Electronic Supplementary Information

# A luminescent lanthanide approach towards direct visualization of primary cilia in living cells

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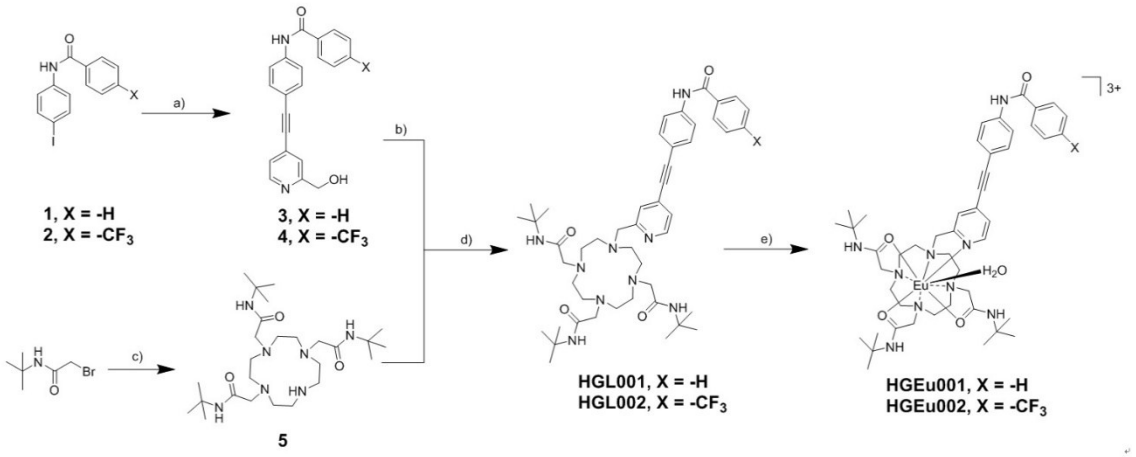
## 1. Synthesis and characterization

**General information for synthesis.** Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), diisopropylamine (DIPA) and acetonitrile ($CH_3CN$) were prepared over calcium hydride ($CaH_2$). All reactions were carried out with anhydrous solvents under a nitrogen atmosphere unless otherwise specified. All the reagents were obtained commercially with high quality and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) which was carried out on silica gel plates (0.25 mm, 60F-254) by using UV light as visualizing method. Flash column chromatography was carried out on 200-300 mesh silica gel. $^1H$ and $^{13}C$ NMR spectra were recorded on a 400 ($^1H$: 400 MHz, $^{13}C$: 100 MHz) spectrometer. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, br = broad. High-resolution mass spectra were obtained from an ESI or MALDI-TOF mass spectrometer.




Scheme S1. The chemical structures and the synthetic route for the primary-cilium-specific probe **HGEu001** and its motif complex **HGEu002**. (unspecific control) a) (4-ethynylpyridin-2-yl)methanol[S1], $Pd(PPh_3)_4$, CuI, DIPEA, THF; b) MsCl, DIPEA, DCM; c) cyclen, $NaHCO_3$, $CH_3CN$; d) $K_2CO_3$, MeCN, 60 $^oC$; e) $EuCl_3{\cdot}6H_2O$, $H_2O$, MeOH, rt., 24 hours.

### Synthesis of N-(4-iodophenyl)-4-(trifluoromethyl)benzamide (2)

To the solution of 4-iodoaniline (5 g, 46.23 mmol) and DIPEA (13.42 mL, 77.06 mmol) in DCM (200 mL), 4-(trifluoromethyl)benzoyl chloride (5.72 mL, 38.53 mmol) was added dropwise at 0 $^oC$ in 30 min. The resulting solution was stirred for 12 hours at room temperature. The solvent of the resulting mixture was concentrated to 100 mL,

and the white precipitate was collected as product after filtration. (13.41 g, 34.29 mmol, yield = 89 %) $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.57 (s, 1 H), 8.13 (d, *J* = 4 Hz, 2 H), 7.92 (d, *J* = 4 Hz, 2 H), 7.72 (d, *J* = 4 Hz, 2 H), 7.63 (d, *J* = 4 Hz, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 164.5, 138.6, 138.5, 137.3, 131.6, 131.3, 128.6, 125.4, 125.3, 125.2, 122.5, 87.9; HRMS (MALDI-TOF) m/z calcd. for $C_{14}H_{10}F_3INO$ $[M + H]^+$ 391.9759 found 391.9761.

**Synthesis of N-(4-((2-(hydroxymethyl)pyridin-4-yl)ethynyl)phenyl)benzamide (3)**

(4-ethynylpyridin-2-yl)methanol[S1] (0.92 g, 6.8 mmol) was added into the solution of N-(4-iodophenyl)benzamide (**1**)[S2] (3.36 g, 10.4 mmol), $Pd(PPh_3)_2Cl_2$ (136 mg, 0.21 mmol), CuI (80 mg, 0.42 mmol) and DIPEA (20 mL) in freshly distilled THF (200 mL). The resulting mixture was stirred at 45 $^o$C for 6 hours under protection of $N_2$ gas. Silica gel flash column chromatography (DCM : MeOH = 30 : 1) of the concentrated residue gave a white solid as the product. (2.16 g, 6.4 mmol, yield = 94%) $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.49 (s, 1 H), 8.52 (d, *J* = 2 Hz, 1 H), 7.96 (d , *J* = 4 Hz, 2 H), 7.90 (dd, $J_1$ = 4 Hz, $J_2$ = 8 Hz, 2 H), 7.63-7.60 (m, 3 H), 7.57-7.53 (m, 3 H), 7.38 (d, *J* = 2 Hz, 1 H), 5.52 (br, 1H), 4.58 (s, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 165.8, 162.5, 148.9, 140.4, 134.7, 132.4, 131.8, 130.8, 128.4, 127.7, 123.2, 121.4, 120.1, 115.9, 93.6, 86.6, 63.9, 53.5; HRMS (MALDI-TOF) m/z calcd. for $C_{21}H_{17}N_2O_2$ $[M + H]^+$ 329.1290 found 329.1295.

**Synthesis of N-(4-((2-(hydroxymethyl)pyridin-4-yl)ethynyl)phenyl)-4-(trifluoro methyl) benzamide (4)**

(4-ethynylpyridin-2-yl)methanol (1.13 g, 8.52 mmol) was added into the solution of N-(4-iodophenyl)-4-(trifluoromethyl)benzamide (**2**) (4 g, 10.22 mmol), $Pd(PPh_3)_4$ (197 mg, 0.17 mmol), CuI (65 mg, 0.34 mmol) and DIPEA (20 mL) in freshly distilled THF (200 mL). The resulting mixture was stirred at 45 $^o$C for 6 hours under protection of $N_2$ gas. Silica gel flash column chromatography (DCM : MeOH = 30 : 1) of the concentrated residue gave a white solid as the product. (3.10 g, 7.84 mmol, yield = 92%) $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.71 (s, 1 H), 8.52 (d, *J* = 4 Hz, 1 H), 8.15 (d, *J* = 4 Hz, 2 H), 7.93 (d, *J* = 4 Hz, 2 H)， 7.90 (d, *J* = 4 Hz, 2 H)， 7.64 (d, *J* = 4 Hz, 2 H)， 7.55 (s, 1 H), 7.36 (d, *J* = 2 Hz, 1 H), 5.54 (t, *J* = 4 Hz, 1 H), 4.14 (d, *J* = 2 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 164.7, 162.5, 148.9, 140.0, 138.5, 132.5, 131.7, 131.3, 130.70, 128.7, 125.4, 125.2, 123.2, 122.5, 121.4, 120.2,

116.3, 93.4, 86.6, 63.9; HRMS (MALDI-TOF) m/z calcd. for $C_{22}H_{16}F_3N_2O_2$ $[M + H]^+$ 397.1164 found 397.1168.

**Synthesis of 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N-(tert-butyl) acetamide) (5)**

2-bromo-N-(tert-butyl)acetamide [S3] (10.1g, 52.2 mmol) was added into the solution of 1,4,7,10-tetraazacyclododecane (3.0 g, 17.4 mmol) in anhydrous acetonitrile (80 mL), followed by $NaHCO_3$ (21.9 g, 261 mmol). The resulting solution was stirred at room temperature for 24 hours. After filtration of the resulting mixture, filtrate was concentrated and recrystallized from hot water to obtain a white solid as the product. (4.8 g, 8.7 mmol, yield = 50%) $^1$H NMR (400 MHz, $CDCl_3$): δ 6.78 (s, 1 H), 6.68 (s, 2 H), 3.05 (s, 4 H), 3.05 (s, 2 H), 2.70 (m, 16 H), 1.37 (s, 18 H) 1.36 (s, 9 H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 60.5, 59.6, 53.4, 52.9, 52.3, 51.1, 50.9, 46.7, 28.9, 28.8; HRMS (MALDI-TOF) m/z calcd. for $C_{26}H_{54}N_7O_3$ $[M + H]^+$ 512.4288 found 512.4285.

**Synthesis of 2,2',2''-(10-((4-((4-benzamidophenyl)ethynyl)pyridin-2-yl)methyl) -1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N-(tert-butyl)acetamide) (HGL001)**

Methanesulfonyl chloride (0.22 mL, 2.73 mmol) was added into a stirred solution of N-(4-((2-(hydroxymethyl)pyridin-4-yl)ethynyl)phenyl)benzamide (**3**) (300 mg, 0.91 mmol) in anhydrous DCM (150 mL) and DIPEA (1.59 mL, 9.11 mmol). The resulting mixture was stirred at room temperature for 3 hours. The solution was then washed with saturated $NaHCO_3$ solution, saturated $NH_4Cl$ solution and brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated to give a pale yellow solid as the intermediate compound, (4-((4-benzamidophenyl)ethynyl)pyridin-2-yl)methyl methanesulfonate, which was directly used in the next step without further purification. The pale yellow solid was dissolved in dry $CH_3CN$ (50 mL). 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N-(tert-butyl)acetamide) (**5**, 0.50 g, 0.61 mmol) and anhydrous $K_2CO_3$ (1.26 g, 9.1 mmol) were added. The resulting mixture was stirred at 50 $^o$C for 12 hours under $N_2$ gas. The solids were filtered off, and the filtrate was concentrated. Silica gel flesh column chromatography ($CH_2Cl_2$ : MeOH = 12 : 1) of the residue gave a pale yellow solid as the product (378 mg, 0.46 mmol, yield = 75%,). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.53 (s, 1 H), 8.38 (d, $J$ = 2 Hz, 1 H), 7.97 (d, $J$ = 4 Hz, 2 H), 7.94 (d, $J$ = 4 Hz, 2 H), 7.83 (br, 2 H),7.60-7.53 (m,

7 H), 7.38 (d, *J* = 2 Hz, 1 H), 3.66 (br, 2 H), 3.20-2.07 (m, 22 H), 1.30 (s, 9 H), 1.22 (s, 18 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 170.5, 169.9, 165.9, 158.7, 149.0, 140.5, 134.7, 132.3, 131.8, 131.1, 128.5, 128.2, 127.8, 125.1, 123.5, 120.1, 115.9, 93.9, 86.3, 58.1, 57.9, 57.2, 50.4, 50.3, 28.3, 28.1; HRMS (MALDI-TOF) m/z calcd. for $C_{47}H_{68}N_9O_4$ $[M + H]^+$ 822.5394, found 822.5390.

