Electronic Supplementary Information (ESI) for

A BODIPY-based fluorescent probe for rapid detection of NO in cells and zebrafish

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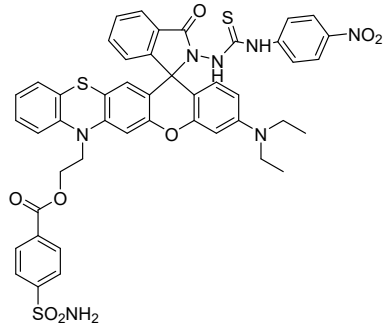
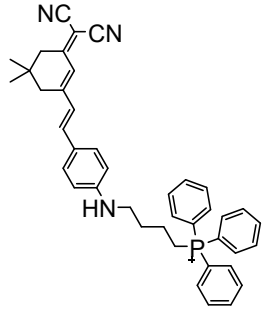
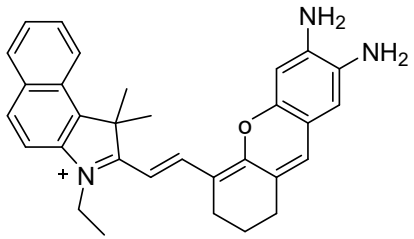
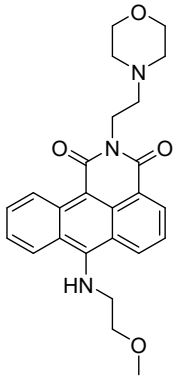
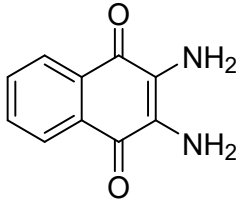
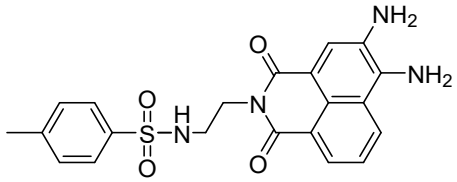
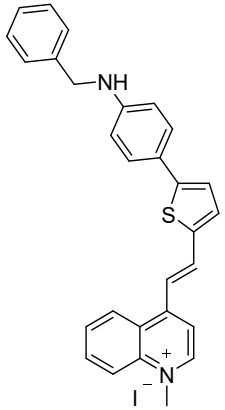
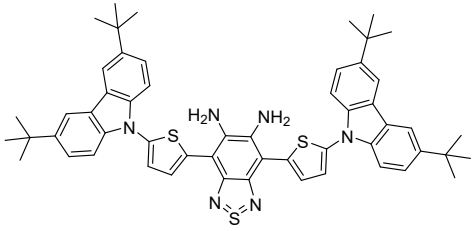
Contents

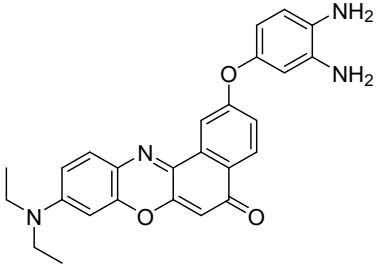
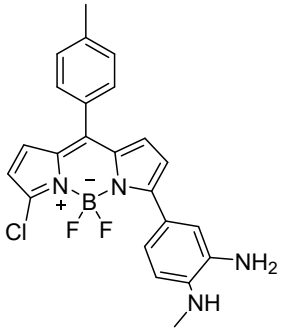
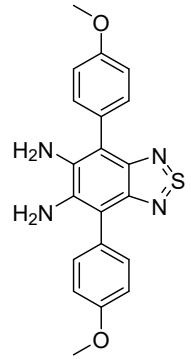
1. Properties of reported probes

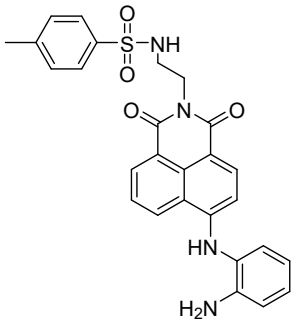
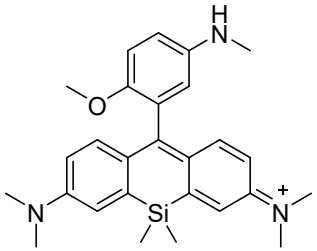
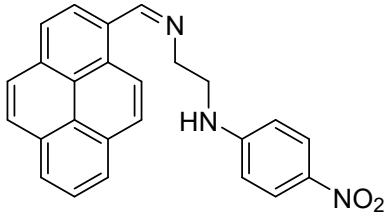
Table S1. Summary of the relevant properties of reported probes

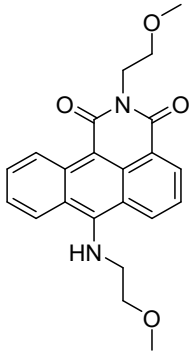
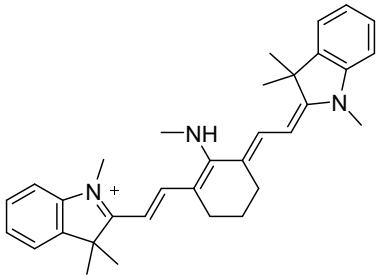
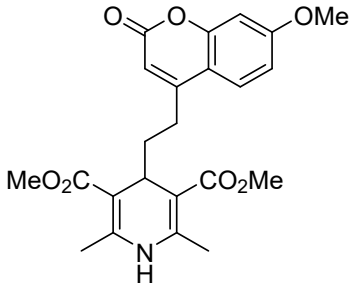
Probe	Solvent	λ_{em}	LOD	Time	Reference
	DMSO: PBS (v/v 1:1)	780 nm	0.54 μM	＜ 1 min	Bioorg. Chem. 2024, **148**, 107476
	EtOH: PBS (v/v 1:1)	547 nm 656 nm	32 nM	20 min	Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 2025, **329**, 125636

