Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Hepatoma-selective imaging of heavy metal ions using a 'clicked' galactosyl rhodamine probe

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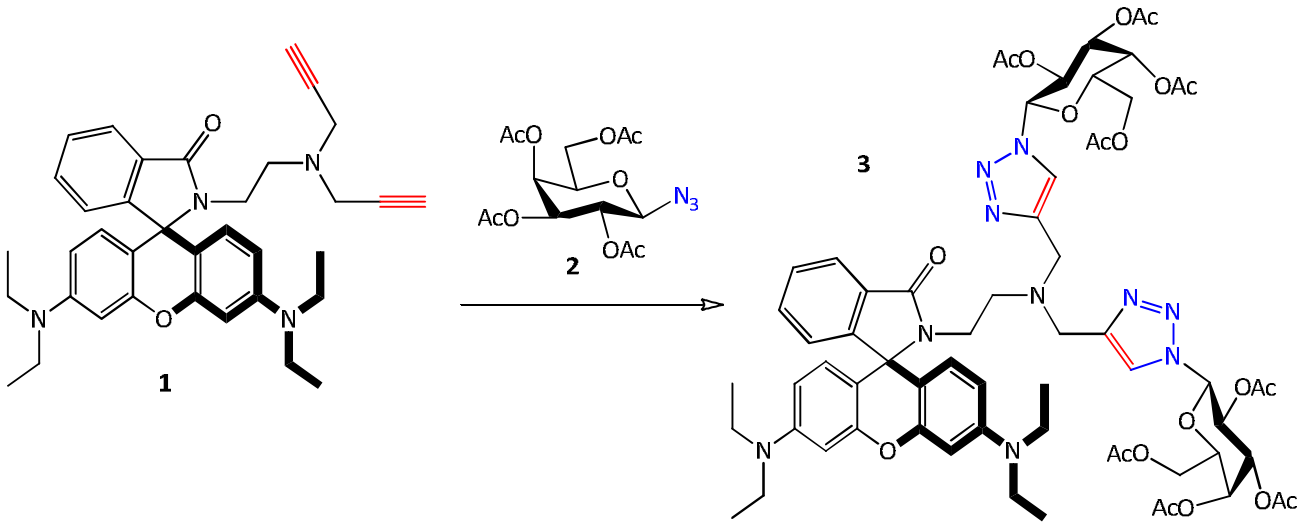
S1. Experimental section

General. All purchased chemicals and reagents are of analytical grade. Solvents were purified by standard procedures. Reactions were monitored by TLC (thin-layer chromatography) using E-Merck aluminum precoated plates of Silica Gel. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethylsilane (TMS) as the internal standard (chemical shifts in parts per million). High resolution mass spectra were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV).

