Electronic Supplementary Information

Immobilising an acid-cleavable dimeric phthalocyanine on gold nanobipyramids for intracellular pH detection and photodynamic elimination of cancer cells

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Contents

3 h and (f) 4 h.

Fig. S6 (A) Bright-field, fluorescence and the merged confocal images of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH 7.4 or 5.0 for 2 h. Scale bar: 25 μm. (B) SERS spectra of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH (a) 7.4, (b) 6.5, (c) 6, (d) 5.5 and (e) 5 for 2 h. (C) Quantified intracellular fluorescence intensities of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at different pH for 2 h. (D) Plots of the ratiometric intensities of I_{1087}/I_{830} and I_{1203}/I_{830} versus the pH of the medium.

Fig. S7 (A) Bright-field, fluorescence and the merged confocal images of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH 5.0 for 4 h. Scale bar: 25 μm. (B) The corresponding Raman images. Scale bar: 10 μm. (C) Raman spectra collected in the (a) red, (b) yellow and (c) green colour regions of the MCF-7 cell shown in (B).

Fig. S8 Cytotoxicity of **1**@AuBP against MCF-7 cells upon incubation for 12 h both in the absence and presence of light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}).

Fig. S9 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **2** in $CDCl_3$.

Fig. S10 ESI mass spectrum of **2**.

Fig. S11 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **5** in $CDCl_3$.

Experimental section

General

N,*N*-Dimethylformamide (DMF) and 1-pentanol were distilled under reduced pressure from barium oxide and sodium respectively. Tetrahydrofuran (THF) was purified using an INERT solvent purification system. All other solvents and reagents were of reagent grade and used as received. All aqueous solutions were prepared using ultrapure water (18 MΩ, Milli-Q, Millipore). All the reactions were performed under an atmosphere of nitrogen and monitored by thin-layer chromatography (TLC; Merck precoated silica gel 60_{F254} plates). Chromatographic purification was performed with column chromatography on silica gel (Macherey-Nagel, 230-400 mesh) and/or size-exclusion chromatography on Bio-Beads S-X1 beads (200-400 mesh) with the indicated eluents. Compounds **3**[R1] and **4**[R2] and AuBPs[R3] were prepared according to the literature procedure.

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker AVANCE III HD 500 NMR spectrometer (^{1}H, 500 MHz; ^{13}C, 125.8 MHz) in $CDCl_3$ unless otherwise stated. Spectra were referenced internally by using the residual solvent (for ^{1}H, $\delta = 7.26$) or solvent (for ^{13}C, $\delta = 77.2$) resonance relative to $SiMe_4$. Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Finnigan MAT 95 XL mass spectrometer. For accurate mass measurements, the lowest *m*/*z* value of the isotopic envelope was reported and compared with the theoretical value. UV-Vis and steady-state fluorescence spectra were taken on a Cary 5G UV-Vis-near-IR spectrophotometer and a Hitachi F-7000 spectrofluorometer respectively.

Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai G2 Spirit transmission electron microscope operated at 120 keV acceleration voltage. The dark-field images were obtained by an inverted microscope (eclipse Ti-U, Nikon, Japan) equipped with a dark-field condenser ($0.8 < NA < 0.95$) and a 40× objective len (NA = 0.8), and a white light source (100 W halogen lamp) was used to excite the plasmon resonance scattering light of AuBPs. The fluorescence images were obtained on the same microscope using a mercury lamp (100 W Epi illuminator) as the excitation light source. Raman spectra were recorded on a Raman microspectrometer with an inverted microscope (Ti2-U, Nikon, Japan) and a Raman spectrograph (HRS-300S, TPI, USA). The system was equipped with a class IIIB laser (785 nm, 80 mW) with a 60× objective lens. The Raman cell images were obtained on a laser scanning confocal microscope (XploRA PLUS, Horiba, Japan). The fluorescence cell images were obtained on an Olympus FV1000 IX81-SIM confocal microscope equipped with a 635 nm diode laser. MTT assay was performed on a Tecan Spark 10 M microplate reader.

Preparation of 2

A mixture of **3** (50 mg, 0.12 mmol) and thioacetic acid (26 μL, 0.37 mmol) in acetone (20 mL) was heated under reflux for 1 h. The solvent was then removed under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/Et_3N (100:1 v/v) as the eluent to afford the product as a colourless oil (42 mg, 85%). ^{1}H NMR (400 MHz): δ 7.34 (d, J = 8.8 Hz, 2 H, ArH), 6.90 (d, J = 8.8 Hz, 2 H, ArH), 5.46 (s, 1 H, acetal-H), 4.10 (t, J = 6.4 Hz, 2 H, OCH_2), 3.57-3.63 (m, 2 H, OCH), 3.47-3.53 (m, 2 H, OCH), 3.42 (t, J = 6.8 Hz, 4 H, CH_2N_3),

3.27 (t, J = 6.4 Hz, 2 H, SCH_2), 2.37 (s, 3H, CH_3) 1.86 (quintet, J = 6.4 Hz, 4 H, CH_2). $^{13}C\{^1H\}$ NMR (100.6 MHz): δ 195.5, 158.7, 131.1, 128.0, 114.4, 101.7, 66.6, 62.1, 48.6, 30.7, 29.2, 28.6. HRMS (ESI): *m*/*z* calcd for $C_{17}H_{24}N_6NaO_4S$ $[M+Na]^+$ 431.1472, found 431.1468.

Preparation of phthalonitrile 5

A mixture of **4** (900 mg, 3.87 mmol), 4-nitrophthalonitrile (805 mg, 4.65 mmol) and K_2CO_3 (803 mg, 5.81 mmol) in DMF (50 mL) was stirred at 70 °C overnight. The solvent was then removed under vacuum. The residue was redissolved in CH_2Cl_2. The solution was then loaded onto a silica gel column using CH_2Cl_2/MeOH (20:1 v/v) as the eluent to afford the product as a colourless oil (300 mg, 22%). ^1H NMR (400 MHz): δ 7.70 (d, J = 8.0 Hz, 1 H, ArH), 7.31 (d, J = 1.6 Hz, 1 H, ArH), 7.23 (dd, J = 1.6 and 8.0 Hz, 1 H, ArH), 4.19-4.23 (m, 4 H, CH_2), 3.87-3.90 (m, 2 H, CH_2), 3.65-3.72 (m, 12 H, CH_2), 2.42 (t, J = 2.0 Hz, 1 H, C≡CH). $^{13}C\{^1H\}$ NMR (100.6 MHz): δ 162.1, 135.3, 119.9, 119.7, 117.5, 115.8, 115.4, 74.7, 71.1, 70.8, 70.7, 70.5, 69.4, 69.3, 68.8., 58.5. HRMS (ESI): *m*/*z* calcd for $C_{19}H_{22}N_2NaO_5$ $[M+Na]^+$ 381.1421, found 381.1417.

Preparation of phthalocyanine 6

A mixture of alkynyl phthalonitrile **5** (300 mg, 0.84 mmol), phthalonitrile (1.07 g, 8.35 mmol) and anhydrous zinc acetate (767 mg, 4.18 mmol) in 1-pentanol (150 mL) was heated to 80 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (375 μL, 2.51 mmol) was then added, and the mixture was stirred at 140 °C overnight. After brief cooling, the volatiles were removed under

reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL), and the solution was filtered. The filtrate was collected and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (50:1 v/v) as the eluent, followed by size-exclusion chromatography using THF as the eluent. The crude product was further purified by silica gel column chromatography using CH_2Cl_2/MeOH (50:1 v/v) as the eluent. The purified product was recrystallised from CH_2Cl_2 and hexane to give a dark green solid (66 mg, 10%). ^{1}H NMR (500 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.02-9.17 (m, 6 H, Pc-H$_\alpha$), 8.82 (d, *J* = 8.0 Hz, 1 H, Pc-H$_\alpha$), 8.36 (d, *J* = 1.5 Hz, 1 H, Pc-H$_\alpha$), 7.95-8.04 (m, 6 H, Pc-H$_\beta$), 7.48 (dd, *J* = 1.5 and 9.0 Hz, 1 H, Pc-H$_\beta$), 4.61 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.18-4.21 (m, 4 H, CH_2), 3.96 (t, *J* = 4.5 Hz, 2 H, CH_2), 3.85 (t, *J* = 4.5 Hz, 2 H, CH_2), 3.78-3.80 (m, 2 H, CH_2), 3.72-3.75 (m, 6 H, CH_2), 2.44 (t, *J* = 2.5 Hz, 1 H, C≡CH). ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 160.5, 153.5, 153.3, 153.2, 152.8, 152.7 ,140.3, 138.4, 138.3, 138.1, 131.6, 128.9, 128.8, 128.7, 123.5, 122.5, 122.4, 118.4, 105.4, 79.8, 74.7, 71.2, 71.0, 70.9, 70.8, 70.6, 70.2, 69.3, 68.2, 58.6 (some of the signals were overlapped). HRMS (ESI): *m*/*z* calcd for $C_{43}H_{34}N_8NaO_5Zn$ $[M+Na]^+$ 829.1836, found 829.1830.

