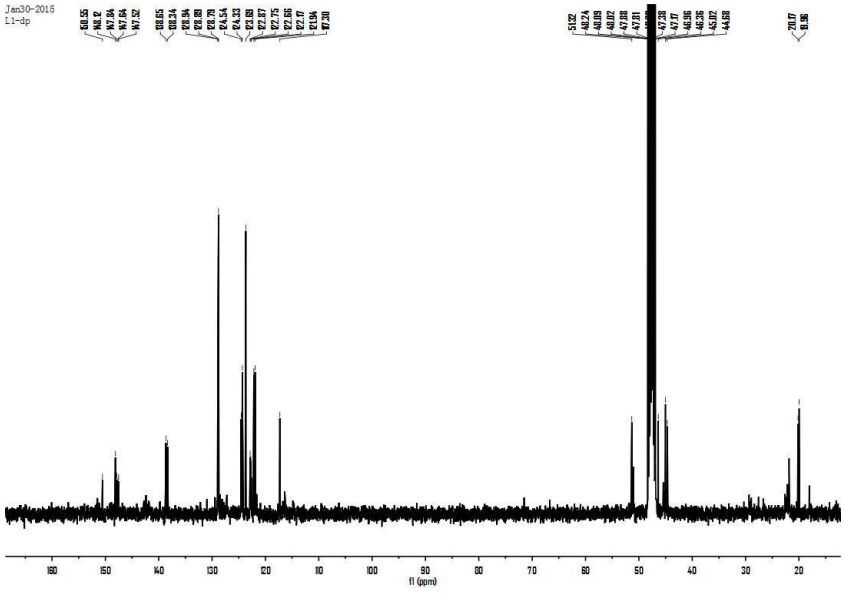


Figure S20 ^{13}CNMR spectra of TAB-Br in CD_3OD.

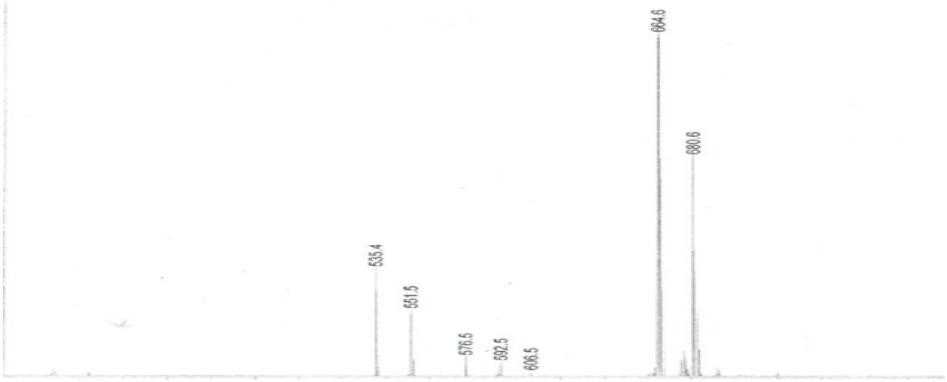


Figure S21 Mass spectra of TAB-Br.

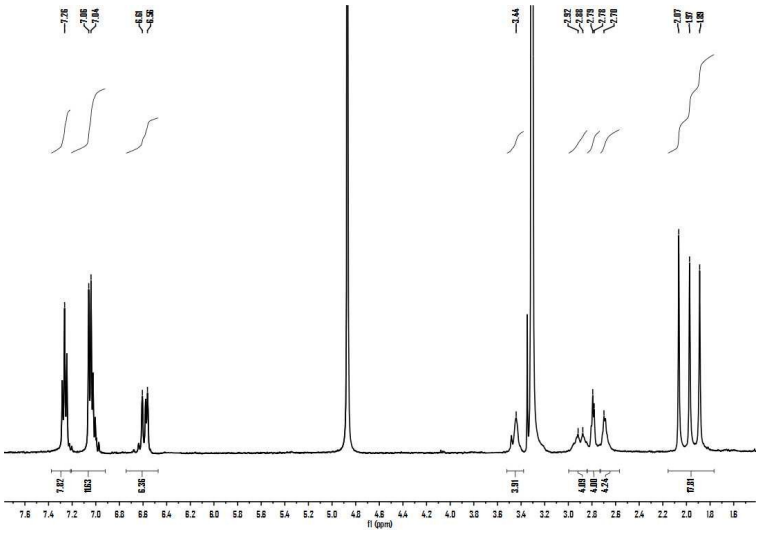


Figure S22 ^{1}HNMR spectra of TAB-Br in CD_3OD.

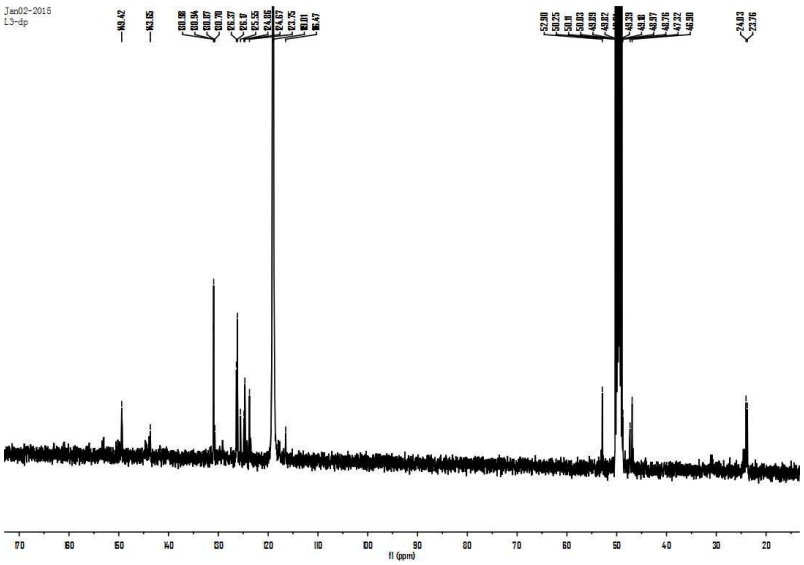


Figure S23 ^{13}CNMR spectra of TAB-3 in CD_3OD.

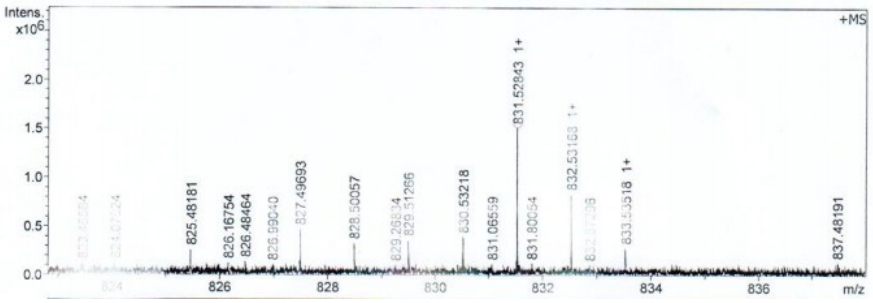


Figure S24 Mass spectra of TAB-3.