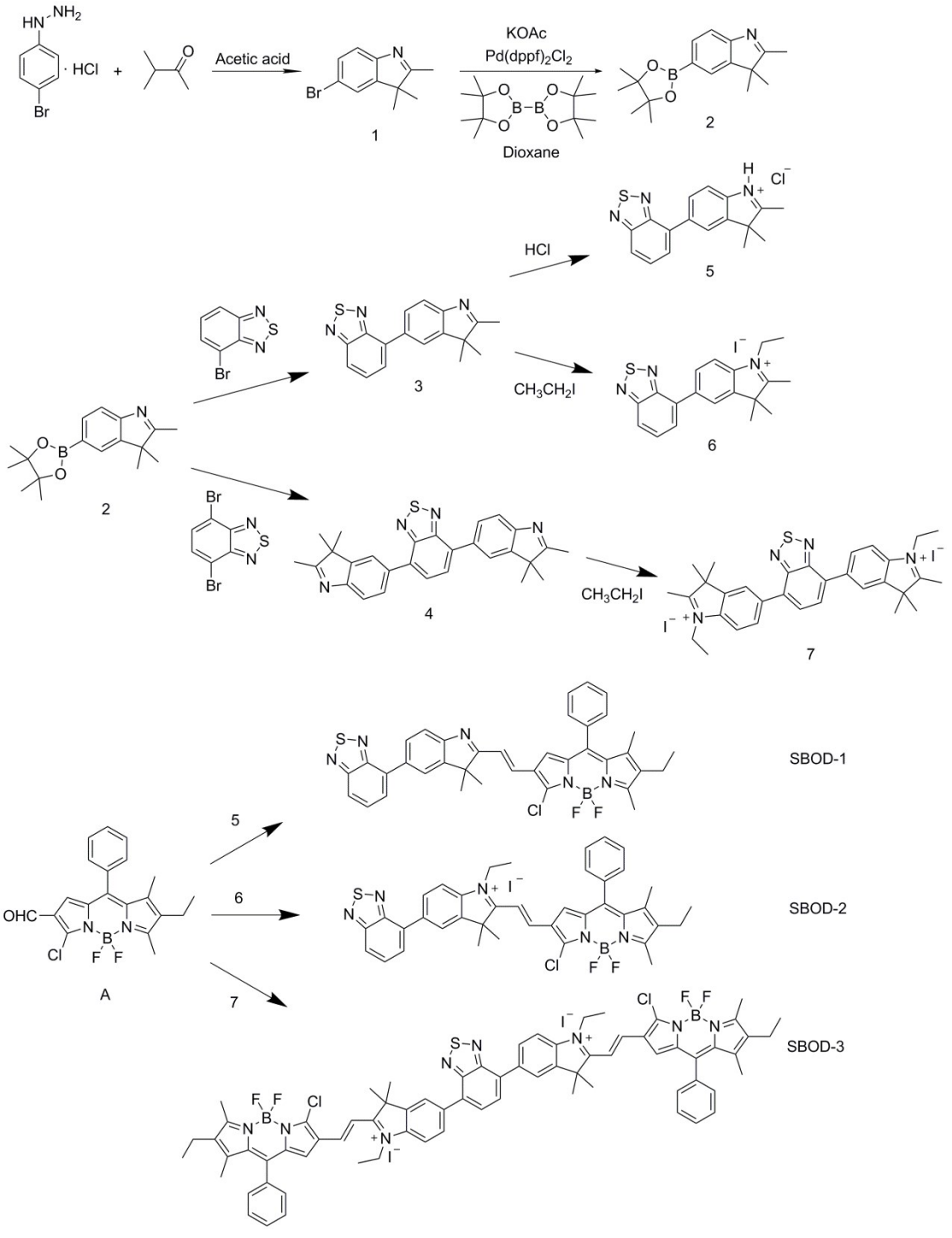
Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

An electron-deficiency-based framework for NIR-II fluorescence probes

Jinzhu Gao,[a,†] Rongchen Wang,[a,†] Tianli Zhu,[a] Jiahui Tan,[b] Xianfeng Gu,*[b] Chunchang Zhao *[a]

1. The rational design of three H_2S-activated probes.



Scheme S1. The rational design of H_2S-activated probes. The design strategy based on our assumption the electron deficiency of substituent in monochlorinated BODIPY greatly affects the thiol−halogen aromatic nucleophilic substitution. As shown in Figure S1b, SBOD-1 showed H_2S-activated NIR-I emission (PBS/CH_3CN, v/v, 1:1, 10 μM, pH 7.4, room temperature). Increasing the electron withdrawing ability of substituent achieved bright light-up NIR-II emission and rapid response to H_2S. Probe SBOD-3 was obtained through further extending the conjugate chain. Both SBOD-2 and SBOD-3 showed fluorescence response to H_2S in NIR-II region.

2. Synthesis.

Compound A was obtained based on our previously reported procedures.[1, 2]

Synthesis of compound 1. 25.55 g (0.114 mol) of ρ-Bromophenylhydrazine hydrochloride, 32 mL (0.3 mol) of 3-methyl-2-butanone and 160 mL of acetic acid were heated under reflux for 3 h. Stop heating and cool to room temperature. Remove solvent under vacuum, dilute with DCM, combine organic phases, wash three times with deionized water, dry over anhydrous sodium sulfate, and remove solvent under vacuum. A red oil was obtained (10 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 (d, 1H), 7.42 (d, 1H), 7.38 (s, 1H), 2.27 (s, 3H), 1.28 (s, 6H).

Synthesis of compound 2. Put 3.01g (12.6 mmol) compound 1, 4.80 g (18.9 mmol) bis(pinacolato)-diboron, 3.72 g (37.9 mmol) anhydrous potassium acetate and 0.37 g (0.5 mmol) dichloro[1,1'-bis(diphenylphosphino)-ferrocen]-palladium(II) in a 500 mL flask, and degassed three times with argon. Under the protection of argon, 200 mL of dry dioxane was added, and the reaction was stirred at 90°C for 5 hours. The remaining solid was filtered off and the solvent was removed under vacuum. The resulting crude product was purified by column chromatography on silica gel to give the product as a pink powder (3.31g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (dd, 1H),

7.73 (s, 1H), 7.55 (d, 1H), 2.30 (s, 3H), 1.36 (s, 12H), 1.31 (s, 6H).

Synthesis of compound 3. 50 mg (0.25 mmol) of 4-bromobenzothiadiazole, 90 mg (0.3 mmol) of compound 2, 6 mg (0.005 mmol) of tetrakis(triphenylphosphine)palladium(0), one drop of Aliquant 336 were placed in a flask, then degassed with argon for three times. Under argon protection, 2.5 mL of degassed toluene and 2.5 mL of a 2M aqueous potassium carbonate solution were added. The reaction was refluxed overnight. It was poured into 20 mL of water and extracted three times with DCM, combined organic phases and washed three times with water. Dry over anhydrous sodium sulfate and remove the solvent under vacuum. The resulting crude product was purified by column chromatography on silica gel to give the product as a yellow-orange oil (58 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.00 (dd, 1H), 7.90 (dd, 1H), 7.85 (d, 1H), 7.72-7.70 (m, 2H), 7.68 (d, 1H), 2.32 (s, 3H), 1.40 (s, 6H).

Synthesis of compound 4. 292 mg (1 mmol) of 4,7-Dibromobenzothiadiazole, 740 mg (2.6 mmol) compound 2, 25 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0), one drop of Aliquant 336 were placed in a flask, then degassed with argon for three times. Under argon protection, 10 mL of degassed toluene and 10 mL of degassed 2M potassium carbonate aqueous solution were added dropwise. The reaction was refluxed overnight. It was poured into 100 mL of water and extracted three times with DCM, combined organic phases and washed three times with water. Dry over anhydrous sodium sulfate and remove the solvent under vacuum. The resulting crude product was purified by column chromatography on silica gel to give the product as a yellow oil (533 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95 (d, 1H), 7.93 (d, 1H), 7.92 (d, 2H), 7.81 (s, 2H), 7.73 (s, 1H), 7.71 (s, 1H), 2.31 (s, 6H), 1.42 (s, 12H).

Synthesis of compound 5. Add 1.5 mL of concentrated hydrochloric acid to 293 mg (1.0 mmol) 3,

stir at room temperature for 30 min, remove excess solvent by rotary evaporation.

Synthesis of compound 6. 50 mg (0.17 mmol) of compound 3, 265 mg (1.7 mmol) of iodoethane and 8 mL of acetonitrile were heated and stirred under reflux overnight. Cool to room temperature, remove the solvent under vacuum, add a small amount of DCM to dissolve, add 30 mL of ether, then the white solid was precipitate out. The solid was recovered by suction filtration and washed with cold ether (21 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.16 (d, 1H), 8.13 (s, 1H), 8.08 (dd, 1H), 7.86 (d, 1H), 7.75 (m, 2H), 4.88 (q, 2H), 3.20 (s, 3H), 1.76 (s, 6H), 1.68 (t, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 196.19, 155.21, 152.72, 142.27, 140.56, 138.96, 131.82, 130.37, 129.32, 127.95, 123.79, 121.23, 115.01, 54.99, 47.61, 43.18, 21.39, 11.75. HRMS (ESI, m/z): calculated for $C_{19}H_{20}N_3S^+$ $[M-I]^+$: 322.1372, found: 322.1375.

Synthesis of compound 7. This compound was prepared in a manner analogous to the synthesis of compound 6 using 76.5 mg compound 4 (0.17 mmol) and 265 mg iodoethane (1.7 mmol) to give a powder of compound 7 (23.3 mg, 18%). ^{13}C (151 MHz, CD_3OD) δ 196.52, 153.34, 142.80, 140.34, 138.70, 131.94, 130.30, 128.40, 124.32, 115.08, 109.93, 54.63, 43.23, 21.51, 12.03.