Preparation of dimeric phthalocyanine 1

A mixture of CuI (2 mg, 11 μmol) and Et_3N (14 μL, 0.1 mmol) in DMF (2 mL) was prepared. This mixture (20 μL) was then added to a dispersion of **2** (10 mg, 24 μmol) and **6** (49 mg, 61 μmol) in DMF (5 mL). The resulting mixture was stirred at room temperature for 24 h. The

solvent was then removed in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (20:1 v/v) as the eluent, followed by size-exclusion chromatography using THF as the eluent. The crude product was further purified by silica gel column chromatography using CH_2Cl_2/MeOH (20:1 v/v) with a small amount of Et_3N as the eluent. The purified product was recrystallised from CH_2Cl_2 and hexane to give a dark green solid (15 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 8.87-9.03 (m, 12 H, Pc-H$_\alpha$), 8.59 (d, J = 4.4 Hz, 2 H, Pc-H$_\alpha$), 8.09 (s, 2 H, Pc-H$_\alpha$), 7.84-7.98 (m, 12 H, Pc-H$_\beta$), 7.42 (s, 2 H, triazole-H), 7.34 (d, J = 4.4 Hz, 2 H, Pc-H$_\beta$), 7.12 (d, J = 6.8 Hz, 2 H, ArH), 6.76 (d, J = 6.8 Hz, 2 H, ArH), 5.18 (s, 1 H, acetal-H), 4.53-4.57 (m, 4 H, CH_2), 4.47-4.50 (m, 4 H, CH_2), 4.23 (t, J = 4.0 Hz, 4 H, CH_2), 4.17-4.19 (m, 4 H, CH_2), 3.96-3.98 (m, 6 H, CH_2), 3.86 (t, J = 4.0 Hz, 4 H, CH_2), 3.78 (t, J = 4.0 Hz, 4 H, CH_2), 3.71 (t, J = 4.0 Hz, 4 H, CH_2), 3.63-3.66 (m, 8 H, CH_2), 3.30-3.32 (m, 2 H, CH_2), 3.17-3.19 (m, 4 H, CH_2), 2.31 (s, 3 H, CH_3), 1.92-1.94 (m, 4 H, CH_2). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 195.5, 160.2, 158.6, 153.0, 152.9, 152.8, 152.4, 152.3, 152.2, 144.9, 139.9, 138.2, 138.1, 138.0, 137.8, 131.3, 128.8, 128.7, 128.6, 128.5, 127.8, 125.6, 122.9, 122.4, 122.3, 122.2, 118.0, 114.3, 105.0, 101.7, 71.2, 70.9, 70.8, 70.6, 70.1, 69.8, 68.1, 66.5, 64.5, 61.6, 47.2, 34.3, 30.7, 30.4, 30.2, 28.5 (some of the signals were overlapped). HRMS (ESI): m/z calcd for $C_{103}H_{92}N_{22}NaO_{14}SZn$ $[M+Na]^+$ 2047.5351, found 2047.5344.

Preparation of 1@AuBP

A suspension of AuBPs (2.3 nM, 500 μL) was mixed with **1** in phosphate-buffered saline (PBS)

(3 μM, 500 μL) in the presence of 0.3% Tween 80 (v/v), and the mixture was stirred at room temperature overnight. PBS (0.1 mL) containing 2 M NaCl (5 μL) was then added to stabilise the nanoparticles. The mixture was centrifuged, and the nanoparticles collected were washed with PBS twice. The resulting nanoparticles were resuspended in PBS (1 mL) for further use.

pH-Responsive studies in solution

A stock solution of **1**@AuBP in PBS (2.3 nM) at pH 7.4 was prepared. Its pH was adjusted to different values (6.5, 6.0, 5.5 and 5.0) with 0.1 M HCl. The fluorescence spectra of these solutions were then recorded (from 630-800 nm) upon excitation at 610 nm. For the solution at pH 5.0, the fluorescence spectra were also recorded at different time points over a period of 24 h. The SERS spectra were recorded with a detection time of 10 s.

Density functional theory calculations

DFT calculations were performed using the Gaussian 09 suite of programme[R4] at the level of B3LYP/3-21g to simulate the Raman spectra. Regarding the effect of the aqueous environment, the integral equation formalism version of polarisable continuum model (IEF-PCM)[R5] implemented in Gauss 09 was included for the geometry optimisation and the vibrational frequency calculations of the most stable geometry. The Raman shifts were achieved based on all the calculated vibrational frequencies which were scaled by a factor of 0.967[R6] according to the utilised functional and basis set. The vibrational modes corresponding to the calculated peaks were subsequently analysed.

Cell lines and culture conditions

MCF-7 and Huh7 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (ThermoFisher Scientific, cat. no. 12100-046) supplemented with fetal bovine serum (FBS) (10%, Invitrogen, cat. no. 10270-106) and penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively). The cells were grown at 37 °C in a humidified 5% CO_2 atmosphere.

In vitro pH-responsive studies

Approximately 1×10^6 MCF-7 cells in DMEM (0.5 mL) were seeded on a confocal dish and incubated overnight at 37 °C with 5% CO_2. **1**@AuBP in PBS at pH 7.4 (2.3 nM, 200 μL) was then added into each cell-adhered dish for incubation at 37 ℃ for different periods of time. The cells were examined with an inverted microscope with an external triple channel optical system for confocal imaging. The detection time for all the SERS spectra was 10 s. To examine the pH effect, approximately 5×10^5 MCF-7 cells per well in the culture medium were seeded on 6-well plates and incubated overnight at 37 ℃ under 5% CO_2. The cells were first incubated with nigericin (10 nM) for 30 min. After being rinsed with PBS twice, the cells were incubated with **1**@AuBP in PBS at 7.4, 6.5, 6.0, 5.5 and 5.0 (2.3 nM, 200 μL) for 2 h. The confocal images and Raman spectra were then recorded as described above.

Photocytotoxicity assay

The cytotoxicity of **1**@AuBP against MCF-7 cells was examined by a MTT assay.[R7] Approximately 3×10^5 MCF-7 cells in DMEM were inoculated per well in 96-well plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. **1**@AuBP was diluted to different concentrations with the culture medium. The cells were incubated with 100 μL of these solutions for 12 h at 37 °C under 5% CO_2. The cells were then rinsed with PBS and refed with 100 μL of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter

(Newport) cut on at λ = 610 nm. The fluence rate (λ > 610 nm) was 40 mW cm^{-2}. Illumination of 20 min led to a total fluence of 48 J cm^{-2}. After illumination, the cells were incubated at 37 ^{o}C under 5% CO_2 overnight. The medium was discarded, and a fresh medium (50 μL) containing MTT (0.5 mg mL^{-1}) was added to each well, followed by incubation for 4 h under the same environment. The medium was removed, and the purple formazan crystals were dissolved with dimethyl sulfoxide (100 μL). The absorbance at 540 nm was measured using a Bio-Rad microplate reader. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of other wells. The relative cell viability (%) was calculated by $[\Sigma (A_{test}/A_{control} \times 100)]/n$, where A_{test} is the absorbance of the i^{th} datum (i = 1, 2, ..., n), $A_{control}$ is the average absorbance of control wells in which the photosensitiser was absent, and n (= 4) is the number of data points.

CCK-8 assay and EdU incorporation assay

The cell viability against Huh7 cells was assessed using the cell counting kit-8 (CCK-8, Dojindo). The cells were seeded in 96-well plates and cultured overnight. They were then incubated with different concentrations of **1**@AuBP for 12 h, followed by light irradiation (λ > 610 nm, 20 min). After that, 10 μL of CCK-8 solution diluted with 90 μL of DMEM was added into each well. The optical density at 450 nm was then measured by a spectrophotometer. Cell proliferation was further determined by incorporation of 5-ethynyl-2'-deoxyuridine (EdU) with an EdU cell proliferation assay kit (RiboBio). Briefly, 4×10^4 Huh7 cells per well were seeded in a 24-well plate and left to adhere for 12 h. Culture medium with or without **1**@AuBP (0.23 nM) was added, and then the cells were incubated for 8 h. The cells were then rinsed with fresh medium before being illuminated using the above-mentioned light source for 20 min. After 12

h, the culture medium was replaced with a fresh medium containing 50 μM EdU. After incubation for 4 h, the cells were rinsed with PBS twice and fixed using 4% (w/v) paraformaldehyde in PBS. The analysis was then conducted according to the manufacturer's protocol. The images were obtained on a EVOS cell imaging system (Life Technologies) automatically with 8-10 frames taken per well. The images were analysed using the ImageJ software.

TUNEL assay

Cell apoptosis was studied using a TUNEL FITC apoptosis detection kit (Vazyme). In brief, 1 × 10^5 Huh7 cells per well were seeded in a 24-well plate and left to adhere for 12 h. Culture medium with or without **1**@AuBP (0.23 nM) was added, and then the cells were incubated for 8 h. After replacing the medium with a fresh medium, the cells were illuminated for 20 min using the light source described above. After 12 h, the cells were rinsed with PBS twice and fixed using 4% (w/v) paraformaldehyde in PBS. The analysis was then conducted according to the manufacturer's protocol. The images were obtained on a EVOS cell imaging system (Life Technologies) automatically with 8-10 frames taken per well. The images were analysed using the ImageJ software.

