CHEMISTRY
A European Journal

Supporting Information

# Hyperpolarized $^{129}Xe$ Magnetic Resonance Imaging Sensor for $H_2S$

Shengjun Yang,[a] Yaping Yuan,[a] Weiping Jiang,[a] Lili Ren,[a] He Deng,[a] Louis S. Bouchard,[b] Xin Zhou,*[a] and Maili Liu*[a]

chem_201605768_sm_miscellaneous_information.pdf

# Supplementary information

**Contents:**

**Materials and instruments**

All the chemicals were purchased from commercial supplier and used as-is. Thin-layer chromatography (TLC) analysis was carried out on pre-coated silica plates. Column chromatography was performed using silica gel (200-300 mesh) using eluents in the indicated v:v ratio.

$^{1}H$ and $^{13}C$ NMR spectra were acquired on a Bruker AMX-500 NMR spectrometer at room temperature. Chemical shifts ($\delta$) are given in parts per million (ppm) relative to internal TMS (0 ppm for $^{1}H$) or $CDCl_3$/DMSO-$_{d6}$ (77 ppm/39.5 ppm for $^{13}C$). High-resolution mass spectrometry (HR MS-ESI) spectra were taken on a Agilent technologies 6530 Accurate-Mass Q-TOF LC/MS. pH were measured by Mettler Toledo SevenEasy pH meter. The fluorescence measurements were performed on a Edinburgh Instruments FS5 fluorescence spectrometer. The UV-visible absorption spectra were recorded on an Evolution 220 spectrophotometer (Thermofisher scientific). Quartz cuvettes with a 1-cm path length and 3-mL volume were used for optical spectra measurements. The cells were cultured in Heracell 150i incubator (Thermofisher Scientific), centrifuged by Legend Mircro 17 centrifuge (Thermofisher scientific) and counted by Nexcelom bioscience cellometer. Solutions and buffers were prepared with distilled water passed through Milli-Q ultrapurification system.

**Experimental section**

**Optical experiments.** Sensor 1 was dissolved into DMSO (HPLC grade) to prepare the stock solution with a concentration of 1.0 mM, which was diluted to the desired concentration for measurement. All fluorescence and absorption measurements were carried out at room temperature. For fluorescence experiments, samples were excited at 435 nm and the slit widths of excitation and emission is 2 nm and 2 nm, respectively. The emission spectrum was scanned from 450 nm to 750 nm.

**$^{129}$Xe NMR and MRI experiments.** A homebuilt continuous-flow apparatus was used in this study to produce hyperpolarized $^{129}Xe$ fluid by spin-exchange optical pumping method using 86%-enriched $^{129}Xe$ (26.4% natural abundance of $^{129}Xe$) gas mixture consisting of 2% Xe, 10% $N_2$ and 88% He.[1,2] The average value of the $^{129}Xe$ nuclear-spin polarization generated by this setup was approximately 20%. The temperature in the pumping cell was 418 K and the pressure was 47 PSI.

All $^{129}Xe$ NMR and MRI measurements were carried on a Bruker Avance 400 MHz (9.4 T) NMR spectrometer (Ettlingen, Germany) equipped with microimaging gradient coils. RF pulse frequency for $^{129}Xe$ was 110.7 MHz. Unless otherwise stated, $^{129}Xe$ NMR spectra were acquired using a 10 mm double resonant probe ($^{129}Xe$ and $^{1}H$, PA BBO 400 W1/S2 BB-H-D-10Z) with rectangle pulse of flip angle (90°).

A 10 mm tailor-made NMR tube containing test sample was placed in the magnet's bore and held at a temperature of 298 K controlled by a flow of heated $N_2$ gas. The hyperpolarized gas mixture was directly transferred into tailor-made NMR tube at the rate of 0.08 standard liters per minute for 60 s. Following a delay of 3 s was given to allow bubbles collapse before signal acquisition. During the measurements, degassing of the Xe content of the solution during the course of the experiments was minimized by maintaining the partial pressure of hyperpolarized

xenon to a stable level. All spectra were acquired using 2 signal averages. All the $^{129}$Xe NMR chemical shifts in solution were referenced to the xenon gas signal, which was calibrated at 0 ppm. Unless stated otherwise, all the $^{129}$Xe NMR spectra were processed with a line broadening of 10 Hz.

Images were obtained with matrix size 32 × 32 point images (field of view: 4 cm × 4 cm) using a gradient echo sequence with a Gaussian pulse 20 ms long and slice thickness of 17 mm, echo time of 12.92 ms, repetition time of 26.94 ms and a 10 mm Bruker probehead (MIC WB40 RES 400 $^{1}$H/$^{129}$Xe).

For $^{129}$Xe NMR experiments in A549 cells, each sample included L-81 (0.1% final concentration) to reduce foaming caused by gas bubbling before $^{129}$Xe NMR experiments. [3]

**Synthesis and characterization:**

**Scheme S1.** Synthesis of sensor 1.

**Synthesis of 4-azido-1,8-naphthalic anhydride (3)**[4]

To a suspension of 4-bromo-1,8-naphthalic anhydride (505 mg, 1.82 mmol) in 50 mL round-bottom flask, the suspension of sodium azide (0.24 g, 3.71 mmol) in DMF (8.0 mL) and water (3.0 mL) was added. The mixture was heated to 80 ℃ with vigorously stirring for 4 h, and then the solution was slowly poured into ice water after cooling. The precipitated solid was filtered, washed with water and dried under vacuum to give compound 3 as pale yellow solid (0.28 g, 64%). $^{1}$H NMR (500 MHZ, DMSO-$d_6$): δ 8.53 (d, 1 H, $J$ = 7.0 Hz), 8.46 (t, 2 H, $J$ = 7.2 Hz), 7.88 (t, 1 H, $J$ = 8.0 Hz), 7.76 (d, 1 H, $J$ = 8.0 Hz), 7.42 (s, 1 H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) : δ 161.0, 160.4, 144.5, 133.7, 133.7, 131.1, 129.9, 128.0, 123.9, 119.5, 116.7, 115.0. HR MS (ESI): m/z calcd for $C_{12}H_6N_3O_3^+$ [M + $H^+$] 240.0404, found 240.0406, m/z calcd for $C_{12}H_5N_3NaO_3^+$ [M + $Na^+$] 262.0223, found 262.0230.

