Supporting information

Near-infrared BODIPY photosensitizers for two-photon excited singlet oxygen generation and tumor cell photodynamic therapy

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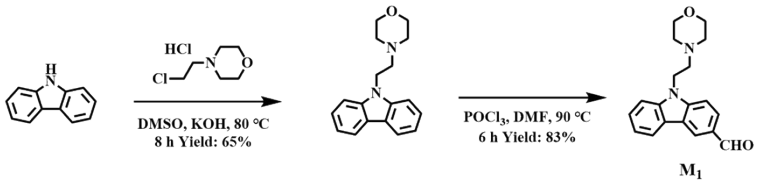
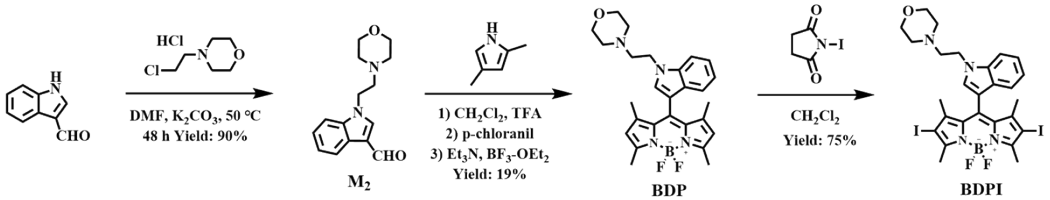
1. Synthesis of M_1, M_2 and BDPI





Scheme S1. The synthesis route of M_1, M_2 and BDPI

Synthesis of 9-(2-morpholinylethyl)-9H-carbazole-3-carboxaldehyde (M_1)[1]: 1.67 g (10.0 mmol) of carbazole and 3.71 g of N-(2-chloroethyl)morpholine hydrochloride (10.0 mmol) were thoroughly dissolved in 30.0 mL of dimethyl sulfoxide solvent. The solution was heated to 80 °C and refluxed with stirring for 8 h. The mixture was then poured into water, extracted with ethyl acetate and dried to concentrate the organic phase. Under the condition of ice-water bath, 2.01 mL (24.0 mmol) of phosphorous trichloride was slowly dripped into 2.00 mL of N, N-dimethylformamide, and then the above organic phase was added and heated to 90 ℃ for 6 h. The crude product was washed and dried, and purified by column chromatography to give 2.56 g of white solid product in 83% yield.

Synthesis of 1-(2-morpholinylethyl)-1H-indole-3-carboxaldehyde (M_2)[2]: to 20 mL of DMF was added indole-3-carboxaldehyde (1.45 g, 10.0 mmol), N-(2-chloroethyl) morpholine hydrochloride (1.49 g, 10.0 mmol) and potassium carbonate (1.57 g, 12 mmol). The above mixture was refluxed with stirring in an oil bath at 50 °C for 48 h. After completion of the reaction, the mixture was extracted with ethyl acetate and saturated saline and dried over anhydrous sodium sulfate. Finally, 2.32 g of the yellow solid product was purified by silica gel column chromatography in 90% yield.

Synthesis of intermediate BDPI[2]: 475 mg (5 mmol) of 2,4-dimethylpyrrole and 516 mg (2 mmol) of compound M_2 were added to 100 mL of CH_2Cl_2, followed by the addition of 50 μL of TFA, and the reaction was carried out for 6 h at room temperature. Then 516 mg (2.1 mmol) of p-chloranil was added slowly, and after 40 min of reaction, 6 mL of Et_3N and 9 mL of BF_3-OEt_2 were added quickly, and the reaction was continued for 8 h. At the end of the reaction, the crude product was washed and dried, and then separated and purified by silica gel column chromatography, and finally 181 mg of orange-red powder BDP was obtained. Next, 150 mg (0.31 mmol) of BDP and 141 mg (0.62 mmol) of NIS were added to the dichloromethane solution, and the mixture was stirred at room temperature for 30 min. At the end of the reaction, the crude product was washed and dried, and 169 mg of red powder solid BDPI was obtained by

column chromatography purification in 75% yield.

