Activatable Near-Infrared Emission-Guided On-Demand Administration of Photodynamic Anticancer Therapy with a Theranostic Nanoprobe

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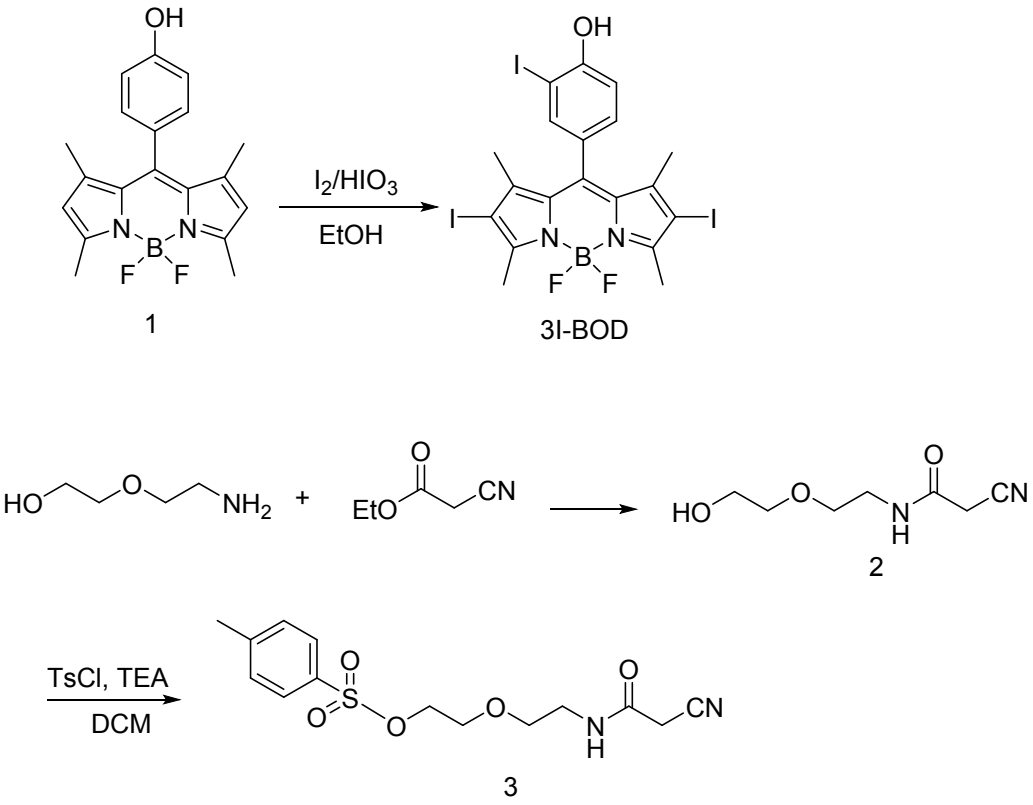
1. Synthesis.



Scheme S1. Synthesis of the requisite intermediates.

Synthesis of compound 3I-BOD. To a solution of Compound 1 (100 mg, 0.29 mmol) and I_2 (186.5 mg, 0.73 mmol) was added dropwisely HIO_3 (100 mg, 0.57 mmol) in 1 mL H_2O. The mixture was heated to 60 ^{0}C and the reaction was monitored by TLC. After completion, saturated NaS_2O_3 was added to quench the reaction, followed by extraction with CH_2Cl_2. Purification by silica gel flash chromatography gave compound 3I-BOD (55 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.57 (s, 1H), 7.14 (d, 2H), 2.64 (s, 6H), 1.50 (s, 6H). HRMS (ESI, m/z): calculated for $C_{19}H_{15}N_2OF_2I_3[M-H]^-$: 716.8380, found: 716.8384.

Synthesis of compound 2. The solution of Ethyl cyanoacetate (10.2 g, 90 mmol), and 2-(2-Aminoethoxy)ethanol (9.45 g, 90 mmol) in ethanol was refuxed for 3 h. The solvent was removed and the crude product was washed with ether to afford 2 (12.5 g, 81%) which was used for next

reaction without further purification.

Synthesis of compound 3. To a solution of compound 2 (1 g, 5.8 mmol) in CH_2Cl_2 (25 mL) was added 4-toluene sulfochloride (1.3 g, 6.8 mmol), followed by addition of 2 mL Et_3N. The resulted mixture was stirred for 4 h at room temperature. Purification by silica gel flash chromatography gave compound 3 (600 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) : δ= 7.81-7.79 (d, 2H), 7.38-7.36 (d, 2H), 4.24-4.22 (t, 2H), 3.71-3.69 (t, 2H), 3.60-3.57(t, 2H), 3.51-3.47 (q, 2H), 3.42 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ= 161.54, 145.20, 132.93, 129.99, 127.80, 125.02, 114.71, 69.32, 69.30, 68.50, 39.85, 25.95, 21.69. HRMS (ESI, m/z): calculated for $C_{14}H_{18}N_2O_5SNa$ $[M+Na]^+$: 349.0834, found: 349.0821.

Synthesis of compound 4. To a solution of compound 3 (95 mg, 0.29 mmol) and 3I-BOD (100 mg, 0.14 mmol) in acetone (25 mL) was added K_2CO_3 (39 mg, 0.28 mmol). The reaction mixture was refluxed for 8 h, followed by removing the solvent, extraction with EtOAc, and washing with H_2O. Purification by silica gel flash chromatography afforded compound 4 (60 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) : δ= 7.69 (d, 1H), 7.21-7.19 (dd, 1H), 6.95-6.93 (d, 1H), 4.26-4.24 (t, 2H), 3.99-3.97 (t, 2H), 3.82-3.80 (t, 2H), 3.62-3.58 (q, 2H), 3.40 (s, 2H),2.64 (s, 6H), 1.48 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ= 162.20, 158.10, 156.02, 144.73, 140.09, 138.03, 131.13, 129.44, 127.66, 116.20, 112.86, 112.84, 69.22, 68.78, 68.54, 31.11, 29.00, 25.26, 16.99, 15.73. HRMS (ESI, m/z): calculated for $C_{26}H_{26}BF_2I_3N_4O_3Na$ $[M+Na]^+$: 894.9098, found: 894.9105.

Synthesis of compound 6. Compound 4 (100 mg, 0.11 mmol) was dissolved in EtOH (20 mL), followed by addition of compound 5 (80 mg, 0.17 mmol) and piperidine. The reaction was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with EtOAc, washed with water, and dried over Na_2SO_4, purified by silica gel flash chromatography to afford

compound 6 (70 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ= 8.43 (s, 1H), 7.64 (d, 1H), 7.52-7.50 (m, 3H), 7.36-7.33 (m, 2H), 7.29-7.27 (d, 2H), 7.17-7.14 (dd, 1H), 7.12 (s, 1H), 7.06-7.04 (d, 2H), 6.92-6.90 (d, 1H), 4.23-4.20 (t, 2H), 3.95-3.92 (t, 2H), 3.79-3.76 (t, 2H), 3.63-3.59 (q, 2H), 2.69 (s, 3H), 2.64 (s, 6H), 2.41-2.36 (q, 2H), 2.26 (s, 3H), 1.49 (s, 3H), 1.45 (s, 6H), 1.06-1.02 (t, 3H) . ^{13}C NMR (101 MHz, $CDCl_3$) δ= 168.34, 160.66, 158.32, 156.66, 145.29, 144.43, 143.65, 138.63, 137.08, 132.74, 132.24, 131.50, 130.06, 129.94, 129.10, 128.82, 128.68, 126.39, 122.07, 117.45, 112.31, 100.37, 87.13, 70.21, 69.22, 69.10, 40.08, 31.93, 29.78, 29.35, 27.23, 25.55, 22.72, 21.13, 17.55, 17.25, 16.08, 14.17, 14.02, 12.61. HRMS (ESI, m/z): calculated for $C_{53}H_{49}B_2F_4I_3N_6O_3SNa$ $[M+Na]^+$: 1351.0741, found: 1351.0724.