**Synthesis of *N*-ethylamino-4-azido-1,8-naphthalic anhydride (2)**

To 4-azido-1,8-naphthalic anhydride 3 (0.2 g, 0.84 mmol) placed in a 50 mL round-bottom flask, the solution of 1,2-diaminoethane (0.103 g, 1.72 mmol) in 15 mL ethanol was added and heated to 80 ℃ for 7 h. After the mixture was cooled to room temperature, the yellowish sediments were collected by filtration and then dried overnight in a vacuum oven at room temperature to give a yellow solid (0.08 g, yield: 35.4%). $^{1}$H NMR (600 MHZ, $CDCl_3$): δ 8.64 (d, 1 H, $J$ = 7.2 Hz), δ 8.59 (d, 1 H, $J$ = 8.4 Hz), 8.45 (d, 1 H, $J$ = 8.4 Hz), 7.75 (t, 1 H, $J$ = 7.8 Hz), 7.47 (d, 1 H, $J$ = 7.8 Hz), 4.27 (t, 2 H, $J$ = 6.6 Hz), 3.07 (t, 2 H, $J$ = 7.2 Hz). $^{13}$C NMR (150 MHz, $CDCl_3$) : δ 164.3, 163.9, 143.6, 132.3, 131.8, 129.2, 128.9, 126.9, 124.4, 122.5, 118.8, 114.7, 43.1, 40.5. HR MS (ESI): m/z calcd for $C_{14}H_{12}N_5O_2^+$ [M + $H^+$] 282.0986, found 282.0997.

**Synthesis of sensor 1**[5]

In a 50 mL round-bottom flask, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride

(EDC·HCl; 16.2 mg, 0.085 mmol), 1-hydroxy-1H-benzotriazole hydrate (Hobt; 11.5 mg, 0.085 mmol) and *N*,*N*-Diisopropylethylamine (DIPEA; 22 mg, 0.17 mmol) were added to the solution of cryptophane 4 (52 mg, 0.055 mmol, used in racemic form) in dry dichloromethane (10 mL) with stirring at room temperature for 30 min. Then compound 2 (20 mg, 0.071 mmol) was added with stirring. The reaction was monitored by TLC until starting material consumed completely. Then the reaction mixture was poured into water and extracted with dichloromethane. The organic layer was washed with water and brine three times respectively and dried over $Na_2SO_4$. After filtration, the mixture was concentrated in vacuum and purified by silica gel column chromatography (dichloromethane/methanol: 100/1 to 20/1) to give sensor 1 (42 mg, 63%) as a yellow solid. $^1$H NMR (600 MHZ, $CDCl_3$): δ 8.70 (dd, 1 H, *J* = 9.0 Hz), 8.59 (d, 1 H, *J* = 7.8 Hz), 8.45 (d, 1 H, *J* = 8.4 Hz), 7.75 (t, 1 H, *J* = 7.8 Hz), 7.47 (d, 1 H, *J* = 7.8 Hz), 4.27 (t, 2 H, *J* = 6.6 Hz), 3.07 (t, 2H, *J* = 7.2 Hz). $^{13}$C NMR (150 MHz, $CDCl_3$) : δ169.0, 164.3, 163.9, 149.9, 149.7, 149.6, 149.5, 147.8, 147.5, 146.9, 146.8, 146.6, 146.6, 146.4, 143.8, 134.6, 134.2, 134.2, 134.1, 134.0, 133.9, 133.6, 132.5, 132.1, 131.8, 131.7, 131.7, 131.4, 131.2, 129.3, 129.0, 126.9, 124.4, 122.3, 121.2, 121.2, 121.0, 120.6, 120.5, 118.6, 118.0, 114.8, 113.7, 113.6, 69.8, 69.4, 69.4, 69.2, 56.0, 55.7, 39.6, 38.4, 36.2, 36.1, 36.0. HR MS (ESI): m/z calcd for $C_{69}H_{64}N_5O_{15}^+$ [M + $H^+$] 1202.4393, found 1202.4373.