**Synthesis of 2,2',2''-(10-((4-((4-(4-(trifluoromethyl)benzamido)phenyl)ethynyl) pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N-(tert-butyl)acetamide) (HGL002)**

Methanesulfonyl chloride (0.18 mL, 2.28 mmol) was added into a stirred solution of N-(4-((2-(hydroxymethyl)pyridin-4-yl)ethynyl)phenyl)-4-(trifluoromethyl)benzamide (**4**) (300 mg, 0.76 mmol) in anhydrous DCM (150 mL) and DIPEA (1.33 mL, 7.61 mmol).The resulting mixture was stirred at room temperature for 3 hours. After that the solution was then washed with saturated $NaHCO_3$ solution, saturated $NH_4Cl$ solution and brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated to give a pale yellow solid as the intermediate compound, (4-((4-(4-(trifluoromethyl)benzamido)phenyl)ethynyl)pyridin-2-yl)methyl methanesulfonate, which was directly used in the next step without further purification. The pale yellow solid was dissolved in dry $CH_3CN$ (50 mL). 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N-(tert-butyl)acetamide) (**5**, 0.42 g, 0.51 mmol) and anhydrous $K_2CO_3$ (1.05 g, 7.6 mmol) were added. The resulting mixture was stirred at 50 $^o$C for 12 hours under $N_2$ gas. The solids were filtered off, and the filtrate was concentrated. Silica gel flesh column chromatography ($CH_2Cl_2$ : MeOH = 12 : 1) of the residue gave a pale yellow solid as the product (354 mg, 0.40 mmol, yield = 78%). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.78 (s, 1 H), 8.38 (d, *J* = 2 Hz, 1 H), 8.18 (s, 1 H), 8.16 (s, 1 H), 7.91 (d, *J* = 4 Hz, 4 H), 7.82 (br, 2 H), 7.62 (d, *J* = 8 Hz, 2 H), 7.57 (d, *J* = 4 Hz, 2 H), 7.39 (d, *J* = 2 Hz, 1 H), 3.71 (br, 2 H), 2.87-2.17 (m, 22 H), 1.29 (s, 9 H), 1.27 (s, 18 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 170.5, 169.8, 164.7, 158.6, 148.9, 140.1, 138.4, 132.3, 131.6, 131.3, 131.0, 128.7, 125.4, 125.2, 125.1123.4, 122.5, 120.2, 116.2, 93.7, 86.4, 58.1, 57.9, 57.2, 54.9, 50.4, 50.3, 49.4, 28.3, 28.1; HRMS (MALDI-TOF) m/z calcd. for $C_{48}H_{67}F_3N_9O_4$ $[M + H]^+$ 890.5268 found 890.5264.

**Synthesis of complex HGEu001**

Europium (III) chloride hexahydrate (77 mg, 0.21 mmol) was added to the solution of the ligand (**HGL001**, 0.20 mmol) in MeOH/$H_2O$ (100 mL, v : v = 1 : 1). The resulting

solution was maintained in a pH range of 6.0-6.5 with NaOH solution (0.4 M) and stirred at room temperature for 24 hours. The solvents were removed under vacuum; the residue was dissolved in 1 mL of methanol and dropped into ethyl ether (50 mL). The precipitates were filtered, washed with diethyl ether and dried under vacuum at room temperature. White solids were collected as the products. (222 mg, 0.19 mmol, yield = 90%). HRMS (+ESI) m/z calcd. for $C_{47}H_{65}EuN_9O_4$ $[M - H_2O - 2H]^+$ 972.4372, found 972.4378, calcd. for $C_{47}H_{66}ClEuN_9O_4$ $[M - H_2O - H + Cl^-]^+$ 1008.4139, found 1008.4119, calcd. for $C_{47}H_{67}Cl_2EuN_9O_4$ $[M - H_2O + 2Cl]^+$ 1044.3905, found 1044.3882 (Fig. S2) HPLC characterization: Retention time = 15.20 min. (Table S1 and Fig. S1)

**Synthesis of complex HGEu002**

**HGEu002** was obtained from ligand **HGL002** with same procedures as **HGEu001** (204 mg, 0.19 mmol, yield = 95%). HRMS (+ESI) m/z m/z calcd. for $C_{48}H_{65}EuF_3N_9O_4$ $[M - H]^{2+}$ m/z = 1041.4324/2 = 520.7162, found 520.7186. (Fig. S3); HPLC characterization: Retention time = 15.36 min. (Table S1 and Fig. S1)

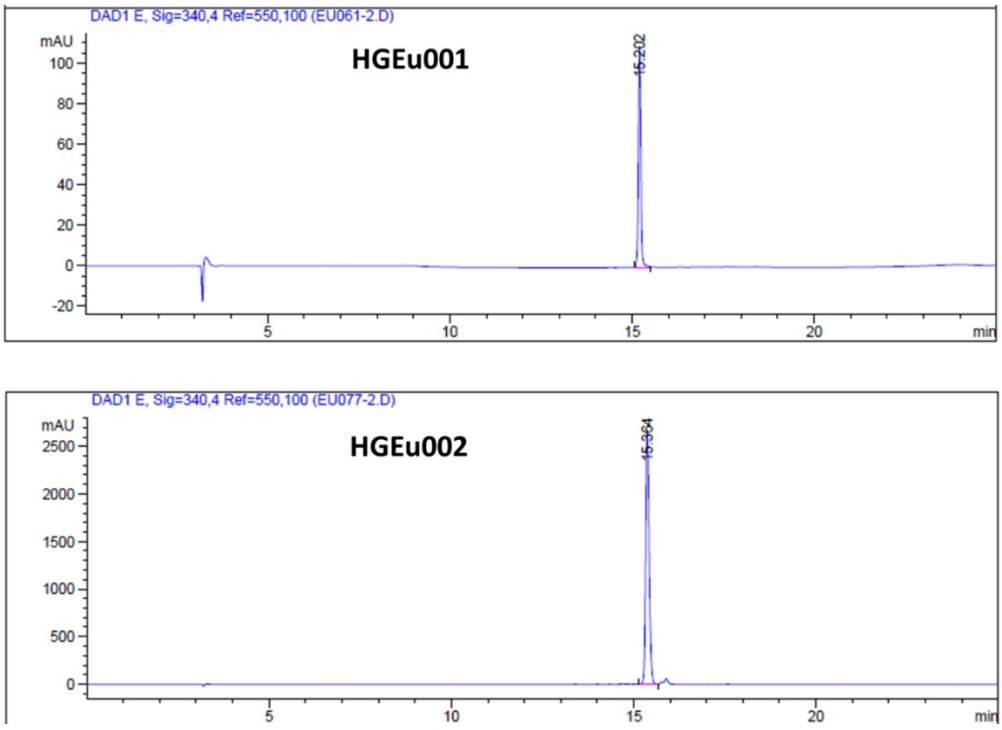



**Fig. S1** HPLC characterization of **HGEu001** and **HGEu002**. The HPLC analysis was performed on Agilent 1290 Infinity Quaternary LC System coupled with a DAD detector. The column used was a Hypersil GOLD Analytical Column (250 × 4.6 mm, 5μm) analytical column. The LC elution profiles were shown in Table S1.

**Table S1** Solvent gradient of HPLC for the characterization of **HGEu001** and **HGEu002**.

| Time /min | 0.05% TFA in water /% | 0.05% TFA in $CH_3CN$ /% |
|---|---|---|
| 0.0 | 90 | 10 |
| 5 | 90 | 10 |
| 15 | 40 | 60 |
| 20 | 90 | 10 |
| 25 | 90 | 10 |

The column Flow rate = 1 mL/min

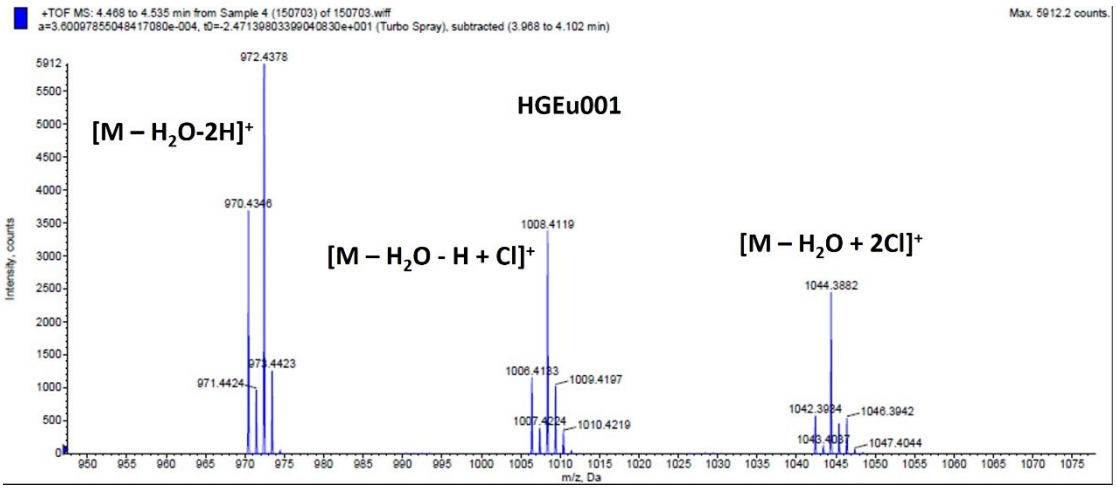



**Fig. S2** HRMS(+ESI) spectrum of the complex **HGEu001**. (m/z calcd. for $C_{47}H_{65}EuN_9O_4$ $[M - H_2O - 2H]^+$ 972.4372, found 972.4378, calcd. for $C_{47}H_{66}ClEuN_9O_4$ $[M - H_2O - H + Cl^-]^+$ 1008.4139, found 1008.4119, calcd. for $C_{47}H_{67}Cl_2EuN_9O_4$ $[M - H_2O + 2Cl]^+$ 1044.3905, found 1044.3882.)