Synthesis of compound TNP-SO. Compound 6 (80 mg, 0.06 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and cooled to 0 ^{0}C. m-CPBA (15 mg, 0.08mmol) was then added to the aforementioned solution which was further stirred for 3 h. Then saturated K_2CO_3 was added to quench the reaction. The mixture was washed with water, dried over Na_2SO_4. Purification by silica gel flash chromatography gave compound TNP-SO (45 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) : δ= 8.90 (s, 1H), 7.80-7.78 (d, 2H), 7.64-7.63 (d, 1H), 7.52-7.46 (m, 3H), 7.45-7.29 (m, 4H), 7.17-7.14 (dd, 1H), 7.04 (s, 1H), 6.93-6.91 (d, 1H), 4.22 (s, 2H), 3.95 (s, 2H), 3.79 (s, 2H), 3.63-3.59 (d, 2H), 2.74 (s, 3H), 2.64 (s, 6H), 2.44-2.38 (q, 2H), 2.36 (s, 3H), 1.51 (s, 3H), 1.45-1.43 (d, 6H), 1.09-1.05 (t, 3H) . ^{13}C NMR(101 MHz, CDCl3) δ= 175.34, 160.52, 158.45, 145.29, 142.40, 141.09, 140.35, 138.63, 132.41, 131.50, 130.24, 130.14, 129.10, 128.96, 128.89, 128.81, 128.68, 128.45, 124.48, 117.21, 112.33, 102.33, 87.07, 70.13, 69.13, 57.22, 40.06, 35.92, 31.86, 29.53, 29.22, 27.83, 27.22, 26.60, 25.53, 22.67, 17.44, 17.23, 16.03, 14.11, 13.90, 12.72. HRMS (ESI, m/z): calculated for $C_{53}H_{49}B_2F_4I_3N_6O_4SNa$ $[M+Na]^+$: 1367.0690, found: 1367.0688.

2. Cells culture and imaging.

Human colorectal cancer HCT116 cells and/or human hepatocellular liver carcinoma cells (HepG2 cells) were cultured at 37 °C in a humidified atmosphere of 5/95 CO_2/air incubator within Dulbecco's Eagle Medium (DMEM) which was supplemented with 10% fetal bovine serum (FBS). These cells were seeded in glass bottom dishes and allowed to adhere for 24 h prior to experiments. For visualization of cellular H_2S, HCT116 cells or HepG2 cells were loaded with Nano-TNP-SO for 1 h. For assay of H_2S generation by the inhibitor and activator: (1) cells pretreated with 1 mM aminooxyacetic acid (AOAA) for 1h were loaded with Nano-TNP-SO for 1 h; (2) cells were treated with S-adenosyl-L-methionine (SAM) (3mM) for 1 h, then were stained with Nano-TNP-SO for 1 h. The confocal imaging was performed using Nikor AIR with a 60 × oil objective. The excitation wavelength was 561 nm, the emission collected at 680-750 nm.

For examination of the cytotoxicity of Nano-TNP-SO toward HCT116 cells, HCT116 cells were incubated with various concentrations of Nano-TNP-SO and then exposed to 530 nm light (100 mW/cm^2) for 10 min, and MTT assay was then performed.

Staining experiments with calcein acetoxymethyl ester/propidium iodide (calcein-AM/PI), four groups HCT116 cells were cultured and treated separately: 1) untreated cells; 2) cells were incubated with Nano-TNP-SO (TNP-SO 5 μM) for 1 h; 3) cells were incubated with Nano-TNP-SO (TNP-SO 5 μM) for 1 h and then exposed to 530 nm light (100 mW/cm^2) for 10 min; 4) cells were exposed to 530 nm light (100 mW/cm^2) for 10 min. All the four group of cells were stained with Calcein-AM (2 μM) and PI (4.5 μM) for 20 min at 37 ^{0}C. The confocal imaging was performed. The excitation wavelengths for Calcein-AM and PI were 488 nm and 561 nm, respectively. And the emission was collected between 490-540 nm and 592-642 nm for Calcein-AM and PI, respectively.

3. In vivo imaging.

All animal experiments were performed in compliance with the relevant laws and institutional guidelines for the Care and Use of Research Animals established by East China University of Science and Technology's Animal Studies Committee, and the experiments were approved by the committee.

The tumor regions and normal site of HCT116 tumor-bearing mice were intravenous injection of Nano-TNP-SO (25 nmol TNP-SO) in PBS at a total volume of 100 μL. Fluorescent images for mice anesthetized with isoflurane were taken at various time points after subcutaneous injection of Nano-TNP-SO into tumor and normal regions.

4. In Vivo PDT.

HCT116 tumor-bearing mice were divided into four groups (4 mice per group): 1) control group (no treatment), 2) mice with only Nano-TNP-SO injection into tumors (25 nmol TNP-SO), 3) mice with only light irradiation, 4) mice with both Nano-TNP-SO injection and irradiation. For PDT, the tumor sites were irradiated with 530 nm light for 30 min. After PDT treatment, the tumor size and body weight were monitored for 10 days. Finally, tumors and major organs (heart, liver, spleen, lung, and kidney) in different groups were collected and sectioned for H&E staining.

5. HRMS analysis.

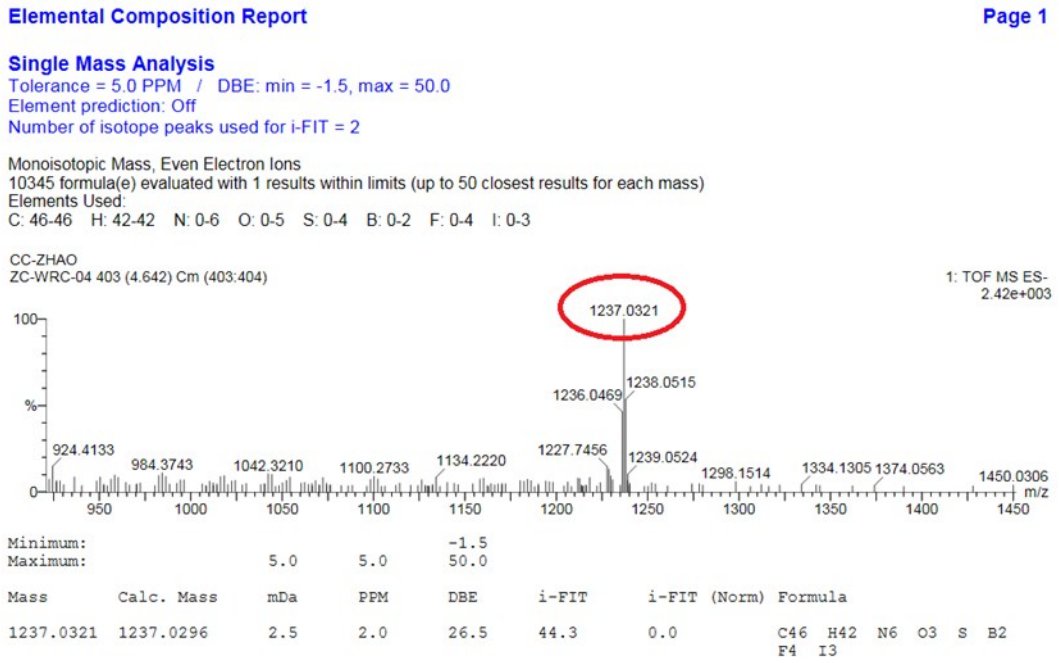


Figure S1. HRMS analysis for demonstration of the conversion of TNP-SO to TNP-HS in the presence of H_2S.

