Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2022

Supporting Information

Biotin-guided near-infrared fluorescent probe for imaging hydrogen sulfide and differentiating cancer cells

Xuekang Cai,[a,b] Zhuochen Zhang,[b] Yalun Dong,[a] Tingting Hao,[b] Long Yi[a*], and Xing Yang[b*]

[a]Beijing University of Chemical Technology (BUCT), Beijing 100029, China.
[b]Department of Nuclear Medicine, Peking University First Hospital, Beijing 100034, China.

[*]Corresponding author: Xing Yang, e-mail address: yangxing2017@bjmu.edu.cn;
Long Yi, e-mail address: yilong@buct.edu.cn.

Table of contents

1. General chemicals and instruments

All solvents and chemicals were purchased from commercial sources, with purity of analytical grade or better, and were used without further purification. The progress of the reaction was monitored by TLC on precoated silica plates (Merck 60F-254, 250 μm in thickness), and spots were visualized by basic $KMnO_4$, UV light or iodine. Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification. NMR spectra were recorded on a Bruker 400 spectrometer and chemical shifts (δ) were reported in ppm using solvent residual peak as an interior label. High-resolution mass spectra (HRMS) were recorded on an Agilent 6540 UHD Accurate-Mass Q-TOFLC/MS (Santa Clara, CA). The UV-visible spectra were recorded on a UV-6000 UV-VIS-NIR-spectrophotometer (METASH, China). Fluorescence studies were performed using a F-280 spectrophotometer (Tianjin Gangdong Sci & Tech., Development. Co., Ltd). HPLC analysis was performed using a Phenomenex C18 Luna 4.60 × 250 mm^2 column on an ANGELA TECHNOLOGIES HPLC LC-10F system. HPLC purifications were performed using a Phenomenex C18 Luna 10.0 × 250 mm^2 column on a Bonna-Agela Technologies Co., Ltd. FL-H050G preparative chromatography system (Tianjin, China). The products were eluted using eluent A (water with 0.1% trifluoroacetic acid) and eluent B (acetonitrile with 0.1% trifluoroacetic acid).

2. Synthetic procedure of probes

Synthesis of 2

2,3,3-Trimethylindolenine (**1**, 600 mg, 3.77 mmol) and 6-indo-1-hexyne (1.00 g, 4.81 mmol) were dissolved in 12 mL acetonitrile, and then the mixture was heated to 80 ℃ and refluxed for 36 h.[1] The reaction solution was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The resulted residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:2), and 908 mg of **2** was obtained with the yield of 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.95 (m, 1H), 7.88–7.82 (m, 1H), 7.67–7.59 (m, 2H), 4.49 (t, J = 7.7 Hz, 2H), 2.85 (s, 1H), 2.85–2.81 (m, 2H), 2.26 (td, J = 7.0, 2.6 Hz, 2H), 1.98–1.88 (m, 2H), 1.66–1.58 (m, 2H), 1.54 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.6, 141.9, 141.1, 129.4,

128.9, 123.5, 115.4, 83.9, 71.8, 54.2, 47.1, 26.4, 24.8, 22.0, 17.3, 14.1. HRMS calcd for $C_{17}H_{22}N^+$ $[M]^+$ 240.1747, found 240.1784.

Synthesis of 3

Compound **2** (908 mg, 2.47 mmol), triethyl orthoformate (366 mg, 2.47 mmol), and *N,N'*-diphenylformamidine (581 mg, 10.0 mmol) were dissolved in 10 mL ethanol. The mixture was stirred and refluxed for 2 h. Then, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:1) to give a red solid **3** (850 mg, 73%).[2] ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (bs, 1H), 8.67 (d, J = 12.2 Hz, 1H), 7.68 (d, J = 7.0 Hz, 1H), 7.59–7.44 (m, 6H), 7.36–7.28 (m, 2H), 6.20 (d, J = 12.2 Hz, 1H), 4.19–4.11 (m, 2H), 2.82 (s, 1H), 2.31–2.23 (m, 2H), 1.90–1.80 (m, 2H), 1.71 (s, 6H), 1.64–1.53 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.5, 151.9, 141.5, 141.0, 138.5, 129.8, 128.5, 126.1, 125.6, 122.6, 118.3, 111.7, 91.2, 83.9, 71.8, 49.5, 43.7, 27.7, 25.9, 25.3, 17.5.

Synthesis of 6

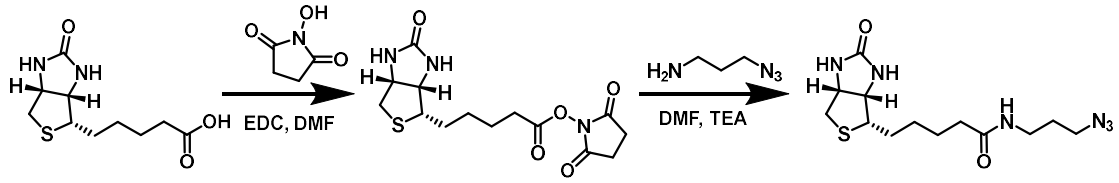
Compound **5** should be freshly prepared beforehand.[3] Cyclohexanone (2.45 mmol, 0.25 mL) was added dropwise to concentrated H_2SO_4 (3 mL) at 0 °C, and then 2-(2-hydroxy-4-(piperidin-1-yl)benzoyl)benzoic acid (**4**, 400 mg, 1.22 mmol) was added in portions with stirring. The reaction mixture was heated at 90 °C for 2 h. The solution was allowed to cool down and poured onto ice (100 g). Perchloric acid (70%, 0.30 mL) was then added, and the resulted precipitate was filtered off and washed with cold water (50 mL) to provide **5** as a red solid. The freshly prepared **5** (244 mg, 0.55 mmol) and **3** (250 mg, 0.53 mmol) were dissolved in 15 mL Ac_2O, and then KOAc was added to the solution. The mixture was allowed to heated to 50 ℃ and stirred for 1 h. Then 15 mL water was added to quench this reaction. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:2) to give **6** (150 mg, 37%) as a green solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.51 (d, J = 13.9 Hz, 1H), 8.23 (d, J = 7.5 Hz, 1H), 7.62–7.56 (m, 1H), 7.54–7.47 (m, 1H), 7.43–7.39 (m, 1H), 7.39–7.34 (m, 1H), 7.24–7.19 (m, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 7.3 Hz, 1H), 6.76–6.70 (m, 3H), 6.00 (d, J = 14.0 Hz, 1H), 4.08 (t, J = 7.2 Hz, 2H), 3.50–3.43 (m, 4H), 2.65–2.57 (m, 2H), 2.31 (td, J = 6.6, 2.4 Hz, 2H), 2.27–2.20 (m, 1H), 2.05–1.95 (m, 4H), 1.82–1.77 (m, 6H), 1.75–1.65 (m, 10H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 171.9, 163.8, 156.0, 154.5, 142.5, 141.5, 140.8, 135.6, 131.9, 131.8, 130.0, 129.2, 128.8, 128.7, 128.6, 124.9, 122.5, 122.2, 116.4, 115.0, 114.2,

110.3, 98.7, 98.2, 83.5, 69.6, 49.2, 48.7, 43.9, 32.1, 28.9, 28.8, 27.4, 26.7, 26.0, 25.6, 24.5, 20.5, 18.2. HRMS calcd for $C_{43}H_{45}N_2O_3^+$ $[M]^+$ 637.3425, found 637.3412

Synthesis of 7

6 (50 mg, 0.06 mmol) was dissolved in DMF (5 mL), and then 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 38 mg, 0.10 mmol) and *N*,*N*′-diisopropylethylamine (DIPEA, 25 mg, 0.20 mmol) were added. The resulted mixture was stirred for 10 min, and 4-nitro-7-piperazinobenzofurazan (NBD-Pz, 20 mg, 0.08 mmol) was added. After stirring for overnight, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:1) to give a green solid **7** (59 mg, 89%). In addition, the solid **7** was dissolved in DMF and further purified by preparative HPLC. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, J = 13.9 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H), 7.65–7.60 (m, 2H), 7.57–7.51 (m, 1H), 7.46–7.37 (m, 2H), 7.23–7.28 (m, 2H), 7.18–7.15 (m, 1H), 7.00–6.95 (m, 1H), 6.88–6.82 (m, 1H), 6.65 (s, 1H), 6.44 (d, J = 8.8 Hz, 1H), 6.08 (d, J = 14.1 Hz, 1H), 4.30–3.92 (m, 6H), 3.83–3.60 (m, 4H), 3.50–3.42 (m, 4H), 2.67–2.56 (m, 2H), 2.40–2.28 (m, 3H), 2.03–1.93 (m, 4H), 1.88–1.82 (m, 2H), 1.81–1.77 (m, 6H), 1.75–1.62 (m, 8H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.6, 168.4, 155.6, 154.4, 148.8, 144.8, 144.7, 142.9, 142.0, 141.1, 135.8, 134.1, 133.0, 130.5, 129.5, 128.9, 128.2, 127.8, 125.7, 123.5, 122.6, 116.0, 114.2, 114.0, 110.9, 104.0, 99.8, 97.9, 83.3, 69.7, 49.7, 48.9, 48.8, 48.5, 46.3, 44.1, 41.7, 31.5, 28.7, 28.6, 27.3, 27.2, 26.1, 25.5, 20.5, 18.1. HRMS calcd for $C_{53}H_{54}N_7O_5^+$ $[M]^+$ 868.4181, found 868.4183.

