Supporting Information

CB[8]- and triarylboron-based supramolecular organic framework for microRNA detection, tumor-targeted drug delivery, and photodynamic therapy

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Experimental Procedures

1. General Information

General chemical reagents were purchased from Beijing InnoChem Science &Technology Co (Beijing, China) and used without further purification. Absorption spectra were recorded on Hitachi UV-3010 (Hitachi, Tokyo, Japan). The fluorescence spectra were obtained on Hitachi F-7100 (Hitachi, Tokyo, Japan). Cells were analyzed using a confocalmicroscope (OLYMPUS FV 1000-IX81 Olympus Corporation, Tokyo, Japan). NMR spectra were obtained on BrukerAvance III 400 H (400 MHz) spectrometers (Bruker, Karlsruhe, Germany). In vivo small animal imaging system (In-Vivo MS FX PRO, Bruker, Germany). RNA and DNA are directly purchased from Beijing InnoChem Science &Technology Co (Beijing, China), which extracted from yeast and salmon white, respectively.

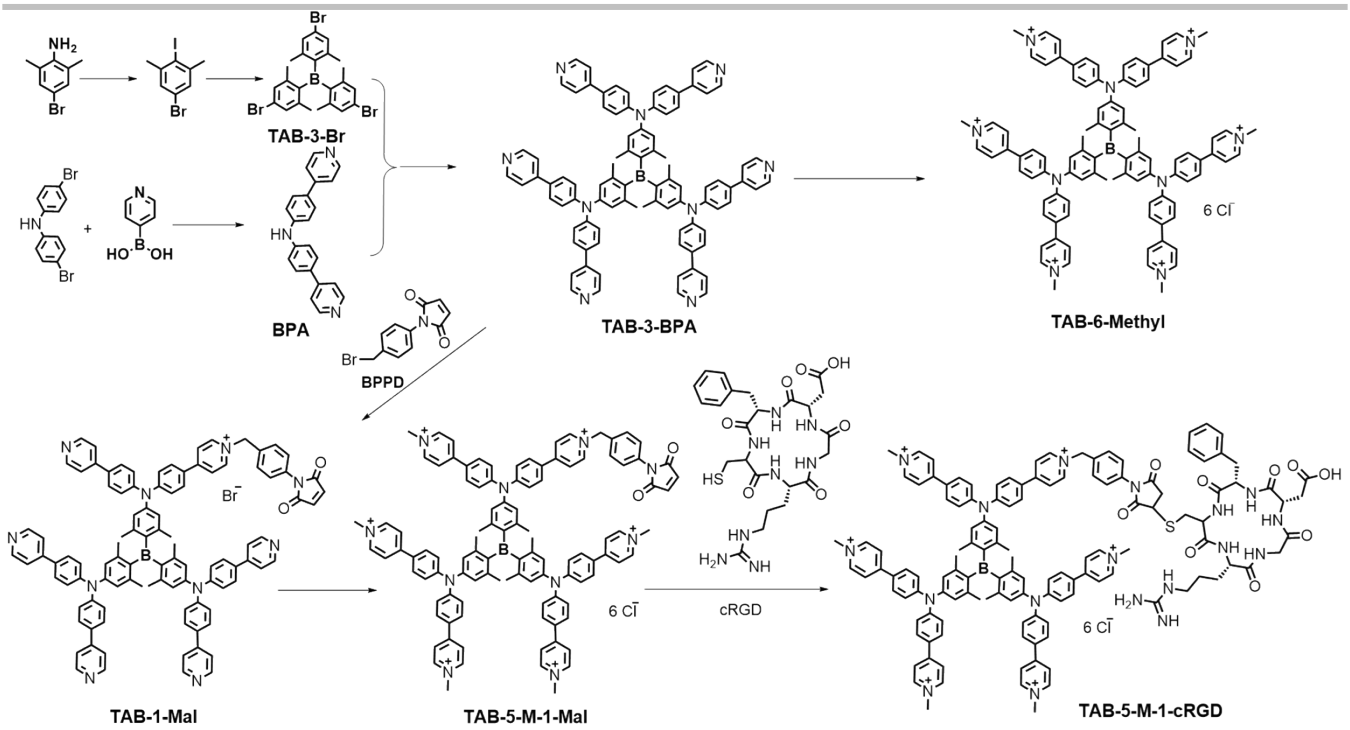
The sequence of miRNA used in our experiment:

miRNA21：UAGCUUAUCAGACUGAUGUUGA

miRNA141: UAACACUGUCUGGUAAAGAUGG

miRNA151: CUAGACUGAAGCUCCUUGAGG

2. Synthesis



Scheme S1. The synthetic route to compounds TAB-6-Methyl and TAB-5-M-1-cRGD.

TAB-3-Br , cRGD, BPA and BPPD were synthesized according to the previous reported procedure.[1, 2, 3]

Synthesis of Compound TAB-3-BPA

TAB-3-Br (560mg, 1mmol),BPA (1.94mg, 6mmol), sodium tert-butoxide (864mg, 9mmol),$Pd(dba)_2$ (69 mg, 0.12mmol), BINAP(150mg, 0.24mmol) and Anhydrous toluene (30 mL)were placed into a three-necked flask under a nitrogen atmosphere and was stirred at 90 °C for 3 days. The reaction mixture was then cooled to 20 °C, evaporated in vacuum. The crude product was purified by column chromatography (SiO_2, EtOAc-Methol5:1) to give compound *TAB-3-BPA*(760 mg, 59%) as yellow solid.^{1}H NMR (400 MHz, CD_2Cl_2) δ: 2.12 (s, 18H), 6.84(s, 6H), 7.27-7.29 (d, 12H,J=8), 7.55-7.57 (d, 12H,J=8), 7.65-7.67 (d, 12H,J=8) ,8.64-8.65(d,12H,J=4)ppm. ^{13}C NMR (100 MHz, CD_2Cl_2) δ:150.10,146.80,142.45,131.59,127.97,124.67,120.68,22.74 ppm. MALDI-TOF (m/z): Calcd. For $[C_{90}H_{72}BN_9]$ (M/Z, Z=3)429.87, found 430.87.

Synthesis of Compound TAB-6-Methyl

A mixture TAB-3-BPA (30mg) and CH_3I (1 ml) was stirred at 25 °C. The reaction mixture turned orange within 5 min and reddish precipitate began to appear.After stirring for overnight, methanol (1mL) was added and continue stirring overnight.The remaining CH3I and methanolwas evaporated in vacuo after 24 h of stirring. The residue was dissolved in water. Potassium hexafluorophosphate wasadded to the solution and the precipitate was dissolved in acetonitrile. The acetonitrile solution was added to a solutionof excess tetrabutyl ammonium chloride in acetonitrile and the deposit was filtered and washed with acetonitrile for five times. By drying the solid in a vacuum oven, desired product compound *TAB-6-Methyl*was obtained as a yellow solid (35mg, 96%).^{1}H NMR (400 MHz, CD_3OD) δ: 2.16 (s, 18H), 4.39 (s, 18H), 6.93(s, 6H), 7.27-7.29 (d, 12H, J=8), 8.06-8.08 (d, 12H, J=8), 8.37-8.39 (d, 12H, J=8) ,8.83-8.85(d,12H, J=8) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ:154.84,150.20,147.08,145.03,143.01,129.36,127.97,125.13,123.98,123.28,22.15 ppm MALDI-TOF (m/z): Calcd. For $[C_{96}H_{90}BN_9]^{6+}$ (M/Z, Z=6)230.12, found 230.12.

Synthesis of Compound TAB-5-M-1-Mal

A mixture TAB-3-BPA (300mg) , BPPD (12.4mg) and DMF (2mL) was stirred at 80 °C for overnight. The DMF was removed. The excessTAB-3-BPA was removed by column chromatography (SiO_2, EtOAc-Methol5:1). The mixture of TAB-5-Maleimide and silica gel was derectly react with CH3I, and the mixture was then disolved into water. The silica gel was removed by centrifugation. Potassium hexafluorophosphate was added to the solution and the precipitate was dissolved in acetonitrile. The acetonitrile solution was added to a solutionof excess tetrabutyl ammonium chloride in acetonitrile and the deposit was filtered and washed with acetonitrile for five times, desired product compound *TAB-5-M-1-Mal* was obtained as a yellow solid (8.2mg, 10%).^{1}H NMR (400 MHz, CD_3OD) δ: 2.17 (s, 18H), 4.39 (s, 15H), 5.76 (s, 2H),6.93(s, 6H), 7.36-7.38 (d,12H,J=8), 7.50-7.52(d,2H,J=8), 7.77-7.79(d,2H,J=8), 8.05-8.07(d,13H,J=8), 8.37-8.38(d,13H,J=4), 8.84-8.85(d,10H,J=4), 8.95-8.96(d,2H,J=4)ppm. ^{13}C NMR (100 MHz, CD_3OD) δ: 154.78, 150.11, 145.03, 142.31, 129.34, 127.85, 123.91, 123.30, 22.18 ppm. MALDI-TOF (m/z): Calcd. For $[C_{106}H_{95}BN_{10}O_2]^{6+}$ ((M+4K)/Z, Z=2) 853.9, found 855.21.