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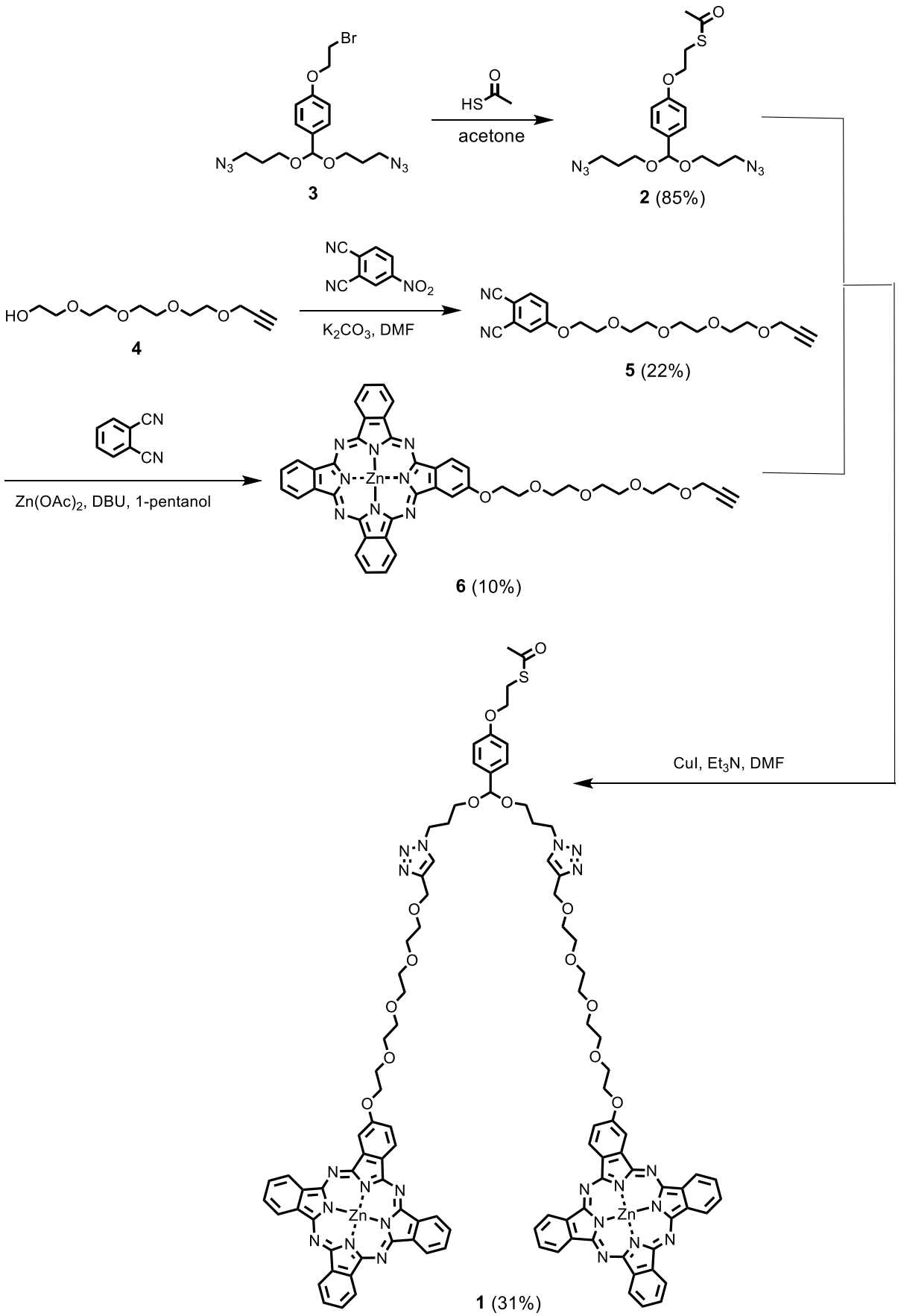

Scheme S1. Synthetic route for dimeric phthalocyanine **1**.

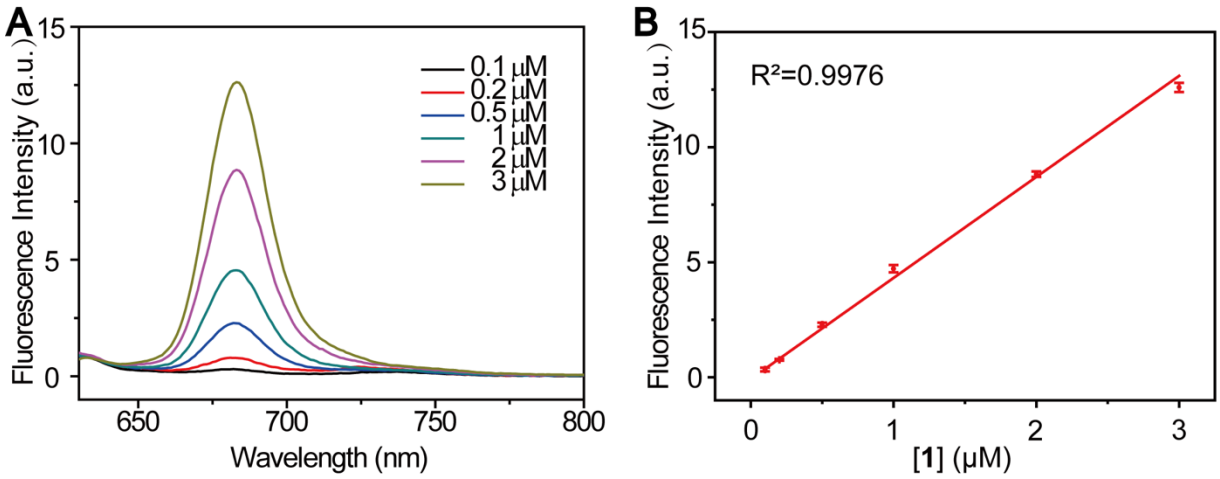

Fig. S1 (A) Fluorescence spectra of **1** in PBS with 0.3% Tween 80 (v/v) at different concentrations (λ_{ex} = 610 nm). (B) Plot of the fluorescence intensity at 690 nm versus the concentration of **1** in PBS.

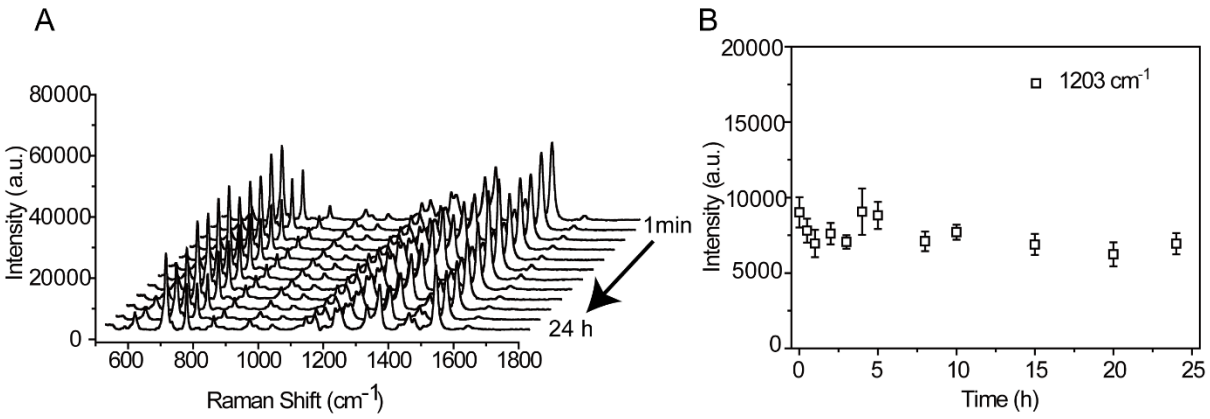

Fig. S2 (A) Change in SERS spectrum of **1**@AuBP (2.3 nM) in PBS with 0.15% Tween 80 (v/v) over a period of 24 h at room temperature. (B) Variation of the SERS intensity of **1**@AuBP at 1203 cm^{-1} over a period of 24 h at room temperature.

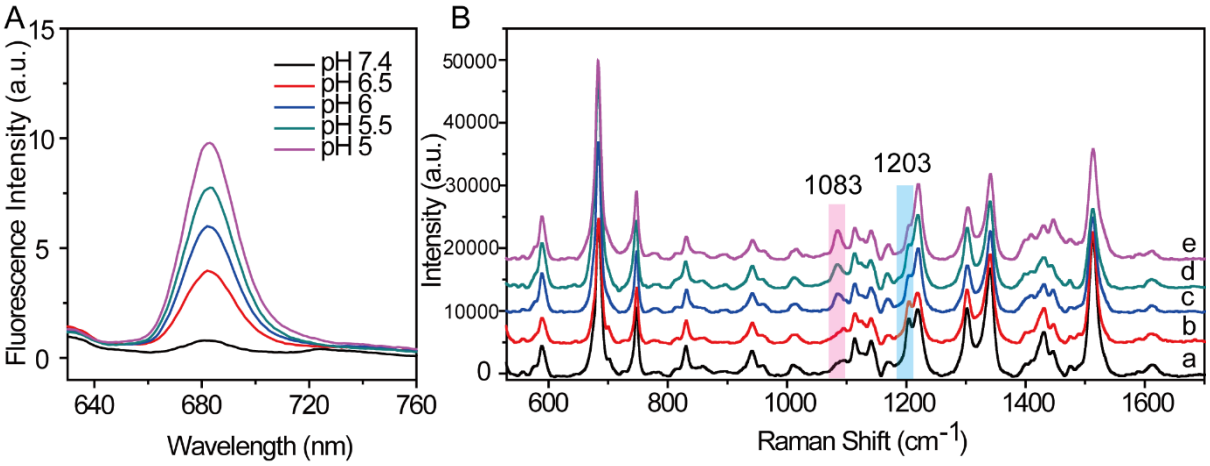

Fig. S3 (A) Fluorescence (λ_{ex} = 610 nm) and (B) SERS spectra of **1**@AuBP (2.3 nM) in PBS at different pH: (a) 7.4, (b) 6.5, (c) 6, (d) 5.5 and (e) 5. The spectra were recorded at 24 h after adjusting the pH.

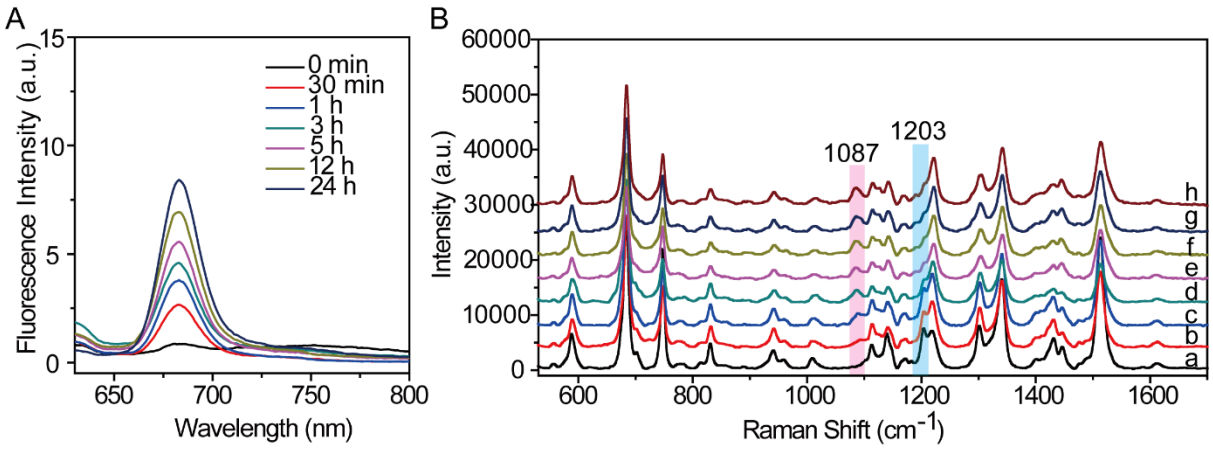

Fig. S4 Changes in (A) fluorescence (λ_{ex} = 610 nm) and (B) SERS spectra of **1**@AuBP (2.3 nM) in PBS at pH 5 over a period of 24 h. The SERS spectra were recorded at (a) 0 min, (b) 30 min, (c) 1 h, (d) 2 h, (e) 5 h, (f) 7 h, (g) 12 h and (h) 24 h after preparing the solution.