Synthesis of Biotin-N_3



D-Biotin (**8**, 200 mg, 0.82 mmol), *N*-hydroxysuccinimide (112 mg, 0.98 mmol) and 3-(ethyliminomethylideneamino)-*N,N*-dimethylpropan-1-amine hydrochloride (EDCI, 190 mg, 0.98 mmol) were dissolved in 10 mL DMF. After stirring for overnight, the solvent was evaporated under reduced pressure. Then 10 mL CH_2Cl_2 was added to break the bulk of solids, and the solid was filtered, washed by a mixed solution (EtOH:AcOH:H_2O = 95:1:4). Compound **9** was obtained without further purification.[4]

9 (60 mg, 0.18 mmol), 3-azido-1-propanamine (18 mg, 0.18 mmol) and triethyl amine (TEA, 19 mg, 0.18 mmol) were dissolved in 2 mL DMF. After stirring for overnight, the reaction solution was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:1) to give a white solid **Biotin-N_3** (30 mg, 53%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.51 (dd, J = 7.9, 4.8 Hz, 1H), 4.33 (dd, J = 7.9, 4.5 Hz, 1H), 3.38 (t, J = 6.7 Hz, 2H), 3.27 (t, J = 6.8 Hz, 2H), 3.25–3.20 (m, 1H), 2.95 (dd, J = 12.7, 4.9 Hz, 1H), 2.73 (d, J = 12.7 Hz, 1H), 2.23 (t, J = 7.4 Hz, 2H), 1.82–1.59 (m, 6H),1.53–1.41 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 176.1, 166.1, 63.4, 61.6, 57.0, 50.1, 41.0, 37.7, 36.8, 29.8, 29.7, 29.5, 26.8.

Synthesis of P1

7 (25 mg, 0.03 mmol), **Biotin-N_3** (20 mg, 0.06 mmol) and CuOAc (7.0 mg, 0.06 mmol) were mixed in 5 mL CH_3CN. After stirring for 4 h at room temperature, the reaction solvent was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2:CH_3OH = 100:5) to give **P1** (15 mg, 54%). The probe **P1** was dissolved in DMF and further purified by preparative HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57–8.46 (m, 2H), 7.90–7.85 (m, 2H), 7.77–7.64 (m, 4H), 7.56–7.53 (d, J = 8.0 Hz, 1H), 7.49–7.44 (m, 1H), 7.41–7.32 (m, 2H), 6.95 (dd, J = 9.2, 2.4 Hz, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.76 (d, J = 9.1 Hz, 1H), 6.56 (d, J = 9.3 Hz, 1H), 6.42–6.29 (m, 2H), 4.37–4.14 (m, 7H), 4.11 (dd, J = 7.8, 4.4 Hz, 1H), 4.04–3.74 (m, 4H), 3.73–3.66 (m, 4H), 3.10–3.04 (m, 1H), 3.01–2.95 (m, 2H), 2.82–2.75 (m, 2H), 2.71–2.67 (m, 2H), 2.35–2.31 (m, 2H), 2.08–1.94 (m, 6H), 1.91–1.85 (m, 2H), 1.83–1.69 (m, 12H), 1.62–1.56 (m, 6H), 1.51–1.41 (m, 4H). HRMS calcd for $C_{66}H_{76}N_{13}O_7S^+$ $[M]^+$1194.5706, found 1194.5704.

3. Spectroscopic measurements

3.1 General spectroscopic measurements

All the absorption and fluorescence spectra were recorded in phosphate buffered saline (PBS, 50 mM, pH 7.4, containing 20% DMSO) buffer solution at 25 ℃. UV-Vis spectra were acquired from 420 to 850 nm (0.2 nm increment). The fluorescence measurements were carried out at an excitation wavelength of 690 nm and emission spectra range from 710 to 850 nm. A stock solution of **P1** (10 mM) was first prepared by dissolving **P1** in DMSO. A stock solution of H_2S (100 mM) was freshly prepared by dissolving Na_2S in degassed 50 mM PBS buffer solution on ice. The stock solution of **P1** and H_2S were diluted into PBS buffer to afford the expected final concentration. In general, the concentration of **P1** was 2 μM (**P1**) and that of H_2S ranged from 1 μM to 200 μM.

3.2 Determination of detection limit and dissociation constant

The detection limit was calculated according to the fluorescence titration tests with the $3\sigma/k$ method,[5] where σ is the standard deviation of fluorescence intensity of only **P1**; k is the slope between the fluorescence intensity and H_2S concentration. Three independent duplication measurements of emission intensity were performed in the presence of low concentration of H_2S, and each average value of the intensities was plotted as a concentration of H_2S for determining the slope (k = 10.51±0.08). The standard deviation was acquired by recording the emission intensities of **P1** ten times (σ = 0.976).

The dissociation constant K_d was calculated according to the fluorescence titration tests with the following equation. Fluorescence intensities at 754 nm (denoted as F in the following formula) were plotted against H_2S concentrations. F_{free}, F_{bound} and K_d denote fluorescence intensity of the H_2S-free and H_2S-bound states and the dissociation constant with H_2S, respectively.[6,7]

$$F = F_{bound} + \frac{F_{free} - F_{bound}}{1 + \frac{[H_2S]}{K_d}}$$

3.3 Determination of fluorescence quantum yield

The quantum yields of **P1** and its H_2S-activated product **P1-p** were determined in

PBS (50 mM, pH = 7.4, including 20% DMSO) with indocyanine green (ICG) in DMSO as the standard ($\Phi = 0.12$).[8] The excitation wavelength was set as 660 nm, and the emission spectra were ranged from 680 to 900 nm. The quantum yield was calculated according to the following equation:

$$\Phi = \Phi_S \times (F/F_S) \times (A_S/A) \times (n^2/n_S^2)$$

where Φ is the quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscript S refers to the standard.

4. HPLC analysis

A mixture of **P1** (400 μM) and Na_2S (1 mM) in PBS buffer (50 mM, pH = 7.4; 50% MeOH) was analyzed by HPLC at different reaction time. Conditions: detection wavelength: 254 nm; flow 1.0 mL min^{-1}; buffer a: water with 0.1% trifluoroacetic acid; buffer b: MeOH; Elution condition: 0-8 min, buffer b: 10-90%; 8-18 min, buffer b: 90%.

5 Cell imaging

5.1 Cell culture

Human hepatocellular carcinoma cells (HepG2), human cervical cancer cells (HeLa), human renal clear cell carcinoma cells (786-O) and human embryonic kidney 293T cells (HEK293T) were purchased from the Chinese Academy of Sciences Typical Culture Collection (Shanghai, China). HepG2, HeLa, HEK293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM) medium containing 10% fetal bovine serum (FBS), 1% penicillin–streptomycin. 786-O cells were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 1% penicillin–streptomycin. The cells were passaged every 2 days under standard cell culture conditions (at 37 °C under 5% CO_2 in air) and used between passages 3 and 8.

5.2 Cytotoxicity assay

HepG2 cells were seeded in a 96-well culture plate at 5000 cells per well and incubated for 24 h. Cells were then treated with culture medium containing probe **P1** (concentrations range 0.1-10 μM) for 24 h. Cell viability was determined by the

thiazolyl blue tetrazolium bromide (MTT) assay using a microplate reader (BioTek, Winooski, VT, USA). The relative viability of the untreated controls was normalized to be 100%, while the medium absorbance set as the background control. Each experiment was performed in quadruple.

5.3 Confocal fluorescence imaging for living cells

HepG2, HeLa, 786-O and HEK293T cells were seeded in glass bottom culture dishes (NEST, San Diego, CA, USA) and cultured under 5% CO_2 atmosphere at 37 °C for 2 days. Cells were washed twice by PBS buffer and then incubated with RPMI 1640 complete medium containing **P1**. The images were captured using FV1000 confocal microscope (Olympus, Japan). The cells were excited by a 635 nm laser diode and detected at BA = 655−755 nm.

For the H_2S imaging in HepG2, HeLa, 786-O and HEK293T cells, cells were treated with **P1** (200 nM) at 37 °C for 30 min, washed by PBS twice, and then imaged. For scavenger-treated imaging, cells were pretreated with **NBD-S8** (200 μM) for 1 h, then incubated with **P1** (200 nM) for 0.5 h, washed and imaged. For biotin-inhibited imaging, cells were pretreated with biotin (2 mM) for 1 h, then incubated with **P1** (200 nM) for 0.5 h, washed and imaged. Random locations on each plate were selected for imaging. Image quantification was performed in ImageJ software (NIH). The results were expressed as the mean ± standard deviation. Comparisons between two sets of data were determined by Student's t-test. For these tests, $P < 0.05$ or $P < 0.01$ was regarded as statistically significant. (n =20 biologically independent cell samples).

