Design and Mechanistic Study of a High-Sensitivity Small-Molecule Fluorescent Probe for the Detection of Microcystin-LR

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Instrument

^{1}H and ^{13}C nuclear magnetic resonance spectra were measured using a Bruker Ultra-shield 400 MHz Fourier transform nuclear magnetic resonance spectrometer in DMSO-d6, with tetramethylsilane (TMS) used as an internal standard for chemical shift calibration. UV/visible absorption spectra were recorded at 293 K using a TECAN microplate reader. Photoluminescence spectra were recorded at 293 K using a Hitachi F4600 fluorescence spectrophotometer. The HRMS was determined in methanol solution using the Thermo Fisher Scientific Q Exactive Focus high-resolution mass spectrometer. The FIRT was measured using the Thermo Fisher Scientific Nicolet iS20 instrument. The XPS test was conducted using Thermo Scientific's K-Alpha.

Mateials

(10-hexanoyl-10H-phenothiazin-3-yl)boronic acid, 5-bromothiophene-2-carbaldehyde, tetrakis(triphenylphosphine)palladium, tetrahydrofuran, potassium carbonate solution, dimethyl sulfoxide solution were purchased from Aladdin Co., Ltd (Beijing, China). Microcystin (LR subtype) and amino acids were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

Synthesis of the Probe

The fluorescent probe was fabricated via a Suzuki coupling reaction with (10-hexanoyl-10H-phenothiazin-3-yl) boronic acid and 5-bromothiophene-2-carbaldehyde. (10-hexanoyl-10H-phenothiazin-3-yl)boronic acid (2.12 g, 6.49 mmol) and 4-triphenylamine boronic acid (0.83 g, 4.33 mmol) were added to 100 mL of THF solvent, followed by the addition of tetrakis(triphenylphosphine)palladium (0.50 g, 0.43mmol) and 2 M potassium carbonate solution (13 mL), after which the mixture was heated at 85 °C for 48 hours. After cooling to room temperature, extraction was performed with ethyl acetate and pure water, and the mixture was evaporated to dryness using a rotary evaporator, followed by purification using silica gel column chromatography with dichloromethane and n-hexane to obtain the probe(1.52 g, yield 89.40%).^{1}H NMR (600

MHz, DMSO) δ 9.88 (q, *J* = 1.3 Hz, 1H), 8.02 – 7.98 (m, 1H), 7.67 (dt, *J* = 2.7, 1.3 Hz, 1H), 7.63 – 7.56 (m, 2H), 7.21 (q, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.05 (t, *J* = 9.1 Hz, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 3.88 (q, *J* = 7.6 Hz, 2H), 1.67 (h, *J* = 7.5 Hz, 2H), 1.38 (p, *J* = 7.2 Hz, 2H), 1.24 (s, 4H), 0.85 – 0.78 (m, 3H). ^{13}C NMR (151 MHz, DMSO) δ 184.25, 152.42, 146.22, 144.33, 141.55, 139.90, 128.32, 127.69, 127.17, 126.29, 124.96 – 124.77, 123.40, 123.07, 116.55, 47.08, 31.27, 26.58, 26.23, 22.54, 14.30. Found: $[M+H]^+$ 394.1294; 'molecular formula $C_{23}H_{23}NOS_2$ ' requires $[M+H]^+$ 394.1294.

Photocurrent measurements

The photocurrent spectra were tested on the electrochemical workstation CHI760E using a three-electrode system (with Ag/AgCl as the reference electrode and platinum wire as the counter electrode) and 0.5 M sodium sulfate (Na_2SO_4) solution as the electrolyte. To prepare the working electrode, 10 milligrams of iridium(III) dye was dispersed in 1 milliliter of ethanol and 20 microliters of Nafion aqueous solution (5 weight%) and then ultrasonically dispersed for 2 hours. Subsequently, 0.1 milliliter of the slurry was dropwise added to the FTO glass substrate (1 square centimeter). After the ethanol evaporated, the iridium(III) dye still covered the surface of the FTO glass. At room temperature, the photocurrent spectra were obtained under a bias voltage of -700 millivolts and an AC amplitude of 5 millivolts in the frequency range of 0.1 hertz to 100 kilohertz. Light was supplied by a 300-watt xenon lamp. The experiment was conducted at room temperature.