**Absorption and fluorescence spectra change upon addition of $HS^-$:**

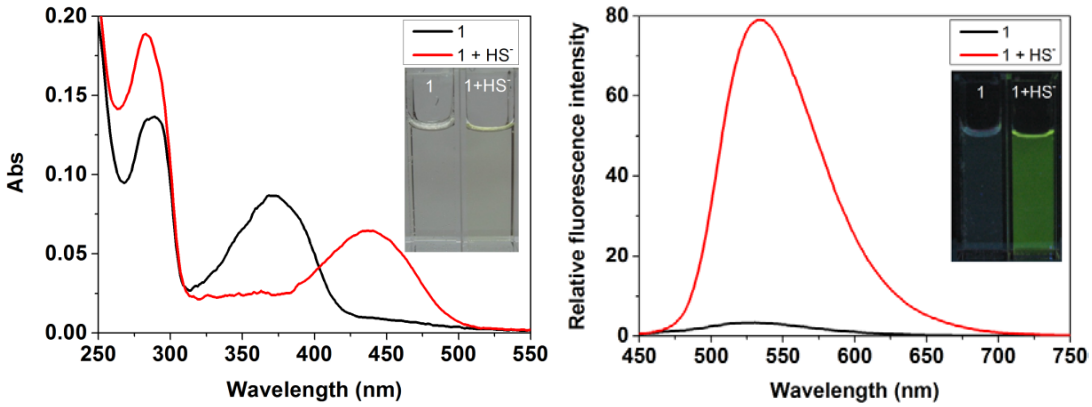



**Figure S1.** (left) UV-vis absorption and (right) fluorescence spectrum change of sensor 1 (10 μM) upon addition of $HS^-$ in HEPES buffer solution (pH 7.4, 20 mM). Inserted graphs show the corresponding visual optical colors of sensor 1 and sensor 1 with $HS^-$. Fluorescence color of sensor 1 and sensor 1 with $HS^-$ were determined under UV lamp at 365 nm.

UV-vis absorption spectrum of sensor 1 shows a main absorption peak at 373 nm. Upon addition of NaHS, the absorption of sensor 1 at 373 nm decreased, along with the simultaneous emergence of a new absorption region around 435 nm. The maximum absorption wavelength is a 62 nm red-shift, along with the solution color change from colorless to light yellow (Figure S8, left, inserted). In this process, a well-defined isobestic point was noted at 403 nm, suggesting a clean chemical transformation.

**The scan kinetics of sensor 1 with $HS^-$:**

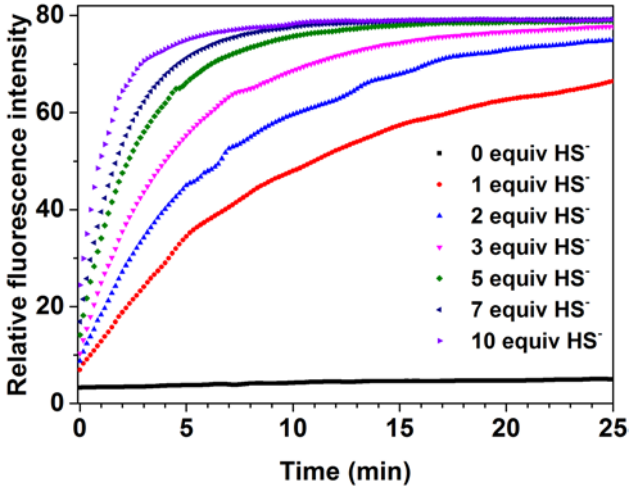



**Figure S2.** Scan Kinetics of sensor 1 (10 μM) treated with 0, 1, 2, 3, 5, 7 and 10 equiv. $HS^-$ in HEPES buffer solution (pH 7.4, 20 mM) at 25 °C.