6. Supplementary figures

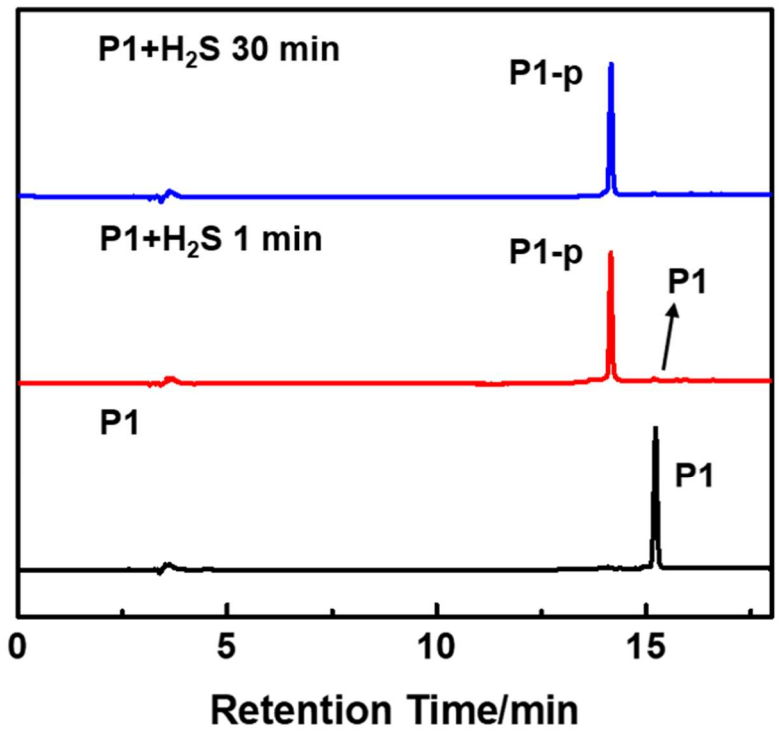


Fig. S1 Time-dependent HPLC analysis of the reaction between **P1** (400 μM) and H_2S (1 mM) in PBS buffer (50 mM, pH = 7.4; 50% MeOH).

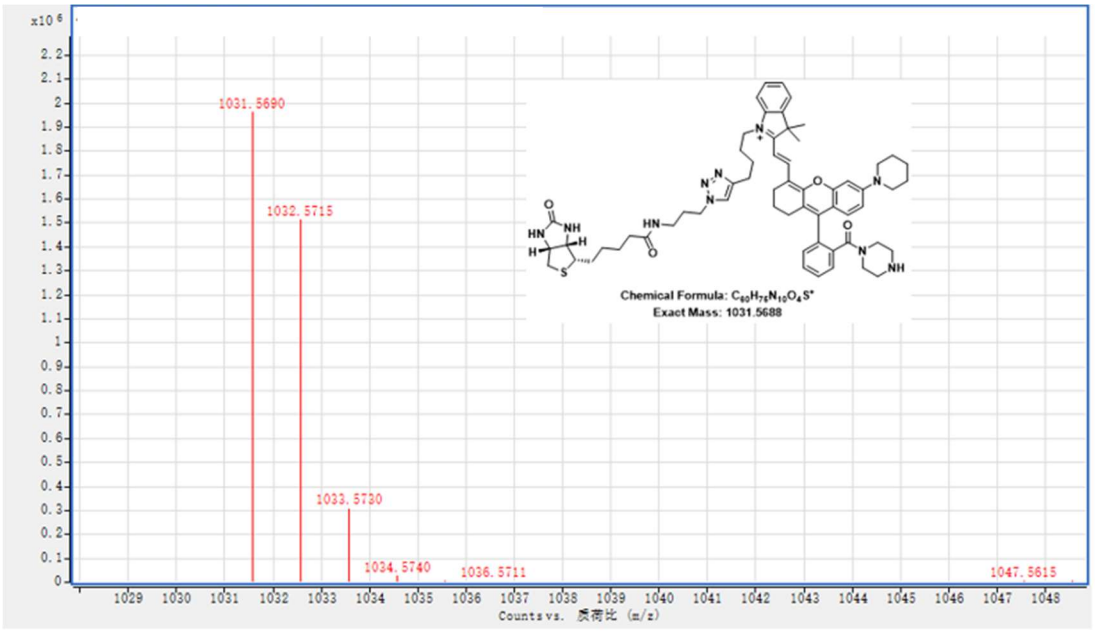


Fig. S2 HRMS analysis for the reaction solution of **P1** (400 μM) and H_2S (1 mM) after 30 min incubation.

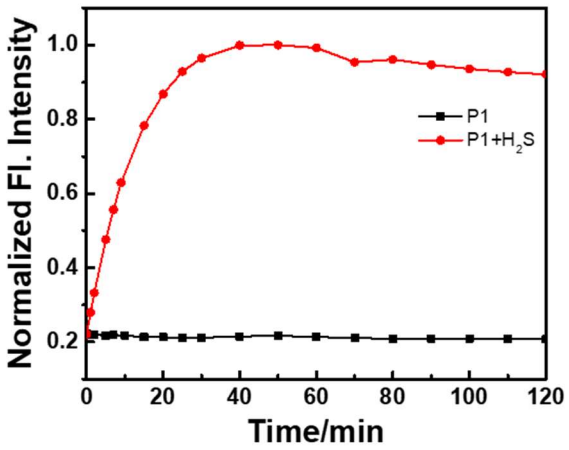


Fig. S3 Time-course fluorescence intensities at 754 nm of 2 μM **P1** in the presence or absence of 100 μM H_2S in PBS (50 mM, pH = 7.4, containing 20% DMSO). λ_{ex} = 690 nm.

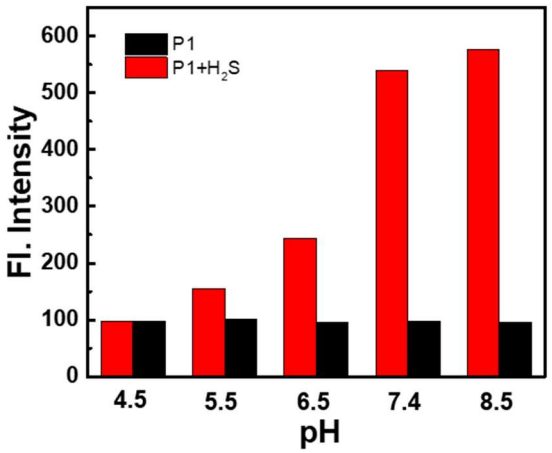


Fig. S4 Fluorescence intensities of **P1** (2 μM) at 754 nm in the presence or absence of H_2S (100 μM) at different pH values. λ_{ex} = 690 nm.

