Supporting Information

for

Gallium and functionalised-porphyrins combine to form potential lysosome-specific multi-modal bio-probes

Jie Pan,[a#] Bethany I. Harriss,[b#] Chi-Fai Chan,[a] Lijun Jiang,[a] Tik-Hung Tsoi,[c] Nicholas J. Long, [b,*] Wing-Tak Wong,[c] Wai-Kwok Wong,[a*] and Ka-Leung Wong[a*]

a. Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Kowloon, Hong Kong SAR. E-mail: wkwong@hkbu.edu.hk, klwong@hkbu.edu.hk

b. Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK. E-mail: n.long@imperial.ac.uk; Fax: +44(0)20 7594 5804; Tel: +44 (0)20 7594 5781

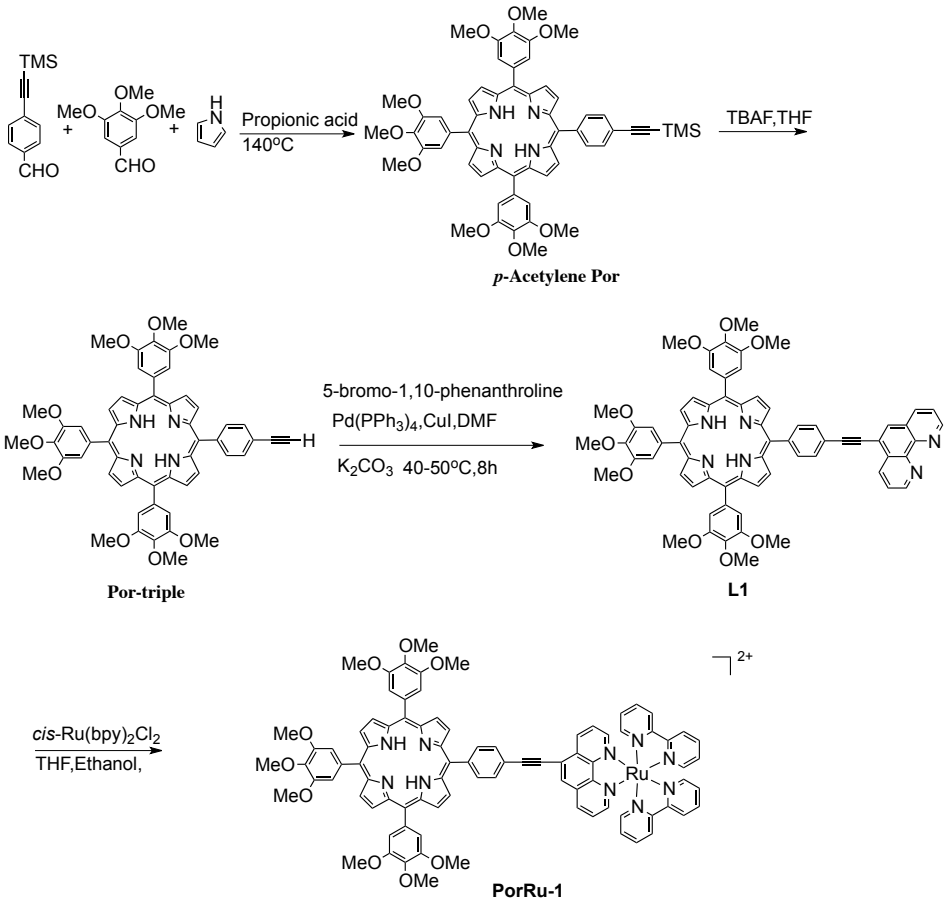
c. Department of applied biology and chemical technology, Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR.

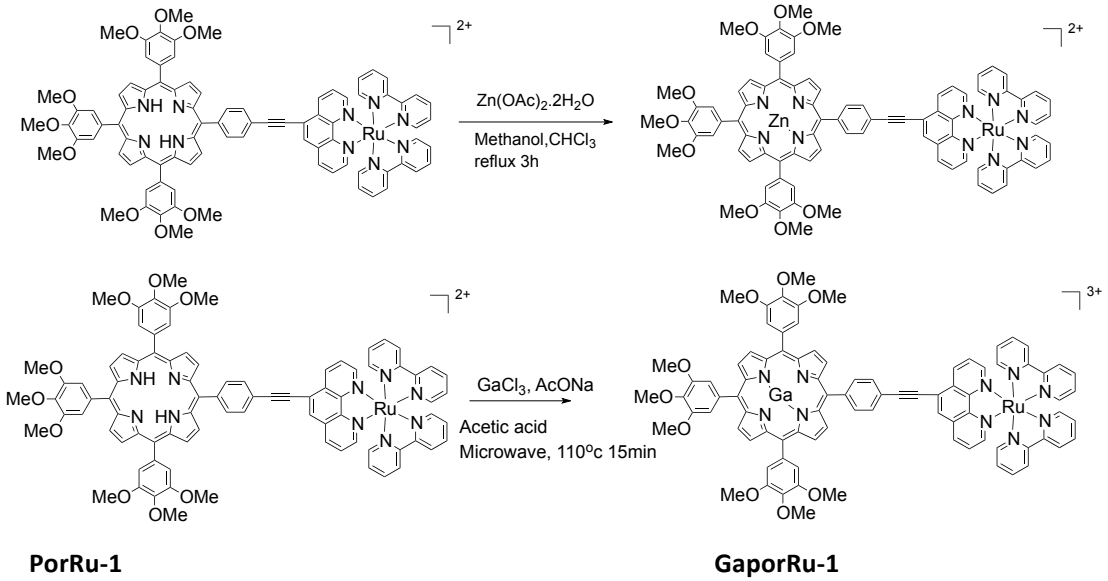
#These authors contributed equally to this work and should be considered co-first authors.

