Electronic Supplementary Information

Enzyme-activated near-infrared fluorogenic probe with high-efficiency intrahepatic targeting ability for visualization of drug-induced liver injury

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Materials and Methods

Reagents and Apparatus.

All chemicals were purchased from Sigma Aldrich and used without further purification. Leucine aminopeptidase (LAP) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. All cell culture media, fetal bovine serum (FBS) and phosphate buffered saline (PBS, pH 7.4) were purchased from HyClone. The ultra-pure water (18 Ω) used in all experiments was obtained from a Milli-Q system (Millipore, USA). High resolution mass spectrometry (HRMS) was obtained on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, USA). The MS parameters are positive ion mode, electrospray ionization (ESI), and the resolution is 30000 (FWHM). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance spectrometer with TMS as an internal standard. Column chromatography was performed using silica gel (200-300 mesh, Qingdao Ocean Chemicals, Qingdao, China). Spectroscopic data was measured on a FS5 Spectrofluorometer (Edinburgh Instruments Ltd., UK) or SpectraMax M4 by Molecular Device. Photostability data was measured on a FS5 Spectrofluorometer with an intermittent excitation of xenon lamp (150 W, once per min, 180 times). High-performance liquid chromatography (HPLC) chromatograms were performed on Agilent, Infinity 1260 with a C18 column (100 Å, 30 × 4.6 mm). Fluorescence images of cells were carried out on a Nikon Ti-e confocal laser scanning microscope. The *in vivo* fluorescence images were acquired with the IVIS spectrum system (PerkinElmer, USA). The fluorescence intensity of region of interest was analyzed by Living Image 4.5 Software.

Determination of the fluorescence quantum yield.

Quantum yields for all the fluorescent compounds were measured by dividing the integrated emission area of their fluorescent spectrum against the area of **ICG** in PBS excited at 730 nm ($\Phi_{ICG} = 0.13$).[1] Quantum yields where then calculated using equation (1), where F represents the integrated emission area of fluorescent spectrum, η represents the refractive index of the solvent, and Abs represents absorbance at excitation wavelength selected for standards and samples. Emission was integrated from 640 nm to 800 nm.

$$\Phi_{flu}^{sample} = \Phi_{flu}^{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{\eta^{sample}}{\eta^{reference}}\right)\left(\frac{Abs^{reference}}{Abs^{sample}}\right) \quad (1)$$

Cell Culture and Cytotoxicity Assay.

Human normal liver cell line (LO2) was purchased from iCell Bioscience Inc. (Shanghai, China) and human hepatocarcinoma cell line (HepG2) was purchased from Cell Resource Centre, Peking Union Medical College. LO2 cells were incubated in Roswell Park Memorial Institute (RPMI)-1640 culture medium containing 20% (*v*/*v*) FBS and 1% (*v*/*v*) penicillin-streptomycin at 37°C in a 5% CO_2 humidified atmosphere. HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) culture medium containing 10% (*v*/*v*) FBS and 1% (*v*/*v*) penicillin-streptomycin at 37°C in a 5% CO_2 humidified atmosphere. The cytotoxicity of **hCy-CA-LAP** and **hCy-LAP** to LO2 and HepG2 cells was measured by using standard Cell Counting Kit-8 system (CCK-8) assays. LO2 and HepG2 cells were respectively plated in a 96-well plates at 1×10^5 cells/well and incubated overnight in the cell incubator before exposure to **hCy-CA-LAP** and **hCy-LAP**. Then, different concentrations of **hCy-CA-LAP** or **hCy-LAP**

probes were incubated with cells for 24 hours. After removing the above solution, CCK-8 reagent diluted with RPMI-1640 medium (20% FBS) or DMEM (10% FBS) was added to each well and incubated for 30 min. Finally, the absorbance was finally measured at 450 nm by a microplate reader.

Cell imaging.

For cell fluorescence imaging, LO2 or HepG2 cells were plated in a glass-bottom dish for 24 h before the experiment. Fluorescence images of the cells were captured by a Nikon Ti-e microscope (λ_{ex} = 639 nm, λ_{em} = 663-738). Cell fluorescence imaging of **hCy-CA-LAP** in normal LO2 cells can be divided into five groups. **hCy-CA-LAP** (1 mM in DMSO) was diluted with culture media and reached a concentration of 10 μM in cell culture wells. In group **hCy-CA-LAP**, **hCy-CA-LAP** (10 μM) was incubated with LO2 or HepG2 cells for 30 min. In group Bestatin + **hCy-CA-LAP**, LO2 or HepG2 cells were pertreated with bestatin (100 μM) for 1 h before incubating with **hCy-CA-LAP**. In group APAP + **hCy-CA-LAP**, LO2 or HepG2 cells were pertreated with APAP (1 mM) for 12 h before incubating with **hCy-CA-LAP**. In group APAP + Bestatin + **hCy-CA-LAP**, LO2 or HepG2 cells were pertreated with APAP (1 mM) for 12 h followed by bestatin (100 μM) for 1 h before incubating with **hCy-CA-LAP**. In group NAC + APAP + **hCy-CA-LAP**, LO2 or HepG2 cells were pertreated with NAC (100 μM) for 1 h followed by APAP (1 mM) for 1 h before incubating with **hCy-CA-LAP**. The cells were washed 3 times with PBS buffer before cell imaging.

***In vivo* fluorescence imaging of acetaminophen (APAP) induced acute liver injury.**

All animal procedures were performed in accordance with the Ethics Committee of Beijing University of Technology, China. Female BALB/c mice (body weight, around 18-20 g) were purchased from Beijing HFK Bioscience Co., Ltd., and maintained under standard conditions. The mice were housed in sterile cages and fed water and food *ad libitum*. For *in vivo* imaging APAP induced acute liver injury, the mice were divided into four groups, three in each group, and the mice were fasted overnight before the experiment. In group PBS + **hCy-CA-LAP**, mice were intraperitoneally injected with PBS (200 μM) for 1 h, and then intravenously injected with **hCy-CA-LAP** (50 μM, 100 μL). In group APAP + **hCy-CA-LAP**, mice were intraperitoneally injected with APAP (300 mg/Kg, 200 μL) for 1 h, and then intravenously injected with **hCy-CA-LAP** (50 μM, 100 μL). In group PBS + **hCy-LAP**, mice were intraperitoneally injected with PBS (200 μM) for 1 h, and then intravenously injected with **hCy-LAP** (50 μM, 100 μL). In group APAP + **hCy-LAP**, mice were intraperitoneally injected with APAP (300 mg/Kg, 200 μL) for 1 h, and then intravenously injected with **hCy-LAP** (50 μM, 100 μL). Subsequently, the mice were immediately performed whole body optical imaging at predetermined time points (0, 1, 2, 3, and 4 h) via IVIS imaging system (Perkin Elmer) equipped with fluorescent filter sets (λ_{ex} = 640 nm, λ_{em} = 740 nm). Data were analyzed with the Living Image 4.5 Software and ROIs of equal area in the regions of interest were obtained for further analysis. The above four groups of mice were all sacrificed 3 h after intravenously injecting probes. Major organs (heart, liver kidney,

spleen, lung) were collected and washed with saline. The images of the organs were taken using IVIS imaging system as described above.

***In vivo* fluorescence imaging of rifampicin (RFP) induced cholestatic liver injury.**

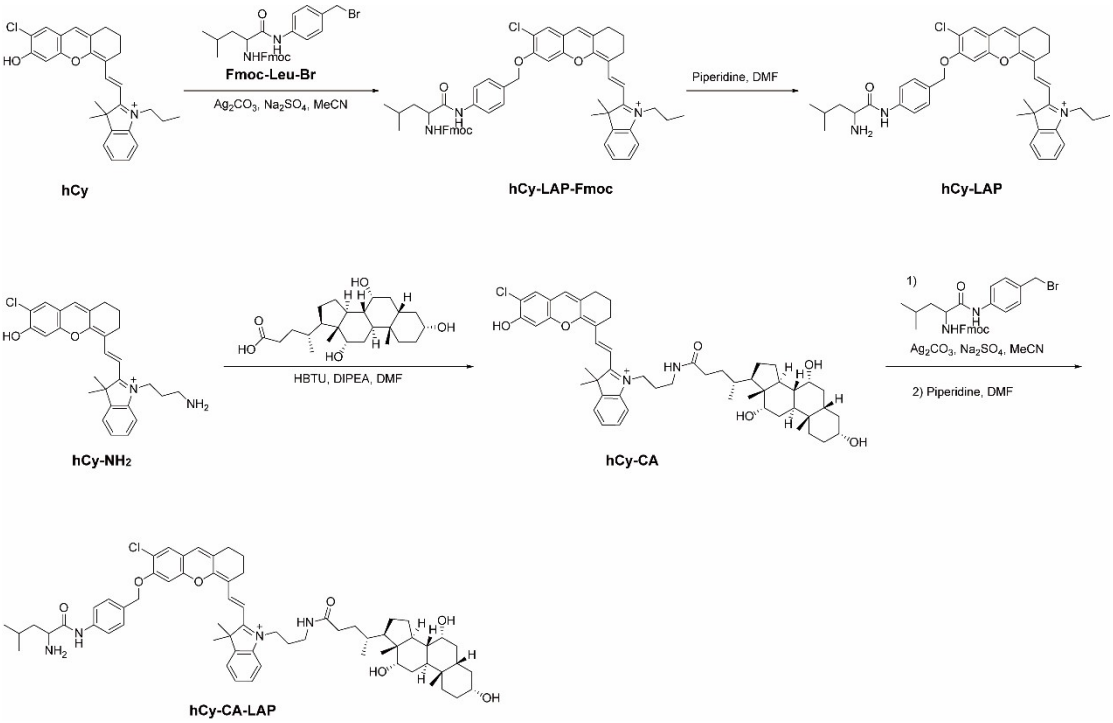
For *in vivo* imaging of RFP-induced cholestatic liver injury, the mice were divided into three groups, three in each group, and the mice were fasted overnight before the experiment. Mice were with RFP at a hepatotoxic dosage (dissolved in aqueous solution of 0.5% carboxymethylcellulose sodium, 300 $mg \cdot Kg^{-1} \cdot d^{-1}$) for one or three days. For the control group, the mice were intragastrically administrated with 200 μL of aqueous solution of 0.5% carboxymethylcellulose sodium. The mice were then intravenously injected with **hCy-CA-LAP** (50 μM, 100 μL) at 6 h after the last drug treatment. Subsequently, the mice were immediately performed whole body optical imaging at predetermined time points (0, 25, 50, 75, and 100 min) via IVIS imaging system equipped with fluorescent filter sets (λ_{ex} = 640 nm, λ_{em} = 740 nm). ROIs of equal area in the regions of interest were obtained for further analysis. The three groups of mice were sacrificed 1 h after intravenously injecting probes. Major organs (heart, liver kidney, spleen, lung) were collected and washed with saline. The images of the organs were taken using IVIS imaging system as described above.