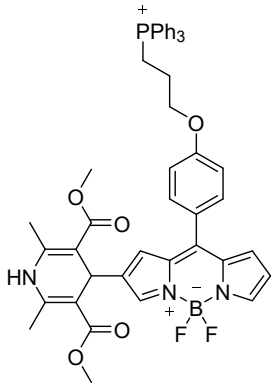
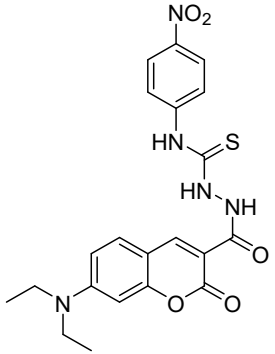
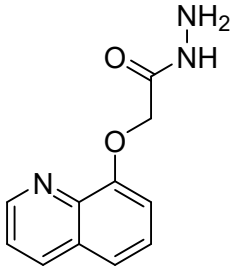
	ethanol solution	705 nm 780 nm	0.536 nM	10 min	J. Med. Chem. 2024, **67**, 4026-4035
	CH_3CN: PBS (v/v 10:1)	521 nm 621 nm	44.3 nM	30 min	Anal. Chim. Acta. 2024, **1297**, 342303
	PBS buffer solution (pH = 7.4)	450 nm	0.08 μM	25 min	Talanta 2024, **272**, 125763

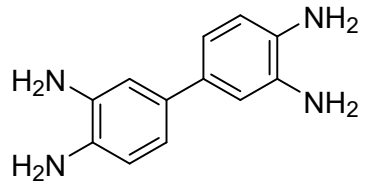
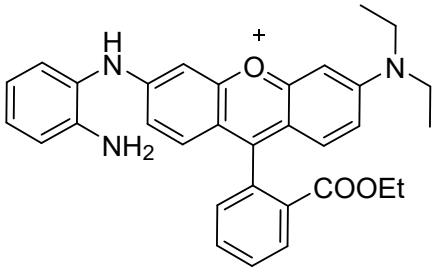
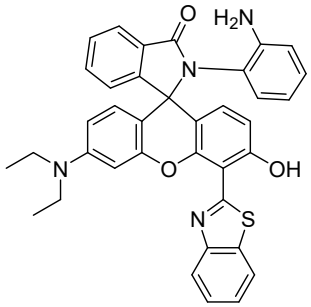
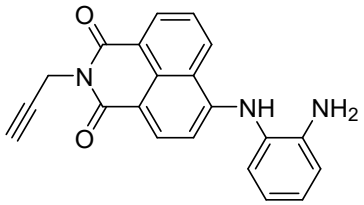
	DMSO: PBS (v/v 20:1)	455 nm	32 nM	10 min	Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 2025, **325**, 125172
	PBS buffer solution (pH = 7.4)	720 nm	15 nM	30 s	Sens. Actuators B Chem. 2024, **421**, 136491
	DMSO: PBS (v/v 10:1)	650 nm	83.10 nM	44 s	Anal. Chim. Acta. 2024, **1296**, 342333

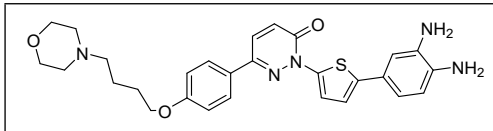
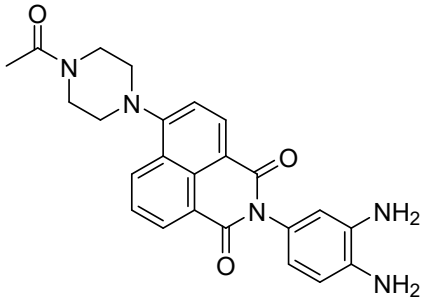
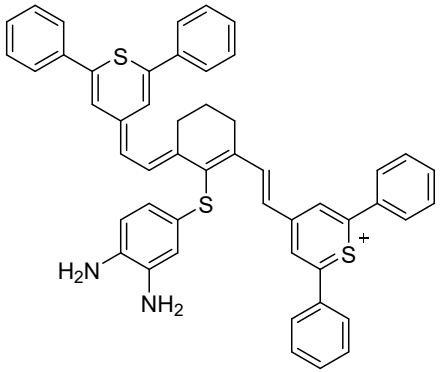
	DMF: PBS (v/v 1:9)	658 nm	9 nM	40 s	J. Mater. Chem. C 2019, **7**, 3246-3252
	CH_3CN: PBS (v/v 1:3)	565 nm	3.4×10^{-8} M	10 min	Anal. Chem. 2021, **93**, 3922-3928
	DMAC: PBS (v/v 1:9)	625 nm 550 nm	1.2 μM	30 min	Anal. Chem. 2018, **90**, 7953-7962

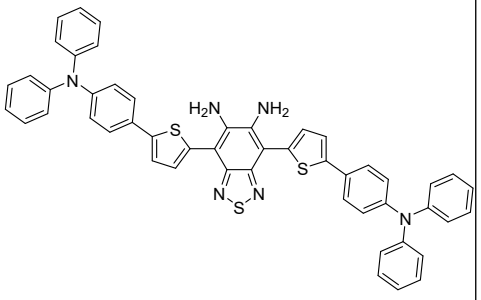
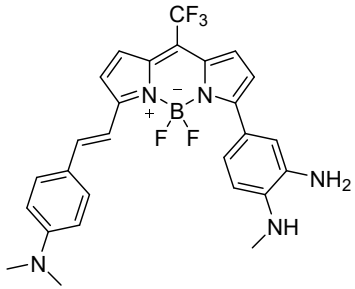
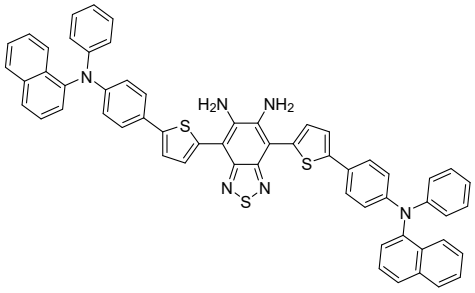
	PBS buffer solution (pH = 7.4)	538 nm	3.3 nM	6 min	ACS Sens. 2018, **3**, 2311-2319
	DMSO: PBS (v/v 1:200)	672 nm	14 nM	30 min	Anal. Chem. 2017, **89**, 9620-9624
	CH_3CN: H_2O (v/v 2:8)	523 nm	2.13 pM	17 min	ACS Omega. 2018, **3**, 10306-10316

