Supplementary Information

A glycol nano-medicine via metal-coordination supramolecular self-assembly strategy for drug release monitoring and chemo-chemodynamic therapy

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Experimental section

Materials and instruments

Materials. All reagents were purchased from commercial suppliers and used without further purification. Water used in this work was ultrapure water. 2,3,4,6-Tetra-O-acetyl-alpha-D-glucopyranosyl bromide, 4-(Diethylamino)salicylaldehyde, diethyl malonate, sodium hydroxide, copper sulfate pentahydrate, copper(II) perchlorate hexahydrate and Imidazole-2-carboxaldehyde were purchased from Adamas Chemical Reagent Co. Potassium carbonate, copper sulfate pentahydrate and acetic acid were purchased from Chron Chemical Reagent Co. Tosyl chloride, sodium methylate and sodium ascorbate were purchased from Aladdin Chemical Reagent Co. Sodium hydride and bis[2-(2-hydroxyethoxy)ethyl] ether were purchased from Xiya Chemical Reagent Co. Hydrazine monohydrate was purchased from J&K Chemical Reagent Co. 3-Bromopropyne was purchased from Jiuding Chemical Reagent Co. Methylene Blue trihydrate, chlorpromazine and genistein were purchased from Adamas Chemical Reagent Co. Phloretin was purchased from Aladdin Chemical Reagent Co. 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine,4-Chlorobenzenesulfonate Salt (DiD) purchased from Shanghai Bioscience Technology Co.

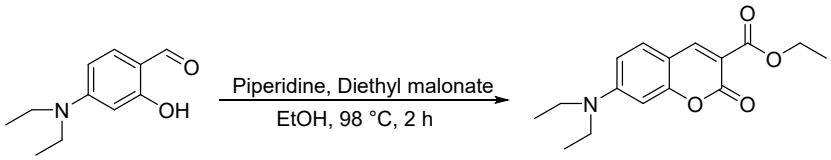
Instruments. The synthetic compounds were characterized with SHIMADZU AXIMA Confidence matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and nuclear magnetic resonance (NMR) spectrometer (Bruker, German). The residual signals from DMSO-d_6 (^{1}H: δ 2.50 ppm; ^{13}C: δ 39.52 ppm) or $CDCl_3$ (^{1}H: δ 7.26 ppm; ^{13}C: δ 77.00 ppm) were used as internal standards. All fluorescence data measurements were obtained using a Shimadzu spectrometer (Japan) equipped with quartz cuvettes of 1 cm path length, and the UV-vis spectra were obtained on a Shimadzu 1750 UV-visible spectrophotometer (Japan). SEM images were obtained using S-4800 instrument (Hitachi Ltd.) with an accelerating voltage of 10.0 kV. DLS measurements were performed on a DelsaTM Nano system (Beckman Coulter, U.S.A.). All pH buffer solutions were prepared on pB-10 digital pH meter

(Sartorius, German). Cell fluorescent images were captured with a confocal laser scanning microscopy (Revolution WD, Andor).

Synthesis and characterization of GluCC

The detailed synthetic route and characterization about GluCCD are described in Fig. S1-S19.

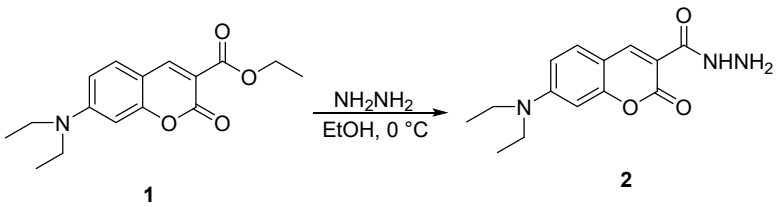
Synthesis of Compound 1



Dissolve 4-(diethyl amino) salicylic aldehyde in ethanol (3.3 mL), add diethyl malonate (1 mL) and piperidine (0.4 mL), the reaction mixture was stirred at room temperature for 2 hours, after reaction, dilute with water (50 mL), extract with ethyl acetate (50 mL x 3), dried with anhydrous Na_2SO_4, concentrate the organic phase, and obtain 1.33 g pure product after column purification, yield 93 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41(s, 1H), 7.33 (d, J = 8.9 Hz, 1H), 6.58 (dd, J = 9.0, 2.5 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.43 (q, J = 7.1 Hz, 4H), 1.37 (t, J = 7.1 Hz, 3H), 1.21 (t, J = 7.1 Hz, 6H) ppm.

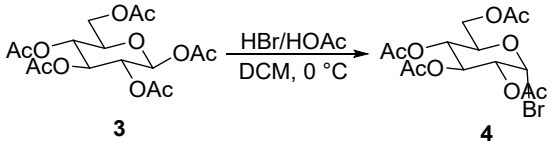
Synthesis of Compound 2



Hydrazine monohydrate (0.311 mL, 6.42 mmol) was added to Compound 1 (465 mg, 1.605mmol) was dissolved in anhydrous EtOH (4.5 mL). The reaction mixture was stirred at room temperature until no further precipitate was observed, at which point it was cooled to 0 ºC and stirred for another 15 min. The precipitate was collected via vacuum filtration to obtain 357 mg pure product, 80.75% yield.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.72 (s, 1H), 8.67 (s, 1H), 7.43 (d, J = 9.0 Hz, 1H), 6.65 (dd, J = 9.0, 2.5 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H), 3.46 (q, J = 7.1 Hz, 4H), 1.24 (t, J = 7.1 Hz, 7H) ppm.

Synthesis of Compound 4



3 **4**

Hydrobromic acid, acetic acid solution (13 mL, 33 % w/w) was added dropwise at 0 ℃ into dichloromethane solution (35 mL) containing compound **1** (7.8068 g, 20 mmol). The mixture was stirred for about 30 min under 0 ℃, then stirred for 3 h at room temperature. After reaction, it was diluted with dichloromethane (40 mL), then the mixture was washed with $NaHCO_3$ (40 mL) twice, H_2O (40 mL) twice, and then dried with anhydrous Na_2SO_4. The solvent was removed under vacuum.

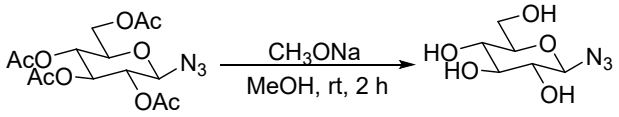
Synthesis of Compound 5

4 **5**

Sodium azide (468 mg, 7.20 mmol) was added to dimethyl sulfone solution (10 mL) containing compound 2, stirred for 10 minutes at room temperature, then diluted with water (30 mL), and then extracted with dichloromethane. The organic phase was washed with brine twice, and the organic phase was concentrated and dried with anhydrous Na_2SO_4. A total of 5 g of pure product was obtained.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.22 (t, J = 9.5 Hz, 1H), 5.11 (t, J = 9.7 Hz, 1H), 4.96 (t, J = 9.2 Hz, 1H), 4.65 (d, J = 8.9 Hz, 1H), 4.28 (dd, J = 12.5, 4.8 Hz, 1H), 4.17 (dd, J = 12.4, 2.3 Hz, 1H), 3.80 (dq, J = 7.7, 2.4 Hz, 1H), 2.06 (dd, J = 28.0, 9.4 Hz, 12H) ppm.

Synthesis of Compound 6

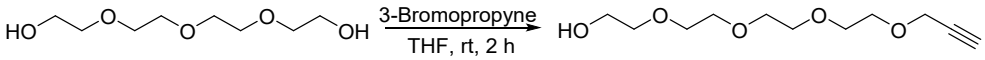


5 **6**

To a solution of Compound **3** (402.8 mg, 0.98 mmol) in dry MeOH (10 mL), CH_3ONa (80 mg, 1.47 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. The solution was then neutralized by addition of ion-exchange resin until pH = 7, filtered, and the solvent was evaporated in vacuum to get the final product as a yellow solid 189.4 mg, yield 85.56 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.45 (d, J = 83.2 Hz, 2H), 4.73 (s, 1H), 4.44 (d, J = 8.6 Hz, 1H), 3.66 (dd, J = 11.9, 2.1 Hz, 1H), 3.45 (dd, J = 11.9, 5.8 Hz, 1H), 3.25 – 3.15 (m, 3H), 3.07 (t, J = 9.2 Hz, 1H), 2.98 (t, J = 8.8 Hz, 1H) ppm.

Synthesis of Compound 7

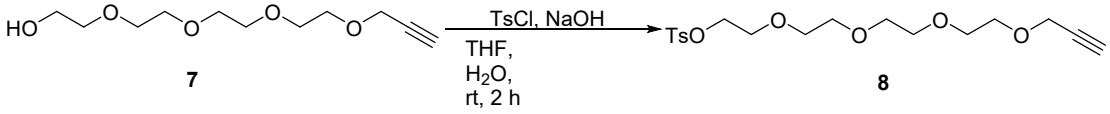


7

NaH (2.41 g，50.4 mmol) was dissolved in THF (40 mL), then it was added into Bis[2-(2-hydroxyethoxy)ethyl] ether (19.56 g, 100.8 mmol). 3-Bromopropyne (1.77 g, 14.85 mmol) was dissolved in THF (7.5 mL), then it was added dropwise into the mixture. The reaction mixture was stirred at room temperature for 2 h, then diluted with water (50 mL) and stirred for 10 min. The THF was evaporated in vacuum. The aqueous solution was extracted with dichloromethane (100 mL) three times, and the organic phase was concentrated and dried with anhydrous Na_2SO_4. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography to yield pure product 2.933 g, 86.50 % yield.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.19 (d, J = 2.4 Hz, 2H), 3.73 – 3.64 (m, 14H), 3.59 (dd, J = 5.4, 3.5 Hz, 2H), 2.42 (t, J = 2.4 Hz, 1H), 2.30 – 2.01 (m, 1H) ppm.

Synthesis of Compound 8

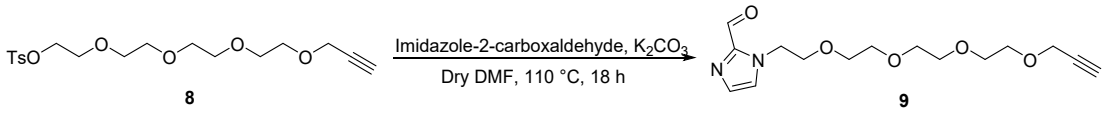


7 8

Compound **7** was dissolved in THF (14 mL), NaOH (1.5 g) was dissolved in water (15 mL), Tosyl chloride (4.15 g, 21.8 mmol) was dissolved in THF (21mL). The mixture of compound **7** and NaOH were added into Tosyl chloride in THF under 0℃. The reaction 3mixture was stirred at room temperature for 2 h. The solvent was evaporated in vacuum, the residue was purified by silica column chromatography to yield pure product 4.6 g, 92.38 % yield.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.19 (d, J = 2.4 Hz, 2H), 4.15 (dd, J = 5.4, 4.3 Hz, 2H), 3.70 – 3.57 (m, 15H), 2.44 (s, 3H), 2.42 (t, J = 2.4 Hz, 1H) ppm.

Synthesis of Compound 9



8 9

Compound **8** (1.55 g, 4.00 mmol), Imidazole-2-carboxaldehyde (422.84 mg, 4.40 mmol), K_2CO_3 (829.26 mg, 6.00 mmol) were dissolved in dry DMF (20 mL). The solution was refluxed for 18 h under 110 ℃, after reaction, dilute with water (20 mL), and then extracted with ethyl acetate. The organic phase was concentrated and purified by silica column chromatography to yield pure product 716.7 mg, 57.82 % yield.