6. The photosensitizing generation of $^1O_2$ induced by TNP-SO.

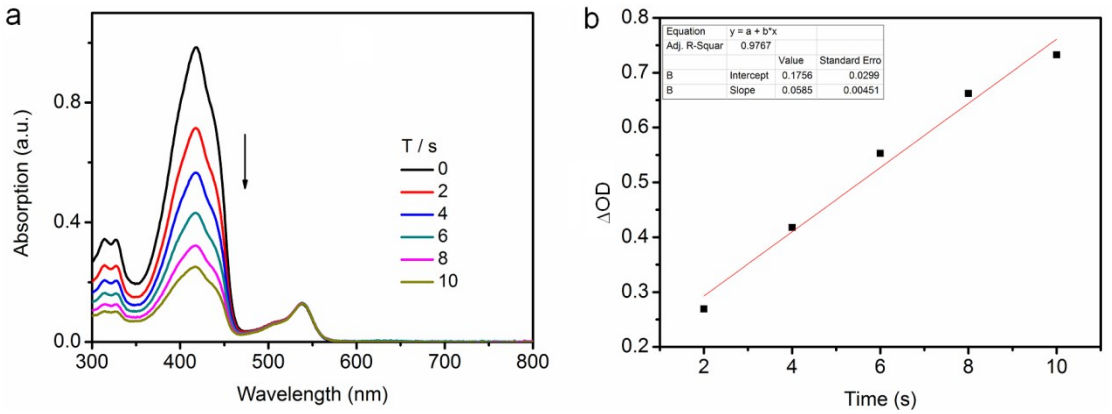


Figure S2. (a) Time-dependent absorption spectra and (b) optical density (OD) changes of DPBF (40 μM) at 418 nm upon 530 nm light irradiation of TNP-SO (2μM) in DMSO solution.

7. The characterization of Nano-TNP-SO.

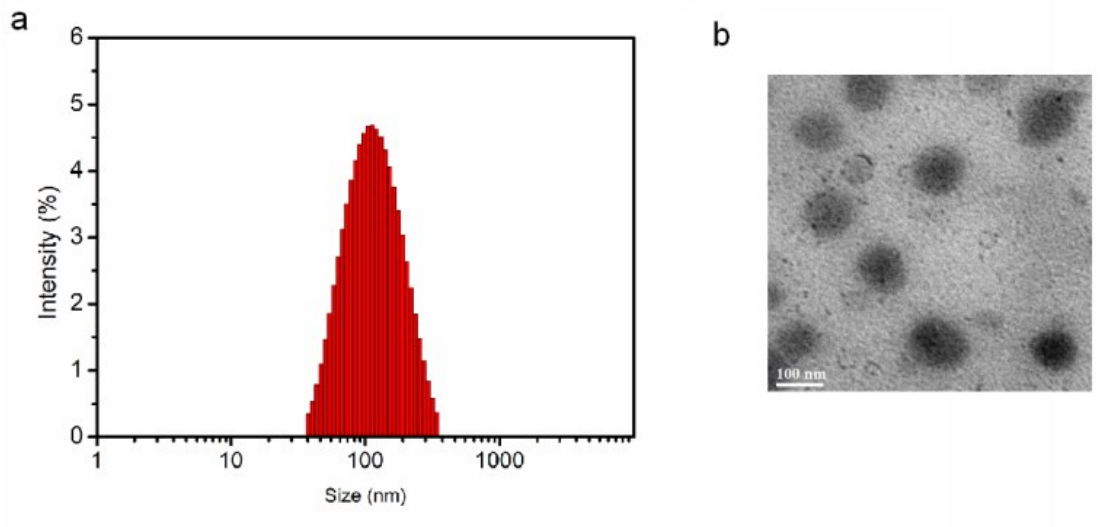


Figure S3. The characterization of Nano-TNP-SO by (a) dynamic light scattering and (b) TEM analyses.

8. The photosensitizing generation of $^1O_2$ induced by Nano-TNP-SO.

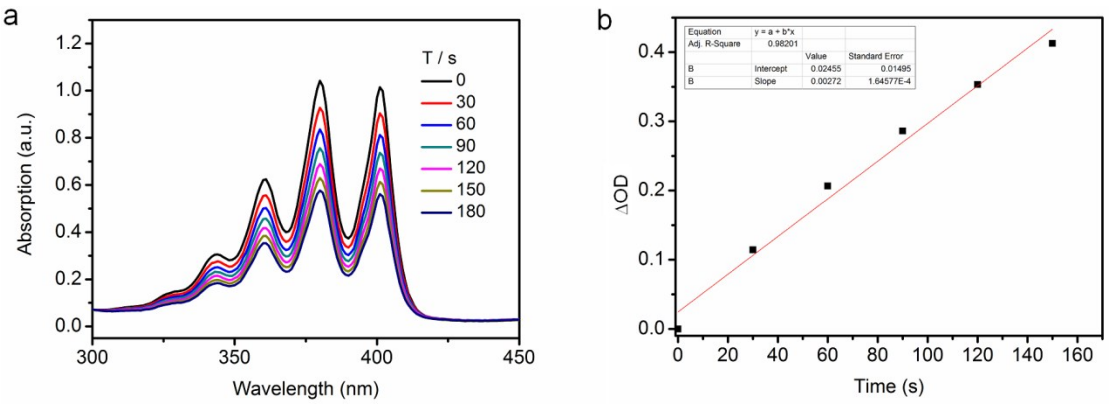


Figure S4. (a) Time-dependent absorption spectra and (b) Optical density (OD) changes of ABDA (50 μM) at 308 nm upon 530 nm light irradiation of Nano-TNP-SO (2μM) in PBS solution.

9. The photostability of Nano-TNP-SO.

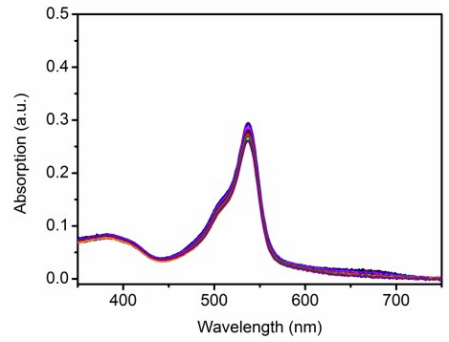


Figure S5. The absorption changes of Nano-TNP-SO under continuous irradiation with an Hg/Xe lamp (Hamamatsu, LC8 Lightningcure, 300 mW).