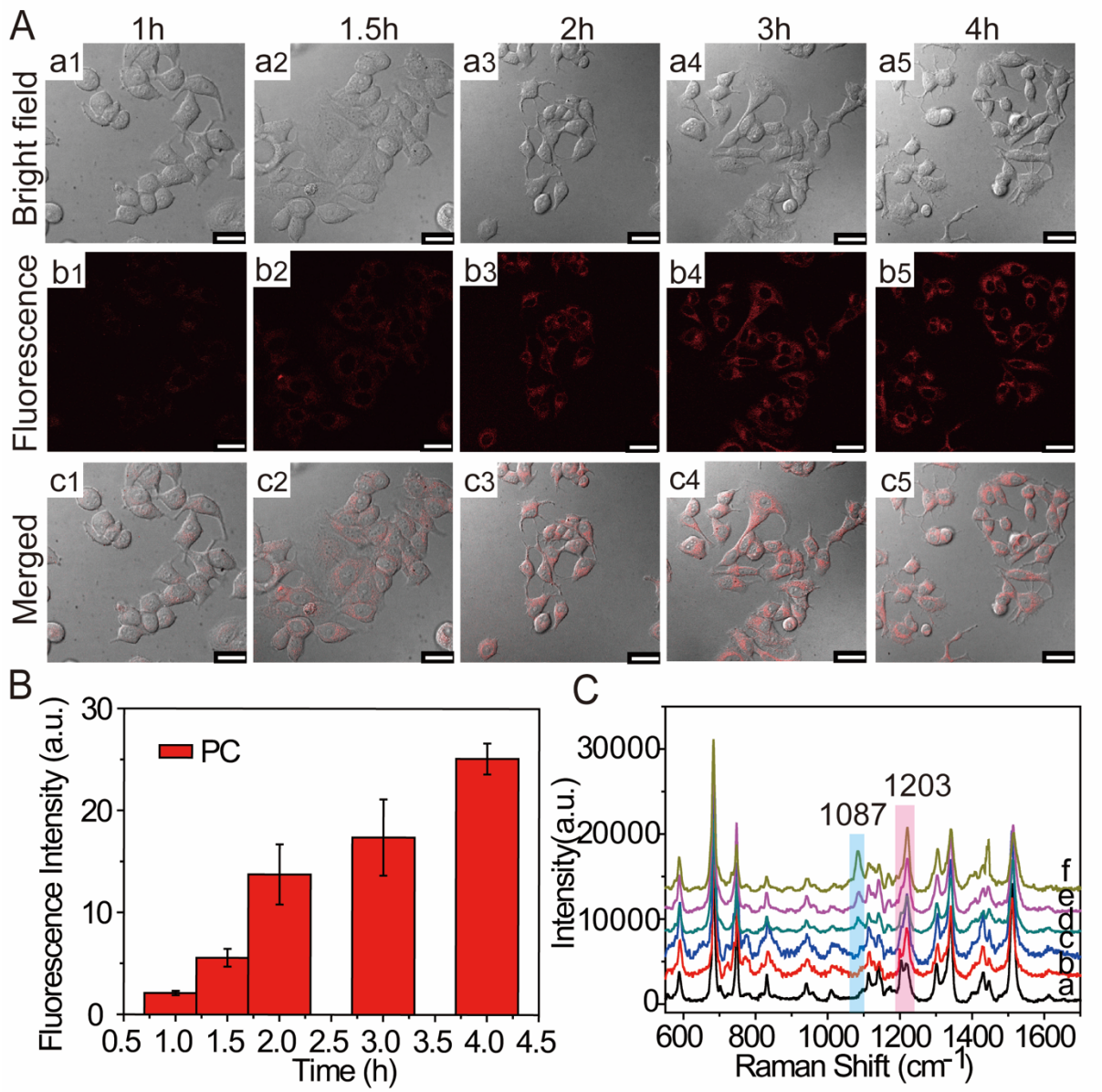

Fig. S5 (A) Bright-field, fluorescence and the merged confocal images of MCF-7 cells after incubation with **1**@AuBP in PBS at pH 7.4 (2.3 nM, 200 μL) for different periods of time. Scale bar: 25 μm. (B) Corresponding quantified fluorescence intensities. Data are reported as the mean ± standard deviation of three independent experiments. (C) SERS spectra recorded after incubating MCF-7 cells with **1**@AuBP in PBS (2.3 nM, 200 μL) for different periods of time: (a) 0 min, (b) 1 h, (c) 1.5 h, (d) 2 h, (e) 3 h and (f) 4 h.

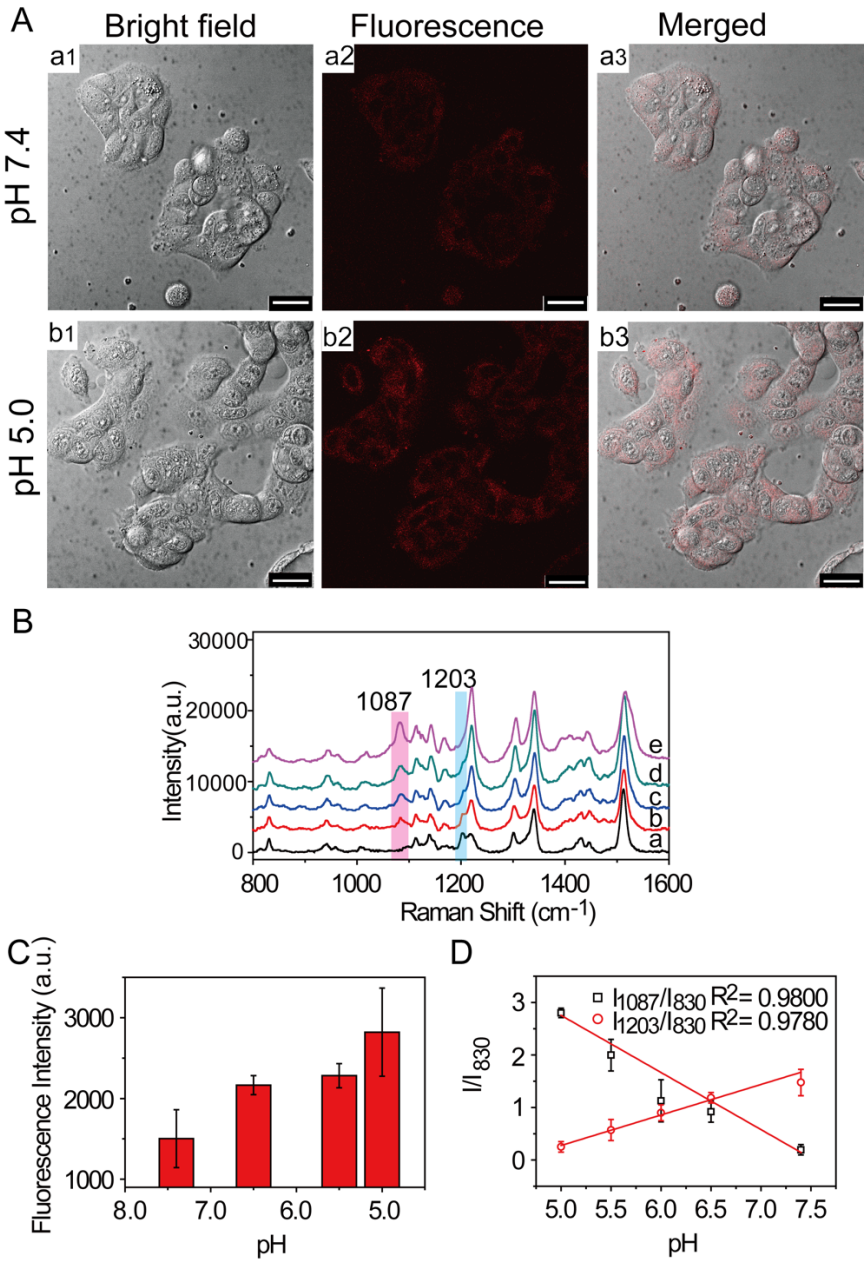

Fig. S6 (A) Bright-field, fluorescence and the merged confocal images of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH 7.4 or 5.0 for 2 h. Scale bar: 25 μm. (B) SERS spectra of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH (a) 7.4, (b) 6.5, (c) 6, (d) 5.5 and (e) 5 for 2 h. (C) Quantified intracellular fluorescence intensities of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at different pH for 2 h. Data are reported as the mean ± standard deviation of three independent experiments. (D) Plots of the ratiometric intensities of I_{1087}/I_{830} and I_{1203}/I_{830} versus the pH of the medium.