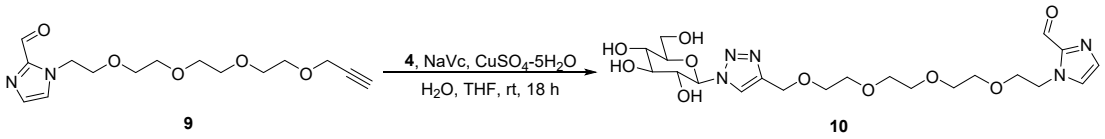
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.79 (d, J = 1.5 Hz, 1H), 7.35 (s, 1H), 7.30 – 7.20 (m, 1H), 4.65 – 4.56 (m, 2H), 4.19 (d, J = 2.4 Hz, 2H), 3.80 – 3.74 (m, 2H), 3.66 – 3.62 (m, 4H), 3.61 (dd, J = 6.1, 3.1 Hz, 4H), 3.57 (s, 4H), 2.44 (d, J = 2.3 Hz, 1H) ppm.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.17, 143.59, 132.32, 127.17, 79.60, 74.59, 70.59, 70.55, 70.52, 70.45, 70.40, 70.34, 70.06, 58.38, 47.48 ppm.

MS (ESI) $C_{15}H_{22}N_2O_5$ calc. for $[M+H]^+$: 311.1602 ; found: 311.1591.

MS (ESI) $C_{15}H_{22}N_2O_5$ calc. for $[M+H+CH_3OH]^+$: 343.1864 ; found: 343.1855.

Synthesis of Compound 10



9 10

$CuSO_4 \cdot 5H_2O$ (52.13 mg, 0.2088 mmol) and sodium ascorbate (207.88 mg, 0.696 mmol) was dissolved in water (4 mL), Compound **4** (238.9 mg, 1.16 mmol) and Compound **9** (592.66 mg, 1.91 mmol) were dissolved in water (13 mL) and THF (11 mL). The reaction mixture was refluxed for 24 h under Ar. After reaction, the solvent was evaporated in vacuum, the residue purified by silica column chromatography to yield pure product 243 mg, 46.00 % yield.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.28 (s, 1H), 7.63 (s, 1H), 7.27 (s, 1H), 5.52 (d, J = 9.2 Hz, 1H), 5.38 (d, J = 6.0 Hz, 1H), 5.28 (d, J = 4.9 Hz, 1H), 5.15 (d, J = 5.5 Hz, 1H), 4.64 (t, J = 5.7 Hz, 1H), 4.59 – 4.45 (m, 4H), 3.79 – 3.65 (m, 4H), 3.60 – 3.51 (m, 4H), 3.51 – 3.40 (m, 10H), 3.22 (td, J = 8.9, 5.6 Hz, 2H) ppm.

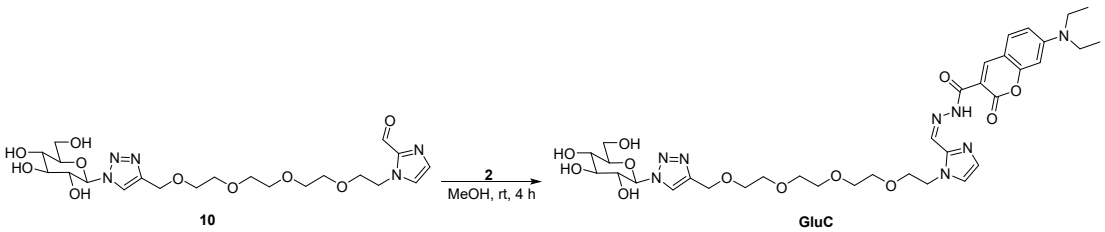
^{13}C NMR (101 MHz, DMSO-d_6) δ 182.38, 144.30, 131.45, 129.04, 123.64, 87.92, 80.39, 77.39, 72.52, 70.23, 70.15, 70.09, 70.05, 69.99, 69.70, 69.55, 63.87, 61.19, 55.40, 49.07, 47.05 ppm.

MS (ESI) $C_{21}H_{33}N_5O_{10}$ calc. for $[M+H]^+$: 516.2300 ; found: 516.2291.

MS (ESI) $C_{21}H_{33}N_5O_{10}$ calc. for $[M+Na]^+$: 538.2119 ; found: 538.2098.

MS (ESI) $C_{21}H_{33}N_5O_{10}$ calc. for $[M+H+CH_3OH]^+$: 548.2562 ; found: 548.2539.

Synthesis of GluC



10 GluC

Compound **10** (243 mg, 0.47 mmol) was dissolved in methanol (4 mL), compound **2** was dissolved in methanol (4 mL), the solution of compound 10 was added into the

solution of compound **2**, and a drop of acetic acid was added. The reaction mixture was stirred at room temperature for 5 h. After reaction, the solvent was evaporated in vacuum, the residue purified by silica column chromatography to yield pure product 327 mg, yield 89.77 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.89 (s, 1H), 8.68 (d, J = 21.3 Hz, 1H), 8.03 (s, 1H), 7.63 (s, 1H), 7.42 (s, 1H), 7.30 (s, 1H), 7.14 (d, J = 36.8 Hz, 1H), 6.62 (d, J = 10.2 Hz, 1H), 6.42 (d, J = 9.3 Hz, 1H), 5.70 (s, 1H), 4.71 – 3.25 (m, 32H), 1.30 – 1.14 (m, 6H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ 161.22, 159.39, 157.47, 152.85, 148.55, 143.86, 140.02, 131.88, 129.21, 128.58, 123.23, 122.73, 110.40, 108.50, 107.86, 96.00, 87.50, 79.98, 76.98, 72.11, 69.82, 69.75, 69.71, 69.67, 69.56, 69.50, 69.11, 63.44, 60.76, 46.58, 44.49, 44.44, 12.38 ppm.

MS (ESI) $C_{35}H_{48}N_8O_{12}$ calc. for $[M+H]^+$: 773.3470 ; found: 773.3453.

MS (ESI) $C_{35}H_{48}N_8O_{12}$ calc. for $[M+Na]^+$: 795.3284 ; found: 795.3280.

Preparation of GluCCD Nanoparticles.

First, DOX (2 mg • mL^{-1}) were mixed with triethylamine (TEA) (2 eq) under ultrasound for 1 h. Then, $CuCl_2$ (10 mg • mL^{-1}) (pH 7.4 – 8) were added to the above mixture. Then, GluC (2 mg • mL^{-1}) was added dropwise and ultrasound for another 3 h at room temperature. After 2 d of dialysis, excess DOX and Cu^{2+} were removed.

Stability of GluCCD

The size of GluCCD was recorded in water and complete medium DMEM by DelsaTM Nano system, respectively.

GSH-sensitive release of DOX from GluCCD

The release profile of DOX in GluCCD was evaluated based on the absorbance of DOX at 495 nm, respectively. Briefly, 1 mL of GluCCD was encapsulated in a dialysis bag (8000 MWCO), and the dialysis bag was immersed into a centrifuge tube with 50

mL PBS (pH 6.0 + GSH or 6.0 or 7.4) and incubated at 37°C with continuous oscillation. 1 mL of dialysate was collected and replaced with 1 mL equal buffers at specified time intervals. The amount of released DOX was determined according to the absorbance standard curves.

Analysis of Hydroxyl Radical (·OH) Generation

·OH was analyzed according to the principle that ·OH can degrade MB and weaken the absorption of MB at 663 nm. Briefly, different mass concentrations of GluCCD solutions (1 mL) were separately added into MB solution (10 μg/L, 100 μL). Then, H_2O_2 solutions (10 mM, 500 μL) were added into the above mixture. After another 10, 20, 40 min of incubation, the mixture was centrifuged (12,000 rpm/min, 20 min) to remove GluCCD, and the absorbance at $\lambda = 663$ nm of supernatant was recorded on a UV−vis spectrophotometer.

Cell Culture.

Human cervical carcinoma cells (HeLa cells) and normal liver cells (HL7702) were cultured in DMEM and 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO_2 in a humidified incubator for 24 h. For all cell-based experiments, cells were harvested from subconfluent (<80%) cultures using a trypsin−EDTA solution and then resuspended in fresh medium. A subculture was performed every two days.

Confocal laser scanning microscopy (CLSM) for the cellular uptake of GluCCD

Confocal laser scanning microscopy was used for investigating the cellular uptake of GluCCD by Hela cells as following, Hela cells (2×10^5) were seeded into 35 mm glassbottom Petri dishes and incubated for 24 h at 37 °C. Next, the cells were treated with GluCCD and further incubated for 0.5, 2, 4 and 6 h. The Hela cells treated with PBS were as a control. Afterwards, the cells were washed with PBS (pH 7.4), and stay in 4 ℃ for imaging by using CLSM (488 nm for DOX, 445 nm for GluC).

Confocal laser scanning microscopy (CLSM) for targeting potential of GluCCD

Hela cells and HL7702 cells were incubated with GluCCD (5 μM DOX

equivalent) for 2 h. Hela cells were preincubated with 55 mM glucose. They were incubated with GluCCD for 2 h in a humidified 5% CO_2 incubator at 37 °C. The cells were washed three times with 1 mL PBS buffer, and then observed with a confocal microscope. The excitation wavelengths of GluC was 445 nm and 488 nm for DOX.

Inhibition of GluCCD by Various Inhibitors

HeLa cells (1×10^6) were seeded in 6-well plates and cultured for 12 h. Then, GluCCD (5 μM DOX equivalent) were added to the wells followed by the treatment with sodium azide (10 mM), phloretin (0.2 mM), genistein (200 μg/ml) or chlorpromazine (50 μg/mL) at 37 °C for 1 h, separately. The cells cultured without inhibitors at 37 °C were set as a positive control. The cells cultured at 4 °C were set as a negative control. The cells were then washed with PBS three times and analyzed with CytExpert software (Beckman, USA).

ROS and GSH evaluation

The fluorescence probe of 2,7-dichlorodi-hydrofluorescein diacetate (DCFH-DA) was used to assess intracellular ROS generation. In brief, cells were incubated with GluCCD (5 μM DOX equivalent), DOX (5 μM) and PBS for 2 h. After that, the old medium was replaced with new medium containing 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, 10 μM), and cultured for another 20 min. Finally, cells were washed and observed by a confocal microscope. Cells images were collected and the fluorescence intensity of ROS was quantified by Image-J. As for intracellular GSH assay, cells were incubated with GluCCD (5 μM DOX equivalent) for 6 h and 12 h. Then cells were washed, digested and centrifuged to obtain the supernatant. Finally, the level of GSH was assessed by the GSH/GSSG kit (Beyotime Biotechnology, Shanghai).

In vitro cytotoxicity

In vitro cytotoxicity was evaluated by MTT assay. In brief, 5×10^3 number of Hela or HL7702 cells were seeded in 96-well plate and cultured overnight. Then, cells were washed and replaced with fresh medum containing various formulations. After 24 h and 48 h, cells were washed again and added by 20 μL of MTT (5 mg/mL), and cultured

for another 4 h. Finally, the absorbance at 490 nm was recorded by a microplate reader. Cells viabilities were determined by the following formula: cells viability = (OD_{sample} - OD_{blank}) / ($OD_{control}$ - OD_{blank}).

