Supporting Information for

Near-Infrared Light Controlled Fluorogenic Labeling of Glycoengineered Sialic Acids *in vivo* with Upconverting Photoclick Nanoprobe

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1.Supporting data for the manuscript.

1.1 Emission spectra of AC_4ManNene with tetrazole (Tz).

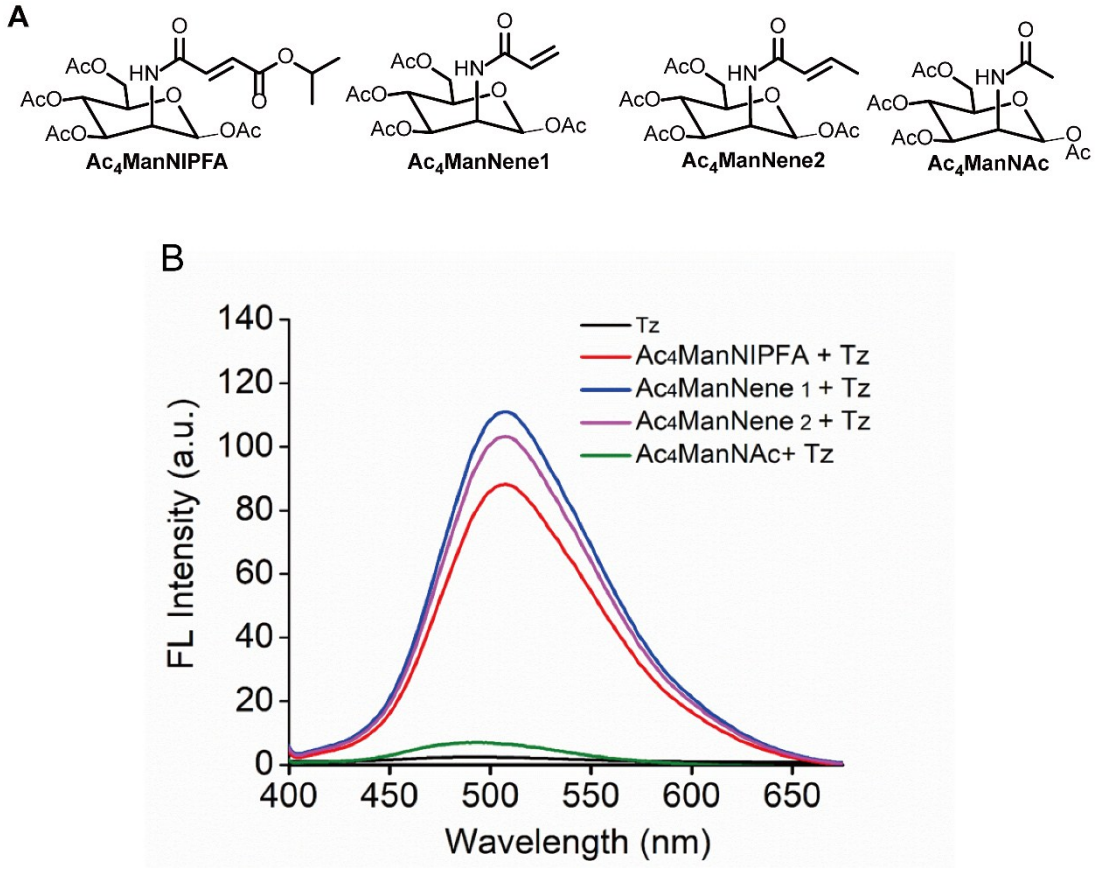


Fig. S1. A The chemical structure of Ac_4ManNIPFA, Ac_4ManNene 1/2 and Ac_4ManNAc. B Emission spectra (λ_{ex} = 375 nm) of Ac_4ManNIPFA, Ac_4ManNene 1/2 and Ac_4ManNAc with tetrazole (Tz) in PBS solution (pH 7.4, 5% DMSO) at a concentration of 10 mM.

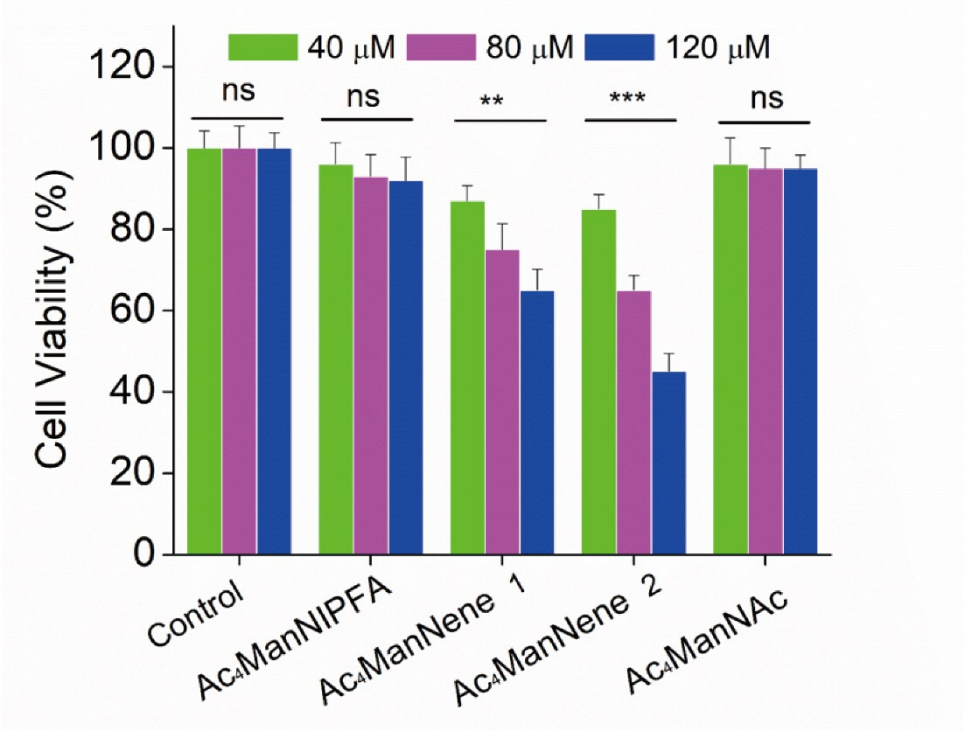


Fig. S2. Cell growth analysis after treatment with different alkene sialic acids analogs. A549 cells were grown in the presence of 40 -120 Mm sialic acids analogs (n=5). Datereprresent mean ±SD; ***p < 0.001, **p < 0.01.