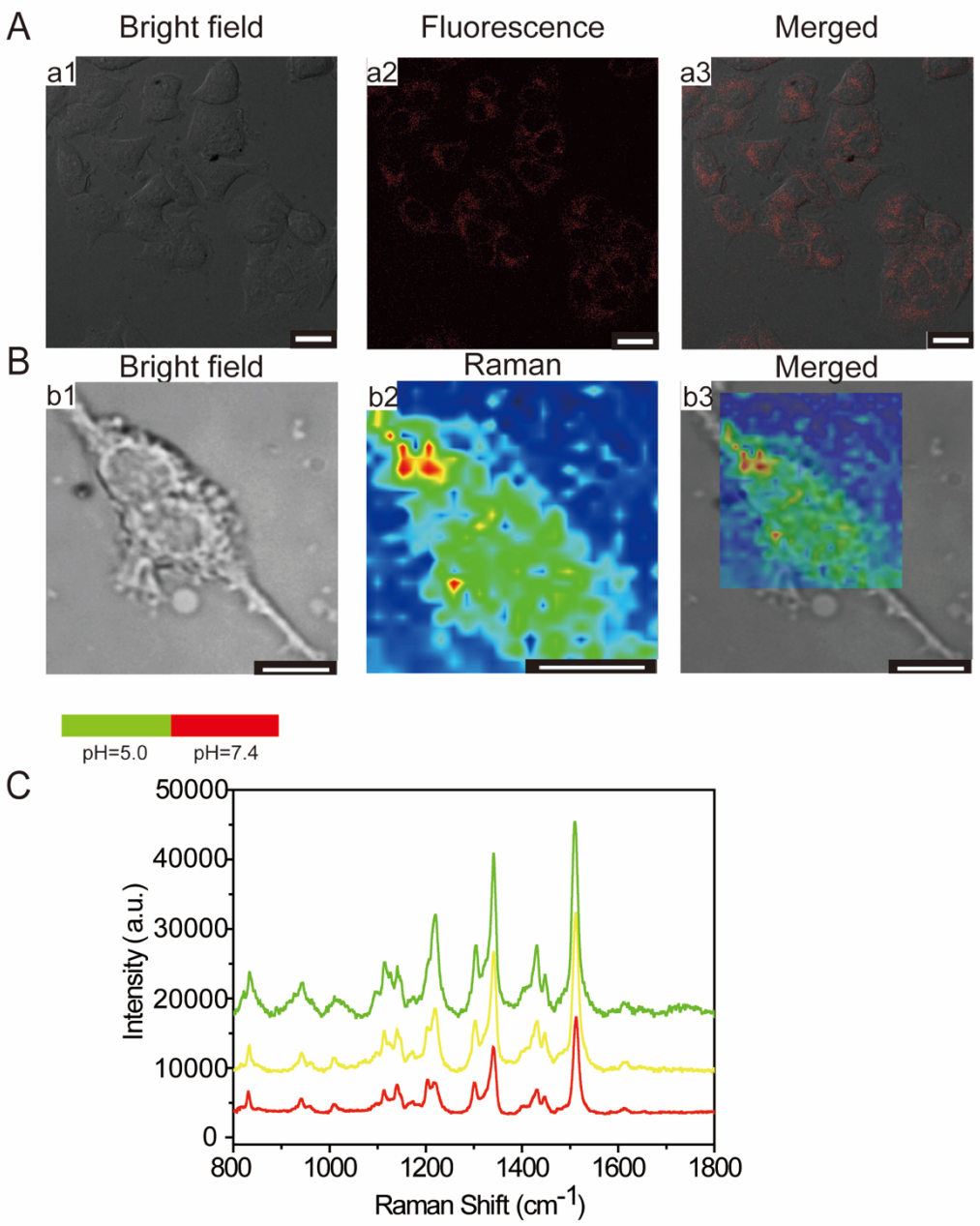

Fig. S7 (A) Bright-field, fluorescence and the merged confocal images of MCF-7 cells after incubation with nigericin (10 nM) for 30 min, followed by incubation with **1**@AuBP (2.3 nM) in PBS at pH 5.0 for 4 h. Scale bar: 25 μm. (B) The corresponding Raman images. Scale bar: 10 μm. (C) Raman spectra collected in the (a) red, (b) yellow and (c) green colour regions of the MCF-7 cell shown in (B).

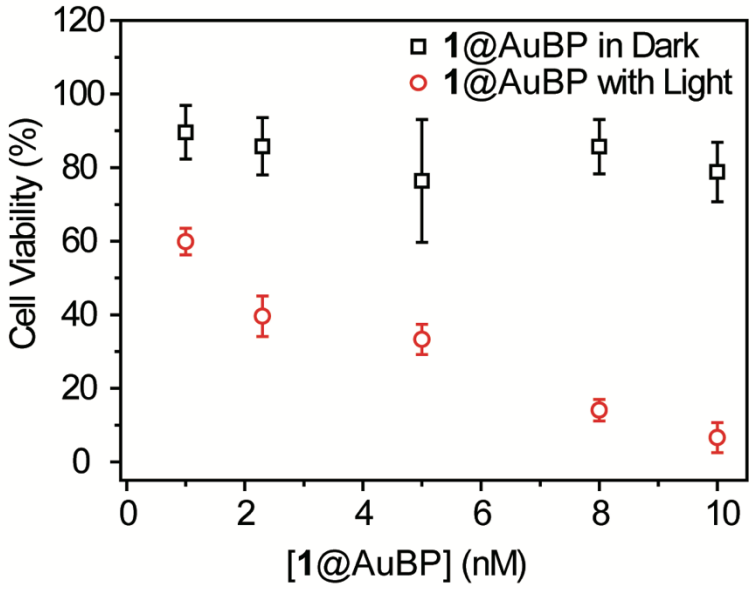

Fig. S8 Cytotoxicity of **1**@AuBP against MCF-7 cells upon incubation for 12 h both in the absence and presence of light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are reported as the mean ± standard error of the mean of three independent experiments, each performed in quadruplicate.

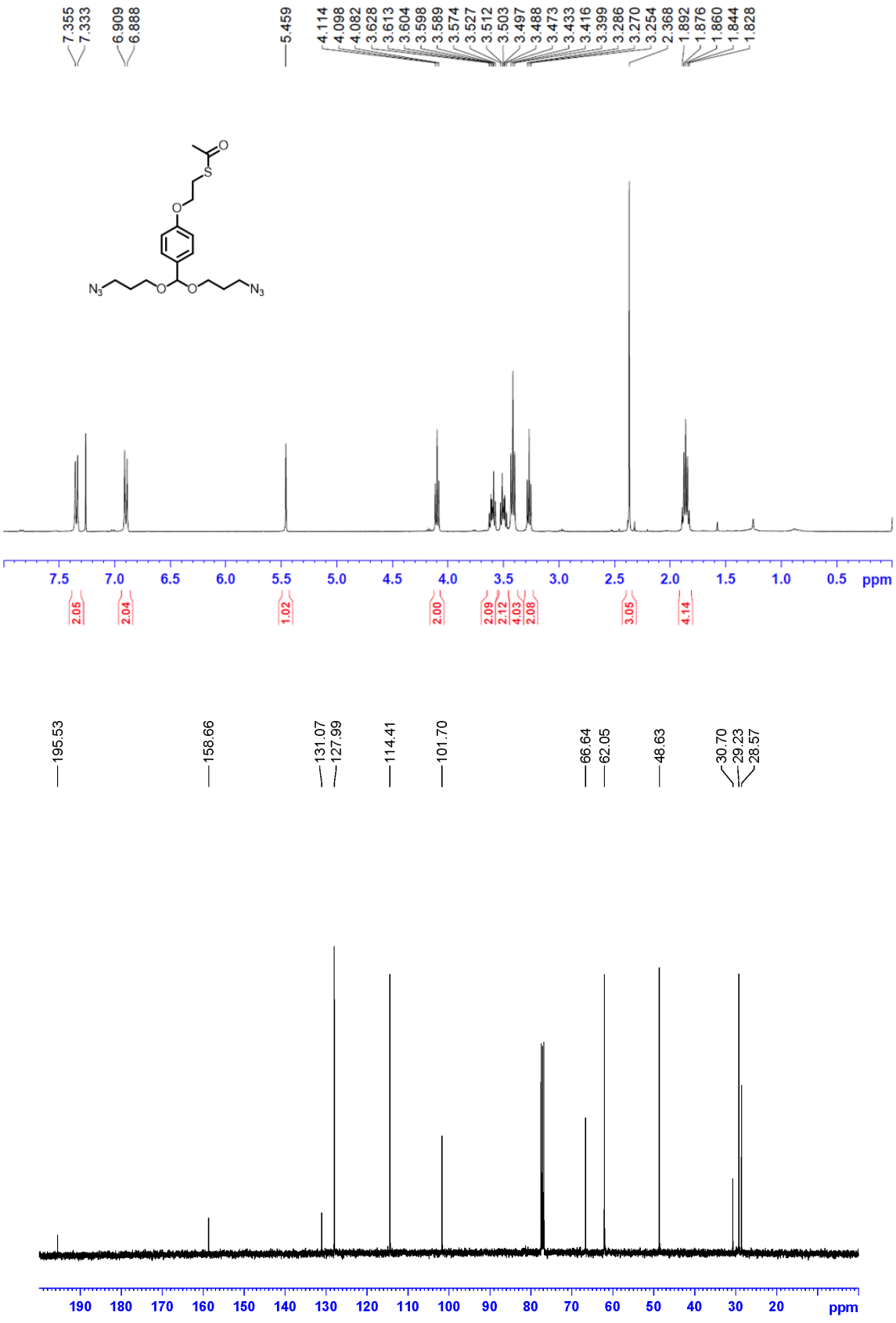

Fig. S9 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **2** in $CDCl_3$.