Synthesis of compound SBOD-1. Compound 4 was dissolved with 10 mL of absolute ethanol, then 425 mg (1.1 mmol) compound A was added, and the mixture was heated and refluxed for 4 h under the protection of argon gas. Cooled to room temperature, diluted with 30 mL DCM, combine organic phases and washed three times with water, dried over anhydrous sodium sulfate, removed the solvent under vacuum. The resulting crude product was purified by column chromatography on silica gel to give the product as powdery solid (483 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (dd, 1H), 7.93 (dd, 1H), 7.89 (s, 1H), 7.73 (d, 1H), 7.71 (t, 1H), 7.67 (d, 1H), 7.59-7.52 (m, 4H), 7.37 (d, 1H), 7.35 (d, 1H), 6.84 (d, 1H), 6.51 (s, 1H), 2.67 (s, 3H), 2.37 (q, 2H),

1.53 (s, 6H), 1.48 (s, 3H), 1.05 (t, 3H). ^{13}C (151 MHz, $CDCl_3$) δ 188.49, 188.25, 182.77, 173.63, 169.75, 161.40, 154.07, 152.26, 152.02, 144.44, 139.23, 133.70, 128.05, 127.74, 126.46, 121.27, 119.18, 118.64, 76.45, 69.92, 58.74, 53.25, 52.73, 51.14, 32.64, 30.29, 28.22, 23.56, 21.97, 21.43, 19.62, 14.34, 13.09, 11.02. HRMS (ESI, m/z): calculated for $C_{37}H_{31}BClF_2N_5S$ $[M+H]^+$: 662.2123, found: 662.2126.

Synthesis of compound SBOD-2. This compound was prepared in a manner analogous to the synthesis of SBOD-1 using 35 mg compound 6 (0.078 mmol) and 25 mg compound A (0.065 mmol) to give a powder of SBOD-2 (21.2 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.14-8.07 (m, 4H), 7.78-7.72 (m, 3H), 7.66 (s, 3H), 7.52-7.35 (m, 4H), 4.98 (q, 2H), 2.71 (s, 3H), 2.40 (q, 2H), 1.61 (s, 6H), 1.51 (s, 3H), 1.07 (t, 3H), 0.88 (t, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.07, 168.63, 155.39, 152.65, 144.38, 142.79, 140.67, 140.31, 139.38, 138.24, 135.97, 135.24, 132.31, 131.38, 130.03, 129.32, 128.64, 128.43, 124.49, 124.10, 123.36, 121.72, 121.09, 114.48, 109.89, 51.79, 44.07, 34.67, 31.73, 29.45, 27.60, 22.37, 17.28, 14.12, 13.91, 12.52. HRMS (ESI, m/z): calculated for $C_{39}H_{36}BClF_2N_5S^+$ $[M-I]^+$: 690.2436, found: 690.2440.

Synthesis of compound SBOD-3. This compound was prepared in a manner analogous to the synthesis of SBOD-1 using 55 mg compound 7 (0.072 mmol) and 55.6 mg compound A (0.144 mmol) to give a powder of SBOD-3 (46.4 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.24-8.14 (m, 7H), 8.00 (b, 2H), 7.82 (b, 3H), 7.66 (b, 6H), 7.44 (b, 4H), 7.35 (d, 2H), 4.92 (q, 4H), 2.72 (s, 6H), 2.43 (q, 4H), 1.91 (s, 12H), 1.62 (t, 6H), 1.51 (s, 6H), 1.07 (t, 6H). ^{13}C (151 MHz, $CDCl_3$) δ 180.02, 168.72, 153.17, 144.34, 143.61, 140.31, 138.04, 135.55, 131.27, 130.54, 129.29, 128.53, 122.99, 121.97, 114.39, 87.76, 80.48, 64.92, 51.36, 43.55, 32.47, 31.74, 30.94, 30.22, 29.46, 28.95, 28.73, 27.48, 22.20, 19.92, 17.15, 13.91, 12.60. HRMS (ESI, m/z): calculated for $C_{72}H_{68}B_2Cl_2F_4N_8S^{2+}$ [M-

2I]$^{2+}$: 622.2388, found: 622.2387.

Preparation of SBOD-P. Add 20 mg mPEG-DSPE to 2 mL deionized water and sonicate until the solution is clear and transparent. Add 0.2 mg SBOD-2 to the system and sonicate for 10 min. To remove unreacted chemicals, the aqueous solution was dialyzed in 500 mL water for 6 h, refreshing water every 2 h. The obtained stock solution of SBOD-P was diluted to the desired concentration for test studies.

3. In vivo imaging. All animal experiments are performed within the scope of relevant laws in Fudan University. In order to obtain HCT116 subcutaneous xenograft nude mice, 200 µl of HCT116 cells (5x106) were injected subcutaneously into designated positions in male nude mice. Three weeks after the subcutaneous injection, SBOD-P in 100 µl PBS was injected intratumorally into the tumor site of the HCT116/HepG2 tumor-bearing mice. Fluorescence images taken at different points in time after the nanoprobe injection. The excitation wavelength was 780 nm.

4. Absorption and emission spectra of SBOD-1.

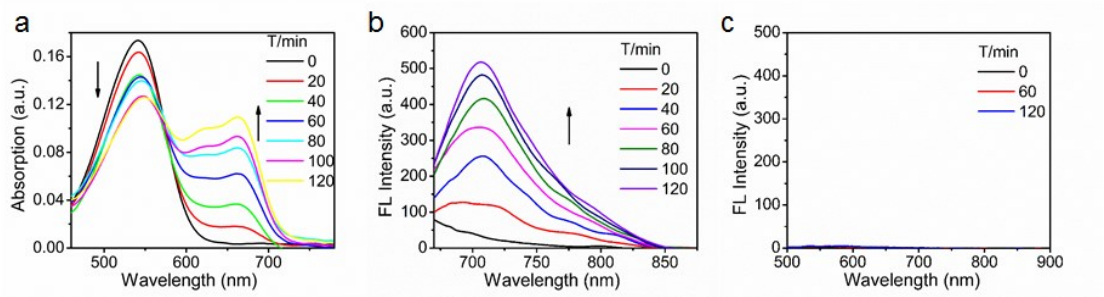


Figure S1. Time-dependent (a) absorption and (b) emission (λ_{ex} = 660 nm) (c) emission (λ_{ex} = 540 nm) spectra of SBOD-1 (10 µM) in the presence of 100 µM NaHS (PBS/MeCN, v/v, 1:1, pH 7.4, 37℃).

5. HRMS analysis of the reaction of SBOD-1 with NaHS.

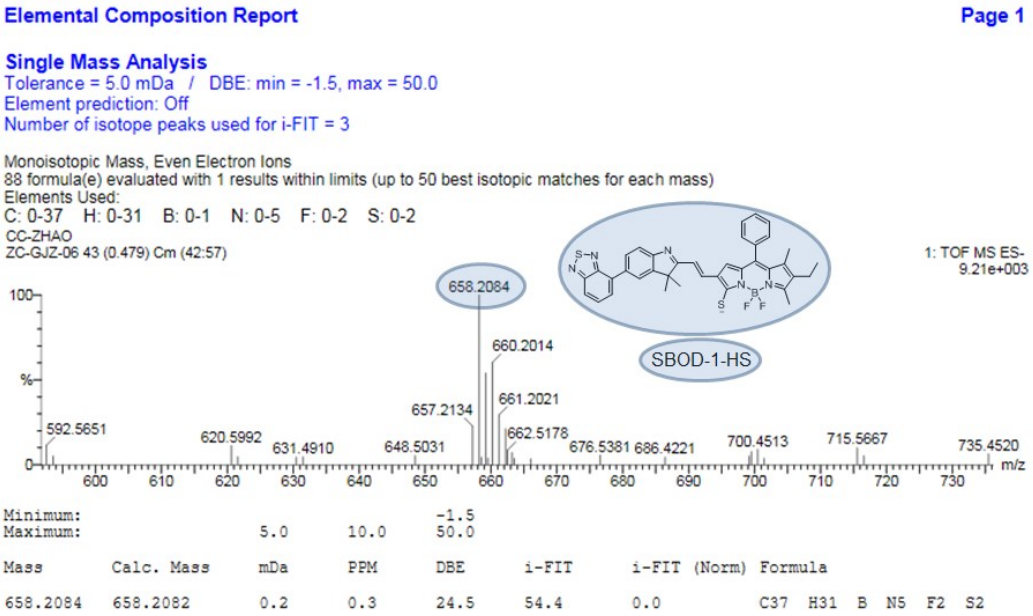


Figure S2. To prove the formation of SBOD-1-HS, the HRMS analysis of the reaction of SBOD-1 with NaHS was performed.

6. HRMS analysis of the reaction of SBOD-2 with NaHS.

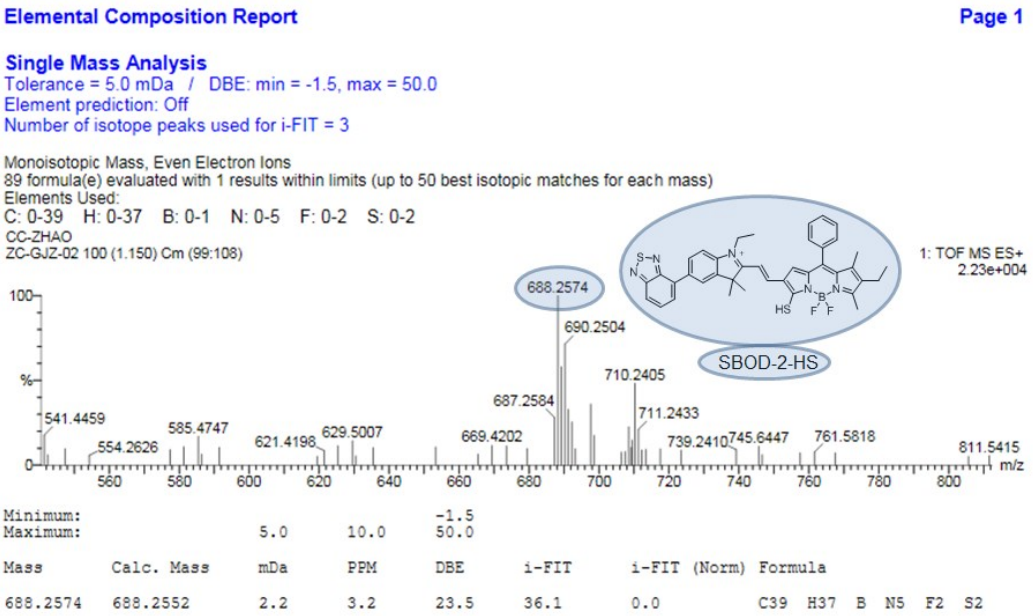


Figure S3. To prove the formation of SBOD-2-HS, the HRMS analysis of the reaction of SBOD-2 with NaHS was performed.