Histology.

Liver tissues of mice were excised and then rinsed with normal saline, and fixed in 10% formaldehyde solution for paraffin embedding. The embedded liver tissues were processed into 10 μm section. Histological examination was performed by staining with hematoxylin and eosin (H&E). All images were obtained from a Nikon ECLIPSE Ti2-U

inverted fluorescence microscope equipped with a IXON-L-888 cameras (ANDOR, UK).

Synthesis and Characterizations



Scheme S1 Synthetic route of **hCy-LAP** and **hCy-CA-LAP**.

Synthesis of hCy7-LAP-Fmoc. NIR fluorophore **hCy7** and compound **Fmoc-Leu-Br** were prepared by the reported methods.[2, 3] A mixture of **hCy7** (28.1 mg, 0.063 mmol), Ag_2CO_3 (34.7 mg, 0.126 mmol) and Na_2SO_4 (17.9 mg, 0.126 mmol) in 3 mL MeCN was stirred at room temperature for 30 min, and then **Fmoc-Leu-Br** (32.8 mg, 0.063 mmol) was added under a nitrogen atmosphere. The mixture was stirred at 40 ℃ for 3 h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (DCM: MeOH = 25:1) to afford **hCy7-LAP-Fmoc** as a blue solid (18 mg, 32%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.82 (s, 1H), 8.36 (d, J = 14.8 Hz, 1H), 8.09 (d, J = 8.0 Hz, 2H), 7.89 – 7.85 (m, 2H), 7.64 (dd, J = 13.3, 6.6 Hz,

4H), 7.35 (dt, *J* = 15.3, 7.4 Hz, 2H), 7.29-7.27 (m, 2H), 7.26-7.18 (m, 7H), 6.97 (s, 1H), 6.68 (s, 1H), 6.25 (d, *J* = 14.8 Hz, 1H), 5.17 (q, *J* = 13.3 Hz, 2H), 4.19-4.16 (m, 3H), 4.05 (t, *J* = 7.5 Hz, 1H), 4.02 – 3.97 (m, 1H), 2.61 (s, 2H), 2.52 (s, 2H), 1.85-1.81 (m, 2H), 1.78 (m, 4H), 1.72 (s, 6H), 0.98 (t, *J* = 7.3 Hz, 3H), 0.90 (t, *J* = 6.0 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.07, 172.91, 160.81, 156.94, 156.56, 152.43, 145.84, 144.27, 141.98, 141.14, 140.11, 131.92, 129.83, 129.06, 128.40, 128.15, 127.79, 127.51, 127.48, 127.21, 127.03, 125.94, 125.84, 124.01, 120.97, 120.59, 119.68, 115.86, 114.63, 112.28, 104.19, 102.37, 72.17, 66.78, 56.40, 51.17, 47.23, 47.10, 41.76, 29.81, 29.19, 28.19, 25.09, 23.17, 22.02, 21.39, 20.22, 11.63. HRMS *m/z* ($C_{56}H_{57}ClN_3O_5^+$) calculated $(M)^+$: 886.3981, found $(M)^+$: 886.4012.

Synthesis of hCy7-LAP. Compound **hCy7-LAP-Fmoc** (18 mg, 0.02 mmol) was added to 20% piperidine in DMF (1.25 mL) and stirred at room temperature for 1 h. Then the mixture was extracted with DCM and washed with saturated brine. The crude product was purified by column chromatography (DCM: MeOH = 10:1) to afford **hCy7-LAP** as a blue solid (8 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.51 (d, *J* = 14.9 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.47 – 7.38 (m, 2H), 7.34 (t, *J* = 4.2 Hz, 4H), 7.03 (s, 1H), 6.86 (s, 1H), 6.43 (d, *J* = 14.9 Hz, 1H), 5.22 (s, 2H), 4.33 (t, *J* = 7.1 Hz, 2H), 3.80 (dd, *J* = 8.2, 5.1 Hz, 1H), 2.71 – 2.55 (m, 4H), 1.98 – 1.89 (m, 2H), 1.88 – 1.81 (m, 2H), 1.77 (d, *J* = 1.5 Hz, 6H), 1.56 – 1.46 (m, 1H), 1.03 (t, *J* = 7.4 Hz, 3H), 0.88 (t, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.18, 173.01, 160.78, 156.90, 152.62, 145.97, 142.11, 141.39, 138.89, 131.80, 130.61, 129.25, 128.60, 127.90, 127.83, 127.66, 123.29, 120.87, 120.21, 115.97, 114.98,

112.77, 104.68, 102.03, 71.81, 54.03, 51.08, 47.25, 43.05, 28.24, 24.91, 24.24, 23.16, 21.97, 21.48, 20.25, 11.62. HRMS *m/z* ($C_{41}H_{47}ClN_3O_3^+$) calculated $(M)^+$: 664.3300, found $(M)^+$: 664.3328.

Synthesis of hCy7-CA. NIR fluorophore **hCy7-NH$_2$** was prepared by the reported method.[4] A mixture of **hCy7-NH$_2$** (54 mg, 0.117 mmol), cholic acid (47.8 mg, 0.117 mmol), HBTU (53 mg, 0.14 mmol) and DIPEA (40.8 μL, 0.234 mmol) in 2 mL DMF was stirred at room temperature for 2 h. Then, excess petroleum ether was added to remove solvent, and the crude product was purified by column chromatography (DCM: MeOH = 10:1) to afford **hCy7-CA** as a blue solid (26 mg, 26%).^{1}H NMR (600 MHz, DMSO) δ 8.17 (d, *J* = 13.8 Hz, 1H), 7.98 (s, 1H), 7.65 (s, 1H), 7.59 (s, 1H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.55 (d, *J* = 2.9 Hz, 1H), 6.03 (d, *J* = 13.8 Hz, 1H), 4.08 (s, 2H), 3.73 (s, 1H), 3.20 – 3.10 (m, 3H), 2.66 (s, 2H), 2.61 (s, 2H), 2.18 (dd, *J* = 25.5, 13.2 Hz, 2H), 2.12-2.08 (m, 2H), 2.00-1.91 (m, 2H), 1.83-1.78 (m, 4H), 1.77 – 1.69 (m, 3H), 1.66 (s, 6H), 1.61-1.58 (m, 2H), 1.43-1.37 (m, 4H), 1.34 – 1.27 (m, 3H), 1.26 – 1.08 (m, 8H), 0.89 (dd, *J* = 13.6, 6.2 Hz, 3H), 0.87 – 0.81 (m, 1H), 0.79 (t, *J* = 5.6 Hz, 1H), 0.74 (s, 3H), 0.57 (s, 1H), 0.50 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 174.94, 172.75, 159.77, 155.78, 155.75, 142.83, 142.49, 140.29, 140.28, 128.33, 127.77, 124.11, 123.75, 122.28, 118.91, 115.03, 114.36, 110.07, 109.87, 102.65, 71.00, 70.42, 66.22, 48.59, 48.14, 48.12, 46.13, 45.70, 41.50, 41.33, 41.11, 36.00, 35.25, 34.99, 34.84, 34.33, 32.56, 31.71, 30.37, 28.52, 27.97, 27.60, 27.25, 26.95, 26.18, 22.74, 22.59, 22.51, 20.54, 17.11, 12.30. HRMS *m/z* ($C_{52}H_{68}ClN_2O_6^+$) calculated $(M)^+$: 851.4760, found $(M)^+$:

851.4789.