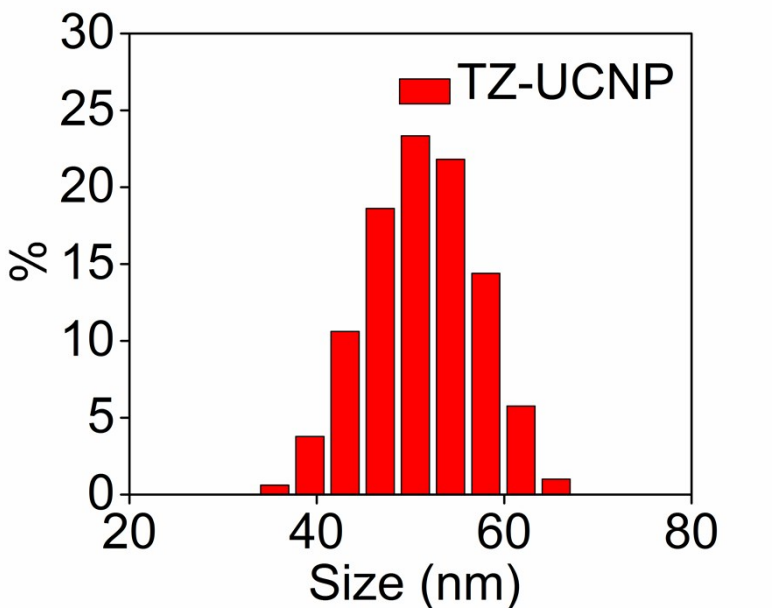


Fig. S3. Dynamic light scattering of Tz-UCNP

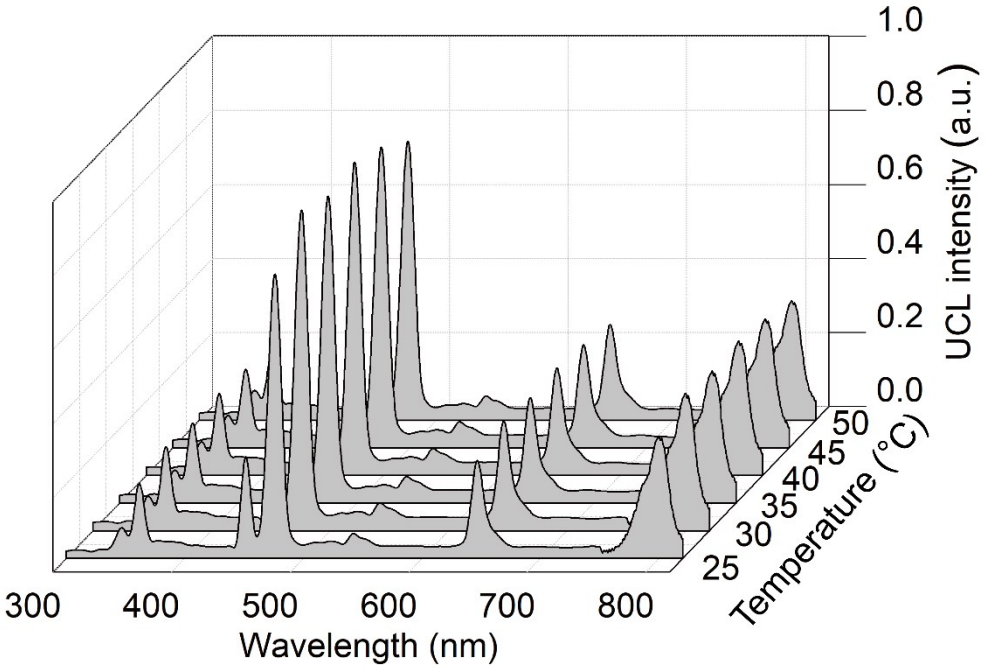


Fig. S4. Tz-UCNP luminescence spectra at different temperatures (25°C -50°C) PBS buffer.