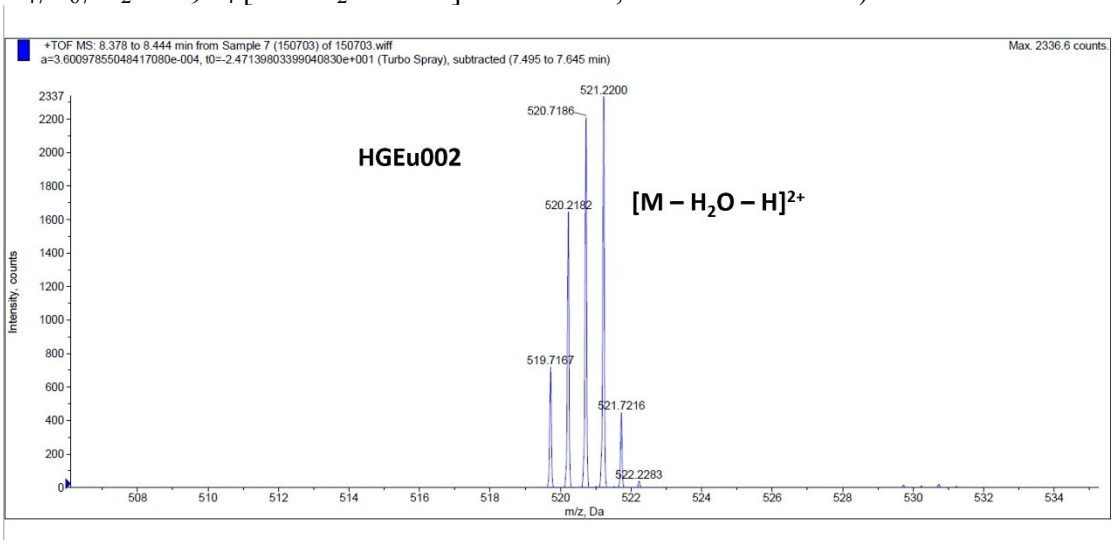



**Fig. S3** HRMS(+ESI) spectrum of the complex **HGEu002**. (m/z calcd. for $C_{48}H_{65}EuF_3N_9O_4$ $[M - H_2O - H]^{2+}$ m/z = 1041.4324/2 = 520.7162 , found 520.7186.

## 2. Photophysical properties studies

UV-Visible absorption spectra in the spectral range 200 to 1100 nm were recorded by an HP Agilent UV-8453 Spectrophotometer. The emission spectra and the emission decay lifetimes of **HGEu001** and **HGEu002** were measured by Horiba Fluorolog-3 spectrophotometer and also measured by Edinburgh instrument FLS920 spectrophotometer for cross checking. (Two spectrophotometers are equipped with a 450W continuous xenon lamp for steady state emission measurement, 60W xenon flashlamp for emission life time measurement and an UV-Vis PMT detector – Hamamatsu - R928 cooled at -20 $^{o}$C.)

The overall quantum yield upon ligand excitation, $Q_{Eu}^{L}$, which we have determined by a demountable integrating sphere supplied by Horiba and Edinburgh Instruments.

For two-photon experiments, the 800 nm pump source was from the fundamental of a femtosecond mode-locked Ti:Sapphire laser system (output beam ~ 150 fs duration and 1 kHz repetition rate). The lasers were focused to spot size ~ 50 μm via an f = 10 cm lens onto the sample. The emitting light was collected with a backscattering configuration into a 0.5 m spectrograph and detected by a liquid nitrogen-cooled CCD detector. A power meter was used to monitor the uniform excitation. The theoretical framework and experimental protocol for the two-photon cross-section measurement have been outlined by Webb and Xu.[S4] In this approach, the two-photon excitation (TPE) ratios of the reference and sample systems are given by:

$$\frac{\sigma_2^S \cdot \phi^S}{\sigma_2^R \cdot \phi^R} = \frac{C_R \cdot n_S \cdot F^S(\lambda)}{C_S \cdot n_R \cdot F^R(\lambda)}$$

where ϕ is the quantum yield, C is the concentration, n is the refractive index, and

$F(\lambda)$ is the integrated emission spectrum. In our measurements, we have ensured that the excitation flux and the excitation wavelengths are the same for both the sample and the reference. The two-photon absorption cross-section $\sigma^2$ of **HGEu001** and **HGEu002** was determined using Rhodamine 6G as reference.

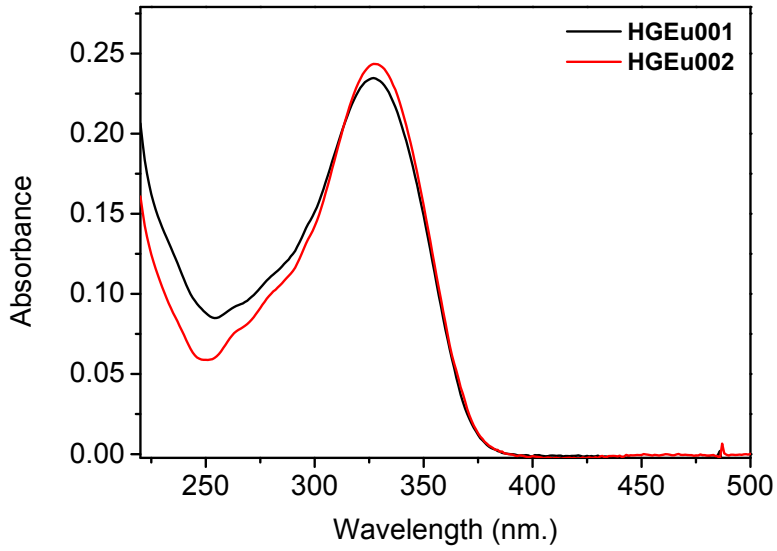



**Fig. S4** The absorption spectra of **HGEu001** and **HGEu002** in aqueous solution (10 μM).

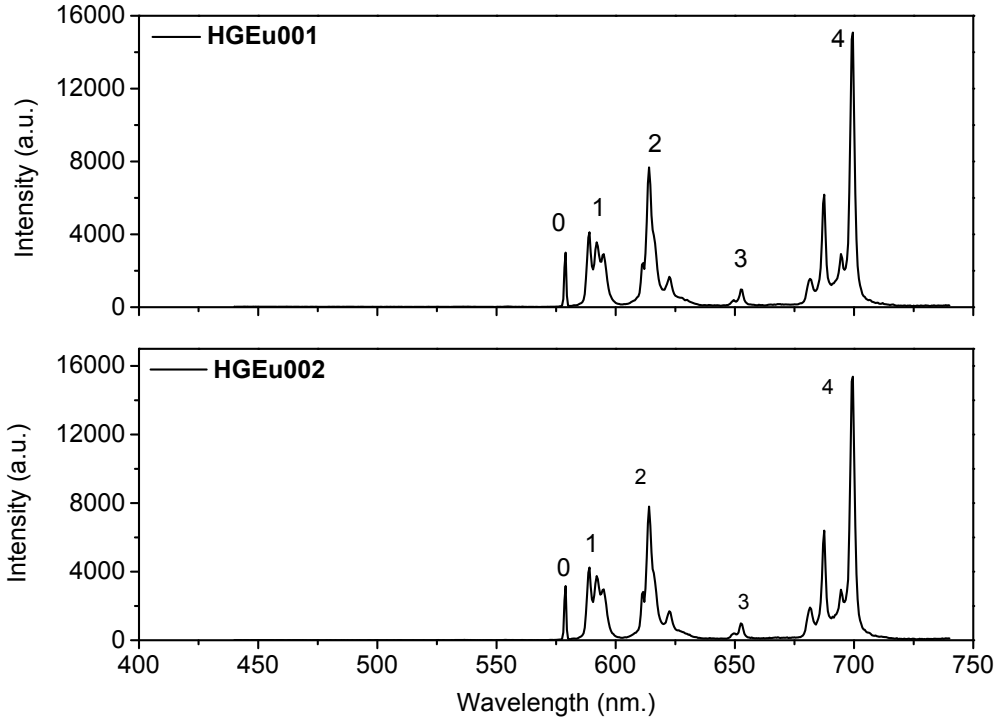



**Fig. S5** The emission spectra of **HGEu001** and **HGEu002** aqueous solution (10 μM, $\lambda_{ex}$ = 340 nm). The spectra were recorded on Horiba Flurolog-3 spectrophotometer. The same emission bands and ratios obtained compared with the emission spectra measured with Edinburgh instrument FLS920 spectrophotometer. (Fig. 1)

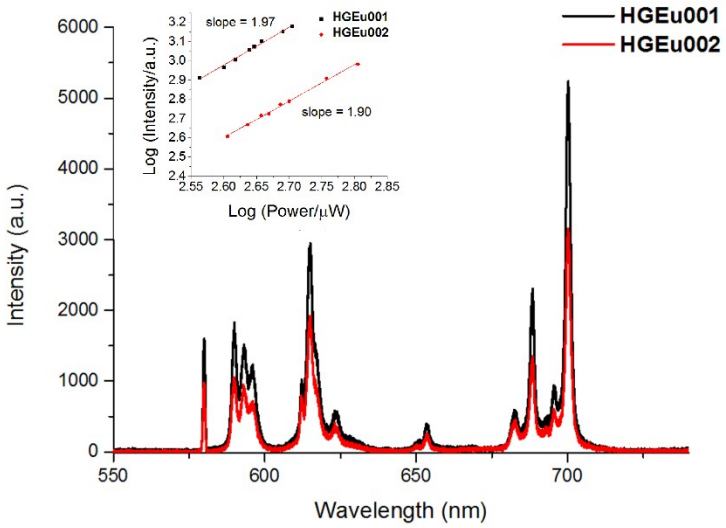



**Fig. S6** The two-photon induced emission spectra of **HGEu001** and **HGEu002** in aqueous solution ($\lambda_{ex}$ = 800 nm, 150 μM). Inset: Quadratic dependence of emission intensity on the excitation power at 800 nm.