2. Singlet oxygen quantum yield measurements of photosensitizers

The $^1O_2$ quantum yield ($\Phi_\triangle$) of photosensitizers were calculated using methylene blue ($\Phi_\triangle$= 0.57, in CH_2Cl_2) as the reference[3] and 1, 3-diphenylbenzofuran (DPPF) as the $^1O_2$ trapping agent[4]. The absorbance of DPBF was kept around 1.0 and the absorbance of photosensitizers was kept between 0.2-0.3. All molecules were excited with red light at 660 nm, and the peak of DPBF at 414 nm was summarized to obtain the decreasing slope, and $\Phi_\triangle$ was calculated according to the following formula.

$$\Phi_{\Delta(sam)} = \Phi_{\Delta(std)} \frac{F_{sam} K_{std}}{F_{std} K_{sam}}$$

Where *F* represents the slope of the absorption value of DPBF at 414 nm decrease speed, *K* is absorption correction factor and $F=1-10^{-OD}$ and *OD* means the absorbance value at the irradiation wavelength, *std* is standard methylene blue.

3. Two-photon imaging and two-photon excitation $^1O_2$ generation assay in zebrafish

The melanin inhibition pretreatment and 24-hour developed zebrafish seedling were purchased from Company EzeRinka. The fry was incubated from eggs to fry at a constant temperature of 28°C for a total of 48-72 h. The fry was transferred to Petri dishes and 2 mL culture medium was added in quantities of 6-8. And then 5 μM of photosensitizer was added to each Petri dish, and fluorescence imaging was taken after 8h of incubation. The two-photon fluorescence imaging was performed using Olympus Confocal Microscope FV 1000 with an IX81 semi-manual inverted microscope. After photosensitizer Cz-BDPI was introduced into zebrafish, appropriate amount of reactive oxygen species indicator 2, 7-dichlorofluorescein diacetate (DCFH-DA, Ex: 488 nm, Em: 510-530 nm) was added and cultured for 30 minutes under dark conditions. The zebrafish were irradiated under 800 nm femtosecond laser for 15 minutes and then fluorescence imaging was performed.

4. Fluorescence imaging experiments of photosensitizers in A549 cells

A549 cells were cultured in 1640 medium containing 10% v/v fetal bovine serum in an incubator with 5% CO_2 for 24 h at 37 ℃. The 2 μM photosensitizer solution was

added for 6 h and then washed with 2 mL PBS buffer three times. The fluorescence images were obtained using Olympus FV3000 confocal laser scanning microscope.

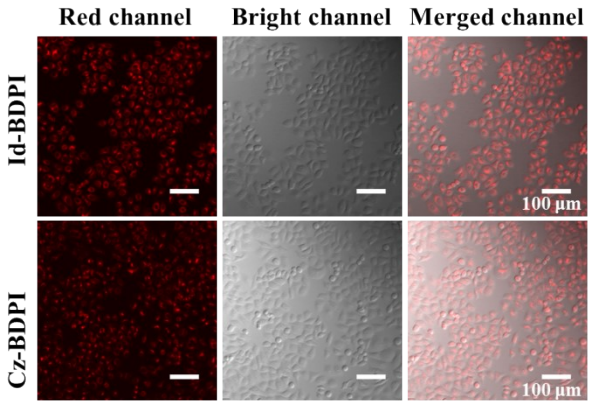


Fig. S1 Fluorescence imaging of photosensitizers Id-BDPI and Cz-BDPI in A549 cells

5. Singlet oxygen generation experiments of photosensitizers in A549 cells

The singlet oxygen generation of Id-BDPI and Cz-BDPI in A549 cells were detected using DCFH-DA (a reactive oxygen species detection probe). A549 cells were treated with Id-BDPI and Cz-BDPI for 6 h and then incubated with DCFH-DA for 10 min. The cells were irradiated with a 730 nm NIR light for 0 or 15 min, and fluorescent images of A549 cells were taken using CLSM, Ex: 488 nm，Em: 510-530 nm.