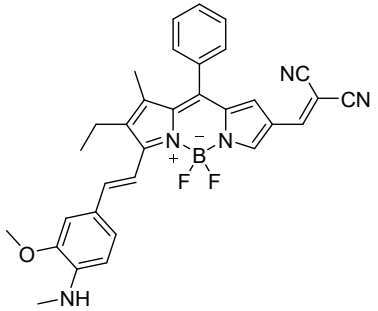
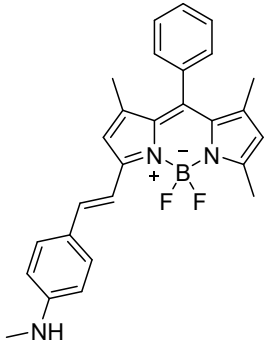
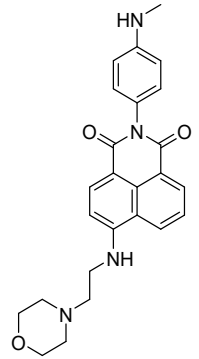
	CH_3CN: PBS (v/v 1:9)	525 nm 625 nm	4.05 nM	15 min	Sens. Actuators B Chem. 2021, **329**, 129147
	EtOH: H_2O (v/v 1:1)	800 nm	11.3 nM	20 min	Dyes. Pigments. 2019, **166**, 211-216
	DMSO: PBS (v/v 5:1)	392 nm	17.0 nM	10 min	RSC Adv. 2016, **6**, 85698

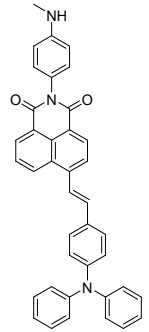
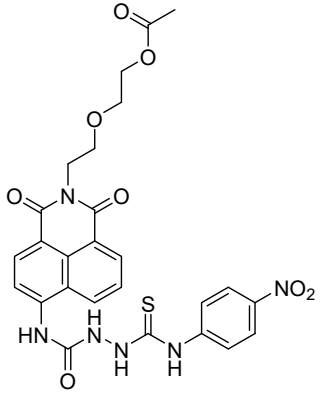
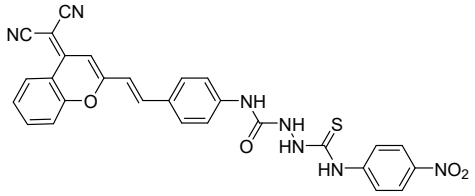
	PBS buffer solution (pH = 7.4)	525 nm	25 nM	10 min	Talanta 2018, **176**, 82-388
	DMSO: HEPES (v/v 3:7)	475 nm	47.6 nM	20 s	ACS Sens. 2019, **4**, 309-316
	HEPES buffer solution (pH = 7.2)	490 nm	45.4 nM	30 min	J. Org. Chem. 2018, **83**, 13287-13295

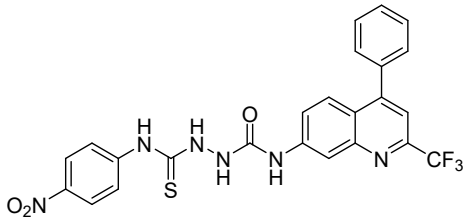
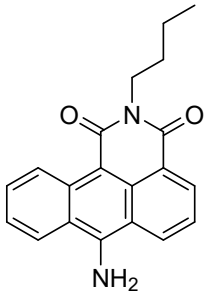
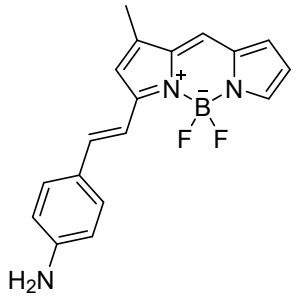
	BR buffer solution	--	40 ppb	35 min	Anal. Bioanal. Chem. 2020, **412**, 2545-2550
	PBS buffer solution (pH = 7.4)	581 nm	2.3 nM	--	J. Mater. Chem. B 2018, **6**, 4096-4103
	DMSO: PBS (v/v 1:9)	457 nm 611 nm	51.3 nM	< 0.33 min	RSC Adv. 2022, **12**, 2721-2728
	PBS buffer solution (pH = 7.4)	520 nm 670 nm	0.25 nM	120 min	Biosens. Bioelectron. 2024, **248**, 116000

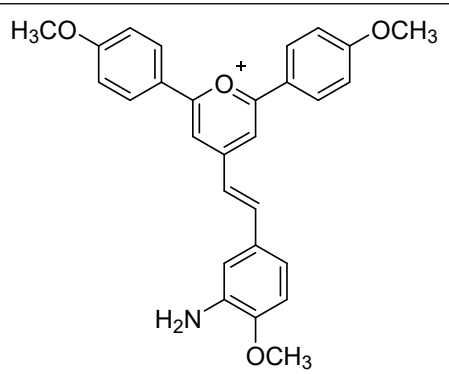
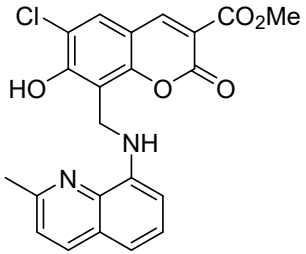
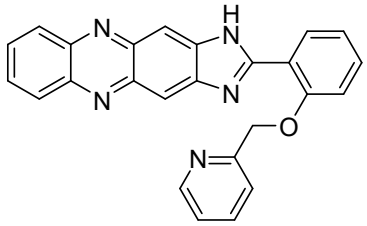
	CH_3CN: PBS (v/v 1:9)	581 nm	242 nM	1 min	Anal. Chem. 2020, **92**, 5064-5072
	Aqueous: DMSO (v/v 87:13)	535 nm	77.8 nM	--	J. Photochem. Photobiol. B Biol. 2022, **234**, 112512
	PBS buffer solution (pH = 7.4)	1050 nm 1550 nm	610 nM	--	Anal. Chem. 2021, **93**, 15279-15287