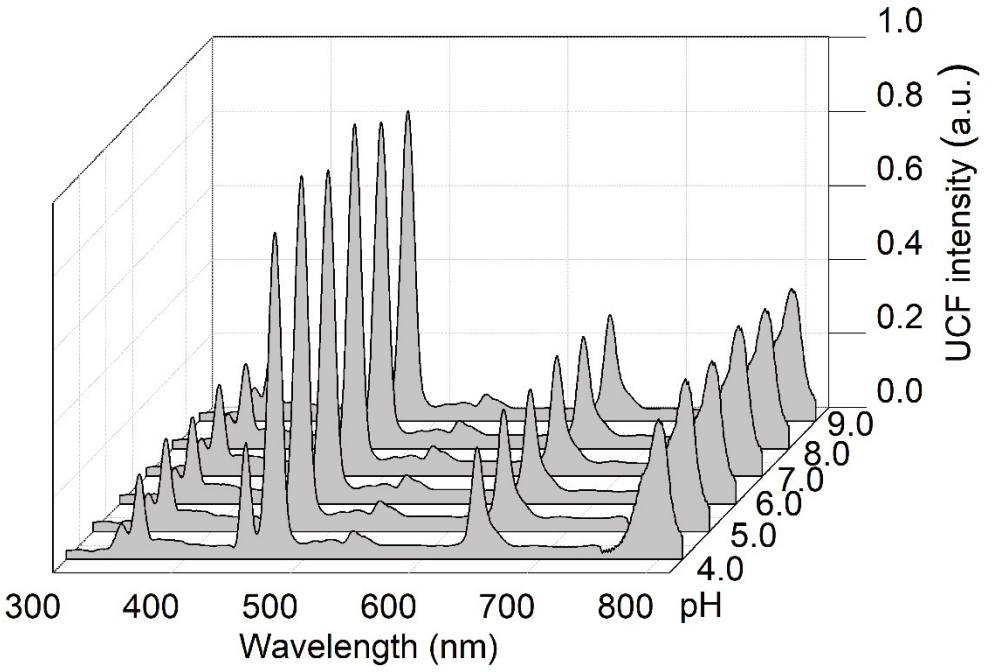
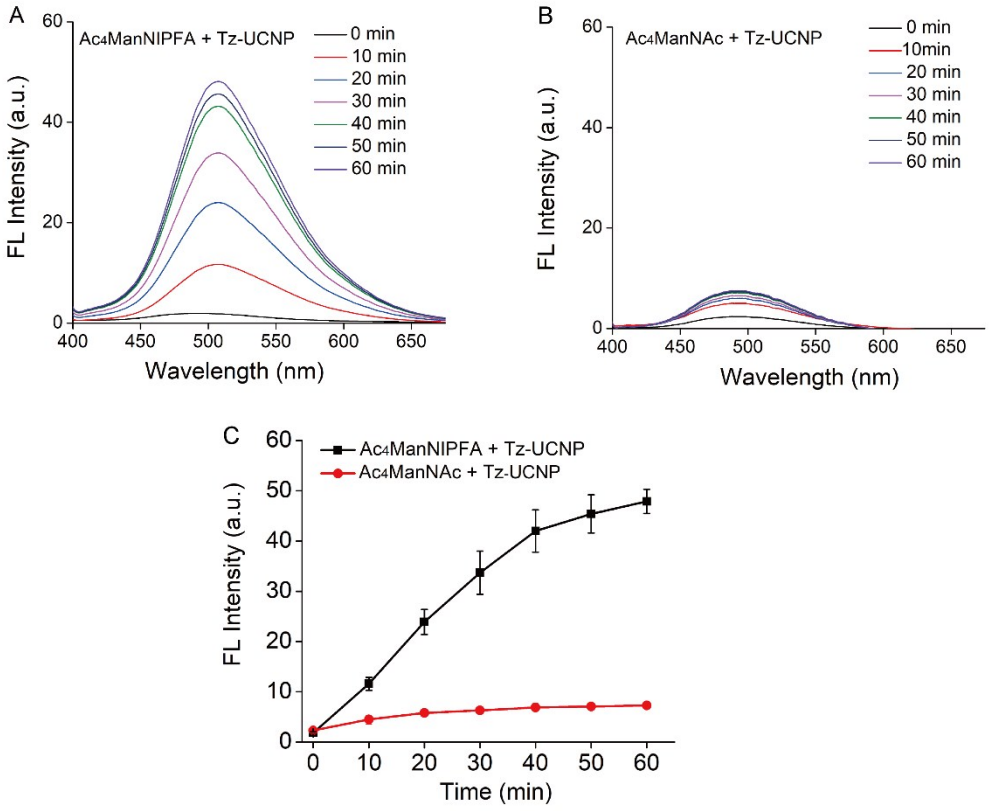


Fig. S5. Tz-UCNP luminescence spectra at different pH (4.0-9.0) PBS buffer.



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ig. S6. A and B. Emission spectra (c, λ_{ex} = 375 nm) of AC_4ManNIPFA, with Tz-UCNP in PBS solution (pH 7.4, 5% DMSO). C. Data statistics of A and B.

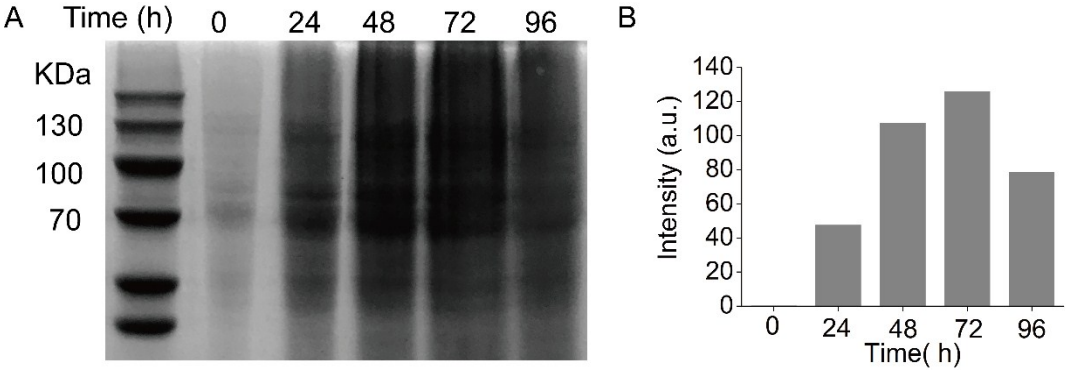


Fig . S7 A. Western blot analysis of lysates from A549 cells A549 cells were pre-incubated with Ac_4ManNIPFA for 0, 24, 48, 72 and 96 h. B. Data statistics of A