10. Costaining experiments.

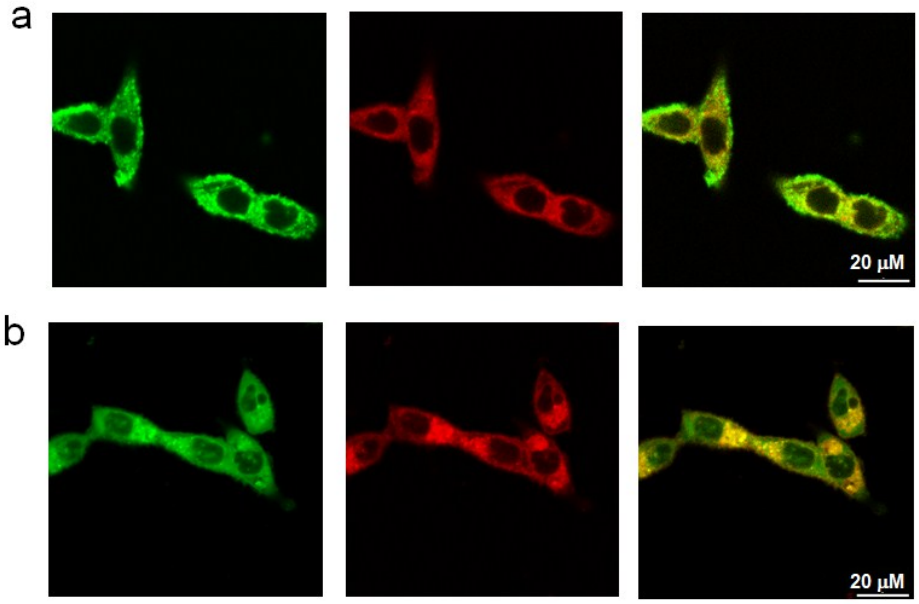


Figure S6. Intracellular localization of Nano-TNP-SO with confocal fluorescence images in HCT116 cells. Cells were stained with Nano-TNP-SO for 1 h and then co-cultured with (a) 5 μM Mito-Tracker Green, (b) 5 μM Lyso-Tracker Green for 5 min.

11. NIR fluorescence visualization of HCT116 tumor-bearing mouse.

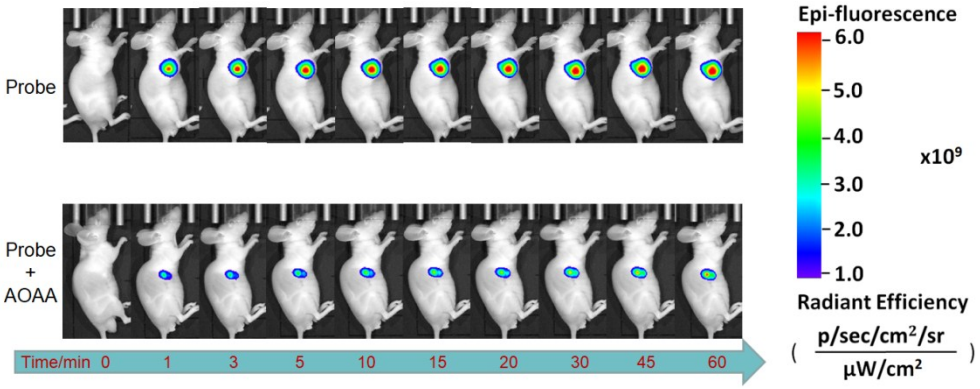


Figure S7. NIR fluorescence visualization of HCT116 tumor-bearing mouse. Fluorescent images were taken at various time points after subcutaneous injection of Nano-TNP-SO (25 nmol TNP-SO) into tumor regions. (a) Nano-TNP-SO treated mice. (b) Mice pretreated with 100 nmol AOAA for 4 h was administrated with Nano-TNP-SO.

12. NMR and HRMS characterization.

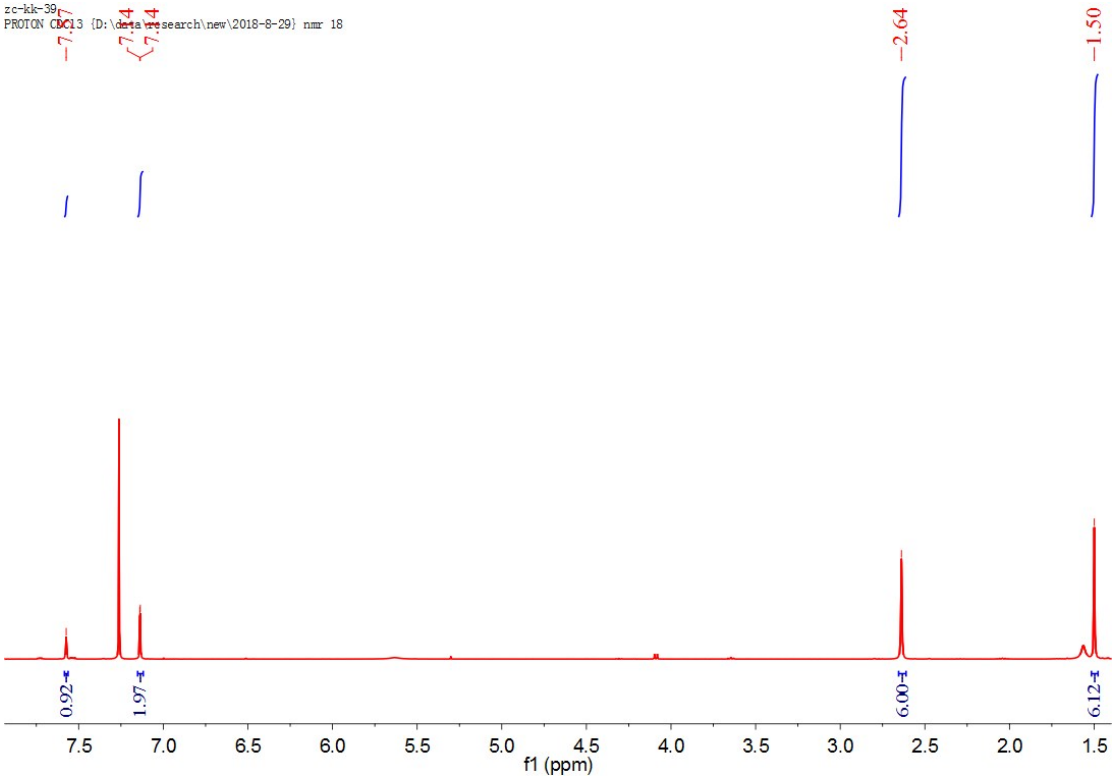


Figure S8. ^{1}H NMR spectrum of compound 3I-BOD.