Synthesis of Compound TAB-5-M-1-cRGD

TAB-5-M-1-Mal (1.76mg,1umol),cRGD(0.7mg,1.2umol),CH3OH (1mL),was added to 2mL PE centrifuge tube and stirred at room temperature over night. CH3OH was removed, The mixture was dissolved into 0.5ml water. Potassium hexafluorophosphate was added to the solution and the precipitate was dissolved in acetonitrile. The acetonitrile solution was added to a solution of excess tetrabutyl ammonium chloride in acetonitrile and the deposit was filtered and washed with acetonitrile for five times. By drying the solid in a vacuum oven, desired product compound *TAB-5-M-1-cRGD* was obtained as a yellow solid (2.3mg, 95%). Because of its complex structure and small amount, it is difficult to analyze by NMR. its generation can be identified by HRMS. MALDI-TOF (m/z): Calcd. For $[C_{130}H_{129}BN_{18}O_9S]^{6+}$ (M/Z, Z=1) 2130.45, found(M/Z+K, Z=1) 2168.99; (M/Z+2Cl+Na, Z=1) 2224.97.

3. Cell culture and imaging

L929, HeLa, and 4T1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with glucose (4.5 g/L), L-glutamine, sodium pyruvate, and 10% fetal bovine serum (FBS). The cells were plated on glass bottomed dishes at 37 °C under 5% CO2 atmosphere before imaging. Cell imag-ing were conducted using a confocal microscope FV1000-IX81 and were analyzed with FV10-ASW software. Cells, pre-washed twice, were incubated with various probes in cultured medium without FBS at 37°C under 5% CO2 for correspond-ing time. Then the cells were washed with PBS to remove unbounded probes for six times before in situ imaging by Olympus Fluorescence confocal microscope.

Cells, pre-washed twice, were incubated with various SOF in cultured medium without FBS at 37°C under 5% CO2 for corresponding time. Then the cells were washed with PBS to remove unbounded probes for six times before in situ imag-ing by Olympus Fluorescence confocal microscope.

4. ROS detection

The of H2DCF-DA (2.0 mM, 100 μL) in DMSO was added into sodium hydroxide solution (0.01 M, 0.8 mL) to activate and placed for 30 min in dark at room temperature, then 4.1 mL PBS solution of probe (1.0 mM, 50.0 μL) was added. The fluorescence signal was measured with the irradiated by LED light (1.5 mW/cm2) , Excitation wavelength: 480 nm.

4T1 cells were stained with TAB-CB[8] and TAB-CB[8]-cRGD-DOX, respectively. Then, the cells were treated with H2DCF-DA (5 μM) aqueous solution and incubated for 20 min in darkness, wrapped in foil in a 37 oC cell incubator. Next, the cells were irradiated by the LED light (1.5 mW/cm2). Detection of H2DCF-DA fluorescence was observed by confocal microscope. H2DCF-DA was excited by a 488 nm laser, and fluorescence emission at 490-530 nm was recorded by confocal laser scanning microscopy using oil objective.

5. Tumor model and In vivo imaging

Nude mice were purchased from the Laboratory Animal Center of North Sichuan Medical College and all experimental procedures about animals were performed according to a protocol approved by the Institutional Animal Care and Treatment Committee of North Sichuan Medical College. We subcutaneously injected with 1×106 4T1 cells in the left anterior axillary. Then they were individually housed until the tumor grow to approximately 2 cm in diameter by measuring caliper. The mice were then placed into the small animal imager after anesthesia and injected with probe through tail vein with a certain amount of probes solution for imaging.

Results and Discussion

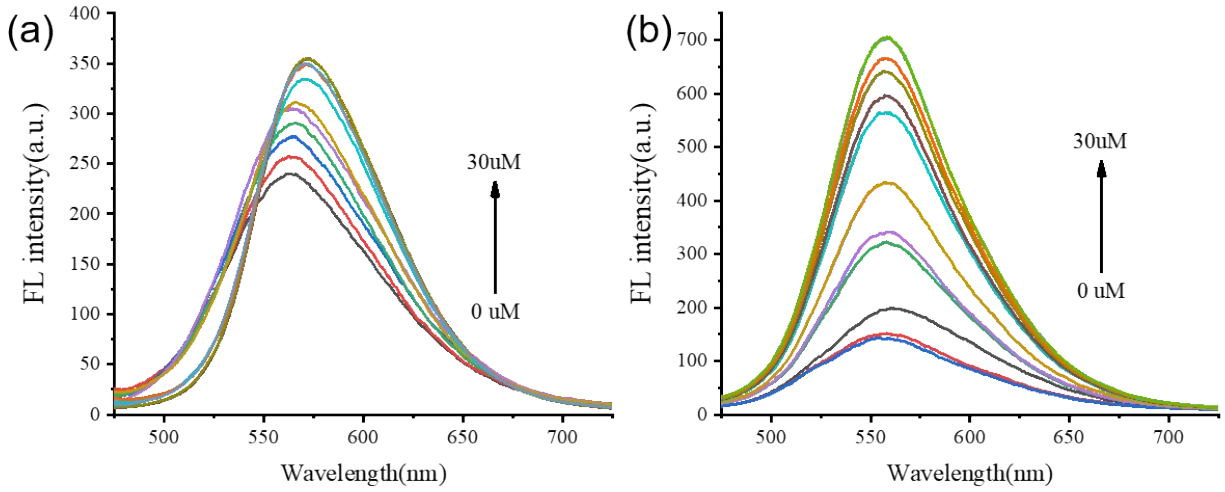


Figure S1. The fluorescence spectra changes of TAB-6-Methyl (10 μM) in water with the addition of different amounts of (a) CB[6] and (b) CB[7]. Ex: 440 nm.

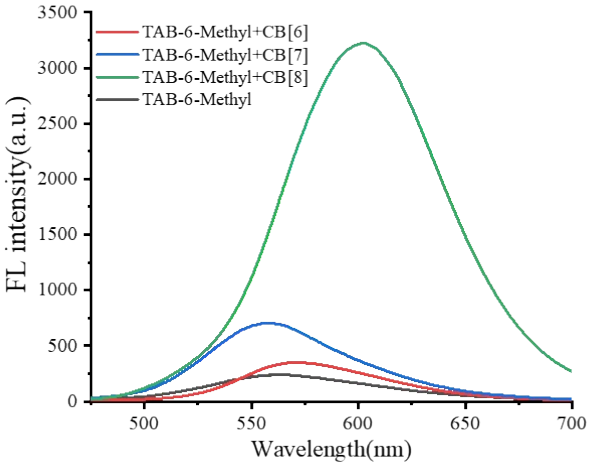


Figure S2. The fluorescence spectra of TAB-6-Methyl (10 μM) in water after the addition of 30 μM CB[6] , CB[7] and CB[8].

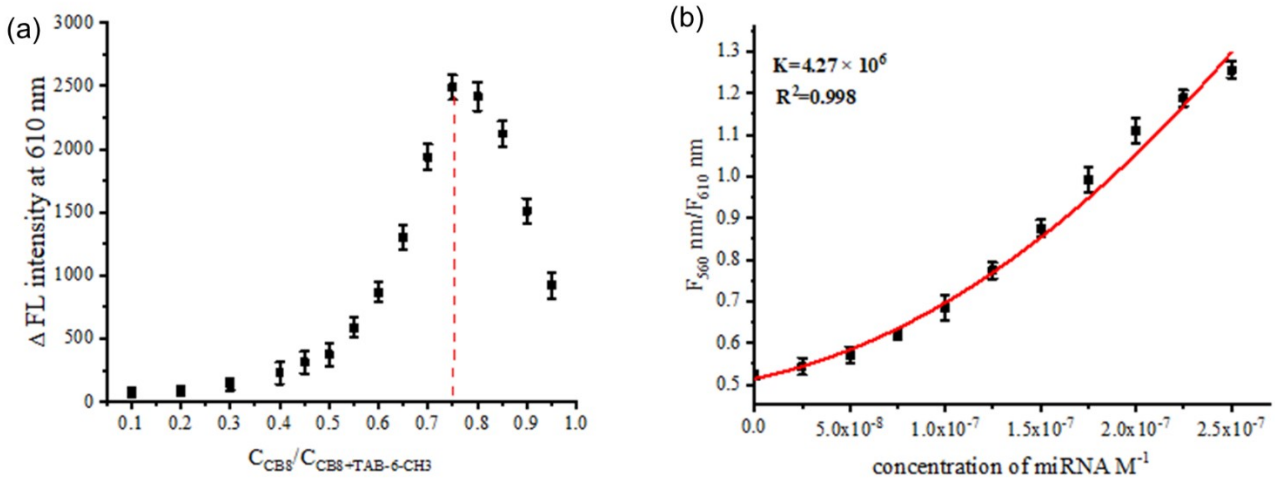


Figure S3. (a) Job plot for TAB-6-Methyl and CB[8] in aqueous solution at 298 K by recording the fluorescence at 610 nm. The total concentration is constant ([TAB-6-Methyl] + [CB[8]] = 2.0×10^{-5} M). (b) Correlation between fluorescence intensity ratio changes (F_{610nm} / F_{560nm}) of TAB-6-Methyl at 560 nm and CB[8] concentration. Excitation wavelength: 440 nm.

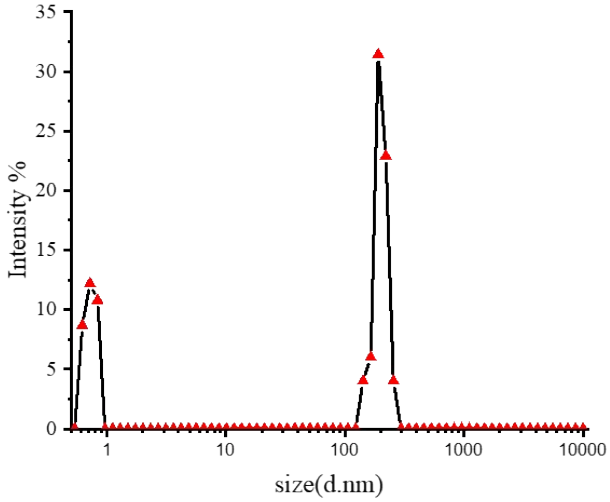


Figure S4. DLS Size distribution of TAB-CB[8] in water.