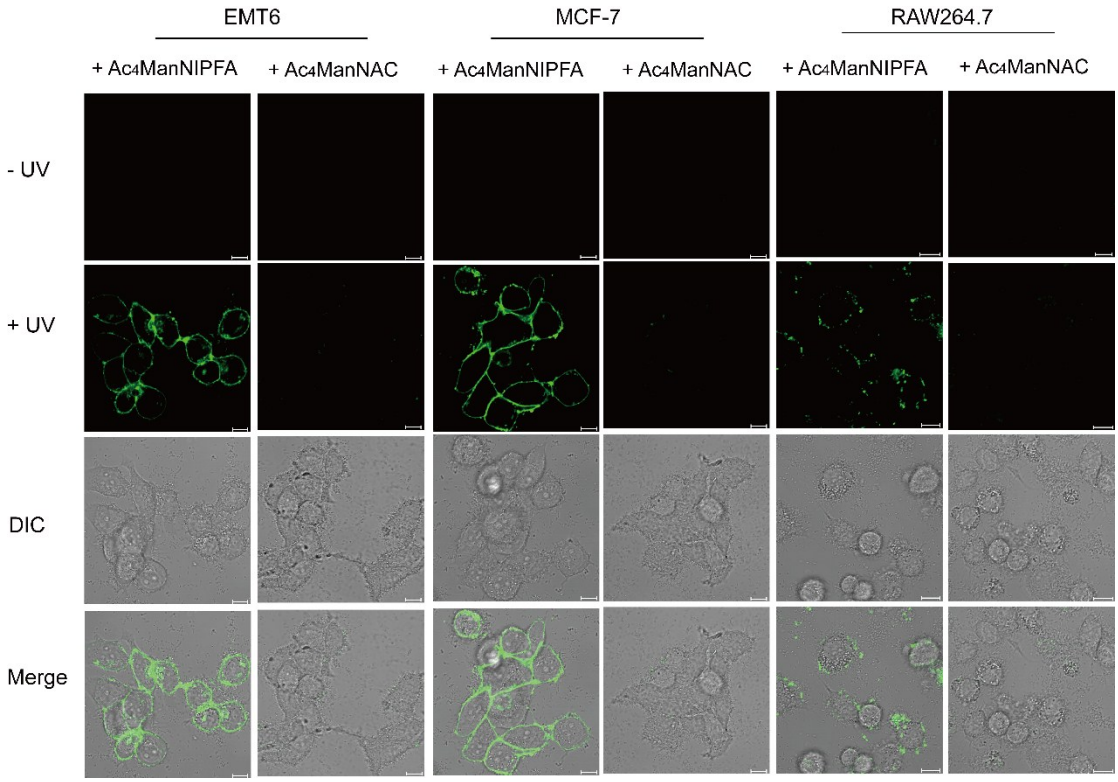


Fig . S8. Confocal images of Tz (30 μM) labeling on the Ac_4ManNIPFA (40 μM) and Ac_4ManNAc (40 μM) pretreated EMT6, MCF-7 and RAW264.7 cells in the absence and presence of UV irradiation for 10 minutes. Scale bar = 10 μm.

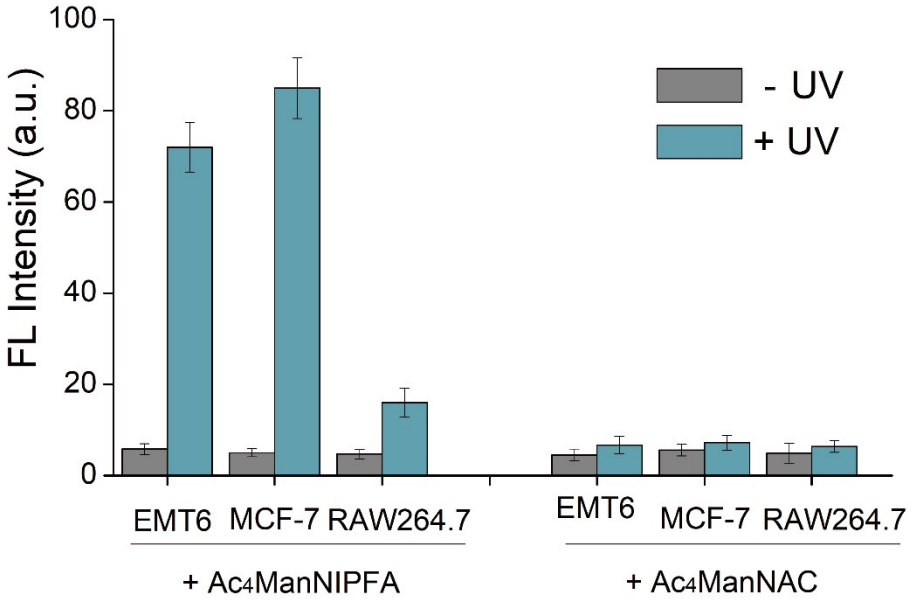


Fig . S9. Fluorescence analysis of EMT6, MCF-7 and RAW264.7, cells treated with Ac_4ManNIPFA and Ac_4ManNAc (40 μM).

1.2 Supporting data for mice mode.

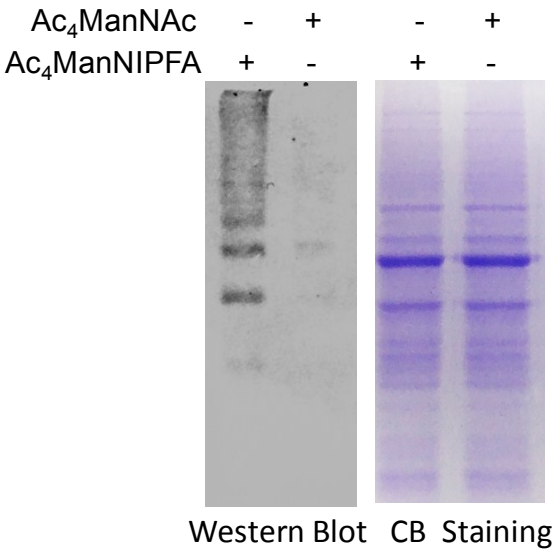


Fig. S10. Western blot analysis of lysates from A549 tumor treated with AC_4ManNIPFA (3 mg/kg) and Ac_4ManNAc (3 mg/kg).

