Supporting Information

NIR-II imaging-guided diagnosis and evaluation of therapeutic effect on acute alcoholic liver injury via a nanoprobe

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1. Materials and instrumentation

All commercial chemicals are analytical reagent grade and can be used without further purification after being purchased from Bide Chemical Reagent Co., Ltd. (China). All reactions were carried out in 100 mL round-bottom flasks with air condensers. Unless otherwise noted, all commercial reagents and solvents were obtained from the commercial provider and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 600 MHz and 400 MHz spectrometers. Chemical shifts were reported relative to internal tetramethylsilane (TMS) (0.00 ppm) or $CDCl_3$ (7.26 ppm) for ^{1}H-NMR, $CDCl_3$ (77.0 ppm) for ^{13}C-NMR. High resolution mass spectra were measured on a BrukerDaltonics SolanX 7.0T FTMS spectrometer. The UV spectra was given by UV/VIS (Jena，Specord 210) spectophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on a Quantamaster 8000 steady state transient modular fluorescence spectrometer (Horiba Co., Ltd, Canada). Transmission electron microscopy images of NTPB-NPs were obtained on a Tecnai G2 20 transmission electron microscope operating. Dynamic light scattering particle size of NTPB-NPs was recorded by an analyzer (Nanobrook Omni, Brookheaven, USA). The photostability tests of NTPB-NPs in water and PBS were recorded under continuous 808 nm laser excitation for 30 min at a power density of 0.5 W cm^{-2}. At the conclusion of the treatment period, blood was collected from the orbital sinus, and then centrifuged at 3500 rpm for 10 min to acquire the serum. ALT and AST levels in the serum were measured by different kits (Jiancheng Bioengineering Institute, Jiangsu, China). The liver tissues were fixed with 10 % formalin solution and embedded in paraffin. After fixation, the tissue sections were cut into 4 μm sections and stained with hematoxylin eosin (H&E). Photomicrographs were taken with a light microscope equipped with a camera (Olympus, Tokyo, Japan). The fluorescence intensity of the mice was detected by an 808 nm excitation laser and a 900 nm band filter. The images of the different groups were recorded by NIR-II small animal imaging system (Series III 900/1700, Suzhou Yingrui Optical Technology Co., Ltd, China)

Animal experiments: The animal experiments were approved by the Institutional Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology (permit number: SCXK (Hubei) 2017–0012). The animal care and experimental procedures were carried out in accordance with the Guidelines of the Institutional Animal Care and Use Committee of Tongji Medical College and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The male BALB/c (20 g-25 g) mice were adaptively fed for one week after arrival. Afterwards they were randomly divided into 3 groups of 6 individuals. The groups were treated as follows: Group 1 (NC): untreated control group; Group 2 (Model): Chinese spirits (3 g kg^{-1}); Group 3 (SILY): SILY (100 mg kg^{-1}) plus Chinese spirits (3 g kg^{-1}). The SILY groups were administered intragastrically with SILY in 2:30 pm for 14 consecutive days and other groups were administered intragastrically with distilled water. During 14 days, all groups were orally administered with Chinese spirits in 9:30 am and 9:30 pm except for NC group. The animals were anesthetized by isoflurane containing 20% O_2 in volume for 20 s in each group, and the temperature for all the experiments was kept at 25 °C.

Measurement of serum alanine aminotransferase (ALT), aspartate transaminase (AST): At the end of the treatment period, blood was collected from the orbital sinus, and then centrifuged at 3500 rpm for 10 min to acquire the serum. Serum ALT, AST, levels were measured by applying different kits (Jiancheng Bioengineering Institute, Jiangsu, China).

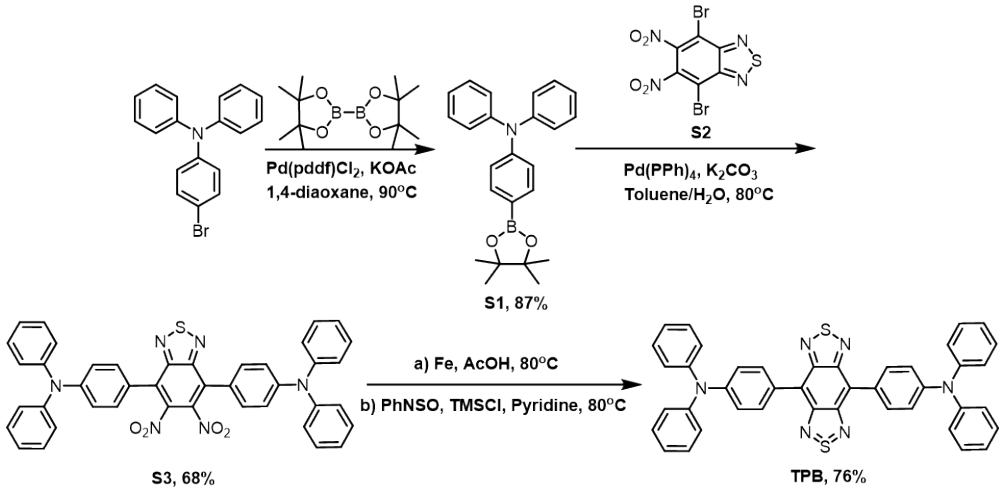
Biochemical analysis of BALB/c mice injected with NTPB-NPs: Blood was collected from the mouse orbit and centrifuged at 3500 rpm for 10 min to obtain serum. Blood biochemical analysis parameters were determined by different kits (Jiangsu Jiancheng Bioengineering Institute).