ESR

ESR measurements were made with a Bruker Elexsys model E500 spectrometer, with a cylindrical cavity model ER 160FC-Q. All measurements were made in X-band frequency (9.85 GHz). Some operating parameters were kept constant, such as power 5.024 mW, modulation frequency at 100 kHz, modulation amplitude of 0.1 mT, scan time 180s, time constant 1.28 ms. The spectrometer was calibrated using a standard strong pitch sample provided and certified by Bruker (Bruker BioSpin GmbH, Bruker, Germany). The strong pitch has factor g = 2.0028.

The experiments took place at room temperature and 4 mm quartz tubes were used, suitable for insertion into the resonant cavity. For simulation and fitting of the spectra, the EasySpin toolbox (Stoll and Schweiger, 2006) was used. The fitting of the experimental spectrum was made by least squares and the difference between the experimental and the simulated spectrum is root-mean-square-deviation, RMSD. The optimization algorithm used was the Nelder-Mead-downhill simplex method.

Ortho view of Z-stack images and reconstructed 3D images of HeLa cells

HeLa cells were seeded into 35 mm plastic bottomed μ-dishes at the density of 10^5 cells/dish containing 1.5 mL complete DMEM medium and cultured for 24 h at 37 °C, 5% CO_2. Subsequently, the medium in the well was removed. Cells cultured with 5 μM GluCCD in complete DMEM medium for 6 h and then cytomembrane was stained with 5 μM DiD in PBS for 20 min at 37 °C, 5% CO_2. Ortho view of Z-stack images at higher magnification (For GluC, λ_{ex} = 445 nm; for DiD, λ_{ex} = 644 nm)

Supplementary Figures

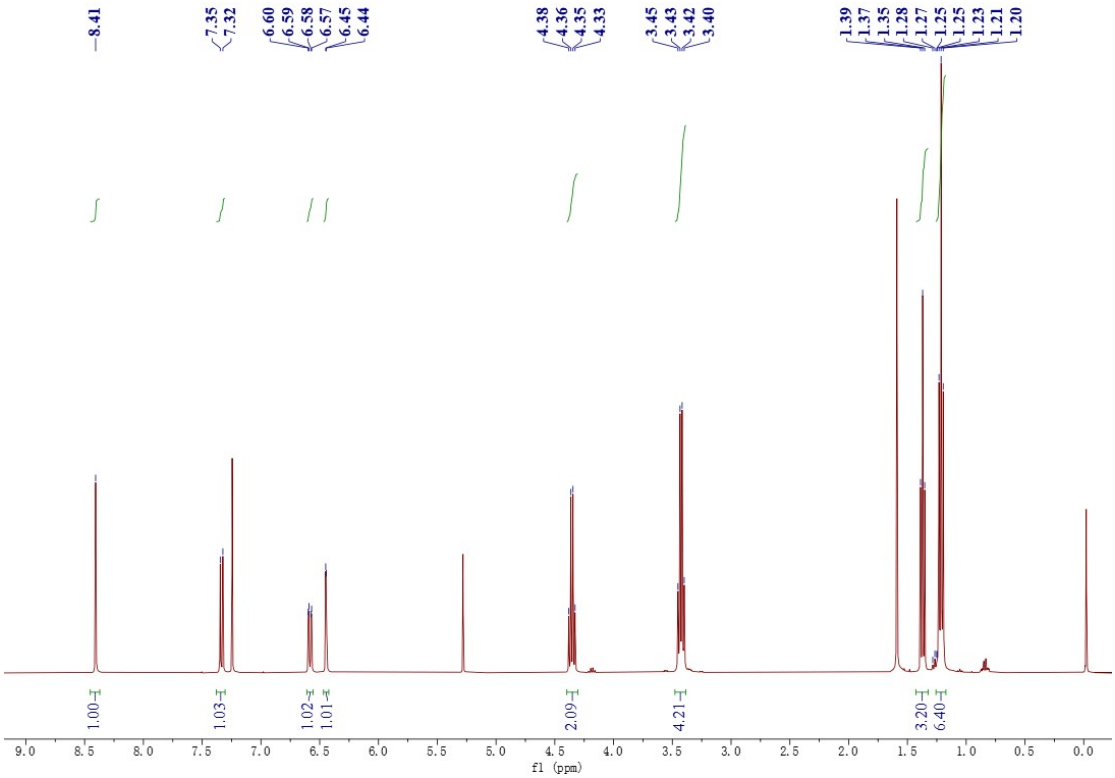


Fig. S1. ^{1}H NMR spectrum of Compound **1**.

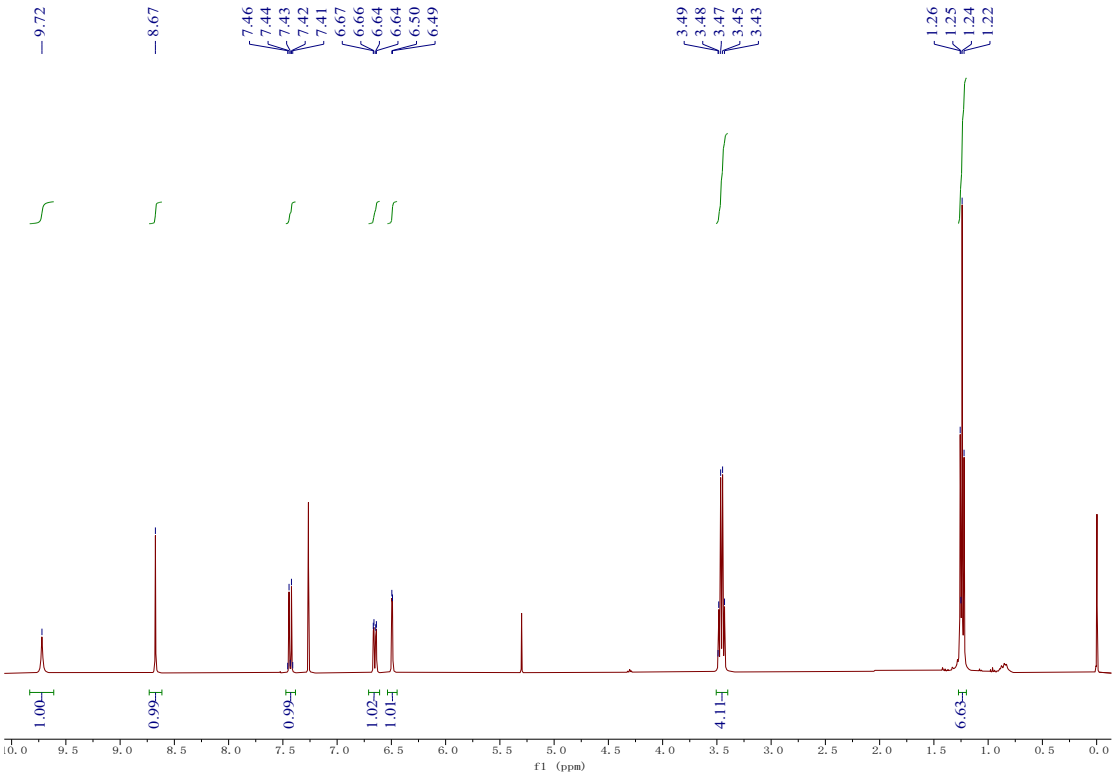


Fig. S2. ^{1}H NMR spectrum of Compound **2**.

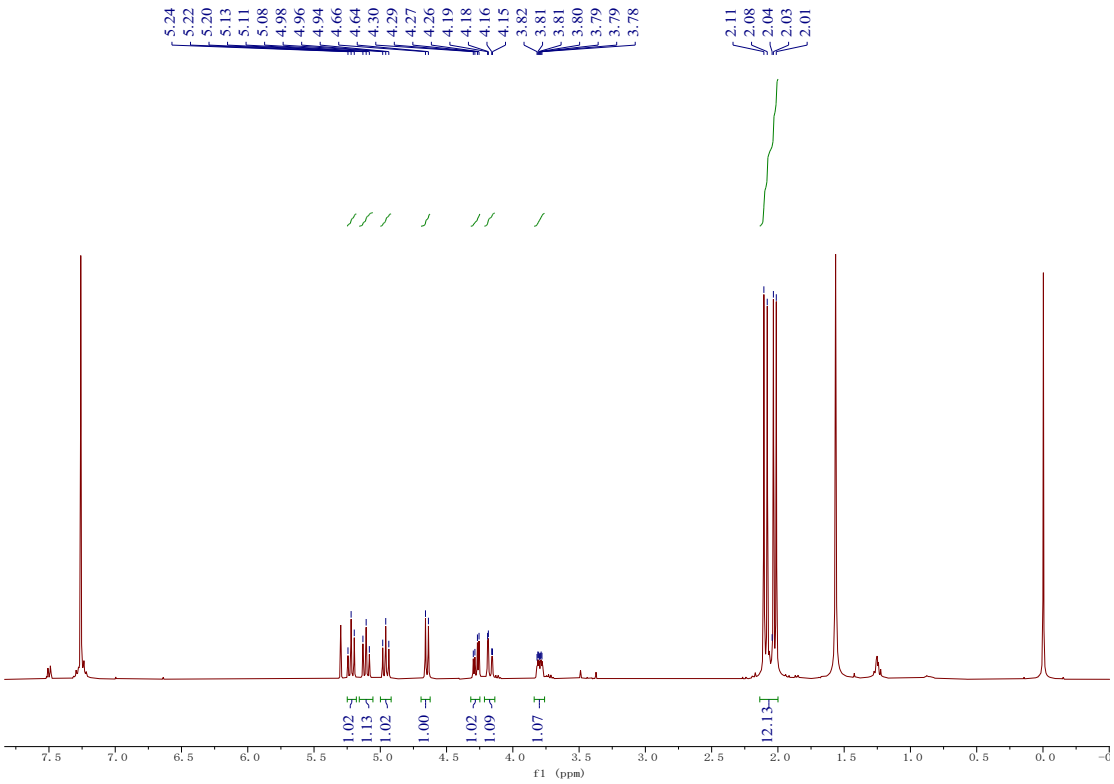


Fig. S3. [1]H NMR spectrum of Compound **3**.

