Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

## A hepatocyte-targeting fluorescent probe for imaging isoniazid-induced hydrazine in HepG2 cells and zebrafish

## 1. Reagents and Equipment

All chemicals and solvents were purchased from local suppliers and used without further purification. Nuclear Magnetic Resonance Spectrum (NMR) were obtained using Bruker 400 MHz or 600 MHz NMR spectrometer. High-Resolution Mass Spectrum (HRMS) were recorded using Varian 7.0 T FTICR-MS. Fluorescence spectra were acquired on the Hitachi F-7000 spectrophotometer. Bioimaging was collected on Olympus Fluoview FV1000.

## 2. Spectroscopic Studies

All spectroscopic measurements were performed in PBS buffer (20 mM, pH 7.4, 50% DMSO) for HP-1/2 and in PBS buffer (20 mM, pH 7.4, 1% DMSO) for GHP. The excitation wavelength is 435 nm. The slit widths for both excitation and emission is 5.0 nm.

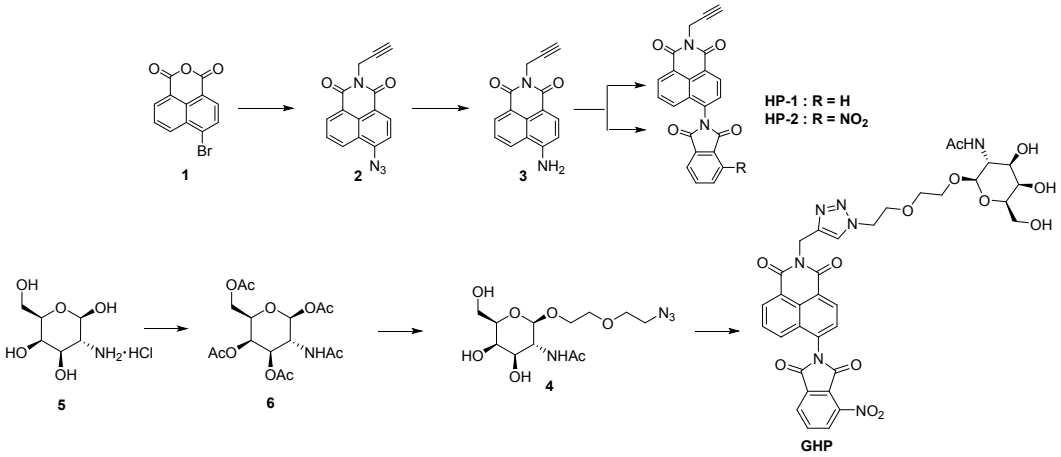
## 3. Cell Imaging

Three sets of experiments were performed. For the cell selectivity experiment, three kinds of cells (HepG2 cells, HeLa cells, and MCF-7 cells) were first treated with **GHP** (10 μM) for 30 min, washed with PBS for three times, and then treated with $N_2H_4$ (500 μM) for another 30 min. For certifying ASGPR-mediated endocytosis, HepG2 cells were given an incubation of **GHP** (10 μM) for 1 h, washed with PBS for three times, and then treated with $N_2H_4$ (500 μM) for another 30 min in the absence and presence of increasing competing free galactose (0-100 μM). To image INH-induced $N_2H_4$, HepG2 cells were first treated with INH (1 mM) for 6 h and then incubated with **GHP** (10 μM) for 30 min. After washing with PBS three times, the above cells were used for imaging immediately. Fluorescence confocal images were taken by using Olympus Fluoview FV1000 with excitation at green channel (Excitation: 488 nm, Emission: 530-582 nm).

## 4. Zebrafish Imaging

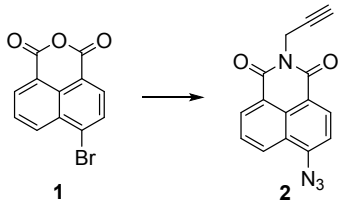
The 5-day-old zebrafish were purchased from Eze-Rinka Company (Nanjing, China). All operation was carried out according to the regulations issued by the Ethical Committee of Hebei University. Three sets of experiments were performed. In a control group, zebrafish were treated with **GHP** (10 μM) for 1 h. For imaging exogenous $N_2H_4$, zebrafish were first given an incubation of **GHP** (10 μM) for 1 h and then treated with $N_2H_4$ (200 μM or 500μM) for 30 min. For visualizing INH-induced $N_2H_4$, zebrafish were first treated with INH (1 mM) for 24 h and then followed by incubation of **GHP** (10 μM) for different times (0 min, 30 min, 60 min). Confocal images were taken by using Olympus Fluoview FV1000 with excitation at green channel (Excitation: 488 nm, Emission: 530-582 nm).

## 5. Synthesis



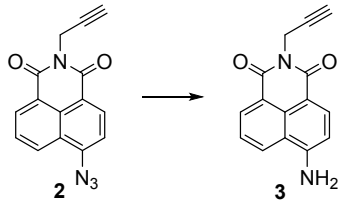

**Scheme S1** Synthetic route of probe **HP-1**, **HP-2**, and **GHP**

**Synthesis of *6-azido-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* (2)**




Compound **1** (2.77 g, 10.0 mmol) and propargylamine (2.74 mL, 40.0 mmol) were dissolved in 50 mL 1,4-dioxane and the solution was refluxed for 5 h. The forming pale yellow precipitate was filtrated and dissolved in 25 mL DMF. After reaction with sodium azide (1.95 g, 30.0 mmol) at 80 °C for 1.5 h, the reaction solution was cooled to 4 °C, the yellow solid was obtained by filtration and recrystallization as compound **2** (2.67 g, yield 97%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 8.66 (d, $J$ = 7.2 Hz, 1H), 8.60 (d, $J$ = 8.0 Hz, 1H), 8.45 (d, $J$ = 8.4 Hz, 1H), 7.74 (t, $J$ = 7.8 Hz, 1H), 7.47 (d, $J$ = 8.0 Hz, 1H), 4.94 (d, $J$ =1.6 Hz, 2H), 2.19 (s, 1H).

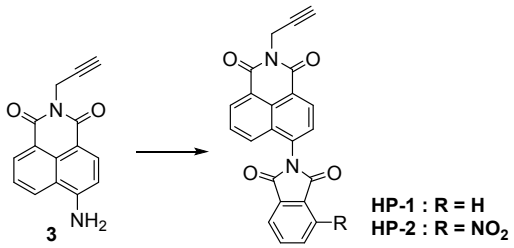
**Synthesis of *6-amino-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* (3)**




Compound **2** (1.38 g, 5.00 mmol) and sodium sulfide nonahydrate (2.40 g, 10.0 mmol) were dissolved in 25 mL DMF. After distirred at room temperature for 2 hours, the reaction solution was extracted with $CH_2Cl_2$, washed with saturated NaCl aqueous solution, and concentrated to obtain an yellow solid as compound **3** (1.21 g, yield 97%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 8.65 (d, $J$ = 8.3 Hz, 1H), 8.44 (d, $J$ = 6.6 Hz, 1H), 8.20 (d, $J$ =

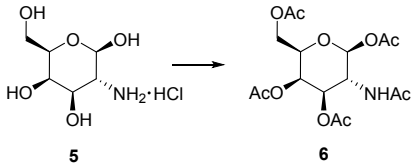
8.4 Hz, 1H), 7.66 (m , 1H), 7.54 (s, 2H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.73 (d, *J* = 2.3 Hz, 2H), 3.04 (t, *J* = 2.3 Hz, 1H).