7. Fluorescent response of SBOD-2, 3, P to H_2S.

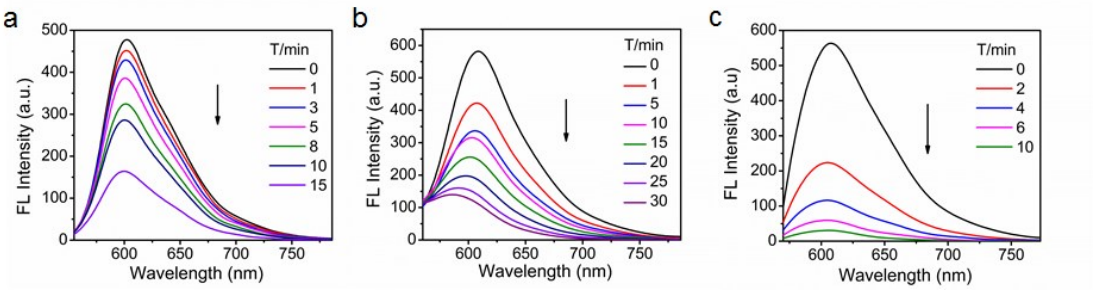


Figure S4. Time-dependent emission spectra of (a) SBOD-2 (10 μM, λ_{ex}=540 nm), (b) SBOD-3 (10 μM, λ_{ex}=540 nm) (PBS/MeCN, v/v, 1:1, pH 7.4, 37℃) and (c) SBOD-P (10 μM, λ_{ex}=550 nm) (PBS, pH 7.4, 37℃) in the presence of 100 μM NaHS. The Fluorescence quantum yield of SBOD-3-2HS was determined to be 0.015% under 808 nm laser irradiation.

8. The stability of SBOD-2 and SBOD-2-HS.

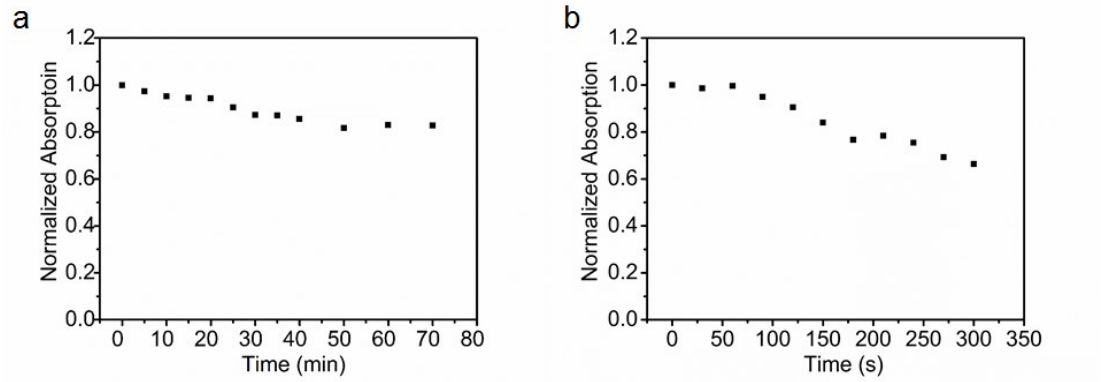


Figure S5. The normalized absorption changes of (a) SBOD-2 at 540 nm and (b) SBOD-2-HS at 780 nm under continuous irradiation with an Hg/Xe lamp (Hamamatsu, LC8 Lightningcure, 6 W).

9. HRMS analysis of the reaction of SBOD-3 with NaHS.

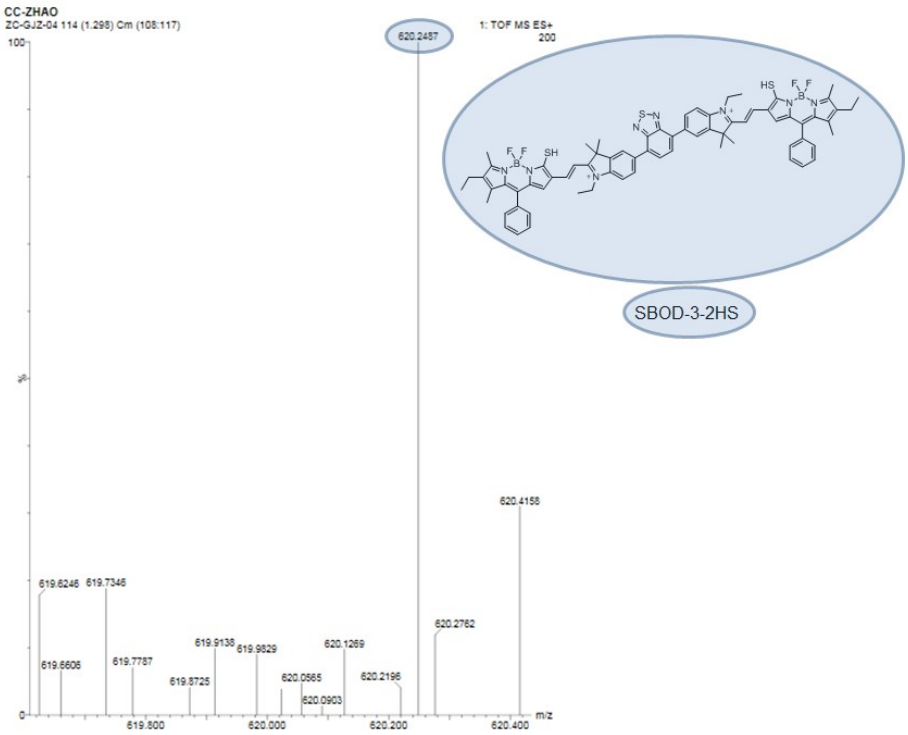


Figure S6. To prove the formation of SBOD-3-2HS, the HRMS analysis of the reaction of SBOD-3 with NaHS was performed.

10. Zeta potential distribution of SBOD-P in PBS.

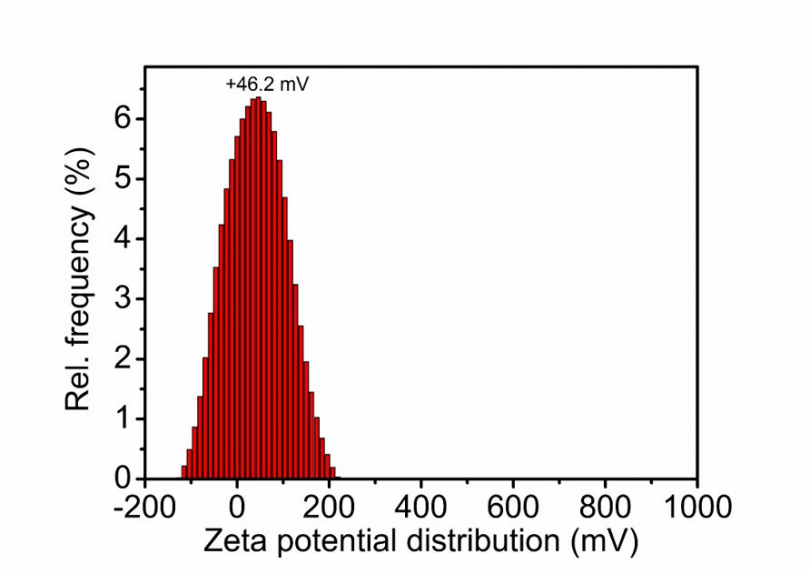


Figure S7. Zeta potential distribution of SBOD-P in PBS.

11. The characterization of SBOD-P.

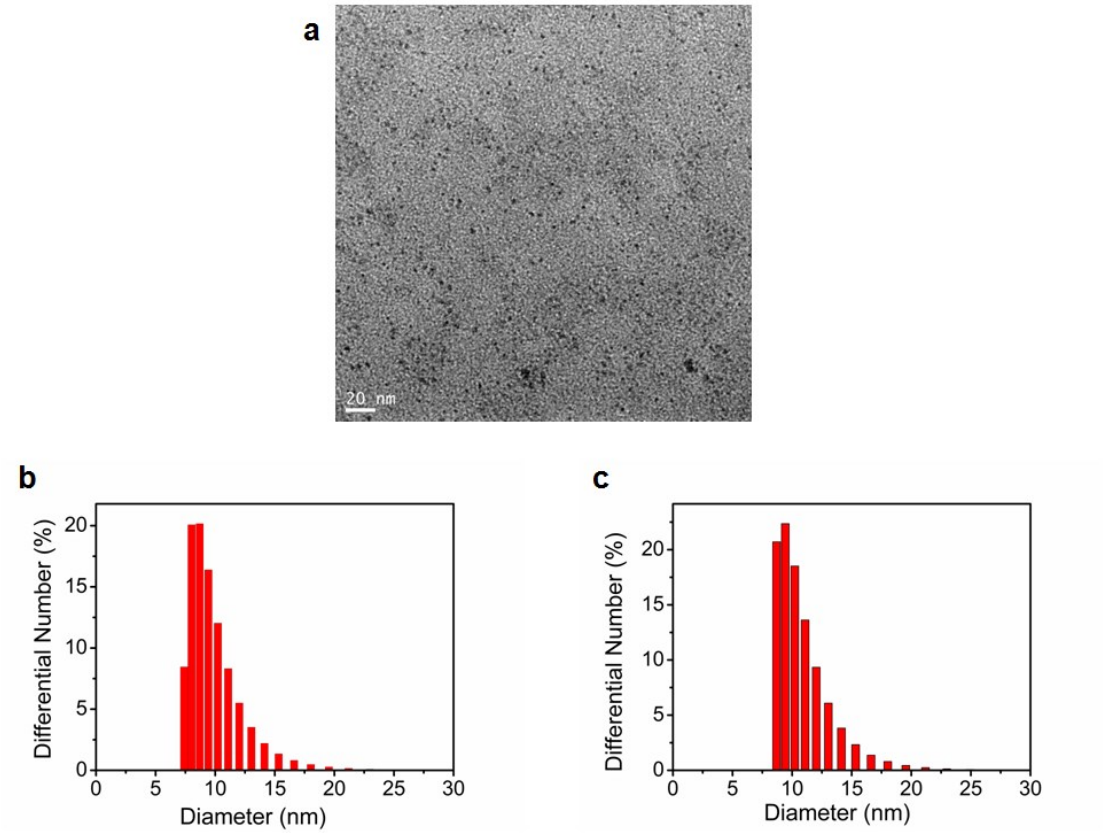


Figure S8. The characterization of SBOD-P by (a) TEM and (b) dynamic light scattering measurement. (c) DLS of SBOD-P after five days in PBS.