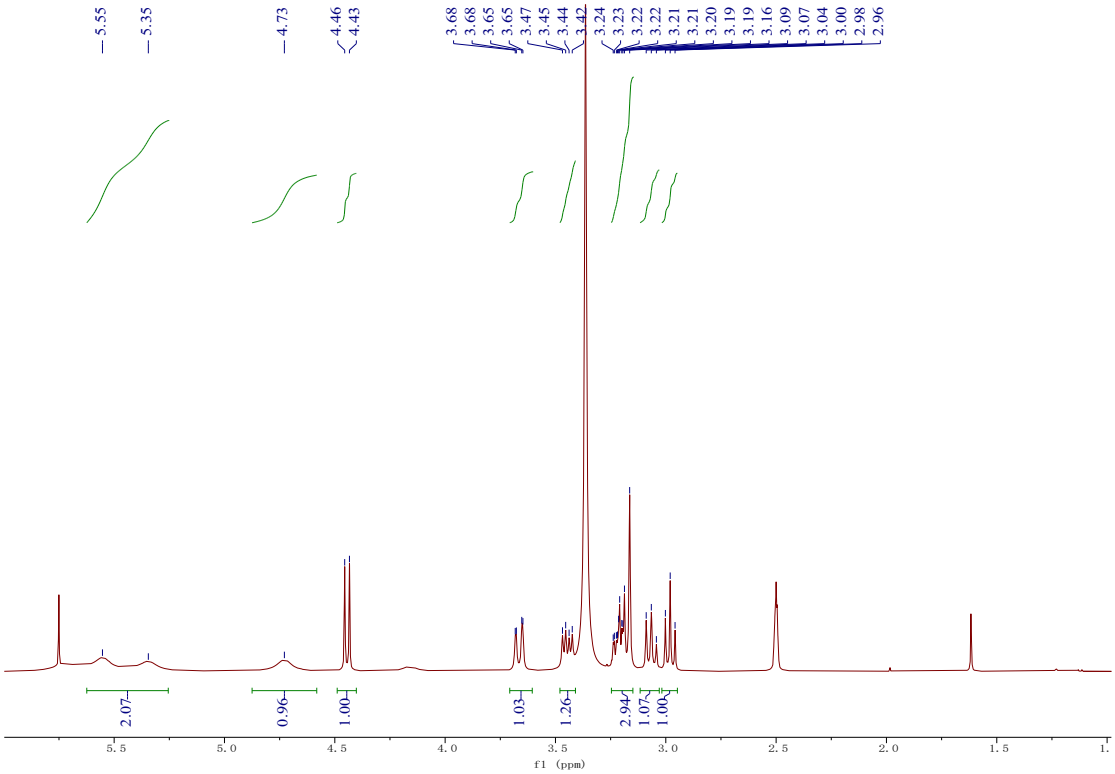


Fig. S4. [1]H NMR spectrum of Compound **6**.

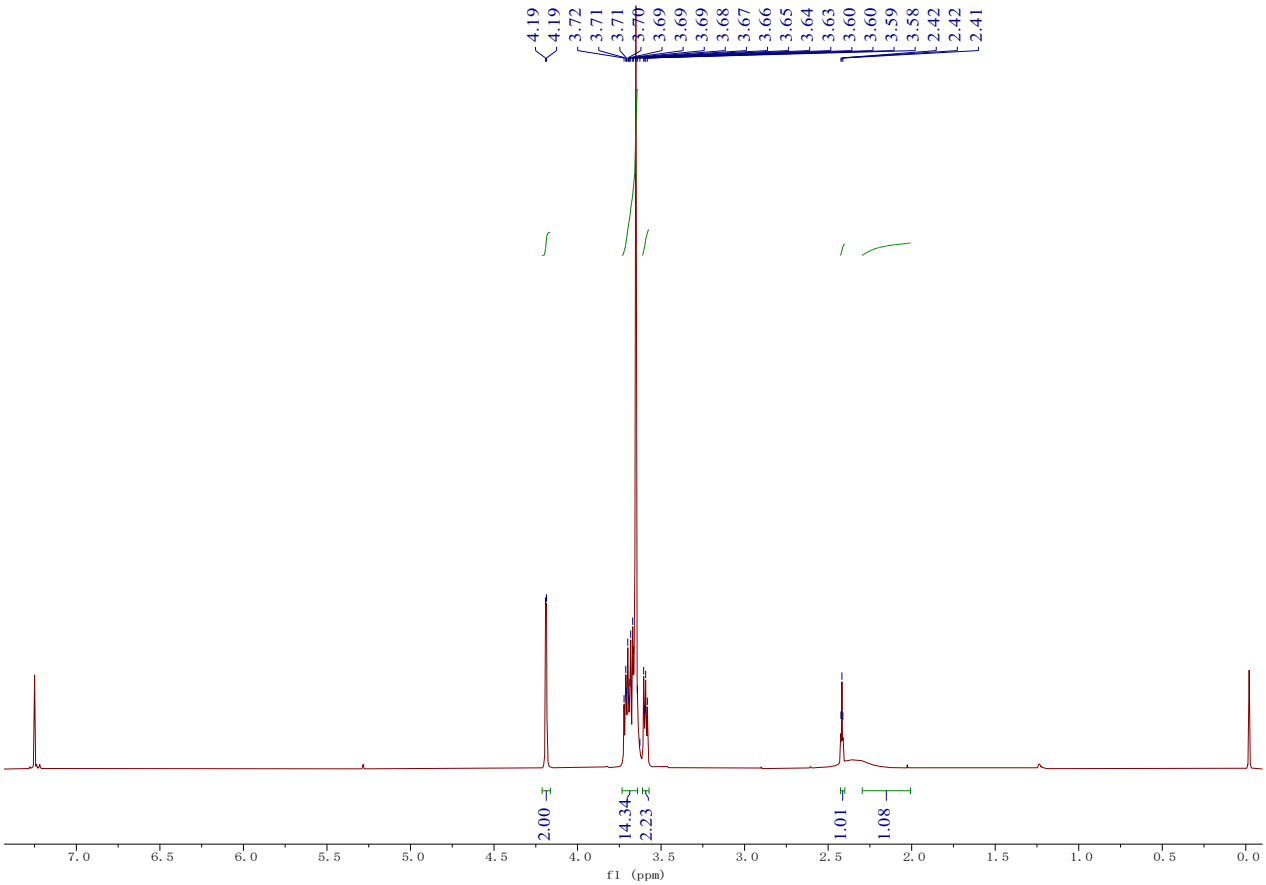


Fig. S5. ^{1}H NMR spectrum of Compound **7**.

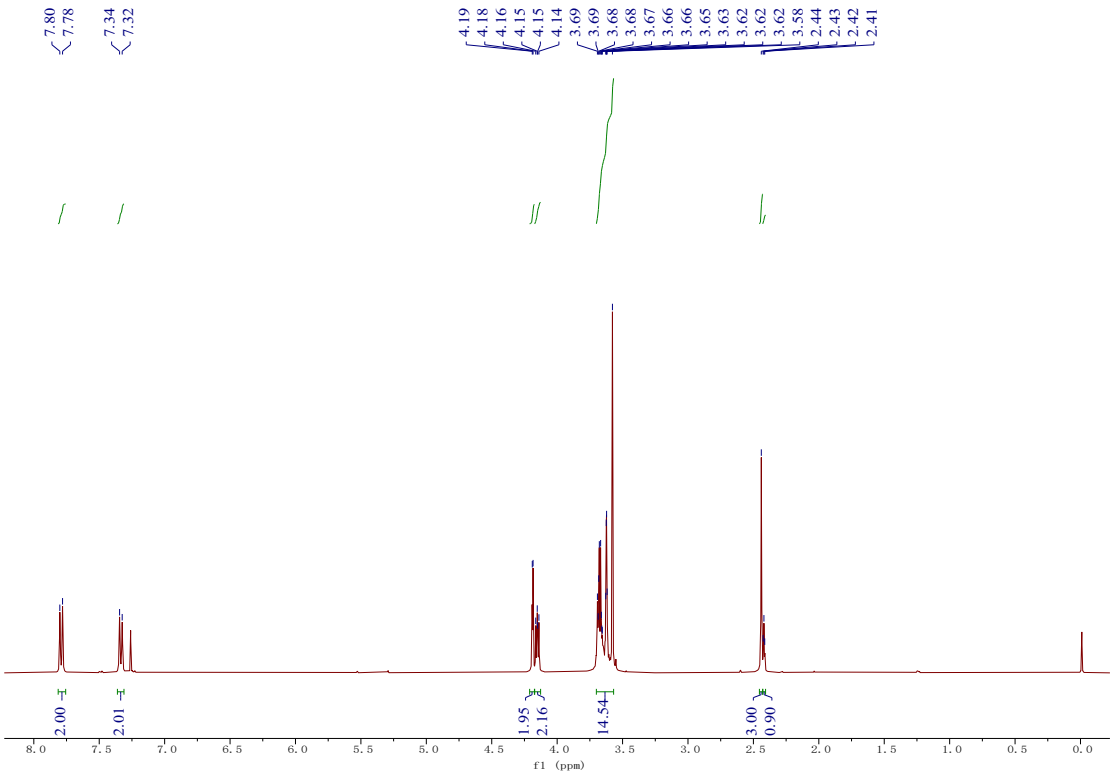


Fig. S6. ^{1}H NMR spectrum of Compound **8**.

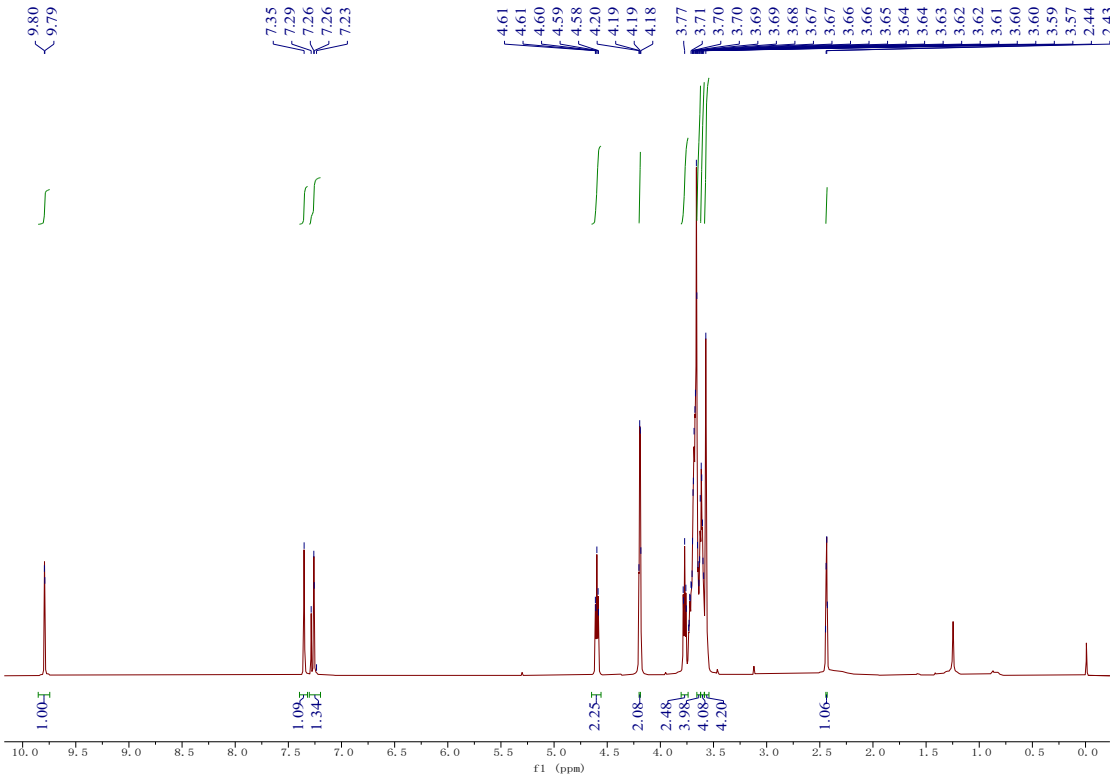


Fig. S7. ^{1}H NMR spectrum of Compound **9**.