**Synthesis of *6-(1,3-dioxoisoindolin-2-yl)-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* (HP-1) and *6-(4-nitro-1,3-dioxoisoindolin-2-yl)-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* (HP-2)**




Compound **3** (387 mg, 1.00 mmol) and *o*-phthalic anhydride (494 mg, 1.30 mmol) or 3-nitrophthalic anhydride (494 mg, 1.30 mmol) were added in glacial acetic acid (20 mL). After refluxing for 8 h, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography to give probe **HP-1** (568 mg, 85 %) and **HP-2** (568 mg, 82 %), respectively, as white solid. For **HP-1**, M.p. 162.4-163.9 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 8.69 (d, *J* = 7.6 Hz, 1H), 8.61 (d, *J* = 7.2 Hz, 1H), 8.46 (d, *J* = 8.8 Hz, 1H), 8.07-8.04 (m, 3H), 7.99-7.97 (m, 2H), 7.91-7.87 (m, 1H), 4.82 (d, *J* = 2.4 Hz, 2H), 3.18 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ = 170.12, 167.67, 163.54, 163.18, 143.16, 135.37, 135.28, 132.51, 129.27, 129.00, 128.74, 128.45, 124.26, 124.13, 123.35, 122.99, 101.05, 70.85, 29.50. HRMS: $C_{23}H_{13}N_2O_4$ [M+H$^+$] calcd. for 381.0869, found 381.0869. For **HP-2**, M.p. 286.2-287.9 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 8.70 (d, *J* = 7.6 Hz, 1H), 8.62-8.59 (m, 2H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.33 (d, *J* = 6.8 Hz, 1H), 8.18 (t, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.91 (t, *J* = 8.0 Hz, 1H), 4.81(d, *J* = 2.0 Hz, 2H)3.18 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ = 165.45, 162.80, 162.59, 162.23, 144.70, 136.52, 134.46, 134.09, 131.69, 130.89, 130.85, 128.88, 128.70, 128.64, 128.21, 128.11, 127.41, 123.70, 122.97, 122.31, 79.30, 73.23, 29.33. HRMS: $C_{23}H_{12}N_3O_6$ [M+H$^+$] calcd. for 426.0720, found 426.0716.

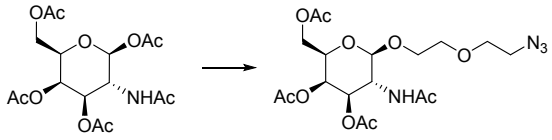
**Synthesis of *(2S,3R,4R,5R,6R)-3-acetamido-6-(acetoxymethyl)tetrahydro-2H-pyran-2,4,5-triyl triacetate* (6)**




Compound **5** (2.16 g, 10.0 mmol) was dissloved in 50 mL of dry pyridine. $Ac_2O$ (5.60 mL, 60.0 mmol) was added dropwise to the solution. After sitrring overnight, white solid was filtered and dried to prepare compound **6** (3.82 g, yield 98%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 7.90 (d, *J* = 8.8 Hz, 1H), 5.63 (d, *J* = 8.8 Hz, 1H), 5.26 (s, 1H), 5.05

(dd, $J$ = 2.2, 11.1 Hz, 1H), 4.22 (t, $J$ = 5.6 Hz, 1H), 3.96-3.88 (m, 2H), 3.80 (d, $J$ = 2.8 Hz, 1H), 4.13-3.96 (m, 3 H), 2.11 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.77 (s, 3H).

**Synthesis of *(2R,3R,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-(2-(2-azidoethoxy)ethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate* (7)**

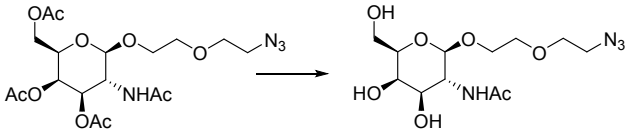



**6** **7**

Compound **6** (1.60 g, 5.00 mmol) was dissolved in 20 mL of 1,2-dichloroethane, then TMSOTf (181 μL, 1.00 mmol) was dropped to the solution at 0 °C. After stirring at room temperature overnight, the reaction solution was quenched by an iced $NaHCO_3$ (420 mg, 5.00 mmol) aqueous solution. A light yellow syrupy-like liquid was obtained by extraction, drying, and concentration, which was further used to synthesis compound **7**.

The above liguid and 2-(2-azidoethoxy)ethan-1-ol (7.86 mg, 6.00 mmol) were dissolved in 20 mL of 1,2-dichloroethane, then TMSOTf (1.08 mL, 6.00 mmol) was dropped to the solution at 0 °C. The reaction solution was stirred at room temperature for 4 h, which was extracted with DCM, dried with $Na_2SO_4$ and concentrated. Compound **7** was purified by column chromatography ($V_{petroleum\ ether}/V_{ethyl\ acetate}$ = 1 / 2) to obtain a white powder (2.04 g, yield: 89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 5.82 (d, $J$ = 8.8 Hz, 1H), 5.33 (d, $J$ = 2.8 Hz, 1H), 5.18 (dd, $J$ = 11.2, 3.2 Hz, 1H), 4.74 (d, $J$ = 8.4 Hz, 1H), 4.15-4.11 (m, 3H), 3.92-3.90 (m, 2H), 3.80-3.74 (m, 1H), 3.63 (s , 4H), 3.42-3.40 (m, 2H), 2.13 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H).

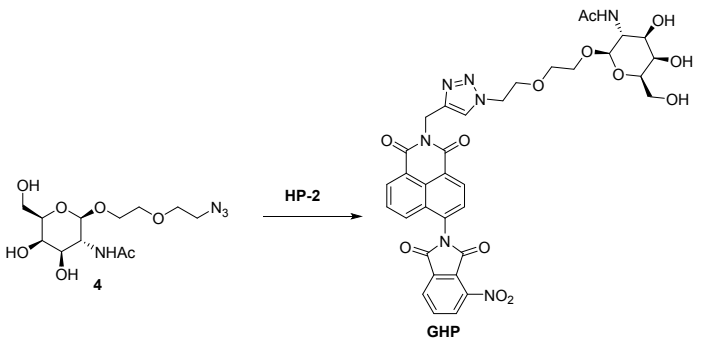
**Synthesis of *N-((2R,3R,4R,5R,6R)-2-(2-(2-azidoethoxy)ethoxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)acetamide* (4)**




**7** **4**

Compound **7** (920 mg, 2.00 mmol) and sodium methoxide (400 mg, 10.0 mmol) were dissolved in 10 mL of methanol and stirred at room temperature for 0.5 h. The reaction solution was neutralized by strong acid cation exchange resinion, filtered, and condensed to obtain compound **4** as a white solid (501 mg, 75%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ (ppm): 4.40 (d, $J$ = 8.4 Hz, 1H), 3.96-3.87 (m, 2H), 3.81-3.80 (m, 1H), 3.74-3.68 (m, 2H), 3.63-3.60 (m, 4H), 3.59-3.55 (m, 1H), 3.48-3.45 (m, 1H), 3.35-3.31 (m, 2H), 3.27 (s, 1H), 1.96 (s, 3H).

**Synthesis of *N-((2R,3R,4R,5R,6R)-2-(2-(2-azidoethoxy)ethoxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)acetamide* (GHP)**




Compound **4** (117 mg, 0.50 mmol), **HP-2** (212 mg, 0.50 mmol) and $CuSO_4 \cdot 5H_2O$ (250 mg, 1.00 mmol) were dissolved in a DMF-$H_2O$ mixed solution (20 mL). After stirring well, sodium ascorbate (396 mg, 2.00 mmol) was added. After further stirred overnight at 50 °C under the atmosphere of nitrogen, the solvent was removed under reduced pressure. The crude product was purified by C18 reversed-phase column chromatography to give probe **GHP** as a light yellow solid (323 mg, 85 %). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 8.62-8.60 (m, 2H), 8.55 (d, $J$ = 7.2 Hz, 1H), 8.50 (d, $J$ = 8.0 Hz, 1H), 8.41-8.35 (m, 2H), 8.01 (s, 1H), 7.95-7.88 (m, 2H), 7.61 (d, $J$ = 8.8 Hz, 1H), 5.32 (s, 2H), 4.44 (s, 2H), 4.25 (d, $J$ = 8.4 Hz, 1H), 3.82 (s, 2H), 3.78-3.67 (m, 5H), 3.64(s, 1H), 3.47-3.40 (m, 2H), 3.31 (t, $J$ = 5.2 Hz, 1H), 1.77 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ (ppm): 169.86, 164.98, 164.15, 163.51, 162.97, 147.04, 143.11, 139.94,135.73, 132.87, 132.06, 131.49, 131.26, 130.49, 128.70, 128.67, 126.99, 123.83, 122.47, 101.51, 75.45, 71.61, 71.57, 69.60, 68.90, 67.70, 67.64, 60.61, 55.06, 53.43, 52.07, 49.32, 35.46, 23.22, 23.17. HRMS: $C_{35}H_{33}N_7O_{13}Na$ ($M+Na^+$) calcd. for 782.2029, found 782.2016.