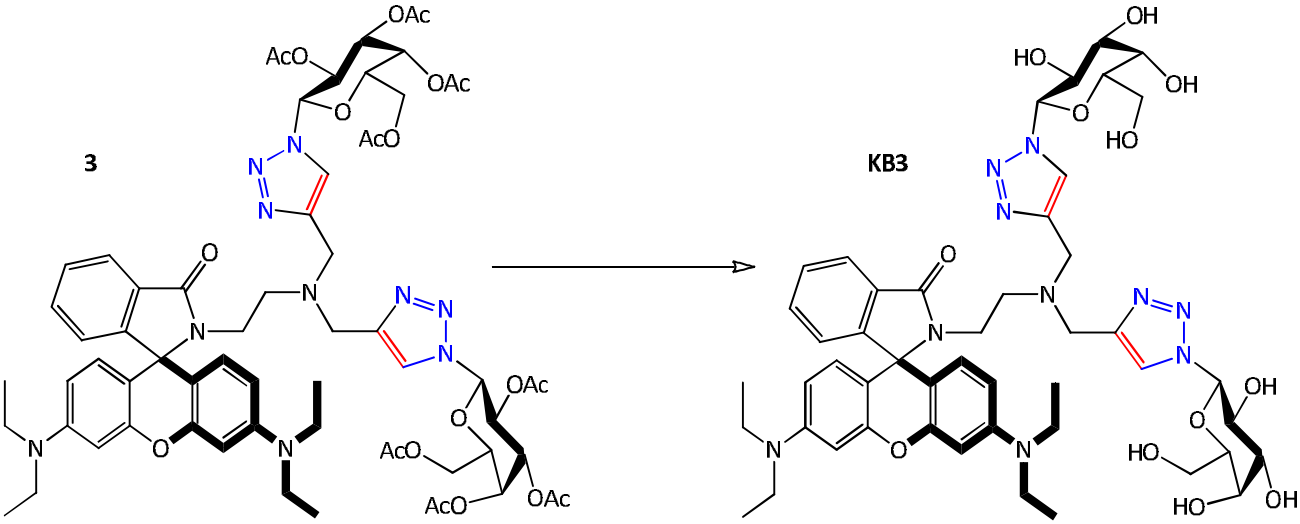
Synthesis of 3



To a solution of **1** (100 mg, 0.18 mmol) and **2** (266 mg, 0.71 mmol) in CH_2Cl_2/H_2O (5 mL/5 mL), were added sodium ascorbate (282 mg, 1.42 mmol) and $CuSO_4 \cdot 5H_2O$ (266 mg, 1.07 mmol), and the mixture was stirred over night at room temperature (r.t.). The resulting mixture was then diluted with dichloromethane and water, washed successively with water and brine, and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuum. The resulting residue was purified by column chromatography on silica gel (DCM/MeOH = 50:1, v/v) to afford compound **3** (150 mg, 65%). ^{1}H NMR (400 MHz, CD_3CN) δ 1.08-1.13 (m, 12H), 1.78 (s, 6H), 1.98 (s, 6H), 2.15 (s, 6H), 2.19 (s, 6H), 3.14-3.21 (m, 2H), 3.32-3.38 (m, 10H), 3.44 (s, 4H), 4.09-4.19 (m, 4H), 4.38 (t, J = 5.2 Hz, 2H), 5.30-5.34 (dd, J = 12.0 Hz, 2H), 5.51 (d, J = 3.2 Hz, 2H), 5.66 (t, J = 9.2

Hz, 2H), 5.93 (d, *J* = 9.2 Hz, 2H), 6.34 (t, *J* = 2.8 Hz, 1H), 6.36 (t, *J* = 2.8 Hz, 1H), 6.39-6.42 (m, 3H), 6.48 (d, *J* = 8.8 Hz, 1H), 7.02-7.05 (m, 1H), 7.48-7.54 (m, 2H), 7.82-7.84 (m, 1H), 7.97 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 12.6, 14.1, 20.3, 20.7, 29.4, 29.7, 31.9, 44.4, 51.5, 61.3, 64.9, 66.9, 67.9, 70.9, 74.0, 86.4, 97.9, 105.5, 105.6, 108.1, 122.4, 122.7, 123.9, 127.9, 128.8, 129.0, 132.3, 148.8, 153.3, 153.4, 168.1, 168.8, 169.8, 170.1, 170.4. HR-ESI-MS *m*/*z*: $[M + H]^+$ calcd. for 1307.5472, found 1307.5463.