Thermo QEFMS Analysis Report

Analysis Info

Sample Name :	3	Reference No.:	Wqkpn081
Instrument :	Q Exactive Focus Orbitrap		
Source :	HESI II	Polarity :	positive
Comment :	ESI pos, 3.5kV, by LC, with sheath gas		

Accurate Mass Measurement

Molecular formula :	$C_{17}H_{24}N_6O_4S$
Experimental Mass $[M+Na]^+$:	431.14681
Theoretical Mass $[M+Na]^+$:	431.14720
Error (ppm) :	0.9

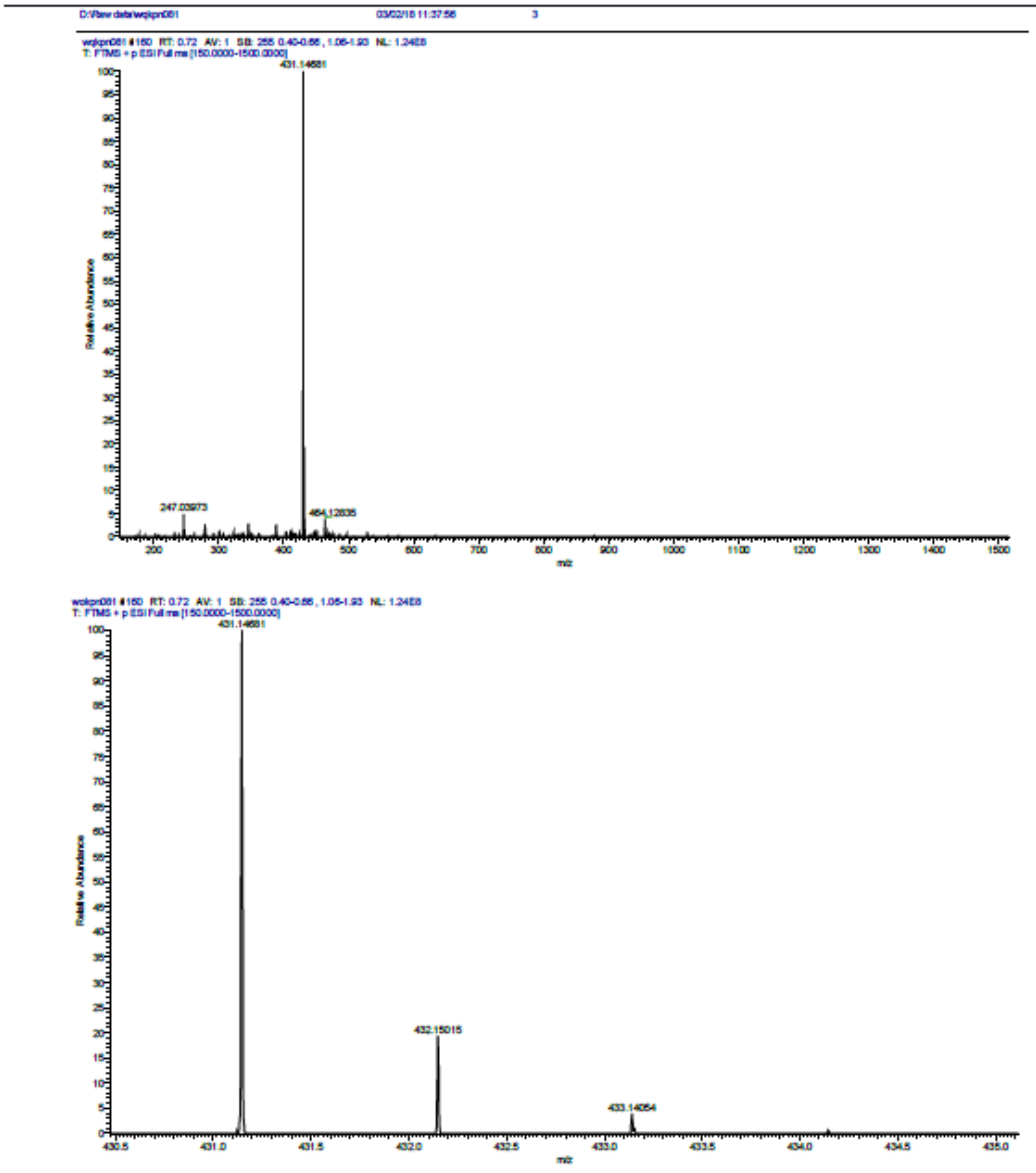

Fig. S10 ESI mass spectrum of **2**. The isotopic pattern of the M^+ envelope is shown in the lower part.

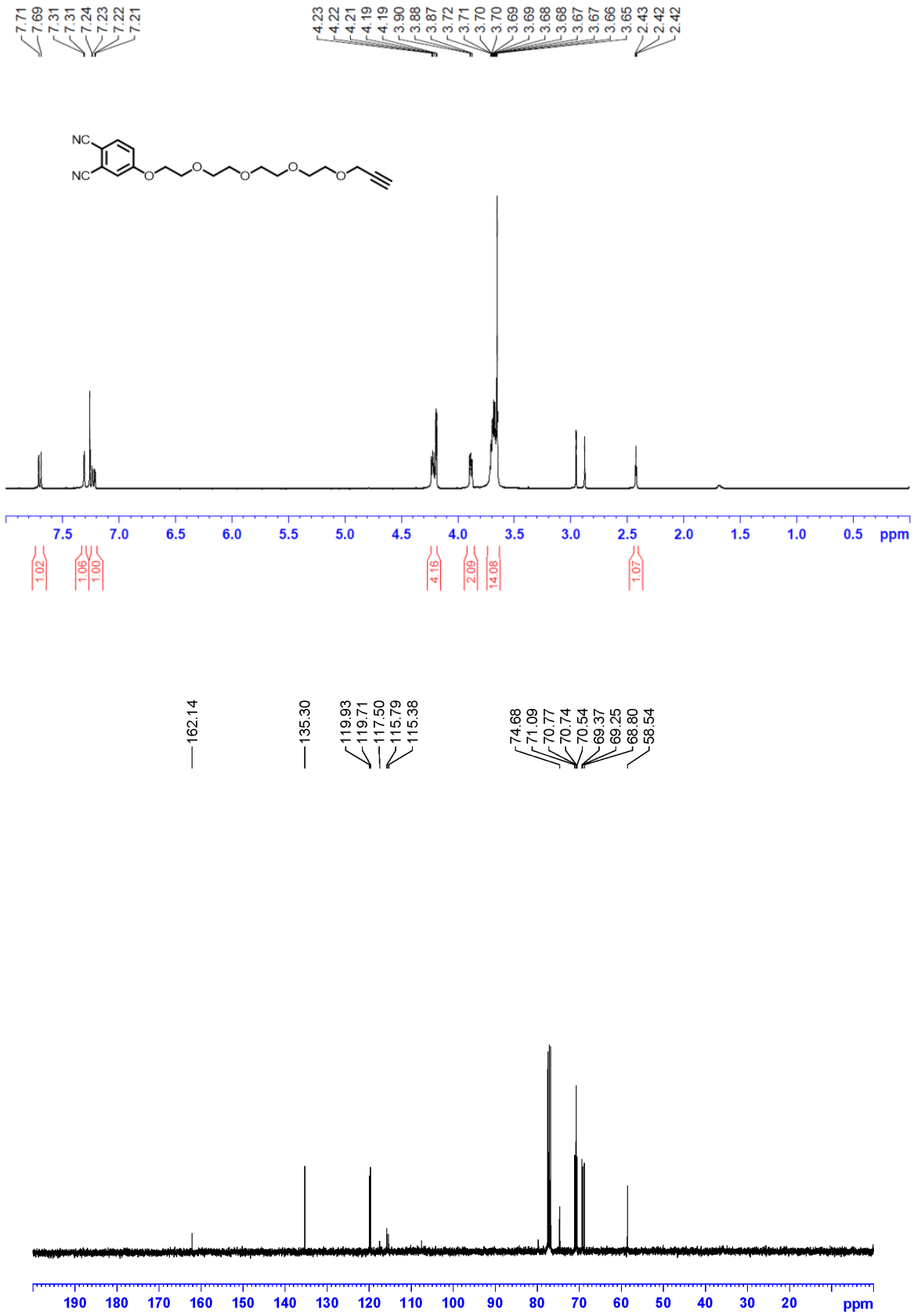

Fig. S11 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **5** in $CDCl_3$.

Thermo QEFMS Analysis Report

Analysis Info

Sample Name :	1	Reference No.:	Wqkpn079
Instrument :	Q Exactive Focus Orbitrap		
Source :	HESI II	Polarity :	positive
Comment :	ESI pos, 3.5kV, by LC, with sheath gas		

Accurate Mass Measurement

Molecular formula :	$C_{19}H_{22}N_2O_5$
Experimental Mass $[M+Na]^+$:	381.14172
Theoretical Mass $[M+Na]^+$:	381.14209
Error (ppm) :	1.0