## 6. Spectroscopic analysis of the probes

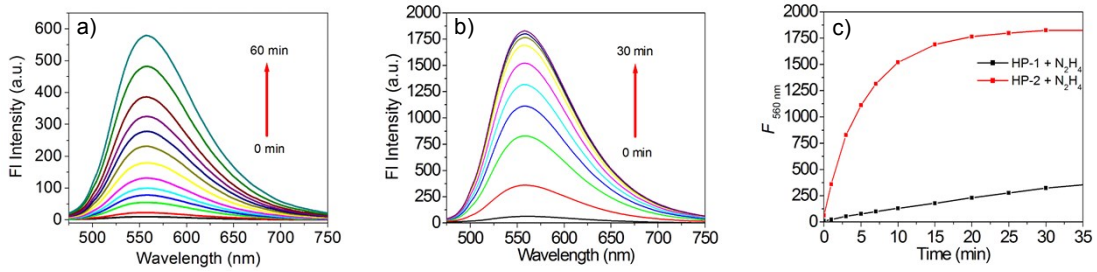



**Fig. S1** Fluorescent spectra of **HP-1** and **HP-2** in PBS buffer (20 mM, pH 7.4, 50% DMSO) at 25 °C. (a-b) Fluorescence change of **HP-1** (5 μM, a) and **HP-2** (5 μM, b) with $N_2H_4$ (500 μM) different reaction times (0-35 min). (c) The relationship between fluorescence intensity at 560 nm and incubation time.

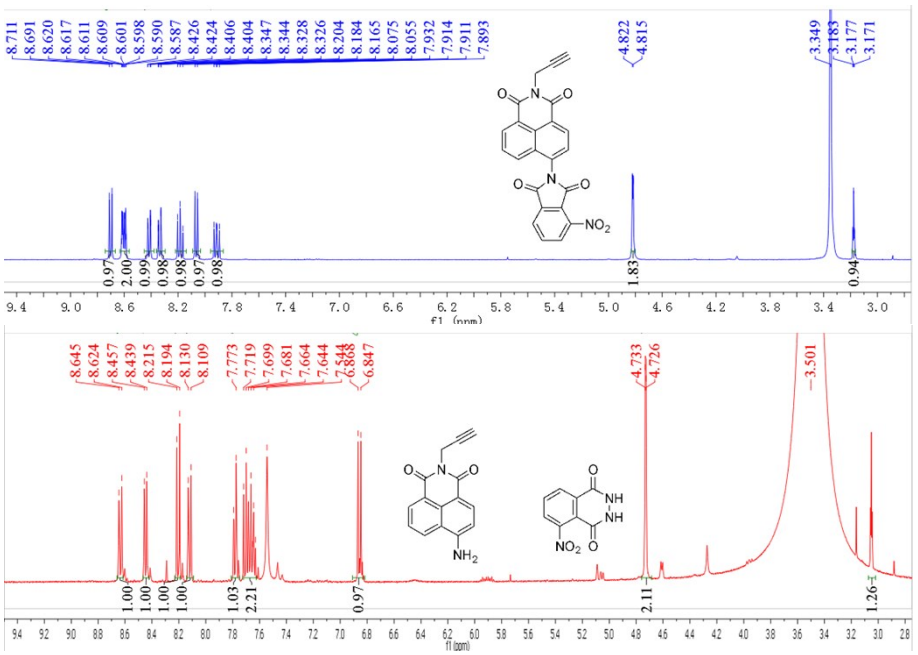


**Fig. S2** The $^1$H NMR of probe **HP-2** before and after reaction with $N_2H_4$ in DMSO-$d_6$ solution.

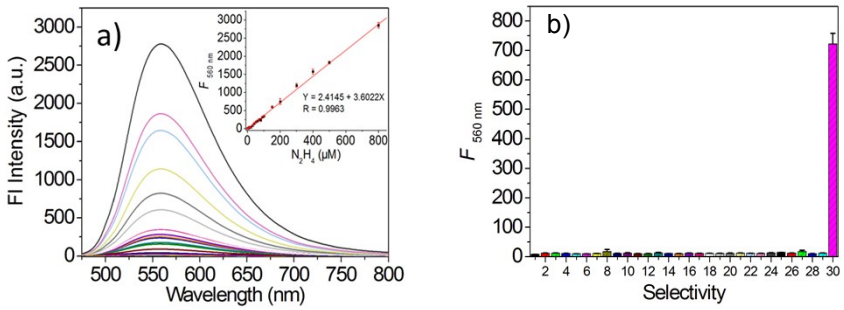



**Fig. S3** (a) Fluorescent spectra of **HP-2** (5 μM) after reaction with different concentrations of $N_2H_4$ (0-800 μM). Inset: the linear relationship between fluorescence intensity at 560 nm of probe **HP-2** and the concentration of $N_2H_4$. (b) Selectivity of **GHP** (5 μM) in the presence of different species (1, probe alone; 2, $Cl^-$; 3, $Br^-$; 4, $I^-$; 5, $SO_4^{2-}$; 6, $Ag^+$; 7, $Mg^{2+}$; 8, $Cu^{2+}$; 9, $Ca^{2+}$; 10, $Zn^{2+}$; 11, $Co^{2+}$; 12, $Zn^{2+}$; 13, $NO_2^-$;14, $SO_3^{2-}$; 15; $S_2O_3^{2-}$; 16, $H_2O_2$; 17, HClO; 18, NO; 19, $H_2S$; 20, Cys; 21, Hcy; 22, GSH; 23, methylamine; 24, aqueous ammonia; 25, hydroxylamine; 26, triethylamine; 27, 1,2-ethylenediamine; 28, *n*-butylamine; 29, phenylhydrazine; 30, $N_2H_4$; 500 μM for 2-19 and 23-30, 1 mM. for 20-21, 5 mM for 22.

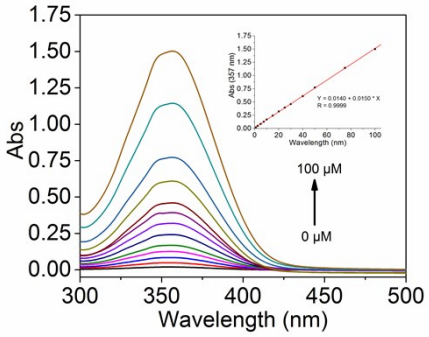


**Fig. S4** The relationship between absorbance at 357 nm and concentration of probe **HGP** in PBS buffer (20 mM, pH = 7.4, 1% DMSO)

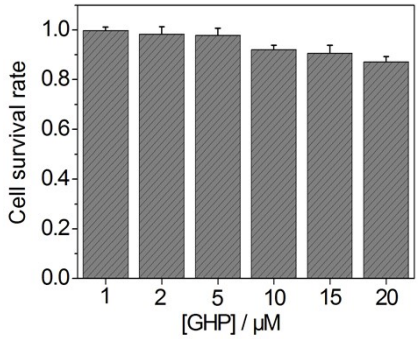



**Fig. S5** The cytotoxicity of the probe **GHP** evaluated by MTT assay. Error bars are ± SEM.

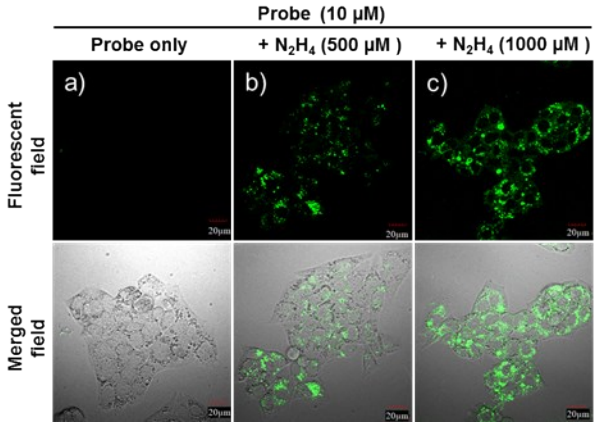



**Fig. S6** Confocal fluorescence imaging of exogenous $N_2H_4$ in HepG2 cells. Cells were given an incubation of **GHP** (10 μM) for 1 h and then treated with $N_2H_4$ (0 μM, 500 μM or 1000 μM) for another 30 min (a-c). Scale bar, 20 μm.