Computation details

The structural optimization of the small molecule was performed using the Gaussian 16 software package at the DFT level with the B3LYP-D3(BJ) functional and the 6-31G* basis set. To intuitively visualize the molecular orbital characteristics, the Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbitalof the optimized structure were extracted using GaussView software, and the corresponding

orbital is surfaces and distributions were generated.

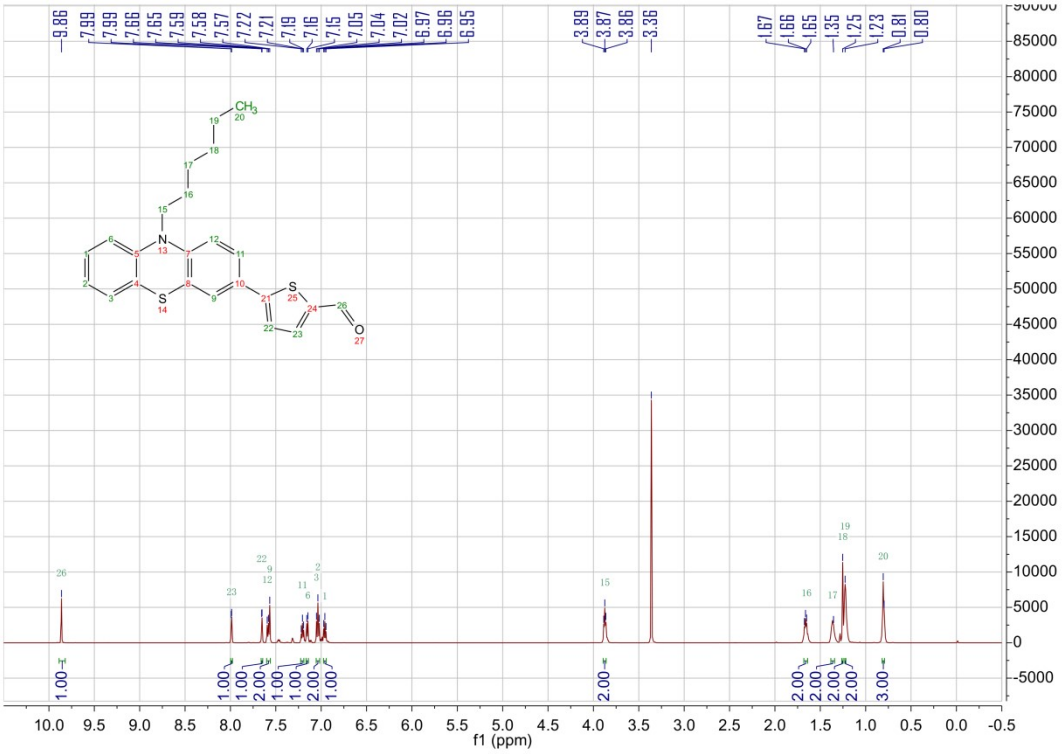