Experimental

General synthesis for the PorRu-1

All the reagents were purchased commercially and used without further purification. All reactions were performed under a nitrogen atmosphere, unless otherwise noted. The anhydrous solvents were dried according to standard protocols in literature. The synthetic route for the preparation of **PorRu-1**, **ZnporRu-1** and **GaporRu-1** is shown in Scheme S1. The intermediates and products were characterized by ^{1}H NMR, ^{13}C NMR, MALDI-TOF MS and UV-Vis spectroscopy. NMR spectra were recorded on either a Brüker Advance 400 (1H: 400 MHz, 13C: 100 MHz) spectrometer. The following abbreviations were used to depict the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, br = broad. **PorRu-1** was synthesized by using our developed method in the literature.[1]





Scheme S1. Synthetic routes of **GaporRu-1** and **ZnporRu-1**.

Preparation of 5,10,15-Tris (3',4',5'-trimethoxylphenyl)-20-[4'-(2i"-trimethylsilylethyl)phenyl]-21H,23H-porphyrin (*p*-Acetylene Por).

3,4,5-Trimethoxylbenzaldehyde (8.00 g, 0.041 mol) and 4-[2-(trimethylsilyl)ethynyl]benzaldehyde (2.75 g, 0.014 mol) were added in 300 mL propionic acid. The reaction temperature was 120 °C and a solution of pyrrole (3.65 g, 0.054 mol) in 50 mL propionic acid was added dropwise. After all the pyrrole was added to the reaction solution, the reaction temperature was increased to 140 °C and refluxed for 3 h. Once the reaction was complete, the propionic acid was distilled off. The residue was purified on silica gel for column chromatography by using DCM as eluent. A purple product was isolated. Yield 2.00 g (15%). ^{1}H NMR ($CDCl_3$) δ -2.68 (s, 2H), 0.45 (s, 9H), 4.05 (d, 18H, *J* = 3.6 Hz), 4.25 (s, 9H), 7.57(d, 6H, *J* = 2.0 Hz), 7.96 (d, 2H, *J* = 8.4 Hz), 8.25 (d, 2H, *J* = 8.0 Hz), 8.92 (d, 2H, *J* = 4.8 Hz), 9.07 (s, 6H). HRMS (MALDI-TOF) ([M]+, m/z): Calcd. for $C_{58}H_{56}N_4O_9Si$, 981.2; Found for [M]+, 981.2.

Preparation of Por-triple

***p*-Acetylene Por** (200 mg, 0.192 mmol) was dissolved in 30 mL DCM and the protecting group (TMS) in the compound was deprotected by 210 μL TBAF (1 M TBAF in THF). The solution mixture was stirred at room temperature for 30 min. The solvent was removed and the residue was purified on silica gel for column

chromatography by using DCM as eluent. Yield 180 mg (98%). ^{1}H NMR ($CDCl_3$) δ -2.8 (s, 2H), 3.06 (s, 18H), 4.15 (s, 9H), 7.46 (s, 6H), 7.89 (d, 2H, *J* = 8.0 Hz), 8.17 (d, 2H, *J* = 7.8Hz), 8.82 (d, 2H, *J* = 4.4 Hz), 8.96 (s, 6H).

Preparation of Phenylacetylene-Linked Porphyrin-Phen . L_1

$Pd(PPh)_4$ (20 mg), CuI (20 mg), K_2CO_3, **Por-triple** (100 mg, 0.11 mmol) and 5-bromo-1,10-phenanthroline (80 mg, 0.313 mmol) were dissolved in 50 mL anhydrous DMF. The reaction temperature was 50 °C and stirred for 12 hours. The organic phase of the reaction mixture was washed by chloroform and water three times. The residue was purified on silica gel for column chromatography by using CH_3Cl/MeOH (v/v = 30:1) as eluent. Yield 64.5 mg, 51%. ^{1}H NMR ($CDCl_3$) δ -2.75(s, 2H), 3.97 (s, 18H), 4.18 (s, 9H), 7.48 (s, 6H), 7.70–7.74 (dd, 1H, *J* = 4.4 Hz), 7.83-7.87 (m, 1H), 8.09 (d, 2H, *J* = 8.0 Hz) 8.27-8.34 (m, 4H), 8.89 (d, 2H, *J* = 4.8 Hz), 8.97-9.00 (m, 6H), 9.01–9.04 (m, 1H), 9.25(d, 1H, *J* = 1.6 Hz), 9.28(d, 1H, *J* = 1.6Hz). ^{13}C NMR (DMSO-d_6), δ 137.42, 136.72, 134.57, 133.23, 132.21, 132.06, 132.03, 131.53, 131.44, 128.83, 128.71, 60.42, 56.20.