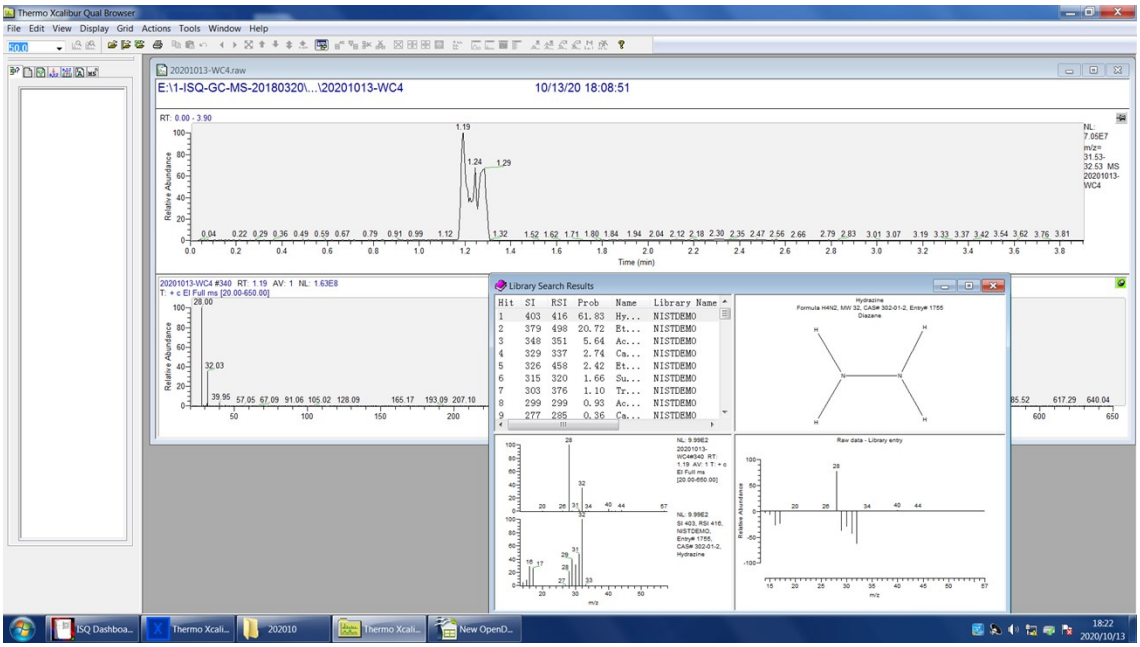


**Fig. S7** Confocal fluorescence imaging of exogenous $N_2H_4$ in HepG2 cells. Cells were given an incubation of **GHP** (10 μM) for 1 h and then treated with $N_2H_4$ (0 μM, 500 μM, or 1000 μM) for another 30 mi

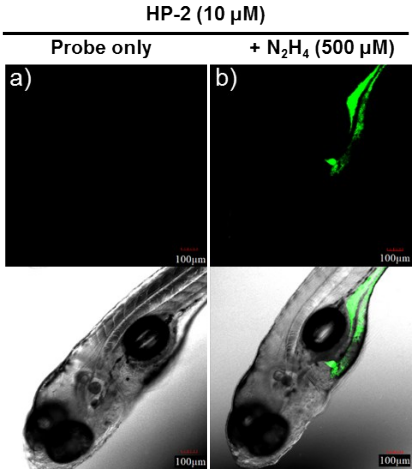



**Fig. S8** Confocal fluorescence imaging of zebrafish (pseudocolor) given an incubation of **HP-2** (10 μM) for 1 h (a) and then treated with $N_2H_4$ (500μM) (b). Images were taken after incubation of $N_2H_4$ for 30 min. (The green fluorescence site is the yolk of zebrafish).

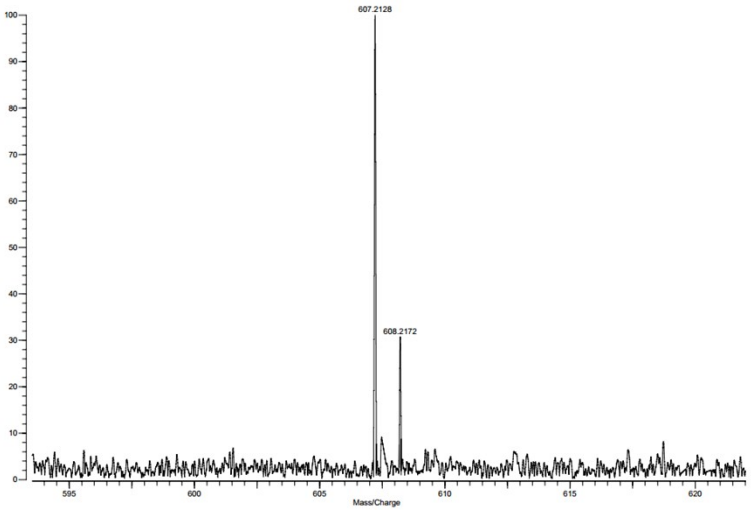



**Fig. S9** HRMS spectra of the reaction of probe **GHP** (5 μM) with $N_2H_4$ (5 mM) in PBS (pH 7.4, 20 mM) at room temperature. The calculation mass $[M+Na^+]$ is 607.2128.