**HR MS spectrum of reaction product between sensor 1 and $HS^-$:**

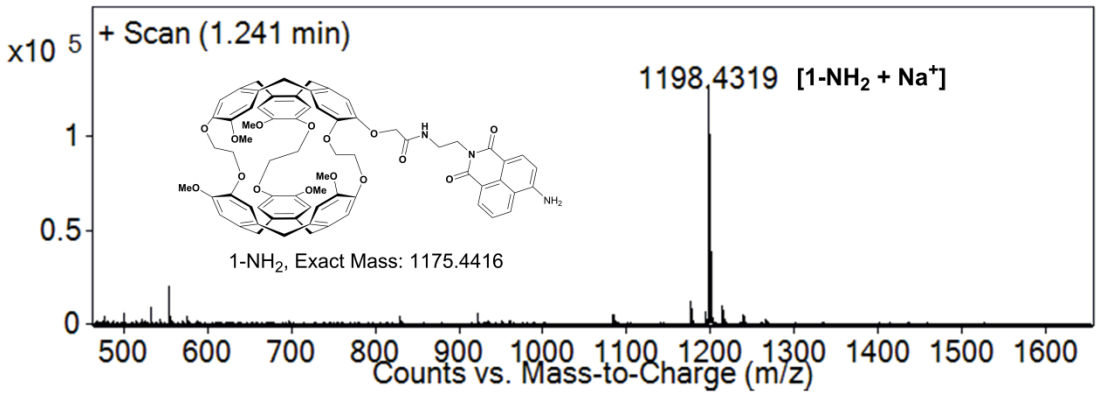



**Figure S3.** HR MS spectrum of reaction product between sensor 1 and $HS^-$ under test conditions.

**$^{129}$Xe NMR selectivity experiments:**

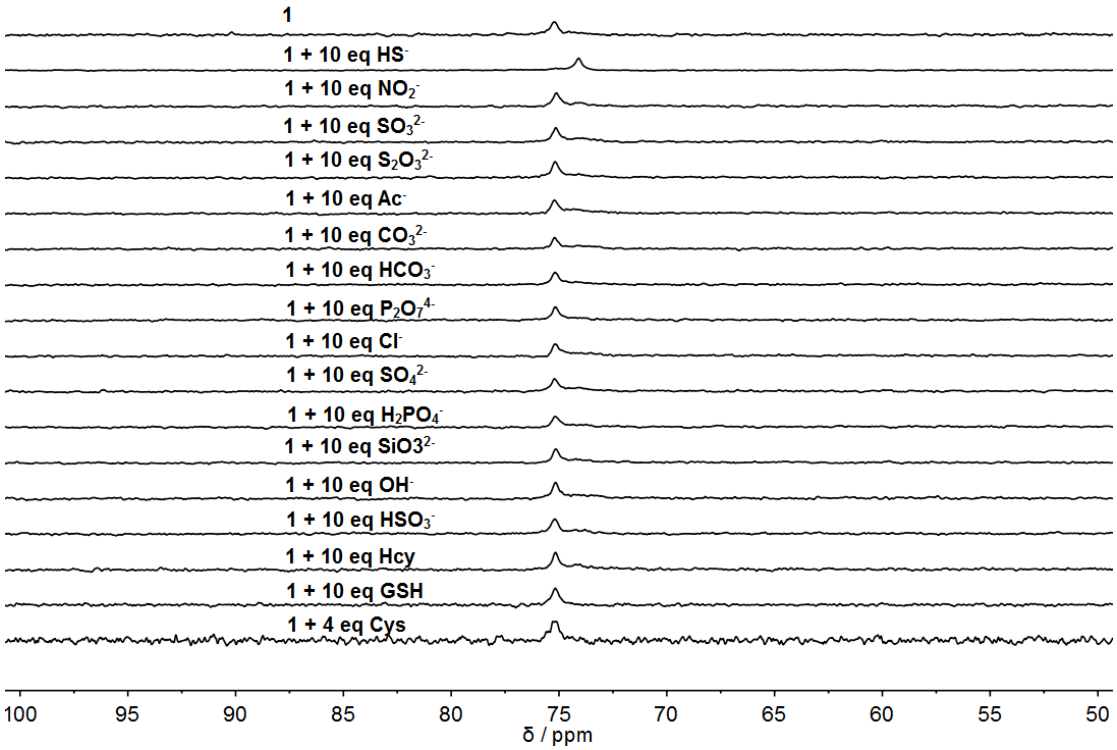



**Figure S4.** High field region of the $^{129}$Xe NMR spectra of sensor 1 (100 μM) in the presence of other species in HEPES buffer solution (pH 7.4, 20 mM) at 25 °C. All spectra were acquired by 2 scans.

**Fluorescence selectivity experiments:**

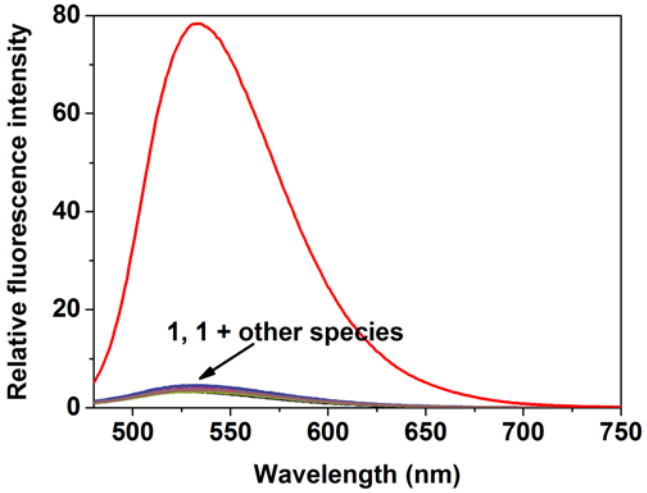



**Figure S5.** Fluorescence spectra of sensor 1 (10 μM) in response to various species in HEPES buffer solution (pH 7.4, 20 mM) at 25 °C. The spectra were obtained after 10 min upon addition of 10 equiv. $NO_2^-$, $SO_3^{2-}$, $S_2O_3^{2-}$, $Ac^-$, $CO_3^{2-}$, $HCO_3^-$, $P_2O_7^{4-}$, $Cl^-$, $SO_4^{2-}$, $H_2PO_4^-$, $SiO_3^{2-}$, $OH^-$, $HSO_3^-$, Hcy, GSH and Cys. (λex = 435 nm, λem = 534 nm)

**Fluorescence microscope experiments in A549 live cells:**

Non-small-cell lung cancer cell A549 (Shanghai, China) was cultured in RPMI 1640 medium (life technologies, China) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in humidified air under 5% $CO_2$. The regular medium was removed when the cell confluence reached about 90%. To measure the cellular uptake, A549 cells were cultured in incubation medium for 24 h in a 6-well chamber slide with one piece of cover glass at the bottom of each chamber.

For $H_2S$ imaging, NaHS (100 μM) pretreated (0.5 h) A549 cells were cultured with 10 μM sensor 1 under 5% $CO_2$ and 37 °C for 2 h. As a control, sensor 1 (10 μM) was added and incubated with A549 cells for 2 h under the same conditions. To achieve fixation, the glass that cells were adhered on was immersed in a solution of 4% paraformaldehyde in PBS for 10 min at room temperature after washed with PBS three times. Following fixation, the glass was washed with PBS three times, and performed using DAPI (blue) for nucleic staining. Then the slides were washed five times with PBS (pH 7.4) and visualized under a laser scanning confocal microscope Nikon A1 (Nikon, Tokyo, Japan). In blue: nuclear staining (DAPI), in green: sensor 1. Images were collected by using 405 nm excitation with emission channel of 408-500 nm (blue), and 488 nm excitation with emission channel of 500-650 nm (green).

**$^{129}$Xe NMR experiments of sensor 1 with $H_2S$ in A549 live cells:**

For $^{129}$Xe NMR experiments of sensor 1 with $H_2S$, NaHS (200 μM, 0.5 h) pretreated A549 cells was cultured in a solution (containing 2% DMSO) of sensor 1 (25 μM) in RPMI 1640 medium at 37 °C. After the incubation of 2 h, A549 cells were washed with PBS buffer three times to remove the remaining sensor 1 and then harvested with PBS. After centrifugation at 2300 rpm/min for 3 min, the collected cells were resuspended in RPMI medium 1640 to a final volume of 2.0 mL and then transferred into the tailor-made NMR tube for $^{129}$Xe NMR experiments. The overall cells density for each experiment was approximately $1 \times 10^7$ cell $mL^{-1}$.