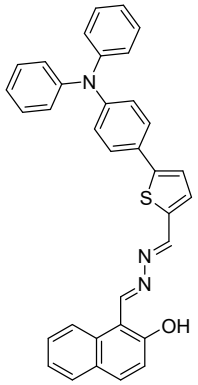
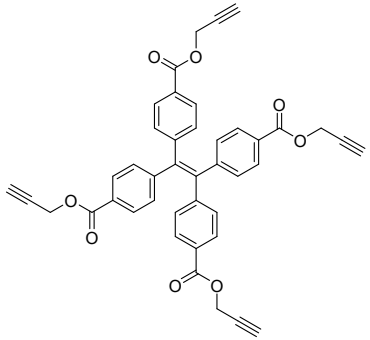
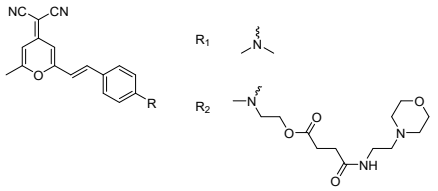
	PBS buffer solution (pH = 7.4)	950 nm	5 μM	--	J. Am. Chem. Soc. 2023, **145**, 7941-7951
	PBS buffer solution (pH = 7)	--	12.7 nM	8 min	J. Am. Chem. Soc. 2023, **145**, 7952-7961
	acetic acid-sodium acetate buffer (containing 5% DMSO)	980 nm	--	30 min	Cell Rep. Phys. Sci. 2022, **3**, 100570

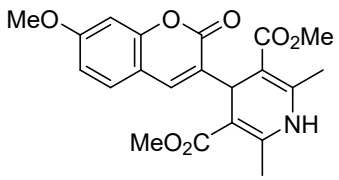
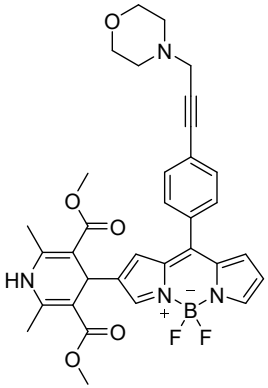
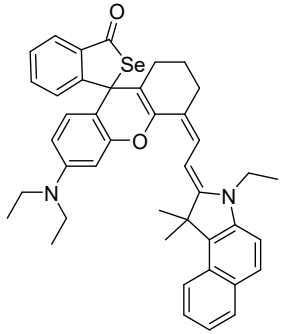
	CH_3CN: PBS (v/v 1:1)	598 nm 720 nm	948 nM	3 min	Med. Chem. Lett. 2022, **59**, 128544
	DMSO: PBS (v/v 3:7)	625 nm 715 nm	56 nM	12 min	Chem. Eng. J. 2023, **463**, 142383
	CH_3CN: PBS (v/v 1:4)	539 nm	3.3 nM	< 1 min	Analyst 2020, **145**, 6125-6129

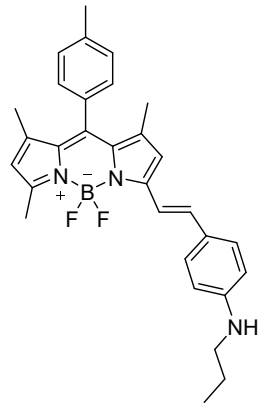
	Dioxane: PBS (v/v 6:4)	638 nm	870 nM	2 min	Chem. Commun. 2020, **56**, 6233-6236
	DMSO: PBS (v/v 99:1)	550 nm	170 nM	10 min	Anal. Chem. 2021, **93**, 4391-4397
	DMSO: PBS (v/v 3:7)	661 nm	17 nM	1 min	Anal. Chem. 2022, **94**, 4072-4077

	HEPES buffer solution (pH = 7)	520 nm	1.6 nM	< 1 min	Sens. Actuators B chem. 2023, **397**, 134654
	CH_3CN: PBS (v/v 1:9)	530 nm	5.52 nM	12 min	Spectrochim. Acta. A Mol. Biomol. Spectrosc. 2022, **277,** 121280
	PBS buffer solution (pH = 7.4)	559 nm	57 nM	0.65 min	Anal. Chem. 2019, **92**, 699-706

	DMF: PBS (v/v 1:9)	585 nm	88 nM	20 min	J. Mater. Chem. B 2021, **9**, 9885-9892
	DMSO: TBS (v/v 1:99)	450 nm	--	> 9 min	J. Phys. Org. Chem. 2019, **32**, e3896
	CH_3CN: HEPES (v/v 1:1)	560 nm	--	--	ACS Appl. Bio. Mater. 2019, **2**, 1944-1955

	DMSO: HEPES (v/v 6:4)	640 nm	12.9 nM	1 min	Anal. Methods 2022, **14**, 4537-4544
	MeOH: H_2O (v/v 1:1)	500 nm	15 ppm	--	Photobiol. A Chem. 2020, **388**, 112132
	Ethanol: PBS (v/v 1:9)	633 nm	340 nM	10 min	Anal. Chem. 2023, **95**, 6279-6286

	DMSO: PBS (v/v 2:8)	423 nm	74 nM	30 min	Photobiol. Sci. 2020, **19**, 1230-1235
	PBS buffer solution (pH = 7)	535 nm	47 nM	20 min	Sens. Actuators B chem. 2021, **339**, 129880
	Ethanol: HEPES (v/v 1:9)	780 nm	28 nM	5 min	Sens. Actuators B chem. 2023, **374**, 132790