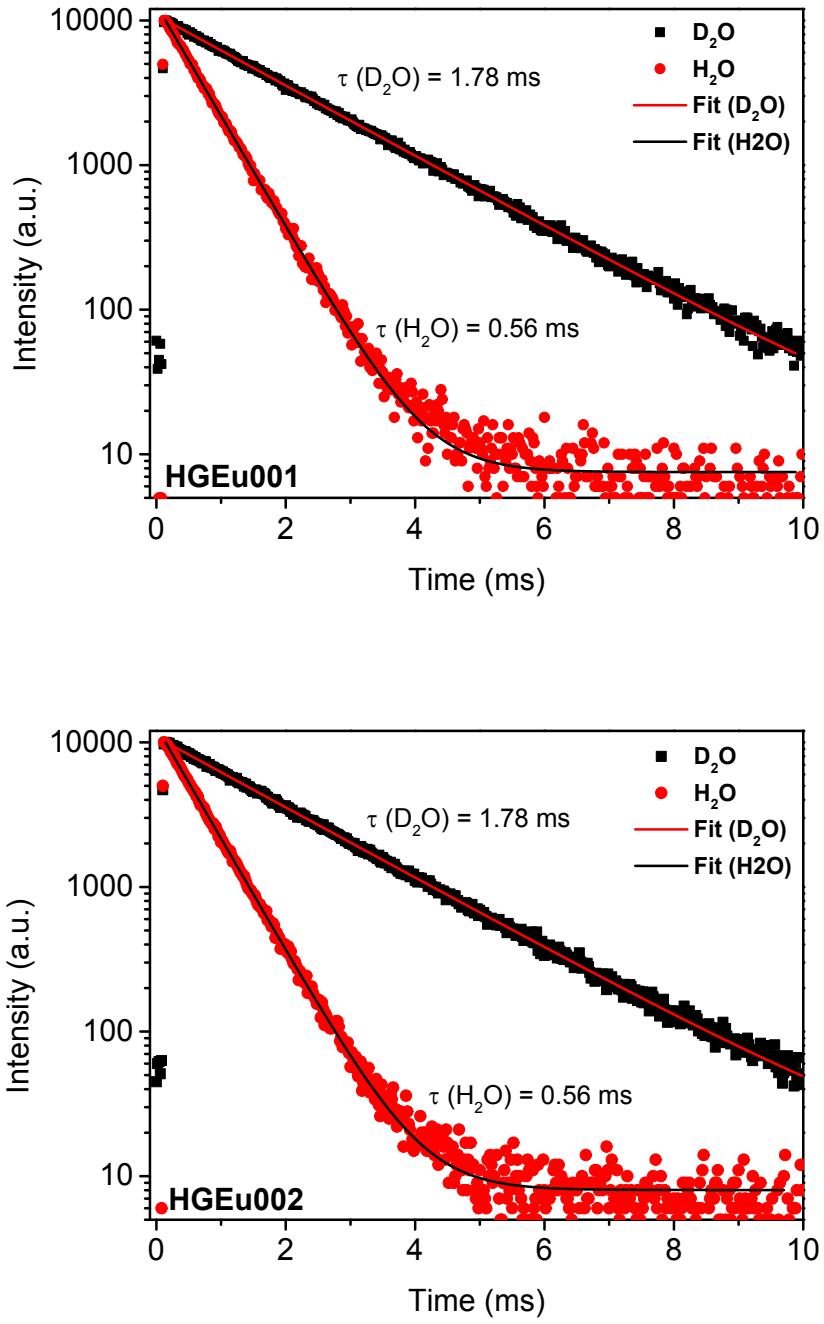



**Fig. S7** The emission decay curve of the complexes **HGEu001** and **HGEu002** in $D_2O$ and $H_2O$. ($\lambda_{em}$ = 614 nm. $^5D_0 \rightarrow {}^7F_2$. $\lambda_{ex}$ = 340 nm)

**Table S2.** Photophysical properties of the europium complexes **HGEu001** and **HGEu002**.

| Complex | $\lambda_{max}$/ nm [a] | $\varepsilon$/ $M^{-1}$ $cm^{-1}$ [a] | $\tau(H_2O)$/ ms [b] | $\tau(D_2O)$/ ms [b] | q[c] | $\Phi_L^{Eu}$/ % [d] | $\sigma^2$/GM [e] |
|---|---|---|---|---|---|---|---|
| **HGEu001** | 327 | 23400 | 0.56 | 1.78 | 0.9 | 10.5 | 10.0 ± 2.5 |
| **HGEu002** | 327 | 22200 | 0.56 | 1.78 | 0.9 | 10.3 | 8.1 ± 2.0 |

[a] Absorption coefficient in $H_2O$, 298K; [b] Europium emission decay ($\lambda_{em}$ = 614 nm. $^5D_0 \rightarrow {}^7F_2$. $\lambda_{ex}$ = 330 nm); [c] Derived hydration numbers, q (± 0.2). q = 1.2[(k($H_2O$) - k($D_2O$))-(0.25 + 0.07x)] (k = $\tau^{-1}$, x = number of carbonyl-bound amide NH oscillators);[S5] [d] Overall europium emission quantum yield in $H_2O$, by integrated sphere;[S6] The two-photon absorption cross-section $\sigma^2$ (GM = $10^{-50}$$cm^4$ s $photon^{-1}$ $molecule^{-1}$ , $\lambda_{em}$ = 550 – 740 nm, $\lambda_{ex}$ = 800 nm).

## 3. Cell-based experiments

### 3.1 Tissue culture

**Cells for MTT cytotoxicity assays:** Human cervical cancer HeLa cells was grown in Dulbecco's Modified Eagle Medium (DMEM). Human lung normal diploid fibroblasts MRC-5 and neuroblastoma cells SK-N-SH were provided by Cell resource center of Shanghai Institute of Biological Sciences, Chinese Academy of Sciences; and cultured in MEM (GIBCO 41500034); Human derived liver cells QSG-7701 cells were grown in RPMI-1640 (GIBCO 31800022); All cells were supplemented with 10% (v/v) fetal bovine serum, 1% penicillin and streptomycin at 37 $^{o}$C and 5 % $CO_2$.

**Ciliated HeLa, MRC-5 and NIH3T3 cells for cilia imaging:** HeLa, MRC-5 and NIH3T3 cells culture and ciliation were performed according to the literature's procedures.[18] Briefly, HeLa, MRC-5 or NIH3T3 cells were grown in DMEM medium supplemented with 10% fetal bovine serum at the confluent of 80%, and then growth medium was replaced by starvation medium and further incubated overnight (~12 hours) to allow ciliation. For immunofluorescences, NIH3T3 cells were rinsed in PBS and fixed in 4% paraformaldehyde for 10 minutes, and then incubated with primary antibodies (anti-acetylated tubulin, #ab24610, Abcam; anti-ARL13B, NeuroMab clone N295B/66) that diluted in 3% BSA+0.1% Trition X-100 and incubated overnight. Secondary antibodies conjugated with Alexa Fluor®488 were used for imaging.

### 3.2 MTT cell cytotoxicity assays

HeLa, SK-N-SH, QSG-7701 or MRC-5 cells treated with testing complexes for 24 hours were further incubated with MTT, 3-(4, 5-dimethylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide (0.5 mg/mL) for 4 hours, to produce formazan during cell metabolism. Then, formazan was thoroughly dissolved by dimethyl sulfoxide

(DMSO), and the absorbance of solutions was measured in Bio-Rad iMark microplate reader (490 nm). Quadruplicates were performed. Data analysis and plotting were operated by the GraphPad Prism 5.0 software.

**3.3 Live cell imaging**

The live cell imaging of **HGEu001** and **HGEu002** were undertaken on a linear fluorescence microscopy under 375 nm UV light excitation or a confocal laser scanning microscope, Leica TCS SP8, equipped with a Ti:sapphire laser (Libra II, coherent). The excitation beam produced by 690 nm to 1080 nm (fs laser) was focused on the adherent cells through a 63x oil immersion objective. Cells were seeded on coverslip in 35-mm culture dishes overnight. And then incubated with **HGEu001** or **HGEu002** (10 μM) for 6 hours, and the cells were washed by PBS 3 times before imaging. For the two-photon time lapses cellular images of **HGEu001** and **HGEu002** were observed with different incubation time (3, 6, 18 and 24 hours). Then the unabsorbed complexes were washed out with PBS buffer and the cells were subject to microscopic imaging. 3D images obtained by the Z-stacks form the two-photon confocal microscopy and the 3D reconstruction was done with built-in programme of Leica TCS SP8 confocal microscope.

**3.4 Co-localization imaging**

**a) Co-localization experiments of HGEu001 with organelles.**

Live cell labeling probes of organelles (mitochondria, lysosome and Golgi apparatus) MitoTracker® Green FM (M-7514), LysoTracker® Green DND-26 (L-7526) and GolgiTracker® Oregon Green (W6748, Wheat Germ Agglutinin) were respectively purchased from ThermoFisher Scientic Inc. and stocked in -20 $^{o}$C.

Three dishes of HeLa cells ($1 \times 10^5$) were first incubated with 10 μM **HGEu001** for 6 hours. Then organelles probes (50 nM, each) were parallelly added to the cells

and further incubated for 15 min. The cells were washed by PBS 3 times before imaging on the linear fluorescence microscopy. Under the excitation of UV light (375 nm) emission from the channel (610 - 630 nm) were collected for the emission signals from **HGEu001**. Under 488 nm blue light excitation, imaging channel in the range between 505 and 555 nm were collected from the emission signal of organelles dyes.

**(b) Co-localization and 3D imaging of HGEu001 and HGEu002 with exogenous expressed cilia makers ARL13B and IFT88.**

Full length ARL13B (ADP-ribosylation factor-like protein 13B) was PCR amplified from cDNA library and inserted into multiple cloning sites of pEGFP-C3 (CLONETECH, #6082-1) using restriction digestion sites of XhoI/EcoRI for GFP-ARL13B expression. The positive recombinant was selected through kanamycin and proved by sequencing. Plasmid mEmerald-IFT88-N-18 (intraflagellar transport protein 88 homolog) expressing GFP-IFT88 was a gift from Michael Davidson (Addgene plasmid # 54125). Plasmids were amplified in E.coli (DH5α) and purified using a StarPrep Plasmid Miniprep Kit (GenStar, #D201-04). Both GFP-ARL13B and GFP-ITF88 for primary cilium tracking were expressed in HeLa cells through lipofectamine2000 (Cat.No.11668-019, Invitrogen) mediated transfection. Briefly, a 3.5 cm dish of HeLa cells (with 70–80% confluent) was transfected with 4 μg DNA plus 4 μL lipofectamine2000 mixture according to the manufacturer instructions. After incubation for 8 hours, 10 μM of **HGEu001** or **HGEu002** were added and incubated for 6 hours. Then the unabsorbed complexes were washed out with PBS buffer and the cells were subject to microscopic imaging.