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
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Elements Used:
C: 19-19 H: 0-20 B: 0-1 N: 0-2 O: 0-1 F: 0-2 127I: 0-3

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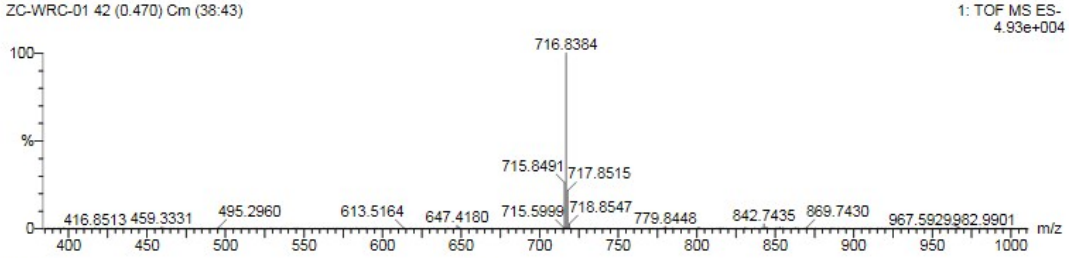
ZC-WRC-01 42 (0.470) Cm (38:43)



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Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
716.8384	716.8380	0.4	0.6	11.5	147.2	0.0	C19 H15 B N2 O F2 127I3

Figure S9. HRMS of compound 3I-BOD.

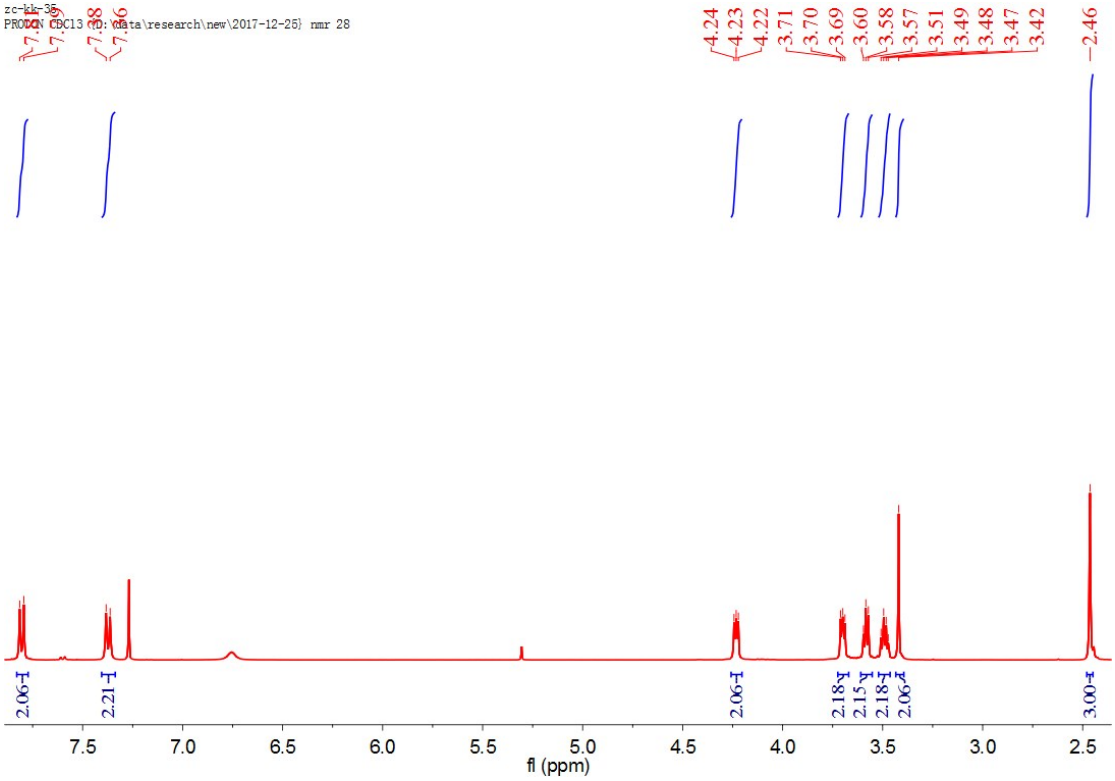

Figure S10. ^{1}H NMR spectrum of compound 3.

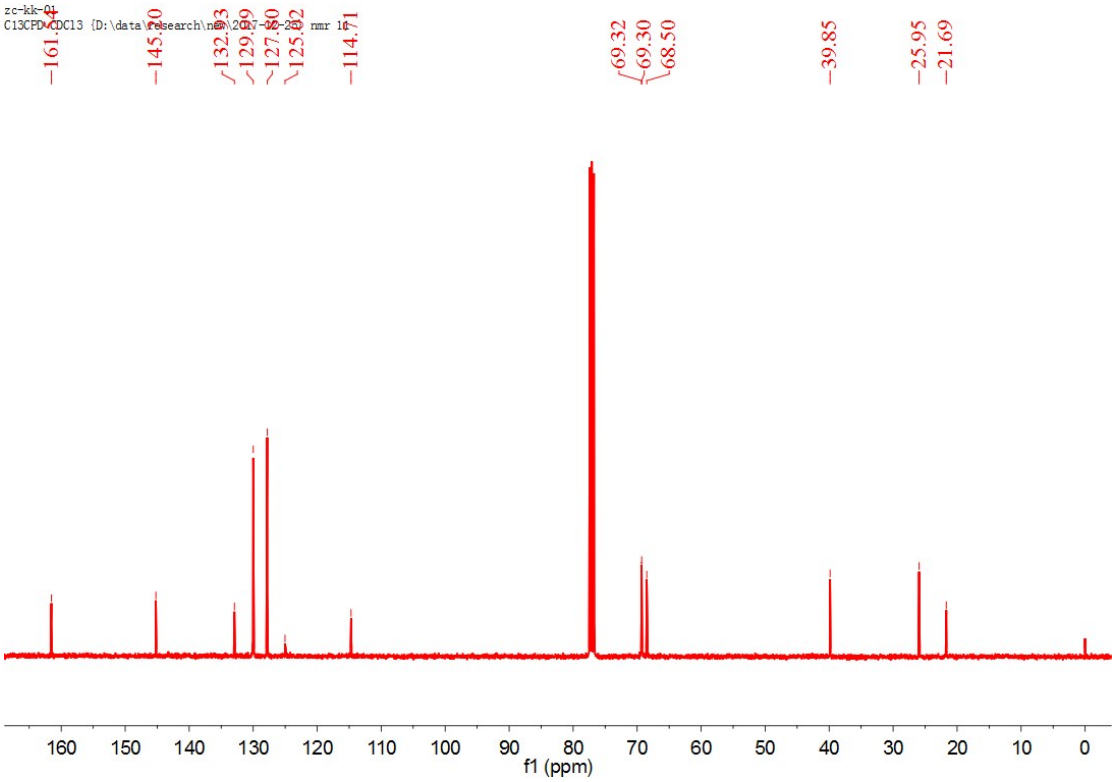

Figure S11. ^{13}C NMR spectrum of compound 3.

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
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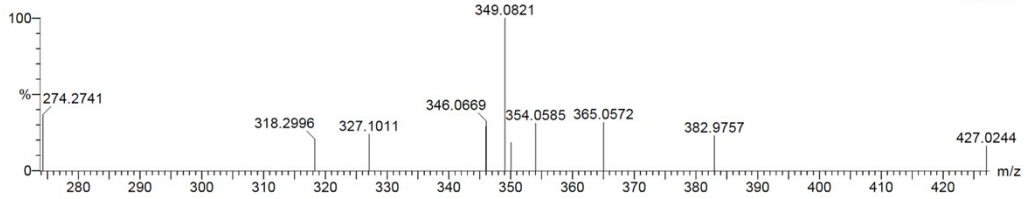

Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
349.0821	349.0834	-1.3	-3.7	6.5	6.6	0.0	C14 H18 N2 O5 S Na

Figure S12. HRMS of compound 3.