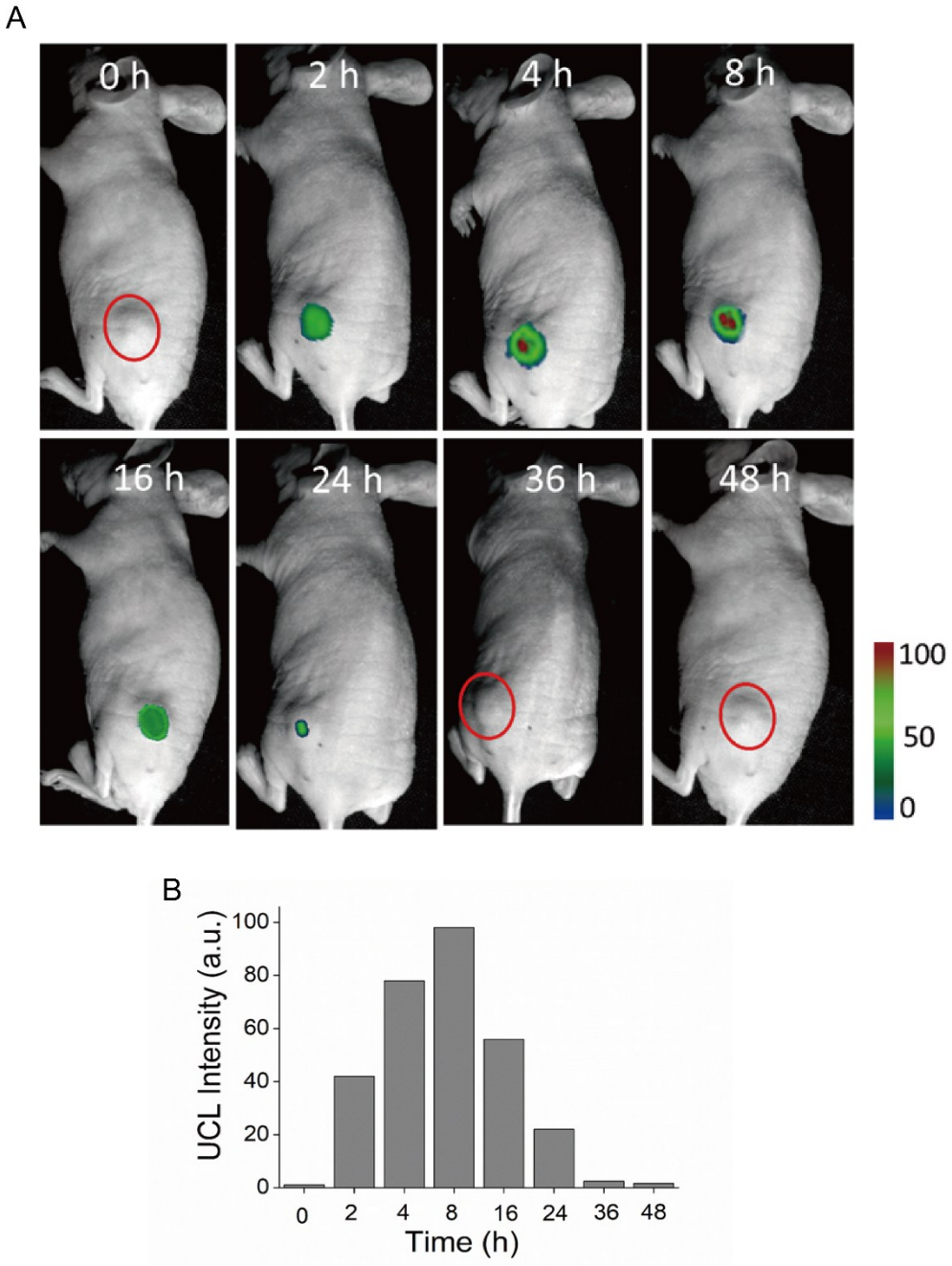


Fig. S11. A. Fluorescence images were acquired after different time of incubation the probe Tz-UCNP, λex = 980 nm. λem = 800-810 nm 30 mg/kg of Tz-UCNP was injected into the tail vein of the above mouse. B. Data statistics of A.

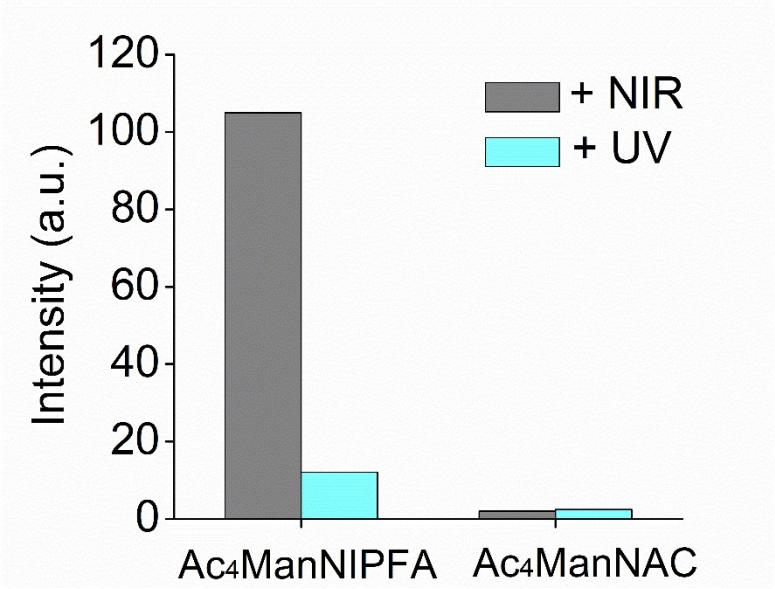


Fig. S12. Data statistics of fluorogenic labeling results of sialic acids in living mice., λex = 980 nm. λem = 505 - 525 nm. Left: irradiation with UV light 350 nm for 10 min; right irradiation with NIR light 980 nm (1.0 w/cm^2) for 1 h.

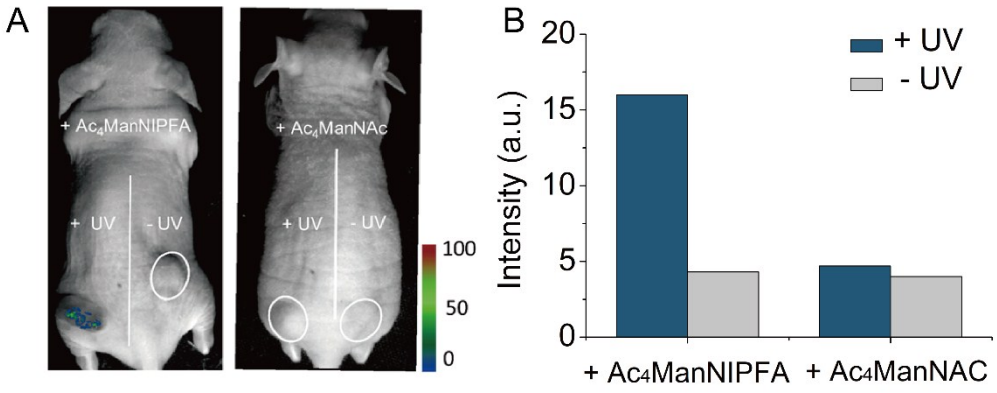


Fig. S13. A. Fluorogenic labeling results of sialic acids in living mice. Ac_4ManNIPFA/Ac_4ManNAc (3 mg/kg) was intratumoral injected to tumor tissues, once a day for 4 days and then 3 mg/kg of Tz was injected into tumor tissues of the above mouse. Fluorescence images were acquired after the left of side irradiation with UV light 350 nm for 10 min., λex = 980 nm. λem = 505 - 525 nm. B. Data statistics of A.