# 3. Supplementary spectra of NMR and HRMS

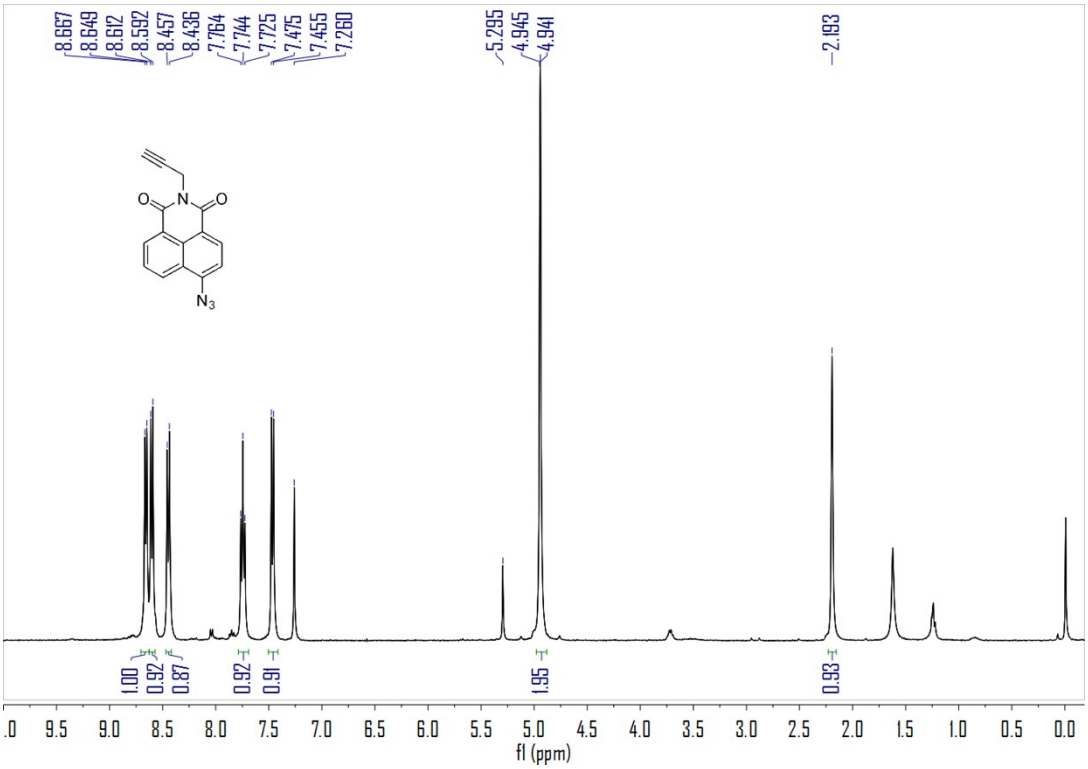



**Fig. S10** $^{1}$H NMR of compound **2**

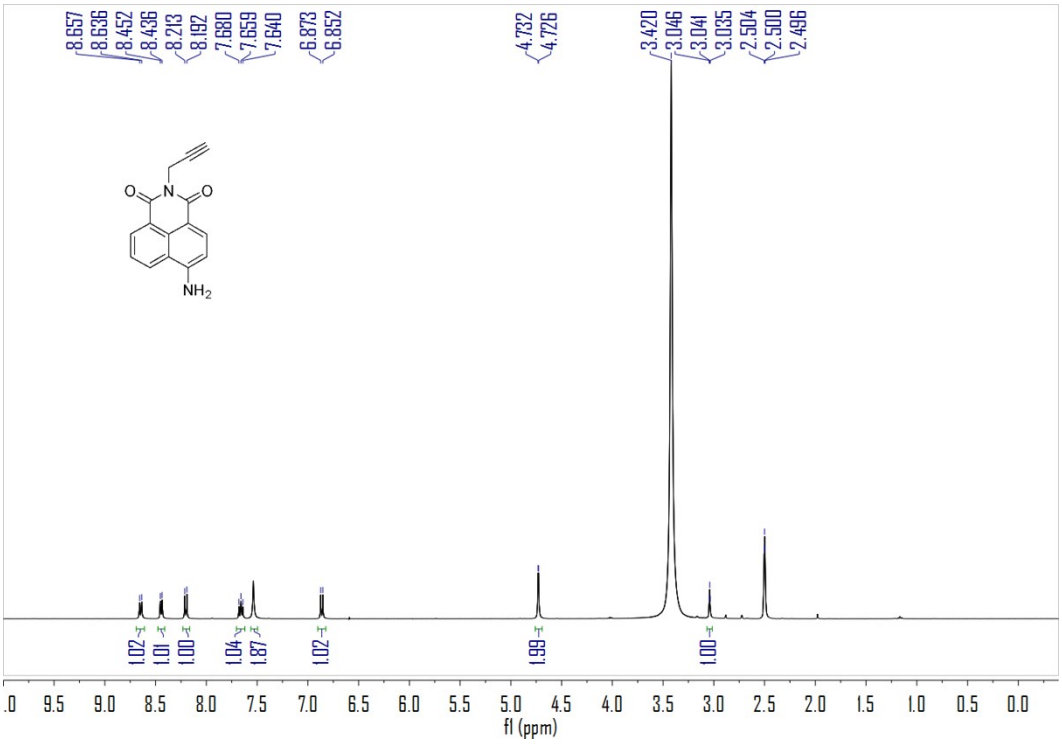



**Fig. S11** $^{1}$H NMR of compound **3**.

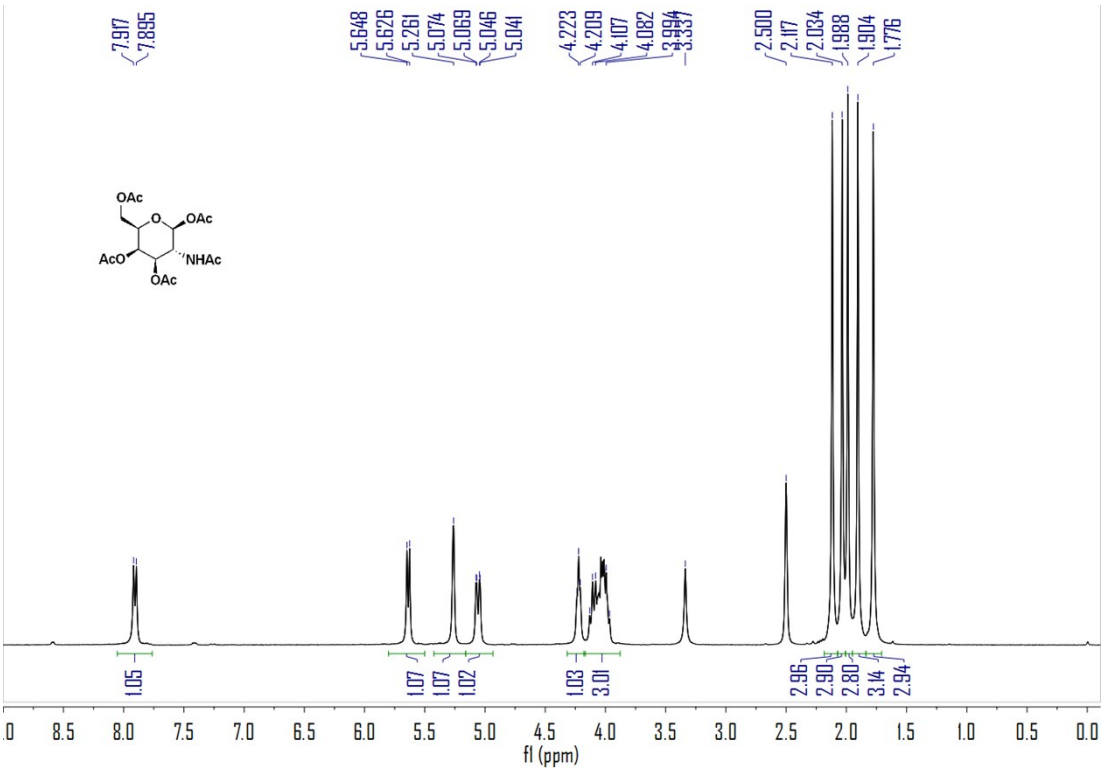



**Fig. S12** $^{1}$H NMR of compound **6**

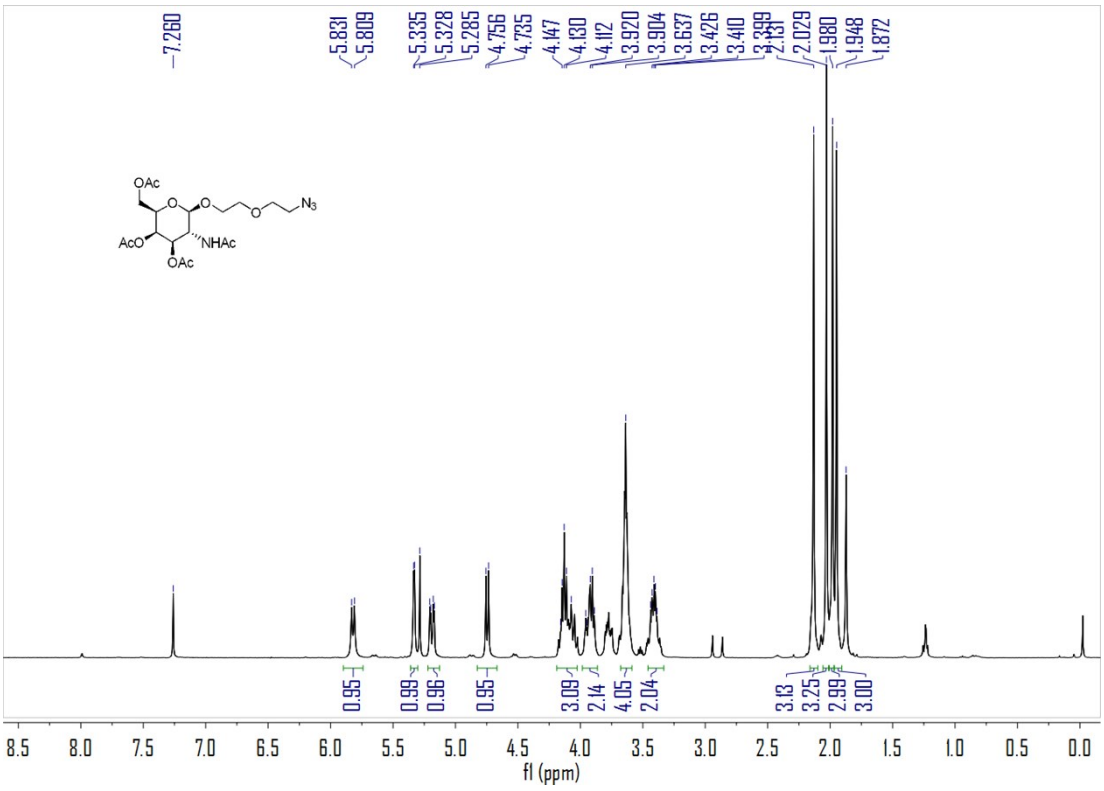