Synthesis of KB3



Compound **3** (200 mg, 0.15 mmol) was dissolved in MeOH/H_2O/Et_3N (8:1:1, v/v/v), and the mixture was stirred over night at r.t. Solvent was then removed in vacuum and the residue was directly purified by column chromatography on silica gel (DCM/MeOH = 4:1, v/v) to afford **KB3** (60 mg, 41%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.14-1.18 (m, 12H), 3.30 (t, *J* = 6.8 Hz, 2H), 3.37-3.42 (m, 10H), 3.55 (s, 4H), 3.70-3.79 (m, 8H), 4.00 (d, *J* = 3.2 Hz, 2H), 4.18 (t, *J* = 9.6 Hz, 2H), 5.55 (d, *J* = 9.2 Hz, 2H), 6.39-6.46 (m, 6 H), 7.11 (d, *J* = 6.4 Hz, 1H), 7.53-7.59 (m, 2H), 7.91 (dd, *J* = 5.6 Hz, 1H), 8.08 (s, 2H); ^{13}C NMR (400 MHz, CD_3OD) δ 11.5, 37.9, 44.0, 50.8, 61.0, 65.7, 69.0, 70.1, 73.9, 78.6, 88.9, 97.6, 104.7, 104.8, 108.2, 122.2, 122.6, 123.7, 128.2, 128.5, 131.0, 132.6, 144.3, 149.0, 153.4, 153.5, 168.5. HR-ESI-MS *m*/*z*: $[M + H]^+$ calcd. for 971.4627, found 971.4713.

Fluorescence spectroscopy. Stock solution of **KB3** (5 mM) was prepared in deionized water. Stock solutions of 5 mM of $LiClO_4$, $Cd(ClO_4)_2$, $Cr(ClO_4)_3$, $Zn(ClO_4)_2$, $Ba(ClO_4)_2$, $Mg(ClO_4)_2$, $KClO_4$, $NaClO_4$,

$Mn(ClO_4)_2$, $Pd(ClO_4)_2$, $Fe(ClO_4)_3$, $Ca(ClO_4)_2$, $AgClO_4$, $Ni(ClO_4)_2$, $Hg(ClO_4)_2$ and $Cu(ClO_4)_2$ were prepared in deionized water. The fluorescence measurements were carried out with a path length of 10 mm and an excitation wavelength at 530 nm by scanning the spectra between 540 nm and 750 nm. The bandwidth for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded in aqueous solution at 25 °C.

RT-PCR. Total RNA was isolated from Hep-G2, Hela or HCT116 cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex Taq™. Real-time PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers for qPCR were as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'

and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'

ASGPR1 forward, 5'-CTGGACAATGAGGAGAGTGAC-3'

and reverse, 5'-TTGAAGCCCGTCTCGTAGTC-3'.

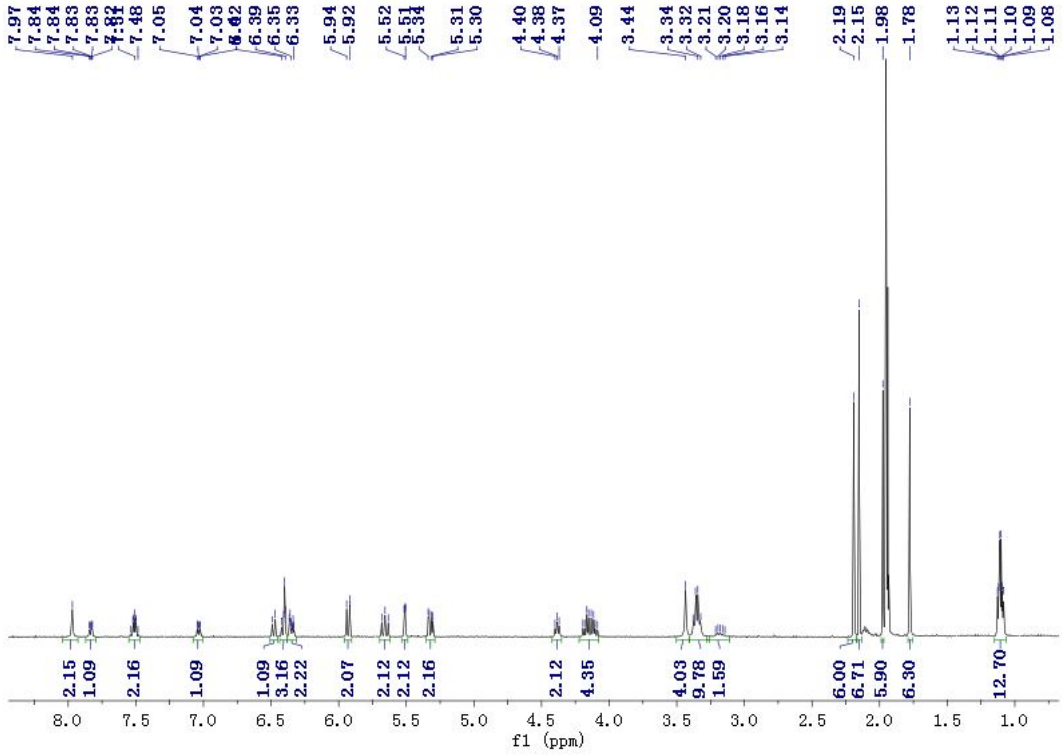
Cell imaging assay. Hep-G2, Hela or HCT116 cells were cultured in DMEM supplemented with 10% FBS. Cells (1.5×10^4/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. After pretreatment with or without 600 or 1500 μM of $Hg(ClO_4)_2$ in 10 mM HEPES for 30 min (followed by three rinses using PBS), the cells were incubated with 20 or 50 μM **KB3** in 10 mM HEPES at different concentrations for another 15 min. Then the cells on the microplate were rinsed in warm HEPES and fixed by 4% paraformaldehyde in HEPES for 15 min at room temperature. After three rinses in HEPES (5 min each time), the fluorescence was eventually detected and photographed with an Operetta high content imaging system (Perkinelmer, US).