Figure S1. ^{1}H NMR spectra of probe

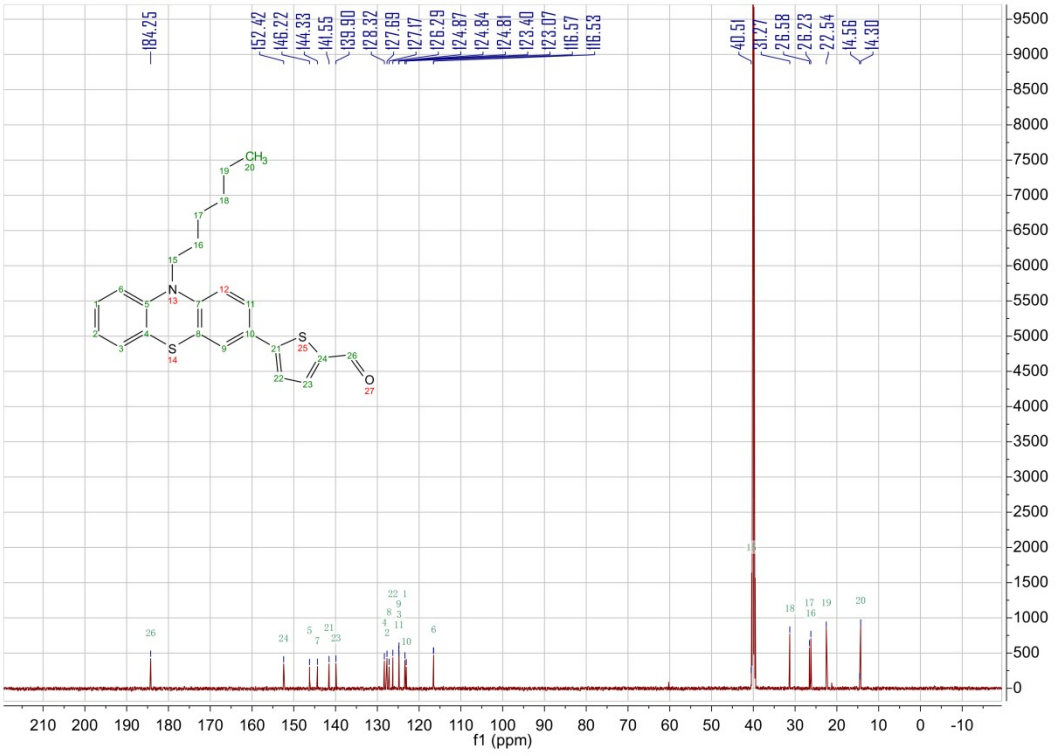

Figure S2. ^{13}C NMR spectra of probe

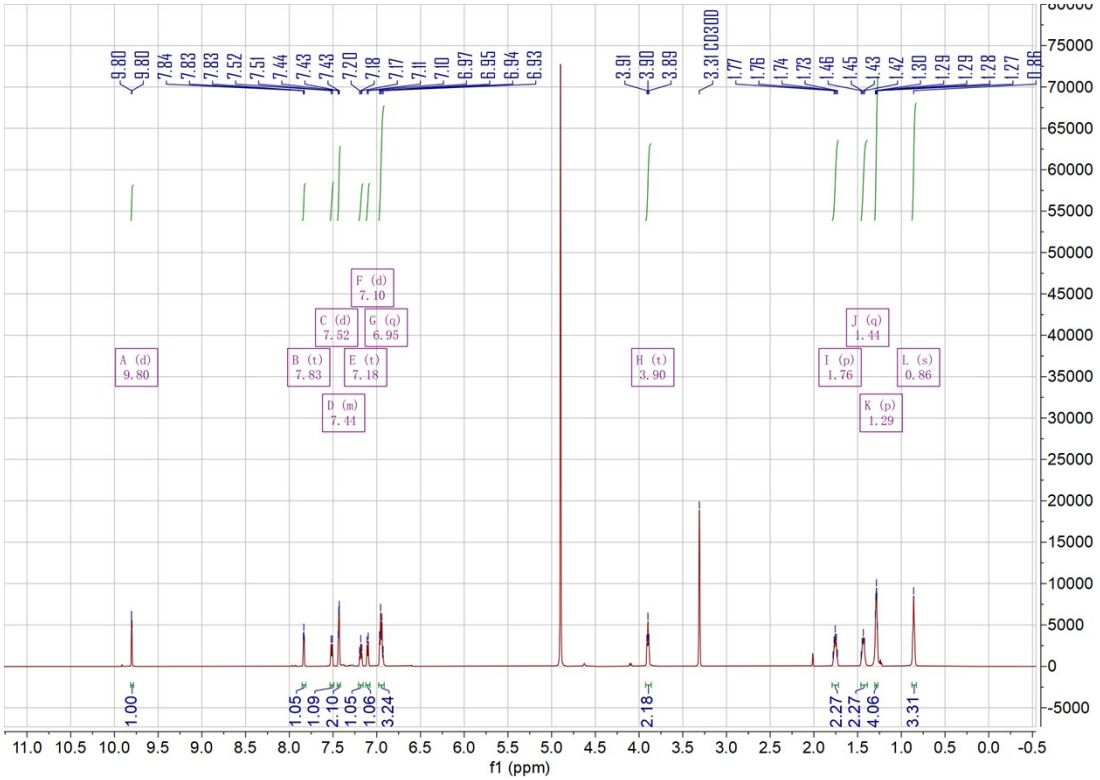

Figure S3. ^{1}H NMR spectra of the probe before reaction with MC-LR

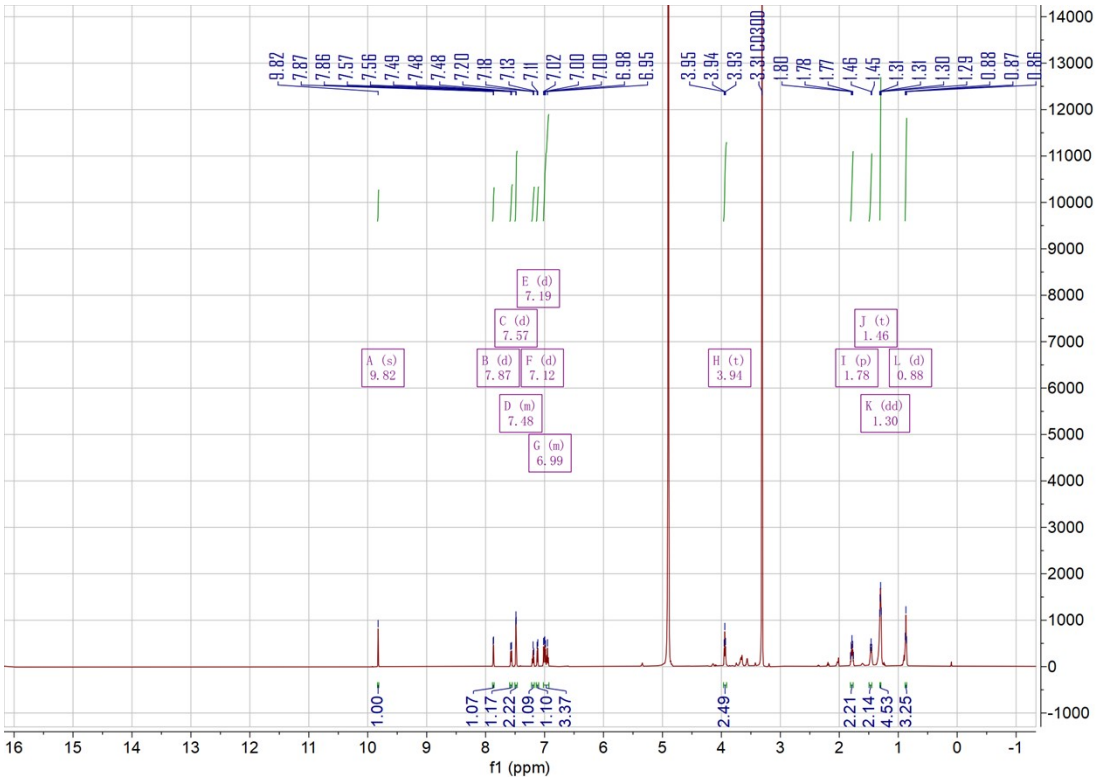