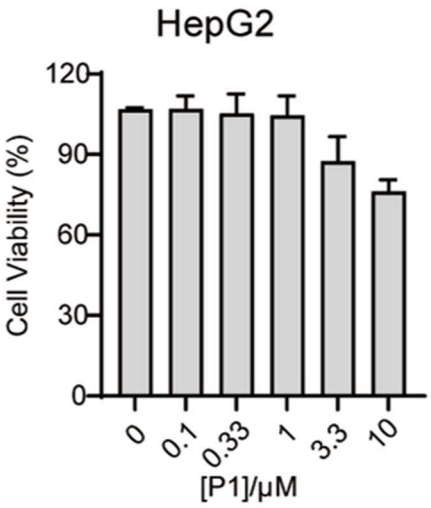
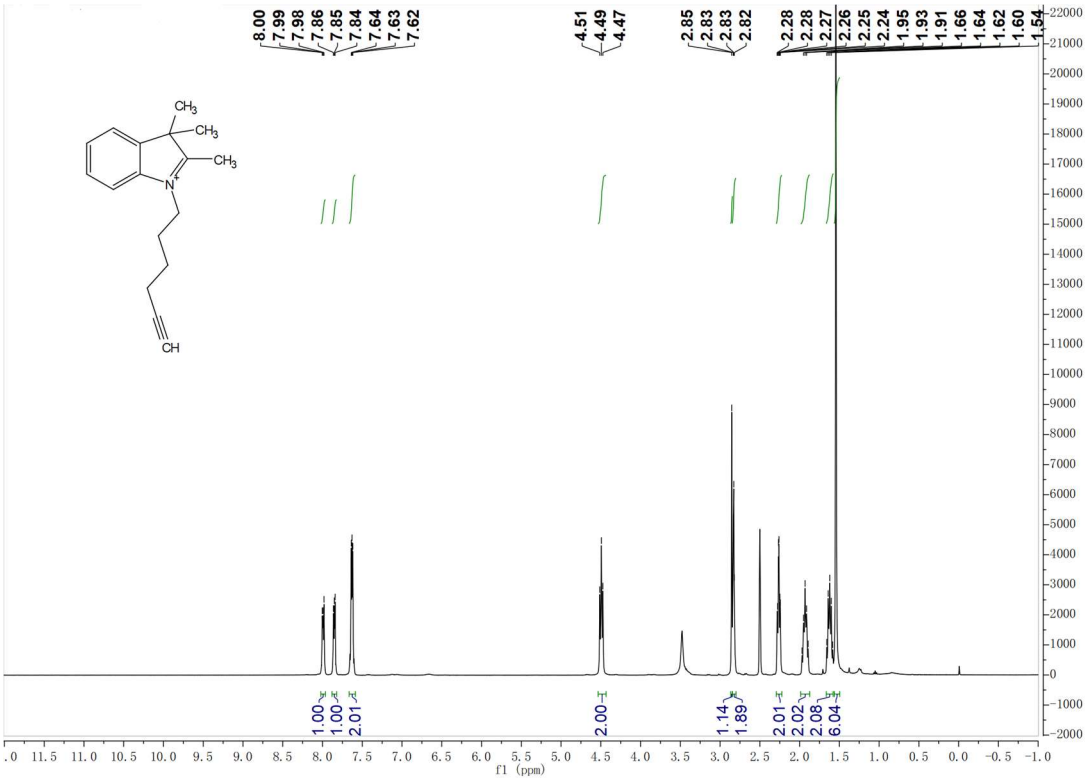
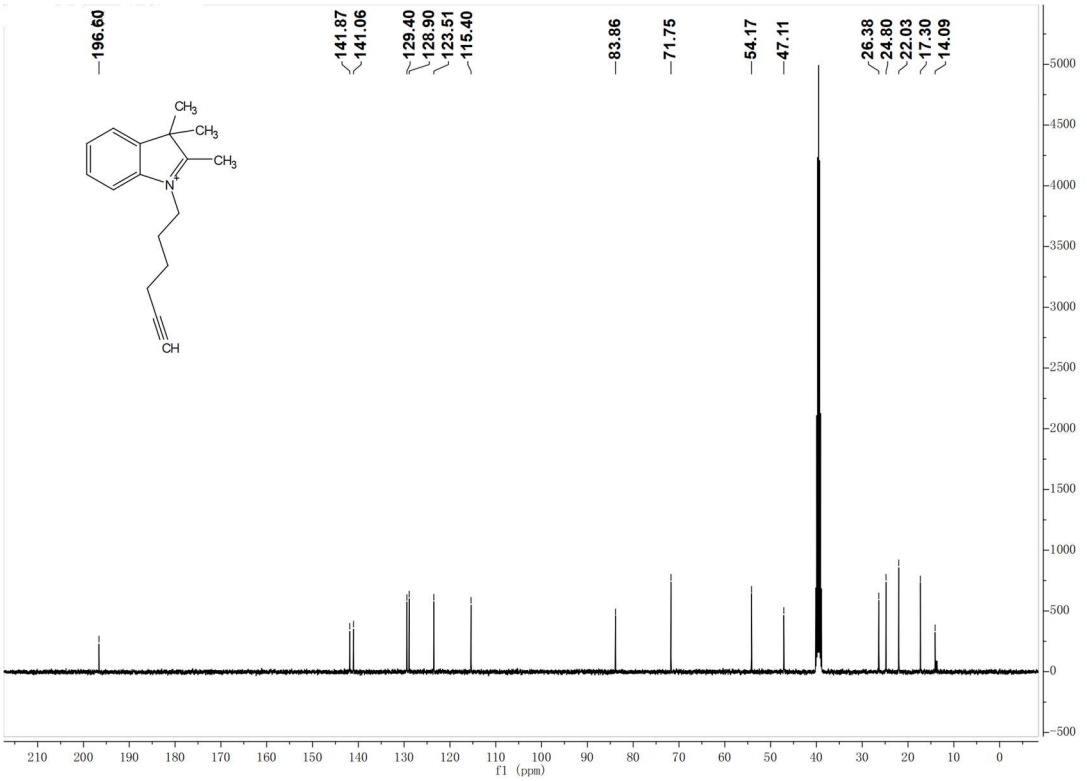
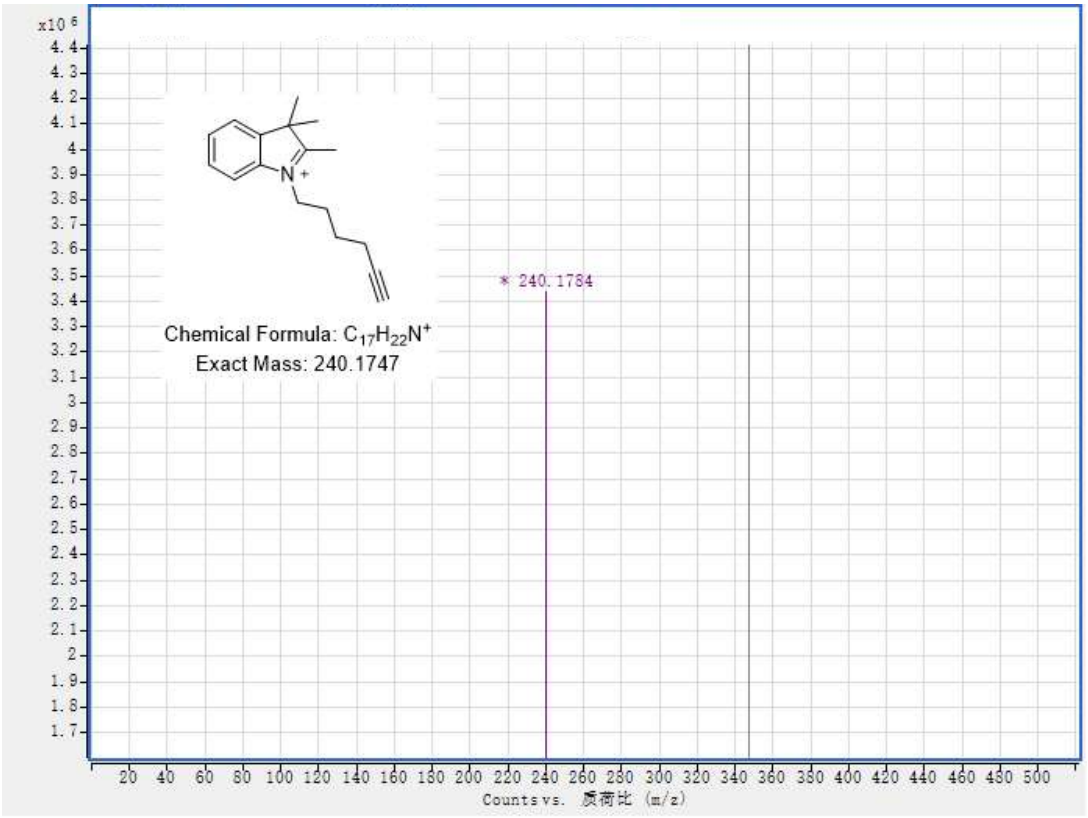


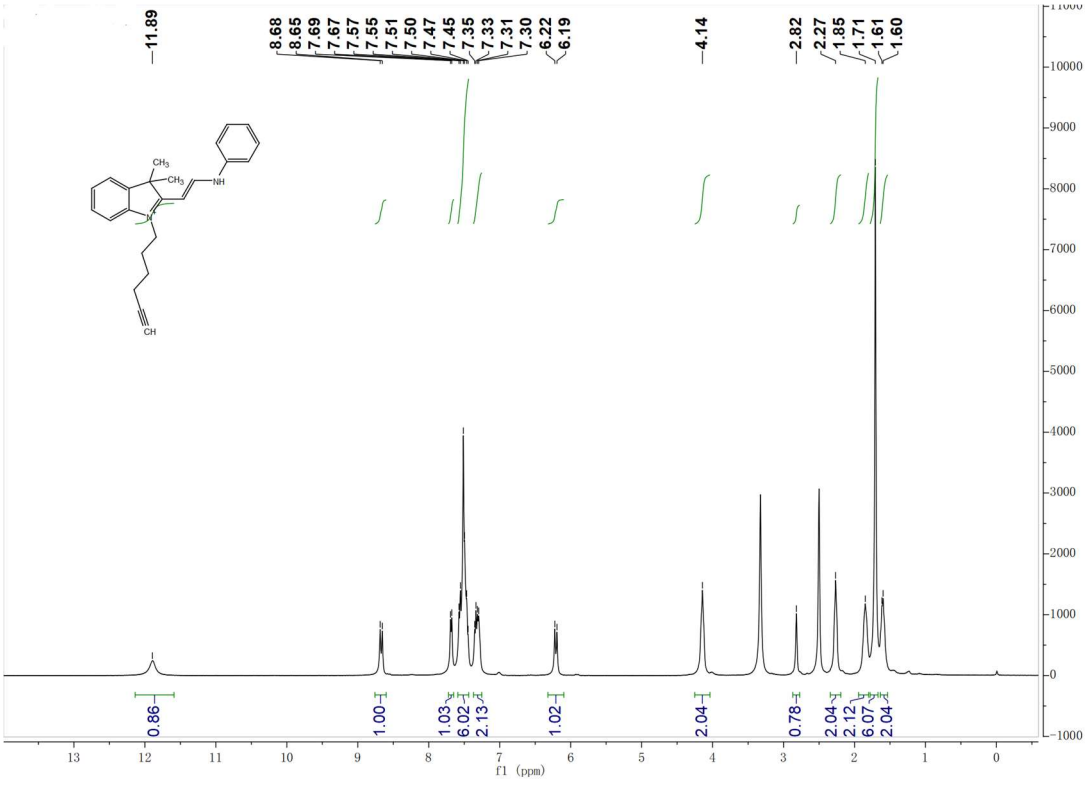
Fig. S5 Cytotoxicity of **P1** determined by the MTT viability assay. Data were presented as mean ± s.d. (n = 4).