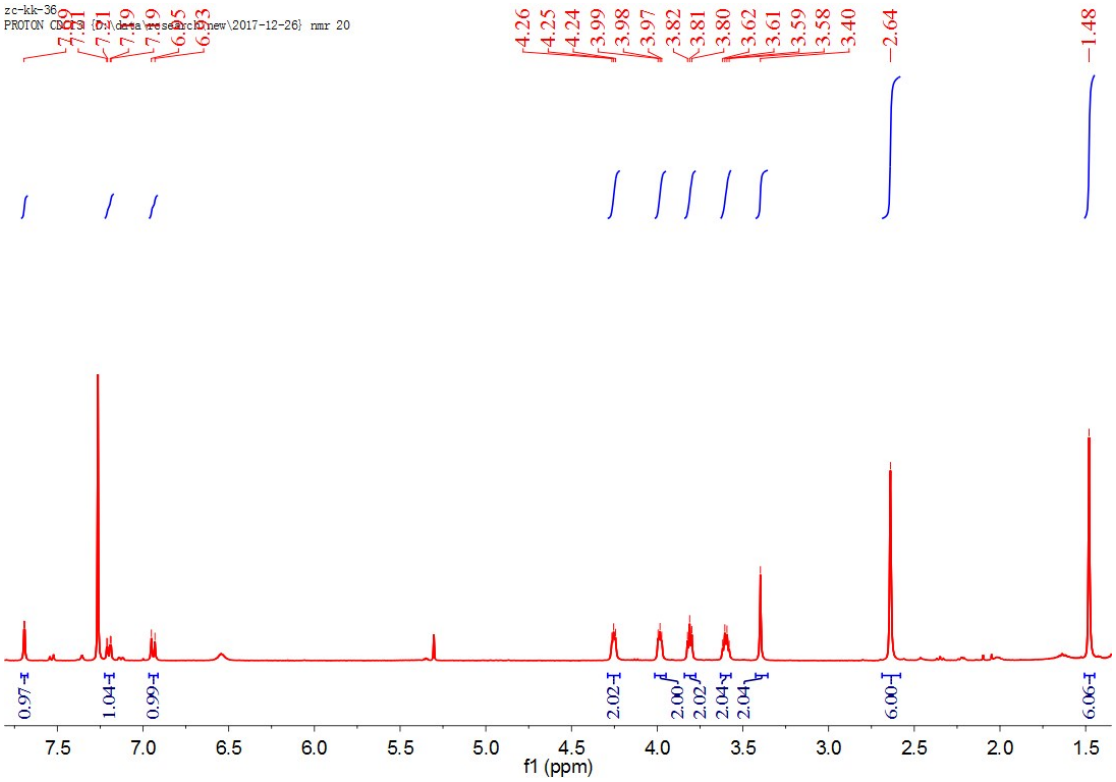

Figure S13. ^{1}H NMR spectrum of compound 4.

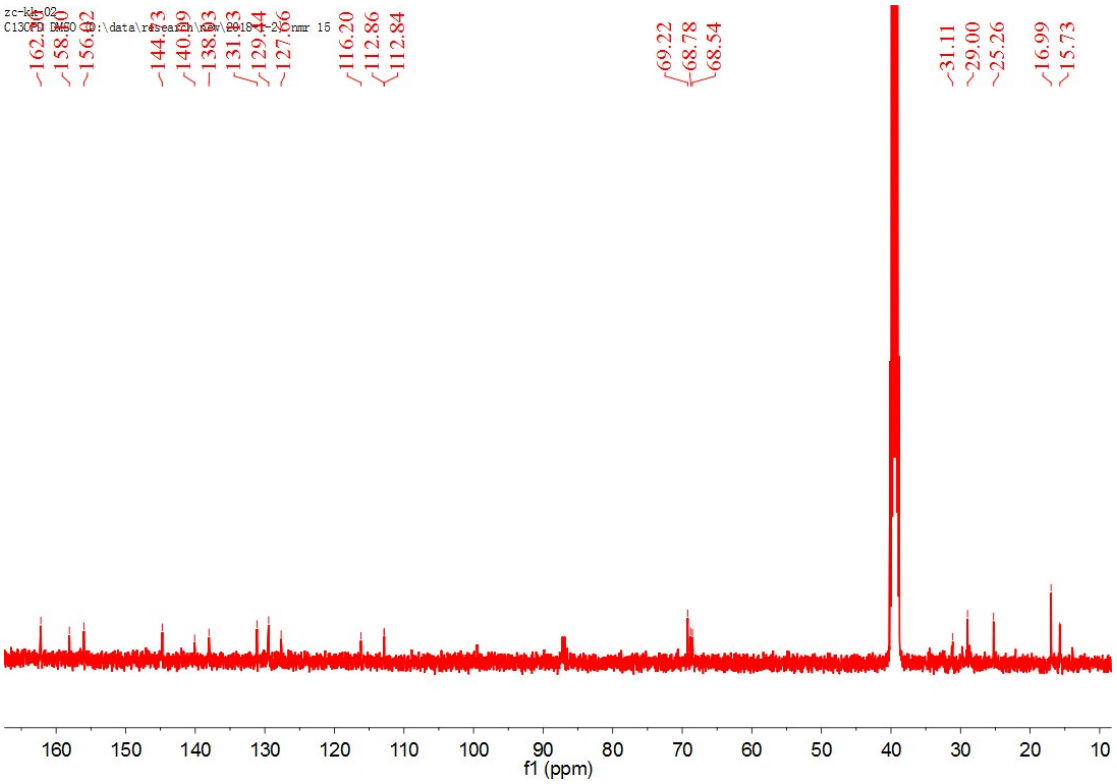

Figure S14. ^{13}C NMR spectrum of compound 4.

Elemental Composition Report **Page 1**

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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
944 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-26 H: 0-26 B: 0-1 N: 0-4 O: 0-3 F: 0-2 Na: 0-1 I: 0-3

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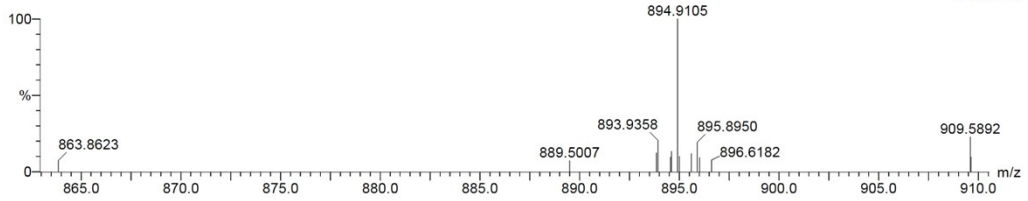

Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
894.9105	894.9098	0.7	0.8	13.5	37.9	0.0	C26 H26 B N4 O3 F2 Na I3

Figure S15. HRMS of compound 4.