**Fig. S13** $^{1}$H NMR of compound **7**

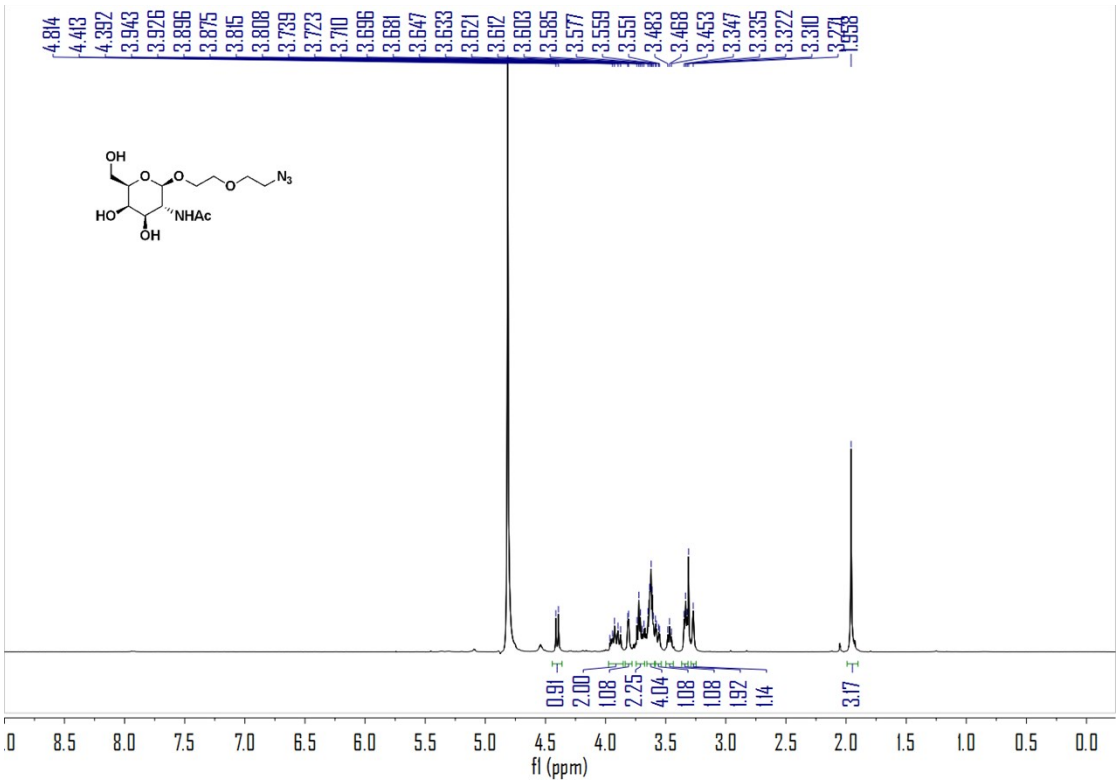



**Fig. S14** [1]H NMR of compound **4**

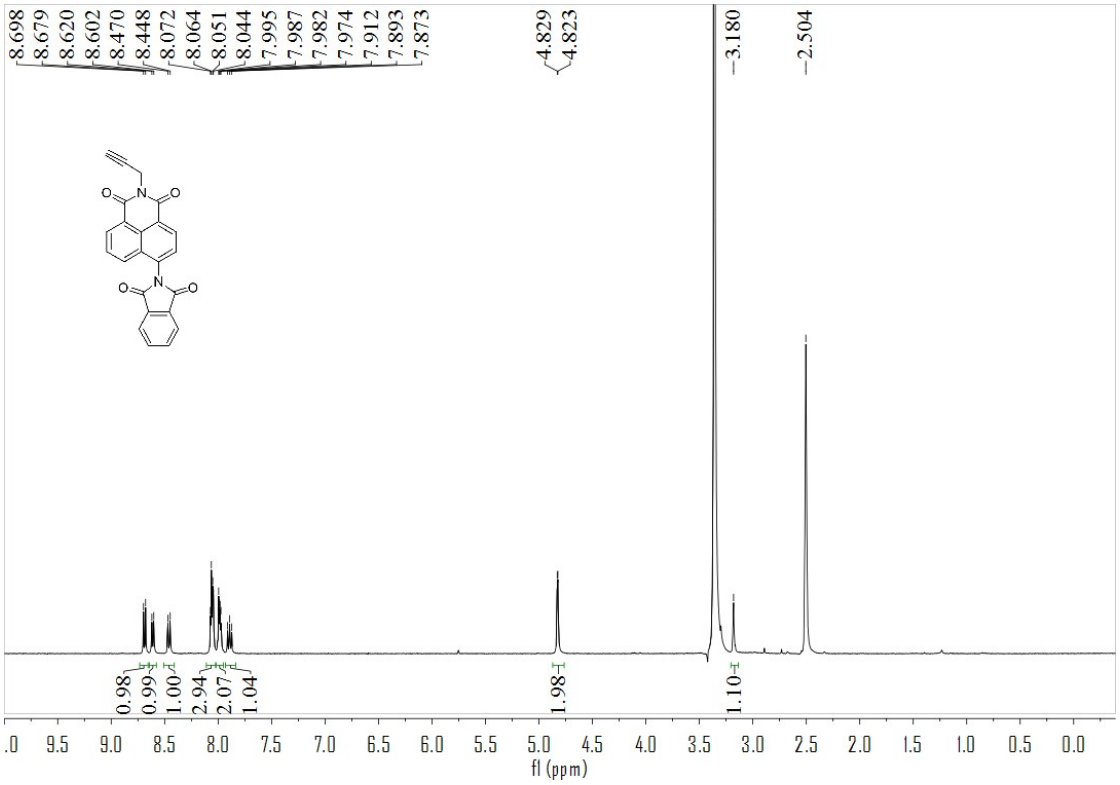



**Fig. S15** [1]H NMR of probe **HP-1**

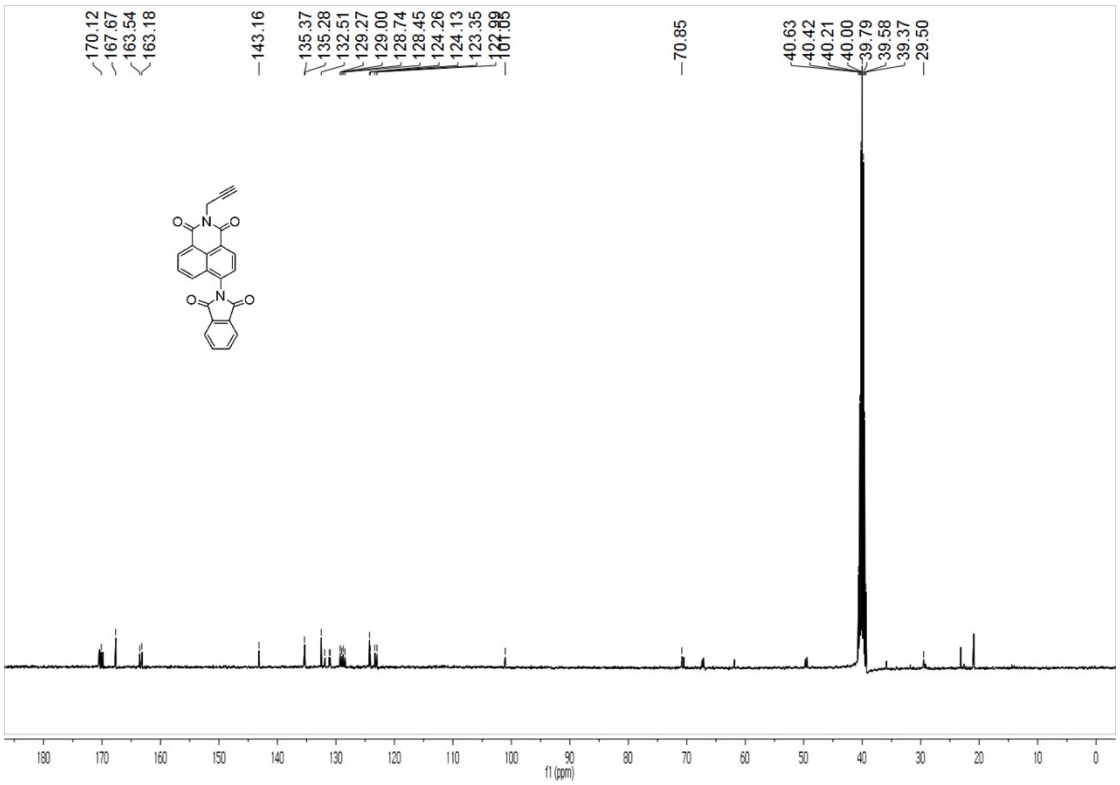
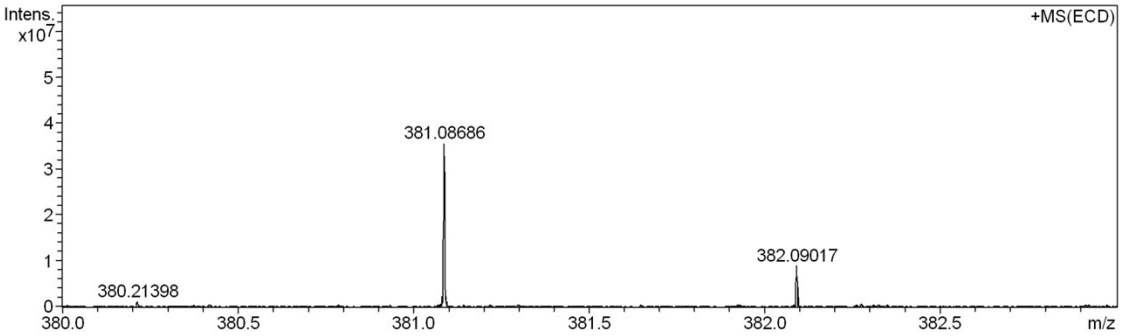