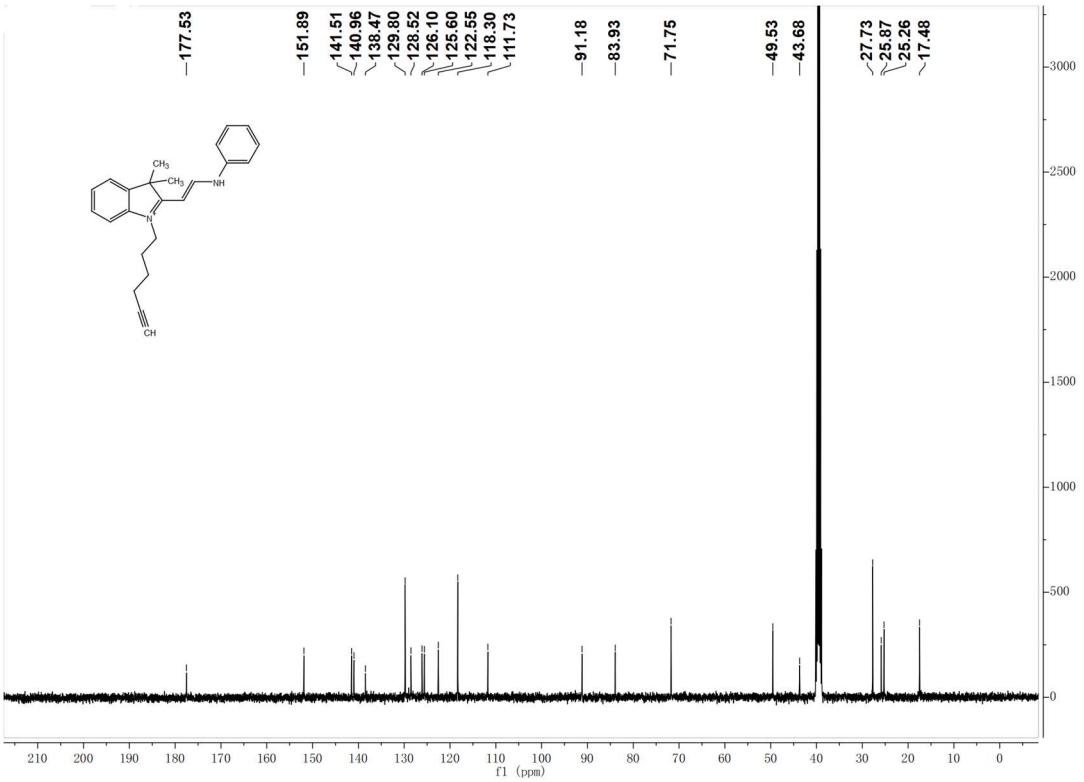
7. Supplementary NMR and HRMS spectra

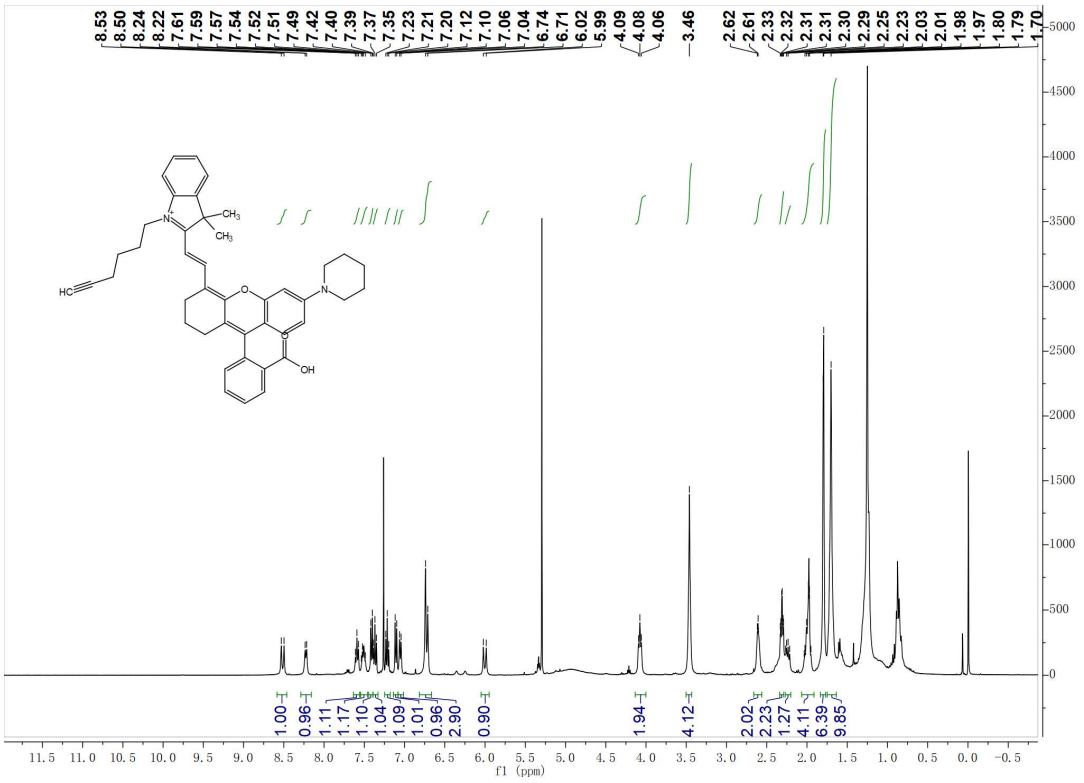


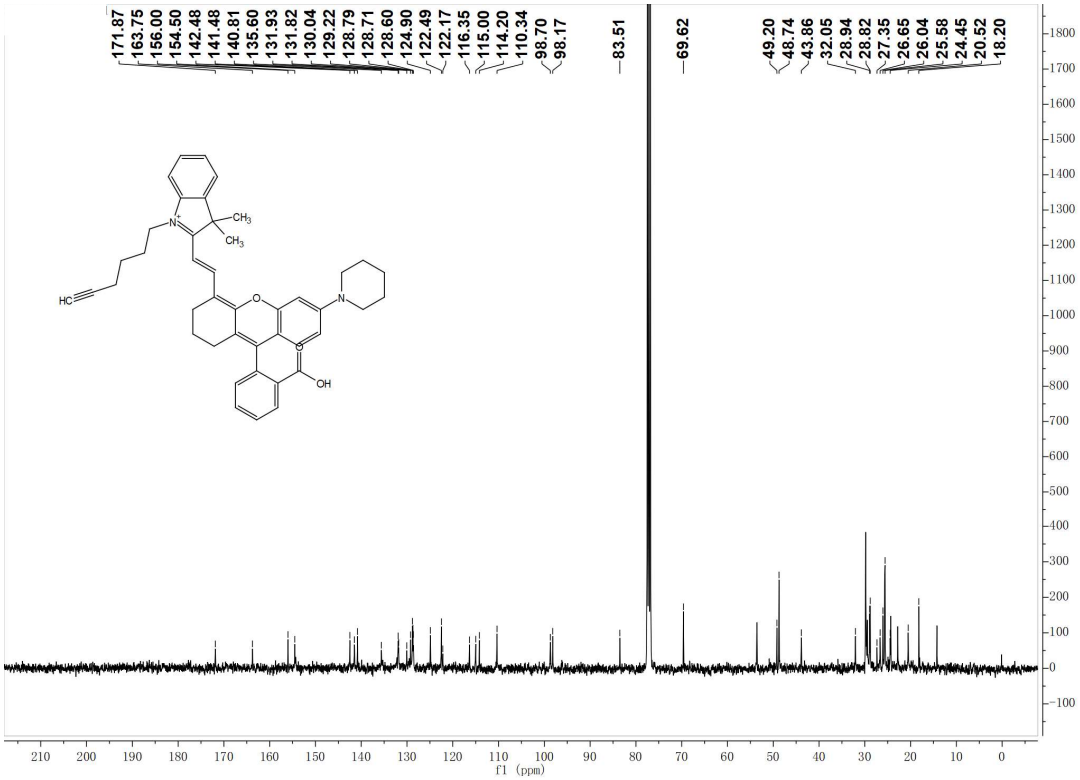


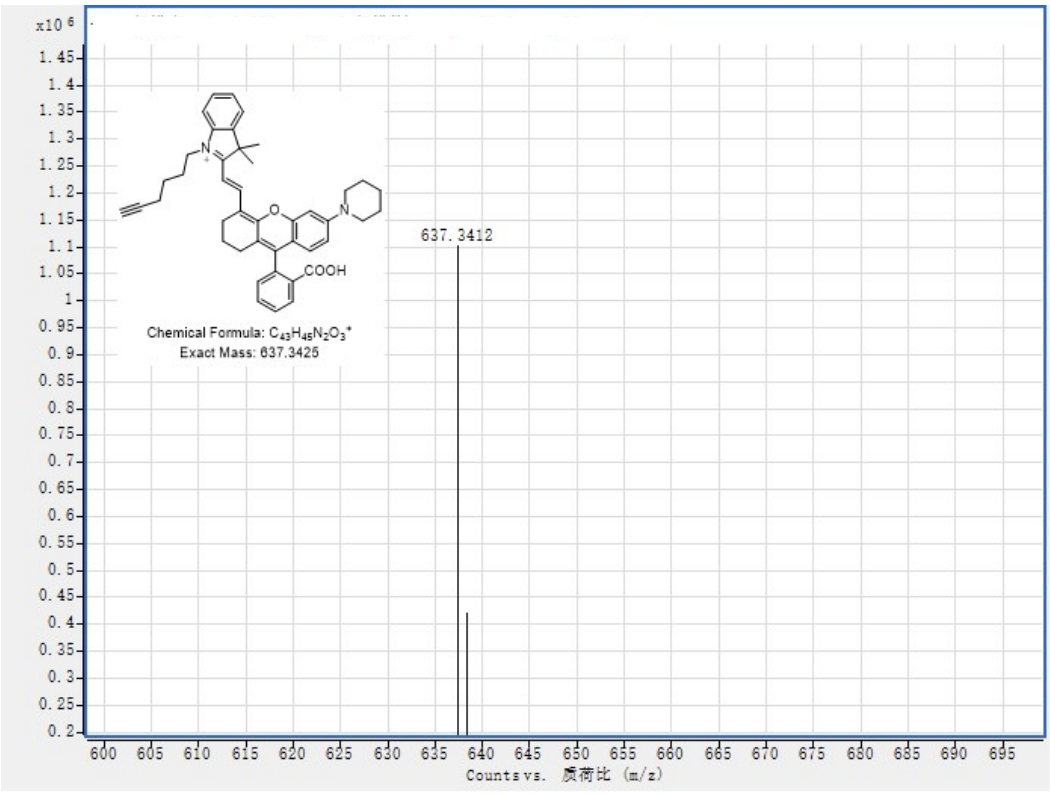

x10 6
* 240.1784
Chemical Formula: C17H22N+
Exact Mass: 240.1747
Counts vs. 质荷比 (m/z)