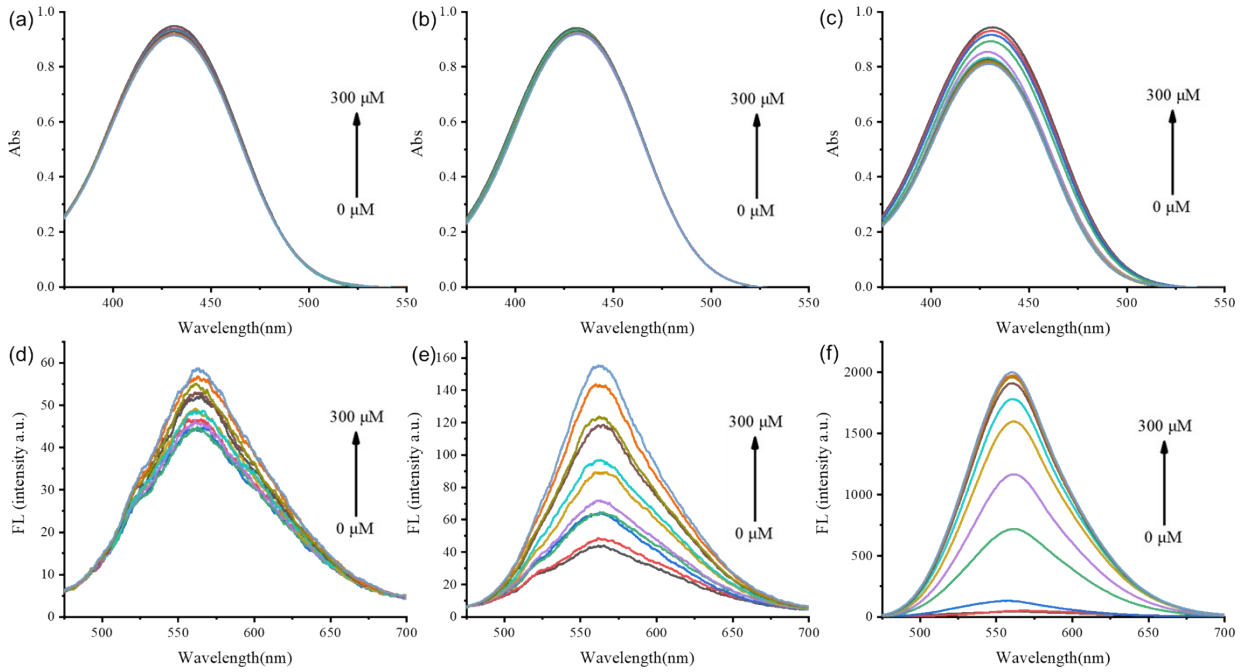


Figure 5. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of TAB-6-Methyl (10 μM) in water with the addition of different amounts of AMP (a, d), ADP (b, e) and ATP (c, f). Ex: 440 nm.

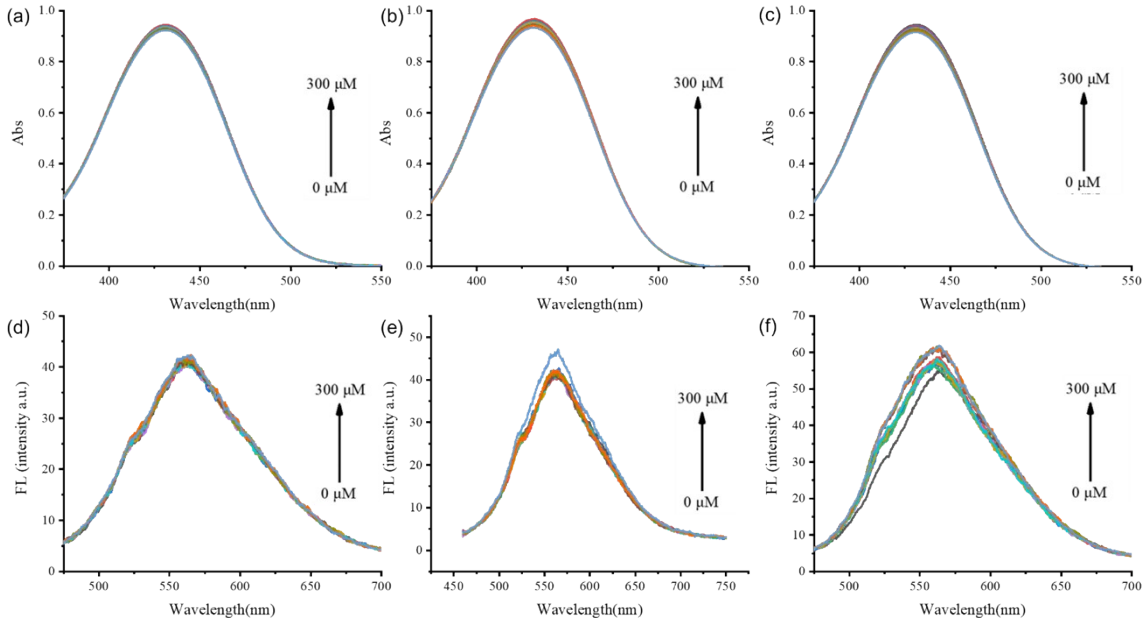


Figure S6. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of TAB-6-Methyl (10 μM) in water with the addition of different amounts of Cys (a, d), Hcy (b, e) and GSH (c, f). Ex: 440 nm.

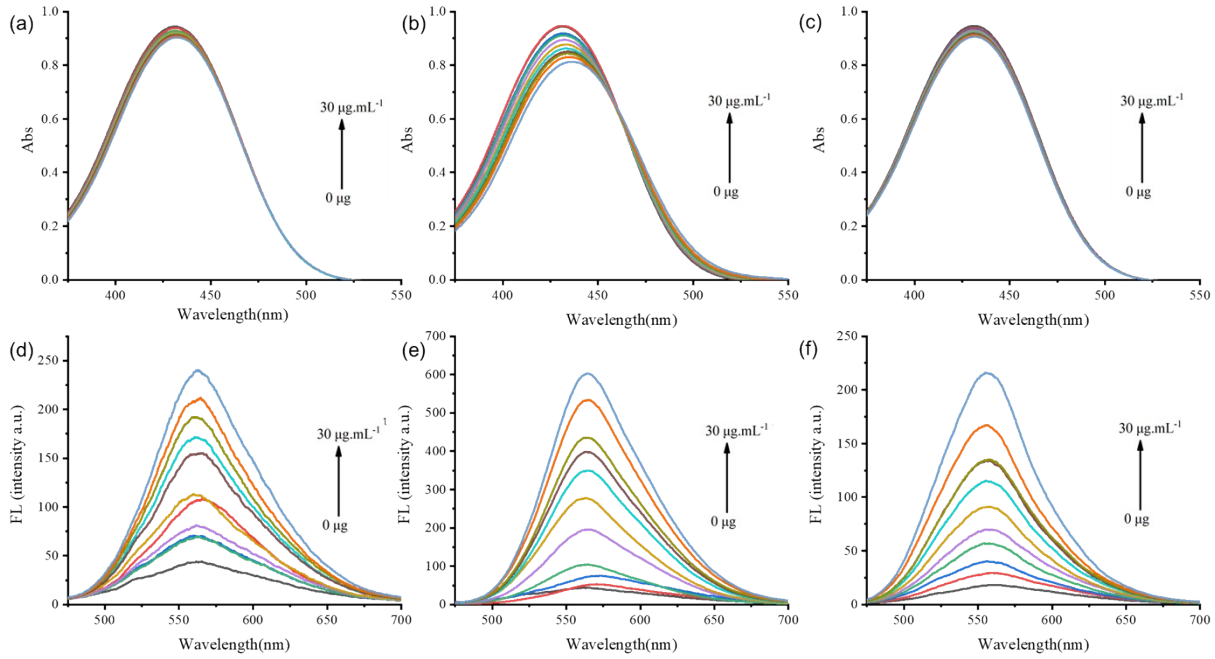


Figure S7. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of TAB-6-Methyl (10 μM) in water with the addition of different amounts of RNA (a, d), DNA (b, e) and BSA (c, f). Ex: 440 nm.