Preparation of Phenylacetylene-Linked [(Porphyrin-Phen)Ru(bpy)$_2$]-[Cl]$_2$ (PorRu-1)

L_1 (20 mg, 0.018 mmol) and *cis*-$Ru(bpy)_2Cl_2$ (34 mg, 0.070 mmol) were dissolved in a mixture of THF (15 mL) and ethanol (15 mL) solution. After refluxing for 12 h, the solvent was removed under reduced pressure. The crude solid was purified on neutral Al_2O_3 for column chromatography with DCM/MeOH (v/v = 20:1) as eluent. Yield 20 mg, 74%. ^{1}H NMR (DMSO-d_6) δ –2.90 (s, 2H), 3.90 (s, 18H), 4.00 (s, 9H), 7.40-7.56 (m, 6H), 7.68–7.96 (m, 12H), 8.13-8.27 (m, 6H), 8.40 (s, 2H), 8.85–9.00 (m, 14H). ^{13}C NMR (DMSO-d_6) δ 156.87, 156.80, 156.57, 156.48, 153.16, 152.95, 151.96, 151.72, 151.51, 151.19, 151.42, 151.19, 147.61, 147.20, 146.93, 142.97, 138.08, 137.96, 137.43, 136.79, 136.70, 131.15, 130.62, 130.15, 129.74, 127.91, 127.86, 127.27, 126.99, 124.58, 124.47, 120.14, 118.64, 112.78, 60.43, 56.21. HRMS (MALDI-TOF, positive mode) ([M]$^+$, m/z): Calcd. for $C_{87}H_{70}N_{10}O_9Ru$, 1500.6; Found for [M]$^+$, 1500.43.

Preparation of Phenylacetylene-Linked [(Zinc-Porphyrin-Phen)Ru(bpy)$_2$]-[Cl]$_2$ (ZnporRu-1)

PorRu-1 (20 mg, 0.013 mmol) and excess amount of $Zn(OAc)_2.2H_2O$ was dissolved in methanol (15 mL) and chloroform (15 mL). After refluxing for 3h, the solvent was removed under reduced pressure. The crude solid was purified on neutral Al_2O_3 for column chromatography with DCM/MeOH (v/v=20:1) as eluent. Yield 17 mg, 83%. ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 18H), 4.00 (s, 9H), 7.45 (d, 2H, J = 5.8Hz), 7.53-7.57 (m, 6H), 7.70 (d, 2H, J = 5.4Hz), 7.74 (d, 4H, J = 4.9Hz), 7.88 (m, 2H, J = 4.2Hz), 7.95–7.99 (m, 1H), 8.15 (s, 1H), 8.16 (d, 2H, J = 1.3Hz), 8.22–8.28 (m, 3H), 8.32-8.35 (m, 1H), 8.82 (d, 2H, J = 4.6Hz), 8.86–8.90 (m, 8H), 8.93–8.96 (m, 4H), 9.28 (d, 1H, J = 9.3Hz). HRMS (MALDI-TOF) ([M–2Cl]+, m/z): Calcd. for $C_{87}H_{68}N_{10}O_9RuZn$, 1562.9; Found for $[M + H]^+$, 1564.35; $[M\text{-bpy}]^+$, 1408.31.

Preparation of GaporRu-1

PorRu-1 (10 mg, 0.0093 mmol) and $GaCl_3$ (4.8 mg, 0.028 mmol) were dissolved in acetic acid (10 mL) and excess amount of NaOAc, heated by microwave at 110 ºC for 10 minutes. The crude product was purified by column chromatography on neutral Al_2O_3 by using $CHCl_3$ as eluent (product yield of **GaporRu-1** ~ 96%).

GaporRu-1 ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 18H), 4.0 (s, 9H), 7.34-7.58 (m, 7H), 7.67-7.80 (m, 4H), 8.10-8.21 (m, 16H), 8.73-8.89 (m, 10H), 9.04-9.14 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 156.88, 156.81, 156.56, 156.50, 153.10, 152.98, 152.72, 151.77, 151.68, 151.50, 151.43, 151.19, 147.62, 147.20, 146.65, 138.09, 137.96, 136.15, 136.07, 131.18, 130.57, 130.16, 129.74, 127.92, 127.80, 127.41, 127.01, 124.59, 124.49, 121.33, 112.62, 60.47, 56.23. HRMS (MALDI-TOF) ($[M]^+$): Calcd for $C_{87}H_{68}ClGaN_{10}O_9Ru$, 1603.78; Found for $[M+H]^+$, 1604.26.

Radiochemistry

Labelling with ^{68}Ga

$^{68}GaCl_3$ was produced by using an Eckert & Ziegler IGG100 ^{68}Ge/^{68}Ga-Generator in a fully-automated Modular-Lab system. $^{68}GaCl_3$ was eluted in 10 mL of a 0.1 M HCl solution onto a cation exchange cartridge (Strata™-XC, 33 μm, Strong Cation, 30 mg/mL, Phenomenex) and was removed using 0.8 mL of 98 % acetone and 2% 0.1 M HCl solution. This was dried at 150 ºC for 20 min before the reaction was performed.

All microwave radiolabelling was performed using a Biotage Initiator + Microwave Synthesizer in high precision glass 0.2 - 0.5 mL reaction vials.