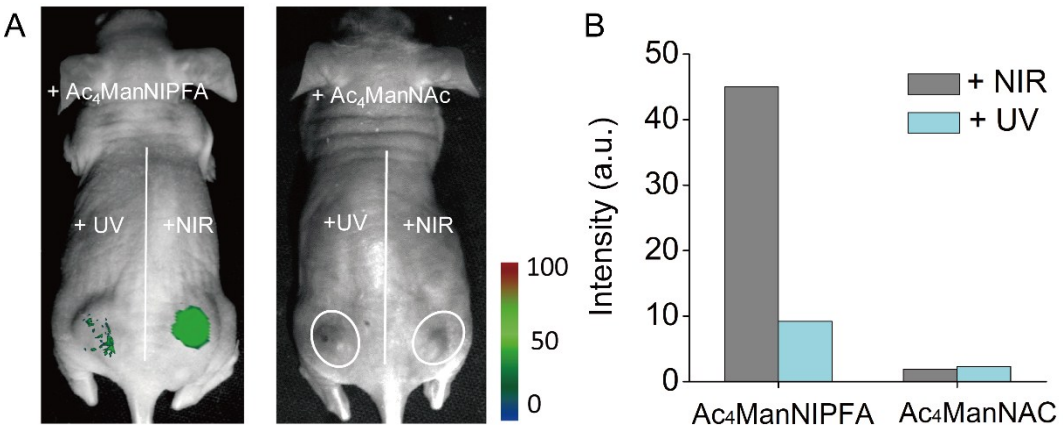


Fig. S14. A. Fluorogenic labeling results of sialic acids in living mice., λex = 980 nm. λem = 505 - 525 nm. Left: irradiation with UV light 350 nm for 10 min; Right: irradiation with NIR light 980 nm (1.0 w/cm^2) for 10 min. B. Data statistics of A..

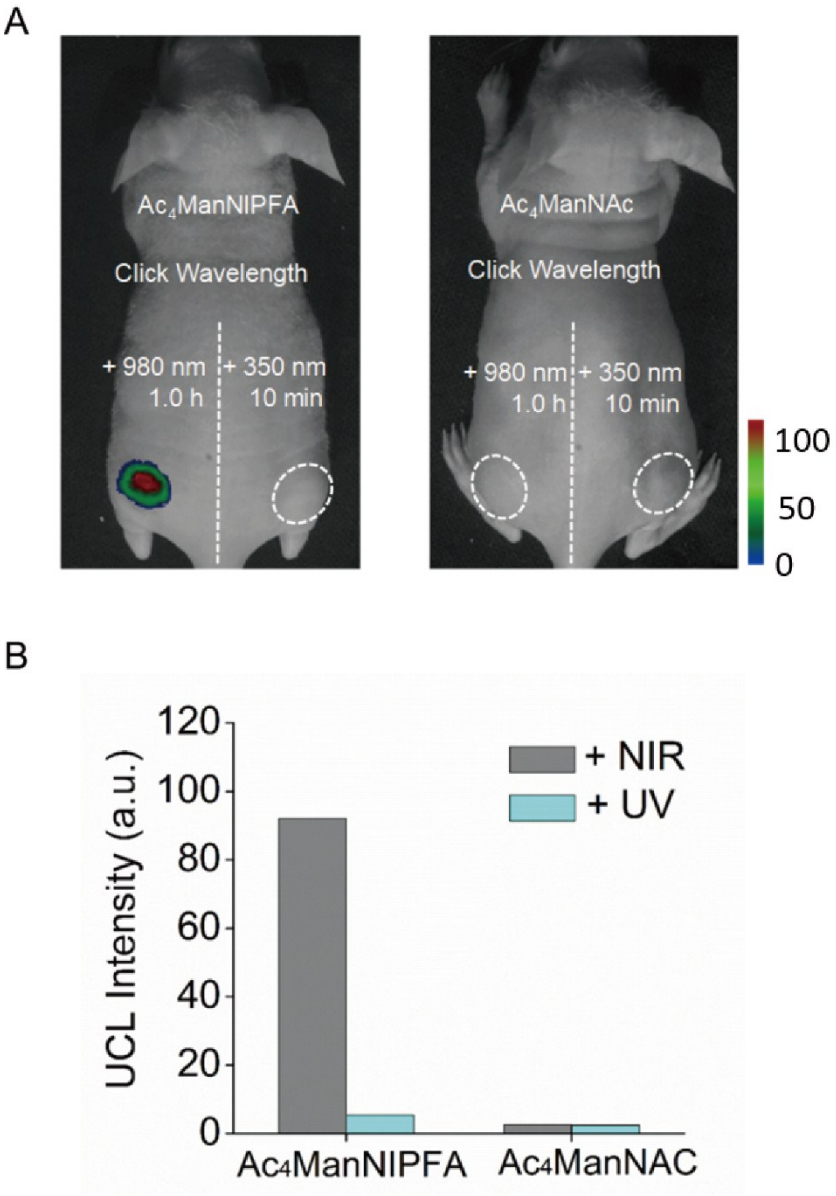


Fig. S15 A. Fluorescence images of UCNPs in living mice that were treated with Ac_4ManNIFPA injection with nanoparticle. (3 mg/kg of Ac_4ManNIPFA and Ac_4ManNAc were injected into the tail vein of A549 tumor-bearing mice once a day for 4 days, then 30 mg/kg of Tz-UCNP was injected into the tail vein of the above mouse. Fluorescence images were acquired after 48 hours of incubation the probe of Tz-UCNP. λ_{ex} = 980 nm (($1.0\ w/cm^2$), λ_{em} = 800 - 810 nm Left: irradiation with NIR light ($1.0\ w/cm^2$) for 1.0 h;Right: irradiation with UV light for 10 min. B. Data statistics of A.