Synthesis of hCy7-CA-LAP. A mixture of **hCy7-CA** (26 mg, 0.03 mmol), Ag_2CO_3 (16.5 mg, 0.06 mmol) and Na_2SO_4 (8.5 mg, 0.06 mmol) in a mixed solution of 1 mL MeOH and 3 mL MeCN was stirred at room temperature for 30 min, then **Fmoc-Leu-Br** (16 mg, 0.03 mmol) was added under nitrogen atmosphere. The mixture was stirred at 40℃ overnight. After reaction, the solvent was evaporated under reduced pressure to obtain a blue solid, which was directly used for subsequent synthesis without purification. The crude intermediate was added to 20% piperidine in DMF (1.25 mL) and stirred for 1 h at room temperature. Then, excess petroleum ether was added to remove solvent, and the crude product was purified by semi-preparative HPLC to afford **hCy7-CA-LAP** as a blue solid (3 mg, 9%). ^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 8.54 (d, *J* = 14.9 Hz, 1H), 8.15 (s, 1H), 7.84 (d, *J* = 7.3 Hz, 1H), 7.76 (dd, *J* = 21.0, 8.2 Hz, 4H), 7.58 – 7.46 (m, 4H), 7.39 (s, 1H), 7.31 (s, 1H), 6.60 (d, *J* = 15.0 Hz, 1H), 5.36 (s, 2H), 4.45 (s, 2H), 4.34 (s, 1H), 4.09 (s, 1H), 3.99 (s, 1H), 3.73 (s, 1H), 3.53 (s, 1H), 3.21 (d, *J* = 7.4 Hz, 1H), 3.19 – 3.10 (m, 3H), 2.78 – 2.58 (m, 4H), 2.17 – 2.08 (m, 2H), 2.01 – 1.90 (m, 4H), 1.79 – 1.74 (m, 6H), 1.72 – 1.65 (m, 4H), 1.64 – 1.58 (m, 5H), 1.45 – 1.38 (m, 4H), 1.28 – 1.17 (m,12H), 0.91 – 0.84 (m, 9H), 0.74 (s, 3H), 0.50 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 177.91, 174.31, 172.86, 159.64, 155.96, 152.24, 144.94, 142.26, 141.31, 138.70, 131.19, 130.88, 129.63, 128.90, 128.70, 128.24, 127.78, 127.44, 122.93, 119.52, 118.83, 115.84, 114.28, 113.37, 105.33, 102.00, 70.97, 70.40, 66.21, 50.63, 46.13, 45.69, 41.50, 41.33, 35.81, 35.26, 35.07, 34.86, 34.33, 32.47, 31.63, 31.27, 30.37, 29.02, 28.69, 28.54, 27.70, 27.44, 27.27,

26.58, 26.19, 25.10, 23.92, 23.64, 22.74, 22.54, 22.16, 22.07, 19.81, 17.12, 13.94, 12.29. HRMS *m/z* ($C_{65}H_{86}ClN_4O_7^+$) calculated $(M)^+$: 1069.6180, found $(M)^+$: 1069.6185.

Table S1 Comparison of LAP-activated imaging probes (**hCy-CA-LAP** and **hCy-LAP**) with corresponding products**.**

	λ_{Abs} (nm)	λ_{em} (nm)	Max Fl. Time (min)	F/F_0	Detection limit (U/mL)	Φ
hCy-CA-LAP	615	-	-	-	-	<0.01
hCy-LAP	603	-	-	-	-	<0.01
hCy-CA-LAP +LAP	680	710	60	27	0.0067	0.20
hCy-LAP +LAP	680	705	40	78	0.002	0.27

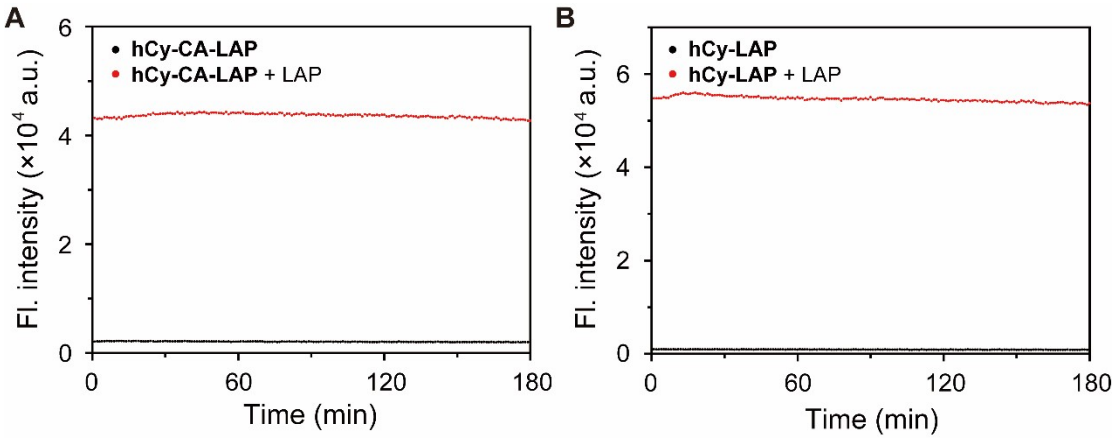


Figure S1 Photostability of **hCy-CA-LAP** and **hCy-LAP** (10 μM) before and after the addition of LAP monitored at 710 nm at 37ºC. λ_{ex}= 680 nm, once per min,180 times, source: xenon lamp (150 W).

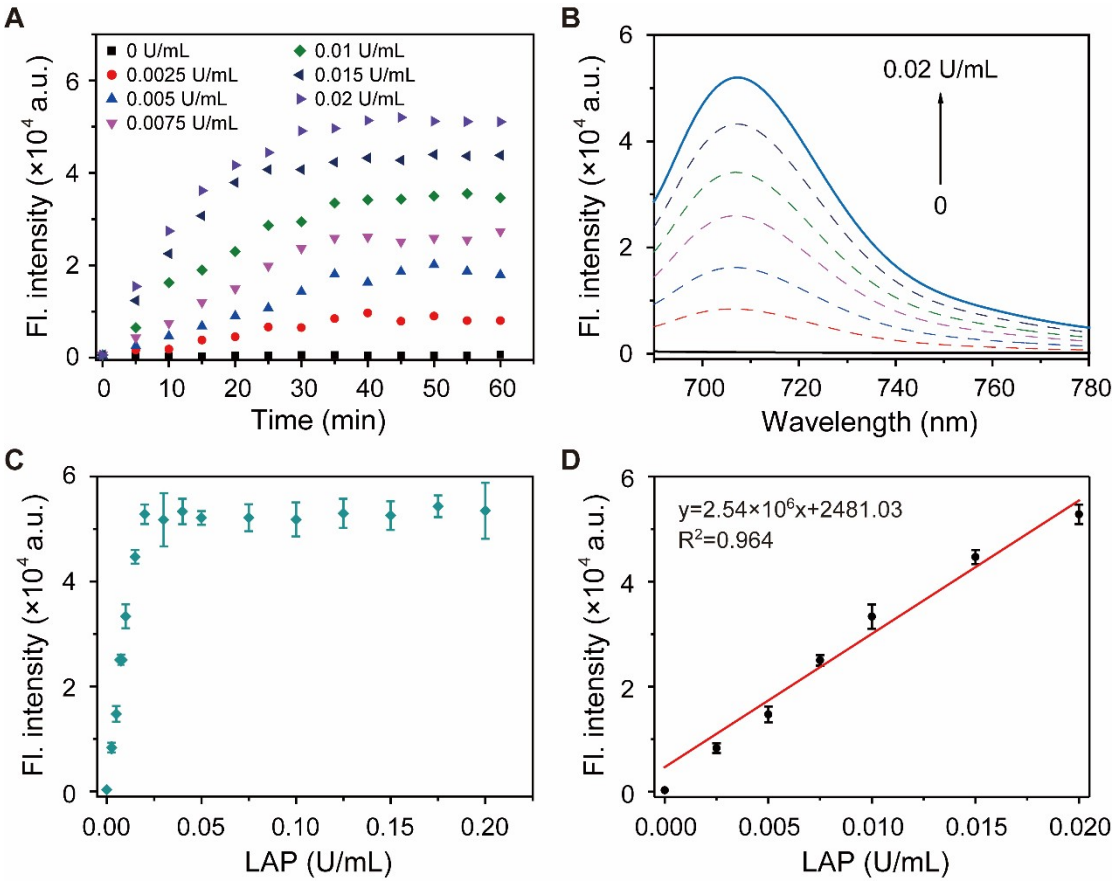


Figure S2 *In vitro* response of **hCy-LAP** toward LAP. (A) The sensing kinetics of **hCy-LAP** ($F_{705\ nm}$) and (B) fluorescence responses of **hCy-LAP** (10 μM) to different concentrations of LAP (0-0.02 U·mL^{-1}). (C) Fluorescence intensity of **hCy-LAP** (10 μM) at 705 nm with different concentrations of LAP (0-0.2 U·mL^{-1}). (D) Linear correlation between fluorescence intensity at 705 nm and low concentrations of LAP (0-0.02 U·mL^{-1}), λ_{ex}= 680 nm. All spectra measurements were recorded in PBS solution (pH = 7.4) at 37ºC.

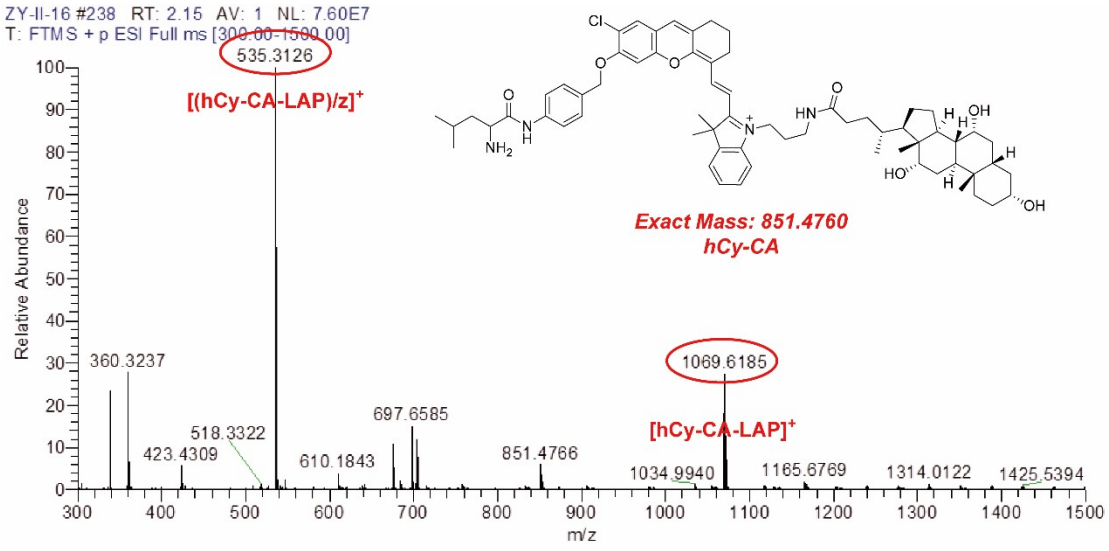


Figure S3 HRMS spectrum of **hCy-CA-LAP**.