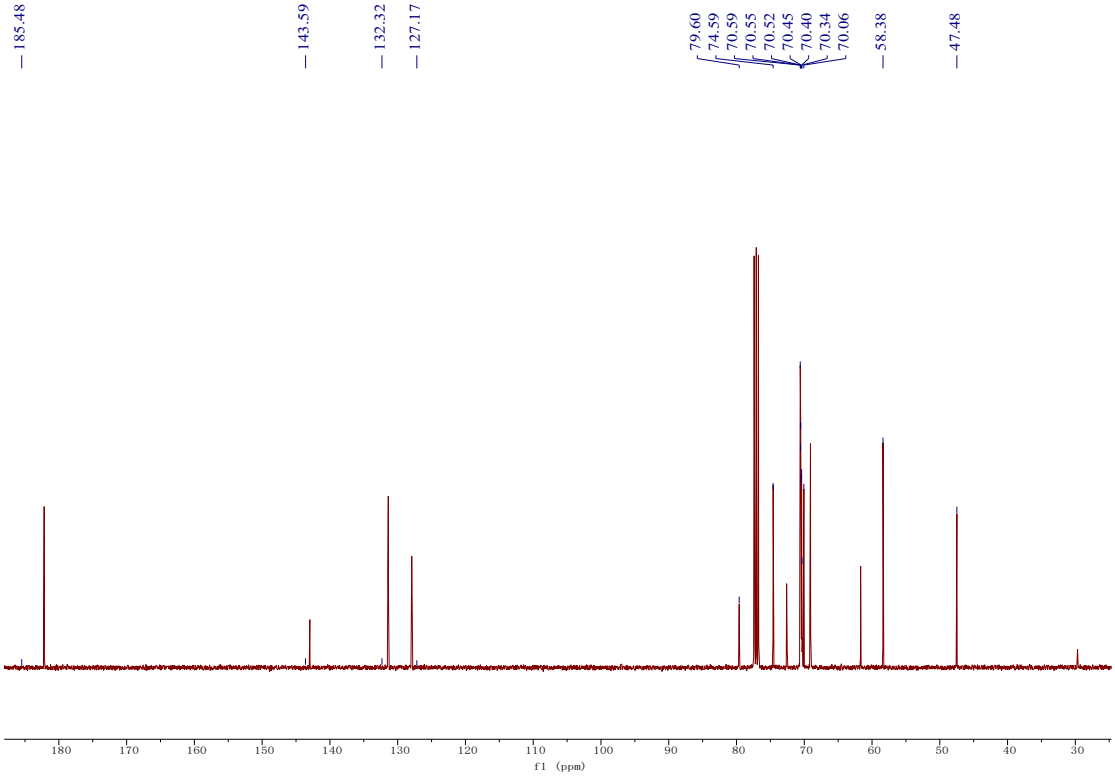


Fig. S8. ^{13}C NMR spectrum of Compound **9**.

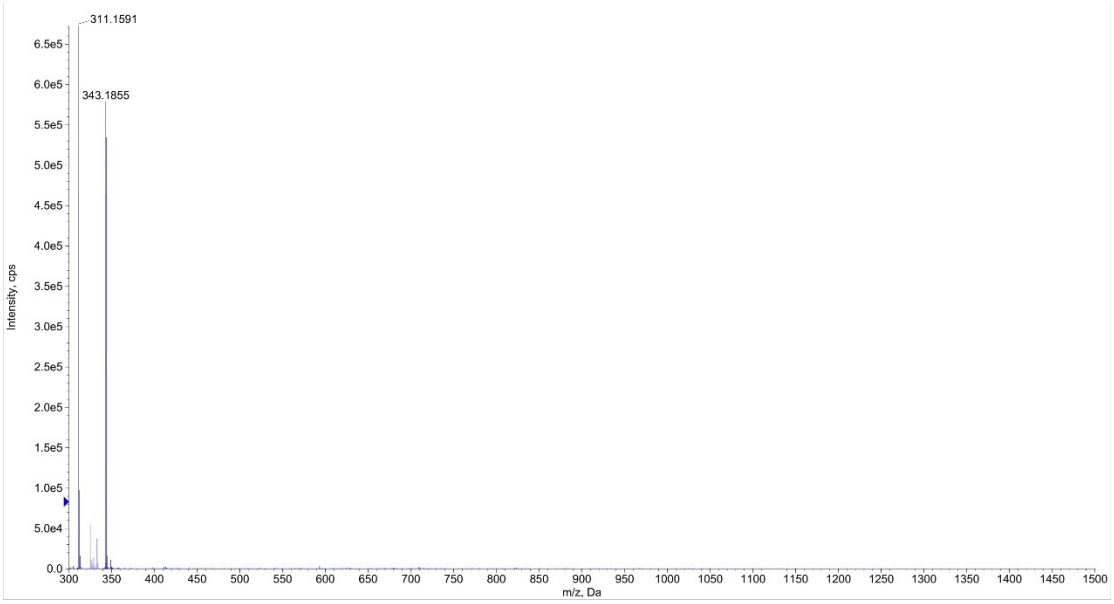


Fig. S9. HRMS spectrum of Compound **9**.

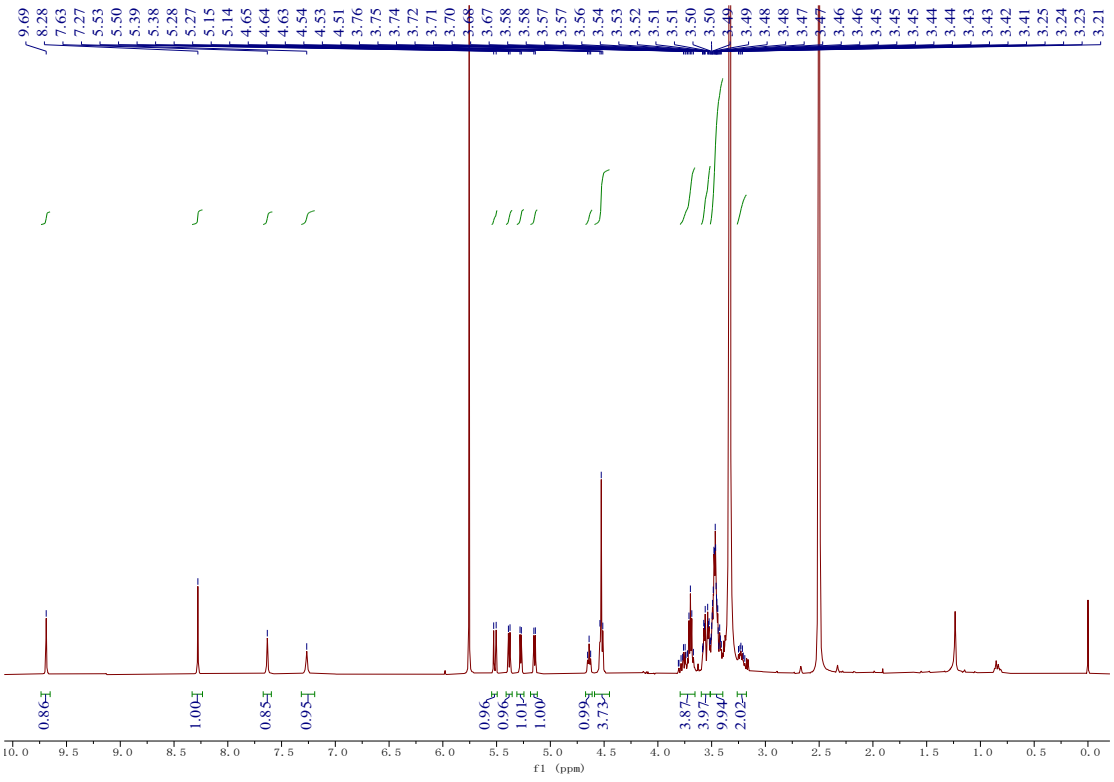


Fig. S10. ^{1}H NMR spectrum of Compound **10**.

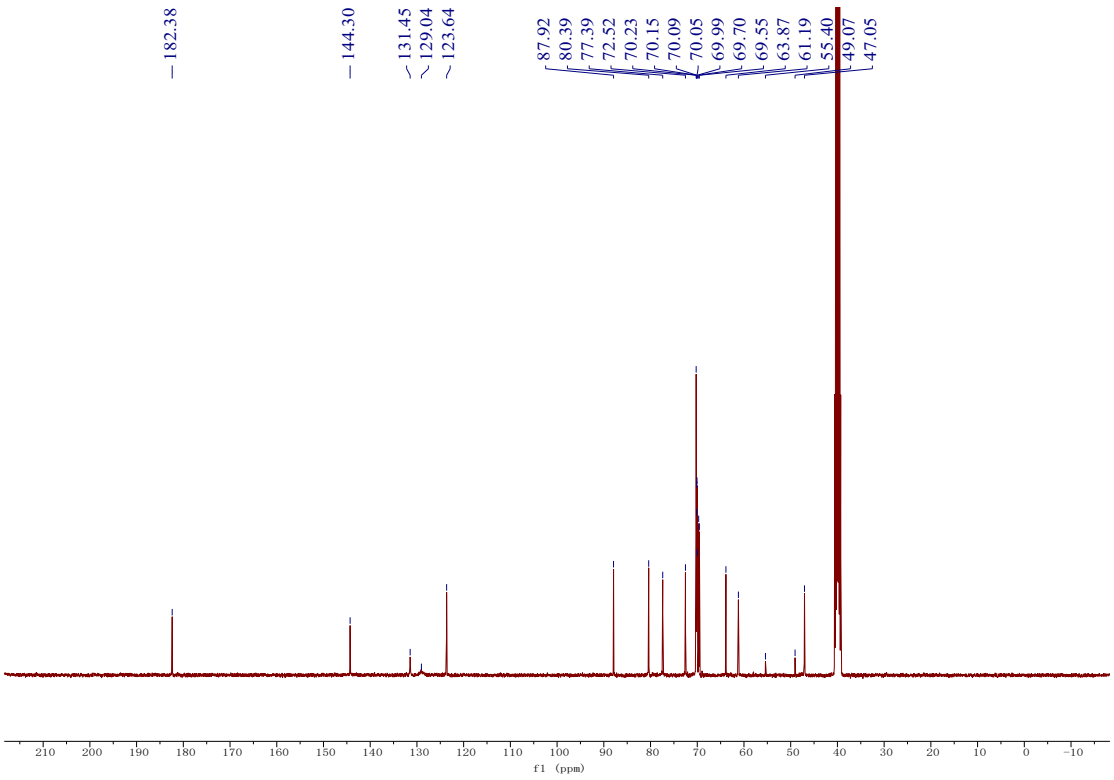


Fig. S11. ^{13}C NMR spectrum of Compound **10**.