Figure S4. ^{1}H NMR spectra of the probe after reaction with MC-LR

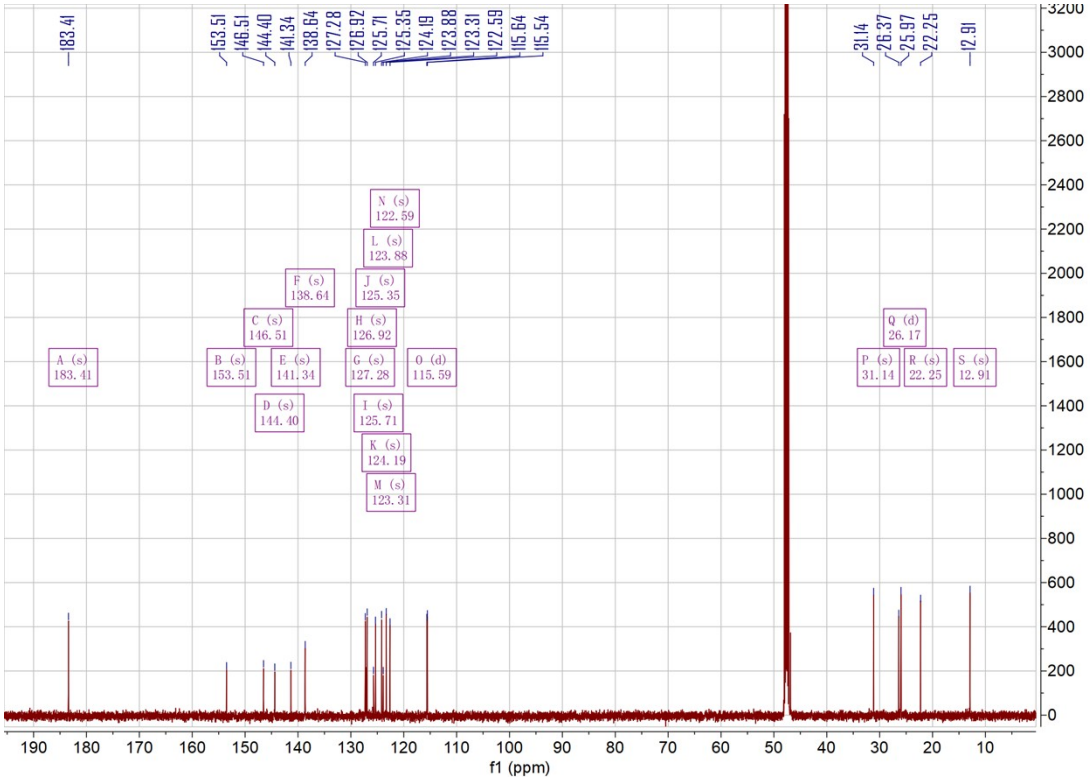

Figure S5. ^{13}C NMR spectra of the probe before reaction with MC-LR

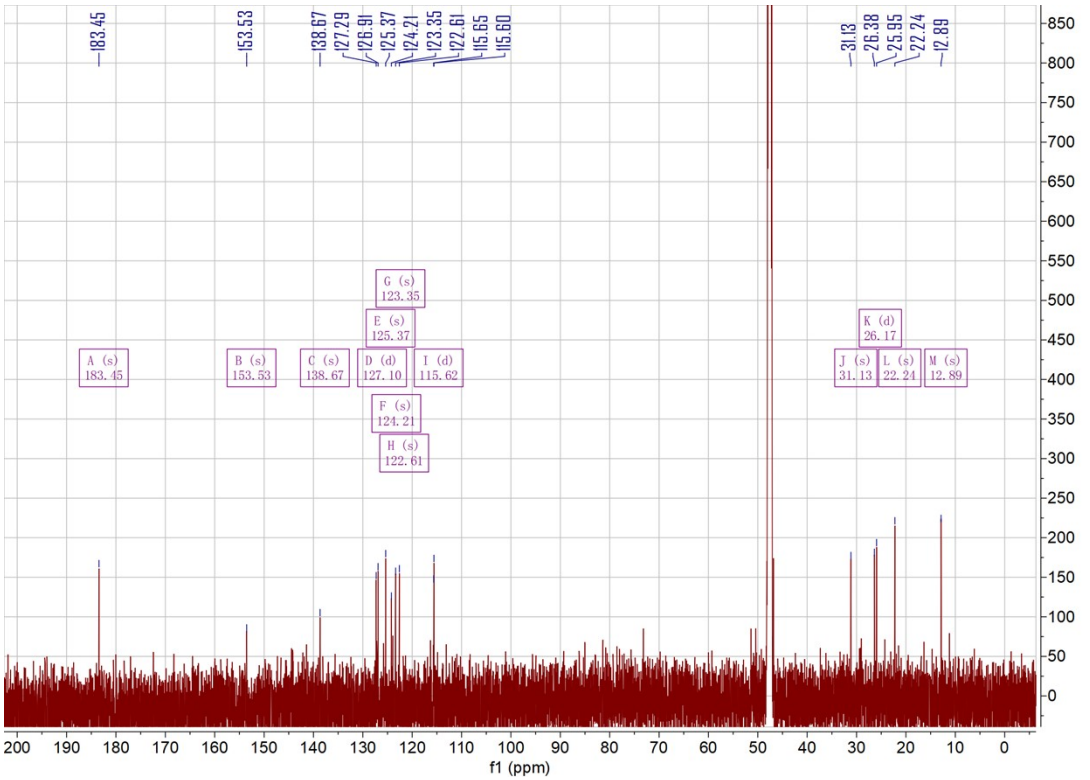