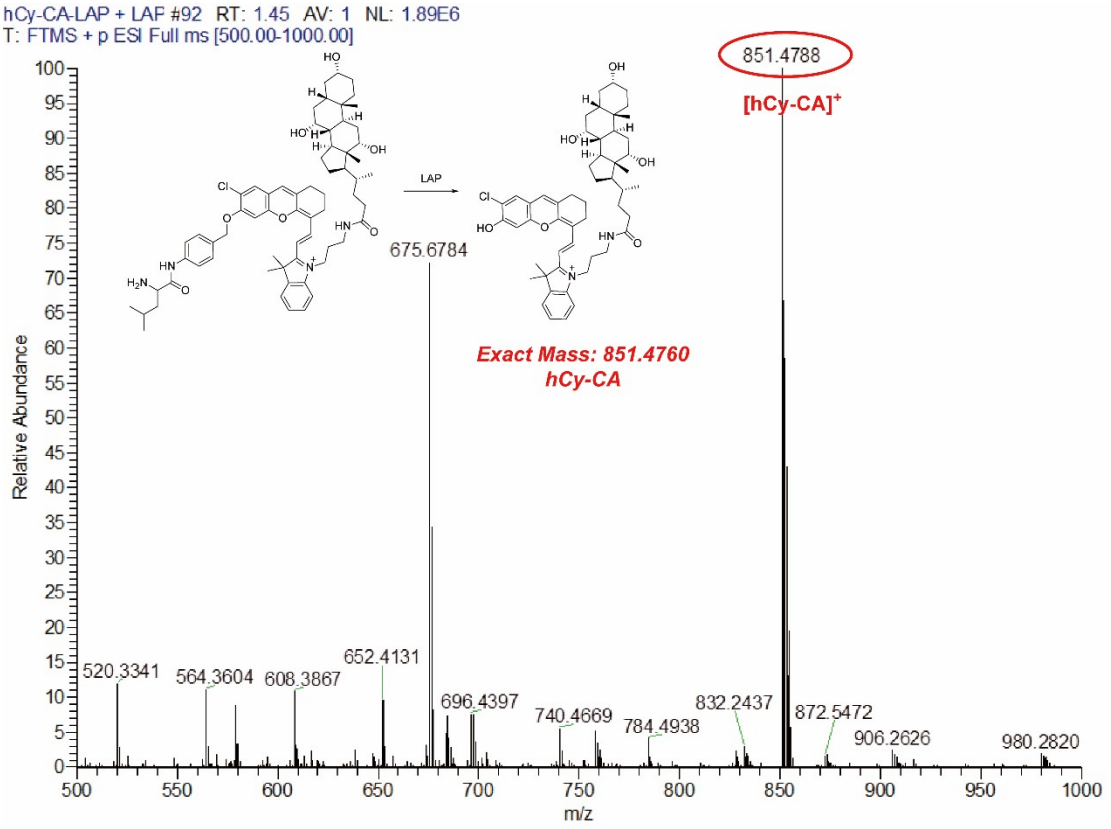


Figure S4 HRMS spectrum of the reaction solution of **hCy-LAP-CA** with LAP.

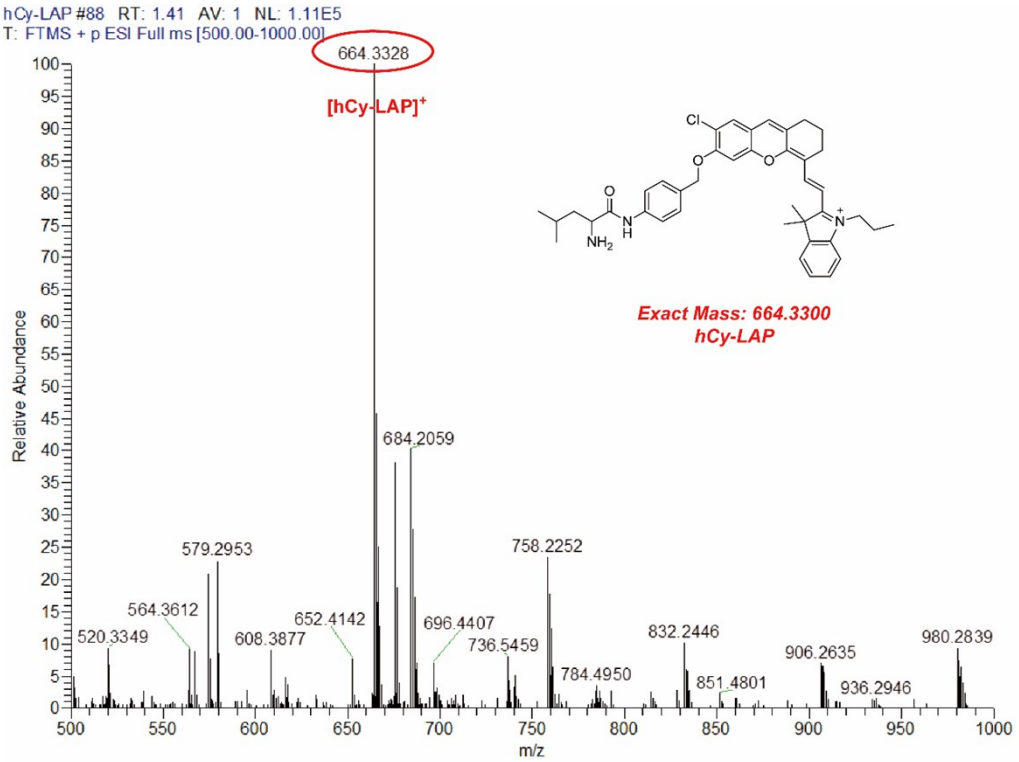


Figure S5 HRMS spectrum of **hCy-CA**.

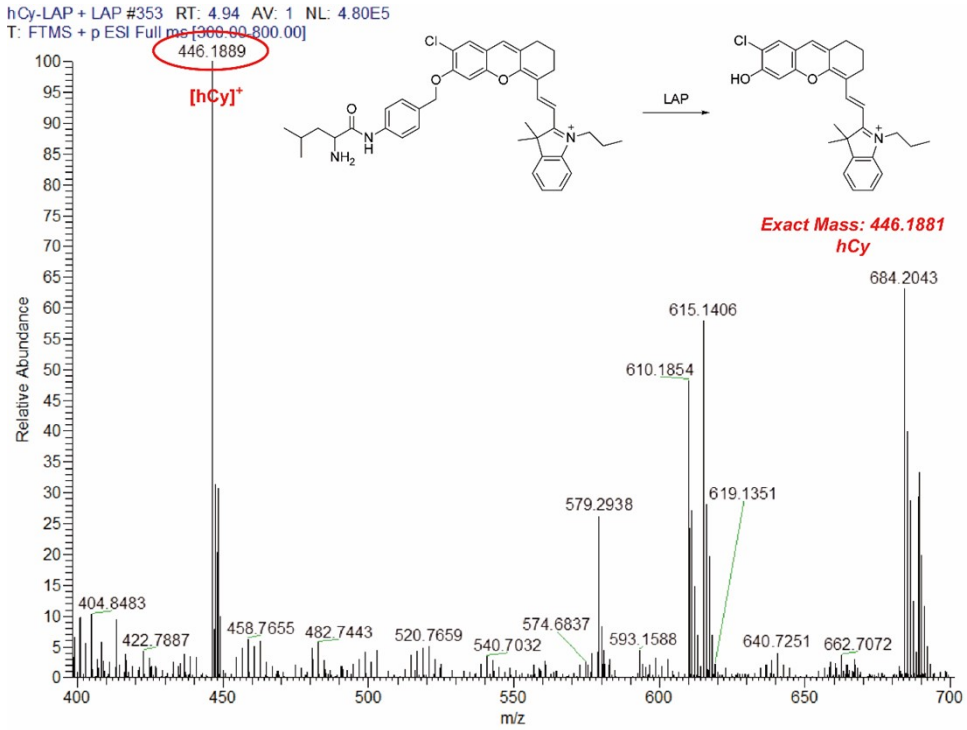


Figure S6 HRMS spectrum of the reaction solution of **hCy-LAP** with LAP.