6. MTT experiments of photosensitizers in A549 cells

MTT assay was used to determine the phototoxicity and dark toxicity of photosensitizers[5]. A range of concentrations of photosensitizer solutions (0 μM -5 μM) were added to 96-well culture dishes lined with A549 cells and incubation was continued for 24 hours. Cells containing photosensitizers were washed three times with PBS buffer and then fresh culture medium was added to continue incubation. For dark toxicity, cells were incubated under light-free conditions for 24 hours. The supernatant was discarded and 100 μL of DMSO solution was added to each well. The absorbance at 490 nm was recorded using an enzyme meter. Cell viability was calculated according to the following formula, where C_v represents cell viability, A_x is the experimental absorbance value, and A_0 is the control absorbance value.

$$C_v = A_x/A_0$$

7. AO/EB staining experiments of photosensitizers in A549 cells

AO/EB staining experiments were used to simulate cell survival and death during photodynamic therapy[6]. A549 cells without photosensitizer were light-exposed using 730 nm for 15 min, and A549 cells containing 2 μM photosensitizers were light-exposed for 0 min, 5 min and 15 min, respectively. 1 μl of Acridine Orange/Ethidium Bromide (AO/EB) dye was added to the above dishes and incubation was continued for 20 min before imaging was taken. To minimize the interference of external light sources, the experiments were performed under dark conditions, and the AO/EB images were obtained by CLSM.

8. Lysosomal localization experiments of photosensitizers in A549 cells

Lysosomal co-localization assays are used to test the lysosomal targeting ability of photosensitizers[7]. A549 cell culture dish containing 2 μM photosensitizer was added with 0.5 μM lysosome tracking green (commercial lysosome trapping agent) and continued for 20 min. Lysosome colocalization images were obtained by CLSM. (Lyso-Tracker Green: Ex: 488 nm, Em: 515-545 nm).