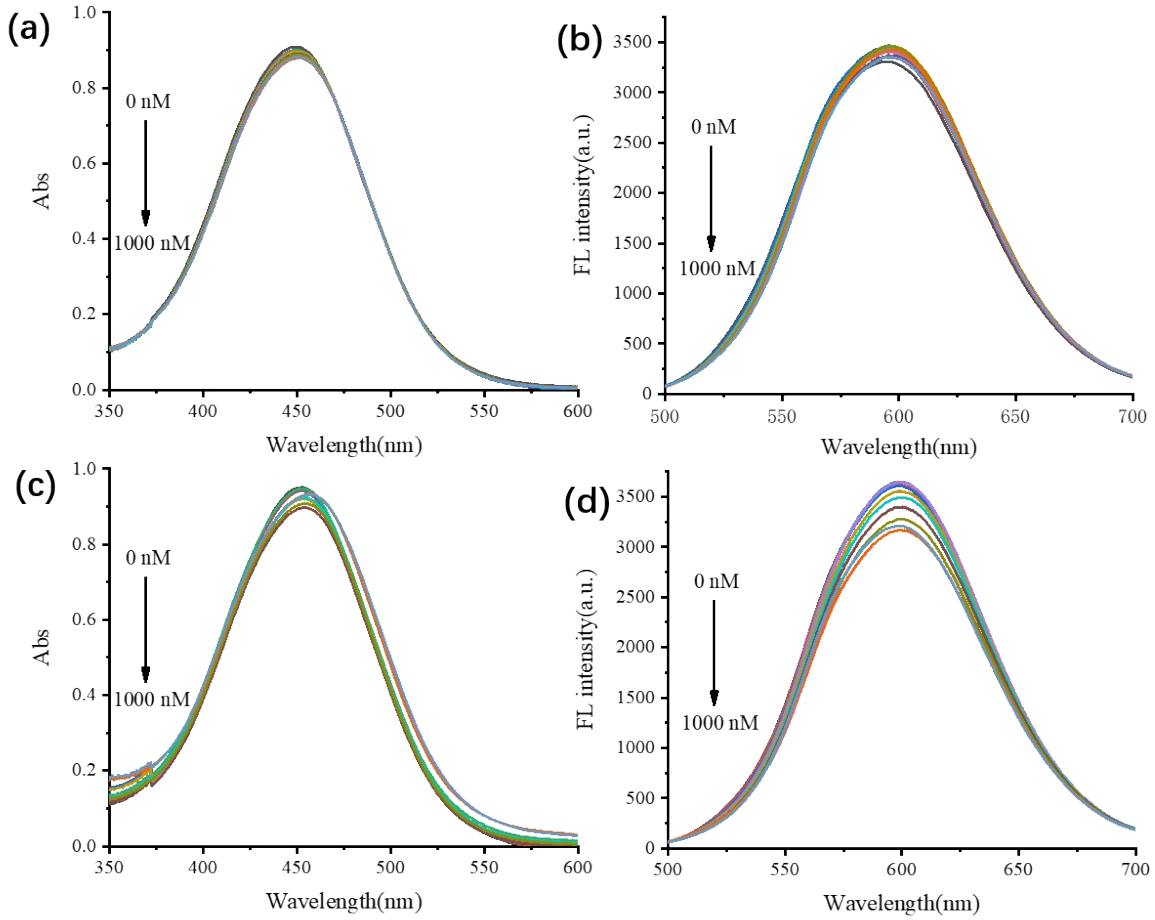


Figure S8. UV absorption and fluorescence spectral changes of TAB-CB[8]-cRGD in water with the addition of different amounts of miRNA 141(a, b) and miRNA 151 (c, d).

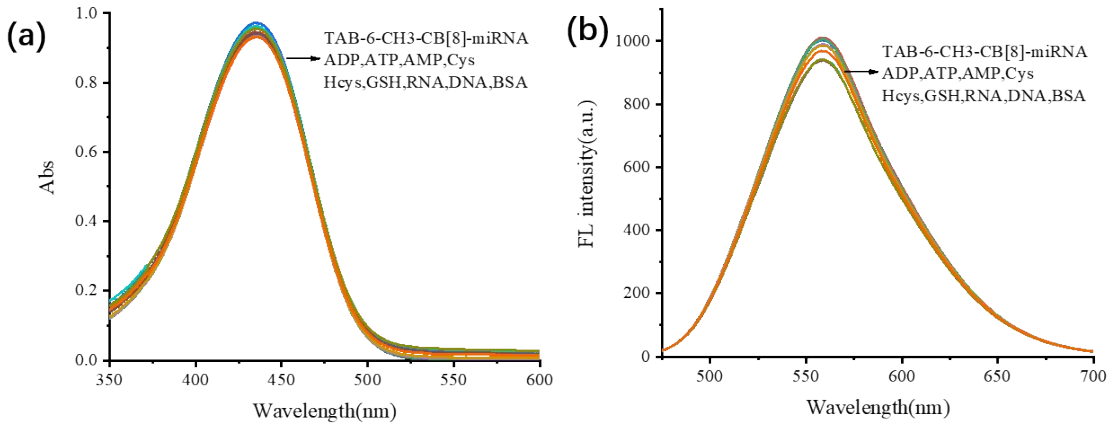


Figure S9. (a) Absorption and (b) fluorescence response for various negatively charged substance of TAB-CB[8] (10 μM) after being disassembled by miRNA21. Ex: 440 nm.

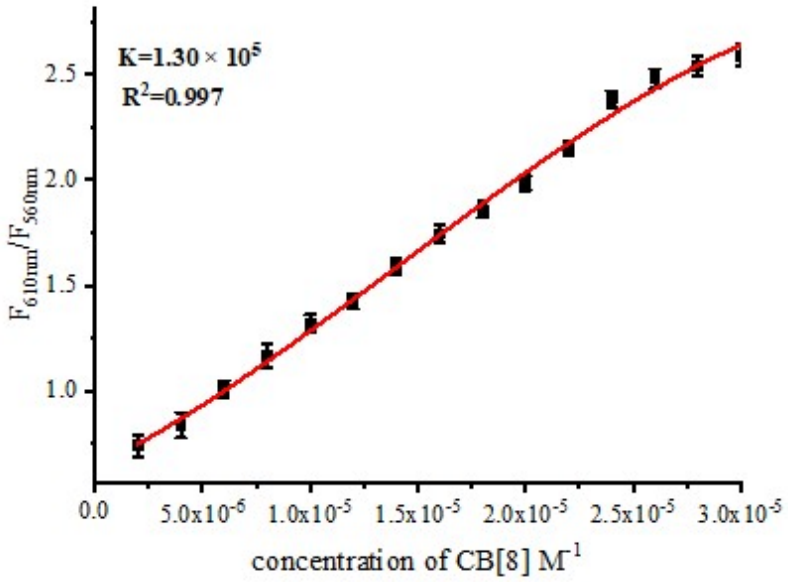


Figure S10. Correlation between fluorescence intensity ratio changes (F_{610nm} / F_{560nm}) of TAB-CB[8] and miRNA concentration.

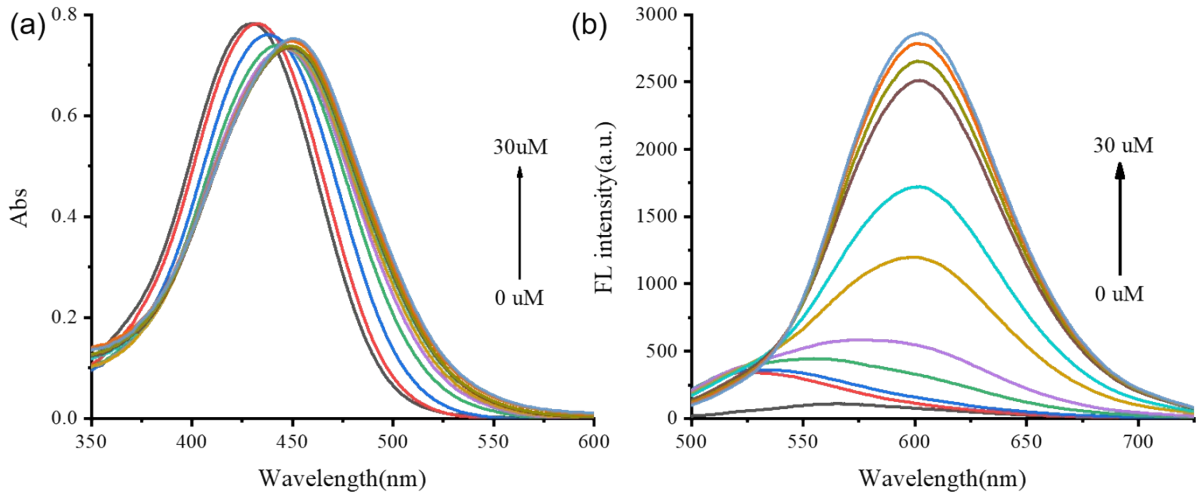


Figure S11. (a) absorption and (b) fluorescence spectra changes of the mixture of TAB-6-Methyl (8 μM) and TAB-5-M-1-cRGD (2 μM) in water with the addition of different amounts of CB[8]

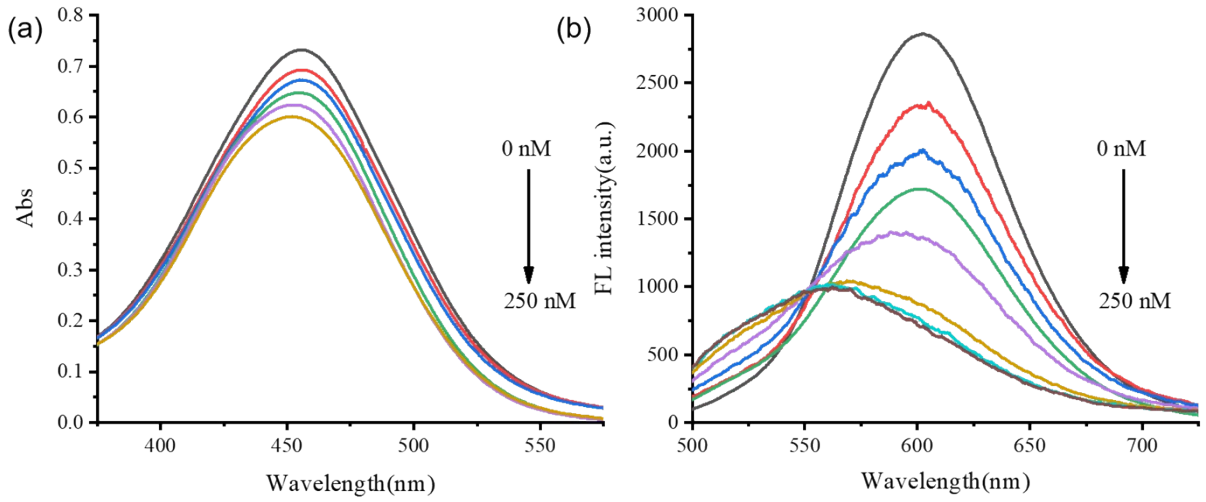


Figure S12. (a) UV absorption and (b) fluorescence spectral changes of TAB-CB[8]-cRGD (10 μM) in water with the addition of different amounts of microRNA.