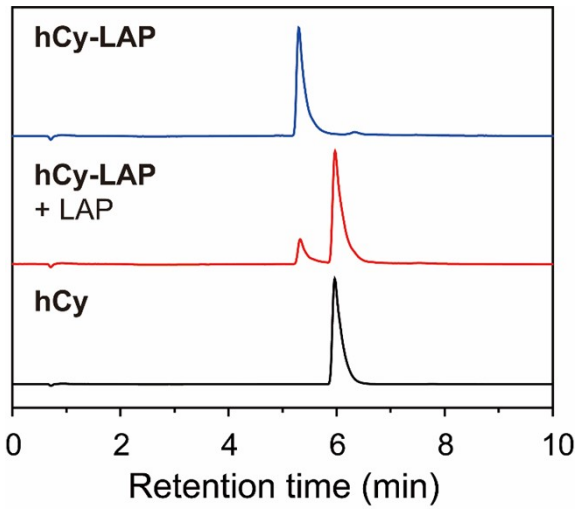


Figure S7 Chromatograms of **hCy-LAP**, **hCy- LAP** reacting with LAP and **hCy**. λ_{abs} = 600 nm.

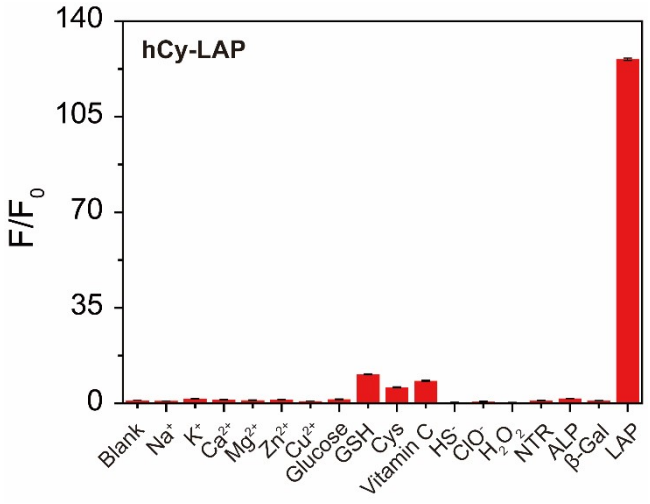


Figure S8 Fluorescence response of **hCy-LAP** (10 μM) to different analytes (analytes: 1 mM, enzyme: 0.1 U·mL^{-1}) in PBS buffer (pH 7.4) at 37℃, λ_{ex} = 680 nm, λ_{em} = 705 nm.

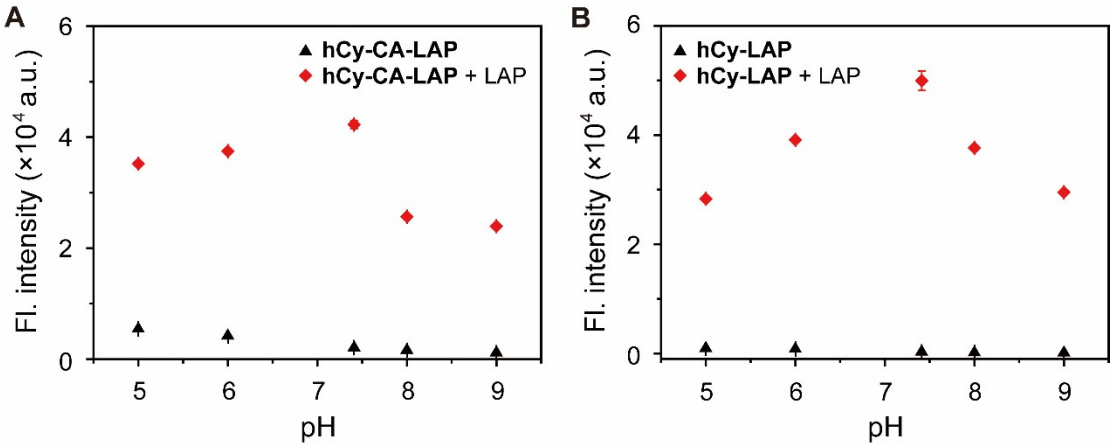


Figure S9 Effects of pH on the fluorescence of (A) **hCy-CA-LAP** and (B) **hCy-LAP** (10 μM) in the absence (black) and presence (red) of LAP (0.2 U·mL^{-1}). All spectra measurements were recorded in PBS solution (pH = 7.4) at 37ºC.

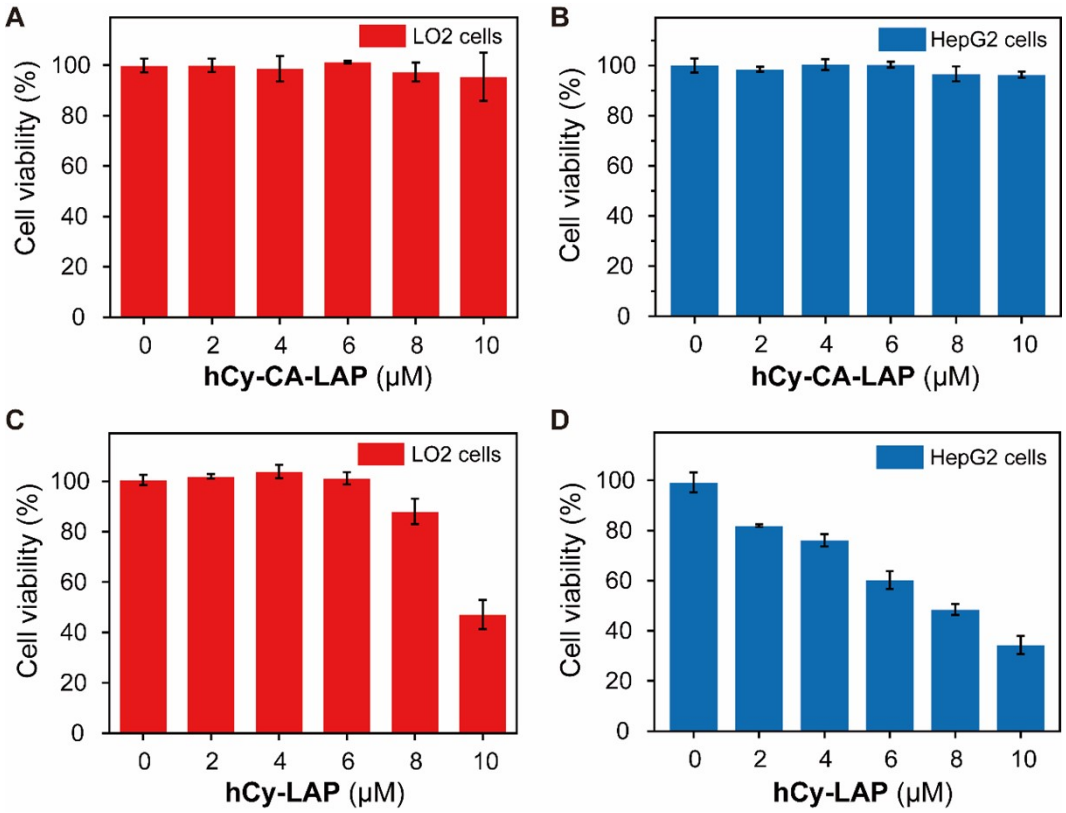


Figure S10 Cytotoxicity of **hCy-CA-LAP** and **hCy-LAP** to LO2 and HepG2 cells. Cells were incubated with the probe at corresponding concentrations for 24 h. The results are reported as percentage relative to untreated cells and the results are the mean standard deviation of three separate measurements.

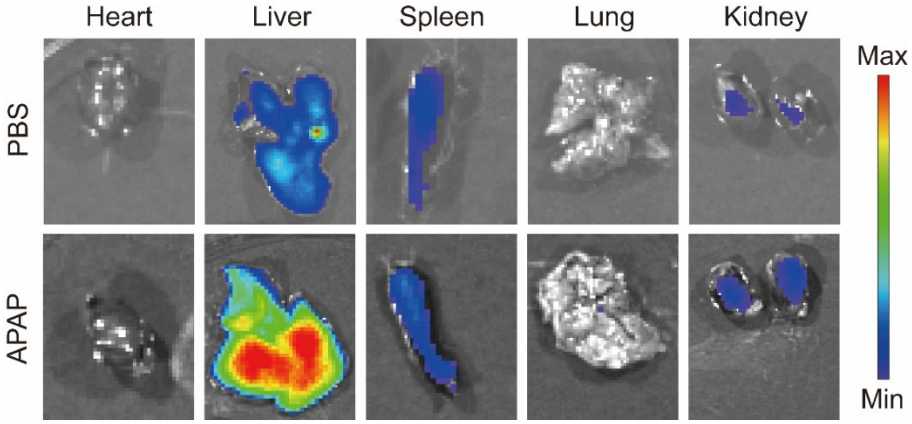


Figure S11 Organs dissected from mice 3 h after intravenous injection of **hCy-CA-LAP** (50 μM, 100 μL). λ_{ex} = 640 nm, λ_{em} = 740 nm.