Histological assessment: The liver tissues were fixed with 10% formalin solution and embedded in paraffin. After fixation, the tissue sections were cut into 4 μm sections and stained with hematoxylin and eosin (H&E). Photomicrographs were taken with a light microscope equipped with a camera (Olympus, Tokyo, Japan).

Statistical analysis: Statistical analysis was performed using SPSS 19.0. All data are

expressed as mean ± SD. The normal distribution of the sample data was evaluated by the nonparametric Kolmogorov Smirnov test, and the differences between groups were analyzed by one-way ANOVA combined with the LSD test (assuming equal variance) or Dunnett's T3 test (assuming unequal variance). P values of less than 0.05, 0.01 were regarded as statistically significant.

2. Synthesis of NIR-II dye TPB



Scheme S1. The synthesis procedures of TPB

Synthesis of compound S1: To a solution of 4-bromotriphenylamine (2.033 mmol, 0.66 g), bis(pinacolato)diboron (2.438 mmol, 0.62 g), potassium acetate (6.095mmol, 0.598 g) in dioxane, were stirred at room temputrue. Degassing the reaction system, flushing argon and quickly adding ($Pd(dppf)Cl_2$) (0.2 mmol, 0.2 g). The reaction system was degassed for three times, filled with argon for protection, and reacted at 90 ℃ for 24 h. The reaction was monitored by TLC (thin layer chromatography).After the completion of the reaction, the mixture was cooled to room temperature. The solvent was evaporated under reduced pressure and the crude product was precipitated, which was further purified by washing repeatedly with an eluent of DCM/ petroleum ether (V:V = 1:1) [1]. Yield: 87 %; white solid; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.6 Hz, 2H), 7.25 (t, J = 7.8 Hz, 4H), 7.12 – 7.08 (m, 4H), 7.07 – 7.00 (m, 4H), 1.33 (s, 12H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 135.84 (s), 129.29 (s), 124.99 (s), 123.35 (s), 121.79 (s), 24.87 (s).

HRMS (ESI^+): m/z found $[M+H]^+$ 372.2132. molecular formula $C_{24}H_{27}BNO_2^+$, requires $[M+H]^+$ 372.2129

Synthesis of compound S3: S1 (870.30 mg, 2.34 mmol) and S2 (300 mg, 0.781 mmol) were dissolved in 2M potassium carbonate solution. and THF (20 mL). Degassing the reaction system, flushing argon and quickly adding ($Pd(PPh_3)_4$) (0.25 mmol, 30 mg). The reaction system was degassed for three times, filled with argon for protection, and reacted at 80 ℃ for 24 h. After cooling, the solvent was removed under reduced pressure to obtain crude product, further purification by column chromatography on silica gel using DCM/ petroleum ether (V:V = 1:3) as eluent [1]. Yield: 68 %; pink solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 8.8 Hz, 4H), 7.33 (t, J = 7.8 Hz, 8H), 7.21 (d, J = 7.6 Hz, 8H), 7.15-7.11 (m, 8H). HRMS (ESI^+): m/z found $[M+H]^+$ 713.1976. molecular formula $C_{42}H_{29}N_6O_4S^+$, requires $[M+H]^+$ 713.1966

Synthesis of compound TPB: Compound S3 (140 mg, 0.196 mmol) iron powder (1.96 mmol, 0.11 g) were dissolved in 10 mL acetic acid. The resulting mixture was stirred at 80 °C for 24 h. Then, the solution was extracted with DCM, washed with saturated $NaHCO_3$ solution twice and saturated NaCl solution once respectively and dried over sodium sulfate. Next, the solvent was removed under reduced pressure to obtain crude product, the resulting brown oil, N-thionylaniline (0.392 mmol, 55 mg) and chlorotrimethylsilane (1.96 mmol, 213 mg) were dissolved in anhydrous pyridine (10 mL). The reaction was kept 80 °C for 24 h under nitrogen atmosphere. After cooling, the solvent was removed under reduced pressure to obtain crude product, washing with water twice, methanol twice and DCM three times and the blue solid TPB was finally obtained [1]. Yield: 76 %.

^1H NMR (400 MHz, CD_2Cl_2) δ 8.19 (d, J = 8.8 Hz, 4H), 7.36 (t, J = 7.8 Hz, 8H), 7.29 – 7.18 (m, 12H), 7.13 (t, J = 7.4 Hz, 4H).