	CH_3CN: PBS (v/v 3:7)	577 nm	98.7 nM	60 s	this work

"--" Not mentioned

2. Materials and Methods

The probe was weighed to 4.84 mg, and a test masterbatch was prepared at a concentration of 1 mM in 10 mL of dimethyl sulfoxide (DMSO). A saturated NO aqueous solution was generated by introducing concentrated sulfuric acid into an aqueous solution of high-concentration sodium nitrite, resulting in the formation of NO, nitrogen dioxide (NO_2), and other gases. This mixture was subsequently filtered through a saturated sodium hydroxide (NaOH) aqueous solution to remove NO_2 gas. The purified NO gas was then bubbled into 5 mL of deoxygenated deionized water for 30 minutes to yield a saturated NO aqueous solution with a concentration of 1.9 mM.[S1]

Hydroxyl radicals (•OH) were generated via the Fenton reaction by dissolving 55.6 mg of $FeSO_4 \cdot 7H_2O$ in 10 mL of phosphate-buffered saline (PBS) and adding 20 mM of hydrogen peroxide (H_2O_2), achieving a final concentration of 10 mM. Superoxide radicals ($O_2^{\bullet -}$) were prepared by dissolving 7.1 mg of potassium superoxide in 10 mL of DMSO, resulting in a concentration of 10 mM. Hypochlorite ions (ClO^-) were prepared by diluting sodium hypochlorite (NaClO) with deionized water, with the concentration corrected by measuring the absorbance at 209 nm ($\varepsilon_{209\ nm} = 350\ M^{-1}\ cm^{-1}$) prior to use.[S2]

Solutions of other small molecule analytes were prepared either by using their corresponding salts or by acquiring pure substances and subsequently diluting them with deionized water. All spectroscopic tests were performed in PBS: CH_3CN (V: V = 7: 3, 0.1 M, pH = 7.4).

References

[S1] J. Hartung, *Chem. Rev*., 2009, **109**, 4500-4517.

[S2] Z. Q. Mao, H. Jiang, Z. Li, C. Zhong, W. Zhang, and Z. H. Liu, *Chem. Sci*., 2017, **8**, 4533-4538.

3. pH stability experiments with BDD

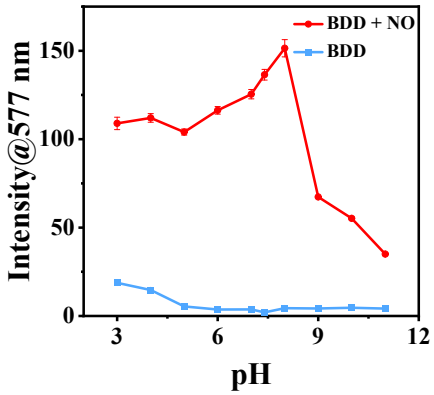


Fig. S1. Fluorescence spectra of 5 μM probe BDD and BDD + NO at different pH (3 - 11).

4. Cytotoxicity of BDD

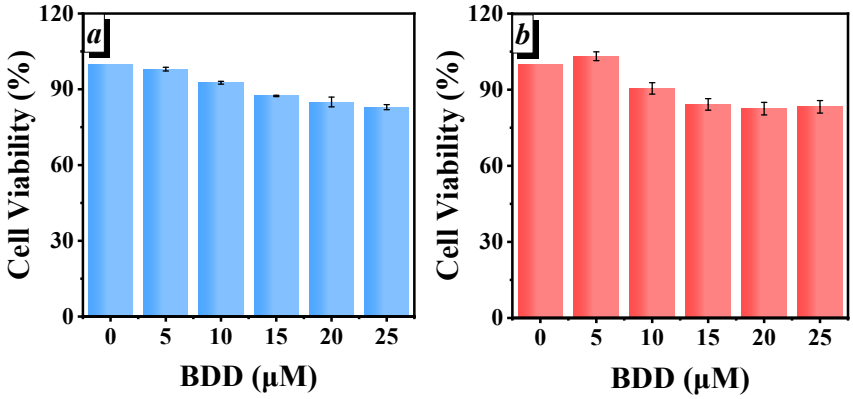


Fig. S2. Toxicity assessment of BDD at different concentrations (0-25 μM) tested by CCK-8 assay on (a) HepG2 cells and (b) RAW 264.7 cells.

5. Mass spectrometry characterization

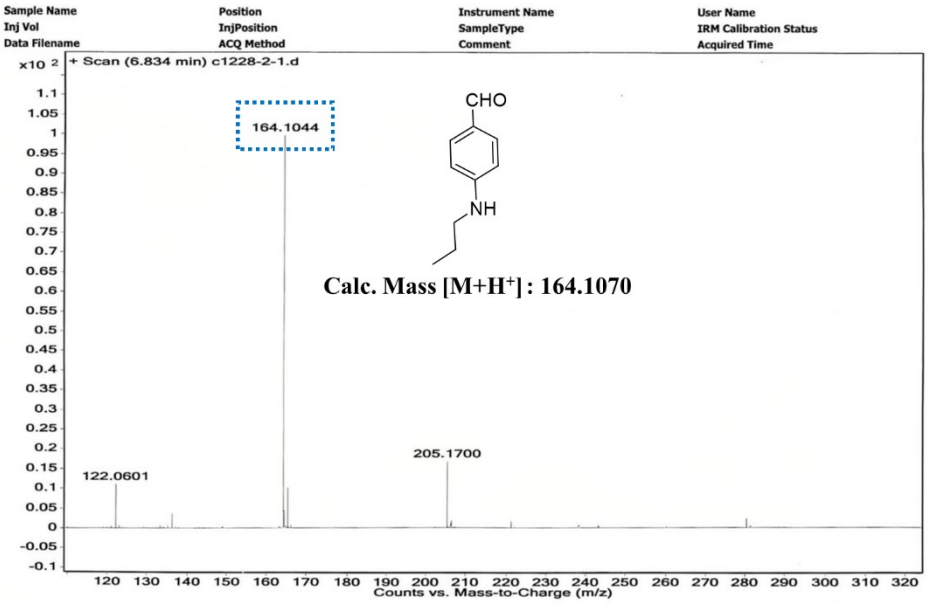


Fig. S3. Mass spectra of compound 3 in solution (pH 7.4).