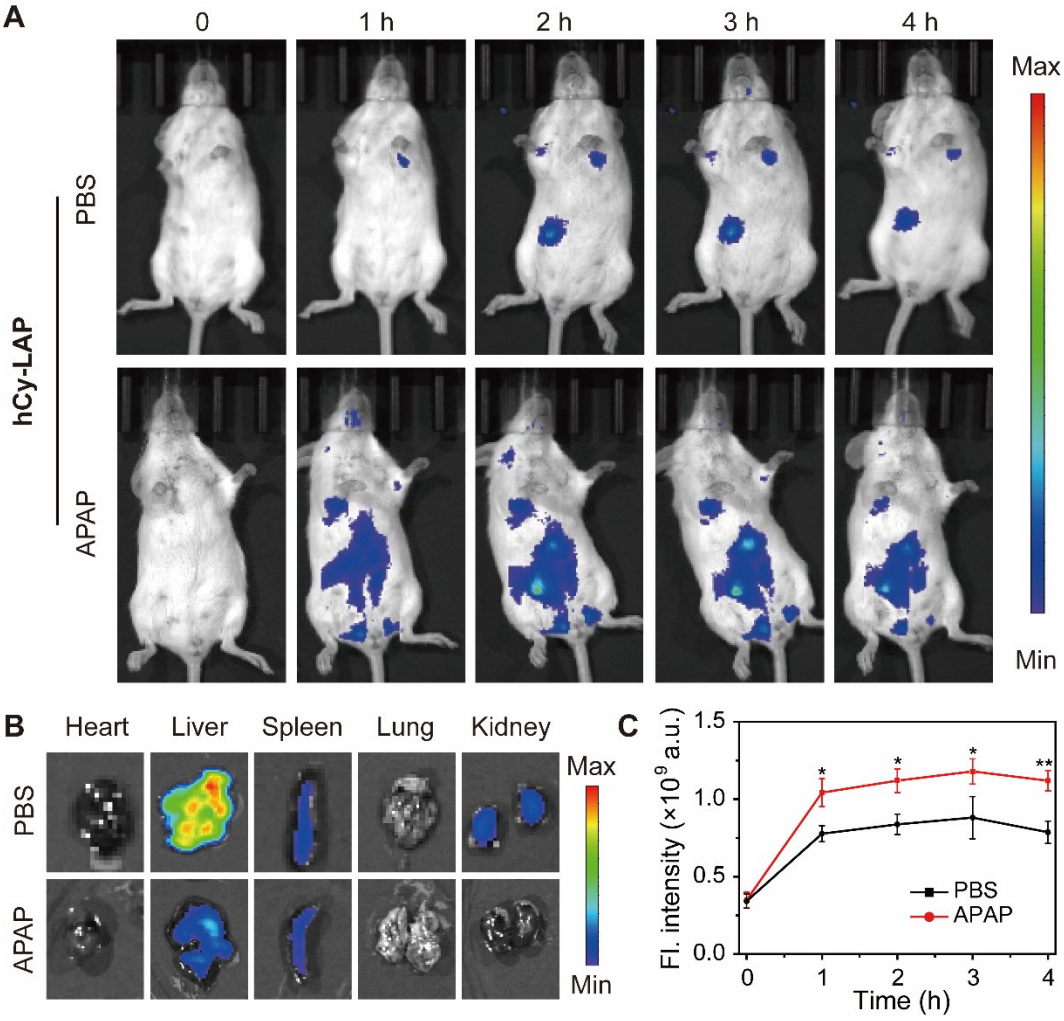


Figure S12 Time-dependent *in vivo* fluorescence imaging. (A) Balb/c female mice were pretreated with APAP (300 mg/Kg, 200 μL) or PBS (200 μL) for 1 h, and then intravenous injected with **hCy-LAP** (50 μM, 100 μL). (B) Organs dissected from mice

3 h after intravenous injection of **hCy-LAP** (50 μM, 100 μL). (C) Quantification of the fluorescence signal in (B) *: $P < 0.05$, and **: $P < 0.01$, compared with the control group (PBS-treated group). λ_{ex} = 640 nm, λ_{em} = 740 nm.

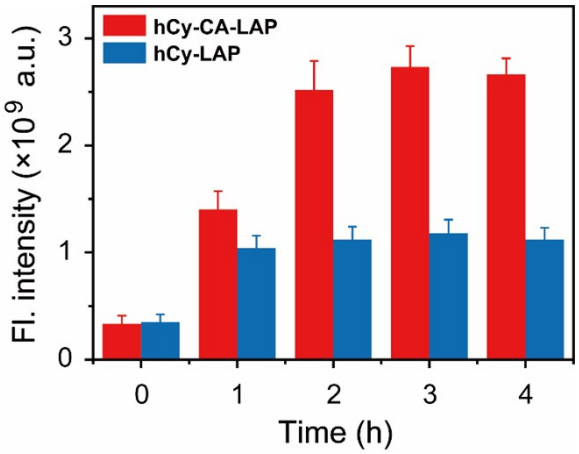


Figure S13 Quantification of the fluorescence signal at the imaging site of APAP-treated mice after intravenous injection of **hCy-CA-LAP** and **hCy-LAP** (50 μM, 100 μL).

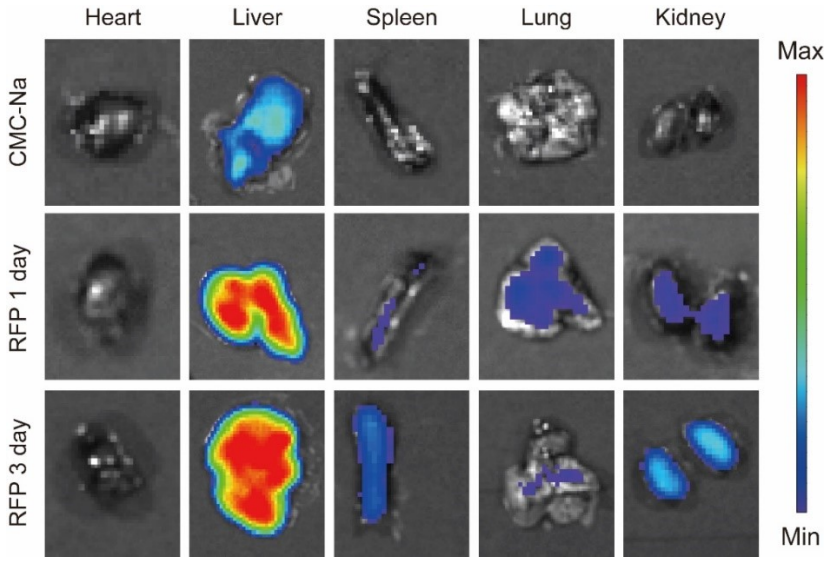


Figure S14 Organs dissected from mice 60 min after intravenous injection of **hCy-CA-LAP** (50 μM, 100 μL). λ_{ex} = 640 nm, λ_{em} = 740 nm.

NMR spectra

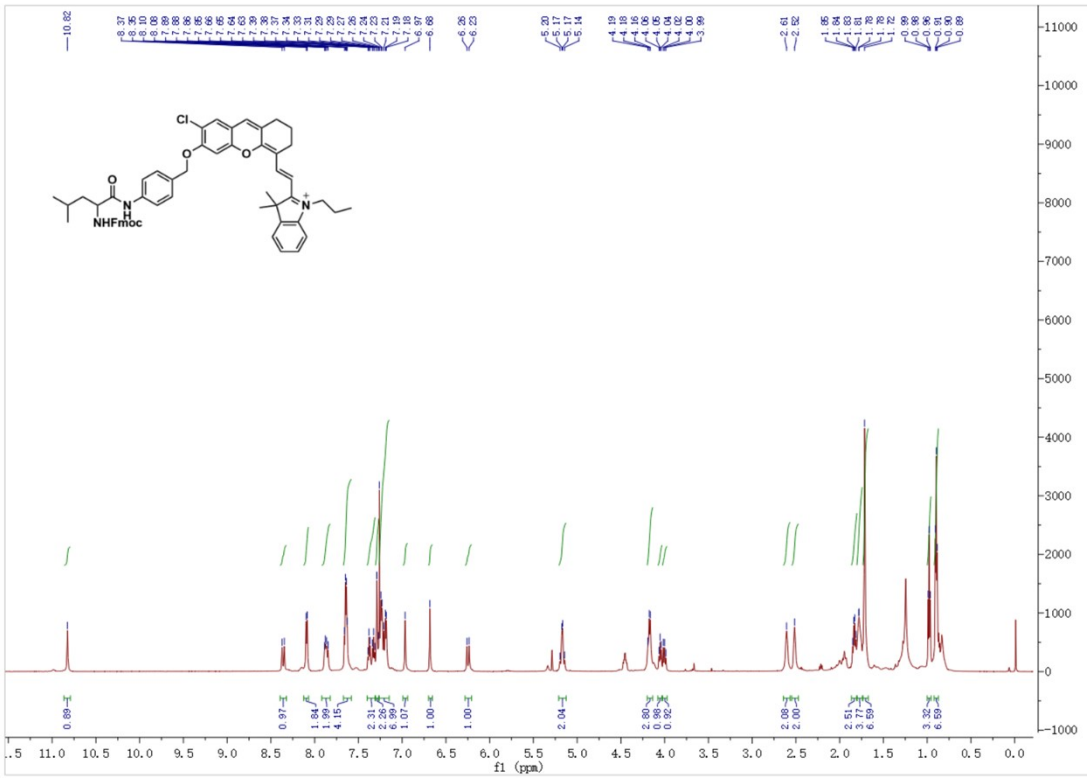


Figure S15 ^{1}H NMR spectrum of **hCy-LAP-Fmoc**.