**Hyper-CEST MRI experiments with A549 live cells:**

For Hyper-CEST spectroscopy and MRI, the hyperpolarized Xe gas mixture was bubbled for 20 s at a total flow rate of 0.08 SLM followed by a 3 s delay before acquisition. After bubbling, a saturation pulse was irradiated at the desired frequency of caged Xe. The continuous wave saturation parameters: $B_1$ field strength 6.5 μT, and saturation time 10 s. The Hyper-CEST spectroscopy data was obtained with one scan and fitted with exponential Lorentzians. The saturation frequency is measured relative to dissolved Xe resonance (194.5 ppm). The on-resonance frequency was at 74.8 ppm, while the off-resonance frequency was at 314.2 ppm. $^{129}$Xe Hyper-CEST MR images were acquired using FLASH sequence, $20 \times 20$ $mm^2$ field of view, $32 \times 32$ matrix size, echo time: 2.5 ms. The MR images were processed using MATLAB (R2014a, MathWorks, Natick, MA). CEST effect of each pixel were calculated by the formula (CEST effect = (offRes - onRes) / offRes) point by point. The image (32 x 32 image matrix) was interpolated to a 64 x 64 image matrix, and the center area (2 x 2 $cm^2$) of FOV was selected as new FOV (32 x 32 image matrix) to display the $H_2S$ image. The mask was used in the post-processing which covers the image areas that do not belong to the sample phantom and the normalized signal intensities

less than 30%.

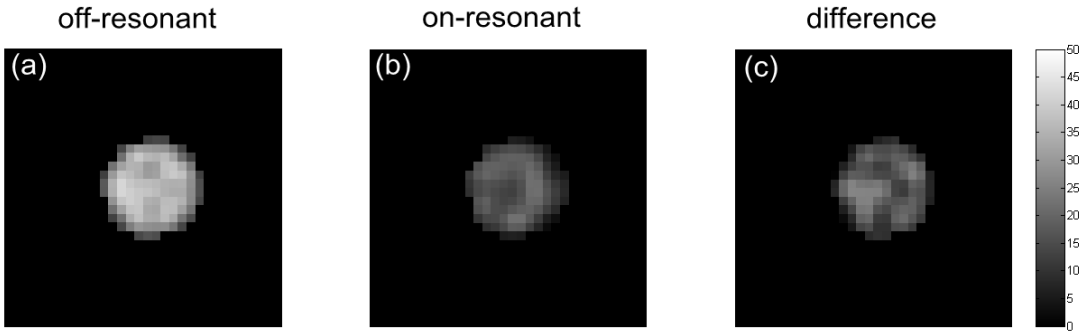



**Figure S6.** $^{129}$Xe Hyper-CEST imaging of the distribution of A549 cells labeled with sensor 1. (a) off-resonant, (b) on-resonant, (c) difference. Images were acquired by employing a Flash sequence.

**$^{129}$Xe NMR experiments of sensor 1 without $H_2S$ in A549 live cells and blank experiment:** For $^{129}$Xe NMR experiments of sensor 1 without $H_2S$, A549 cells was cultured with sensor 1 (25 μM) in RPMI 1640 medium (containing 2% DMSO) at 37 °C for 2 h. After the same post-process as $^{129}$Xe NMR experiments of sensor 1 with $H_2S$ applied, A549 live cells were used for $^{129}$Xe NMR experiments. For the blank experiment, A549 live cells were directly used for $^{129}$Xe Hyper-CEST experiments without treated by sensor 1 and NaHS. The overall cells density for each experiment was approximately $1 \times 10^7$ cell $mL^{-1}$.

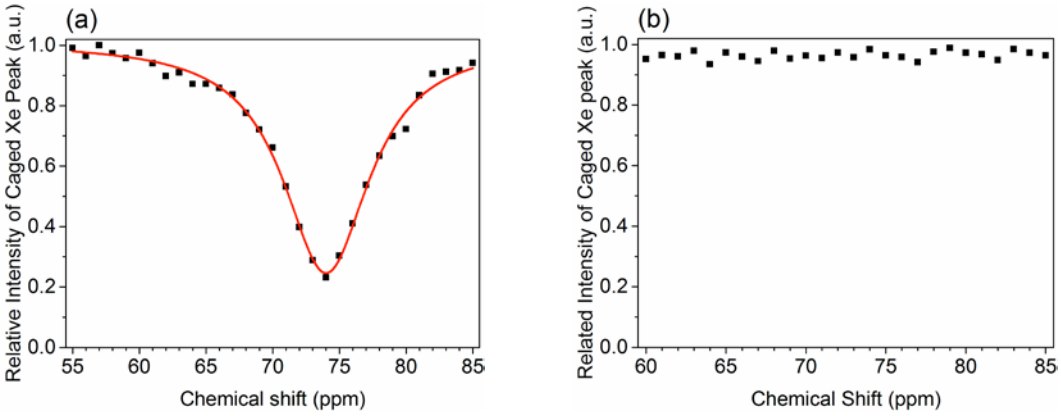



**Figure S7.** (a) Hyper-CEST spectrum of sensor 1 labeled A549 cells (incubated with 25 μM sensor 1 for 2 h). Exponential Lorentzian fits are shown as red colored solid lines. (b) Hyper-CEST spectrum of A549 living cells.