HRMS (ESI^+): m/z found $[M+H]^+$ 681.1881. molecular formula $C_{42}H_{29}N_6S_2^+$, requires

$[M+H]^+$ 681.1890

3. Free TPB in absorbance analysis

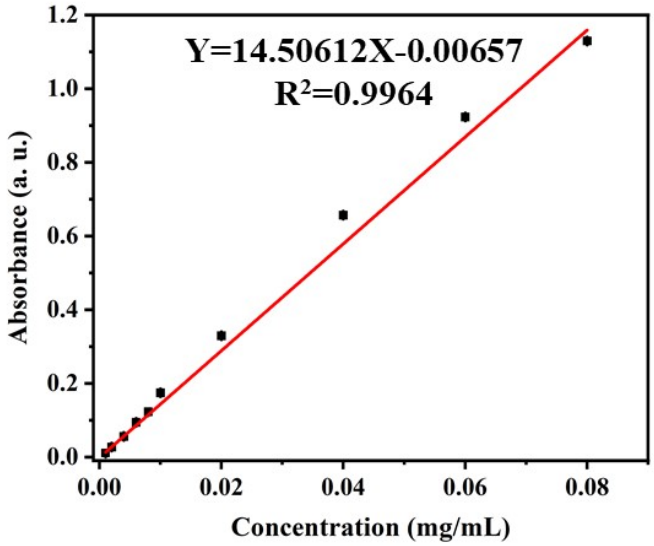


Fig S1. Free TPB calibration curve

4. Photostability of the NTPB-NPs

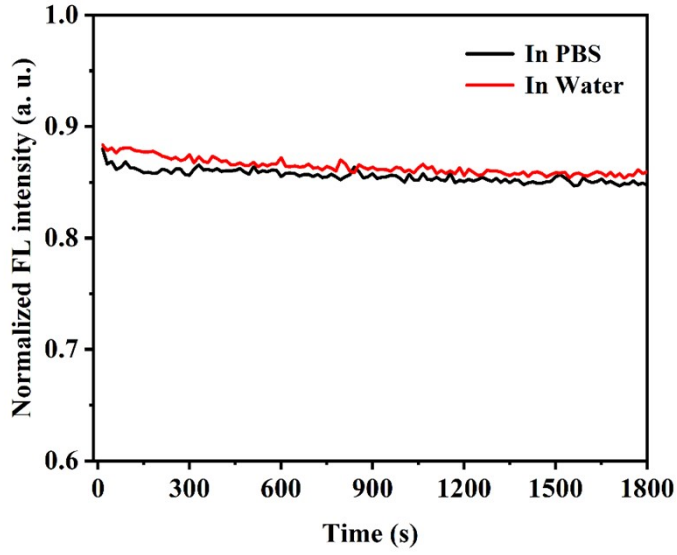


Fig S2. The photostability of the NTPB-NPs in different solution

5. Blood vessels imaging of BALB/c mice with NTPB-NPs

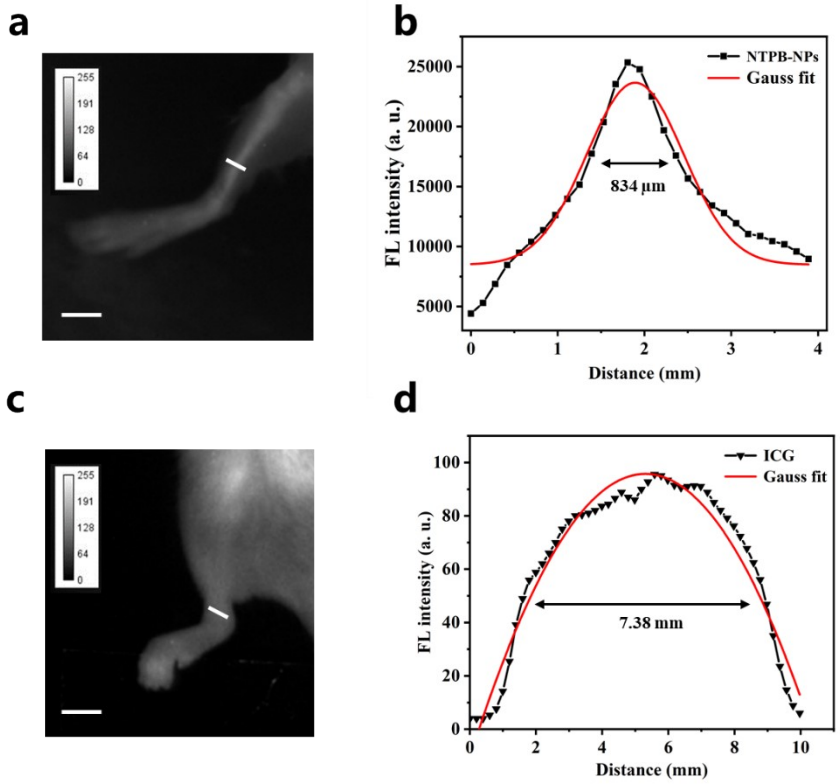


Fig S3. (a). NIR-II fluorescence images of normal BALB/c mice with NTPB-NPs; (b). Cross-sectional fluorescence intensity profile along the white line in (a); (c). NIR-I fluorescence images of normal BALB/c mice with ICG; (d). Cross-sectional fluorescence intensity profile along the white line in (c). The scale bar is 5 mm.