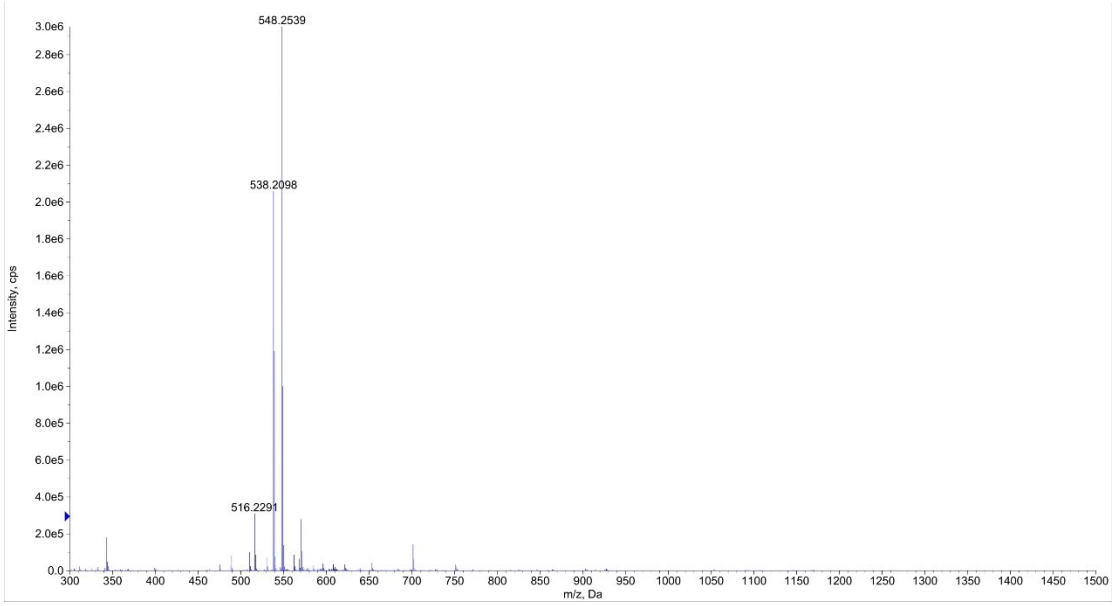


Fig. S12. HRMS spectrum of Compound **10**.

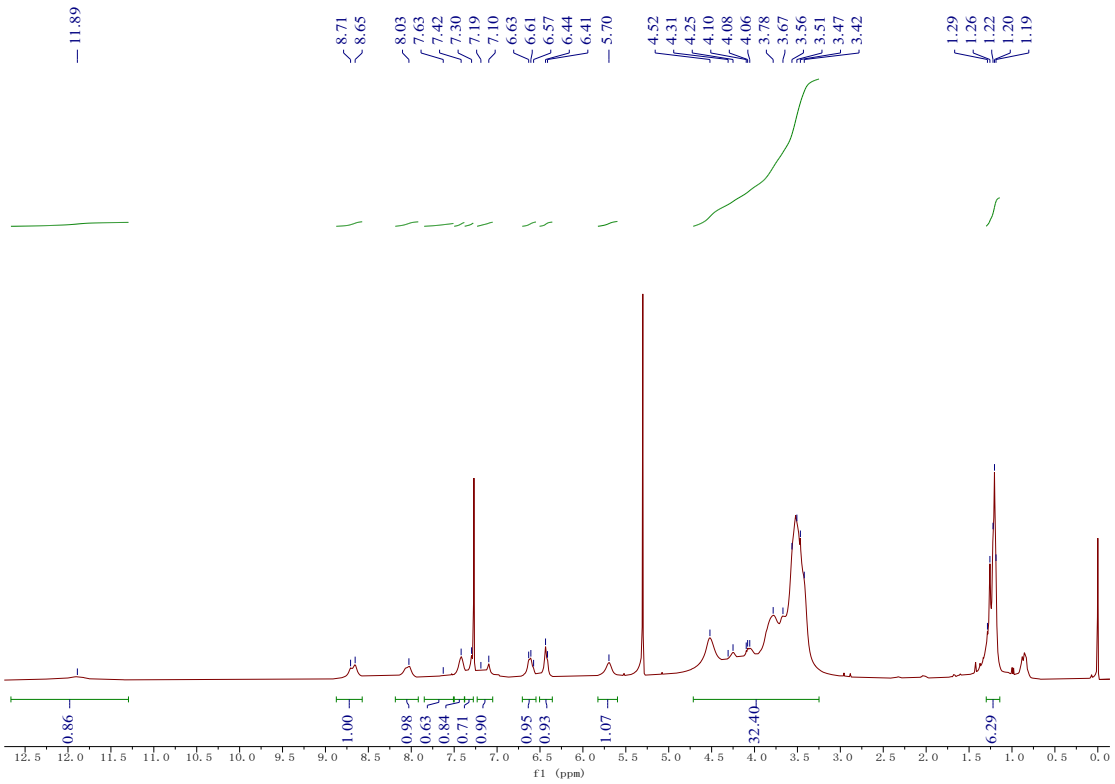


Fig. S13. ^{1}H NMR spectrum of **GluC**.

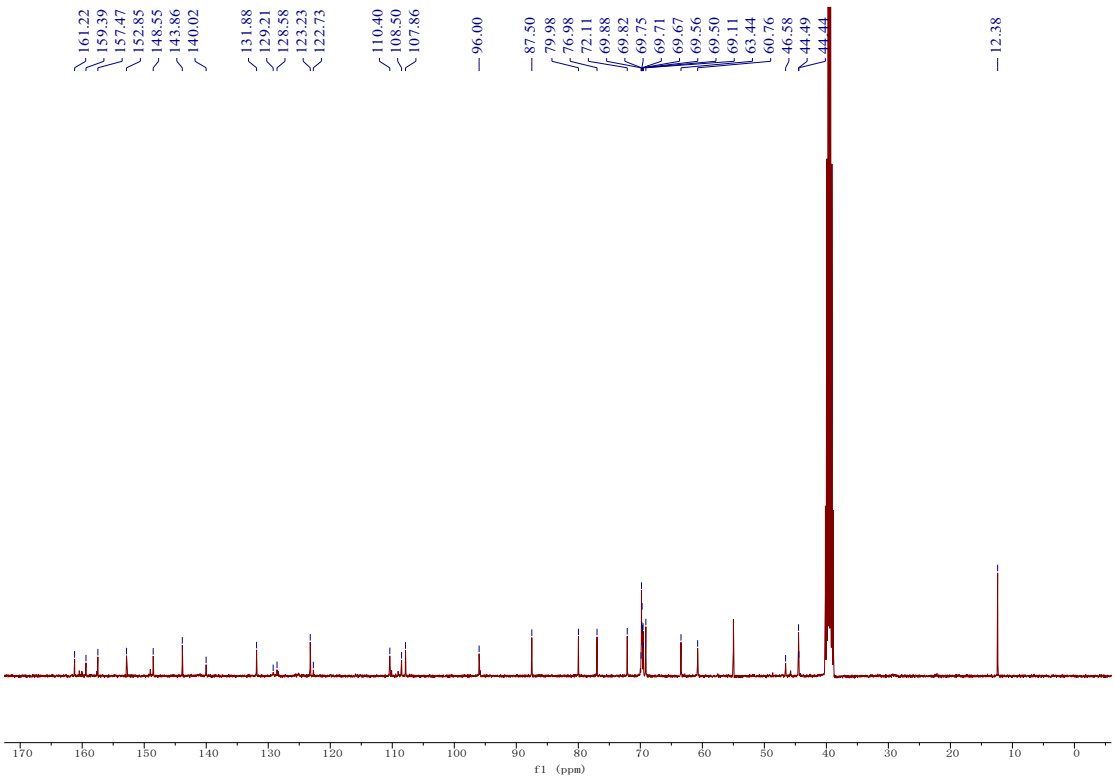


Fig. S14. ^{13}C NMR spectrum of **GluC**.

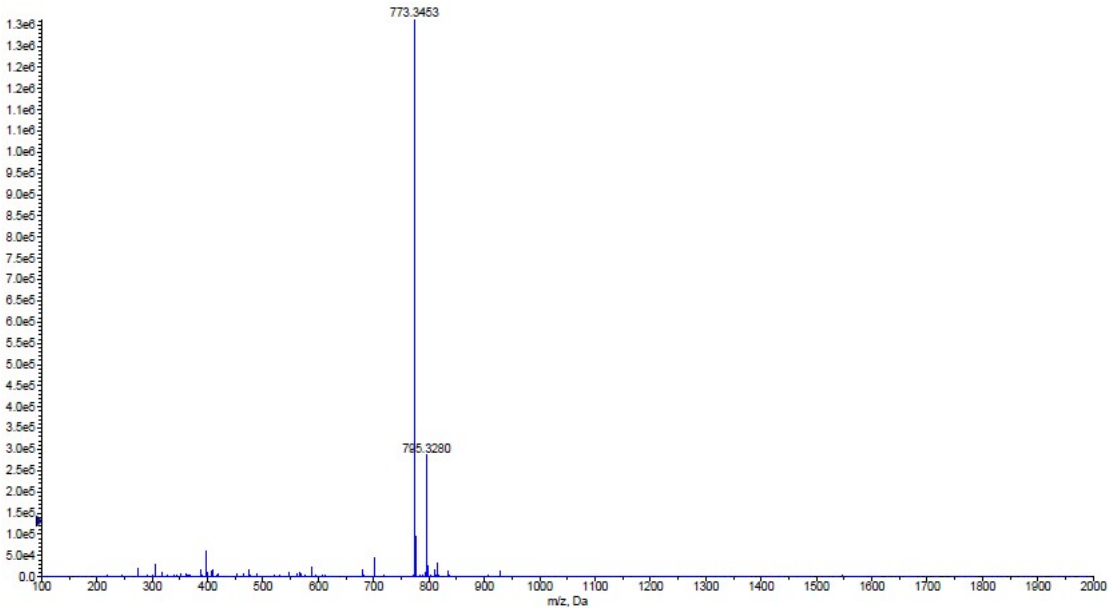


Fig. S15. HRMS spectrum of **GluC**.

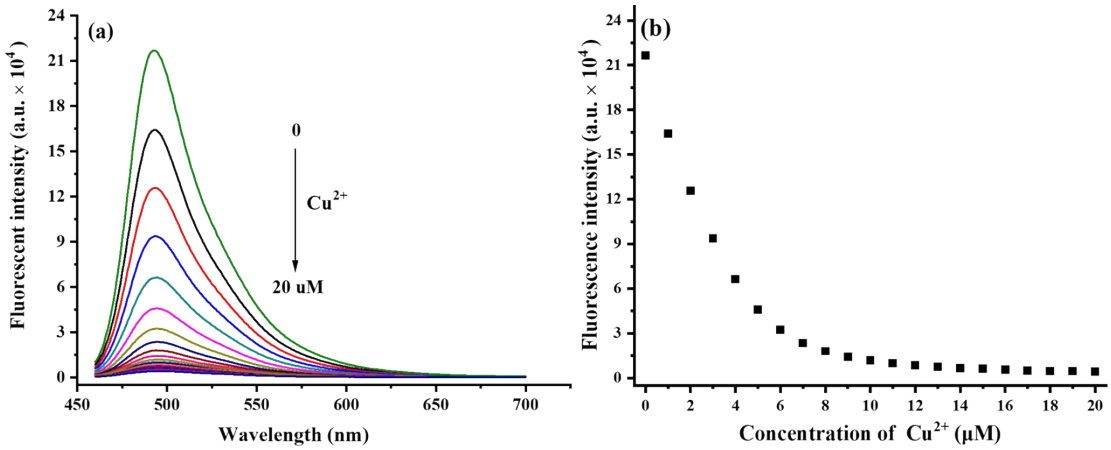


Fig. S16. (a) The UV–vis spectra titration test of GluC (10.0 μM) with Cu^{2+} ions (as ClO_4^- salts) in water. (b) UV–Vis titration profile of GluCC upon addition of Cu^{2+} ions.