**References**

[1] X. Zhou, D. Graziani, A. Pines, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16903-16906.

[2] J. Zhang, W. Jiang, Q. Luo, X. Zhang, Q. Guo, M. Liu, X. Zhou, *Talanta* **2014**, *122*, 101-105.

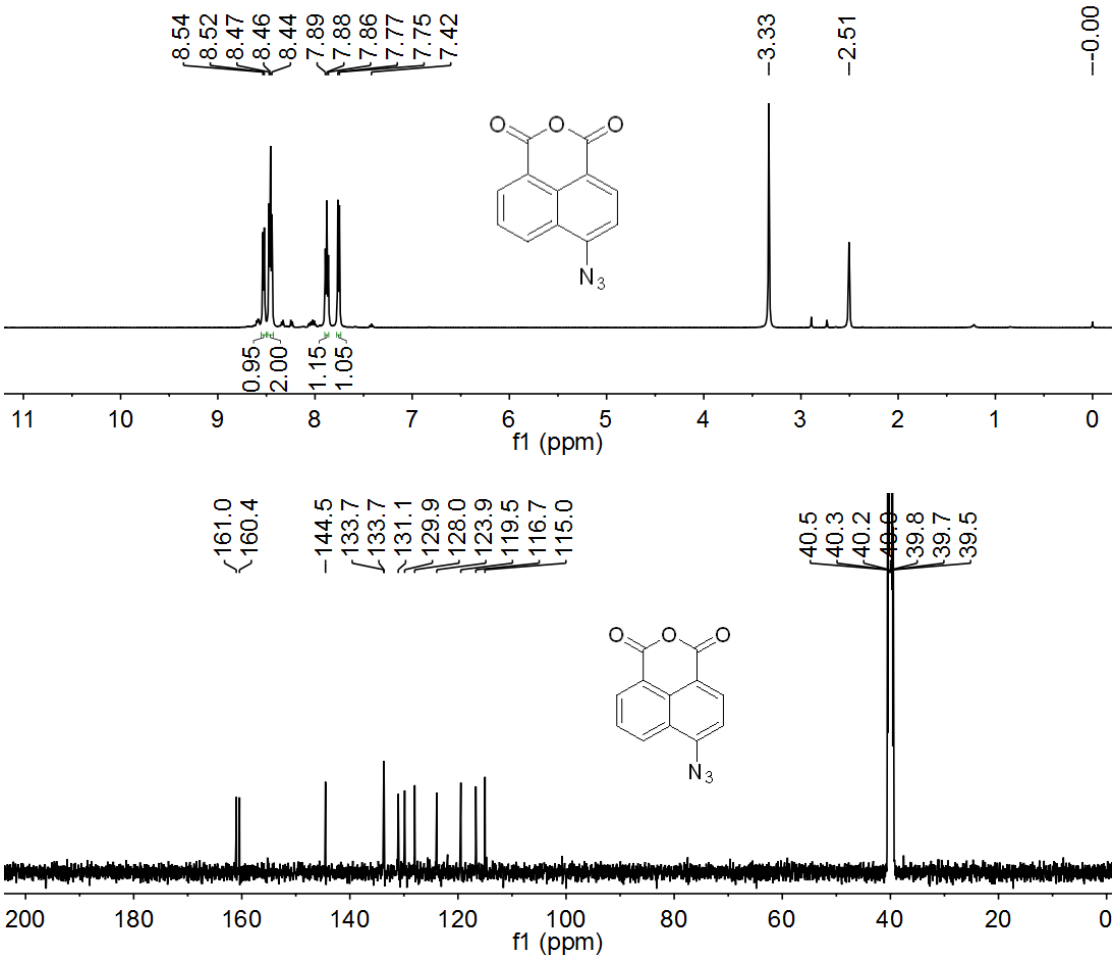
[3] S. Klippel, J. Döpfert, J. Jayapaul, M. Kunth, F. Rossella, M. Schnurr, C. Witte, C. Freund, L. Schröder, *Angew. Chem. Int. Ed.* **2014**, *53*, 493-496; *Angew. Chem.* **2014**, *126*, 503-506.

[4] X. Li, Y. Lin, Q. Wang, Y. Yuan, H. Zhang, X. Qian, *European Journal of Medicinal Chemistry* **2011**, *46*, 1274-1279.

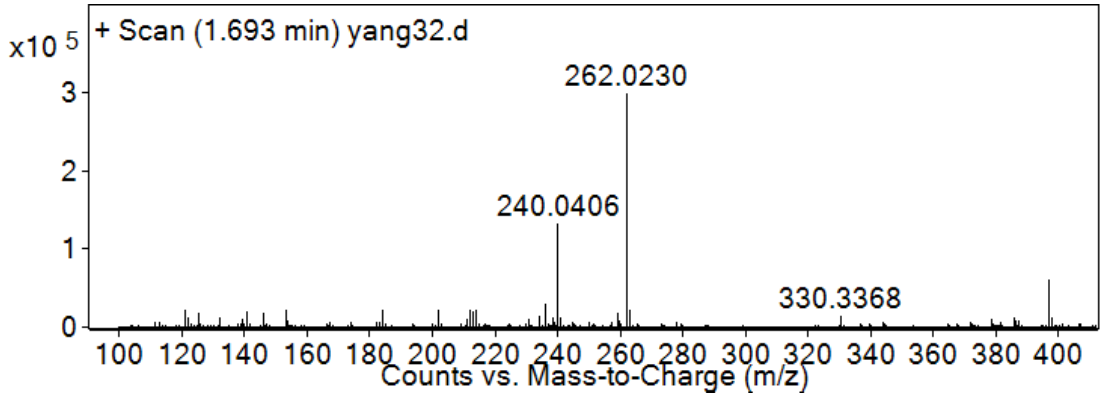
[5] N. Kotera, N. Tassali, E. Léonce, C. Boutin, P. Berthault, T. Brotin, J.-P. Dutasta, L. Delacour, T. Traoré, D. A. Buisson, F. Taran, S. Coudert, B. Rousseau, *Angew. Chem. Int. Ed.* **2012**, *124*,

4100-4103; *Angew. Chem.* **2012**, *124*, 4176-4179.