Cell viability assay. Cells were plated overnight on 96-well plates at 5000 cells per well in growth medium. After seeding, cells were maintained in growth media treated at increasing concentrations (6.25 μM, 12.5 μM, 25 μM, 50 μM, 100 μM) of **KB3** (dissolved in DMSO, final concentration) for 72 h.

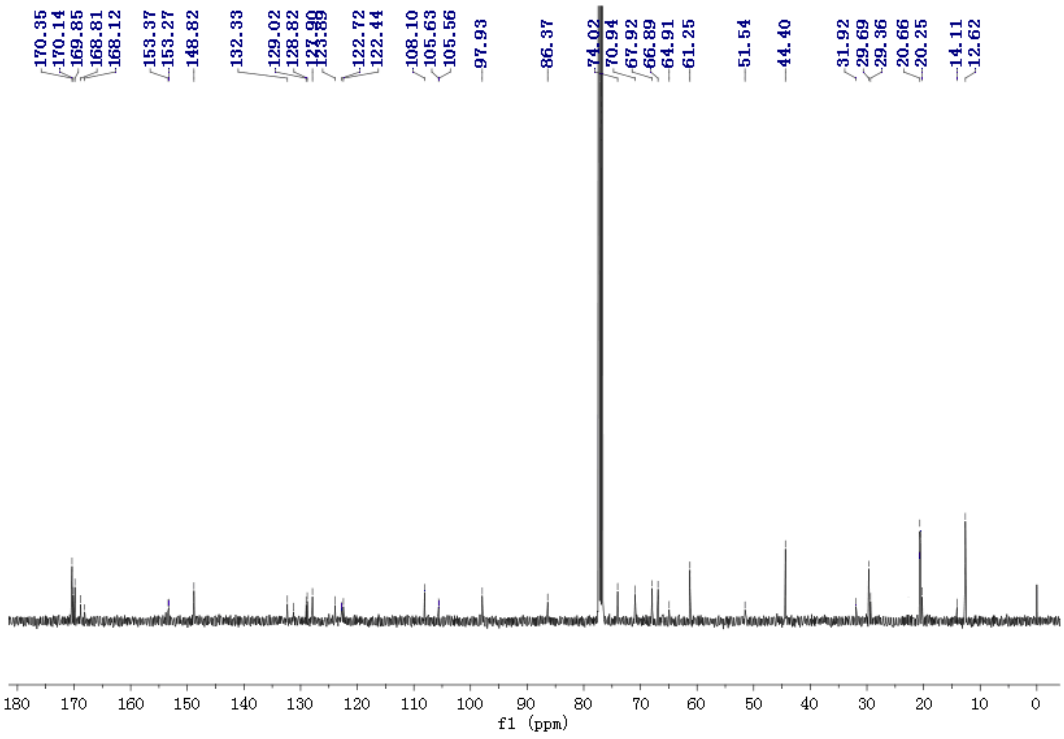
20 µL of MTS (Promega Corp) solution (2 mg/mL) was added to each well for 2 h at 37 °C, and then the absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm with a reference at 690 nm. The optical density of the result in MTS assay was directly proportional to the number of viable cells. Each experiment was done in triplicate.