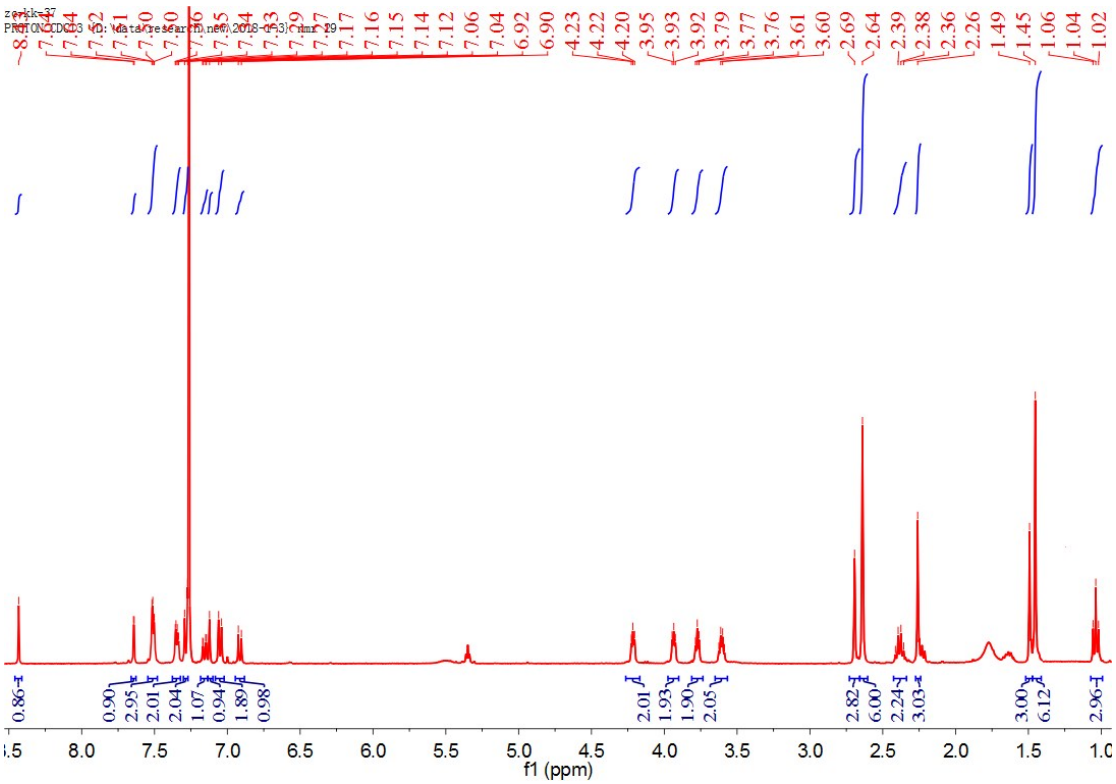

Figure S16. ^{1}H NMR spectrum of compound 6.

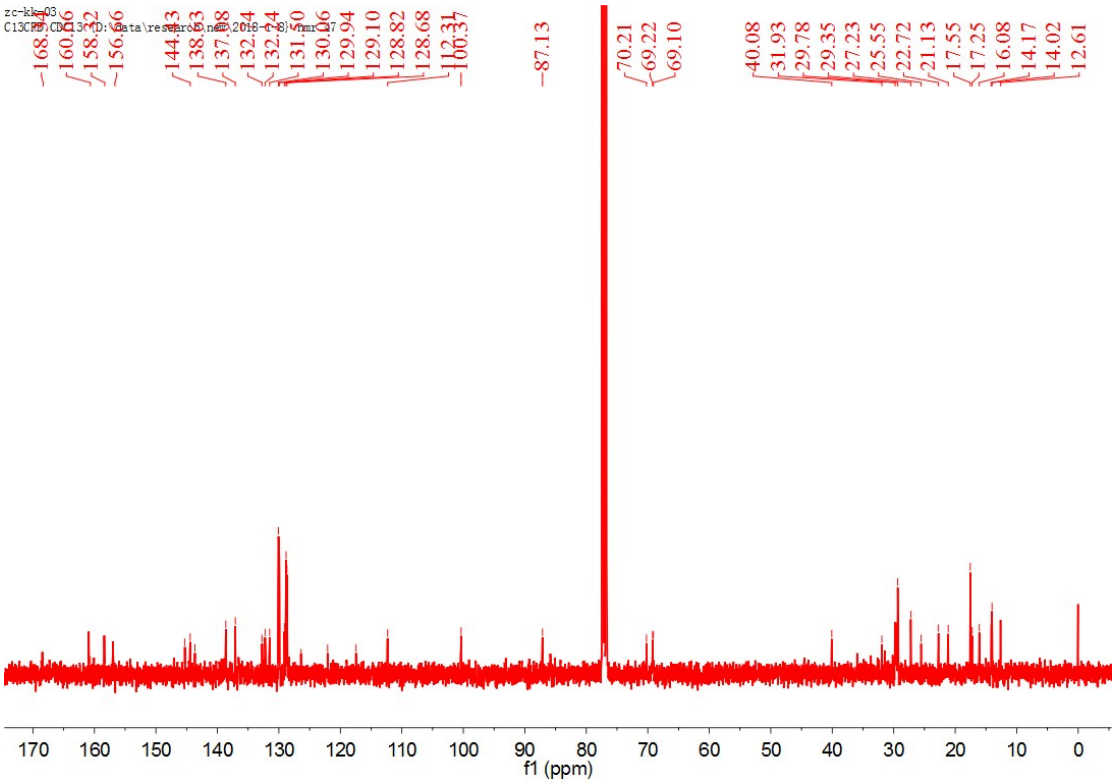

Figure S17. ^{13}C NMR spectrum of compound 6.

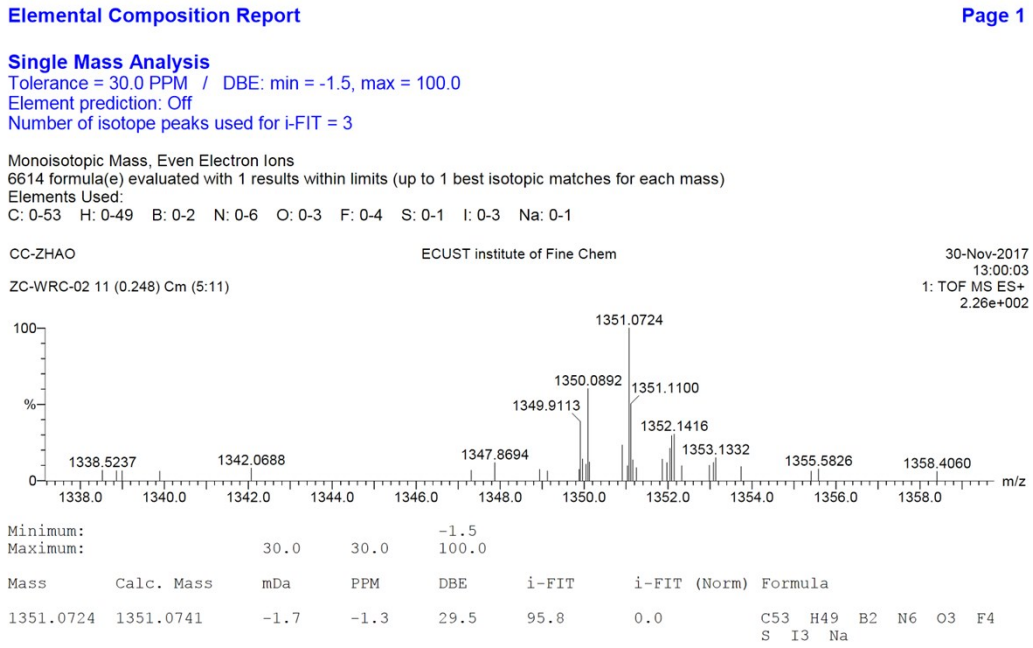

Figure S18. HRMS of compound 6.