2. Supporting experimental section.

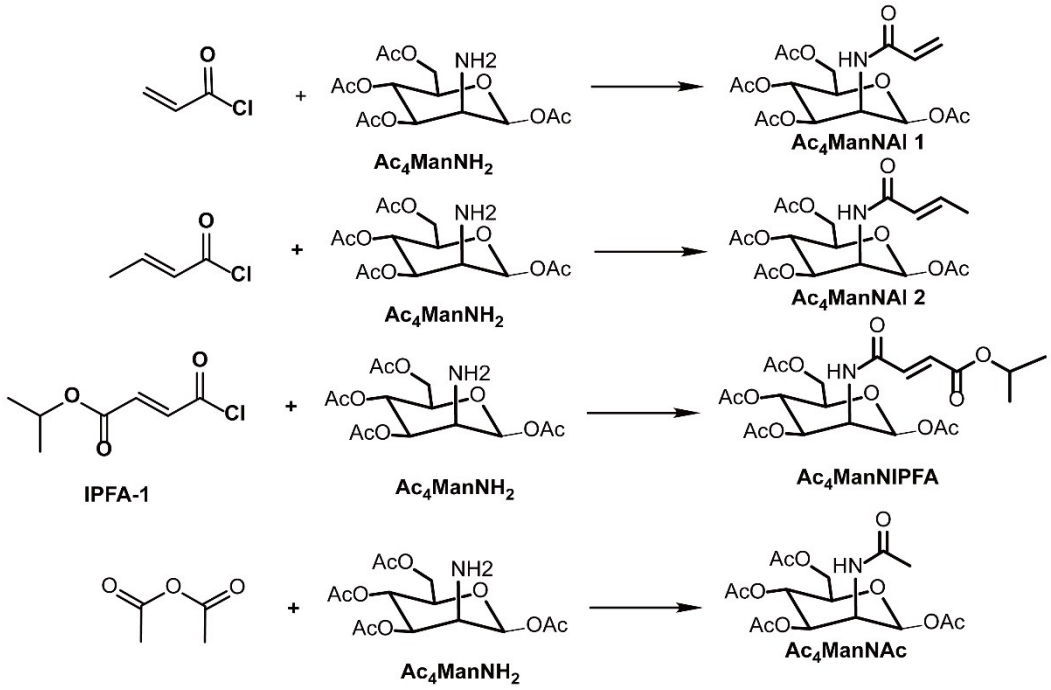
2.1 Materials

$Y(CH_3COOH)_3$, $Yb(CH_3COOH)_3$, $Tm(CH_3COOH)_3$, N-Hydroxysuccinimide (NHS), N, N-dimethylfomamide (DMF), Dimethyl sulfoxide (DMSO) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aladdin Reagent, Ltd. (Shanghai, China); CCK-8 was obtained from Dojindo (Japan). Dulbecco's modified Eagles medium (DMEM) cell culture medium, fetal bovine serum, streptomycin, and penicillin were purchased from Thermo Fisher Scientific Co., Ltd. (China). All chemicals used in this study were of analytical and reagents were used without further purification. All aqueous solutions were prepared using ultrapure deionized water (DI water), which was obtained through a Millipore Milli-Q water purification system (Billerica, USA) and had an electric resistance > 18.2 MΩ.

2.2 Instruments.

The ^1H NMR spectra were recorded on a Bruker Ultrashield 400 Plus NMR spectrometer. All chemical shifts are reported in the standard δ notation of parts per million. The UV–vis absorption spectra were recorded on a UV–vis spectrometer (Lambda 35 UV–vis spectrometer, Perkin-Elmer, USA) at room temperature. The fluorescence spectrum was measured using a LS-55 fluorescence spectrophotometer (Perkin-Elmer, USA). The upconversion fluorescence spectra of upconversion nanoparticles were recorded using a 1.0 W/cm^2 980 nm diode CW laser (Changchun New Industries Optoelectronics Tech. Co., Ltd.) as the excitation source. The TEM images were collected on a feld emission high resolution 2100 F transmission electron microscope (JEOL, Japan) operating at an acceleration voltage of 200 kV. The confocal fluorescence images of cells were collected on a ZEISS LSM 710 META confocal microscope. A 40× objective was used for image capturing. The cell viability was measured using a microplate reader (Infnite 200, TECAN, Switzerland). Quantitative data were expressed as mean ± SD. P values were calculated by two-tailed Student's test (***$p < 0.001$, **$p < 0.01$, or *$p < 0.05$). $P < 0.05$ was considered statistically

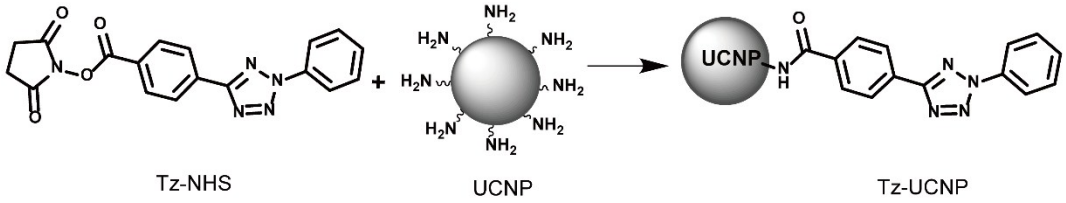
significant. All statistical analyses were carried out using GraphPad Prism Software (Version 6.0, GrapPad Software, San Diego, CA).



Scheme S1. Synthetic procedures of the Ac_4ManNIPFA, AC_4ManNene 1/2 and AC_4ManNAc.