6. The fluorescence quantum yields of NTPB-NPs and ICG

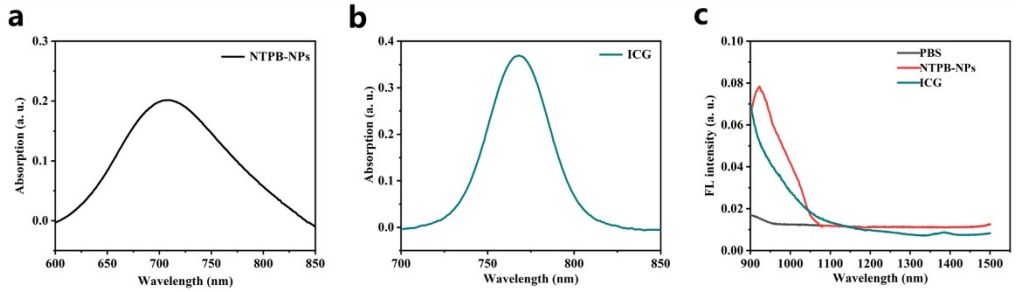


Fig S4. (a). Absorption spectra of NTPB-NPs in PBS; (b). Absorption spectra of ICG in PBS. (c). Fluorescence spectra of PBS, NTPB-NPs and ICG in 900-1500 nm.

7. In vivo NIR-II imaging of ICG and NTPB-NPs

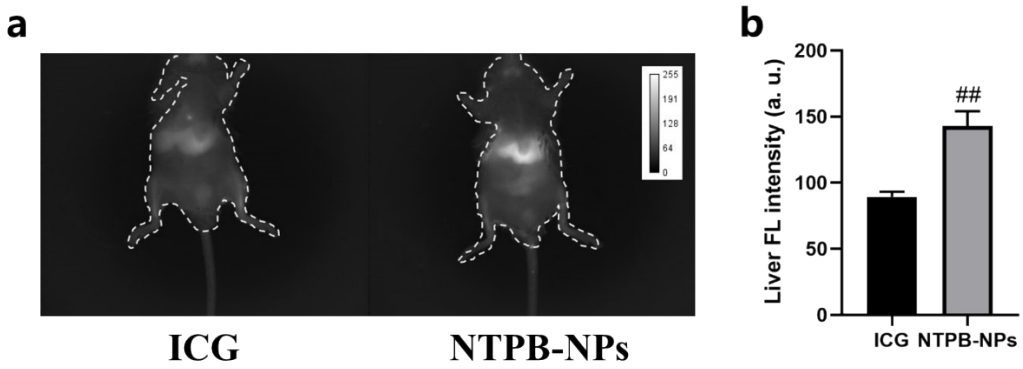
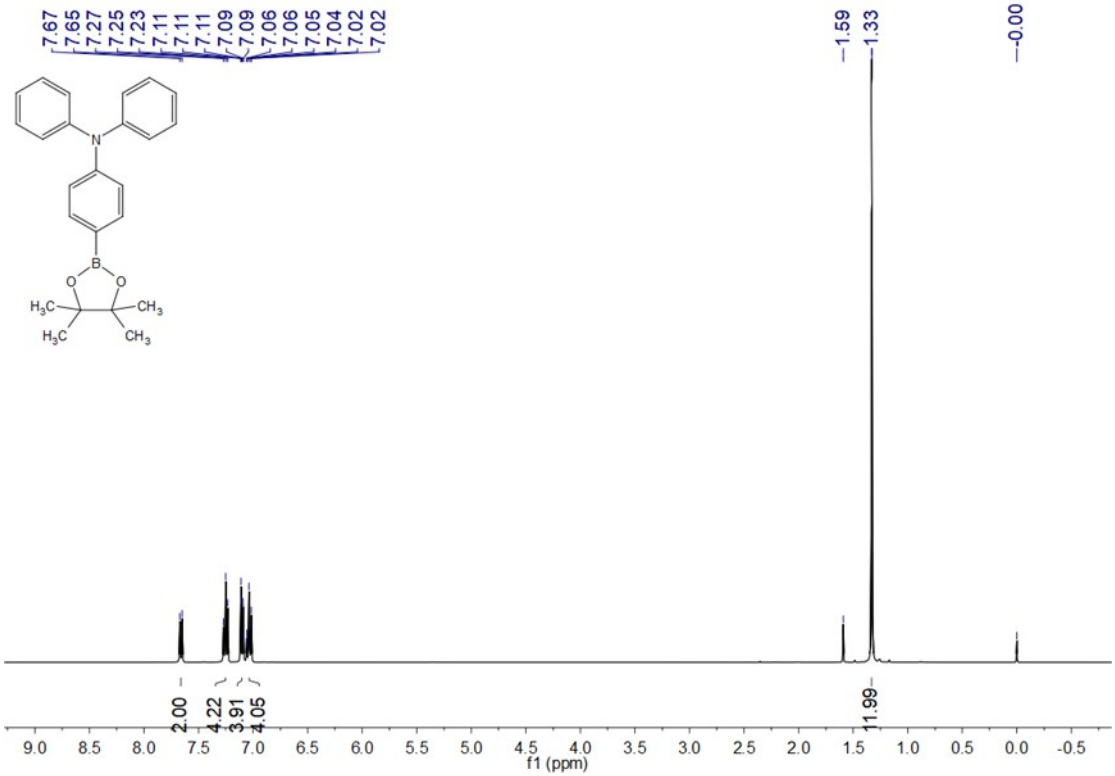