9. ^{1}H NMR, ^{13}C NMR and HRMS spectra of Id-BDPI

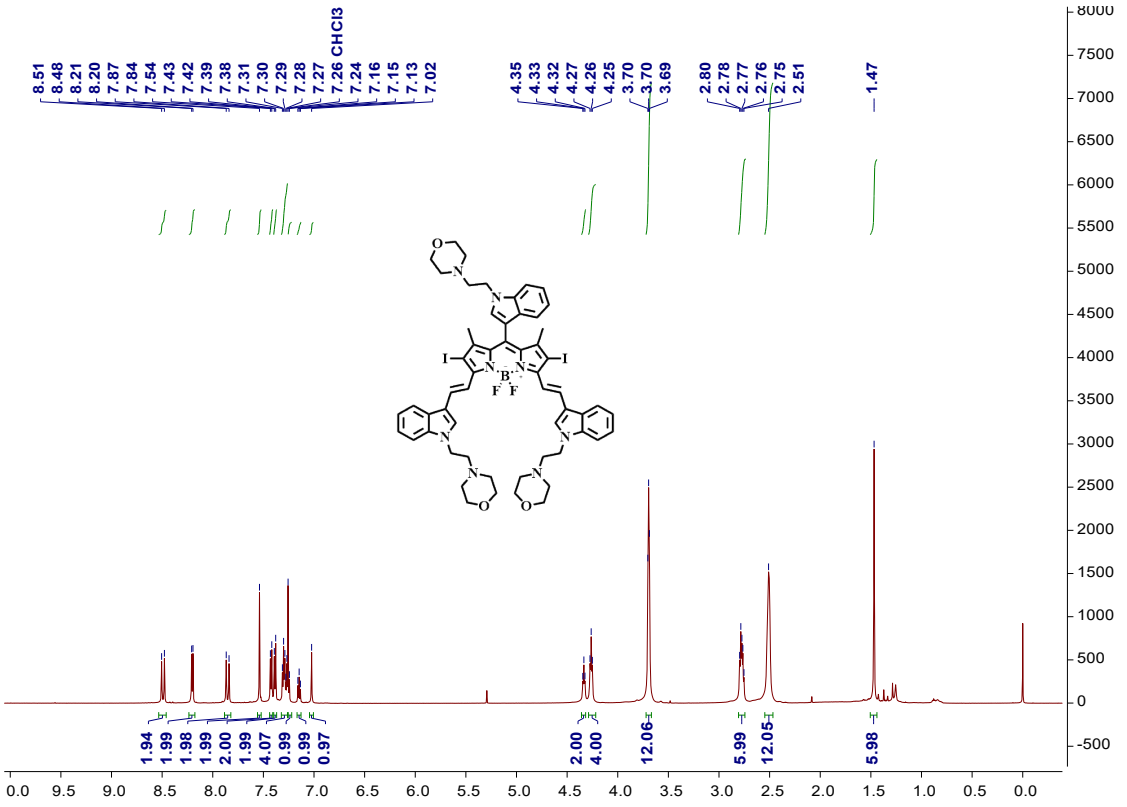


Fig. S2 ^{1}H NMR of photosensitizer Id-BDPI

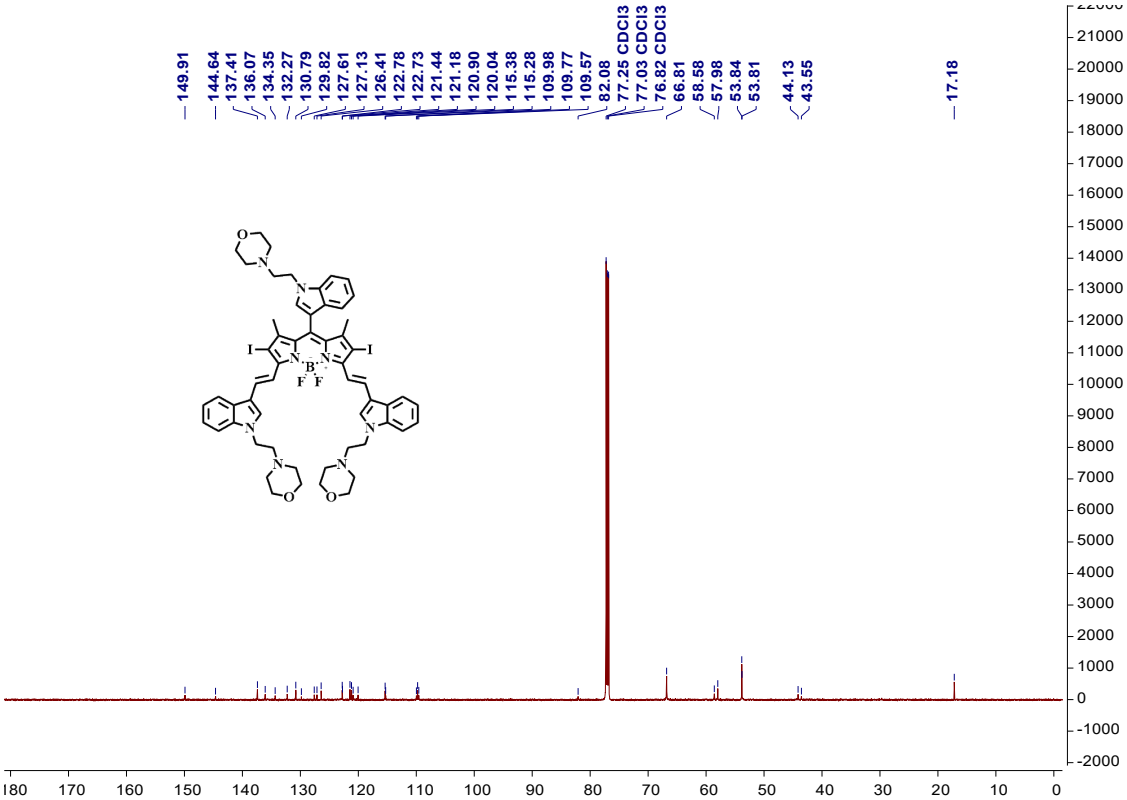


Fig. S3 ^{13}C NMR of photosensitizer Id-BDPI

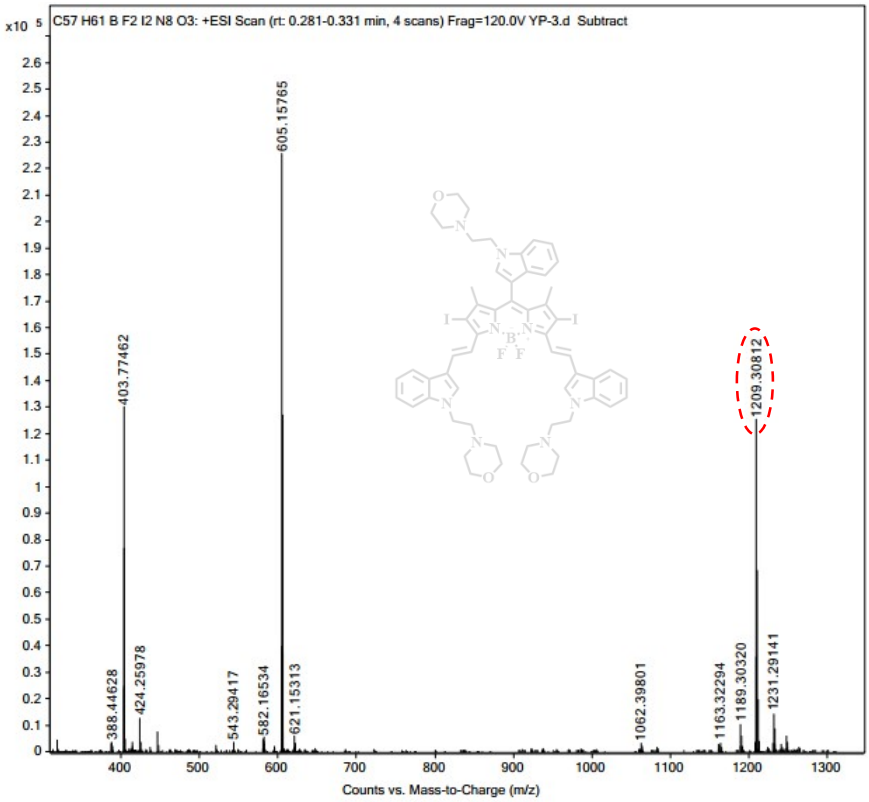


Fig. S4 HRMS of photosensitizer Id-BDPI