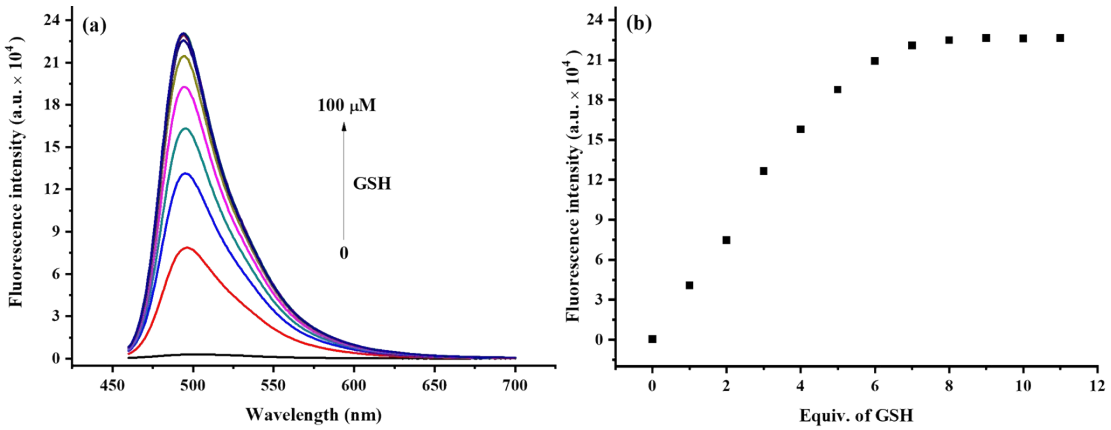


Fig. S17. (a) The fluorescence spectra titration of GluCC (10.0 μM) upon addition of GSH in aqueous solution. (b) Fluorescence titration profile at 494 nm upon the addition of GSH (excited at 450 nm).

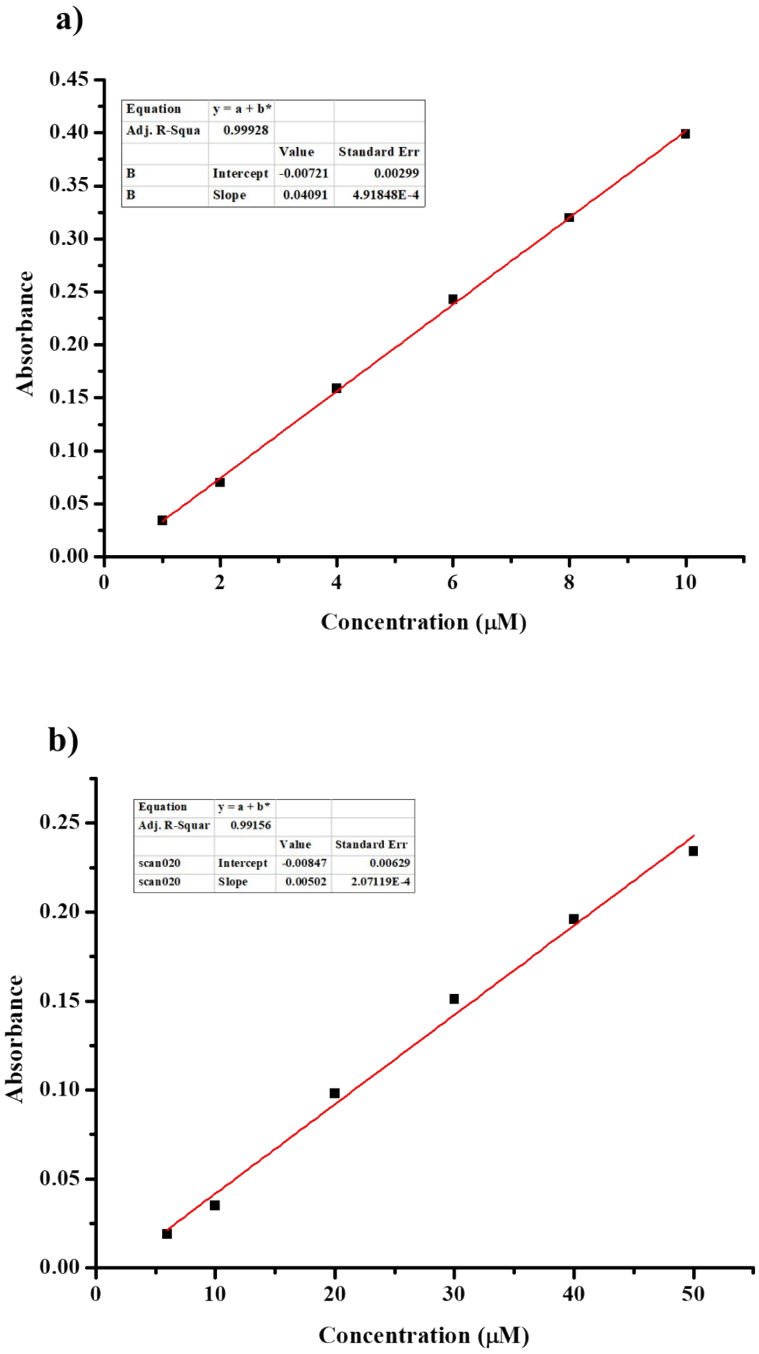


Fig. S18. (a) The absorbance standard curves of GluC. (b) The absorbance standard curves of DOX.

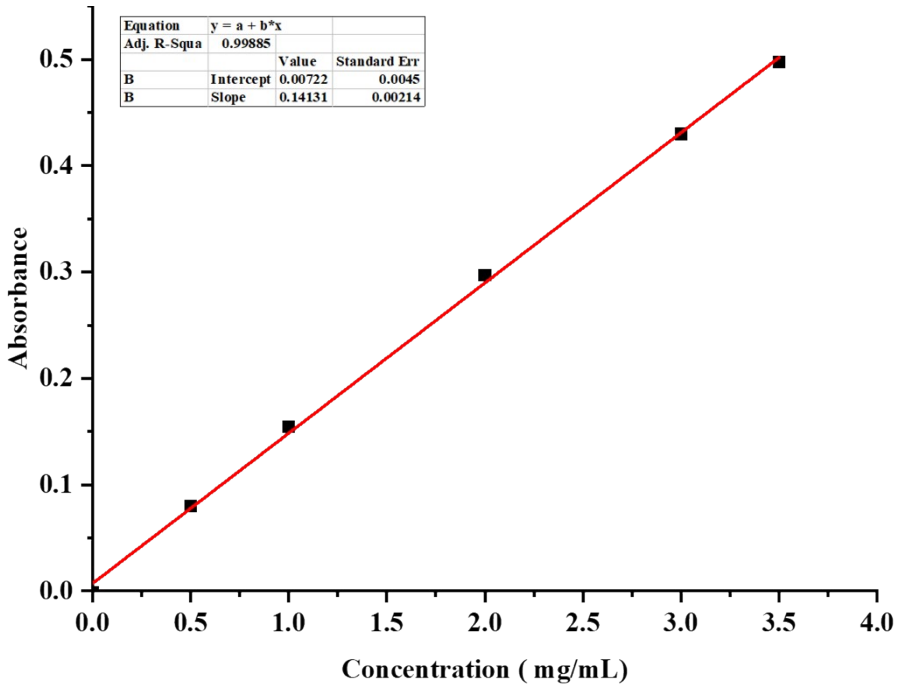


Fig. S19. The AAS standard curves of Cu.

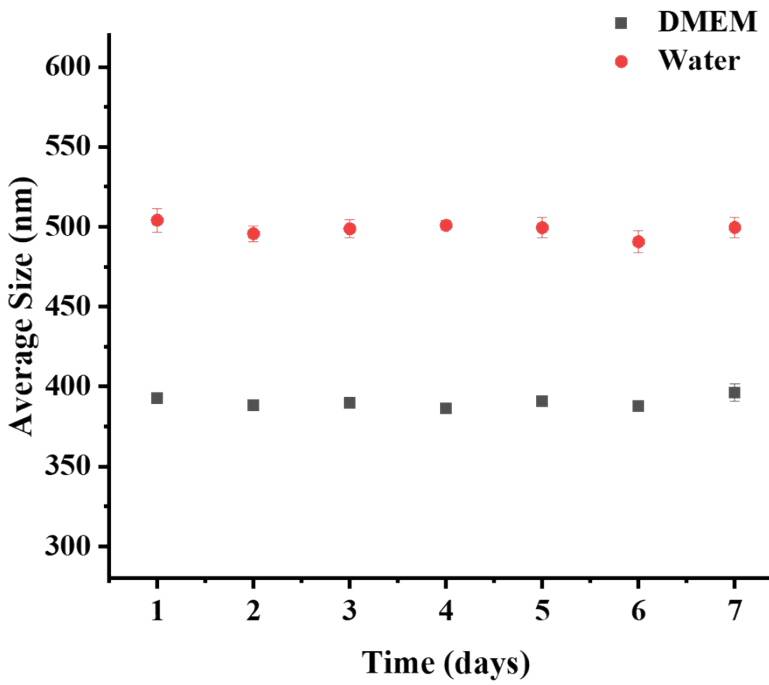


Fig. S20 Particle size of GluCCD in water and in complete medium DMEM within a week.

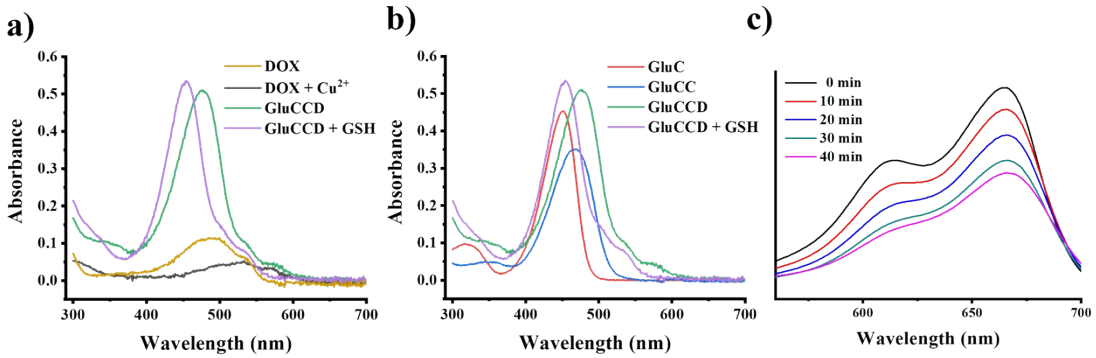


Fig. S21. (a) Absorption spectra of DOX, DOX + Cu^{2+}, GluCCD, GluCCD + GSH. (b) Absorption spectra of GluC, GluCC, GluCCD and GluCCD + GSH in H_2O. The concentrations of DOX, GluC and GSH were 10.0 μM. The concentration of Cu^{2+} was 20 μM. c) Catalytic activity of GluCCD for different incubation time.