**(c) Immunofluorescences co-localization imaging of HGEu001 and HGEu002 with endogenous cilia markers acetylated tubulin and ARL13B.**

The co-staining experiments of **HGEu001** and **HGEu002** with endogenous cilia

markers acetylated tubulin and ARL13B in ciliated NIH3T3 cells with two-photon confocal microscope ($\lambda_{ex}$ = 700 nm, BP = 550 - 665 nm). **HGEu001**/**HGEu002** were dosed in a concentration of 10 μM, incubation for 6 hours. Acetylated tubulin or ARL13B were lablled by immunofluorescence using antibodies and imaged from the emission of Alexa Fluor® 488.

**Table S3.** Cytotoxicity of the complexes **HGEu001** and **HGEu002** against HeLa, SK-N-SH, QSG-7701 and MRC-5 cell line. ($IC_{50}$/ μM)

| Complex | HeLa | SK-N-SH | QSG-7701 | MRC-5 |
|---|---|---|---|---|
| **HGEu001** | 411 | 389 | 395 | 395 |
| **GEu002** | 417 | 437 | 410 | 402 |

Incubation time = 24 hours; Raw data are shown in Fig. S9.

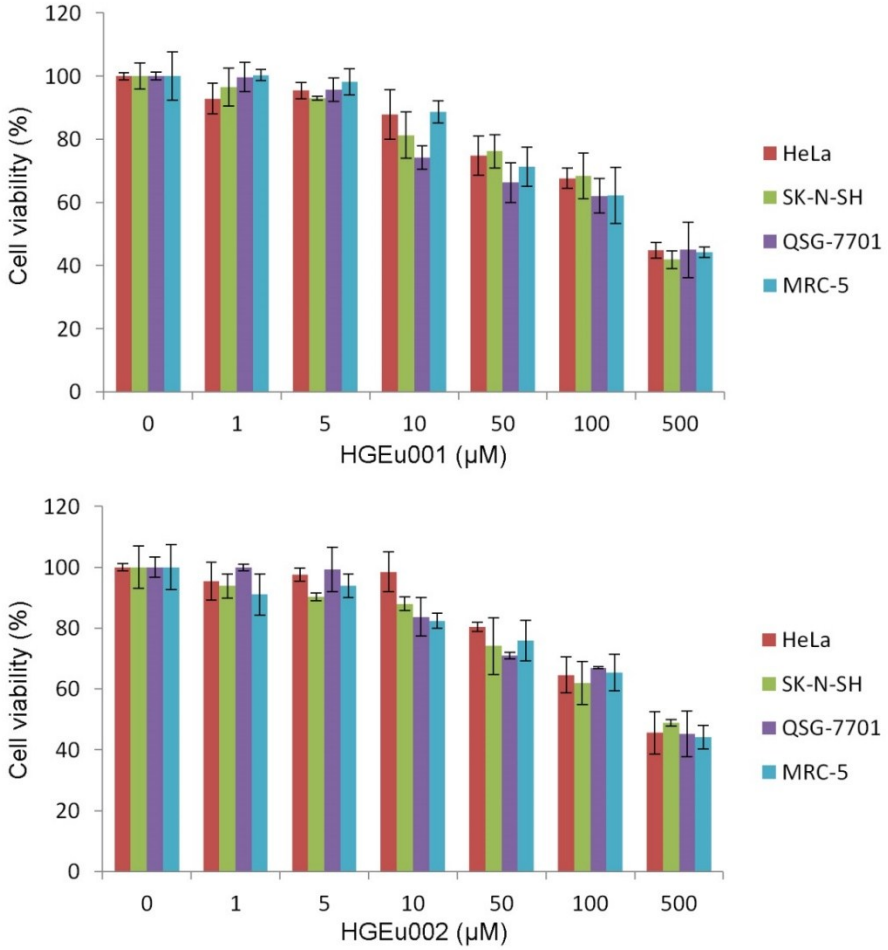



**Fig. S8** Raw data of **c**ytotoxicity of **HGEu001** and **HGEu002** in **Table S2**.

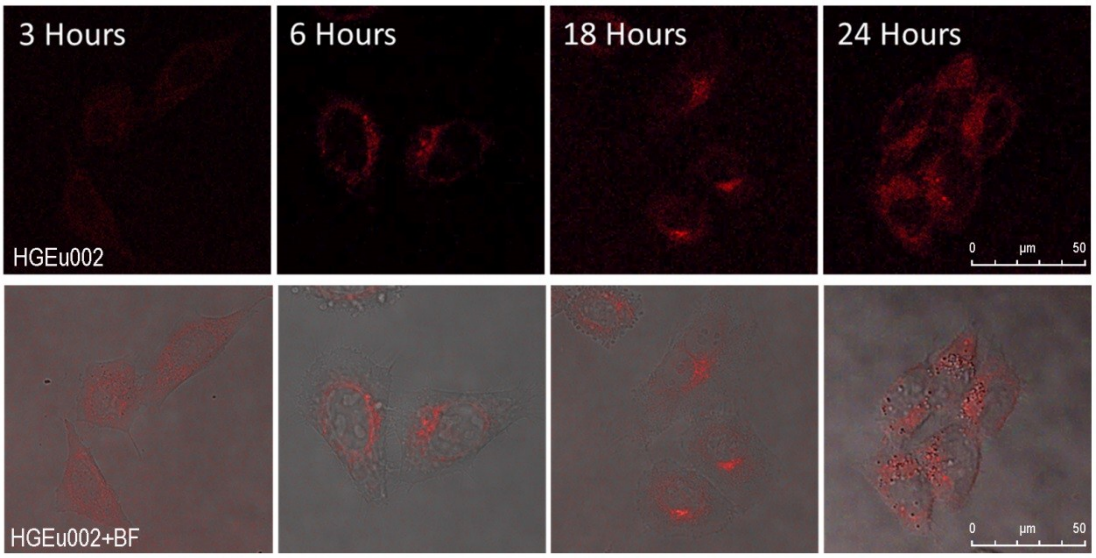
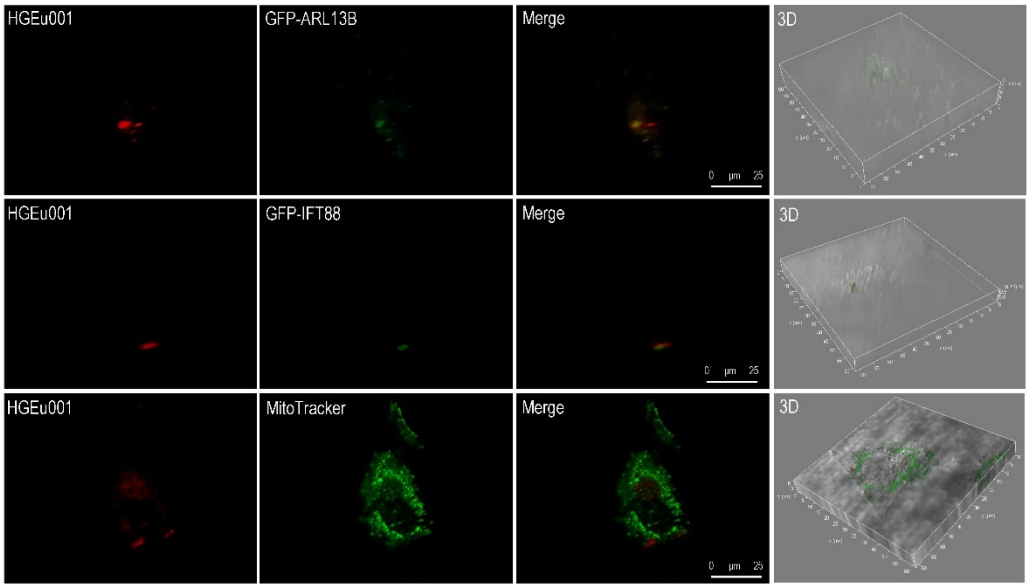



**Fig. S9** The two-photon living cell imaging of **HGEu002** in HeLa cells which the images were taken at 3, 6, 18 and 24 hours incubation time point (Dosed concentration = 10 μM, $\lambda_{ex}$ = 700 nm, filter Bandpass = 550 - 665 nm); The red emission of **HGEu002** is dispersed in cytoplasm. (In parallel with Fig. 3)

A)




B)

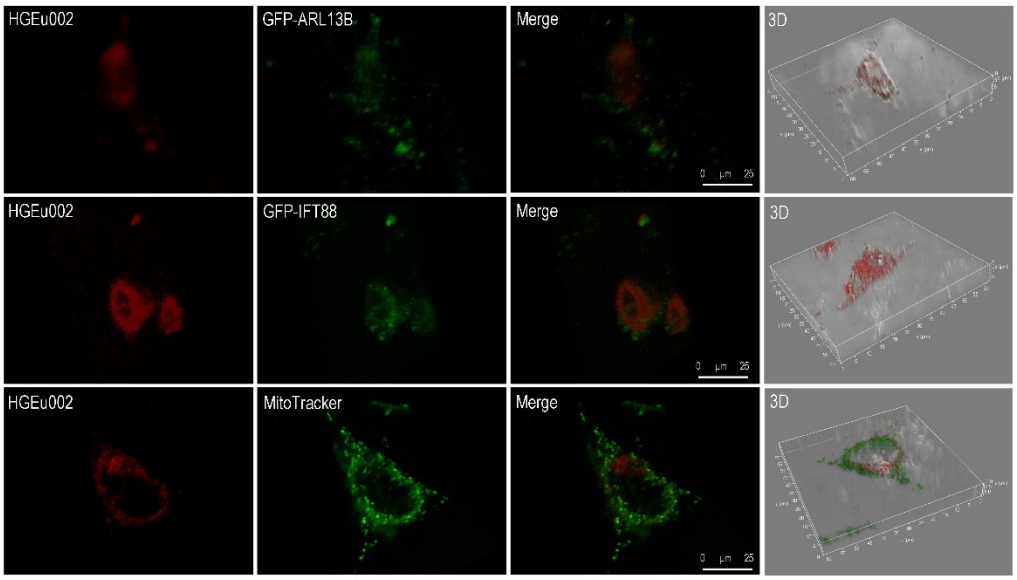



**Fig. S10** Two-photon confocal *in vitro* images of **HGEu001** (Plane A) and **HGEu002** (Plane B**)** with co-localization of green GFP-ARL13B/GFP-IFT88/MitoTracker® Green FM (M-7514) in HeLa cells. ($\lambda_{ex}$ = 700 nm) HeLa cells were firstly transfected with GFP-ARL13B/GFP-IFT88 or incubated with MitoTracker® Green FM (M-7514) for 15 minutes and further incubated 6 hours with 10 μM of **HGEu001/HGEu002**. ARL13B is the cilium-specific protein required for culinary axoneme structure, while IFT88 is the component of IFT complex B that involving in cilium biogenesis.[S7-S8] These two proteins are commonly used as primary cilium marker in the literature, however in our experiments, only GFP-IFT88 shown the rod-like structure of primary cilium nicely merged with **HGEu001**. GFP-ARL 13 B did not show a typical rod-like structure of primary cilium but unspecific staining because of overexpression of the GFP protein and low ciliated population of HeLa cells. In addition, the motif complex **HGEu002** still showed the dispersed distribution in the cytoplasm