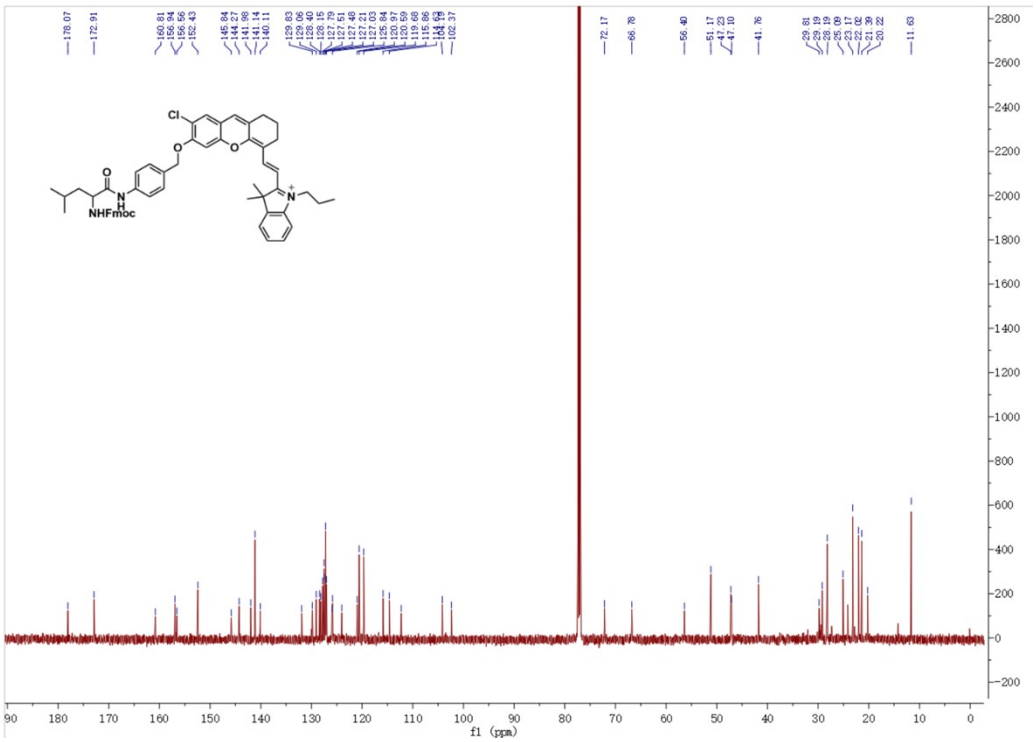


Figure S16 ^{13}C NMR spectrum of **hCy-LAP-Fmoc**.

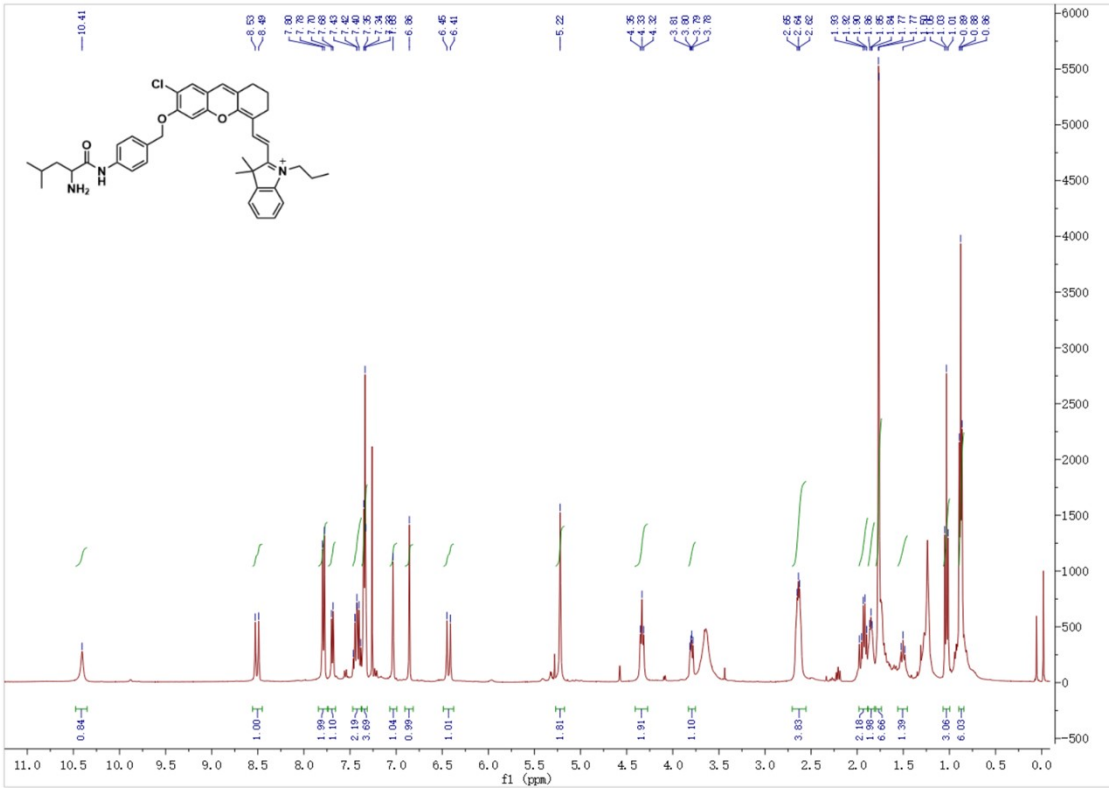


Figure S17 ^{1}H NMR spectrum of **hCy-LAP**.

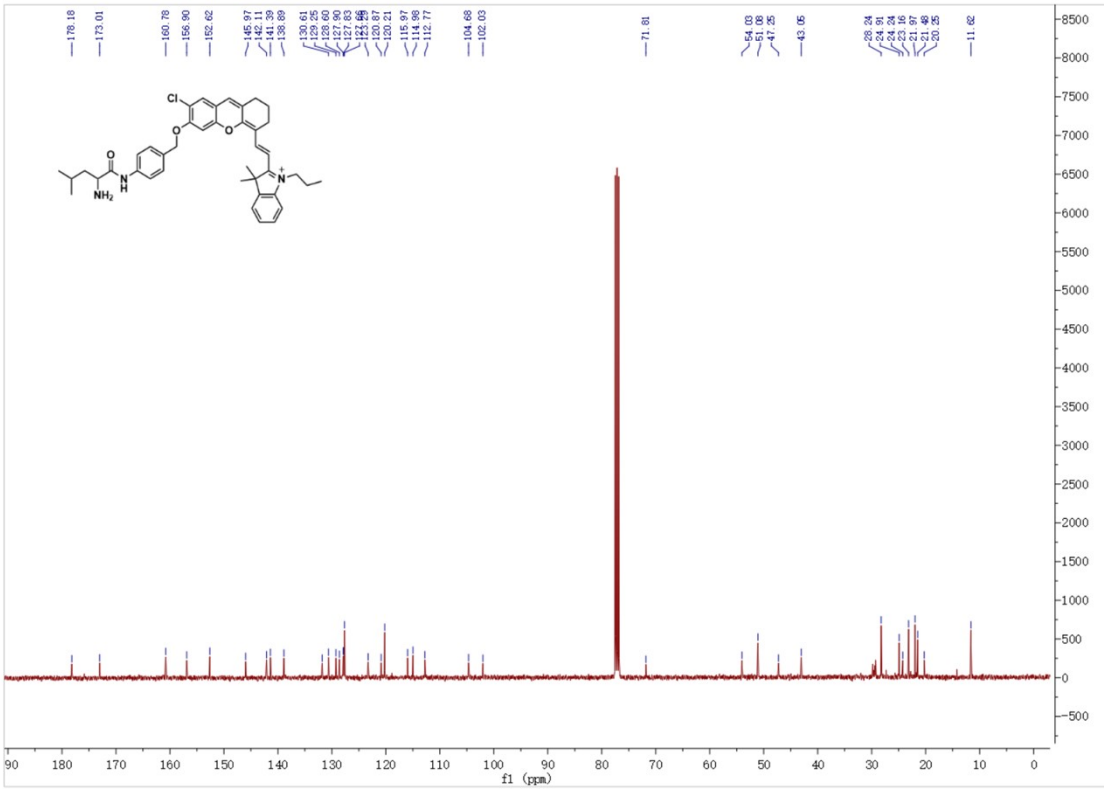


Figure S18 ^{13}C NMR spectrum of **hCy-LAP**.

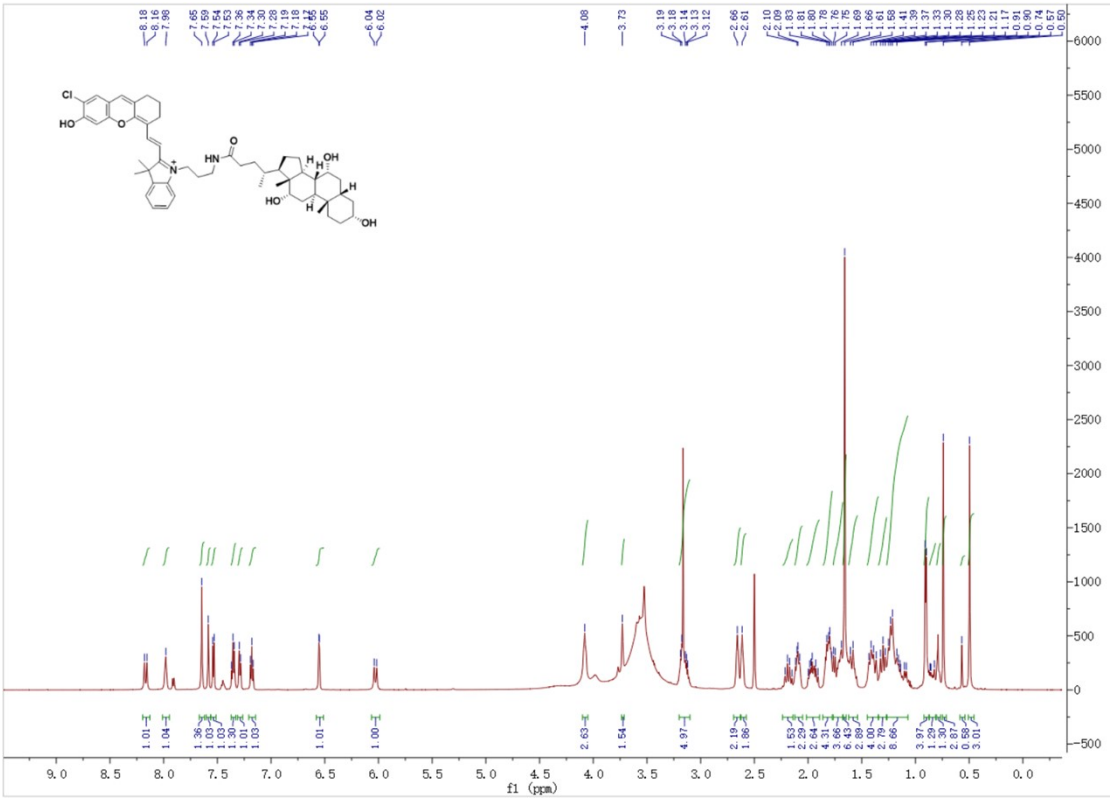


Figure S19 ^{1}H NMR spectrum of **hCy-CA**.