Figure S6. ^{13}C NMR spectra of the probe after reaction with MC-LR

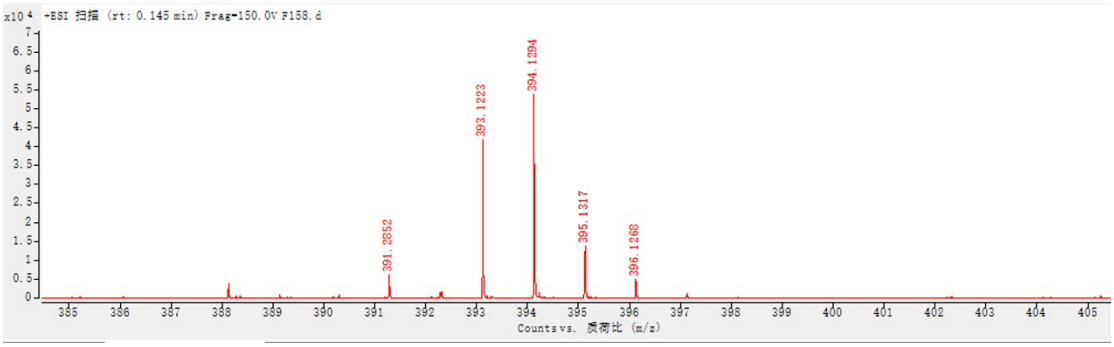

Figure S7. HR-MS result of probe

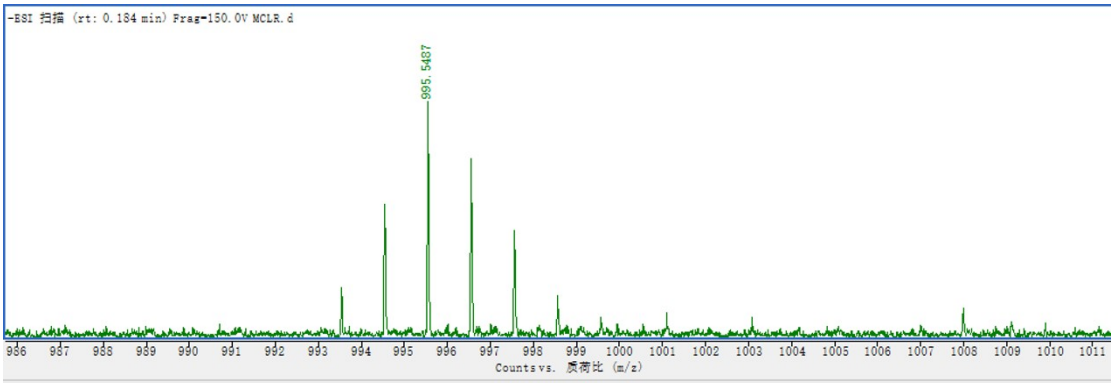

Figure S8. HR-MS result of MC-LR (required [M+H$^+$]:995.5560; found [M+H$^+$]:995.5487)