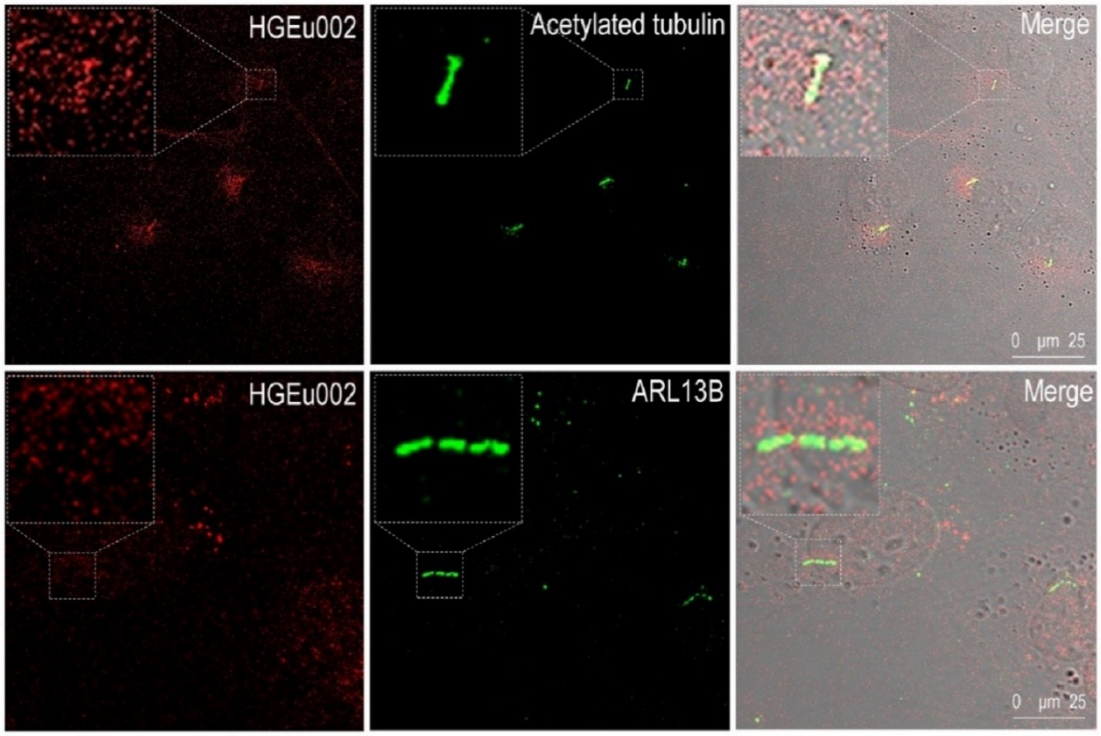



**Fig 11.** The co-staining experiments of **HGEu002** (red) with primary cilium markers acetylated tubulin or ARL13B (green) in ciliated NIH3T3 cells with a two-photon confocal microscope ($\lambda_{ex}$ = 700 nm, BP = 550 - 665 nm). **HGEu002** were dosed in a concentration of 10 µM, incubation for 6 hours. Acetylated tubulin or ARL13B were labeled by immunofluorescence using antibodies and imaged from the emission of Alexa Fluor® 488. (In parallel with Fig 5)

NMR spectra of the intermediates compounds:

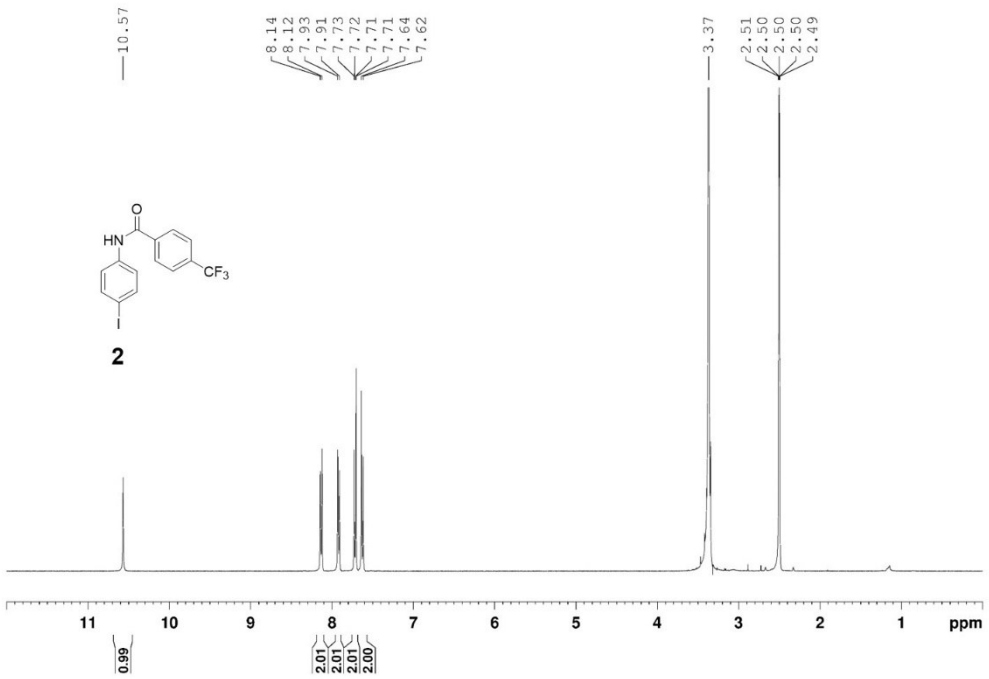



**Fig. S12** $^{1}$H NMR spectrum of compound **2**. (400 MHz, DMSO-$d_6$)

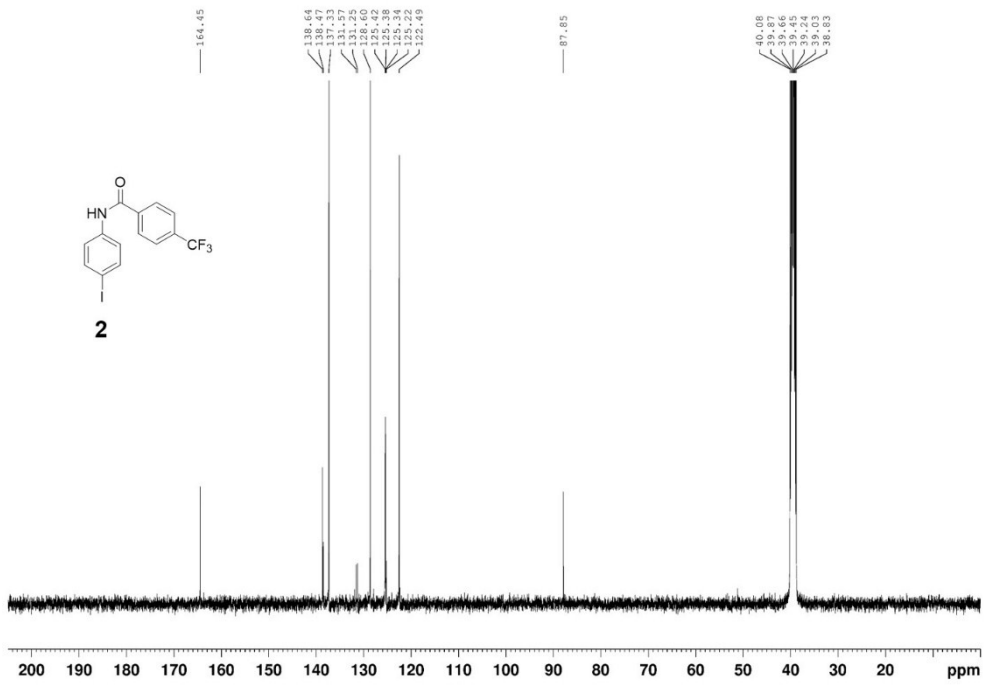



**Fig. S13** $^{13}$C NMR spectrum of compound **2**. (100 MHz, DMSO-$d_6$)

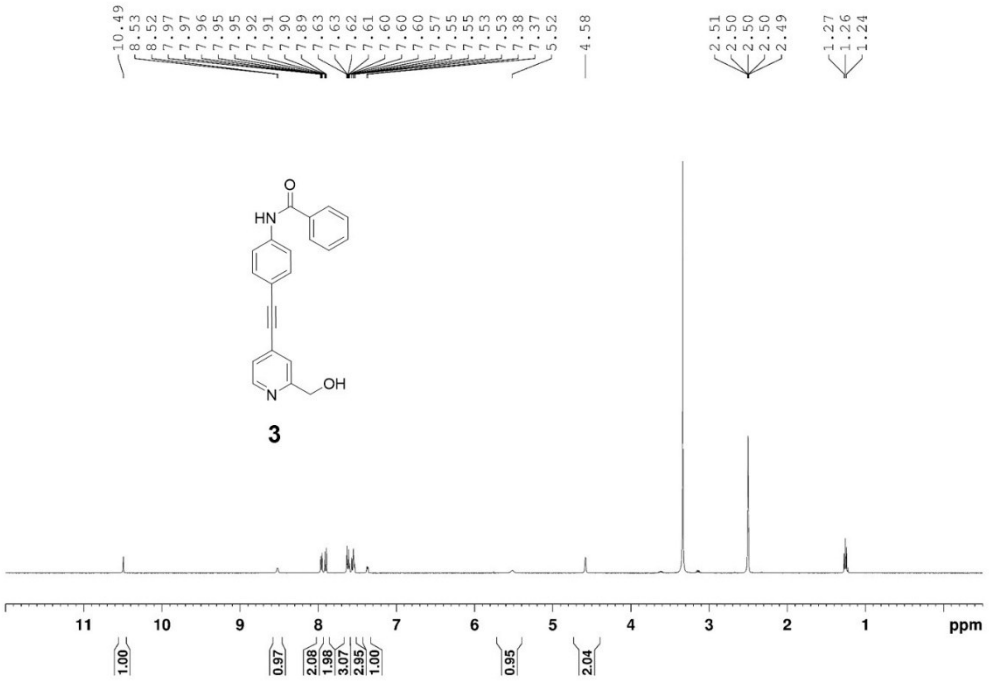



**Fig. S14** $^{1}$H NMR spectrum of compound **3**. (400 MHz, DMSO-$d_6$)

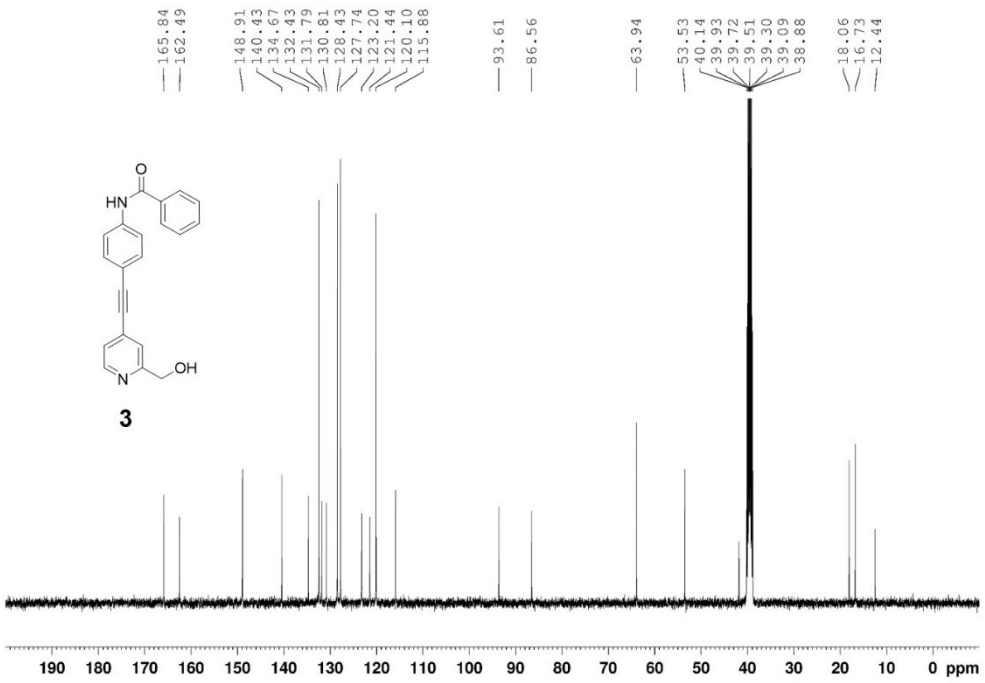



**Fig. S15** $^{13}$C NMR spectrum of compound **3**. (100 MHz, DMSO-$d_6$)

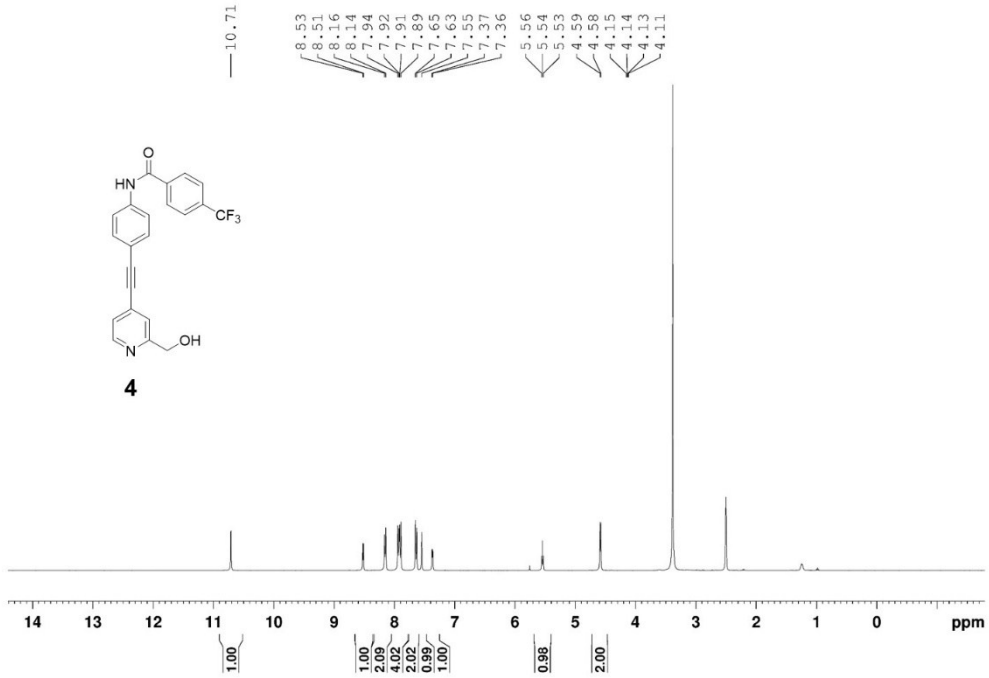



**Fig. S16** $^{1}$H NMR spectrum of compound **4**. (400 MHz, DMSO-$d_6$)

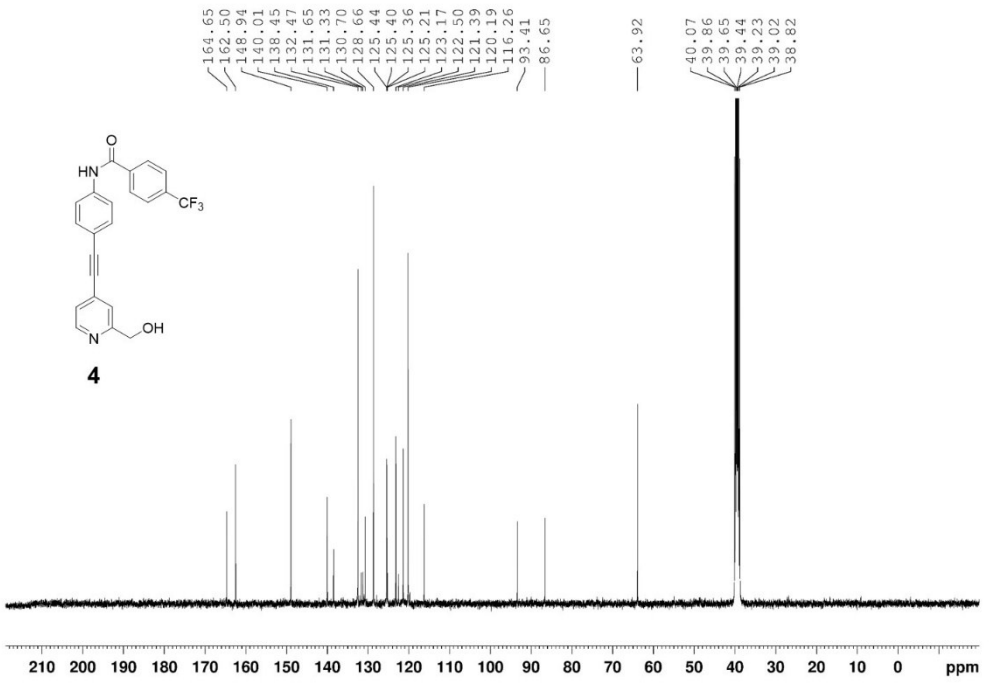



**Fig. S17** $^{13}$C NMR spectrum of compound **4.** (100 MHz, DMSO-$d_6$)

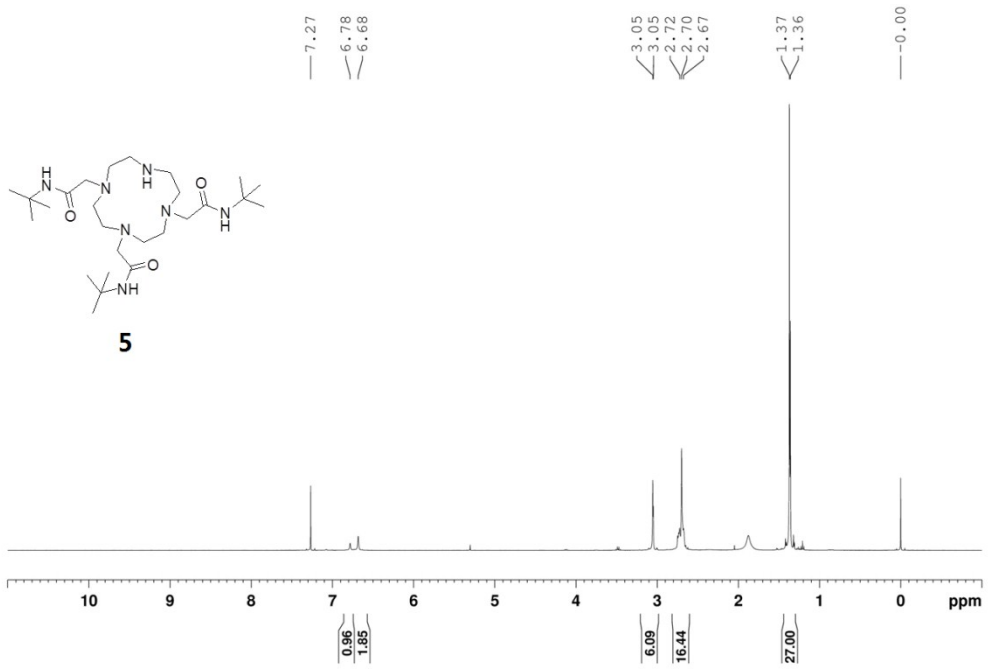