S2. Original NMR spectra of new compounds

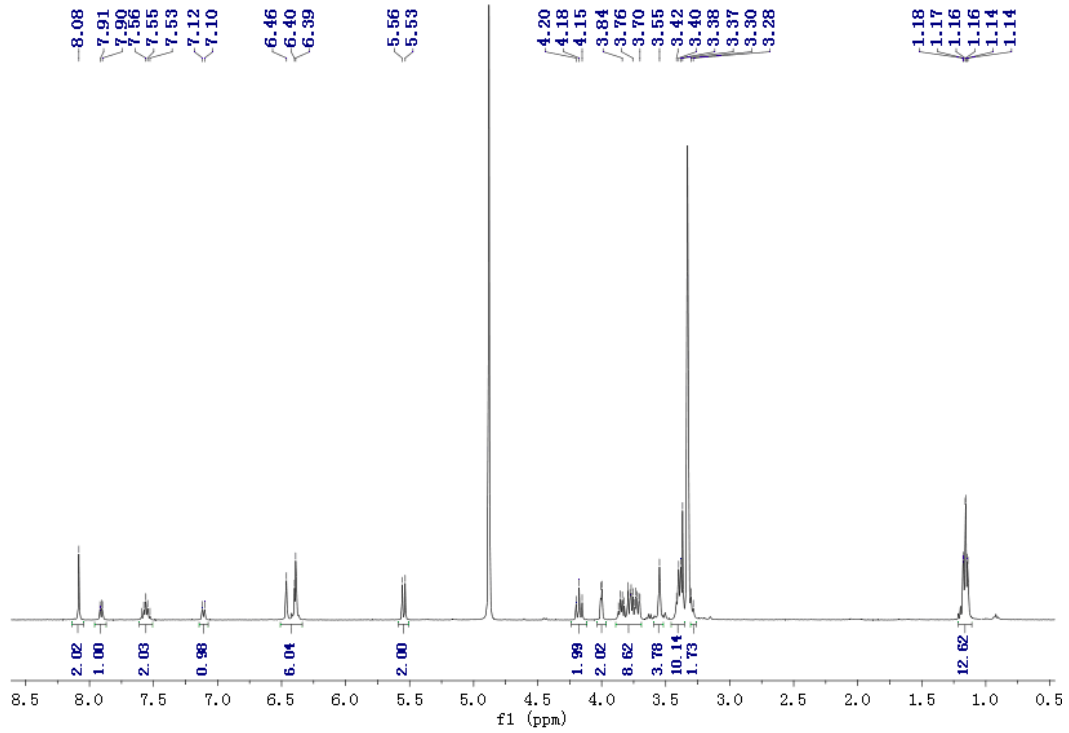
^{1}H NMR of **3**:



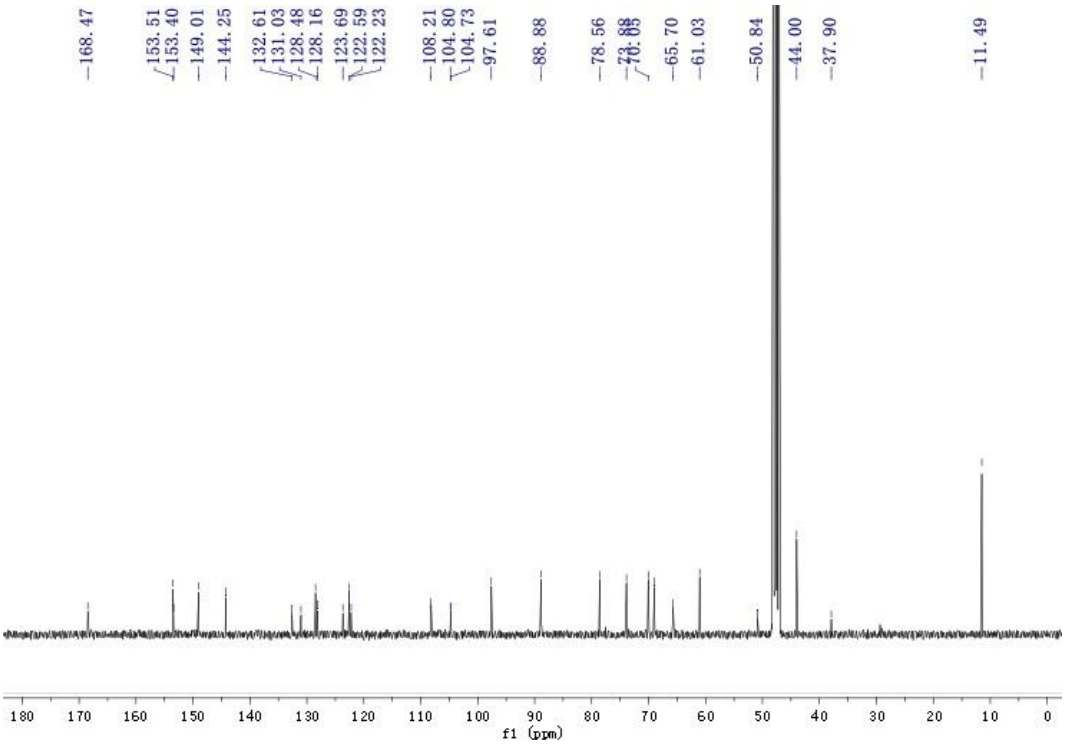
^{13}C NMR of **3**:



^{1}H NMR of **KB3**:



^{13}C NMR of **KB3**:



S3. Fig. S1

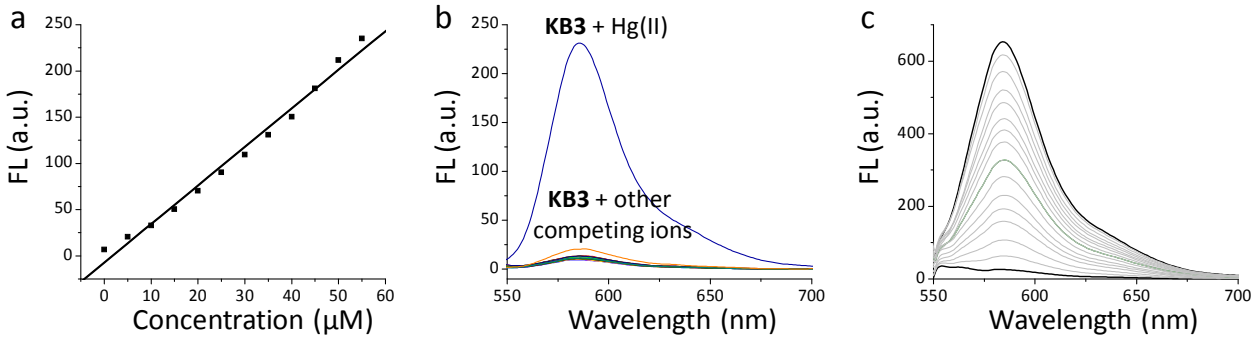


Figure S1. (a) Plotting the fluorescence (FL) intensity of **KB3** (10 μM) as a function of Hg(II) concentration. (b) FL spectra of **KB3** (10 μM) in the presence of various metal cations (100 μM). (c) FL spectra of **3** (10 μM) in the presence of increasing Hg(II) (0-200 μM).