10. ^{1}H NMR, ^{13}C NMR and HRMS spectra of Cz-BDPI

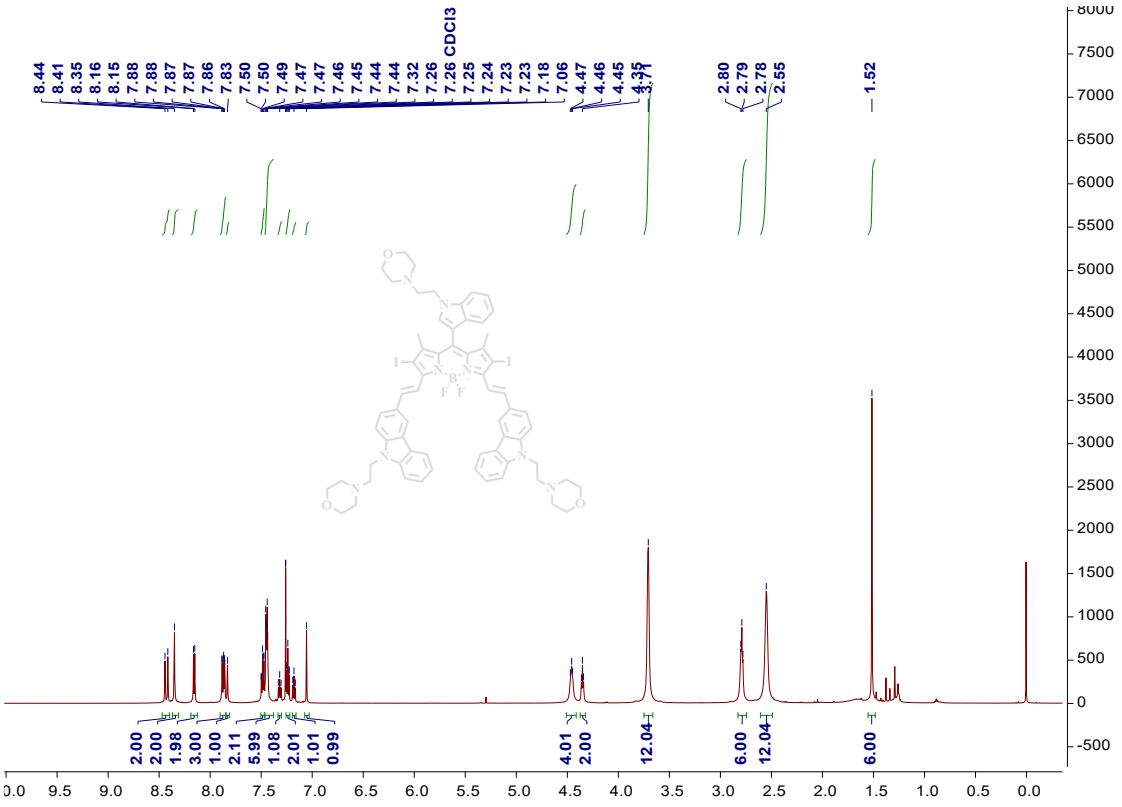


Fig. S5 ^{1}H NMR of photosensitizer Cz-BDPI

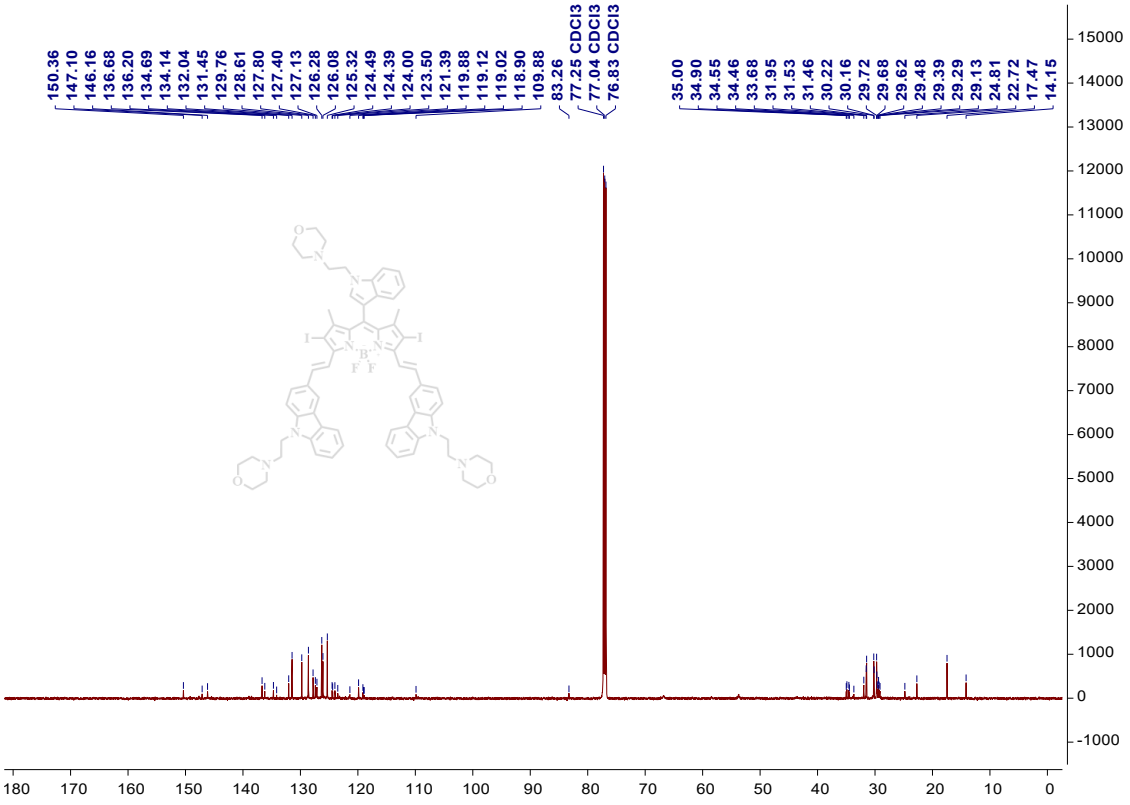


Fig. S6 ^{13}C NMR of photosensitizer Cz-BDPI

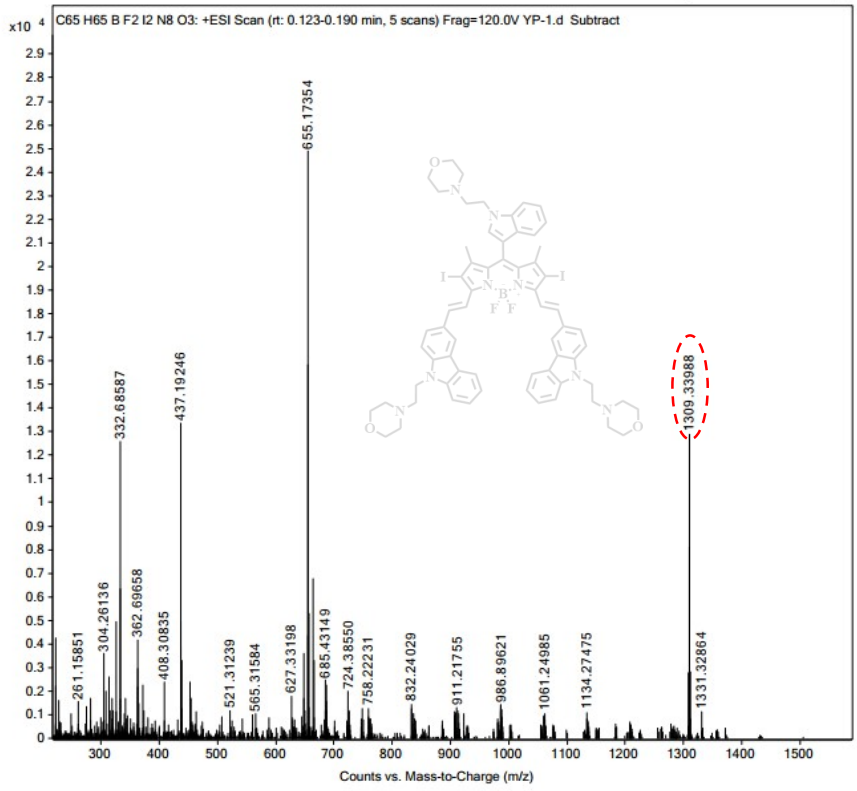


Fig. S7 HRMS of photosensitizer Cz-BDPI

11. References

1. W. Li, W. Feng, B. Liu and Y. Qian, *Org. Biomol. Chem.*, 2024, **22**, 1892-1900.
2. R. Liu and Y. Qian, *Spectrochim. Acta, Part A*, 2024, **304**, 123387.
3. R. W. Redmond and J. N. Gamlin, *Photochem. Photobiol.*, 1999, **70**, 391-475.
4. L. Huang, Z. Li, Y. Zhao, Y. Zhang, S. Wu, J. Zhao and G. Han, *Journal of the American Chemical Society*, 2016, **138**, 14586-14591.
5. X. Xia, R. Wang, Y. Hu, Q. Yao, S. Long, W. Sun, J. Fan and X. Peng, *Science China-Chemistry*, 2022, **65**, 821-828.
6. L. Jiao, F. Song, J. Cui and X. Peng, *Chem. Commun.*, 2018, **54**, 9198-9201.
7. M. Liu, C. Wang and Y. Qian, *New J. Chem.*, 2021, **45**, 18082-18089.