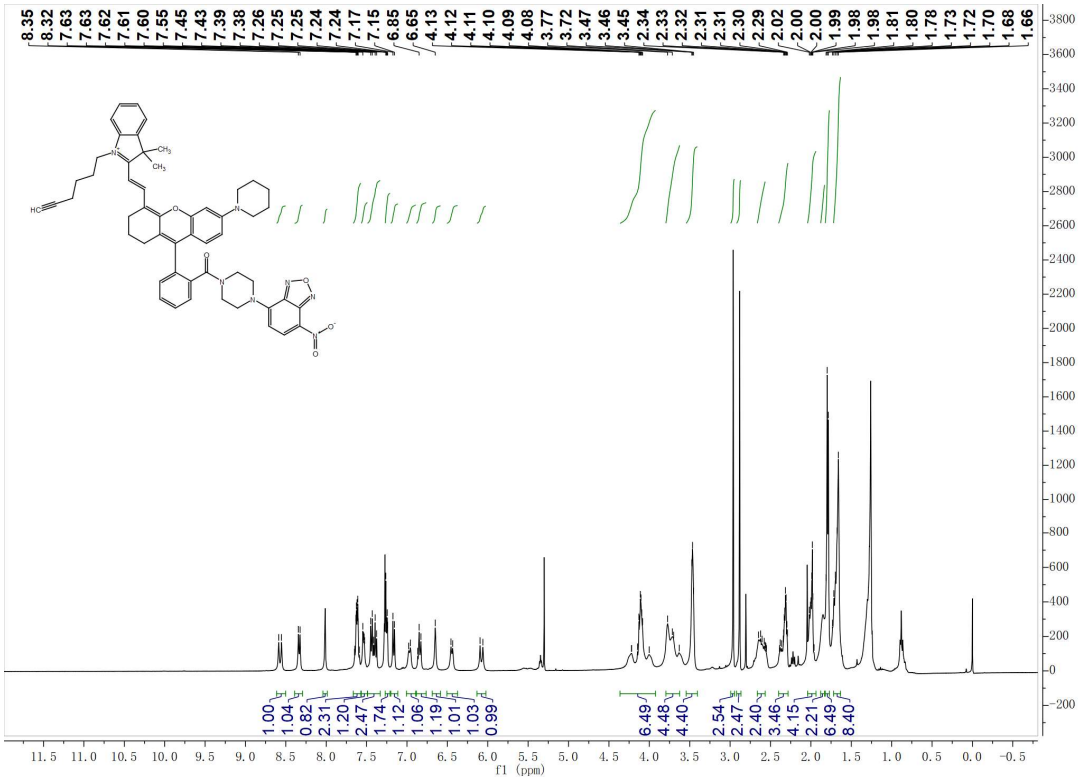

11.89
8.68
8.65
7.69
7.67
7.57
7.55
7.51
7.50
7.47
7.45
7.35
7.33
7.31
7.30
6.22
6.19
4.14
2.82
2.27
1.85
1.71
1.61
1.60
0.86
1.00
1.03
6.02
2.13
1.02
2.04
0.78
2.04
2.12
6.07
2.04
f1 (ppm)

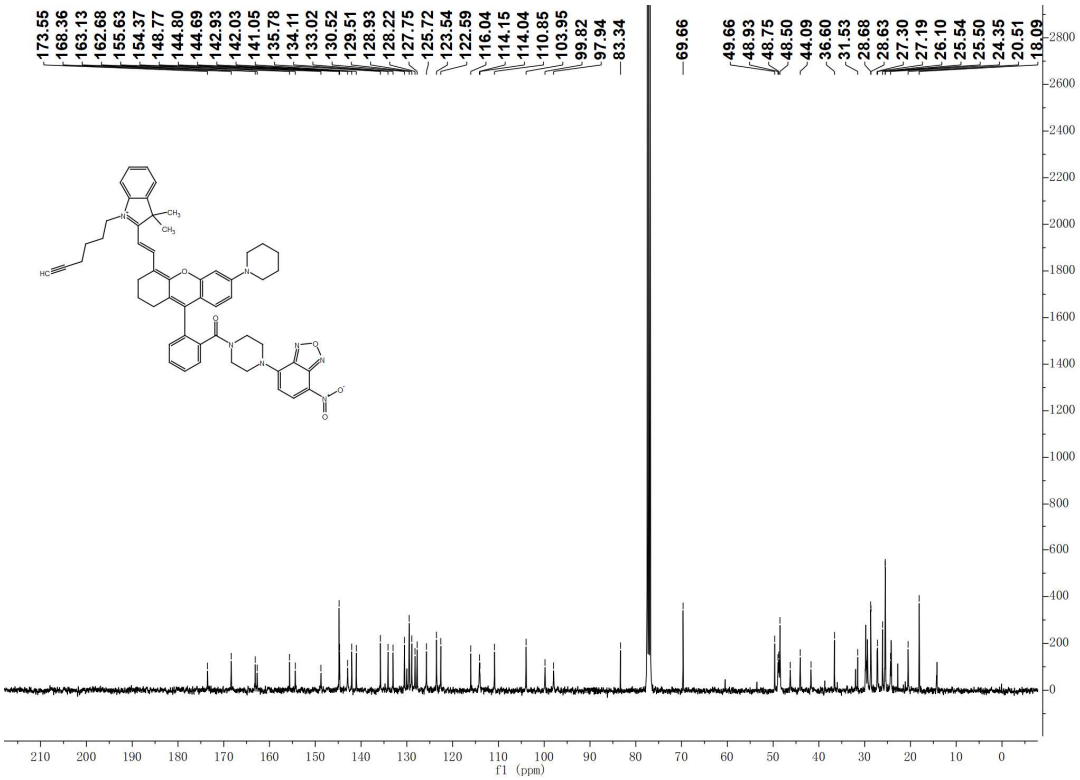

177.53
151.89
141.51
140.96
138.47
129.80
128.52
126.10
125.60
122.55
118.30
111.73
91.18
83.93
71.75
49.53
43.68
27.73
25.87
25.26
17.48
f1 (ppm)

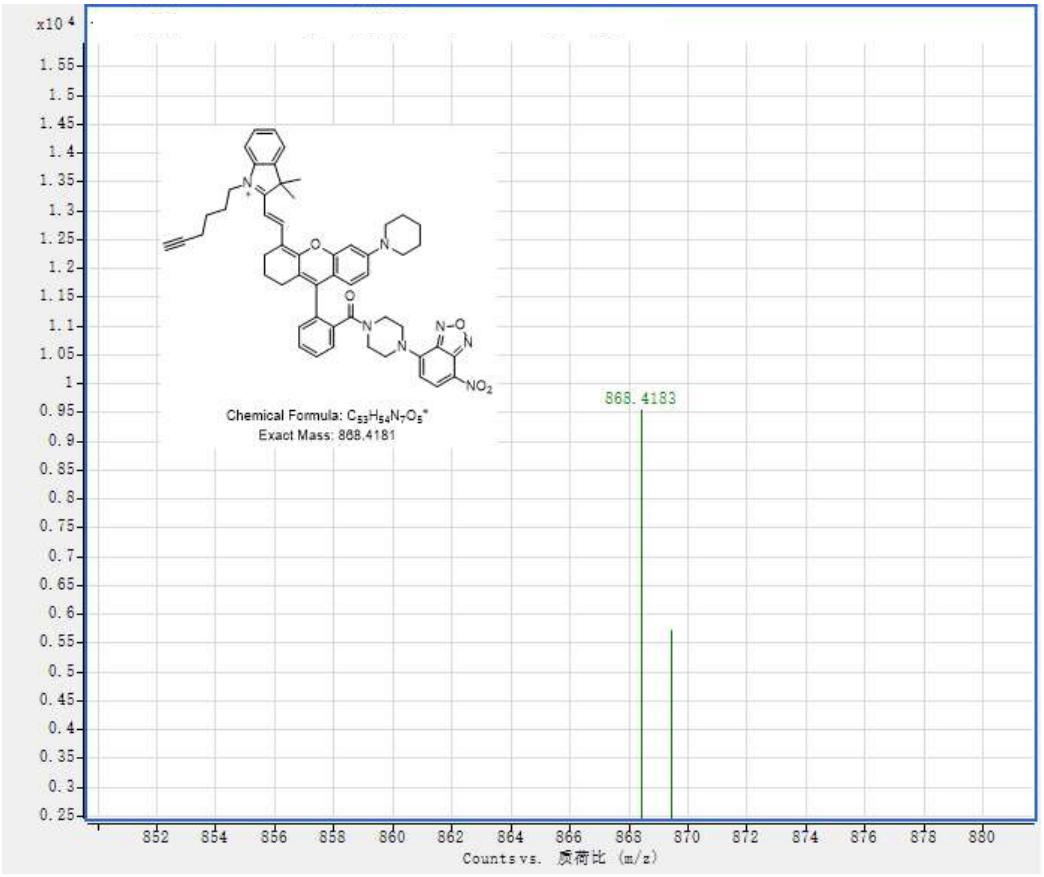

8.53
8.50
8.24
8.22
7.61
7.59
7.57
7.54
7.52
7.51
7.49
7.42
7.40
7.39
7.37
7.35
7.23
7.21
7.20
7.12
7.10
7.06
7.04
6.74
6.71
6.02
5.99
4.09
4.08
4.06
3.46
2.62
2.61
2.33
2.32
2.31
2.31
2.30
2.29
2.25
2.23
2.03
2.01
1.98
1.97
1.80
1.79
1.70
1.00
0.96
1.11
1.17
1.10
1.04
1.09
1.01
0.96
2.90
0.90
1.94
4.12
2.02
2.23
1.27
4.11
6.39
9.85
f1 (ppm)