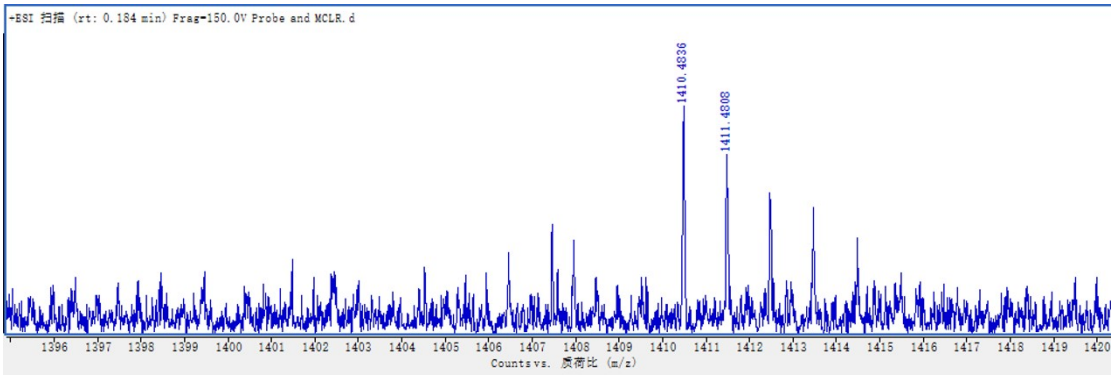

Figure S9. HR-MS result of Probe+MC-LR (required [Probe+MC-LR +Na$^+$]:1410.6601; found [Probe+MC-LR +Na$^+$]:1410.4836)