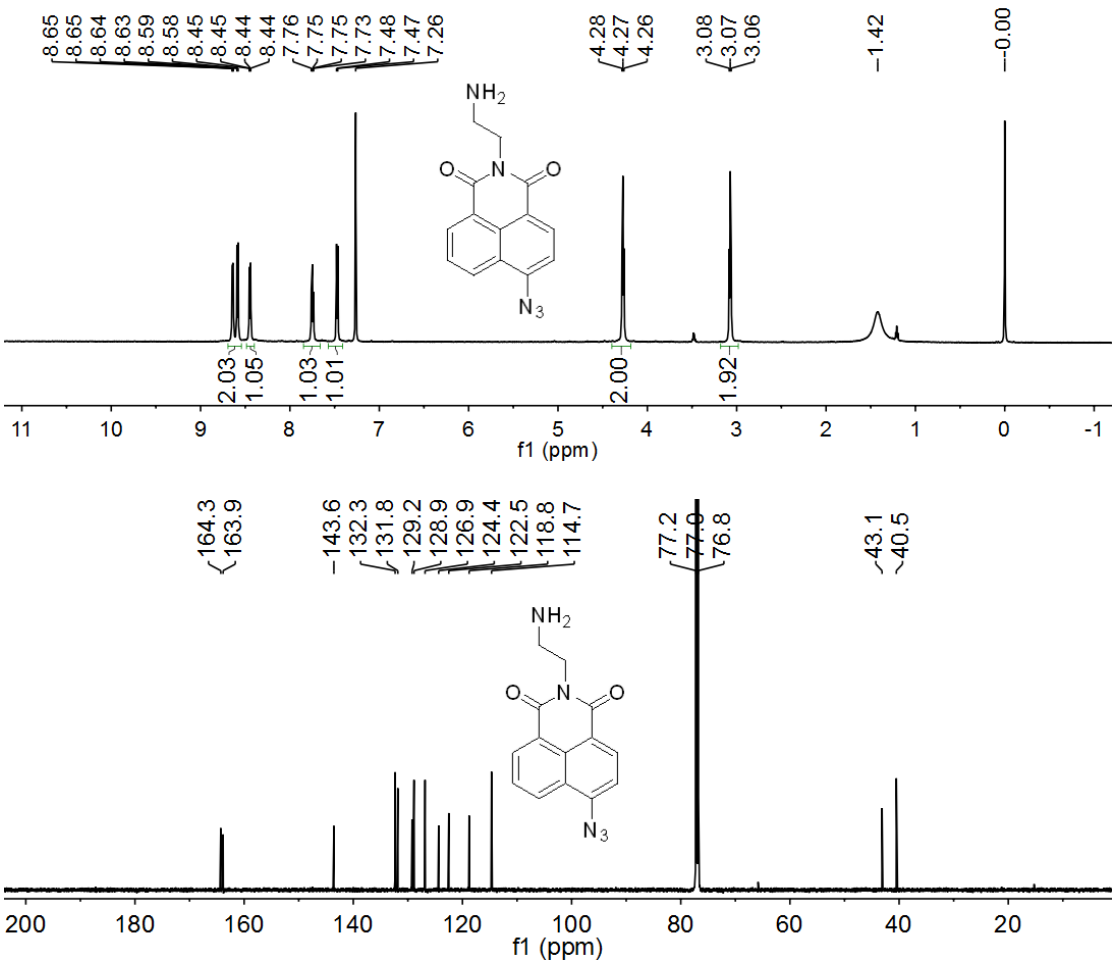
**NMR and HRMS spectra of compound 3, 2 and 1:**



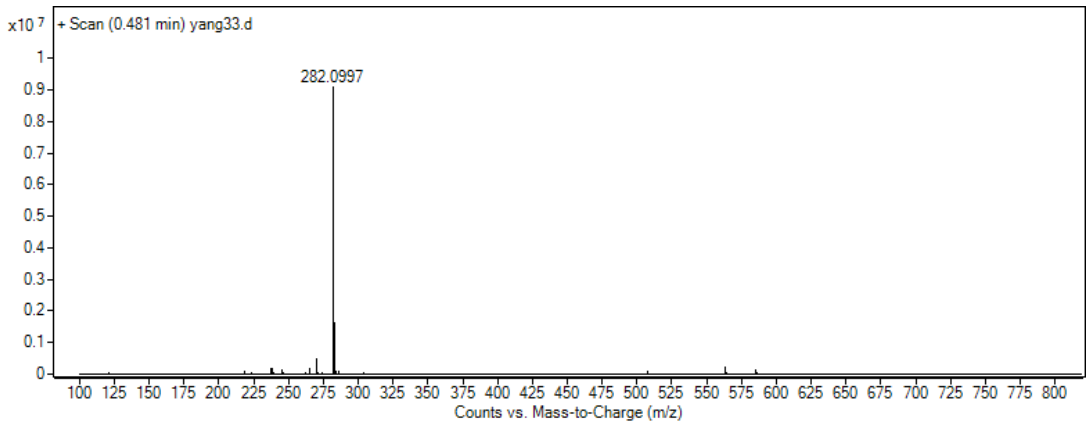

$^{1}H$ and $^{13}C$ NMR of compound 3 recorded in DMSO-d6.