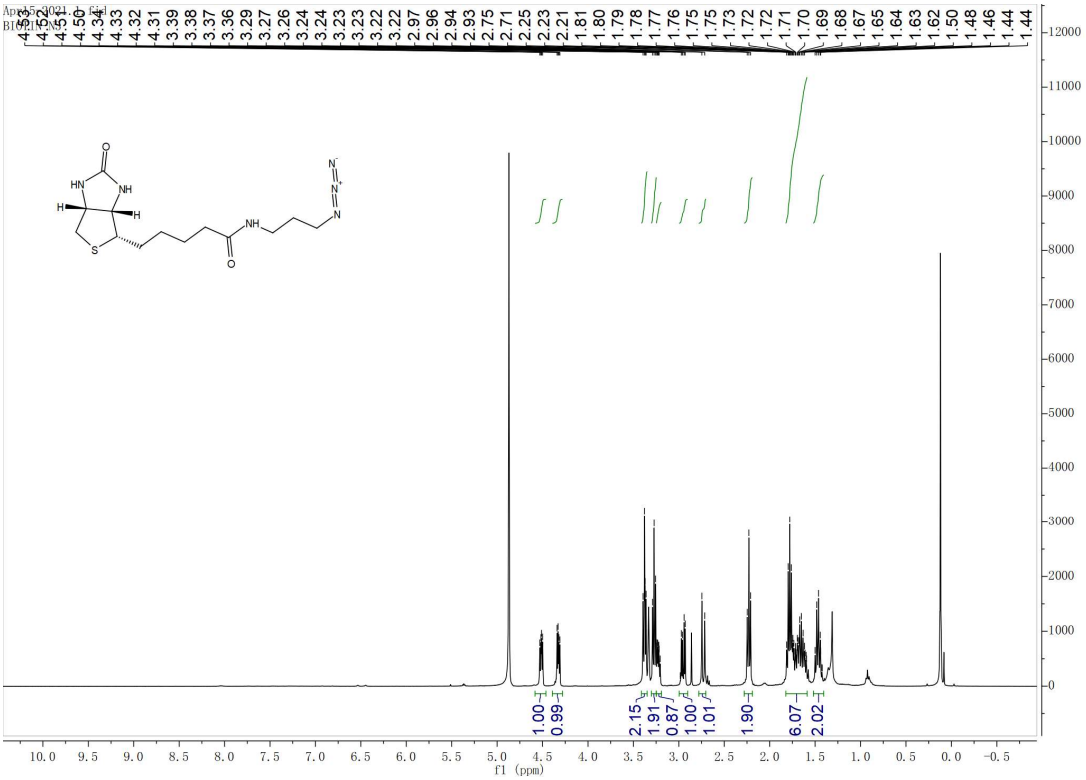

171.87
163.75
156.00
154.50
142.48
141.48
140.81
135.60
131.93
131.82
130.04
129.22
128.79
128.71
128.60
124.90
122.49
122.17
116.35
115.00
114.20
110.34
98.70
98.17
83.51
69.62
49.20
48.74
43.86
32.05
28.94
28.82
27.35
26.65
26.04
25.58
24.45
20.52
18.20
CH3
CH3
HC
O
N
O
OH
f1 (ppm)

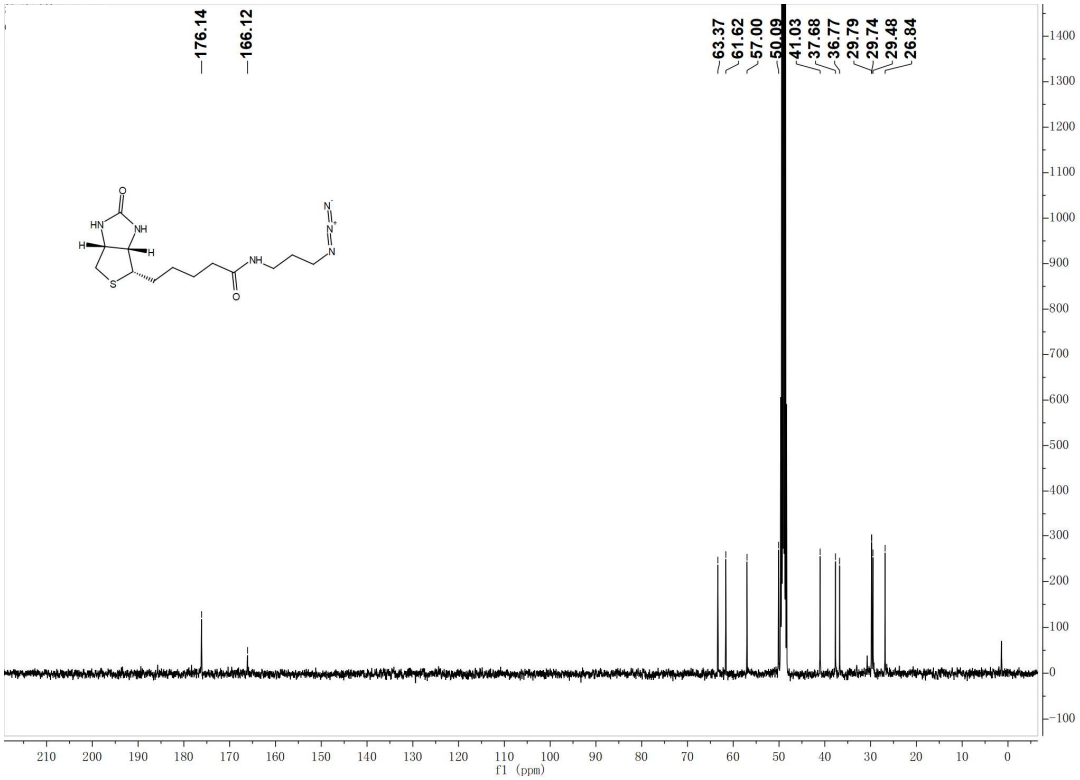

x10 6
637.3412
COOH
Chemical Formula: C43H45N2O3+
Exact Mass: 637.3425
Counts vs. 质荷比 (m/z)