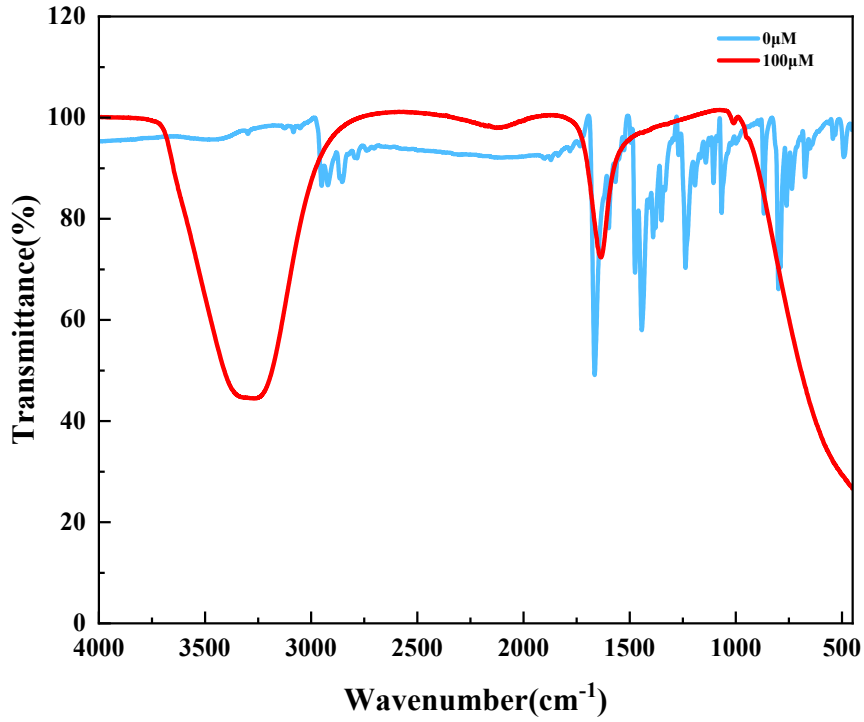

Figure S10. FTIR spectra of the probe before and after reaction

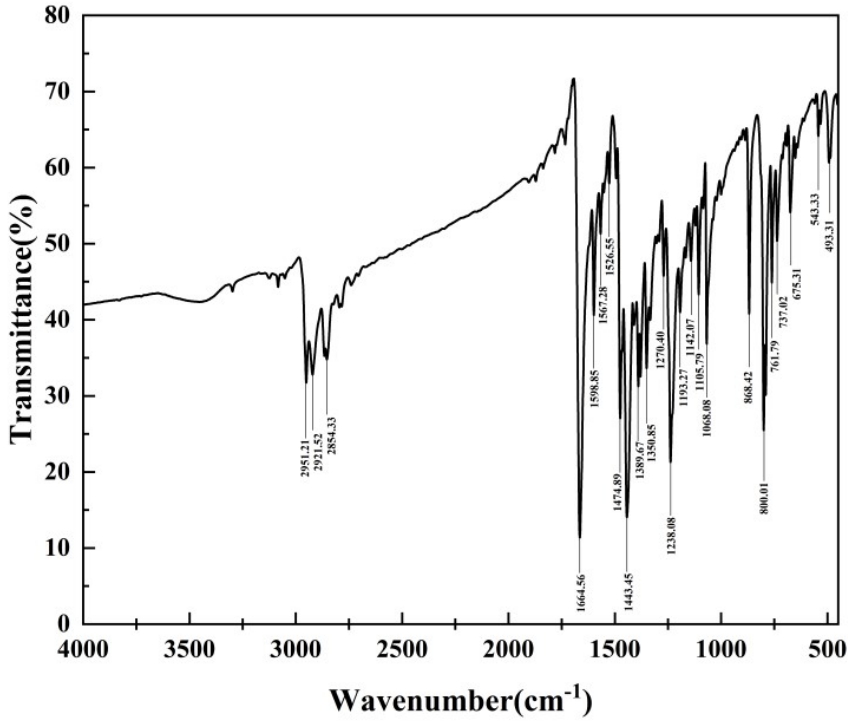

Figure S11. FT-IR result of probe

Table S1. FT-IR result of probe1

Band·(cm^{-1})	Stretching Vibration Assignment	Reference
2951.21	C-H	1
2921.52	C-H	1, 2
2854.33	C-H	1
1664.56	C=O	2, 3
1598.85	C=C	1
1567.28	C=C	1

1526.55	C=C	1
1474.89	C-H	2
1443.45	C-H	2
1389.67	C-H	2
1350.85	C-H	2
1270.40	C-N	1
1238.08	C-N	1, 2
1193.27	C-N	1
1142.07	C-N	2
1105.79	C-N	2
1068.08	C-N	2
868.42	C=C	1, 2
800.01	C=C	2
761.79	C-H	2
737.02	C-H	2
675.31	C=C	2
543.33	C-S	1
493.31	C-S	1

Figure S12. Application comparison chart under 365nm ultraviolet irradiation

Table S2 Photophysical Properties of Various Solvents

Solvent	$E_T(30)$ (Kcal/mol)	ε	n	viscosity (mPa.s)
tetrahydrofuran	37.4	7.58	1.407	0.55
DMSO	45	48.9	1.477 3	1.996

$E_T(30)$ is the molar transition energy expressed in kcal·mol−1 and is a solvent polarity parameter. (Reichardt, C. Empirical parameters of solvent polarity as linear free-energy

relationships. *Angewandte chemie international edition*, 1979, 18(2), 98-110.)

Table S3 Determination of water content in commercial samples

Samples	Fluorescence intensity	Water Content (wt%)	RSD (%, n=3)
tetrahydrofuran	1420.7	0.08	0.07
DMSO	430.7	1.00	1.80

Reference

1. M. F. Amin, P. Gnida, J. G. Małecki, S. Kotowicz, A. K. Pająk, M. Siwy and E. Schab-Balcerzak, *Industrial & Engineering Chemistry Research*, 2024, **63**, 19994–20008.
2. B. Y. K. Hui, R. Tao, K. L. O. Chin, X. Y. D. Soo, A. Sng, S. A. A. Abedi, K. C. Chong, X. Liu, J. Xu and M. H. Chua, *Advanced Optical Materials*, 2026, **14**, e02075.
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