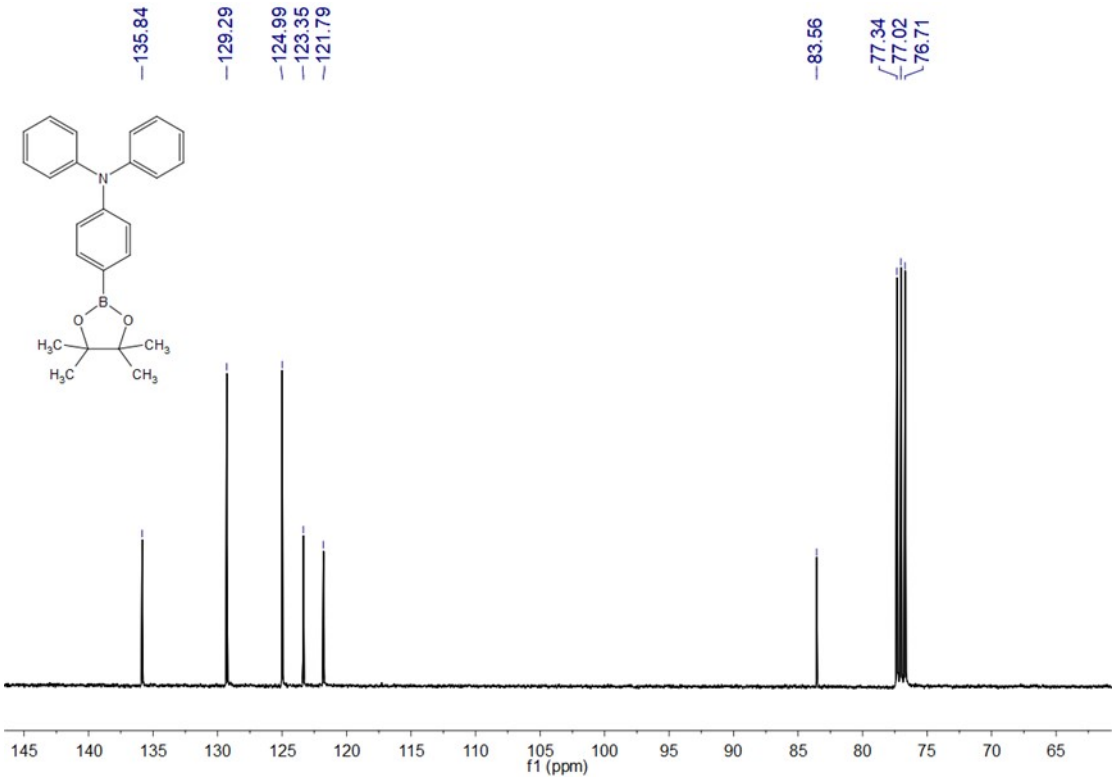
Fig S5. (a). NIR-II fluorescence images of normal BALB/c mice with ICG and NTPB-NPs; (b). In vivo NIR-II fluorescence intensity of livers in mice of (a). The values are given as the mean ± S.D. (n = 3). #p < 0.05, ##p < 0.01 vs. ICG group