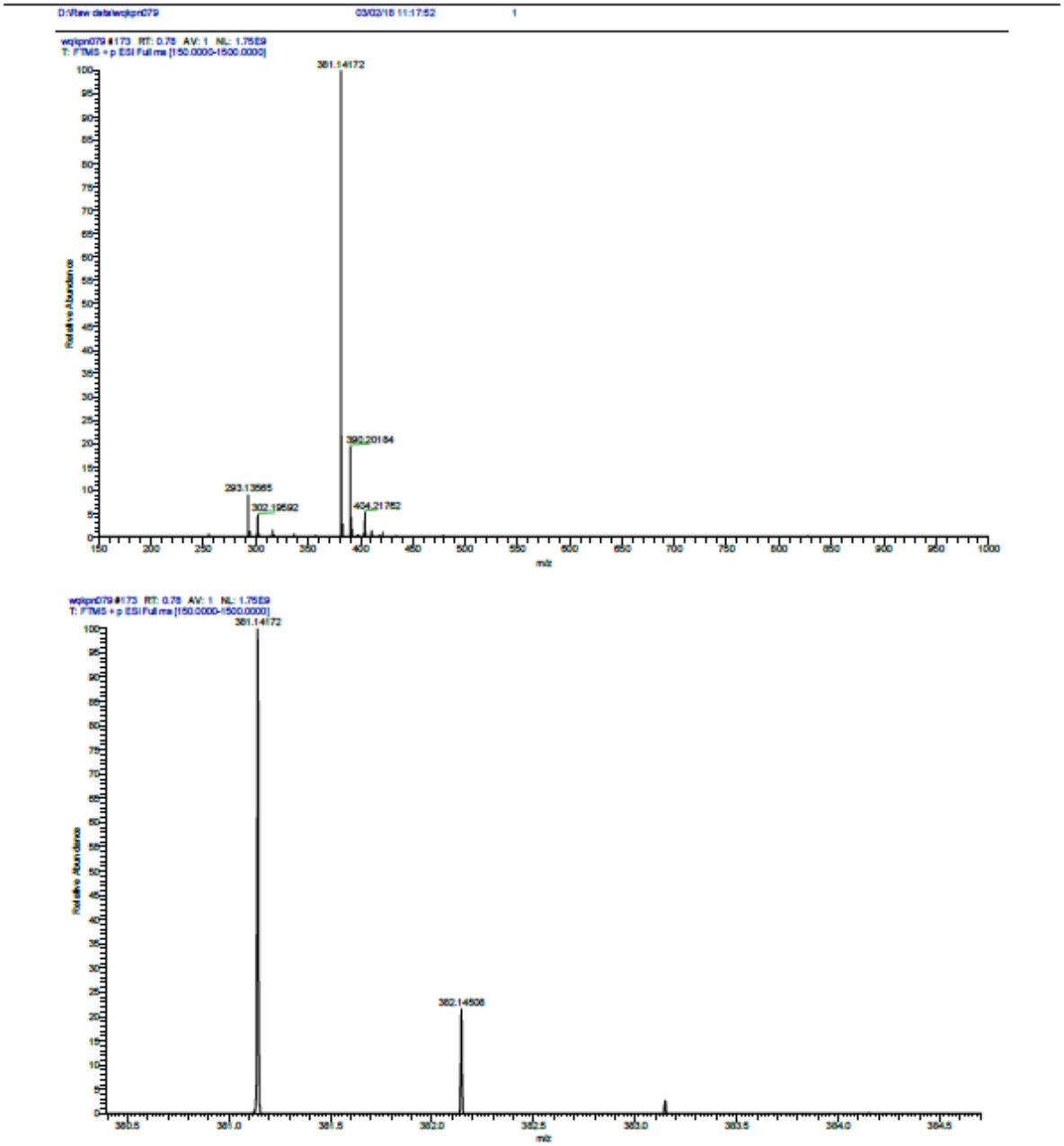

Fig. S12 ESI mass spectrum of **5**. The isotopic pattern of the M^+ envelope is shown in the lower part.

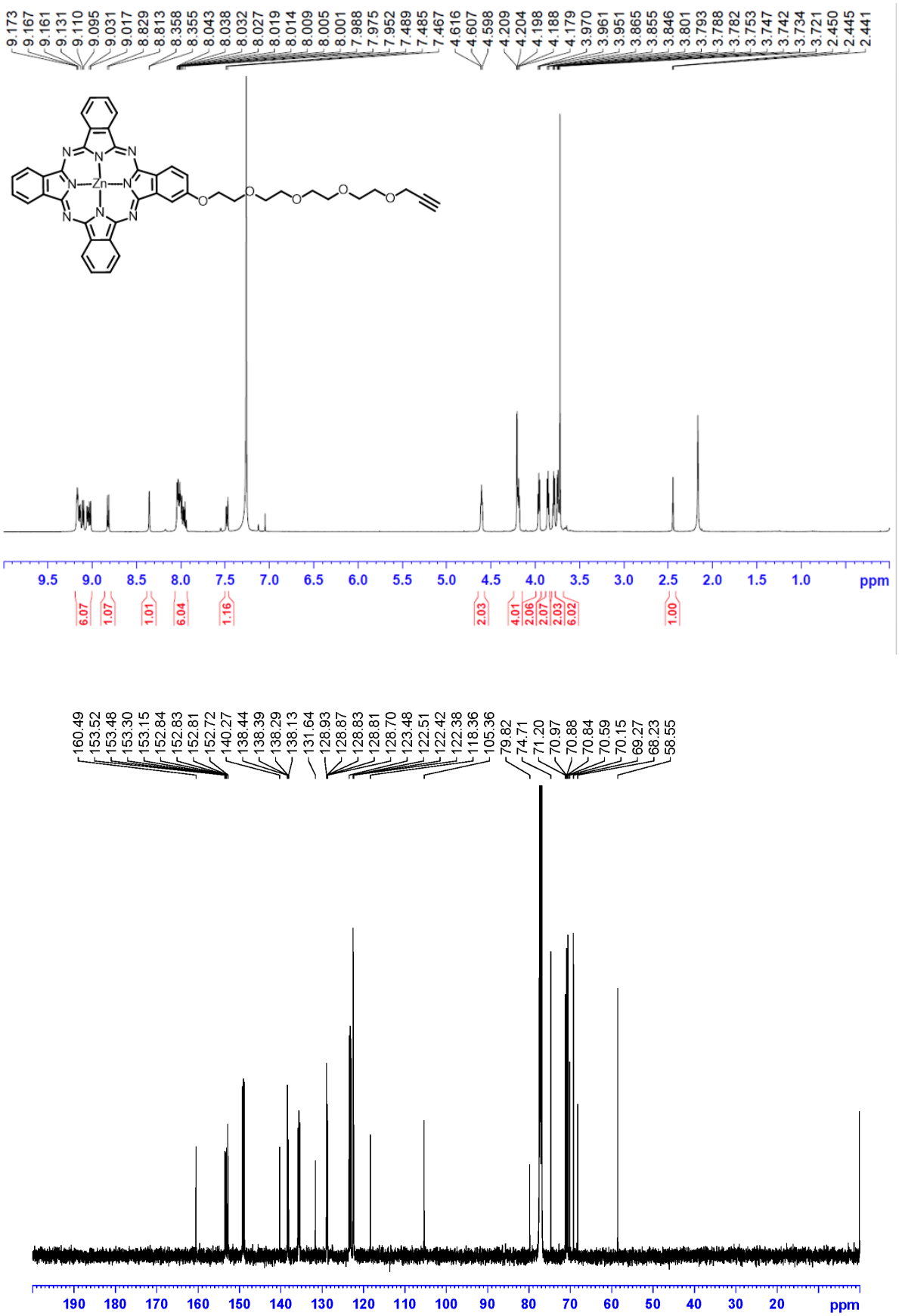

Fig. S13 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **6** in $CDCl_3$ with a trace amount of pyridine-d_5.

Thermo QEFMS Analysis Report

Analysis Info

Sample Name :	2	Reference No.:	Wqkpn080
Instrument :	Q Exactive Focus Orbitrap		
Source :	HESI II	Polarity :	positive
Comment :	ESI pos, 3.5kV, by LC, with sheath gas		

Accurate Mass Measurement

Molecular formula :	$C_{43}H_{34}N_8O_5Zn$
Experimental Mass $[M+Na]^+$:	829.18302
Theoretical Mass $[M+Na]^+$:	829.18358
Error (ppm) :	0.7

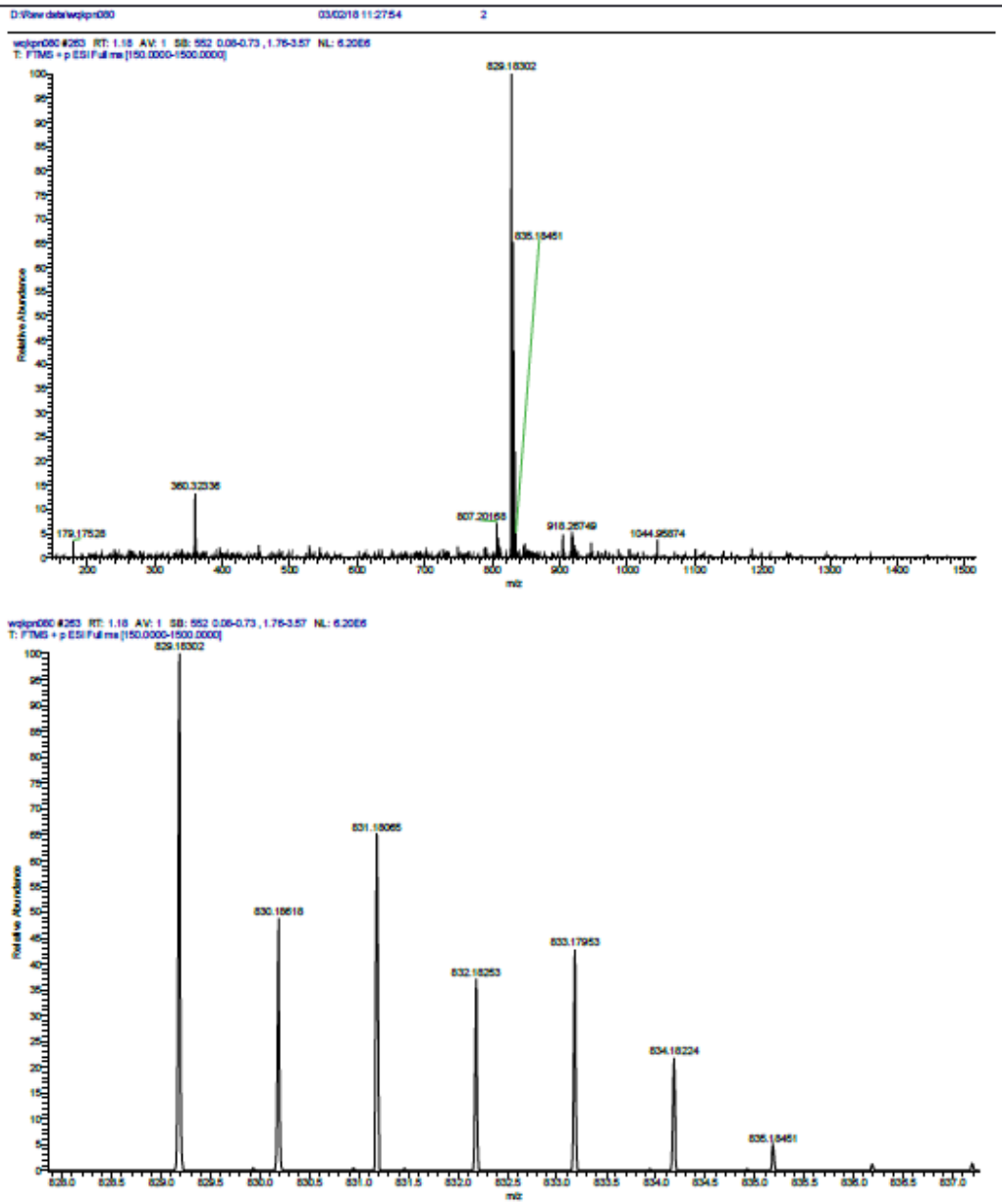

Fig. S14 ESI mass spectrum of **6**. The isotopic pattern of the M^+ envelope is shown in the lower part.