HPLC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Stockport, UK) equipped with a UV (254 nm) detector and a LabLogic Flow-Count radio-detector. A flow rate of 1 mL/ min was used with a Phenomenex Gemini C18 column (150 mm x 4.6 mm) at the following gradient: 95-5 % A over 15 min, 5 % A for 1 min, 5-95 % A over 1 min and 95 % A for 1 min. A = H_2O (0.1% TFA) B = MeCN (0.1% TFA). Laura 3 software (LabLogic, Sheffield, UK) was used to analyze HPLC chromatograms.

Cartridge purification was performed using a SepPac $^{t}C_{18}$ light cartridge. This was preconditioned using EtOH (5 mL) and NaOAc buffer (5 mL 0.2 M pH 4). The sample was then loaded onto the cartridge, washed with NaOAc buffer (5 mL) and eluted in EtOH (0.5 mL).

Preparation of ^{68}GaporRu-1

PorRu-1 (200 μL 2 mg / mL in acetic acid) and NaOAc (1.2 mg) were added to 445 μCi $^{68}GaCl_3$. This solution was microwaved at 150 ºC for 15 min, with 85 % of the activity incorporated into the product (Figure S3). This solution was diluted with NaOAc buffer (1 mL 0.2 M pH 4) and purified using a $^{t}C_{18}$ light cartridge.

The radiolabelling of **^{68}Ga** with **PorRu-1** can also be performed by conventional heating method. **PorRu-1** (200 μL 1 mg/ mL in 0.2 M pH 4 NaOAc buffer) and NaOAc buffer (1 mL, 0.2 M, pH 4) were added to 310 μCi $^{68}GaCl_3$. This solution was heated at 100 ºC for 1 h, with 4 % of the activity incorporated into the product (Figure S4).

Photophysical Measurements. UV-visible absorption spectra (200 - 1100 nm) were recorded by an HP Agilent UV-8453 spectrophotometer. Single-photon luminescence spectra were recorded using an Edinburgh Instrument FLS920 combined steady state spectrophotometer that was equipped with a visible to near-infrared-sensitive photomultiplier in nitrogen-cooled housing. The spectra were corrected for detector

response and stray background light phosphorescence. The quantum yields of the compounds were measured by the comparative method and integrated sphere. Singlet oxygen was detected directly by its phosphorescence emission at 1270 nm using an InGaAs detector on a PTI QM4 luminescence spectrometer. The emission quantum yields (Φ_{em}) of the test compounds were measured by comparative method and integrated sphere (compared with 5,10,15,20-tetraphenyl-zinc-porphyrin, Φ_{em} = 0.033 in toluene, Abs = 0.05). Singlet oxygen quantum yields (Φ_{Δ}) were determined in chloroform by comparing their singlet oxygen phosphorescence emission signals to that of a reference compound (H_2TPP, Φ_{Δ} = 0.55 in chloroform).[2,3]

Cell Culture. Human cervical carcinoma HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ˚C and 5% CO_2.
Breast cancer MCF-7 cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ˚C and 5% CO_2.

In vitro **fluorescence imaging.** HeLa cells were seeded onto a coverslip in 35-mm culture dishes overnight. The cells were then incubated with 10 μM complexes **GaporRu-1** for 20 h at 37 ˚C and 5% CO_2. For colocalization experiments, the cells were then washed with 1X PBS and stained with 50 nM Lyso Tracker Green for 15 min. The emitted fluorescent signals of the complexes and the tracker were examined with an inverted fluorescence microscope (Zeiss Axio Observer Z1, Zeiss, Germany) equipped with a UV lamp, mercury bulbs and a customized fluorescence filter (excitation wavelength = 365 nm, emission wavelength = 610 nm). A 63X oil immersion objective was used for imaging. The same procedure was applied on Mitochondria Tracker for imaging.

Flow Cytometric Cellular Uptake. HeLa cells were seeded onto wells of a 6-well plate overnight. The cells were then incubated with 10 μM complexes **GaporRu-1**

and **ZnporRu-1** for 6 h at 37 ˚C and 5 % CO_2. Cells were then trypsinized and washed with 1X PBS for twice. The uptakes were then monitored with flow cytometer (BD FACSaria Cell Sorting System, BD Biosciences, China). The cells were excited by a 532 nm laser, and the emissions were recorded using a suitable optical filter (APC-Alexa Fluor ® 660nm). At least 10000 events were analyzed for each measurement.

Dark-cytotoxicity assay: The MTT viability assay was performed according to standard methods. In brief, HeLa cells (3×10^3 / well) were seeded in 96 - well plates 24 hours prior to exposure to drugs. The cells were treated with samples **GaporRu-1** and **ZnporRu-1** overnight in the dark. The cytotoxicity was determined by the MTT reduction assay. The cell monolayers were rinsed twice with phosphate-buffered saline (PBS) and then incubated with 50 μL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (0.5 mg / mL) at 37 °C for 3 hours. Then the media were removed, and 100 μL of DMSO solubilizing reagent was added and shaken for 30 minutes to dissolve the formed formazan crystals in living cells. The absorbance was measured at dual wavelength, 540 nm and 690 nm, on a Labsystem Multiskan microplate reader (Merck Eurolab, Switzerland). Each dosed concentration was performed in triplicate wells, and repeated twice for the MTT assay.[4]