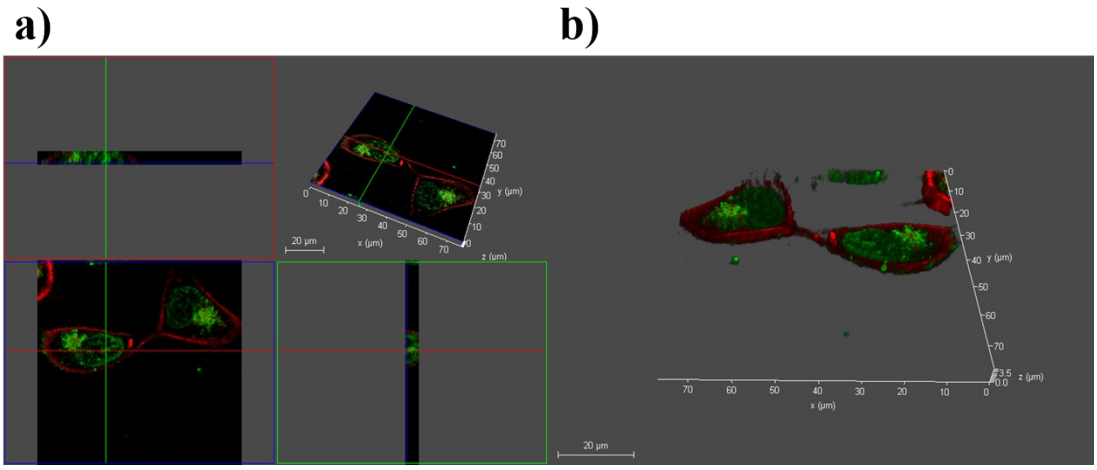


Fig. S22 a) Ortho view of Z-stack images and reconstructed b) 3D images of HeLa cells incubated with GluCCD (5 μM) for 6 h. Cytomembrane was stained with DiD. (DiD: λ_{ex} = 644 nm, λ_{em} = 665 nm; DOX: λ_{ex} = 488 nm; λ_{em} = 590 nm.)

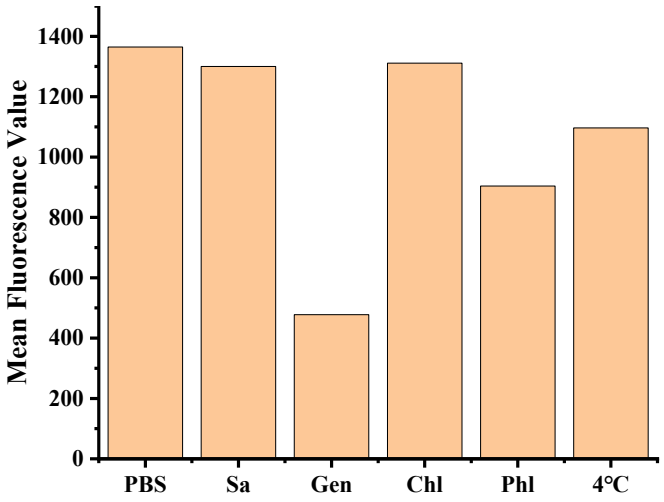


Fig. S23 The uptake inhibition percentage of GluCCD was studied in the presence of various endocytosis inhibitors and at 4°C. Sa: sodium azide; Gen: genistein; Chl: chlorpromazine; Phl: Phloretin.

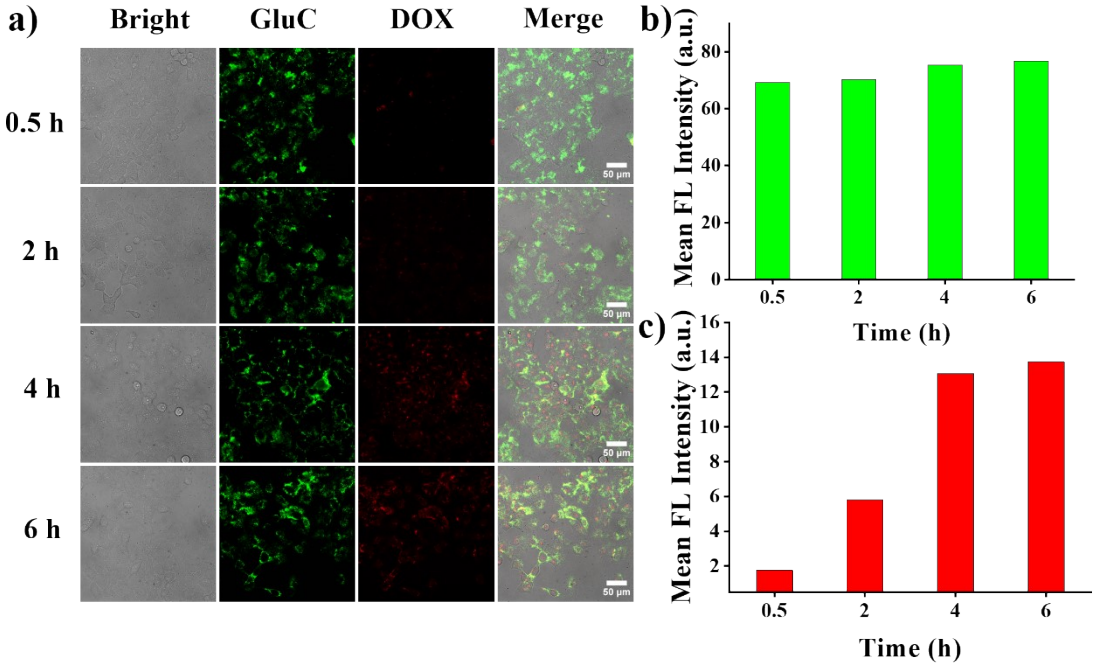


Fig. S24. (a) CLSM images of HeLa cells cultured with GluCCD (5 μM corresponding to DOX) for 0.5, 2, 4, 6 h（GluC: λ_{ex} = 445 nm, λ_{em} = 490 nm; DOX: λ_{ex} = 488 nm; λ_{em} = 590 nm.）Scale bar: 100 μm. (b, c) Quantification of imaging data: relative mean fluorescence with various treatments were quantified in the b) green and c) red channel by the normalized intensity.

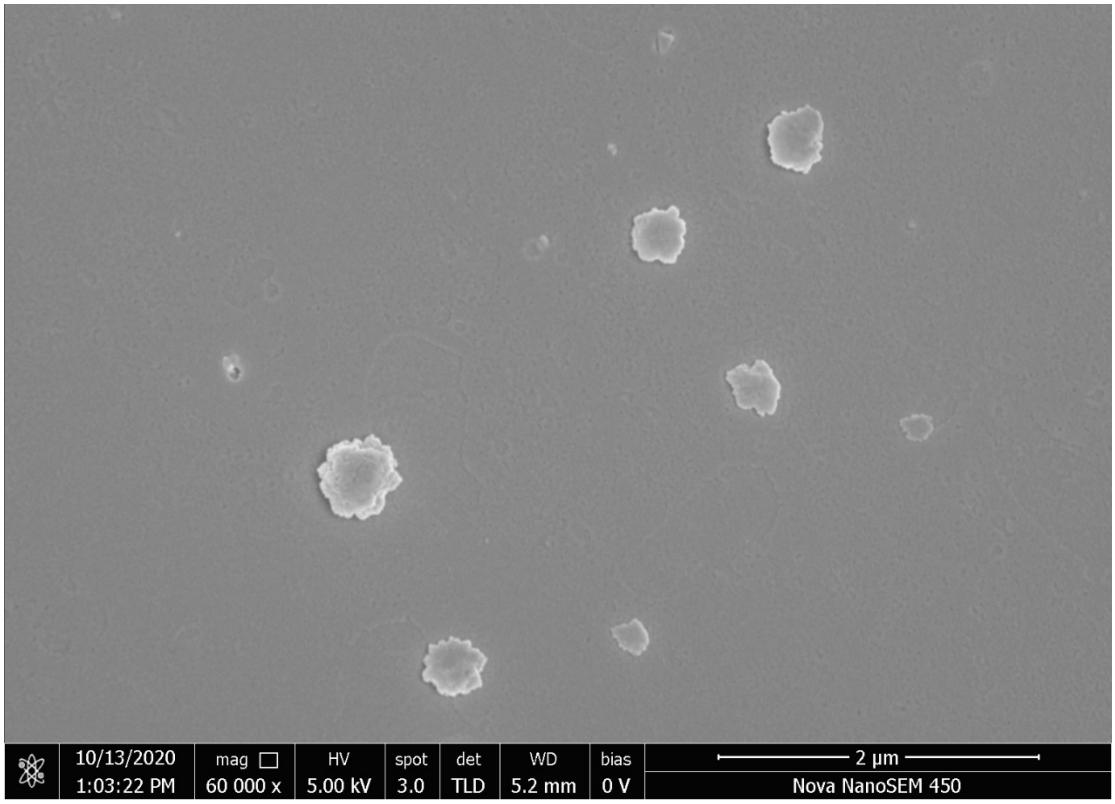


Fig. S25. The SEM image of GluCCC (Curcumin).

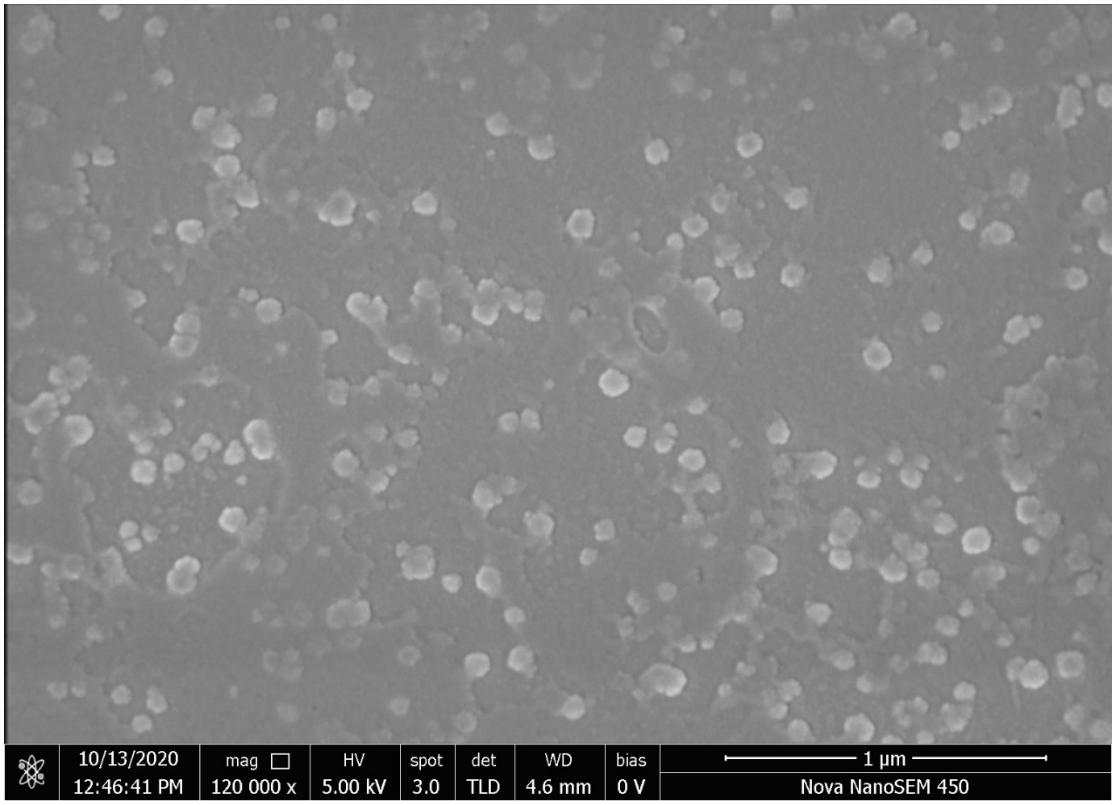


Fig. S26. The SEM image of GluCCG (Gefitinib).