12. pH effect on the reaction of SBOD-P to H_2S.

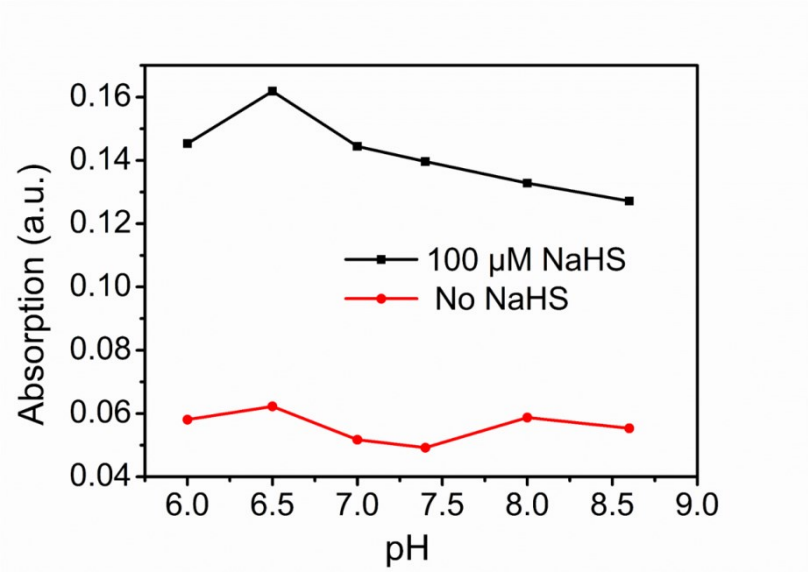


Figure S9. pH effect on the reaction of SBOD-P to H_2S.

13. The detection limit.

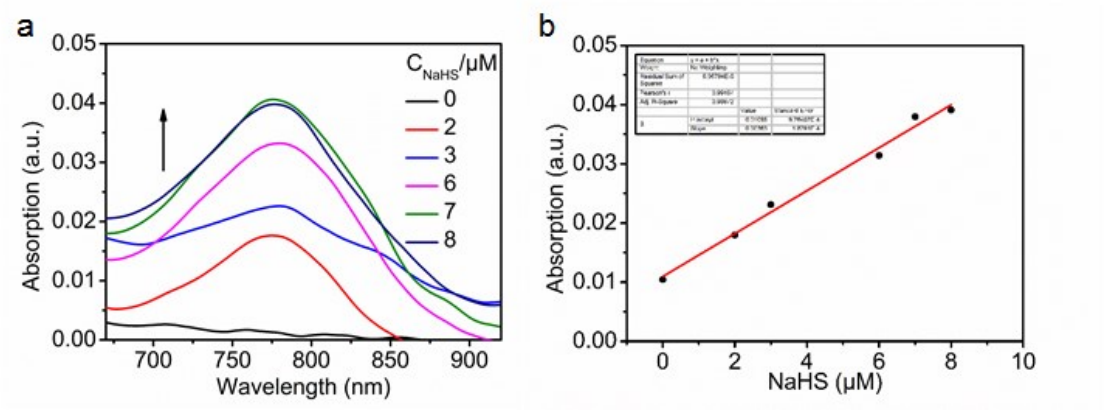


Figure S10. (a) Absorption spectra of BOD-P in the presence of various concentrations of NaHS in PBS buffer (pH 7.4) at 37 °C. Data were recorded 10 min after addition of NaHS. (b)The absorption intensity changes of BOD-P as function of NaHS concentrations, which exhibited good linear relationship with NaHS concentration (0-8 μM), affording a sensitive detection limit of 0.859 μM. The following method was used to calculate the detection limit (DOL): wherein σ is the standard deviation, k is the slope of the linear line. k was determined to be 0.00363 according to the linear correlation, while σ = 0.00104.

DOL = 3σ/k

14. Cytotoxicity Assay.

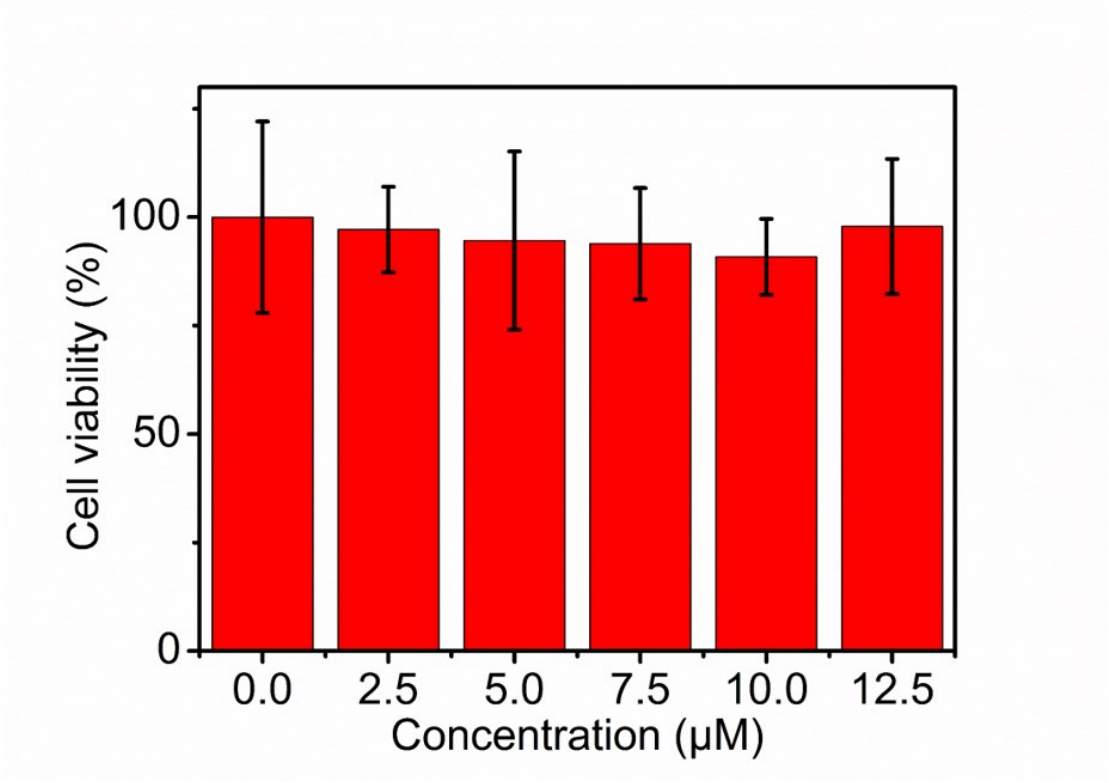
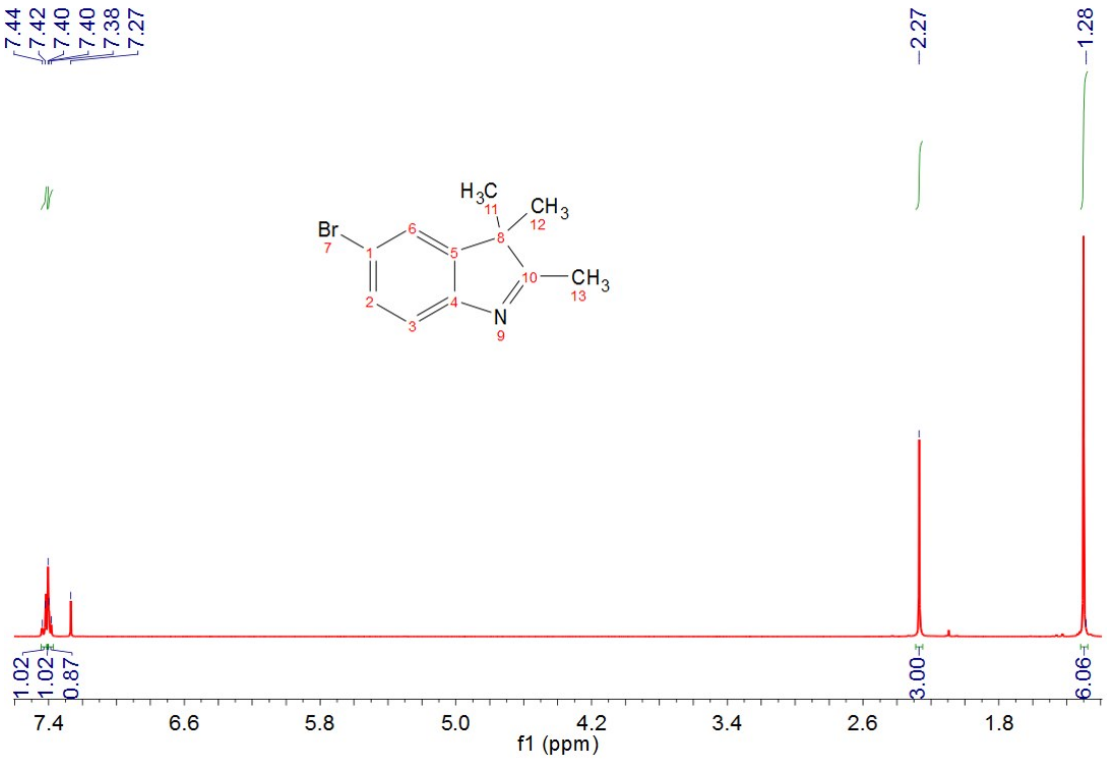
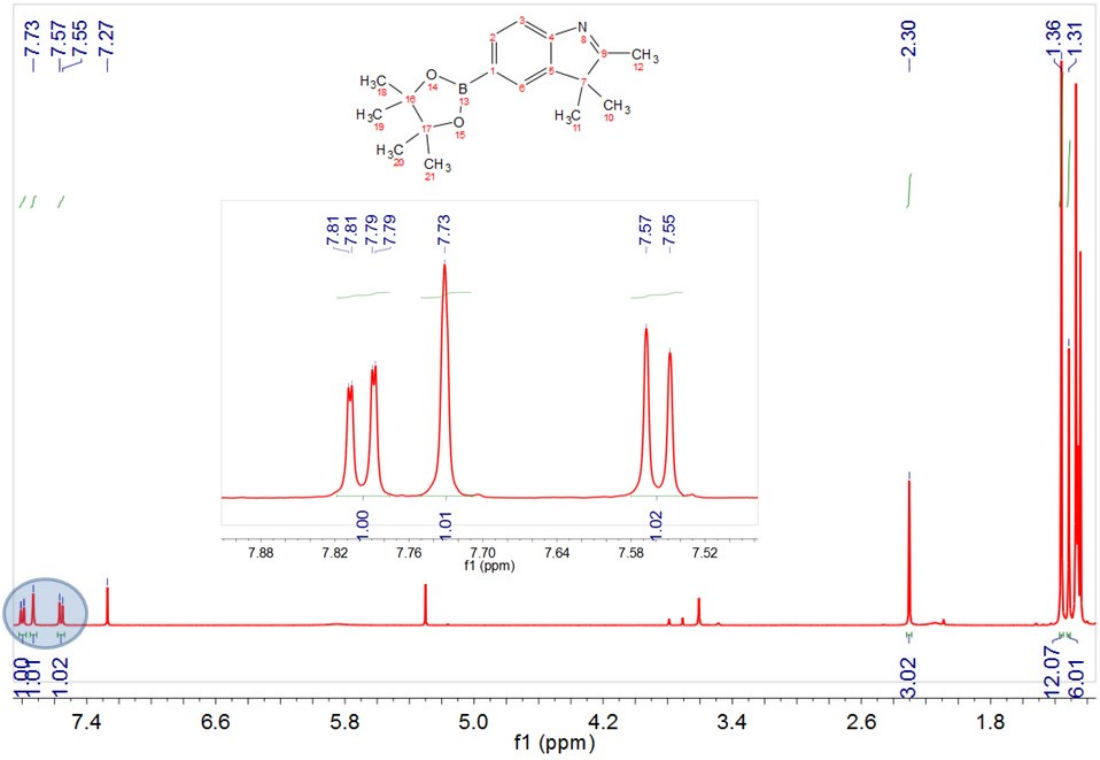