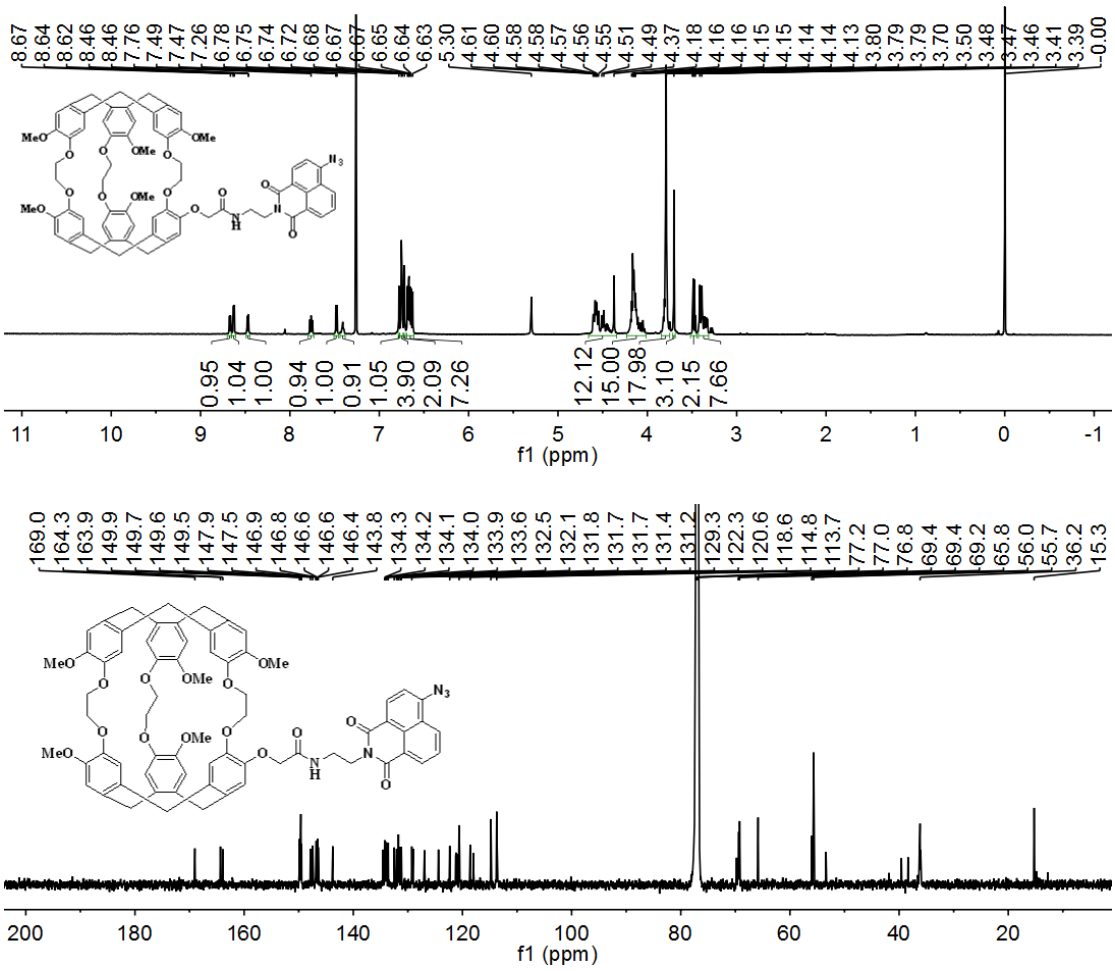

HR MS-ESI of compound 3.



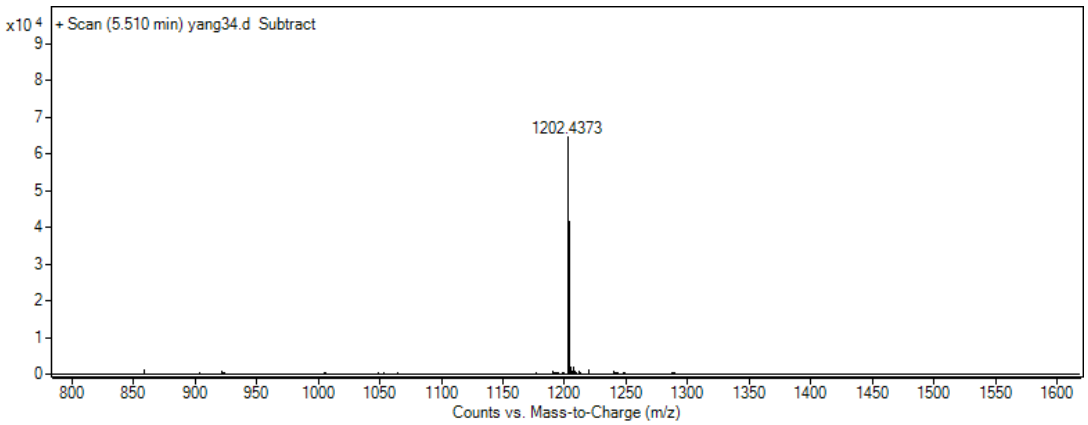

$^{1}H$ and $^{13}C$ NMR of compound 2 recorded in $CDCl_3$.




HR MS-ESI of compound 2.




$^{1}H$ and $^{13}C$ NMR of sensor 1 recorded in $CDCl_3$.




HR MS-ESI of sensor 1.