Photo-cytotoxicity assay: The PDT cytotoxicity assay was performed according to standard methods. In general, HeLa cells (3×10^3 / well) were incubated in 96-well plates 24 hours prior to exposure to drugs. The cells were treated with samples **GaporRu-1** and **ZnporRu-1** in the dark overnight. Afterwards, the cells were exposed to yellow light (1-4 J/ cm^2) produced from a 400 W tungsten lamp fitted with a heat-isolation filter and a 550 nm long-pass filter. The fluence rate was 6 mW/ cm^2. Cell viability was determined by the MTT reduction assay at 24 h post-PDT.[5] The cell monolayers were rinsed twice with PBS and then incubated with 50 μL MTT solution (0.5 mg / mL) at 37 °C for 3 h. Then the media were removed, and 100 μL of DMSO

solubilizing reagent was added and shaken for 30 minutes to dissolve the formed formazan crystals in living cells. The absorbance was measured at dual wavelength, 540 nm and 690 nm, on a Labsystem Multiskan microplate reader (Merck Eurolab, Switzerland). Each dosed concentration at individual light exposure was performed in quadruplicate wells for the PDT assay.

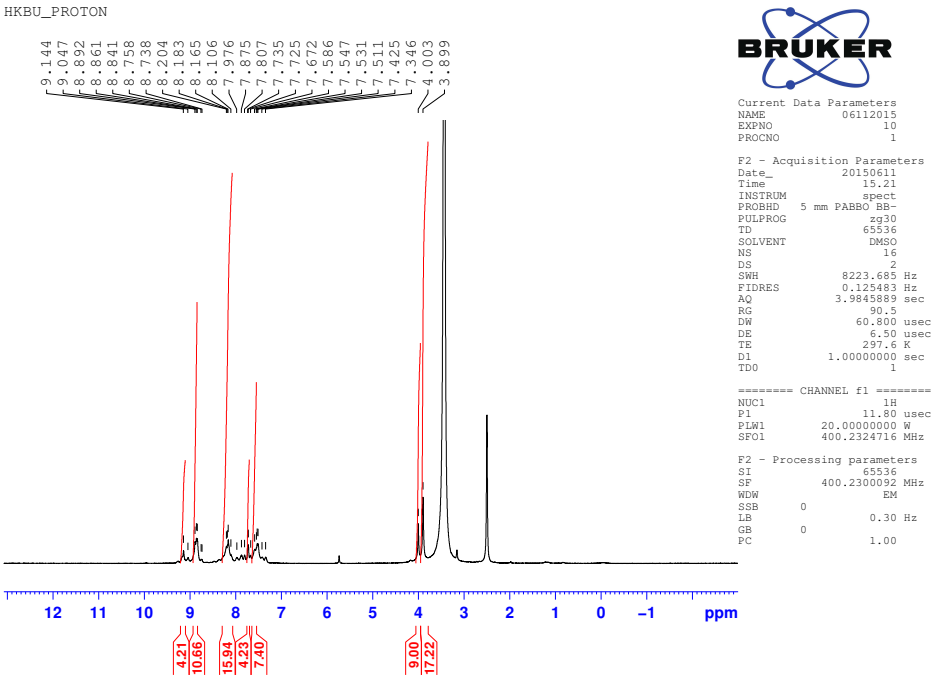


Figure S1. 400 MHz-^{1}H-NMR (DMSO-d_6) spectrum of **GaporRu-1**

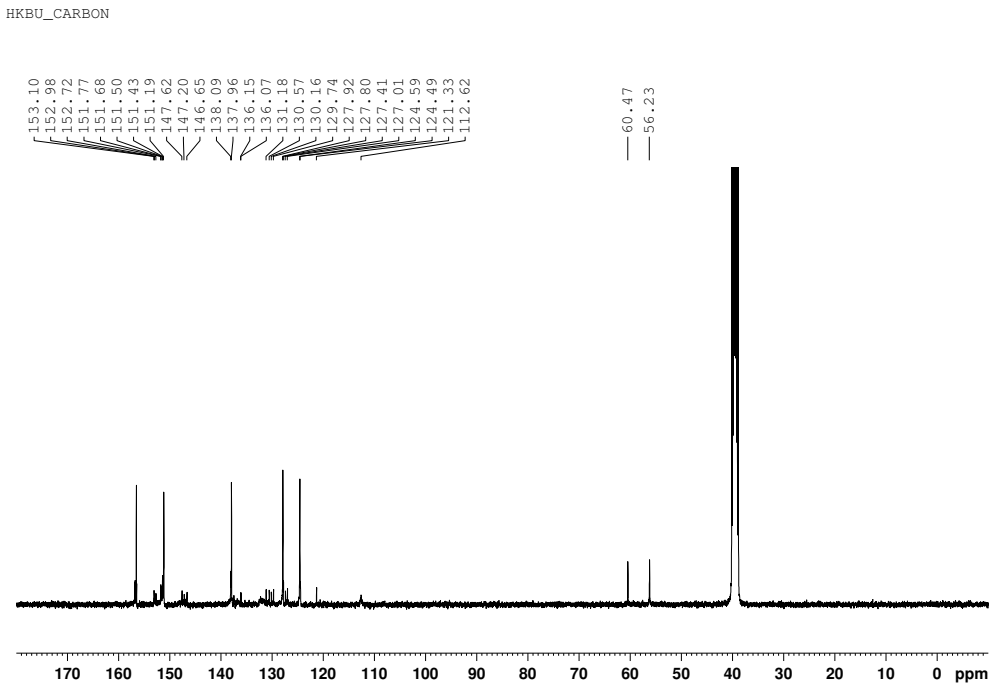


Figure S2. 100 MHz-^{13}C-NMR (DMSO-d_6) spectrum of **GaporRu-1**

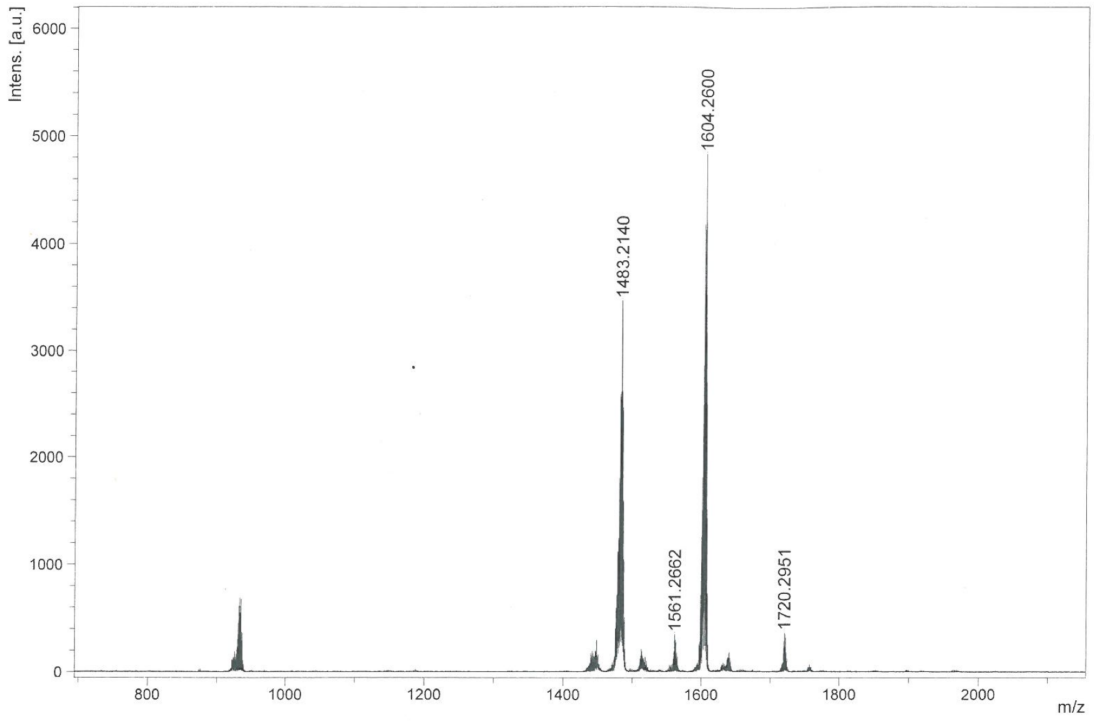


Figure S3. MALDI-TOF mass spectrum of **GaporRu-1**

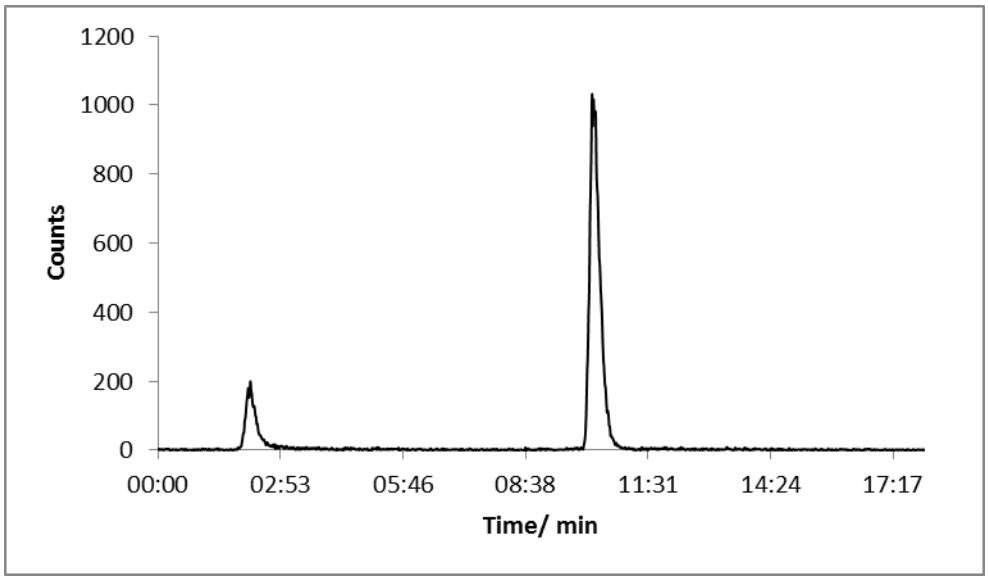


Figure S4. The HPLC spectra of crude **^{68}GaporRu-1** after 15 minutes microwave heating at 150 ºC (85 % incorporation of radiolabel into the desired product).