Figure S11. Cytotoxicity Assay. HCT116 cells were seeded in 96-well microplates in RPMI-1640 medium supplemented with 10% FBS at 37 °C in humidified environment of 5% CO_2. After 24 h of cell attachment, the plates were washed with PBS, followed by addition of various concentrations of probe SBOD-P (0-12.5 μM) in RPMI-1640 medium. The cells were then incubated at 37 °C in humidified environment of 5% CO_2 for 18 h, followed by standard CCK-8 assay.

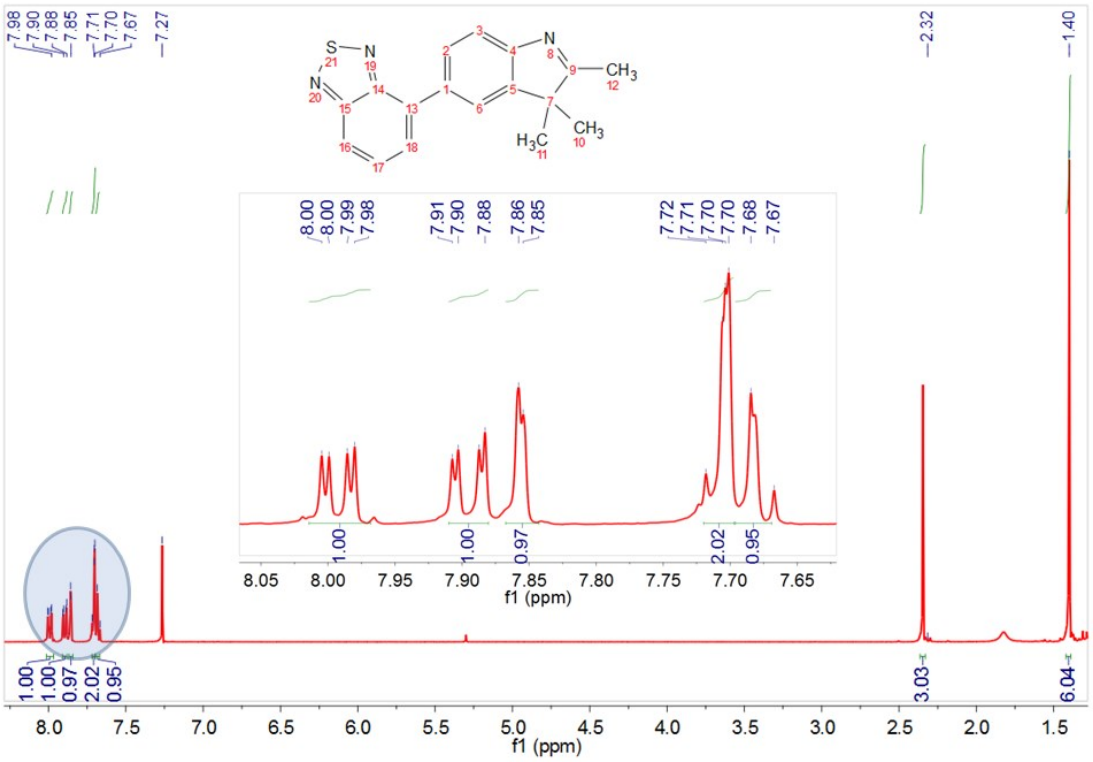
15. NMR and HRMS characterizations.



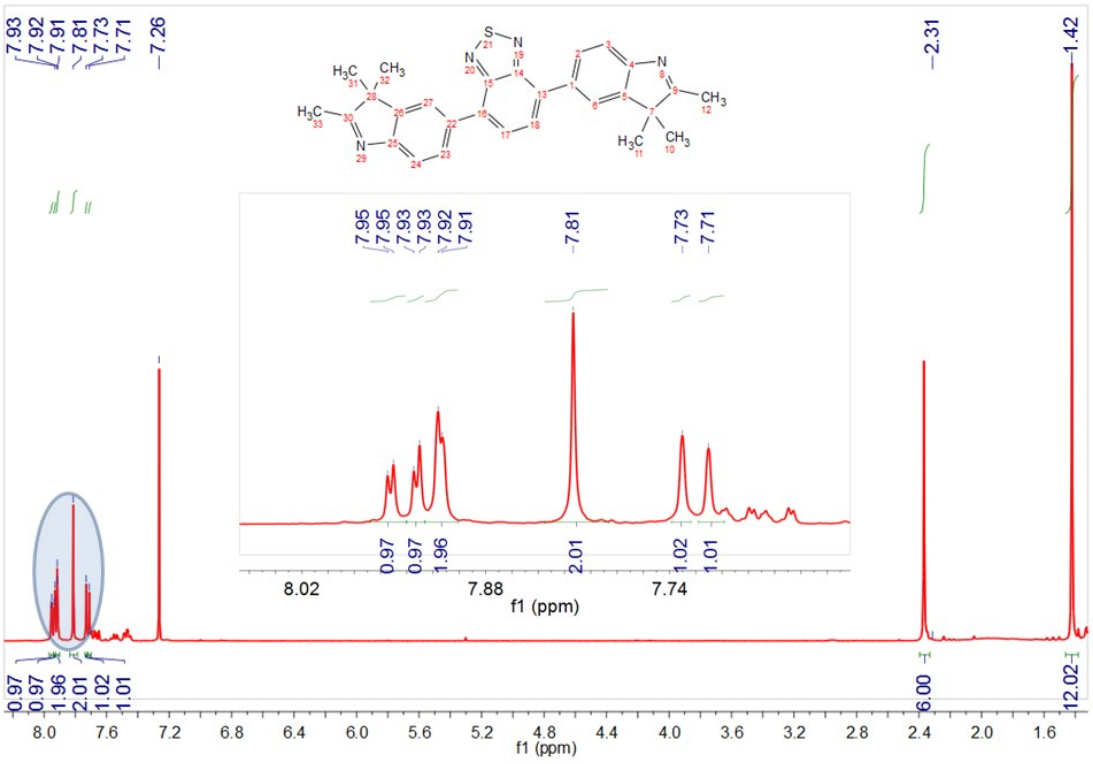
^{1}H NMR spectrum for compound 1



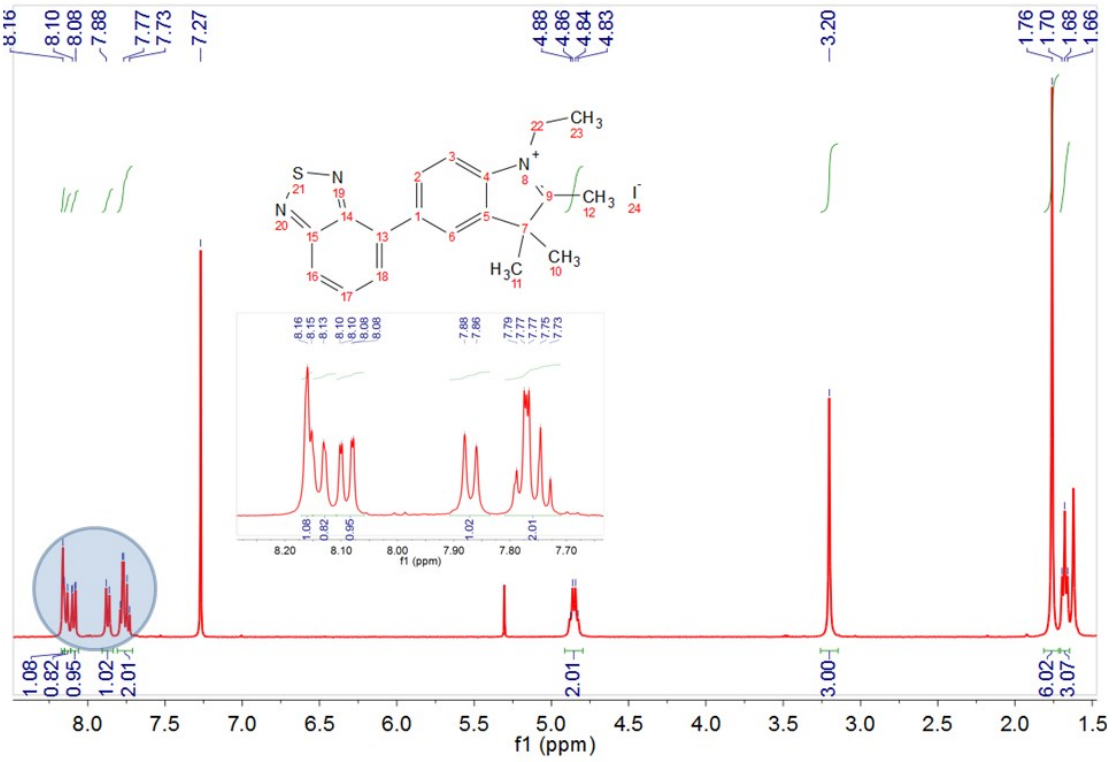
^{1}H NMR spectrum for compound 2.