8. The spectrums of compounds

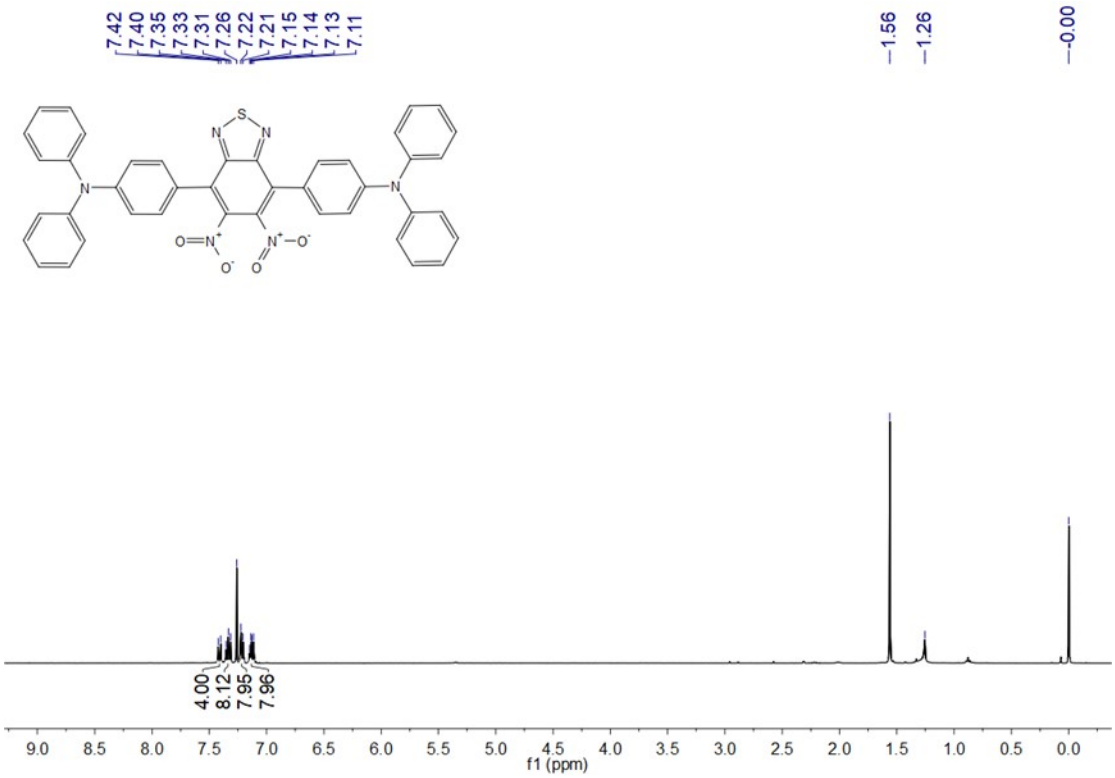
^{1}H NMR spectrum of S1



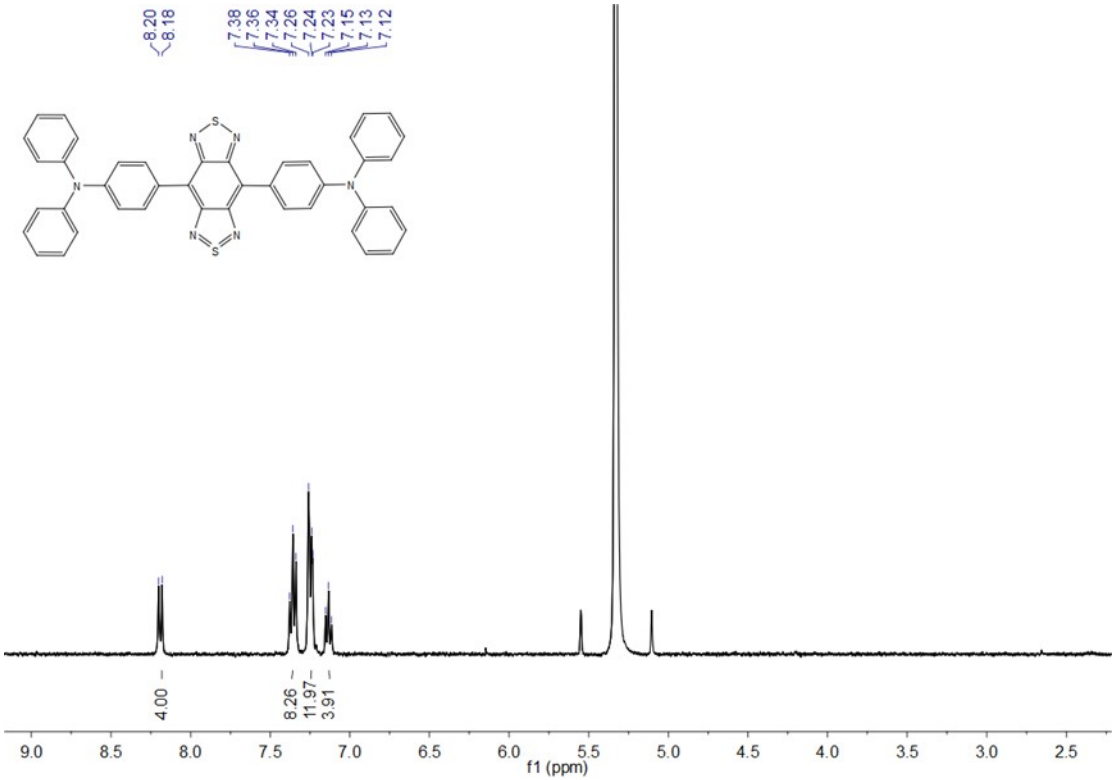
^{13}C NMR spectrum of S1



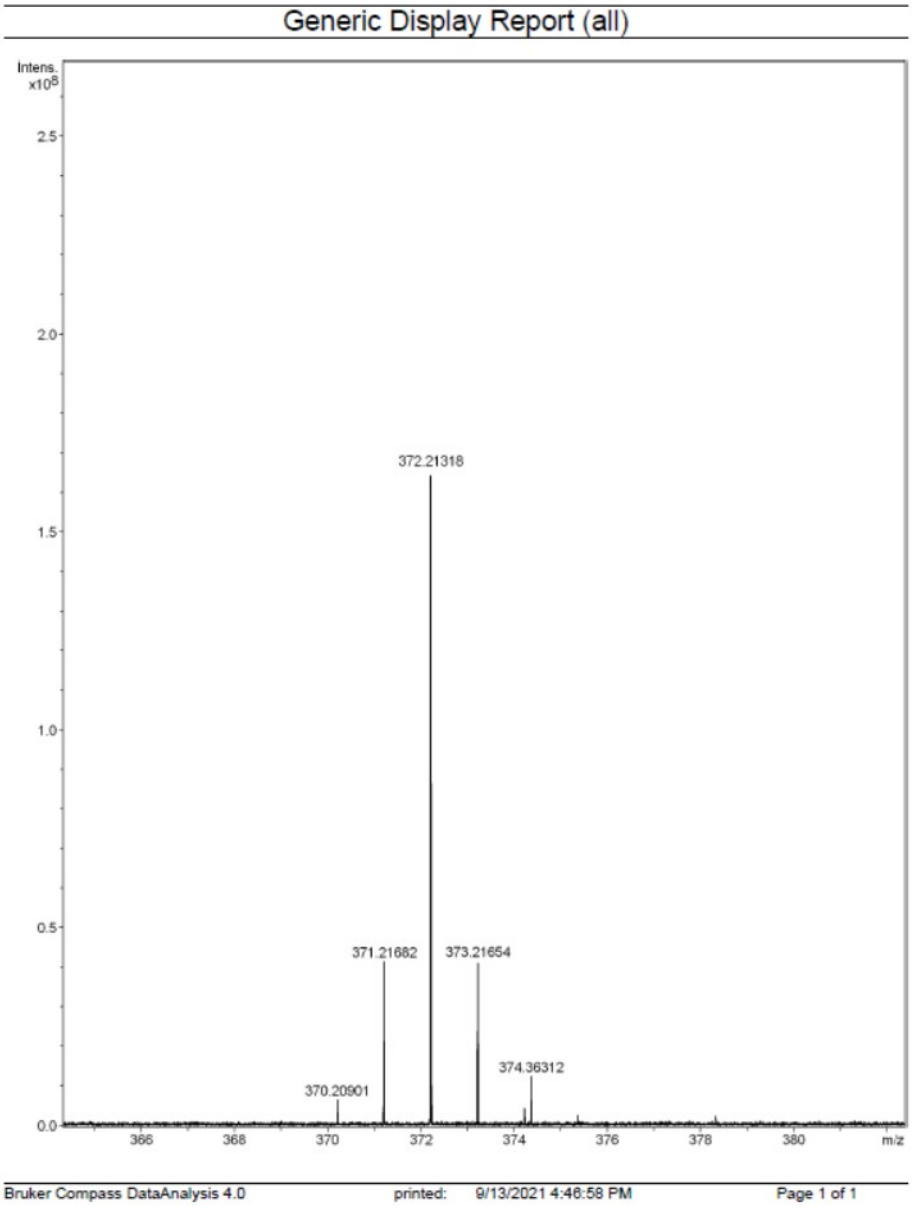