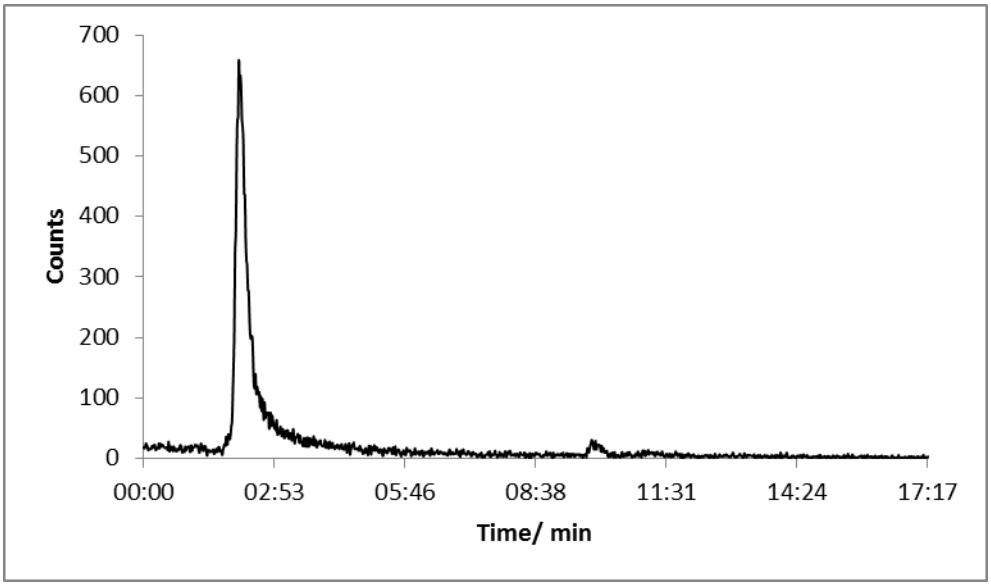


Figure S5. The HPLC spectra of crude ^{68}Ga radiolabelling for **^{68}GaporRu-1** without microwave heating (with only conventional heating up at 100 ºC, 1 h, yield 4 %).

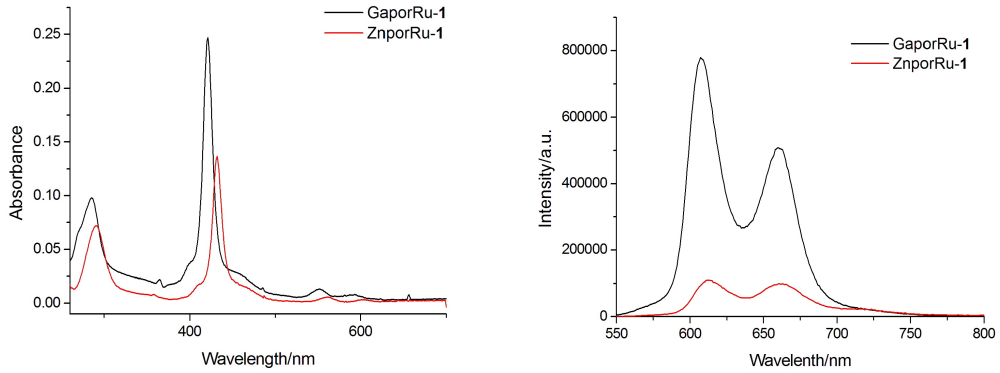


Figure S6. The absorption and emission spectra (λ_{ex} = 420 nm) of **GaporRu-1** and **ZnporRu-1** in aqueous solution with 0.1% DMSO (conc. = 1 μM).

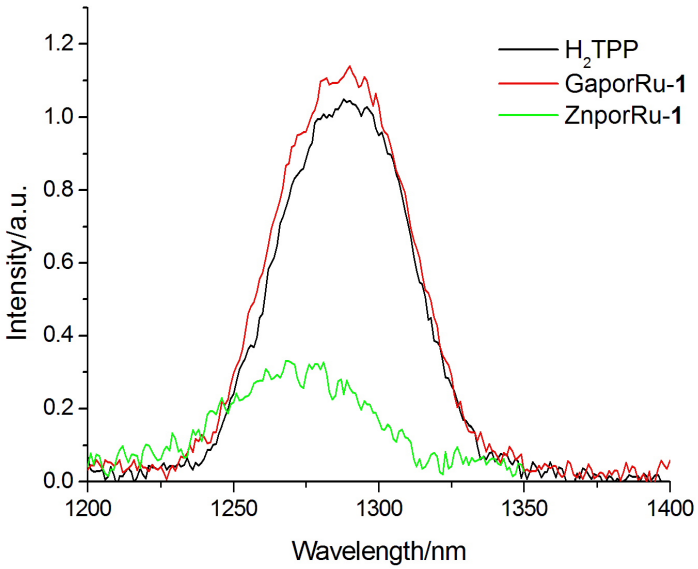


Figure S7. The emission spectra of **GaporRu-1**, **ZnporRu-1** and 5,10,15,20-tetraphenylporphyrin (H_2TPP) in chloroform (λ_{ex} = 420 nm, Absorbance = 0.05).