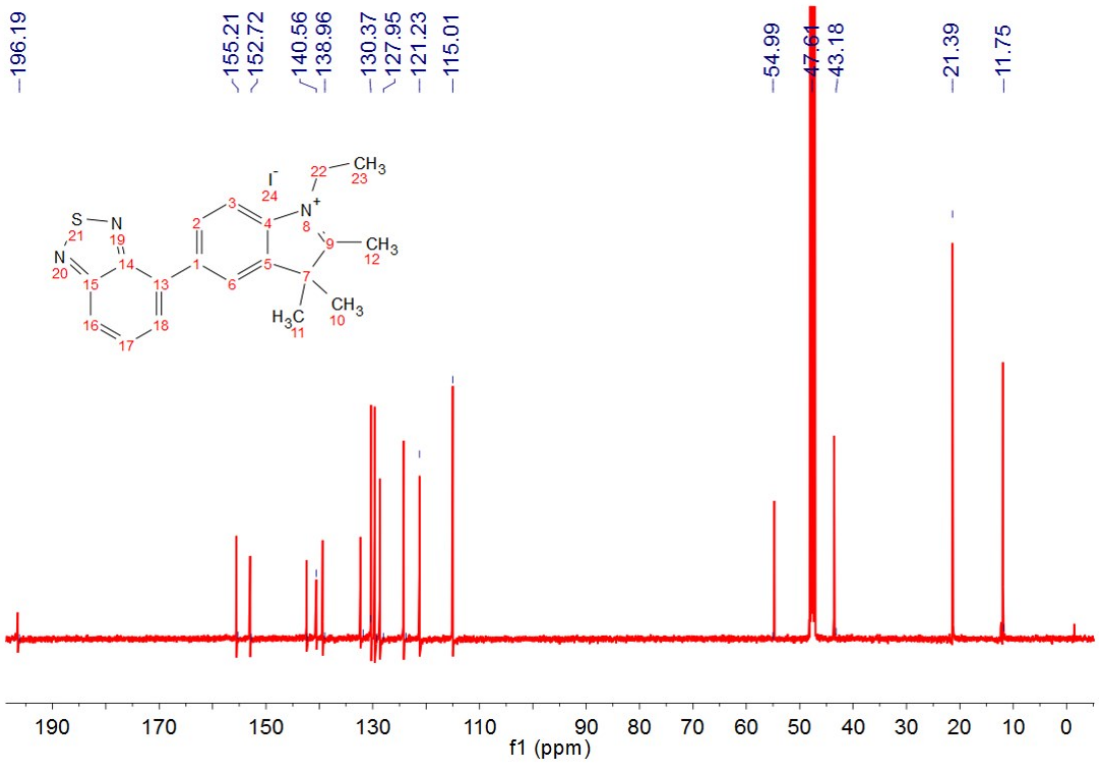
^{1}H NMR spectrum for compound 3.



^{1}H NMR spectrum for compound 4.



^{1}H NMR spectrum for compound 6

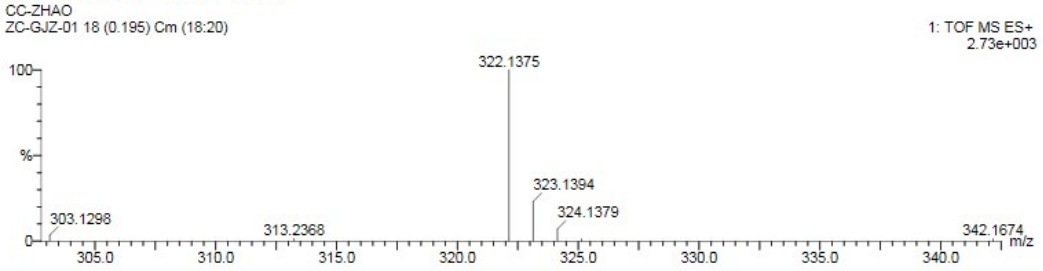


^{13}C NMR spectrum for compound 6

Elemental Composition Report **Page 1**

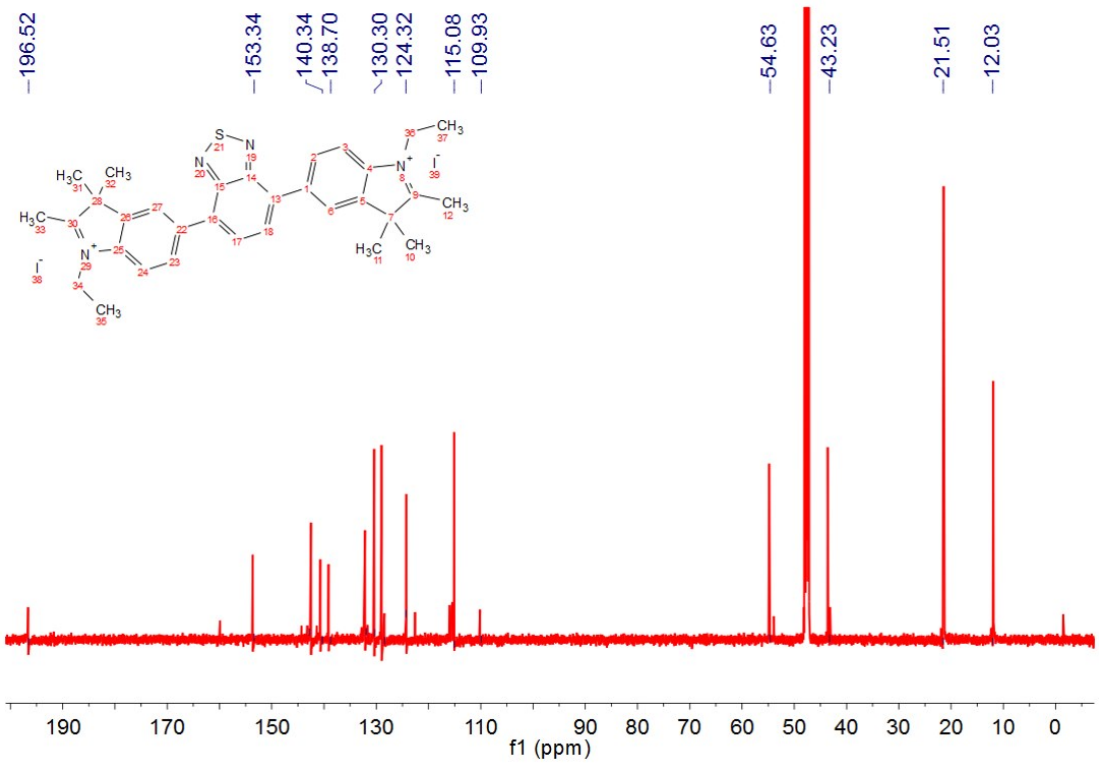
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-19 H: 0-20 N: 0-3 S: 0-1
CC-ZHAO
ZC-GJZ-01 18 (0.195) Cm (18:20)

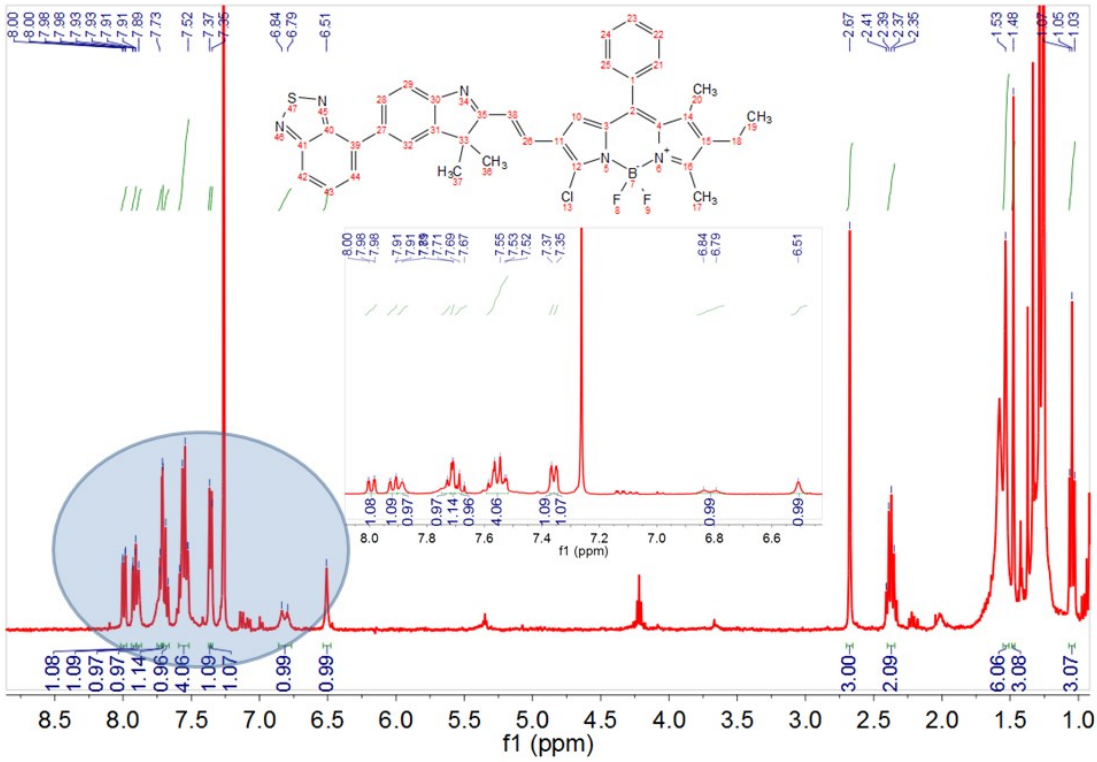


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
322.1375	322.1378	-0.3	-0.9	11.5	7.4	0.0	C19 H20 N3 S