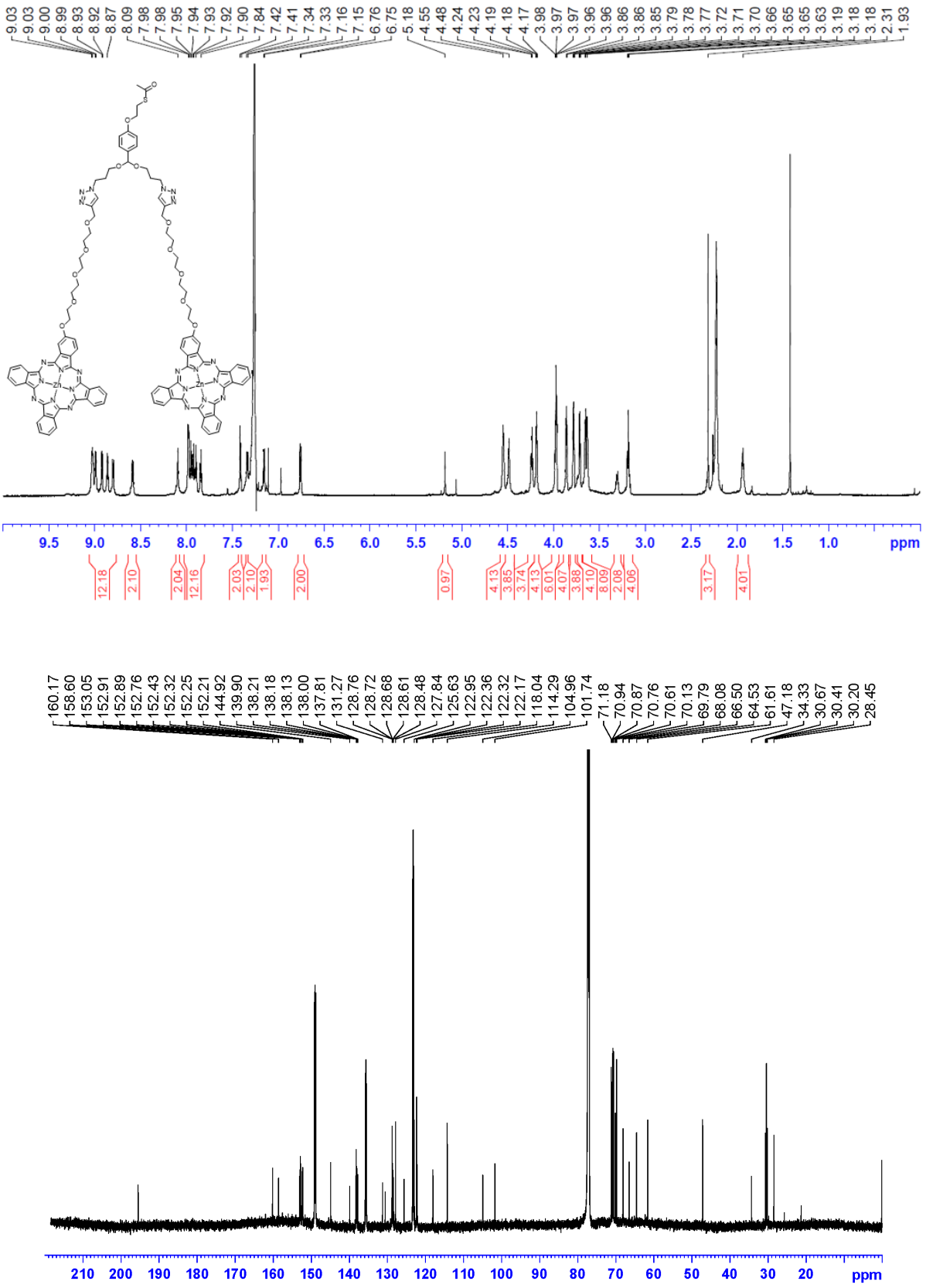

Fig. S15 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **1** in $CDCl_3$ with a trace amount of pyridine-d_5.

Thermo QEFMS Analysis Report

Analysis Info

Sample Name :	1	Reference No.:	Wqkpn087
Instrument :	Q Exactive Focus Orbitrap		
Source :	HESI II	Polarity :	positive
Comment :	ESI pos, 3.5kV, by infusion, with sheath gas		

Accurate Mass Measurement

Molecular formula :	$C_{103}H_{92}N_{22}O_{14}SZn_2$
Experimental Mass $[M+Na]^+$, $[M]^+$:	2047.53444, 2024.54788
Theoretical Mass $[M+Na]^+$, $[M]^+$:	2047.53513, 2024.54536
Error (ppm) :	0.3, 1.2

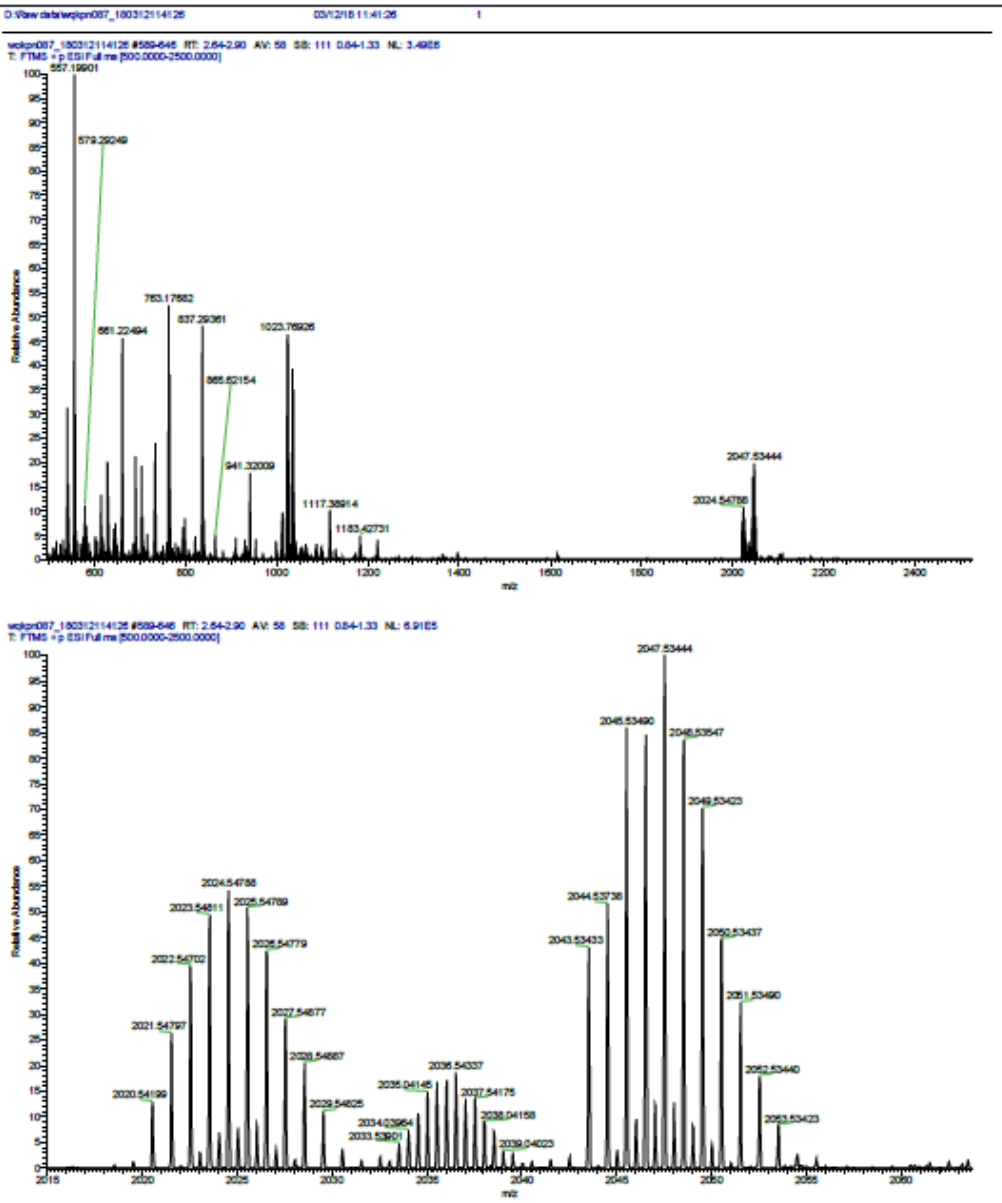

Fig. S16 ESI mass spectrum of **1**. The isotopic pattern of the M^+ envelope is shown in the lower part.

Table S1. Experimental and calculated SERS bands of **1**@AuBP and the corresponding assignments.

Experimental value (cm^{-1})	Calculated value (cm^{-1})	Assignment
588	597	Pc in-plane symmetric breathing; C-O-C rock
683	678	Pc in-plane symmetric stretching
747	759	Pc in-plane symmetric bending
830	840	Pc in-plane symmetric breathing
942	972	C-O-C asymmetric stretching; Pc in-plane asymmetric bending
1018	1029	benzene ring symmetric bending
1113	1145	Pc in-plane symmetric bending; CH_2 rocking
1141	1173	Pc in-plane symmetric stretching
1172	1212	benzene ring symmetric bending
1203	1234	Pc in-plane symmetric bending
1219	1251	C-O-C asymmetric stretching; benzene ring breathing
1302	1312	Pc in-plane symmetric breathing and stretching
1340	1360	Pc in-plane symmetric stretching
1414	1452	Pc in-plane symmetric bending
1445	1479	Pc in-plane symmetric bending
1513	1580	Pc in-plane symmetric stretching
1614	1665	benzene ring symmetric stretching