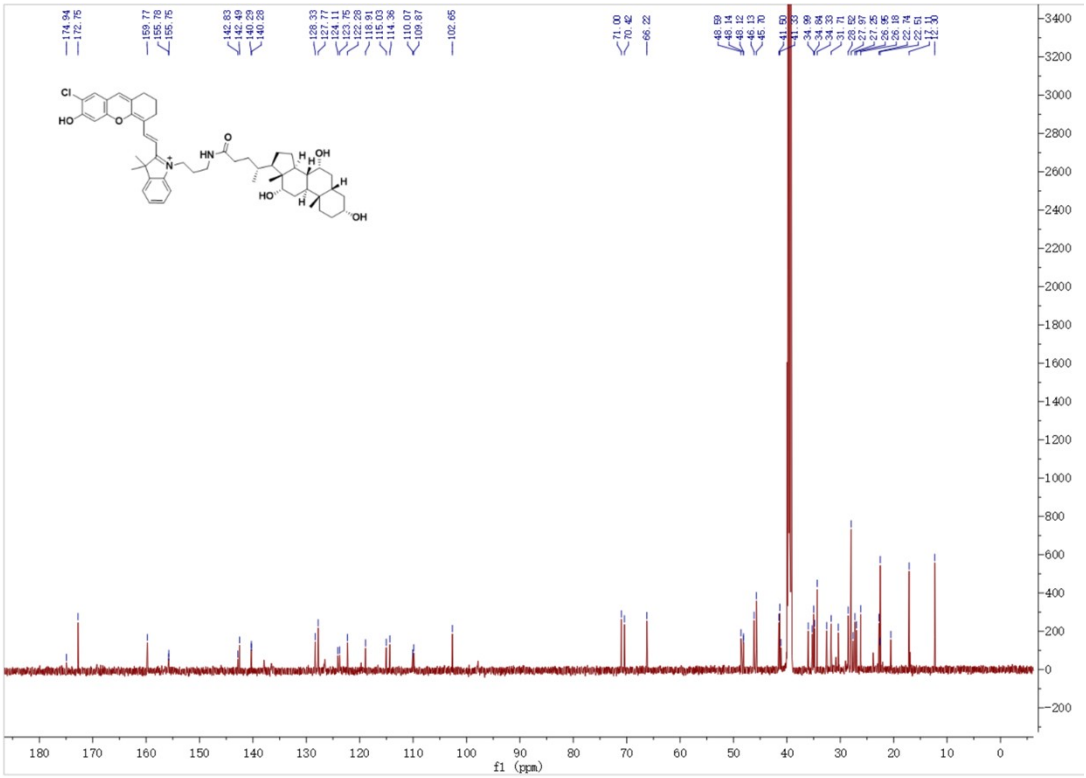


Figure S20 ^{13}C NMR spectrum of **hCy-CA**.

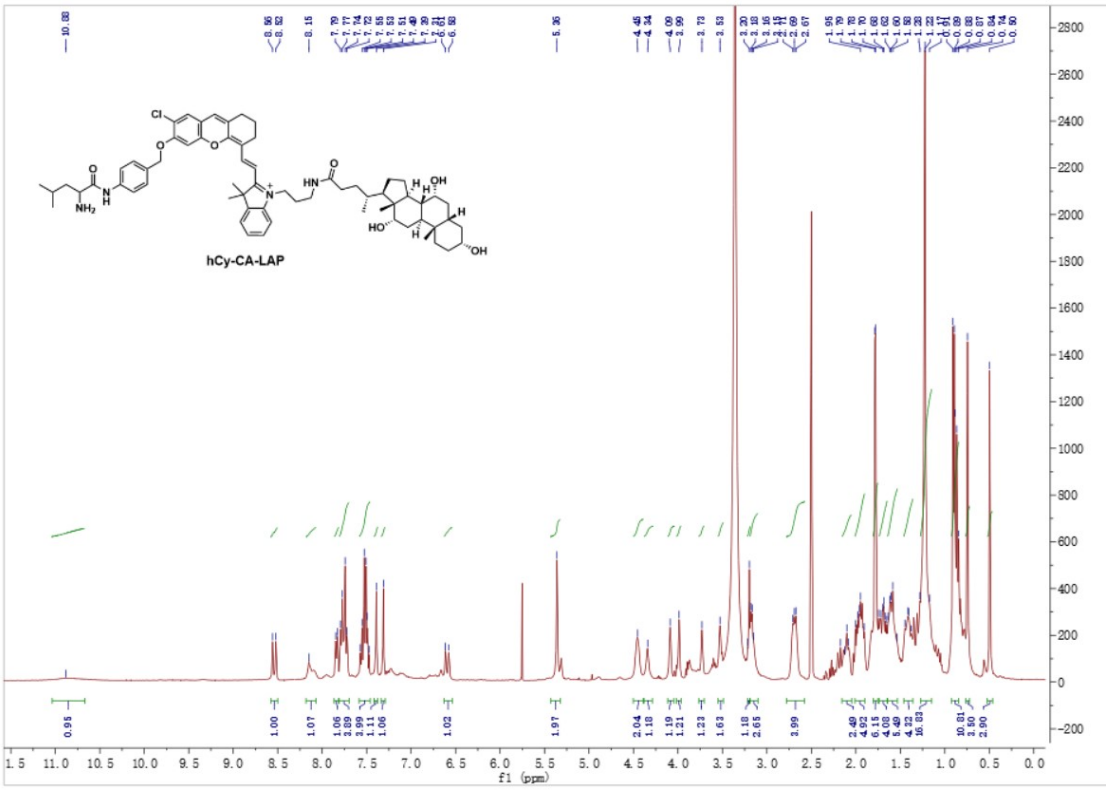


Figure S21 ^{1}H NMR spectrum of **hCy-CA-LAP**.

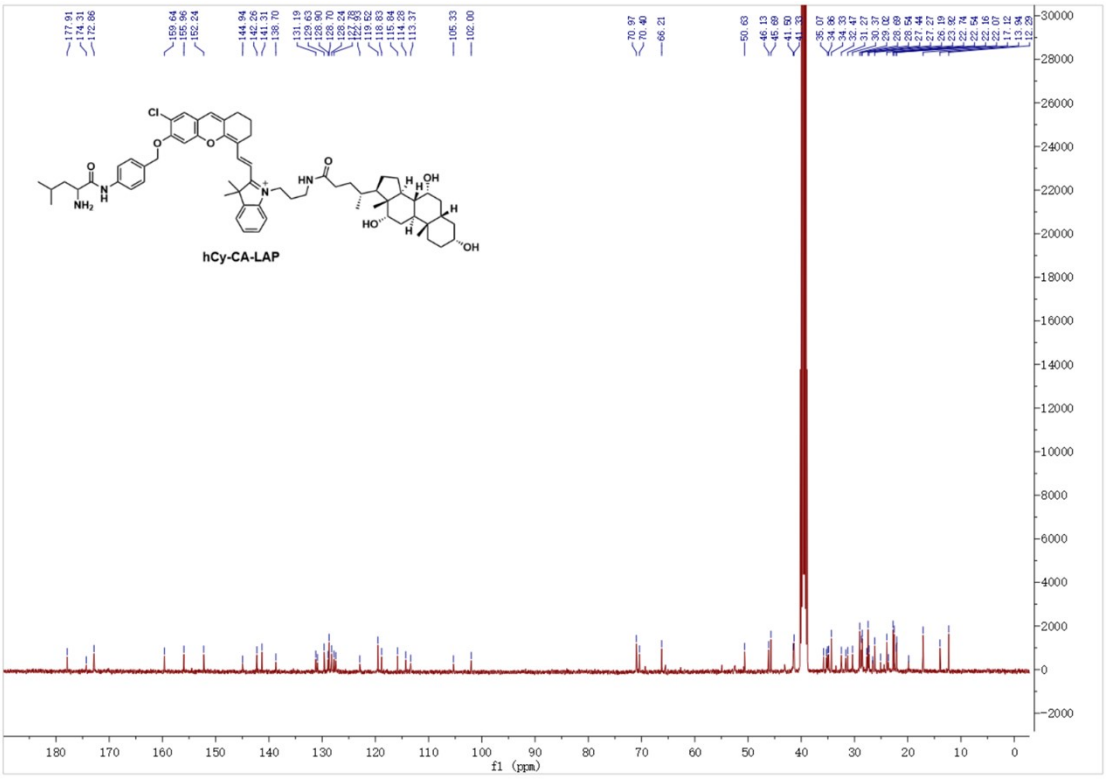


Figure S22 ^{13}C NMR spectrum of **hCy-CA-LAP**.

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