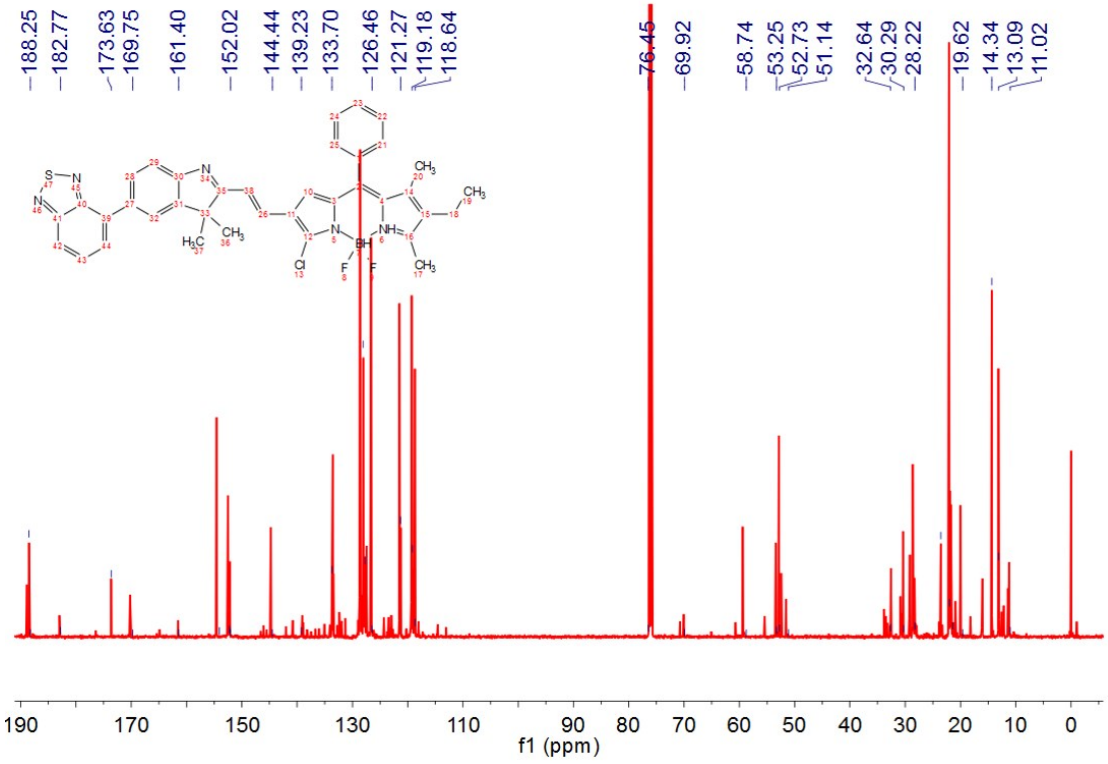
HRMS spectrum for compound 6.



^{13}C NMR spectrum for compound 7



^{1}H NMR spectrum for compound SBOD-1

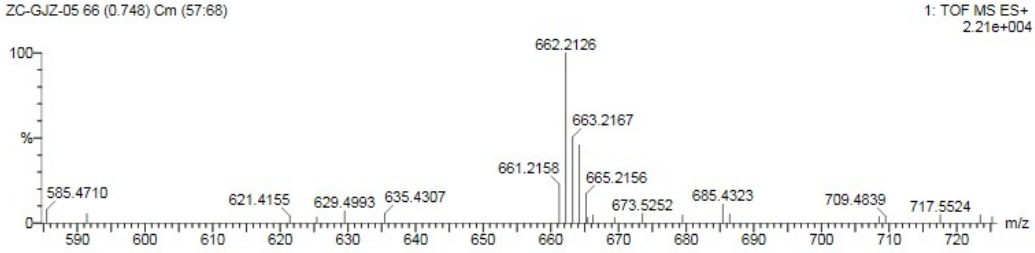


^{13}C NMR spectrum for compound SBOD-1

Elemental Composition Report **Page 1**

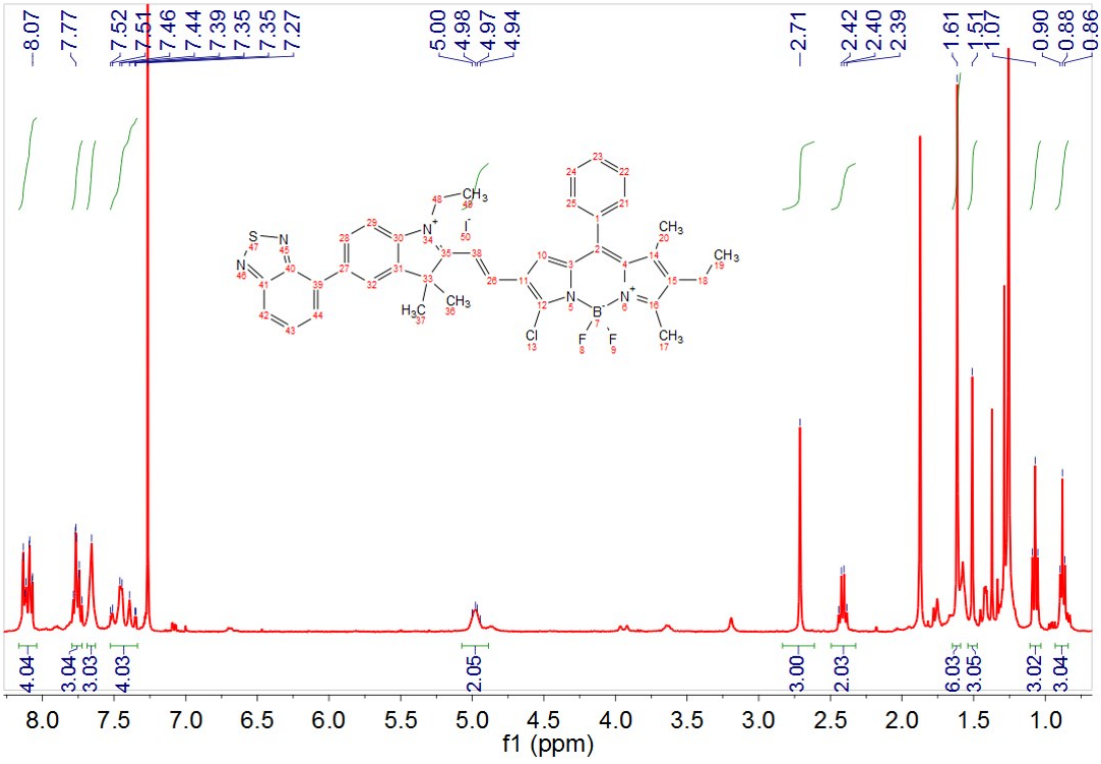
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
121 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-37 H: 0-32 B: 0-1 N: 0-5 F: 0-2 S: 0-1 Cl: 0-1
CC-ZHAO
ZC-GJZ-05 66 (0.748) Cm (57:68)

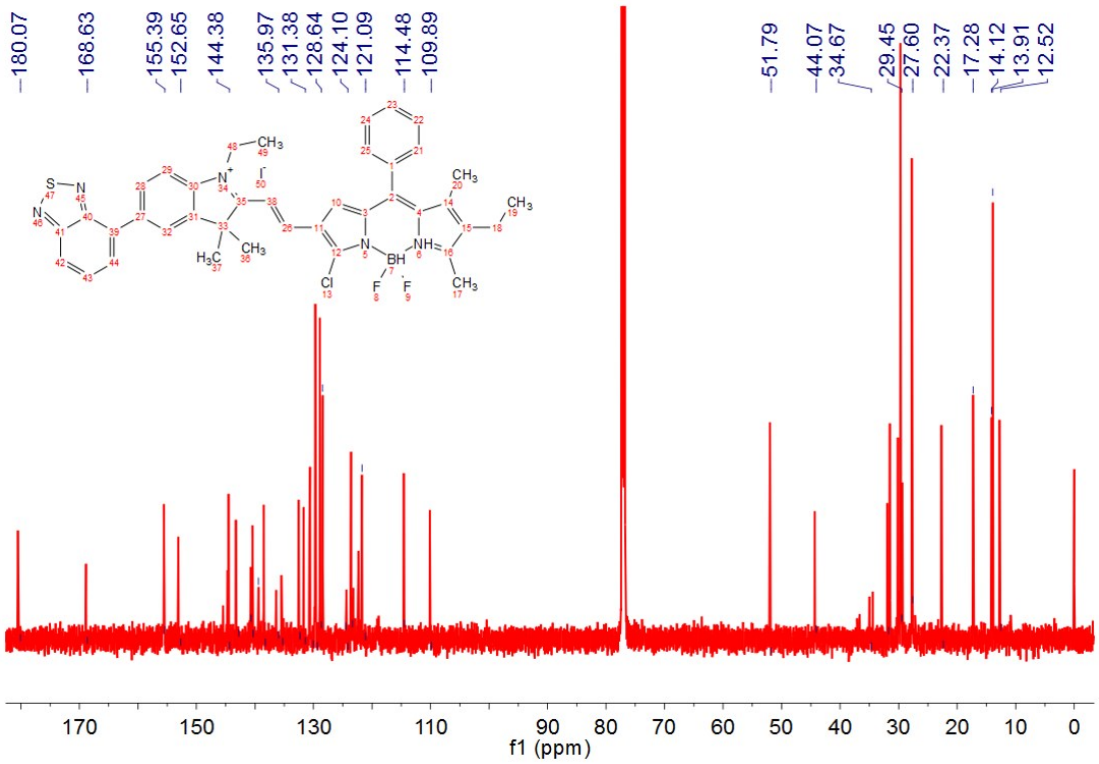


Minimum:		5.0	10.0	-1.5			
Maximum:				50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
662.2126	662.2128	-0.2	-0.3	23.5	28.2	0.0	C37 H32 B N5 F2 S Cl

HRMS spectrum for compound SBOD-1.