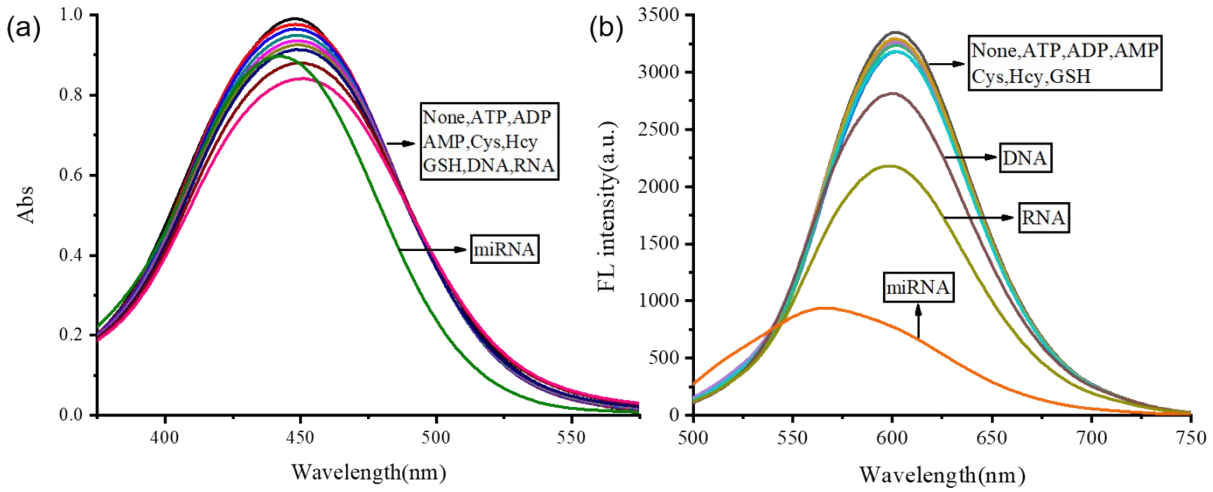


Figure S13. (a) UV absorption and (b) fluorescence spectral changes of TAB-CB[8]-cRGD (10 μM) in water with the addition of various negatively charged substance.

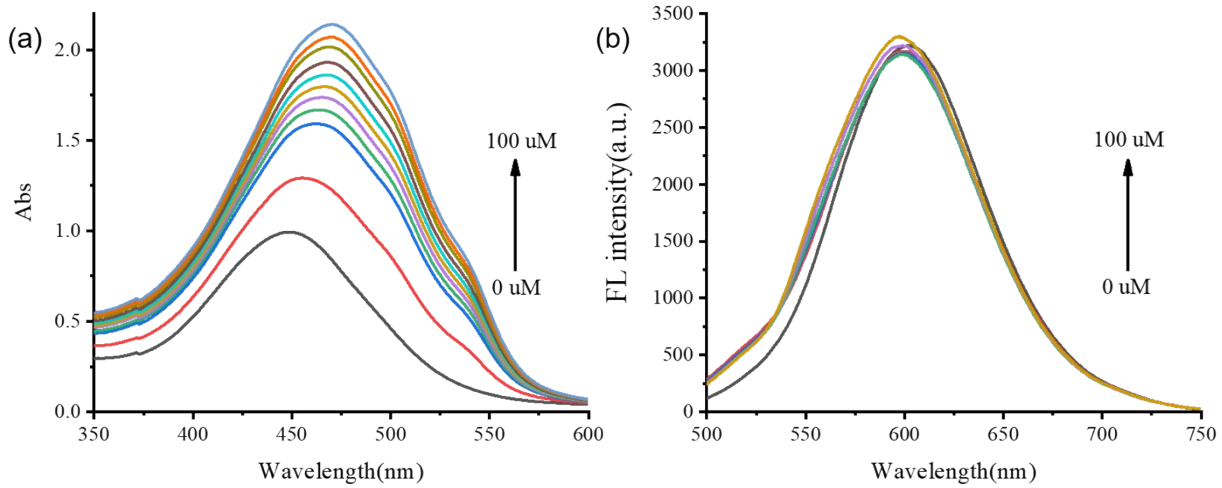


Figure S14. (a) UV absorption and (b) fluorescence spectral changes of TAB-CB[8]-cRGD in water with the addition of different amounts of DOX. Ex: 440 nm.

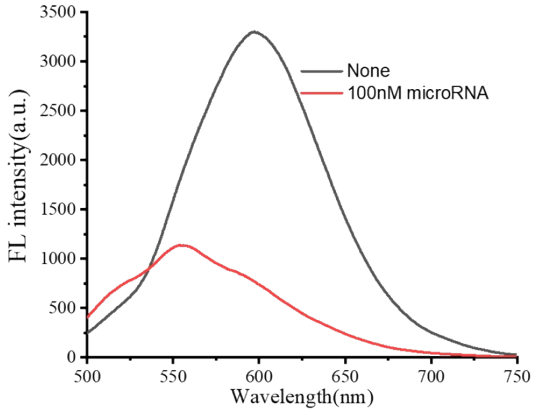


Figure S15 . Fluorescence spectral changes of TAB-CB[8]-cRGD-DOX in water before and after the addition of 100 nM microRNA. Ex: 440 nm.

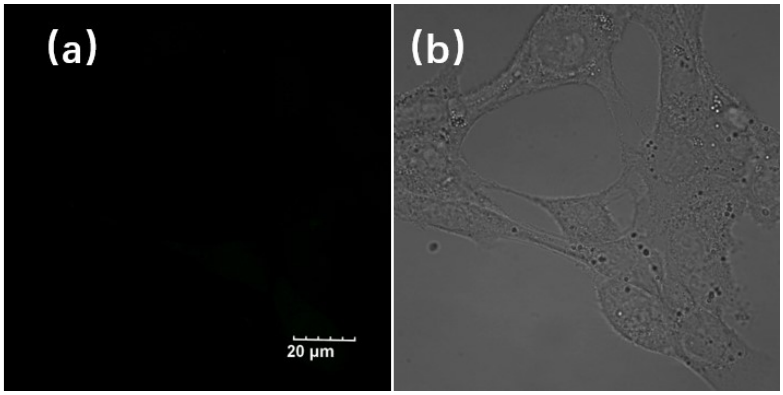


Figure S16. Confocal fluorescence images of 4T1 cells stained with 5 μM H2DCF-DA after light irradiation (1.5 mW/cm^2) for 2min. Ex: 405nm.

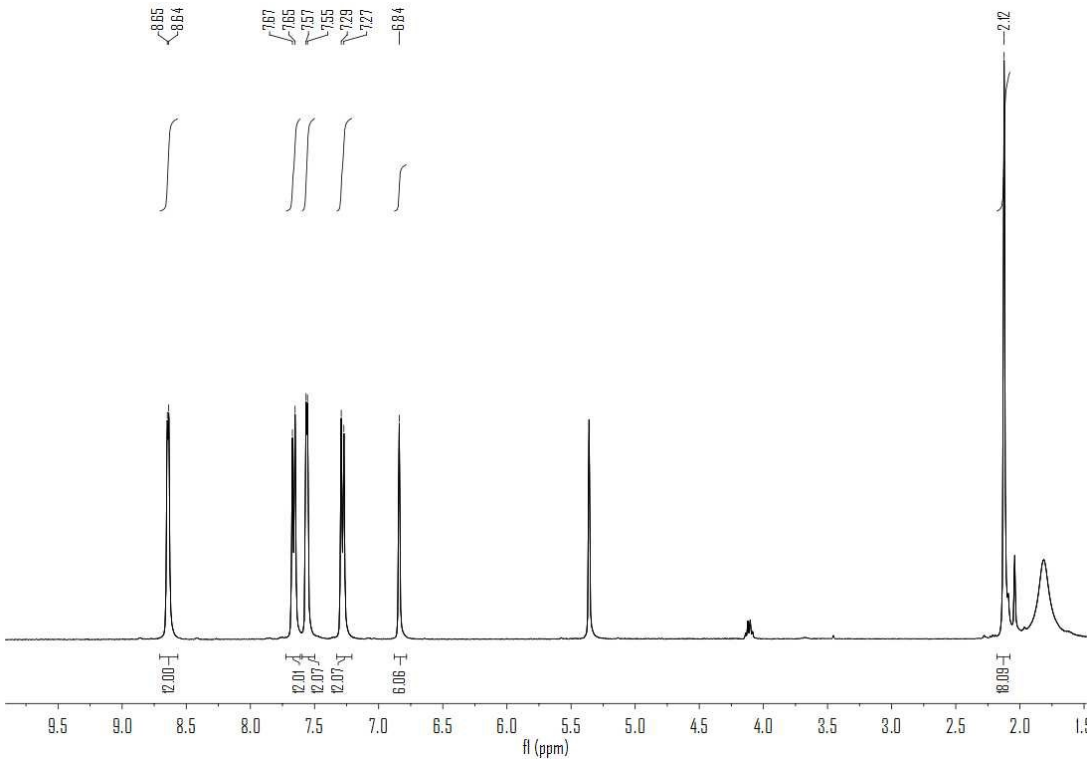


Figure S17. [1]HNMR spectra of TAB-3-BPA.