**Fig. S16** $^{13}$C NMR of probe **HP-1**




| Meas. m/z | # | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 381.08686 | 1 | C 23 H 13 N 2 O 4 | 100.00 | 381.08698 | 0.1 | 0.3 | 8.5 | 18.5 | even | ok |

**Fig. S17** HRMS of probe **HP-1**

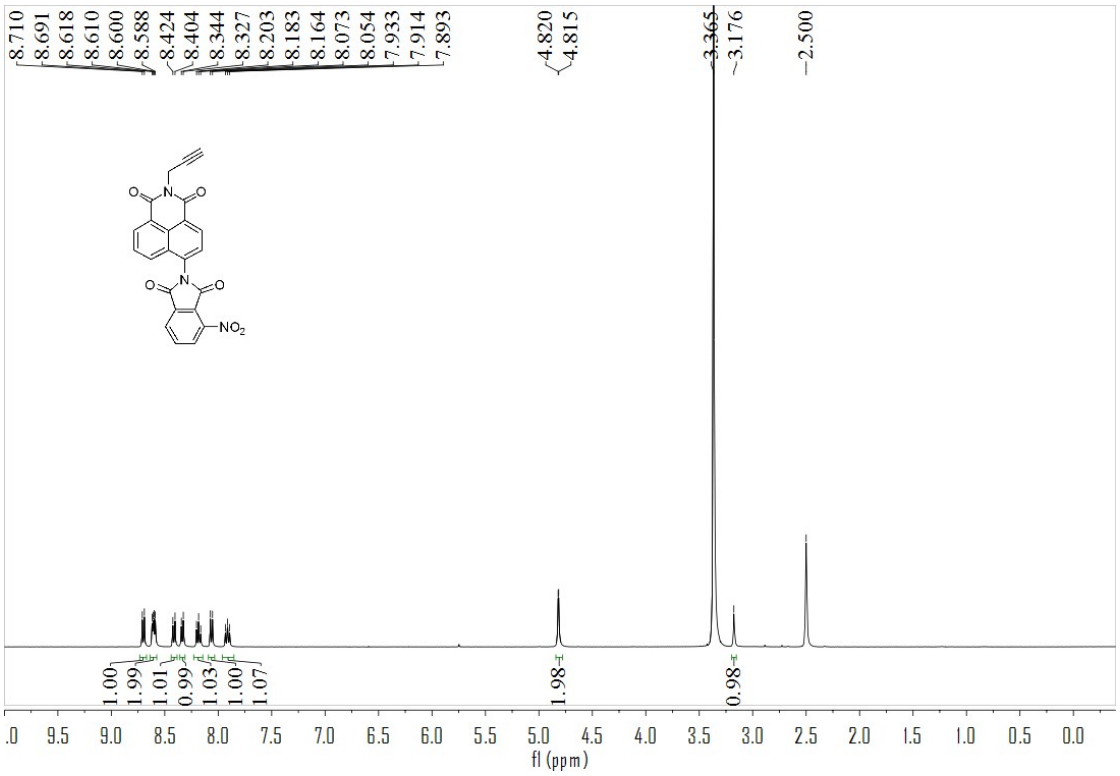



**Fig. S18** $^{1}$H NMR of probe **HP-2**.

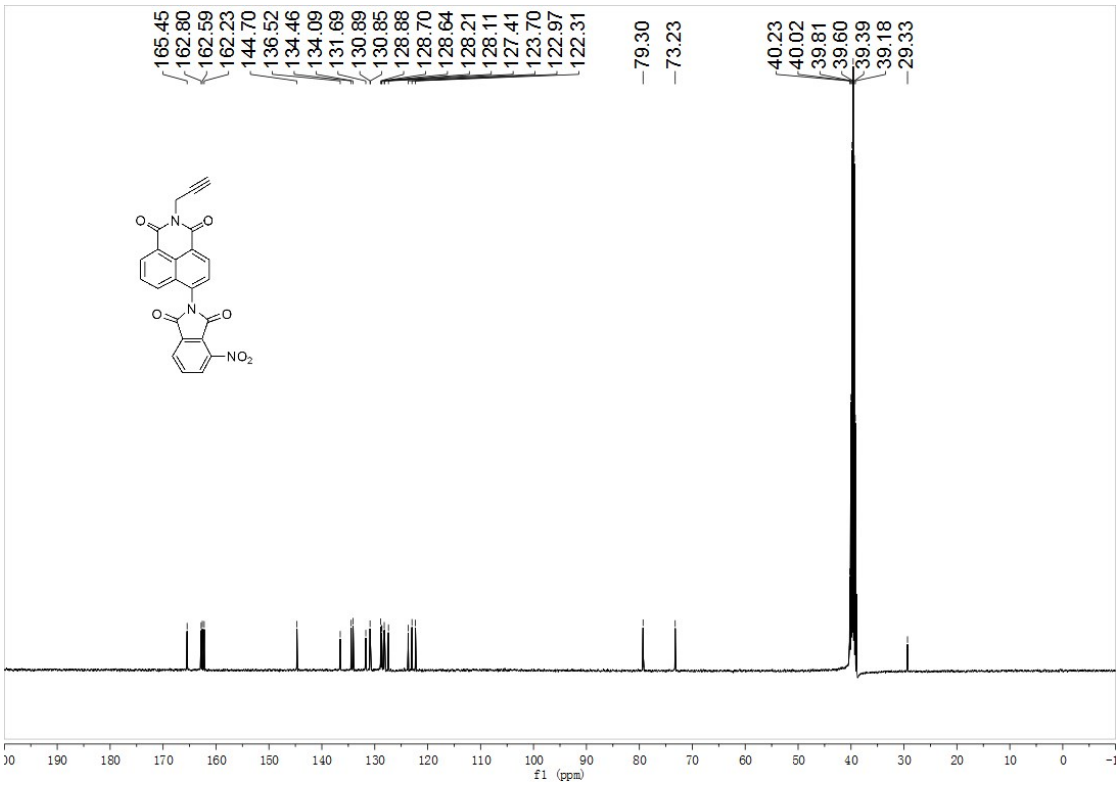
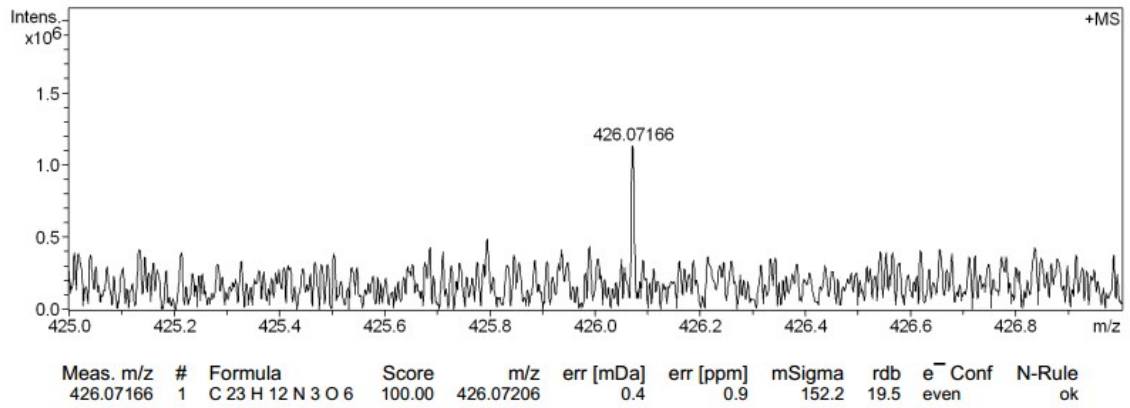