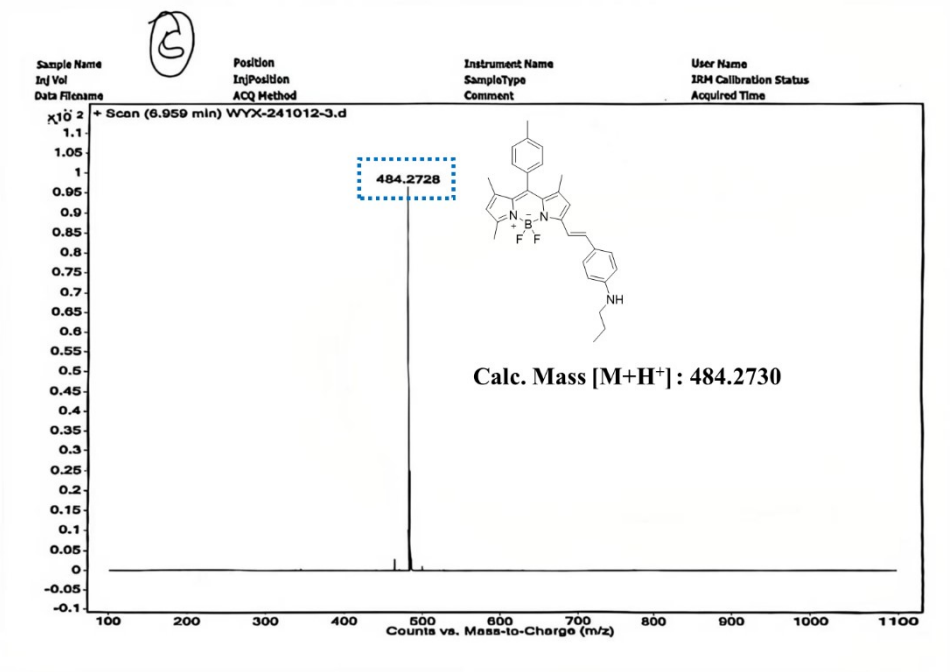
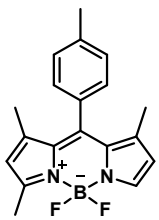
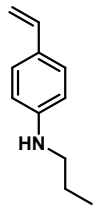


Fig. S4. Mass spectra of BDD in solution (pH 7.4).

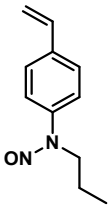
6. Atom coordinates for structures of model compounds



C	-5.034495	-3.901871	-0.000336
C	-1.326750	-1.893790	-0.002269
C	-0.787646	-4.553092	0.000959
C	-3.117136	-5.763325	0.001574
C	0.257130	0.216709	-0.002340
C	-0.737841	2.677693	-0.002257
C	-3.796575	5.546231	-0.004115
H	-5.712166	6.254647	-0.003548
C	-1.501146	6.878576	-0.009588
C	0.453298	5.107531	-0.008930
H	-3.436390	-7.781798	0.003919
H	-1.290533	8.910311	-0.014469
C	3.047731	-0.138528	-0.001777
C	4.376263	-0.323959	-2.276824
C	4.380528	-0.270339	2.276231
C	6.991059	-0.636946	-2.267821
H	3.353869	-0.211538	-4.051708
C	6.993798	-0.583904	2.270743
H	3.360332	-0.116015	4.049264
C	8.342683	-0.776779	0.001680
H	7.999156	-0.770596	-4.051536
H	8.004680	-0.675843	4.055759
C	11.165297	-1.171183	0.008396
H	11.635109	-3.180763	0.199144
H	12.024982	-0.499685	-1.746308
H	12.061535	-0.179814	1.585322
N	-3.956980	-1.601187	-0.002613
N	-3.349022	3.051463	0.000106
B	-5.416240	0.960634	0.004456
C	1.728738	-5.847856	0.004135
H	2.849547	-5.360266	-1.663061
H	2.866484	-5.319634	1.647056
H	1.463533	-7.895821	0.029796
C	3.215550	5.728492	-0.016347
H	4.183543	4.972547	1.646536
H	4.181998	4.939364	-1.664547
H	3.472216	7.777654	-0.036652
C	-7.829240	-4.245323	0.001357
H	-8.320639	-6.248074	0.000123
H	-8.656552	-3.328784	1.659583
H	-8.659328	-3.325793	-1.653751
F	-6.900459	1.158046	-2.151692
F	-6.886835	1.152486	2.170527



C	3.537488	2.020274	0.288278
C	0.952343	2.280827	0.567379
C	-0.664494	0.161570	0.468489
C	0.432052	-2.224453	0.072483
C	3.037982	-2.454045	-0.196317
C	4.660408	-0.361876	-0.100682
H	4.713584	3.698572	0.371858
H	0.139060	4.142562	0.876267
H	-0.742391	-3.898938	-0.023507
H	3.841410	-4.320111	-0.501100
C	7.387731	-0.730968	-0.407226
H	7.950381	-2.690225	-0.701659
C	9.206115	1.029062	-0.363941
H	11.174545	0.516895	-0.615237
H	8.812638	3.022705	-0.079313
C	-5.043923	-1.487065	0.191113
H	-4.760446	-2.189693	-1.753055
H	-4.735791	-3.099250	1.457316
N	-3.238878	0.482283	0.819069
H	-3.854664	2.260764	0.506508
C	-7.762466	-0.553441	0.494190
H	-9.020654	-2.181340	0.263507
H	-8.023599	0.127968	2.434033
C	-8.518531	1.505340	-1.396480
H	-10.506862	2.022637	-1.168550
H	-7.415456	3.242066	-1.160561
H	-8.247832	0.858051	-3.343704