2.3 Synthesis of the Ac_4ManNIPFA.

Ac_4ManNH_2 was produced according to the previous literature. Ac_4ManNH_2 (34.8 mg, 0.1 mmol), IPFA (88 mg, 0.5 mmol) were dissolved in dry methylene chloride (10 mL) under nitrogen. The mixture was stirred for 12 h at room temperature. The reaction mixture was collected with methylene chloride solution and dried with Na_2SO_4, and concentrated. The crude product was purified over a silica gel column chromatograph with methanol/methylene chloride (1:20) as the eluent to afford the pure product as the Ac_4ManNIPFA (30.3 mg, 62.2%). ^{1}H NMR (400 MHz, $CDCl_3$): 6.15 (d, J = 4.0 Hz, 1H), 6.08 (d,J = 8.0 Hz 1H), 5.36 (d, J = 4 Hz, 1H), 4.30 (t, J = 4 Hz, 1H), 4.06 (t, J = 4 Hz, 2H), 2.96 (s, 1H), 2.89 (s, 1H), 2.19 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H) ESI-MS (m/z): calcd for C21H29NO12, 487.46 [M^+Na^+2H]; found, 512



Scheme S2. Synthesis of the Tz-UCNP

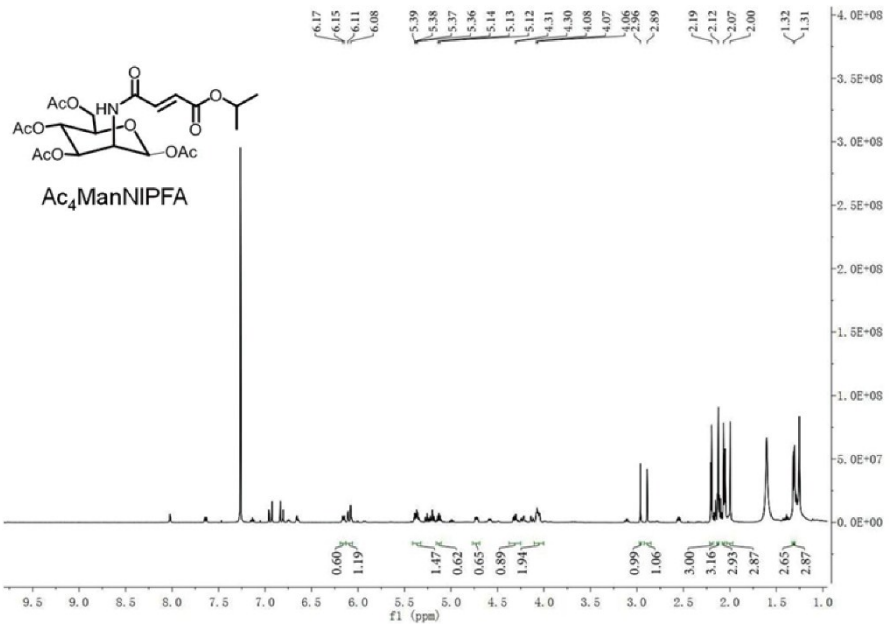
2.4 Synthesis of the UCNP (PEI-Capped $NaYF_4$: Yb^{3+}, Tm^{3+}).

Water dispersed $NaYF_4$:Yb^{3+}, Tm^{3+} nanoparticles were prepared by one step hydrothermal method. Typically, PEI (branched 25 kDa, 1 g),1 mmol of NaCl, and 1 mmol of $Y(CH_3COOH)_3$ (0.5 m), Yb $(CH_3COOH)_3$ (0.5 m), and $Tm(CH_3COOH)_3$ (0.1 m) with a molar ratio of 79.8:20:0.2 were dissolved in 20 mL of ethanol and stirred for 30 min. Then, 10 mL of ethanol containing 5.5 mmol of NH_4F was added to the above solution and stirred for another 30 min. After that, the obtained mixture was transferred into a 50 mL stainless teflon-lined autoclave. The autoclave was then sealed and kept at 190 °C for 24 h. After that, the auto clave was cooled to room temperature naturally. The precipitation was then collected by centrifugation (7000 g, 5 min) and washed with ethanol and deionized (DI) water several times and then dried over vacuum at 60 °C for 12 h to afford the as-prepared $NaYF_4$: Yb^{3+}, Tm^{3+} coated with PEI as a white powder.

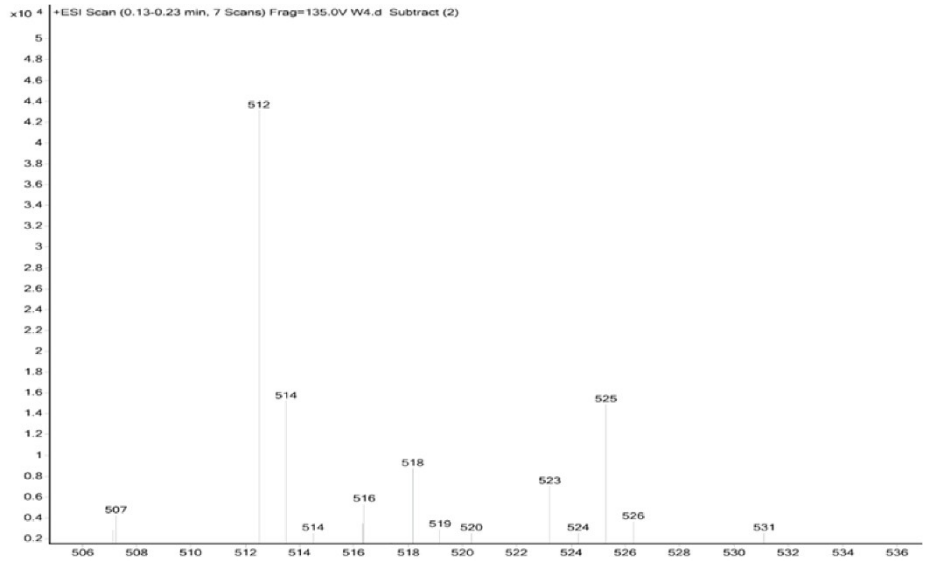
2.5 Cell culture and microscopy.

A549, MCF-7, RAW264.7 and EMT6 cells were cultured in Dulbecco's modified Eagles medium (DMEM). All cell lines were supplemented with 10% fetal bovine serum and 100 U/ml 1% penicillin/streptomycin antibiotics and maintained at 37°C in a 100% humidified atmosphere containing 5% CO_2. Cell density was determined using a hemocytometer before experimentation. Images were acquired on a ZEISS LSM 710 META confocal microscope. A 40× objective was used to capture images. Images were processed and analyzed using the LSM software.

2.6 ^{1}H NMR and MS spectra.



400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of Ac_4ManNIPFA



ESI-MS spectrum of Ac_4ManNIPFA