^{1}H NMR spectrum for compound SBOD-2

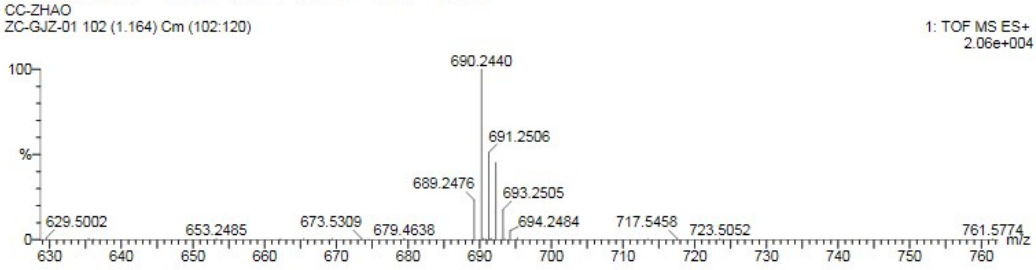


^{13}C NMR spectrum for compound SBOD-2

Elemental Composition Report **Page 1**

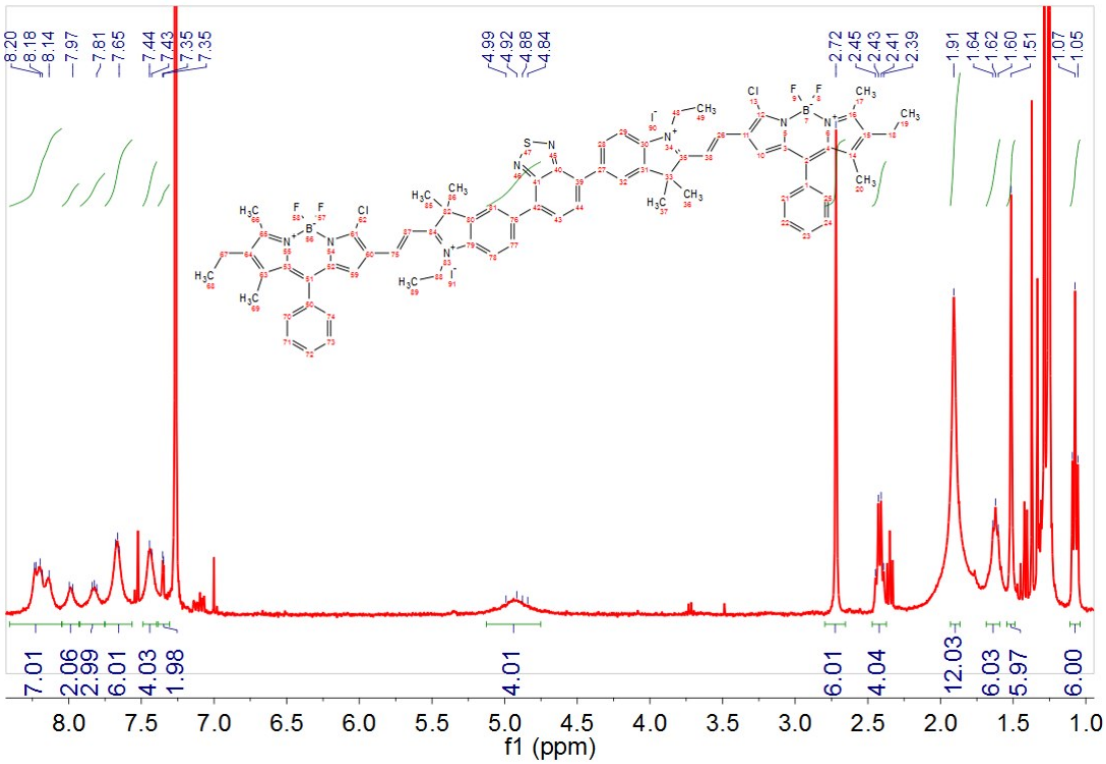
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
121 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-39 H: 0-36 B: 0-1 N: 0-5 F: 0-2 S: 0-1 Cl: 0-1
CC-ZHAO
ZC-GJZ-01 102 (1.164) Cm (102:120)

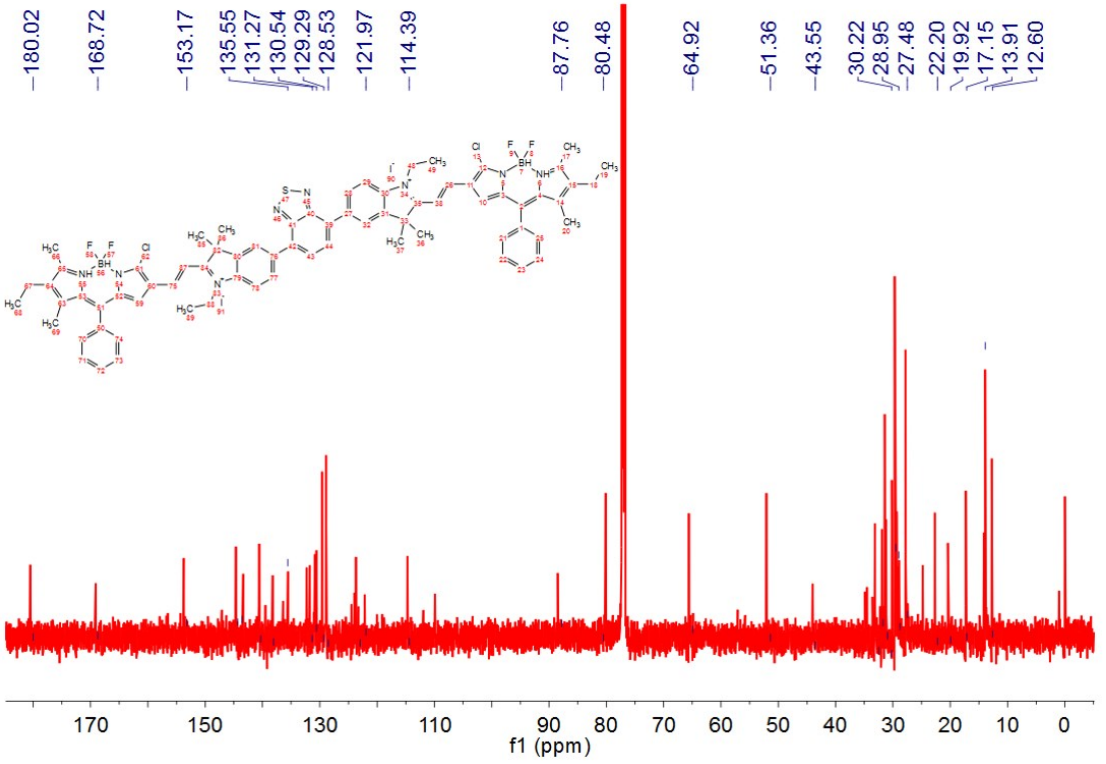


Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
690.2440	690.2441	-0.1	-0.1	23.5	66.3	0.0	C39 H36 B N5 F2 S Cl

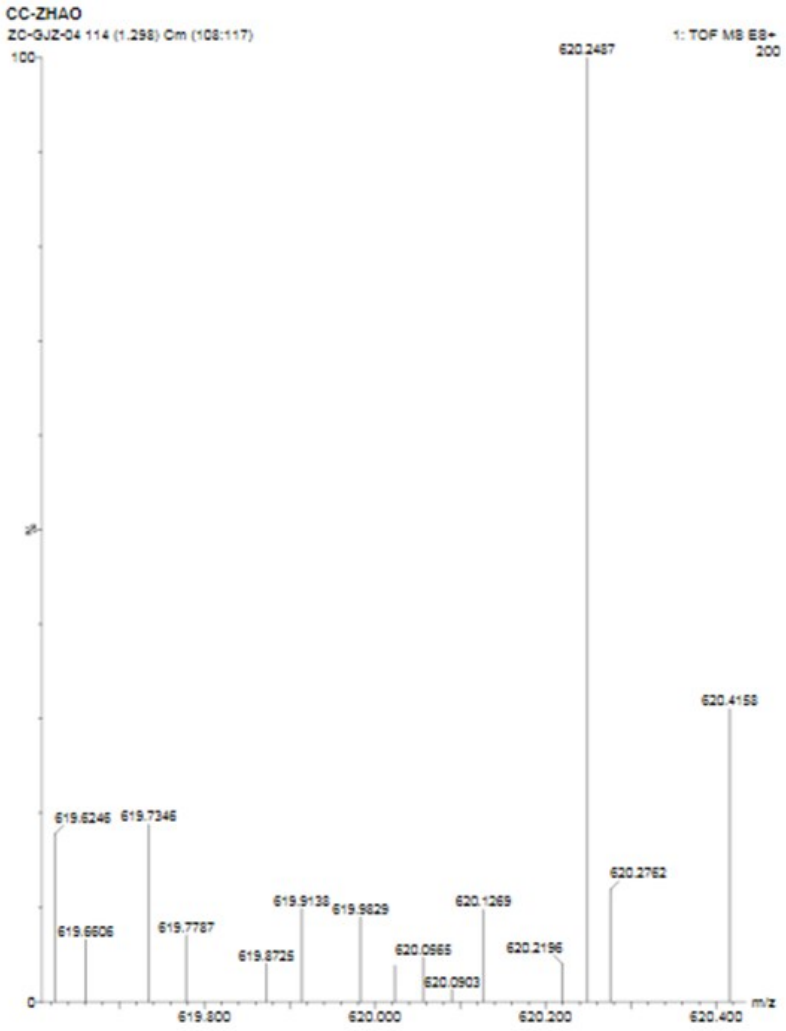
HRMS spectrum for compound SBOD-2.



^{1}H NMR spectrum for compound SBOD-3



^{13}C NMR spectrum for compound SBOD-3



HRMS spectrum for compound SBOD-3.

Notes and references

1 C. Zhao, Y. Zhang, P. Feng, J. Cao, *Dalton Trans.*, 2012, **41**, 831.

2 C. Zhao, J. Zhang, X. Wang, Y. Zhang, *Org. Biomol. Chem.*, 2013, **11**, 372.