C	3.382238	1.173758	1.404395
C	0.805783	1.614629	1.212390
C	-0.596052	0.452830	-0.715758
C	0.627727	-1.155116	-2.418306
C	3.209167	-1.591514	-2.195206
C	4.647788	-0.432046	-0.293400

H	4.425038	2.081792	2.917418
H	-0.126409	2.856811	2.538758
H	-0.429736	-2.058274	-3.922700
H	4.135570	-2.846850	-3.529258
C	7.370845	-0.947339	-0.156711
H	8.053301	-2.314499	-1.535554
C	9.042509	0.076992	1.439823
H	11.022203	-0.448087	1.369912
H	8.517763	1.466220	2.855113
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H	-6.841850	-0.462690	-1.481834
H	-4.563746	-2.094442	-3.290703
N	-3.253925	0.814311	-0.912200
C	-4.810991	-3.366042	0.607645
H	-6.162956	-4.846671	0.084290
H	-2.934467	-4.240568	0.561113
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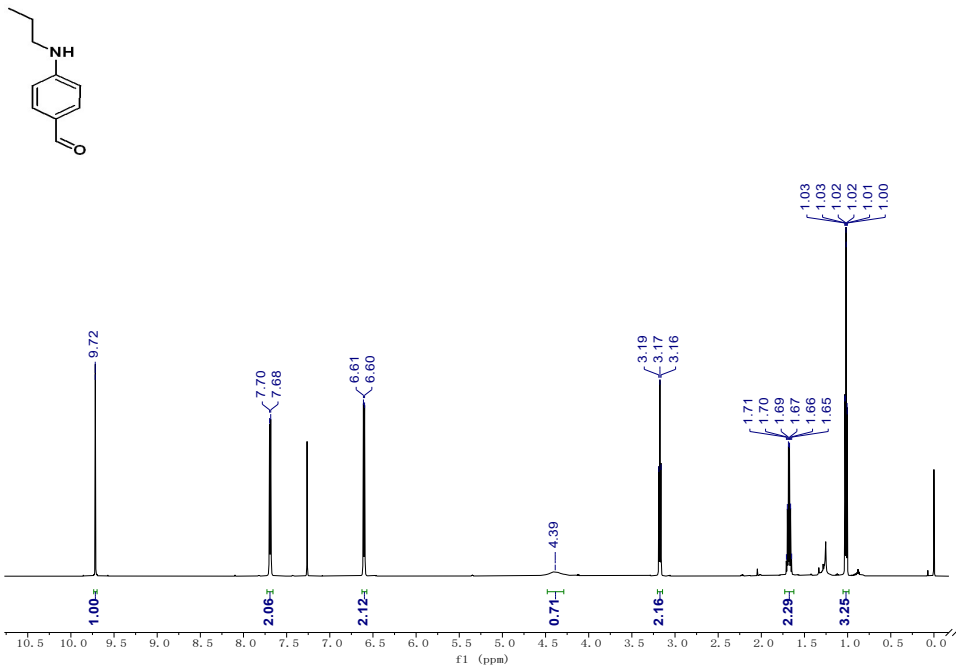
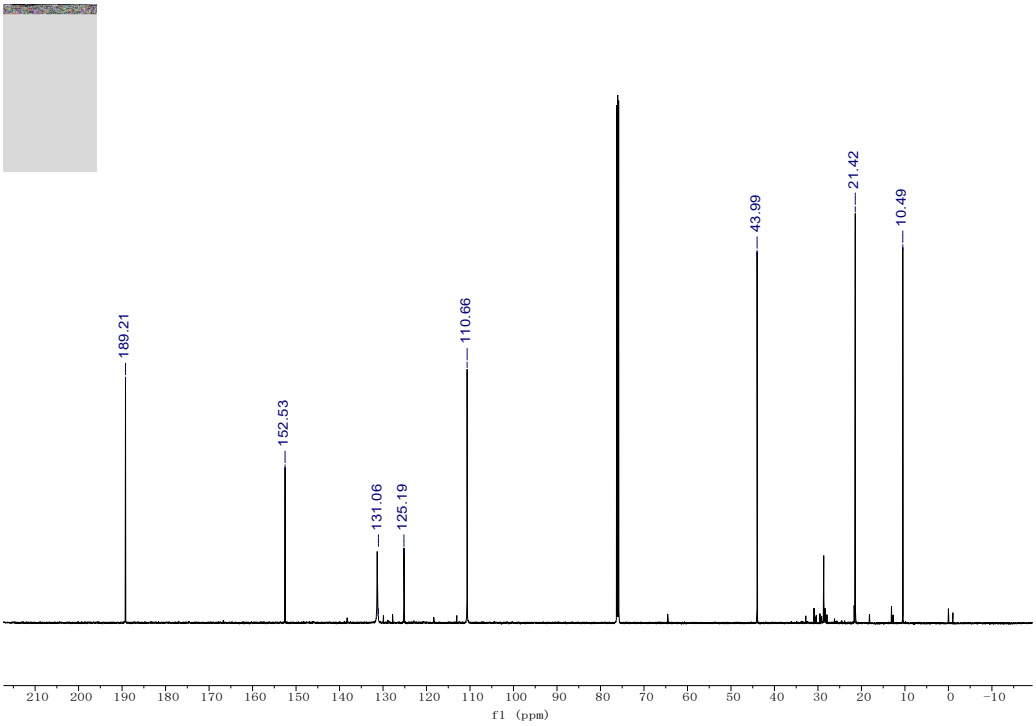
H	-5.350946	-3.919125	4.644122
H	-3.970162	-0.983404	3.852292
H	-7.234500	-1.474790	3.347698
N	-4.445430	3.077293	-0.648795
O	-3.090318	4.899309	-0.234289