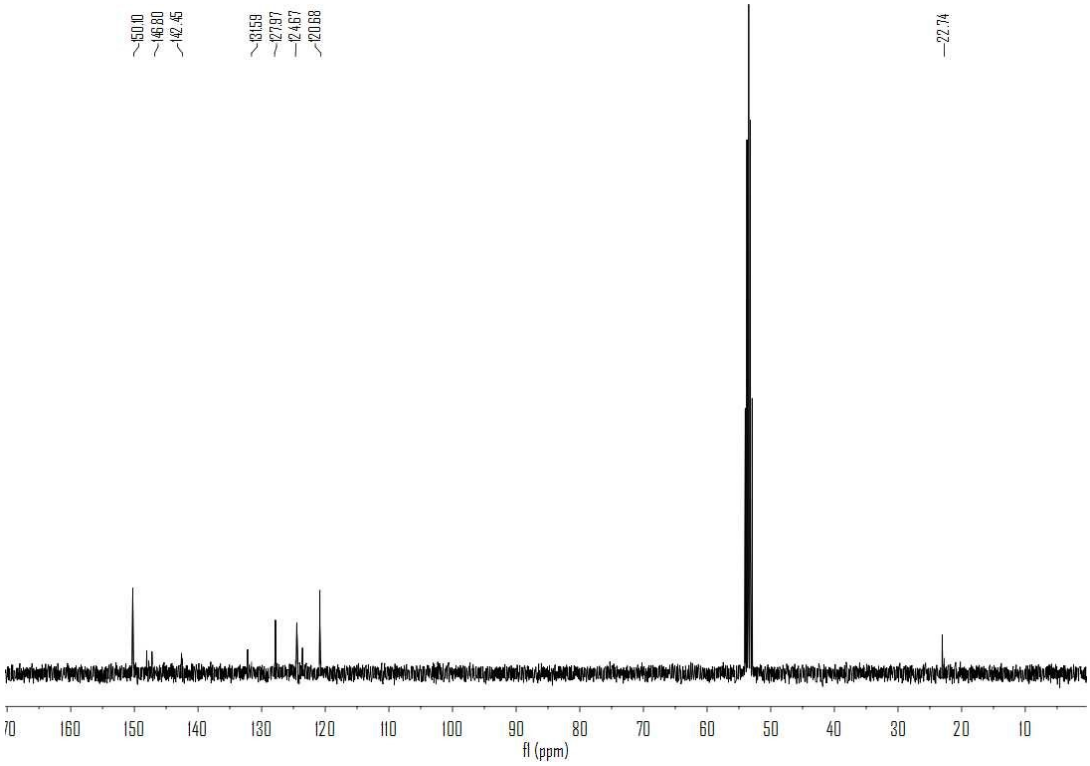


Figure S18. [13]CNMR spectra of TAB-3-BPA.

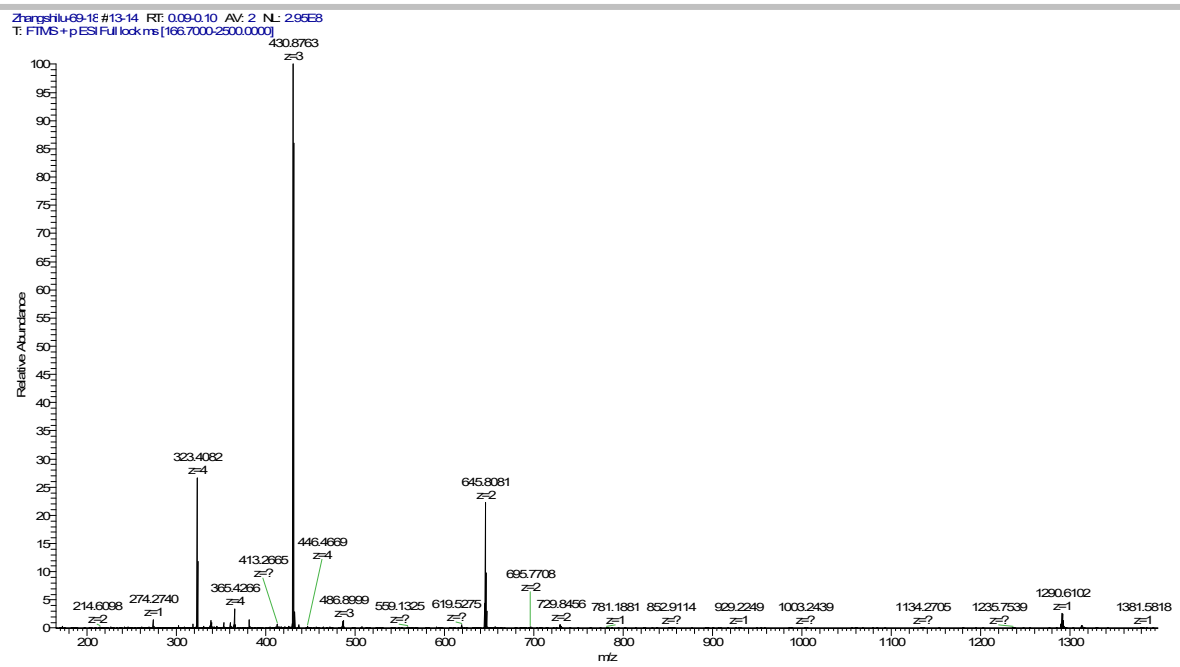


Figure S19. HRMS spectra of TAB-3-BPA.

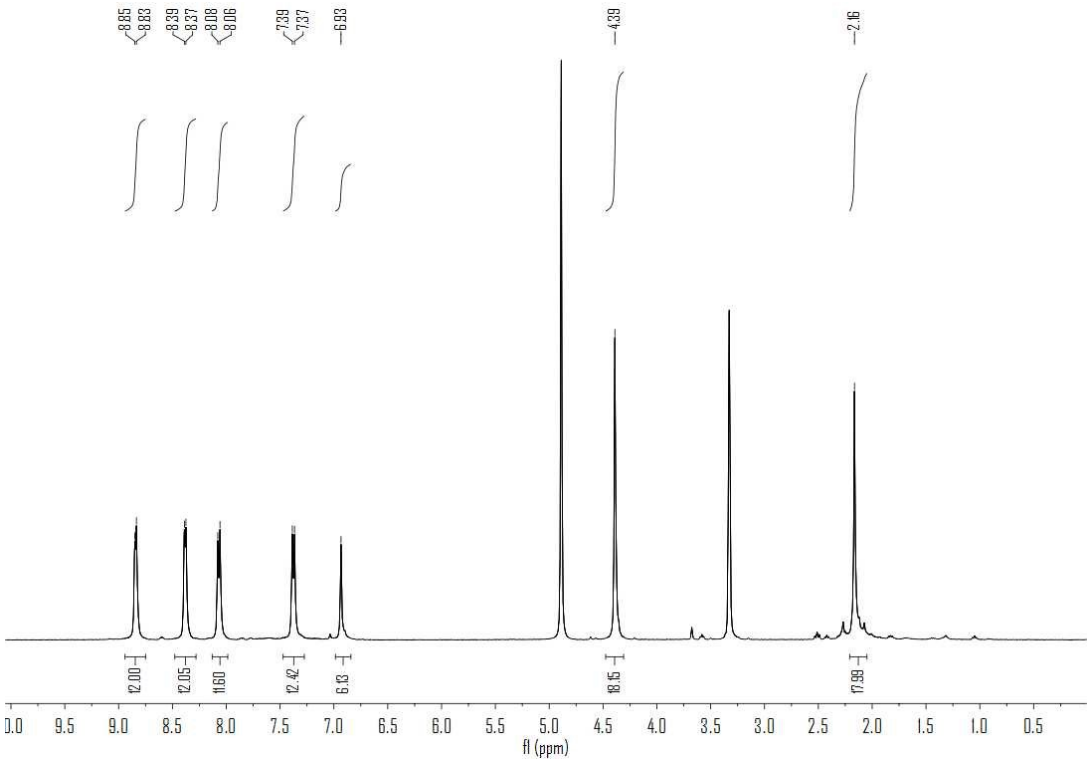


Figure S20. [1]HNMR spectra of TAB-6-Methyl.