**Fig. S19** $^{13}$C NMR of probe **HP-2**




| Meas. m/z | # | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 426.07166 | 1 | C 23 H 12 N 3 O 6 | 100.00 | 426.07206 | 0.4 | 0.9 | 152.2 | 19.5 | even | ok |

**Fig. S20** HRMS of probe **HP-2**.

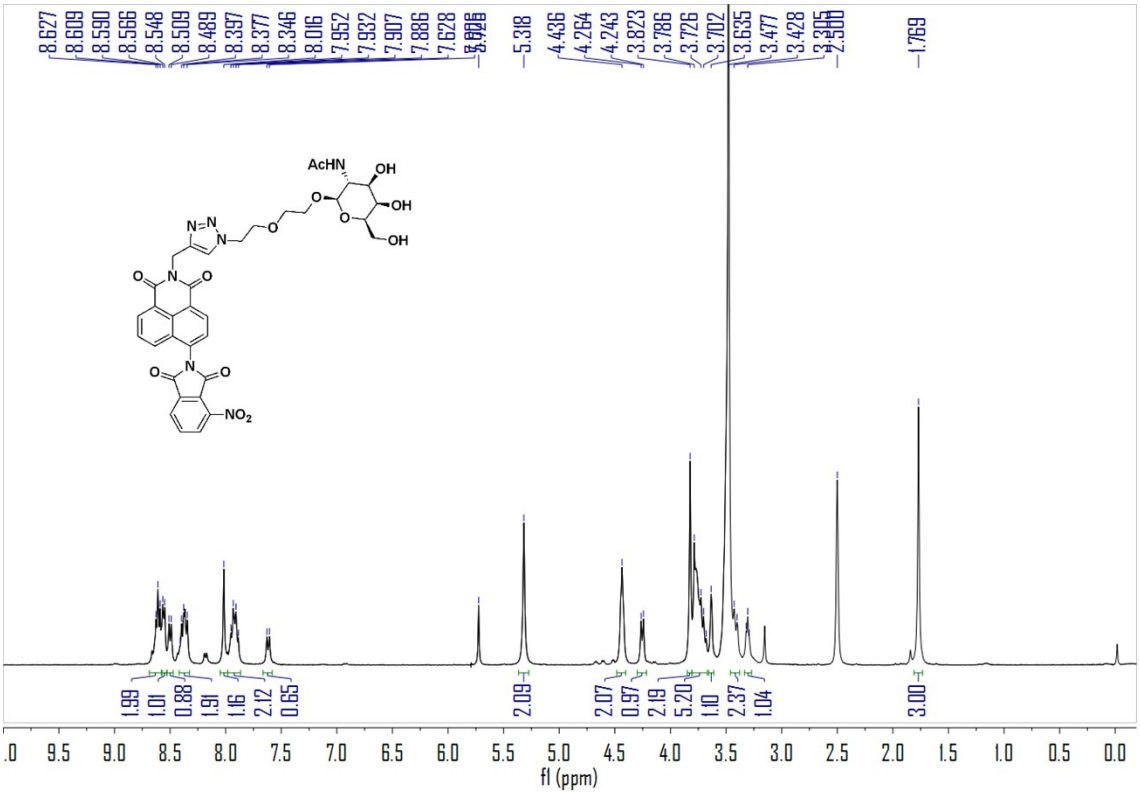



**Fig. S21** $^{1}$H NMR of probe **GHP**

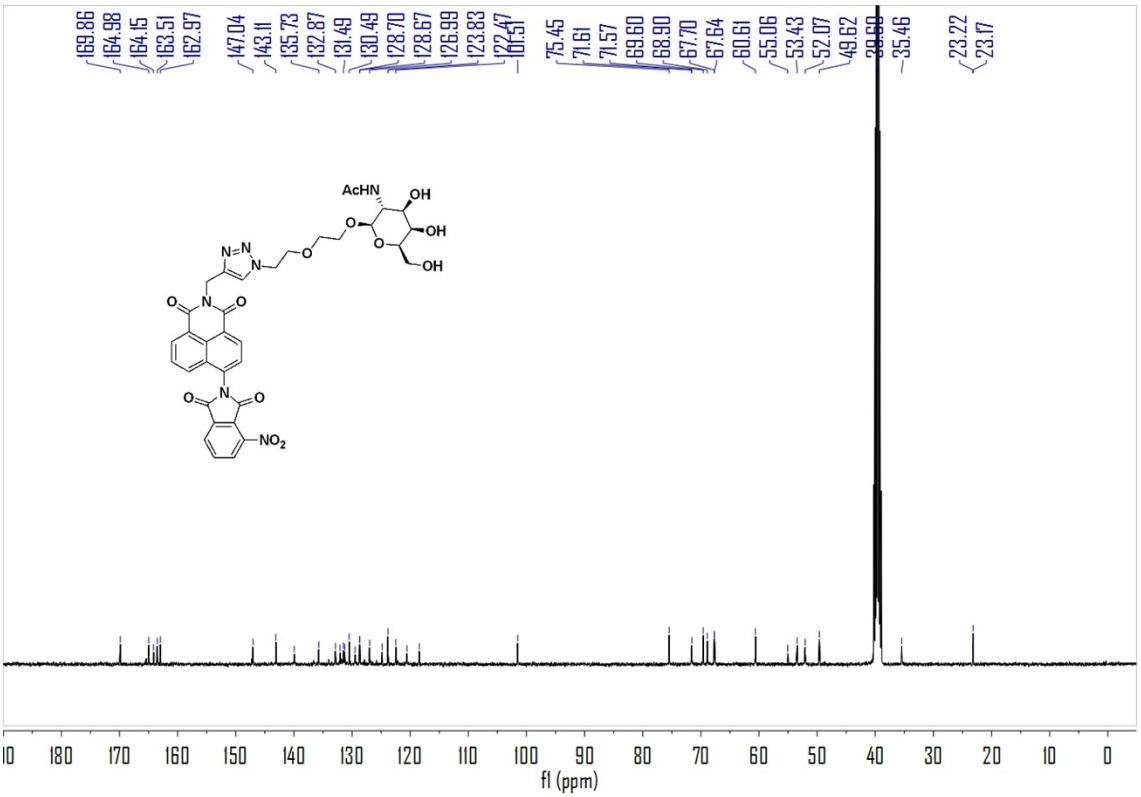



**Fig. S22** $^{13}$C NMR of probe **GHP**

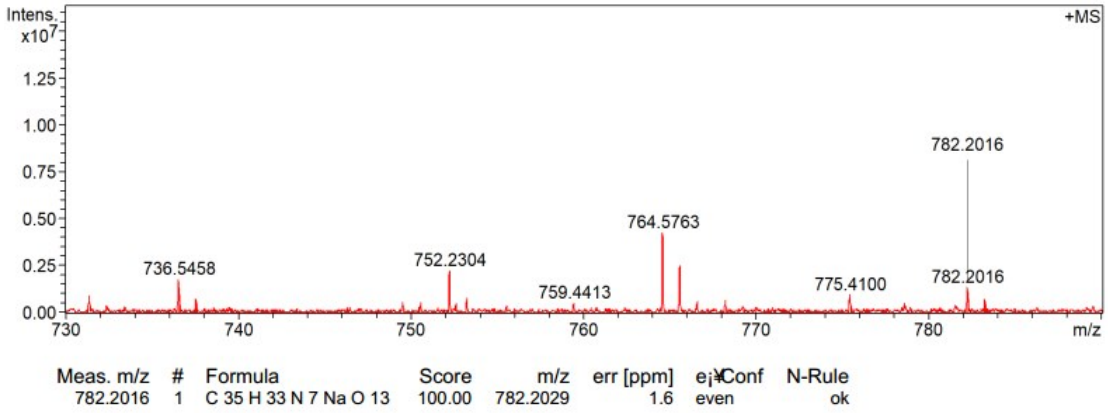



**Fig. S23** HRMS of probe **GHP**