^{1}H NMR spectrum of S3



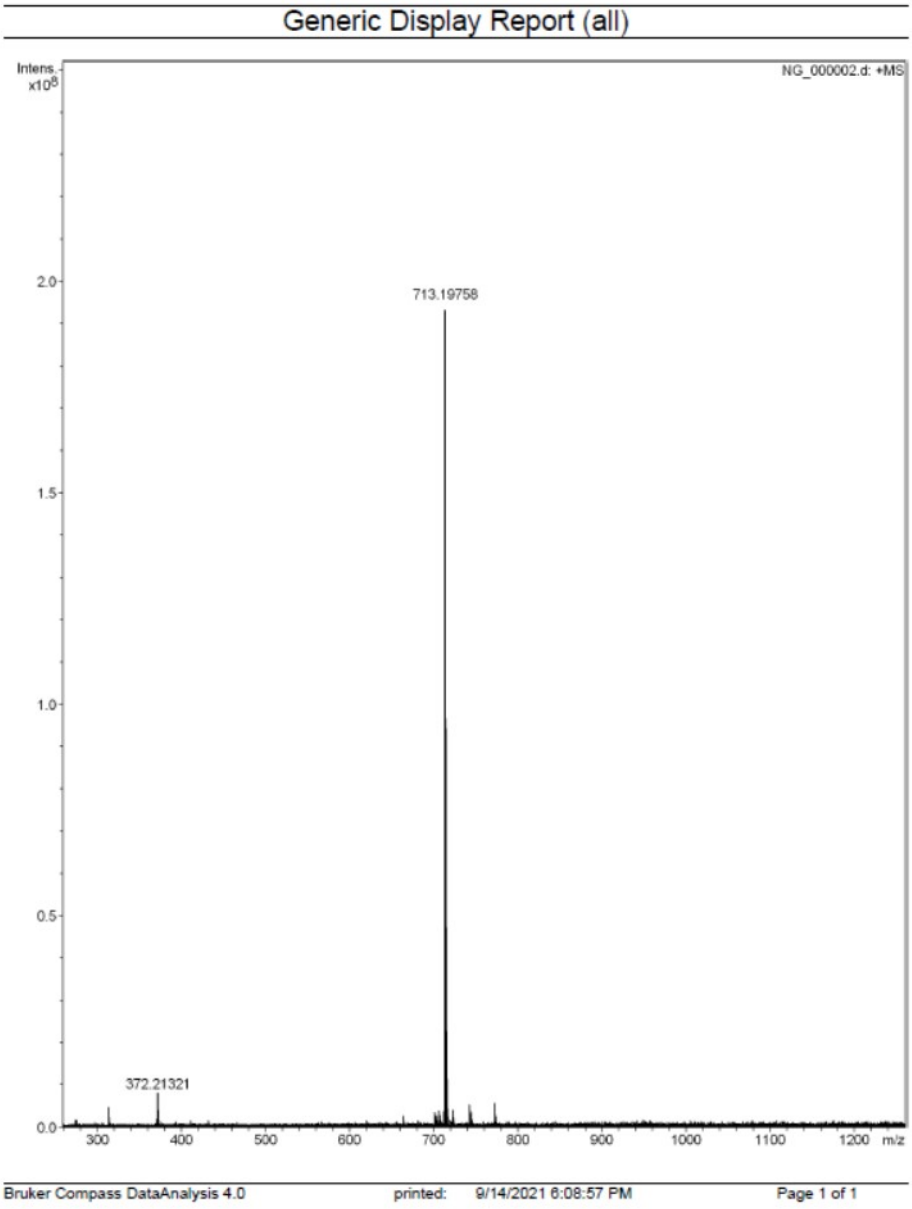
^{1}H NMR spectrum of TPB



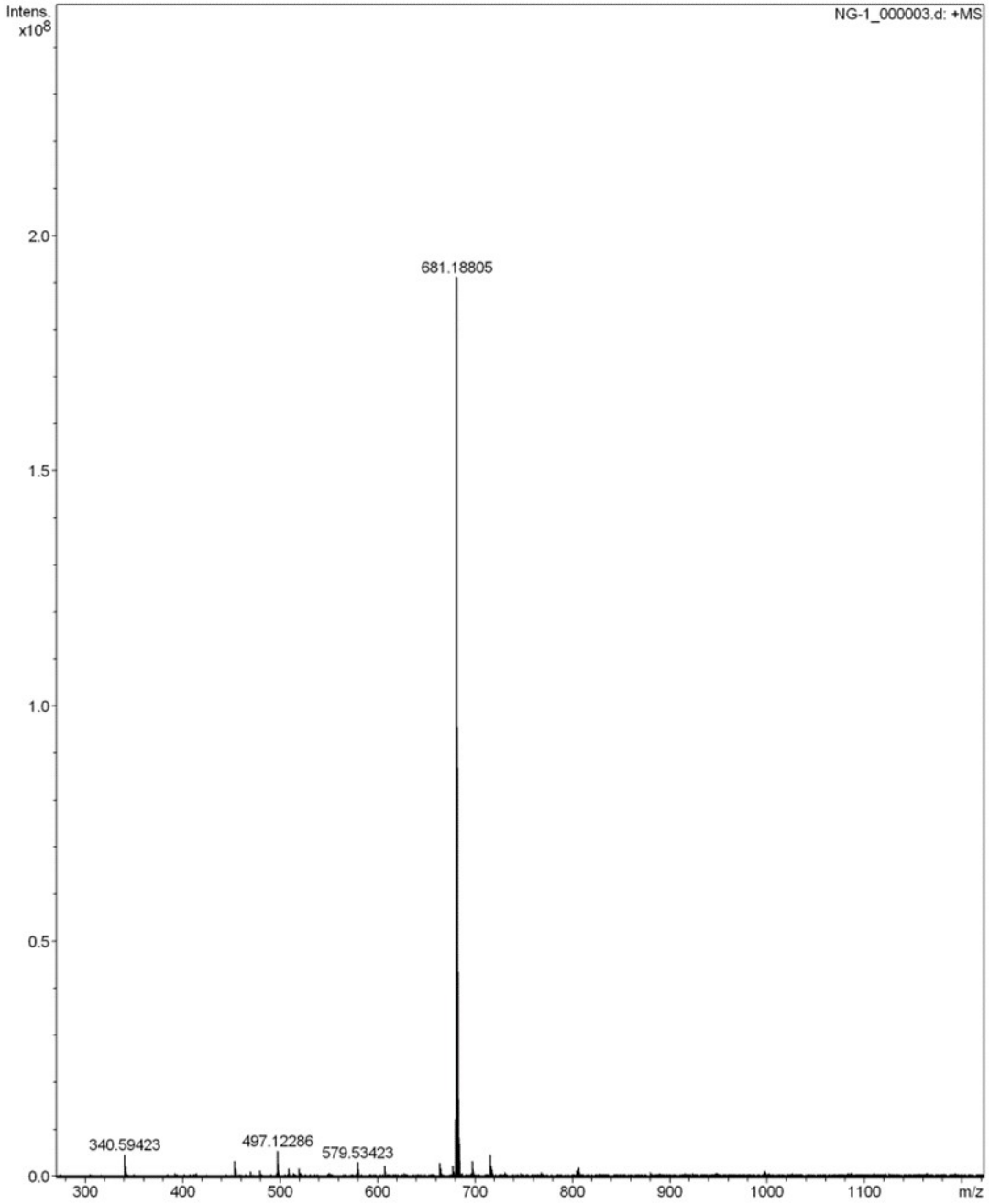
The high resolution mass spectrum of S1



The high resolution mass spectrum of S3



The high resolution mass spectrum of TPB



9. Reference

1. L. Zhang, C. Liu, S. Zhou, N. Wang, Q. Fan, D. Liu, W. Wu, X. Jiang, Improving quantum yield of a NIR-II dye by phenylazo group, *Adv. Healthcare Mater.,* 2020, **9**, 1901470.