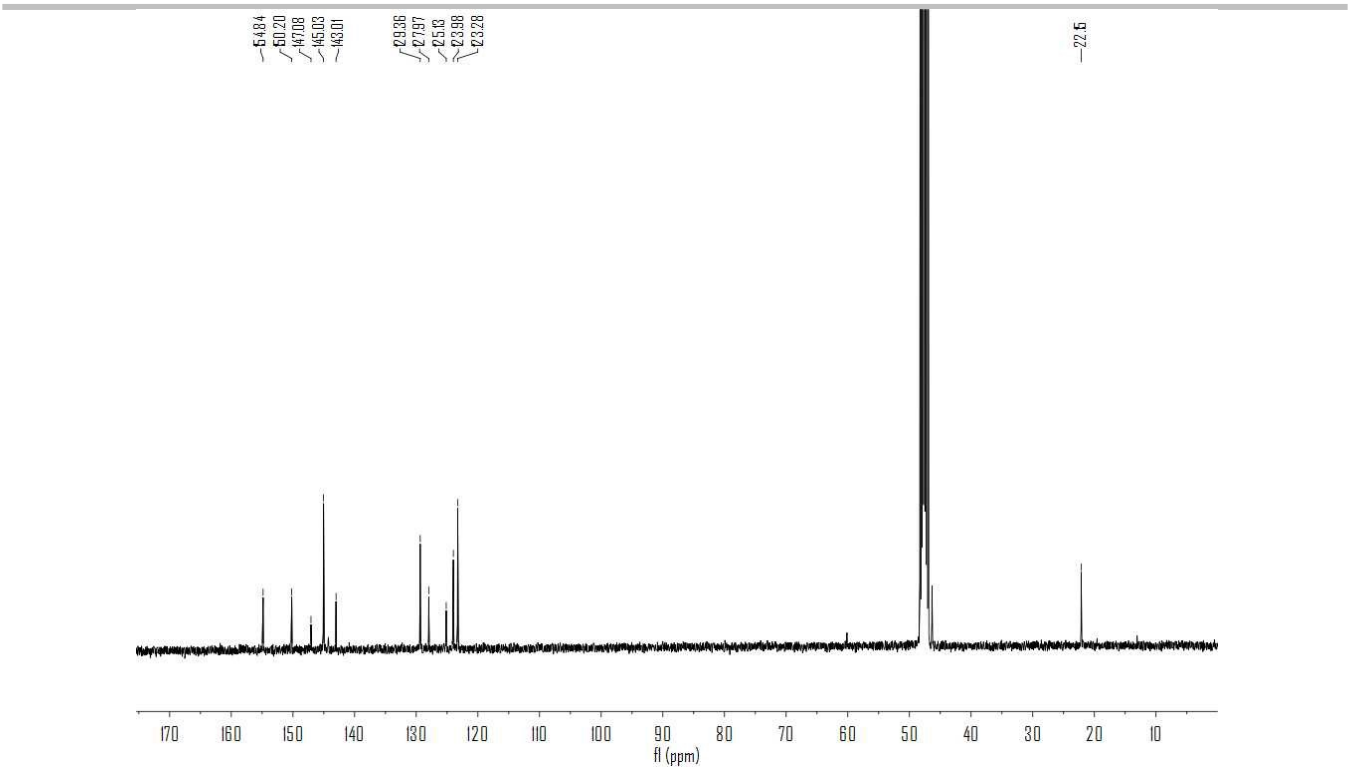


Figure S21. ^{13}CNMR spectra of TAB-6-Methyl.

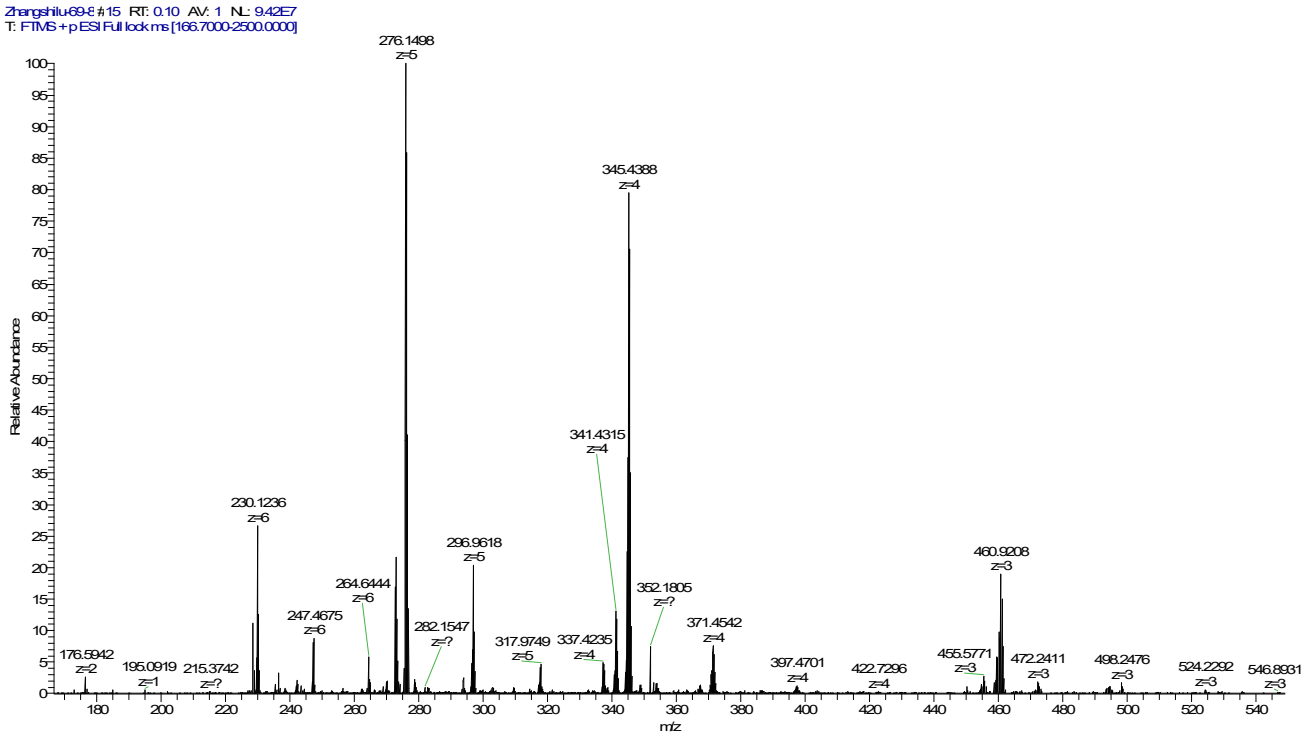


Figure S22. HRMS spectra of TAB-6- Methyl.

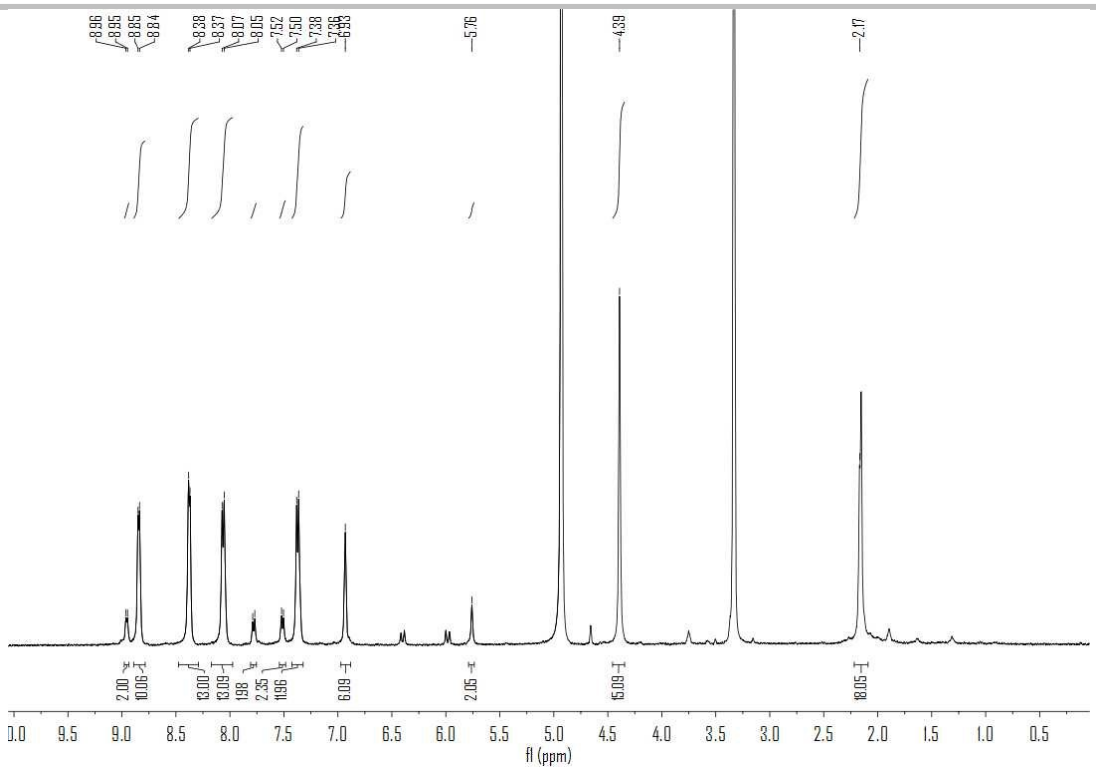


Figure S23. [1]HNMR spectra of TAB-5-M-1-Mal.