**Fig. S18** $^{1}$H NMR spectrum of compound **5**. (400 MHz, $CDCl_3$)

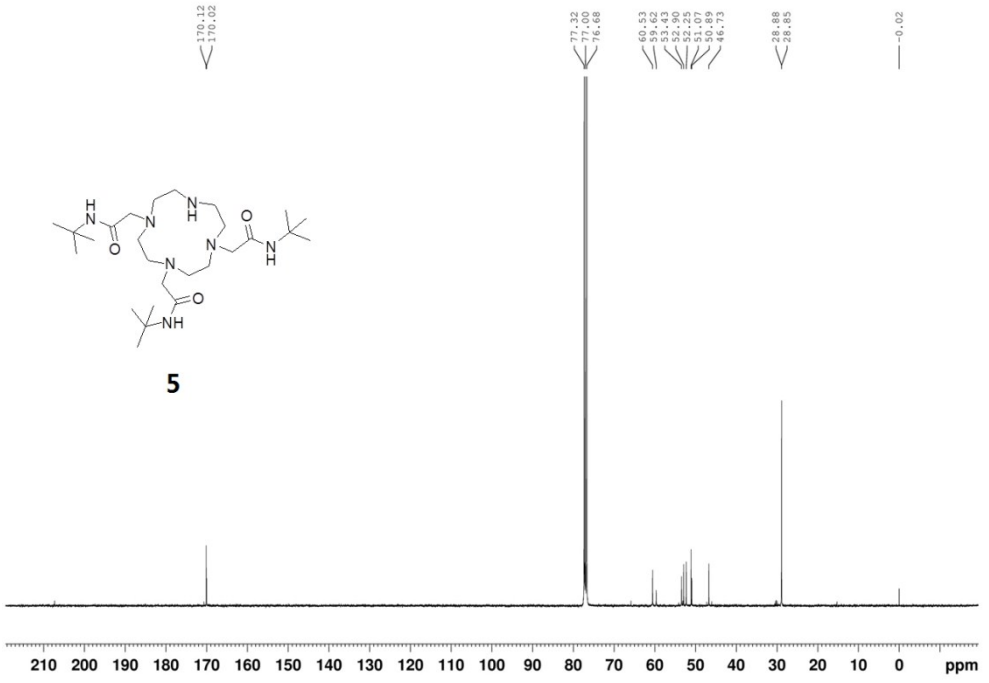



**Fig. S19** $^{13}$C NMR spectrum of compound **5**. (100 MHz, $CDCl_3$)

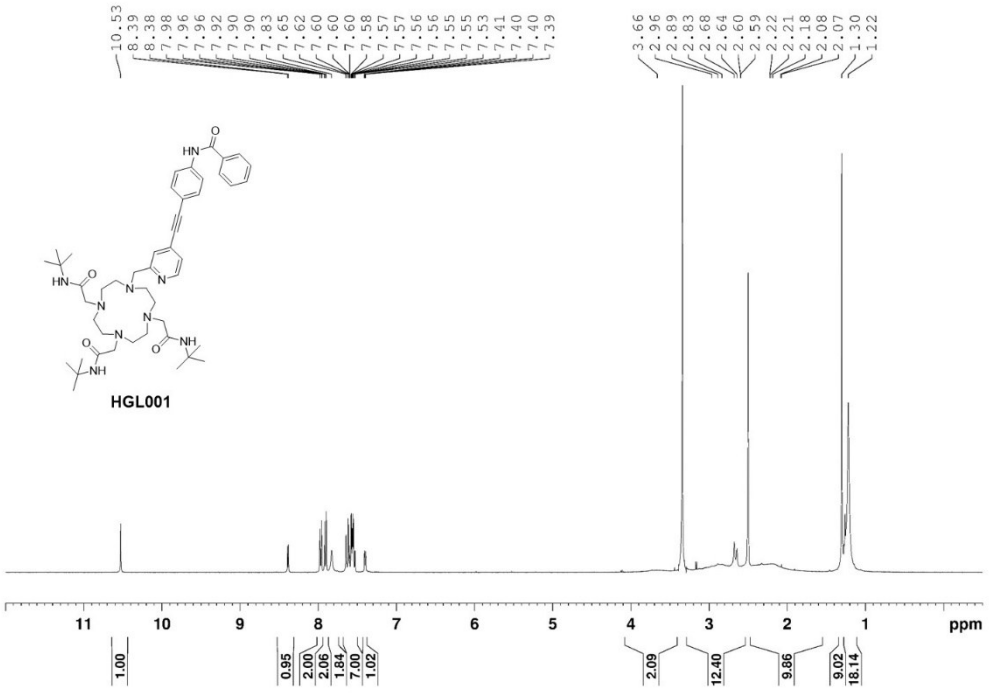



**Fig. S20** $^{1}$H NMR spectrum of **HGL001**. (400 MHz, DMSO-$d_6$)

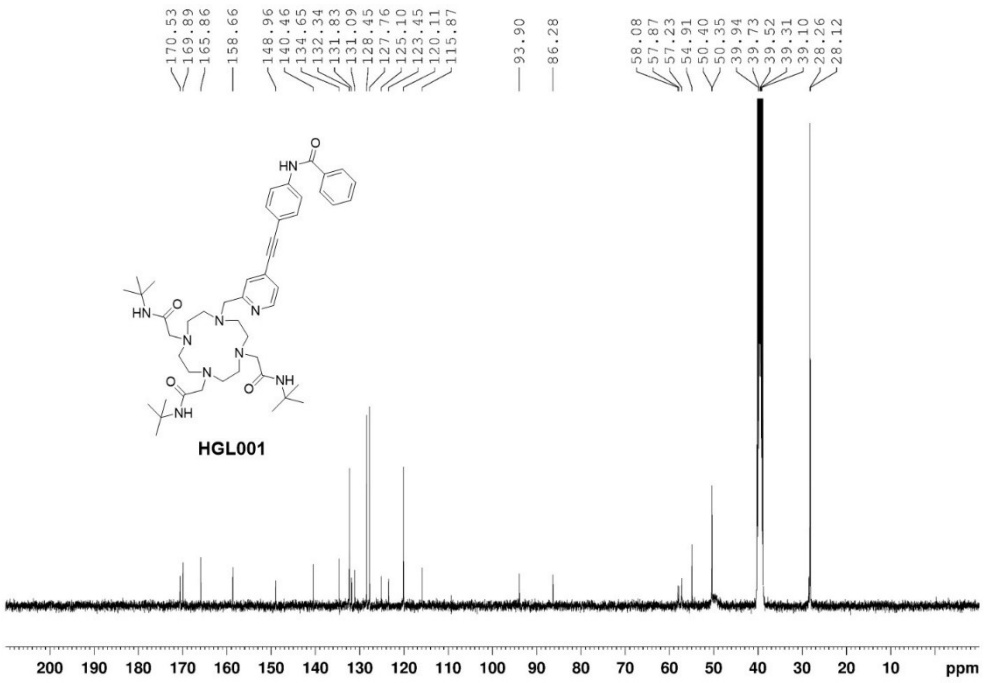



**Fig. S21** $^{13}$C NMR spectrum of **HGL001**. (100 MHz, DMSO-$d_6$)

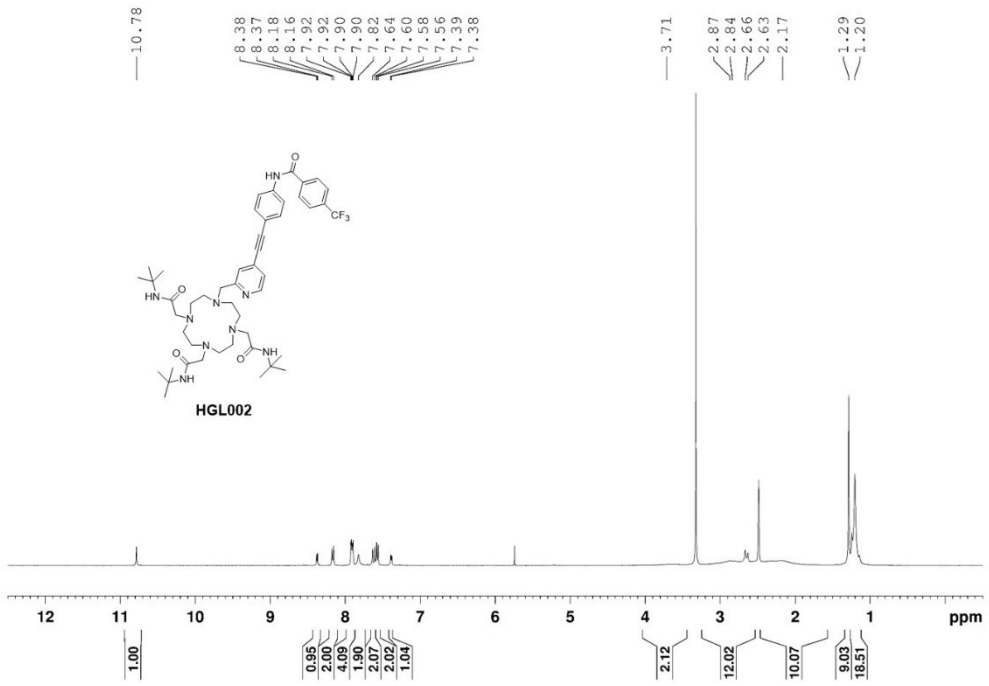



**Fig. S22** $^{1}$H NMR spectrum of **HGL002**. (400 MHz, DMSO-$d_6$)

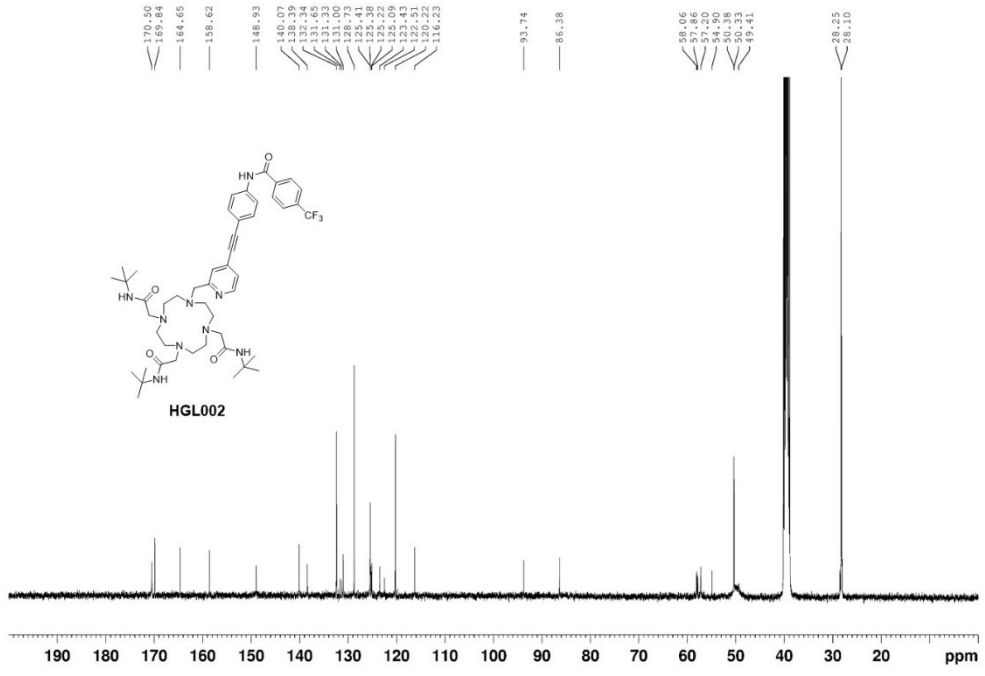



**Fig. S23** $^{13}$C NMR spectrum of **HGL002**. (100 MHz, DMSO-$d_6$)

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