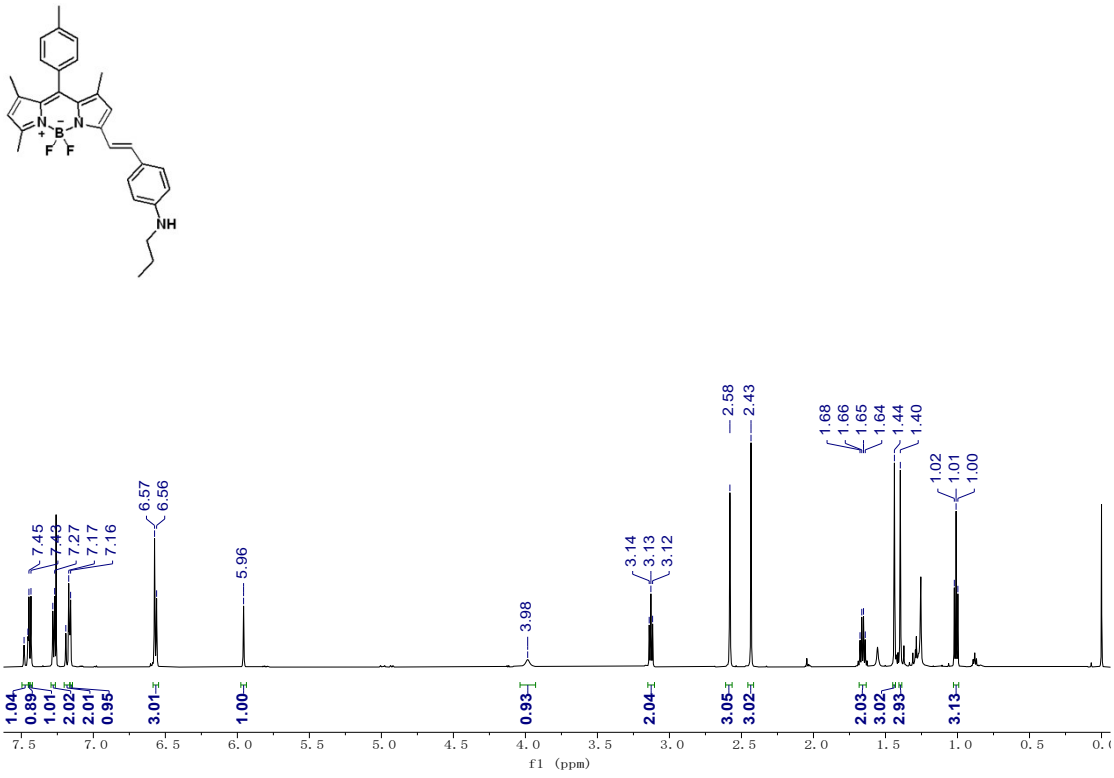
7. Copies of NMR spectra for compounds 3 and BDD



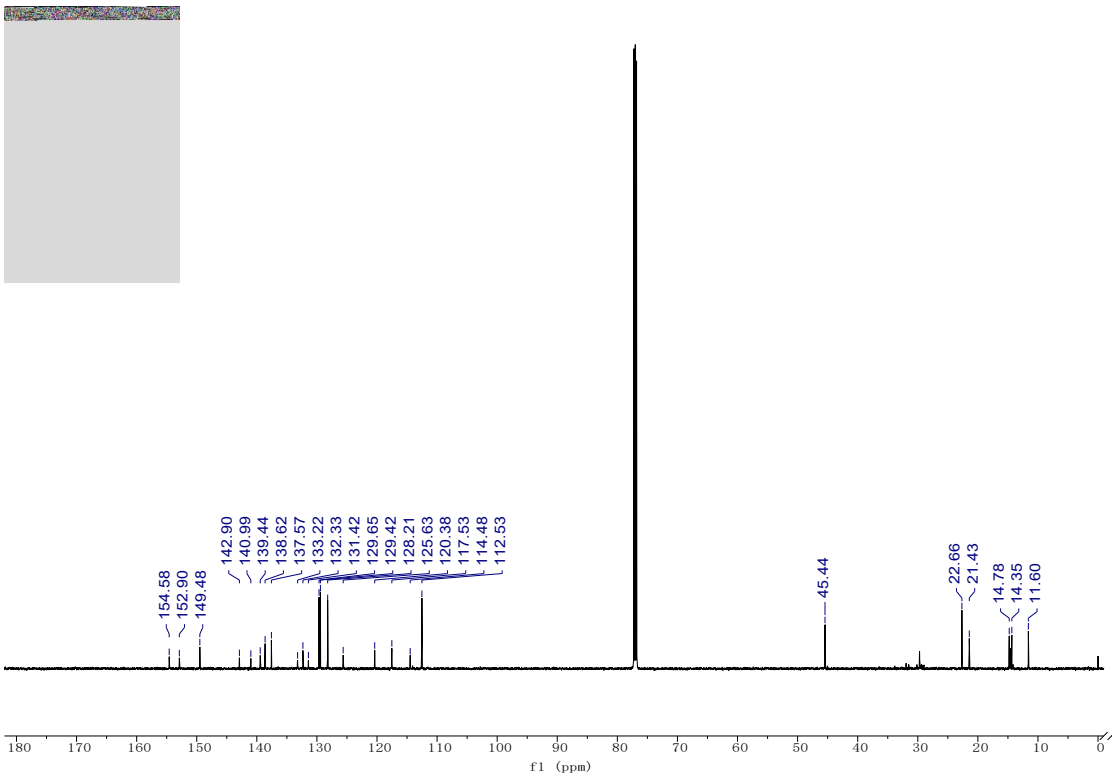
The ^{1}H NMR spectrum of compound 3 ($CDCl_3$, 600 MHz).



The ^{13}C NMR spectrum of compound 3 ($CDCl_3$, 151 MHz).



The ^{1}H NMR spectrum of compound BDD ($CDCl_3$, 600 MHz).



The ^{13}C NMR spectrum of compound BDD ($CDCl_3$, 151 MHz).