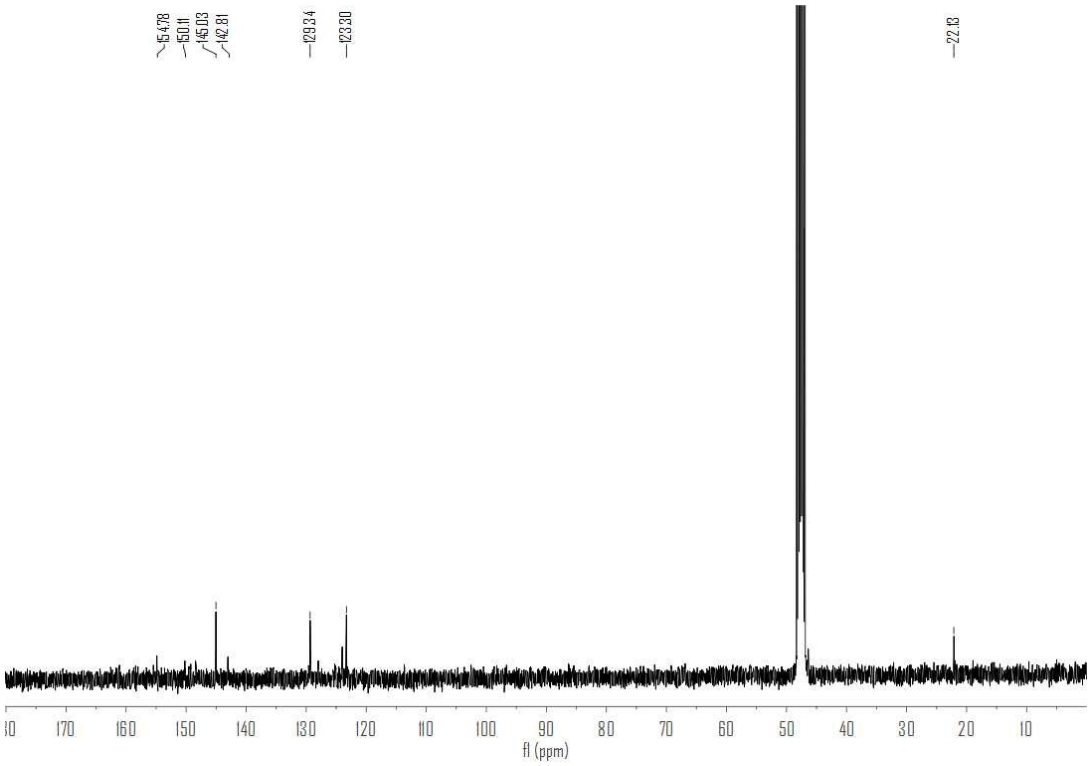


Figure S24. [13]CNMR spectra of TAB-5-M-1-Mal.

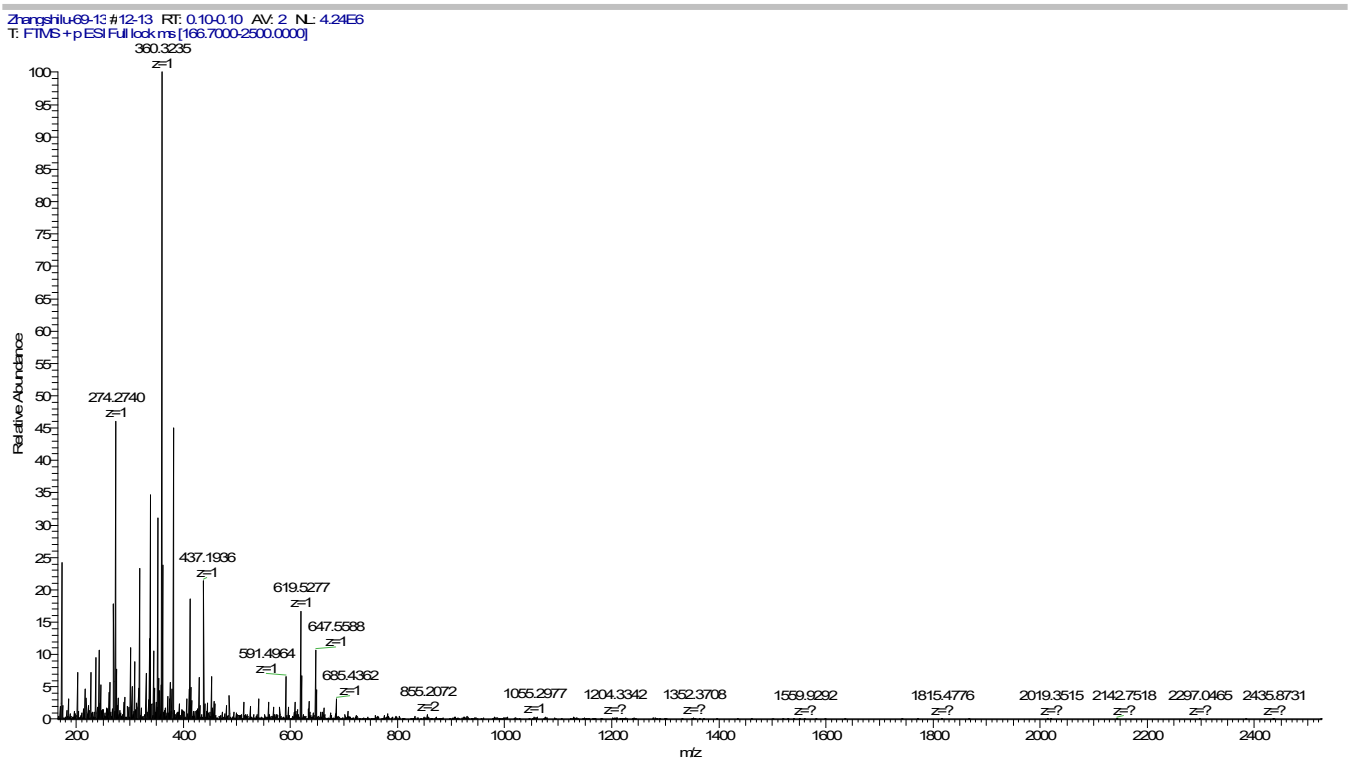


Figure S25. HRMS spectra of TAB-5-M-1-Mal.

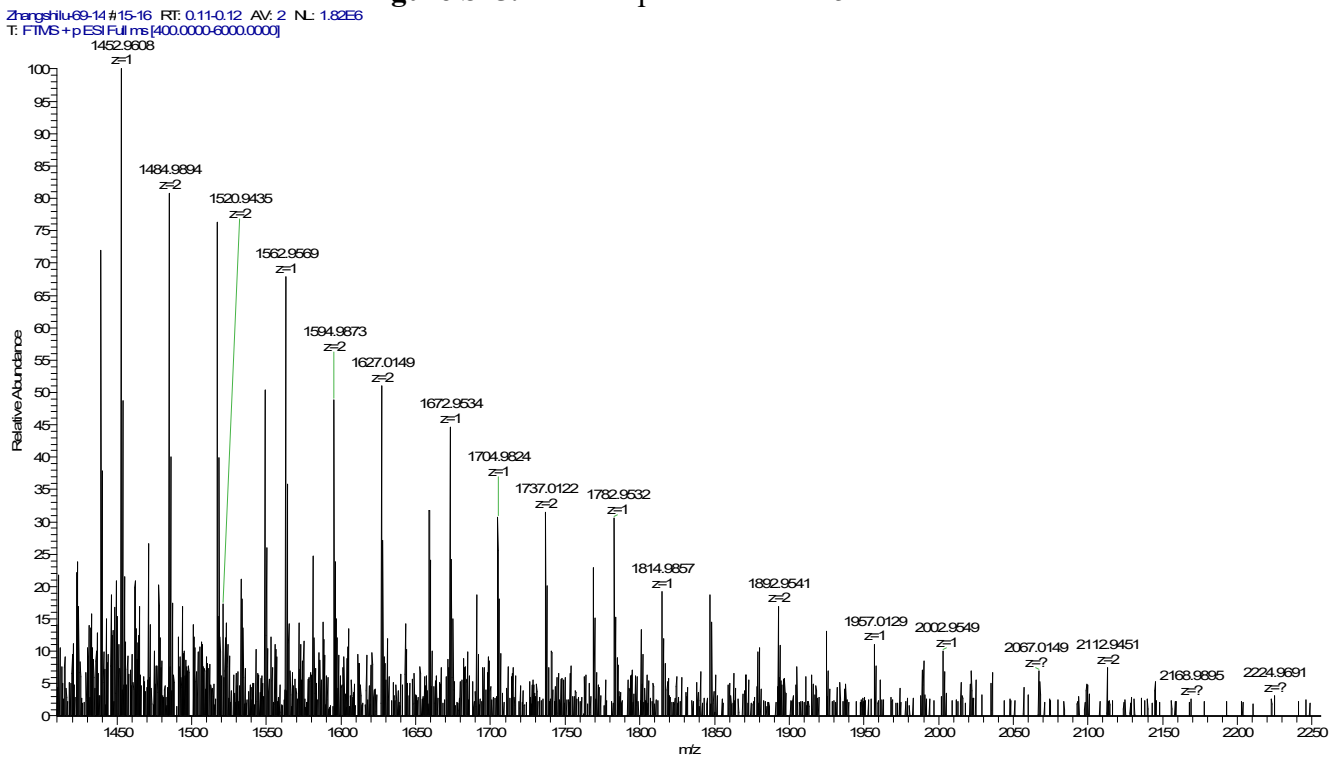


Figure S26. HRMS spectra of TAB-5-M-1-cRGD.

References

(1) Liu, J.; Cheng, K.; Yang, C.; Zhu, J.; Shen, C.; Zhang, X.; Liu, X.; Yang, G. Application of triarylboron substituted with cyclic arginine–Glycine–aspartic acid motifs as a multivalent two-photon fluorescent probe for tumor imaging in vivo. *Anal. Chem.* **2019**, *91*, 6340-6344.

(2) Davie, B. J.; Valant, C.; White, J. M.; Sexton, P. M.; Capuano, B.; Christopoulos, A.; Scammells, P. J. Synthesis and Pharmacological Evaluation of Analogues of Benzyl Quinolone Carboxylic Acid (BQCA) Designed to Bind Irreversibly to an Allosteric Site of the M1 Muscarinic Acetylcholine Receptor. *J. Med. Chem.* **2014**, *57*, 5405-5418.
(3) Cheng, H.-J.; Shen, Y.-L.; Zhang, S.-Y.; Ji, H.-W.; Yin, W.-Y.; Li, K.; Yuan, R.-X. Three Coordination Polymers Constructed with Zinc(II), 3,3′-Thiodipropionic Acid, and Bipyridyl Ligands: Syntheses, Crystal Structures and Luminescent Properties. *Z. Anorg. Allg. Chem.* **2015**, *641*, 1575-1580.