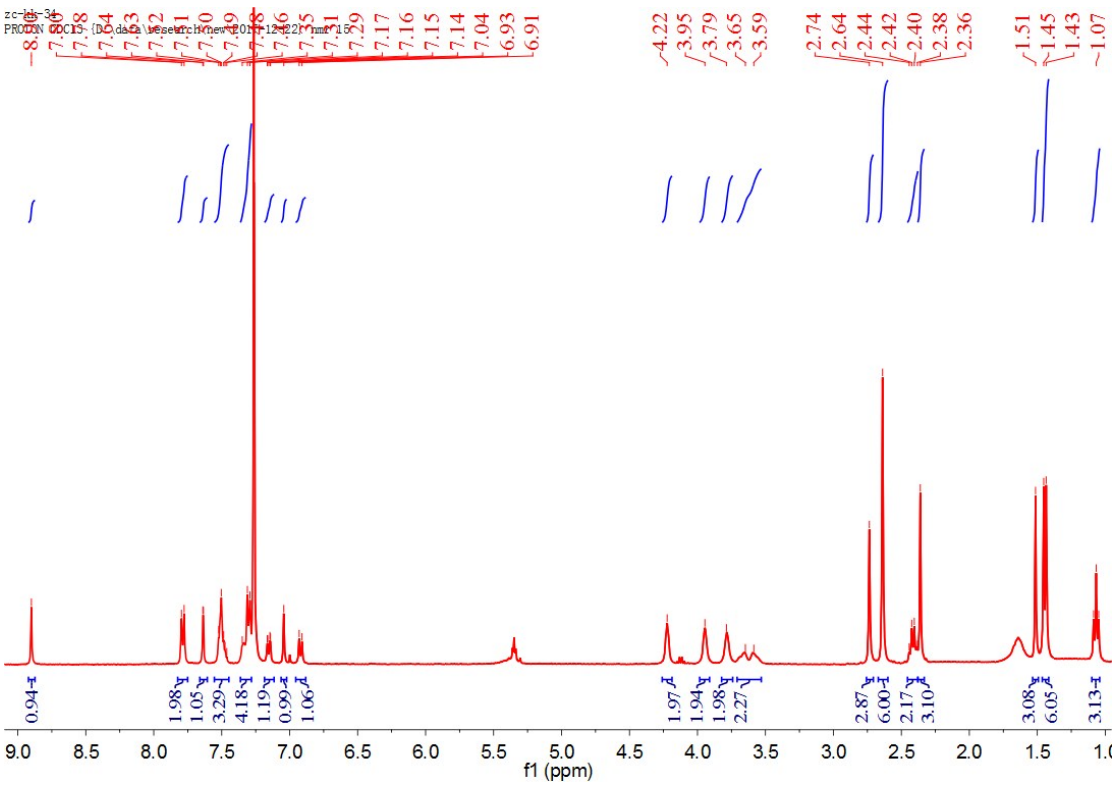

Figure S19. ^{1}H NMR spectrum of compound TNP-SO.

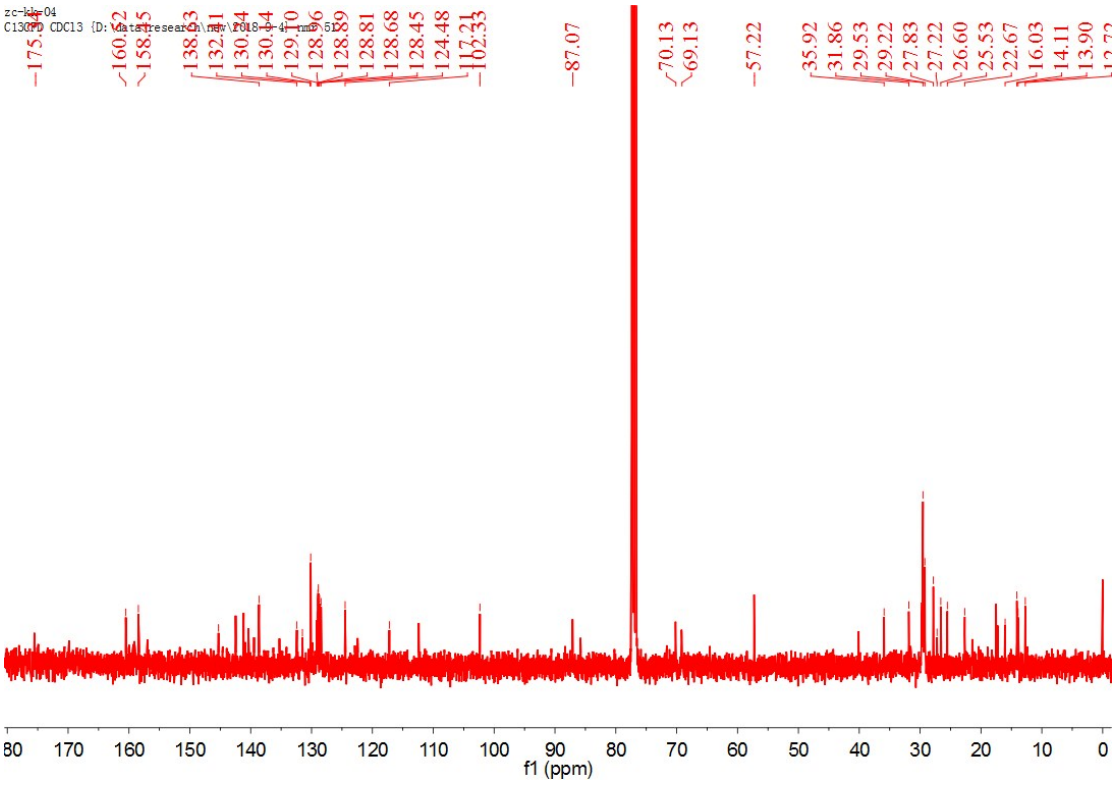

Figure S20. ^{13}C NMR spectrum of compound TNP-SO.

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
8293 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-53 H: 0-49 B: 0-2 N: 0-6 O: 0-4 F: 0-4 Na: 0-1 S: 0-1 I: 0-3

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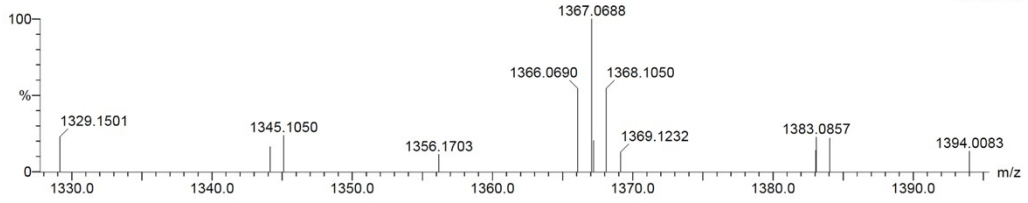

Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1367.0688	1367.0690	-0.2	-0.1	29.5	29.5	0.0	C53 H49 B2 N6 O4 F4 Na S I3

Figure S21. HRMS of compound TNP-SO.