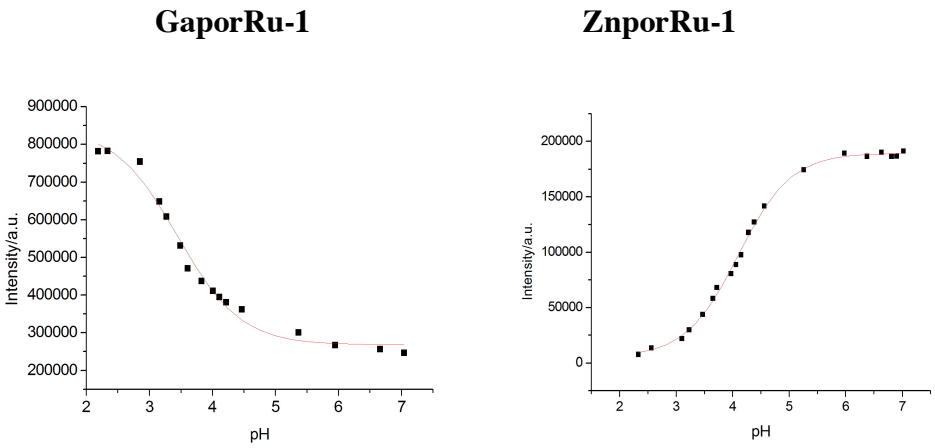


Figure S8. The plot of the emission intensity (λ_{ex} = 430 nm) of **GaporRu-1** (λ_{em} = 607 nm) and **ZnporRu-1** (λ_{em} = 613 nm) in different pH in aqueous solution with 5% DMSO (pKa values of **GaporRu-1** = 3.45 ; **ZnporRu-1** = 4.09).

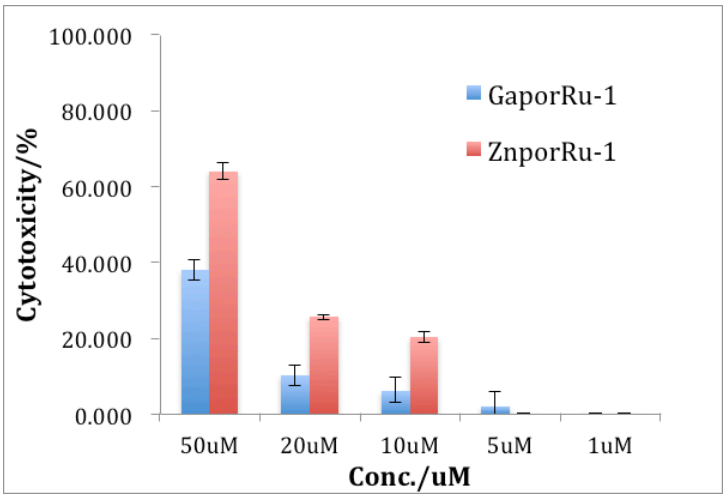


Figure S9. The MTT assay of **GaporRu-1** and **ZnporRu-1** in MCF-7 cells (24 hours incubation, conc. 1-50 μM).

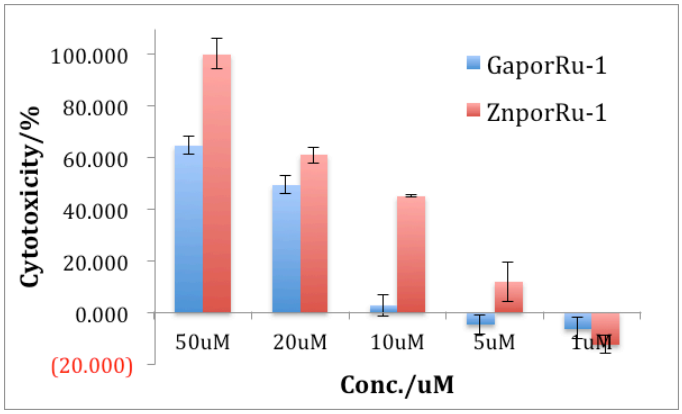


Figure S10. The MTT assay of **GaporRu-1** and **ZnporRu-1** in HeLa cells. (24 hours incubation, conc. 1-50 μM)

Reference

[1] J. X. Zhang, J. W. Zhou, C. F. Chan, T. C. Lau, D. W. Kwong, H. L. Tam, N. K. Mak, K. L. Wong and W. K. Wong, *Bioconjugate Chem.*, **2012**, *23*, 1623-1638.

[2] I. Gupta and M. Ravikanth, *J. Chem. Sci.*, **2005**, *117*, 161-166.

[3] J. Zhang, K. L. Wong, W. K. Wong, N. K. Mak, W. J. D. Kwong and H. L. Tam, *Org. Biomol. Chem.*, **2011**, *50*, 5517-5525.

[4] Y. Li, T. M. Pritchett, J. Huang, M. Ke, P. Shao, and W. Sun, *J. Phys. Chem. A* **2008**, *112*, 7200-7207.

[5] P. A. Wilson, *Oxford University Press*, *eds Masters JRW.*, **2000**.

[6] N. K. Mak, K. M. Li, W. N. Leung, N. S. Wong, D. P Huang, M. L. Lung, Y. K. Lau, C. K. Chang, *Biochem Pharmacol.*, **2004**, *68*, 2387-2396.