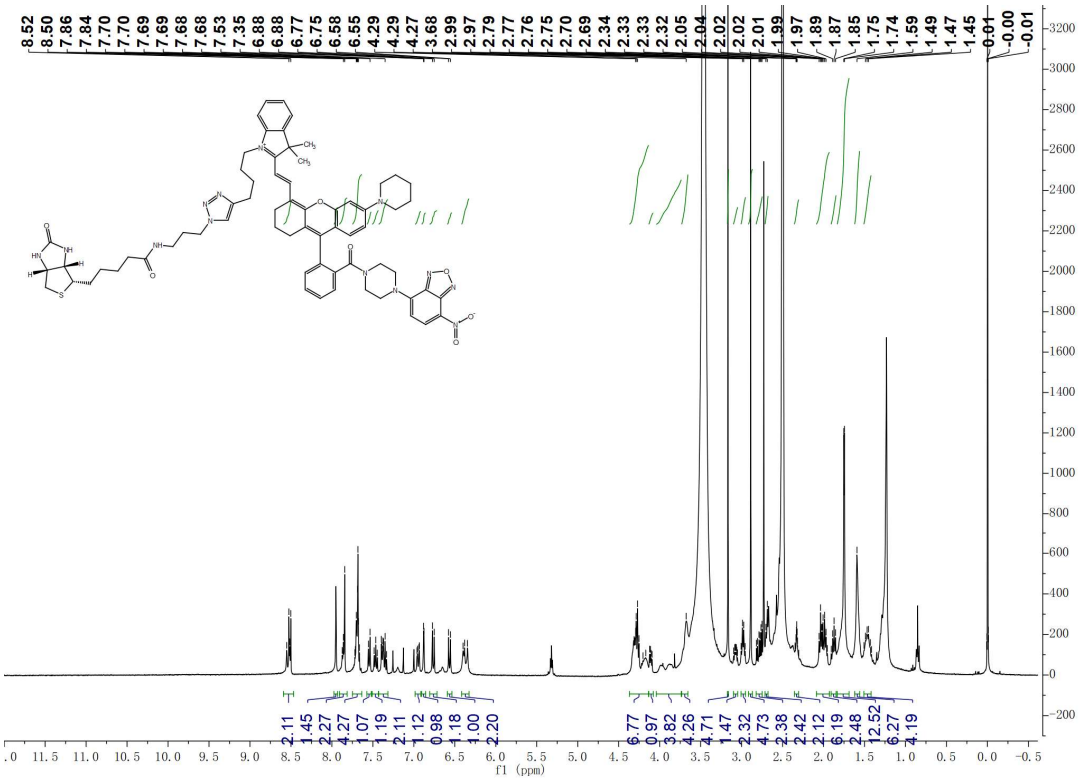

8.35 8.32 7.63 7.63 7.62 7.61 7.60 7.55 7.45 7.43 7.39 7.38 7.26 7.25 7.24 7.24 7.17 7.15 6.85 6.65 4.13 4.12 4.11 4.10 4.09 4.08 3.77 3.72 3.47 3.46 3.45 2.34 2.33 2.32 2.31 2.31 2.30 2.29 2.02 2.00 2.00 1.99 1.98 1.98 1.81 1.80 1.78 1.73 1.72 1.70 1.68 1.66
1.00 1.04 0.82 2.31 1.20 2.47 1.74 1.12 1.06 1.19 1.01 1.03 0.99 6.49 4.48 4.40 2.54 2.47 2.40 3.46 4.15 2.21 6.49 8.40
f1 (ppm)

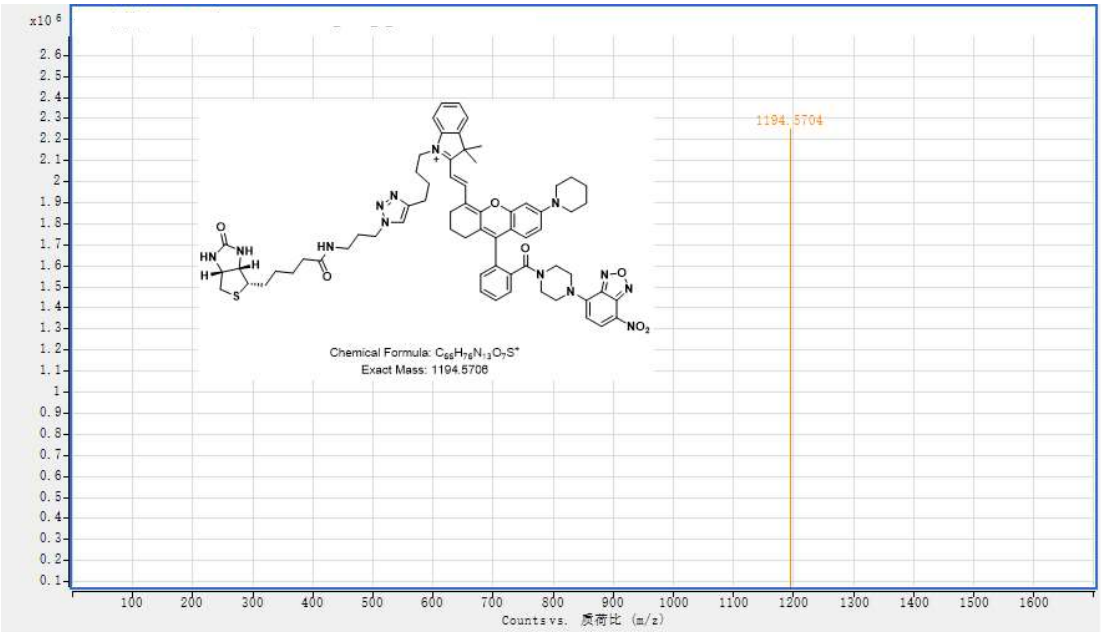

173.55 168.36 163.13 162.68 155.63 154.37 148.77 144.80 144.69 142.93 142.03 141.05 135.78 134.11 133.02 130.52 129.51 128.93 128.22 127.75 125.72 123.54 122.59 116.04 114.15 114.04 110.85 103.95 99.82 97.94 83.34 69.66 49.66 48.93 48.75 48.50 44.09 36.60 31.53 28.68 28.63 27.30 27.19 26.10 25.54 25.50 24.35 20.51 18.09
f1 (ppm)

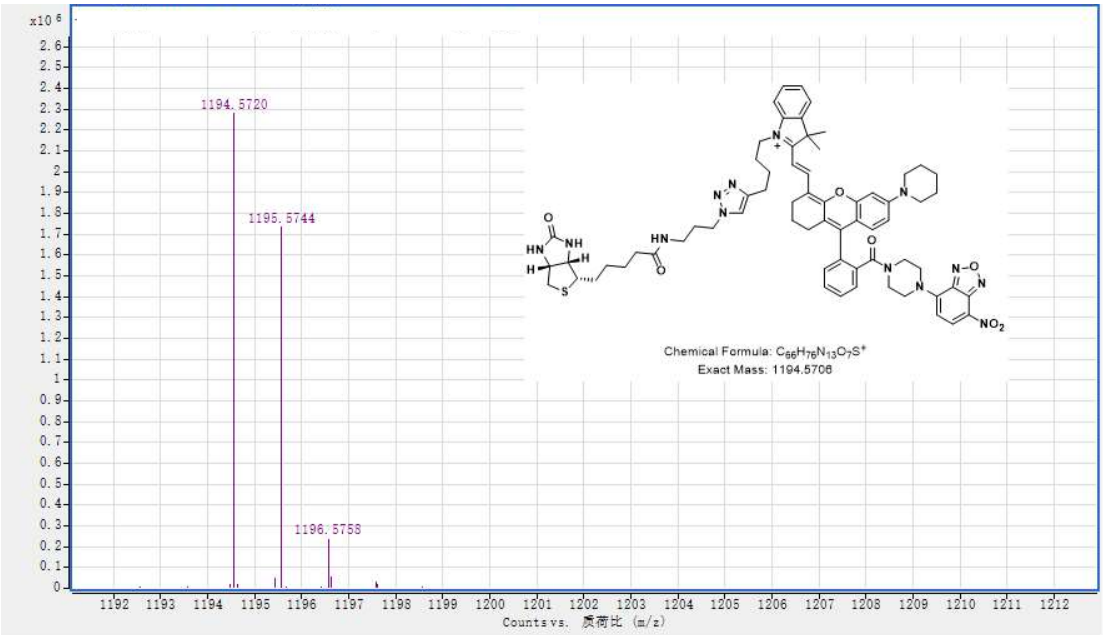

x10 4
Chemical Formula: C53H54N7O5+
Exact Mass: 868.4181
868.4183
Counts vs. 质荷比 (m/z)


f1 (ppm)


176.14
166.12
63.37
61.62
57.00
50.09
41.03
37.68
36.77
29.79
29.74
29.48
26.84
f1 (ppm)


8.52
8.50
7.86
7.84
7.70
7.70
7.69
7.69
7.68
7.68
7.53
7.35
6.88
6.88
6.77
6.75
6.58
6.55
4.29
4.29
4.27
3.68
2.99
2.97
2.79
2.77
2.76
2.75
2.70
2.69
2.34
2.33
2.32
2.05
2.04
2.02
2.02
2.01
1.99
1.97
1.89
1.87
1.85
1.75
1.74
1.59
1.49
1.47
1.45
0.01
0.00
-0.01
2.11
1.45
2.27
4.27
1.07
1.19
2.11
1.12
0.98
1.18
1.00
2.20
6.77
0.97
3.82
4.26
4.71
1.47
2.32
4.73
2.38
2.42
2.12
6.19
2.48
12.52
6.27
4.19
f1 (ppm)


x10 6
1194.5704
Chemical Formula: C66H76N13O7S+
Exact Mass: 1194.5706
Counts vs. 质荷比 (m/z)


x10 6
1194.5720
1195.5744
1196.5758
Chemical Formula: C66H76N13O7S+
Exact Mass: 1194.5706
Counts vs. 质荷比 (m/z)

8. References

1. X. Song, H. Bian, C. Wang, M. Hu, N. Li and Y. Xiao, *Org. Biomol. Chem.*, 2017, **15**, 8091-8101.
2. Y. Liu, Q. Su, M. Chen, Y. Dong, Y. Shi, W. Feng, Z. Y. Wu and F. Li, *Adv. Mater.*, 2016, **28**, 6625-6630.
3. J. Y. Xie, C. Y. Li, Y. F. Li, J. Fei, F. Xu, J. Ou-Yang and J. Liu, *Anal. Chem.*, 2016, **88**, 9746-9752.
4. A. J. Burt, P. Ahmadvand, L. K. Opp, A. T. Ryan, C. Kang and R. J. Mancini, *ChemMedChem*, 2020, **15**, 2004-2009.
5. J. Radford-Knoery and G. A. Cutter, *Anal. Chem.*, 1993, **65**, 976-982.
6. K. Umezawa, M. Yoshida, M. Kamiya, T. Yamasoba and Y. Urano, *Nat. Chem.*, 2017, **9**, 279-286.
7. X. Tu, L. He, H. Huang, H. Ye, L. Sun and L. Yi, *Chem. Commun.*, 2021, **57**, 8802-8805.
8. R. C. Benson and H. A. Kues, *Phys. Med. Biol.*, 1978, **23**, 159-163.