Supporting Information

A turn-on bis-BODIPY chemosensor for copper recognition based on *in-situ* generation of benzimidazole–triazole receptor and its applications in bioimaging

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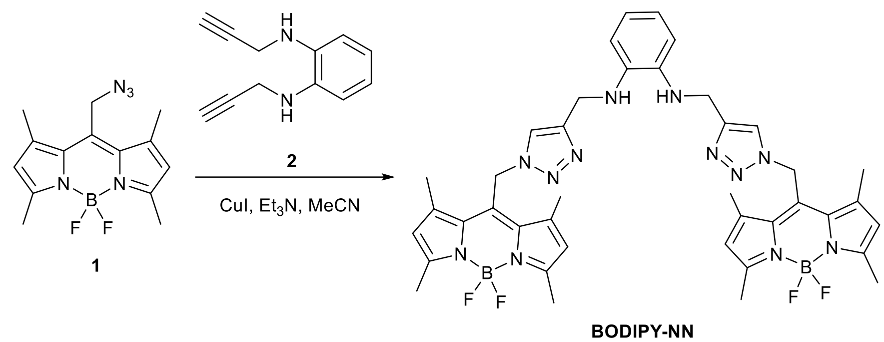
Contents

1. Synthesis and Characterization

1a. Synthesis



Scheme 1. Synthesis of **BODIPY**-**NN**.

BODIPY-**NN**

In the schlenk tube, **1** (140 mg, 0.46 mmol) and **2** (40 mg, 0.22 mmol) were stirred in 5 mL of MeCN at room temperature under inert atmosphere. To this mixture was added 10 mol% of CuI and 40 mol% of Et_3N. The reaction mixture was stirred for 18 h, poured into water, and extracted with 3 × 30 mL CH_2Cl_2. The organic layer was then collected, washed with brine, and dried over anhydrous Na_2SO_4. After solvent removal, the crude product was purified by column chromatography using a gradient elution (5–30% EtOAc in CH_2Cl_2) containing 1% of Et_3N. **BODIPY**-**NN** was obtained as a red solid in 63% yield (110 mg, 0.140 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (s, 2H), 6.71–6.68 (m, 2H), 6.63–6.59 (m, 2H), 6.08 (s, 4H), 5.77 (s, 4H), 4.33 (s, 4H), 3.90 (br s, 2H), 2.54 (s, 12H), 2.17 (s,12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.80, 146.45, 141.59, 136.97, 132.39, 130.04, 123.16, 120.77, 120.01, 113.20, 45.73, 40.41, 15.85, 14.91. HRMS (ESI) m/z: calcd. for $C_{40}H_{45}B_2F_4N_{12}$ $[M+H]^+$, 791.4007; found, 791.4010.

1b. NMR Spectra

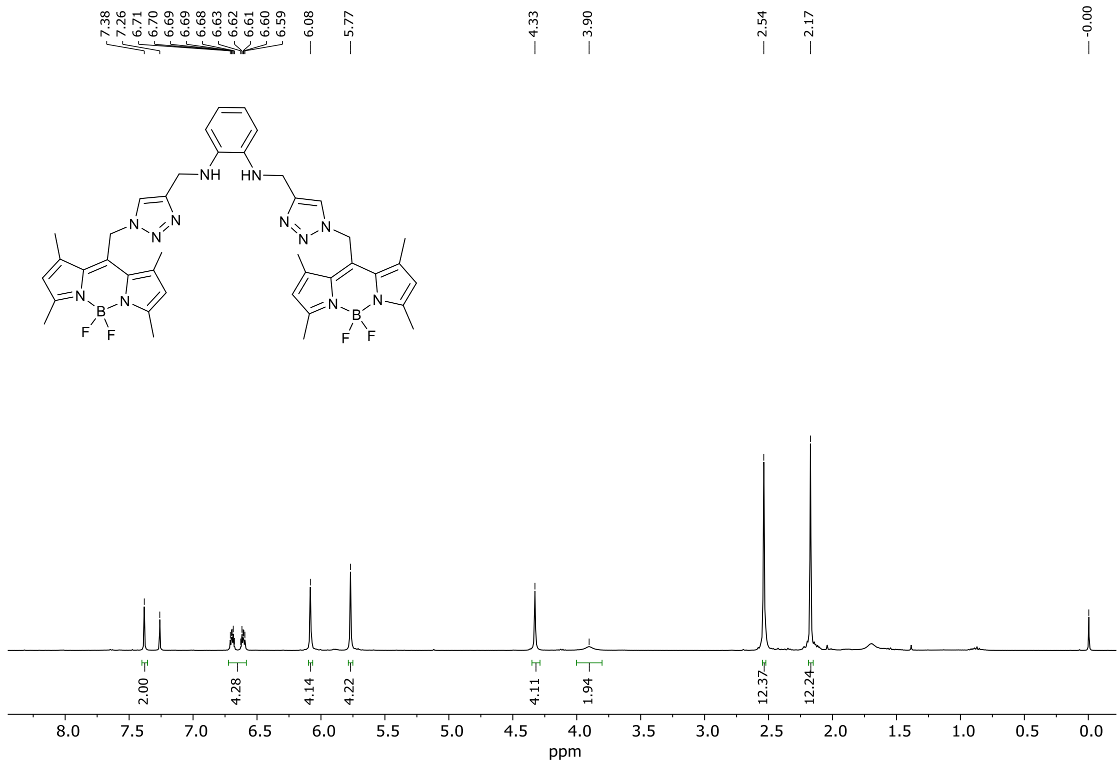


Figure S1. ^{1}H NMR spectrum (400 MHz) of **BODIPY**-**NN** in $CDCl_3$.

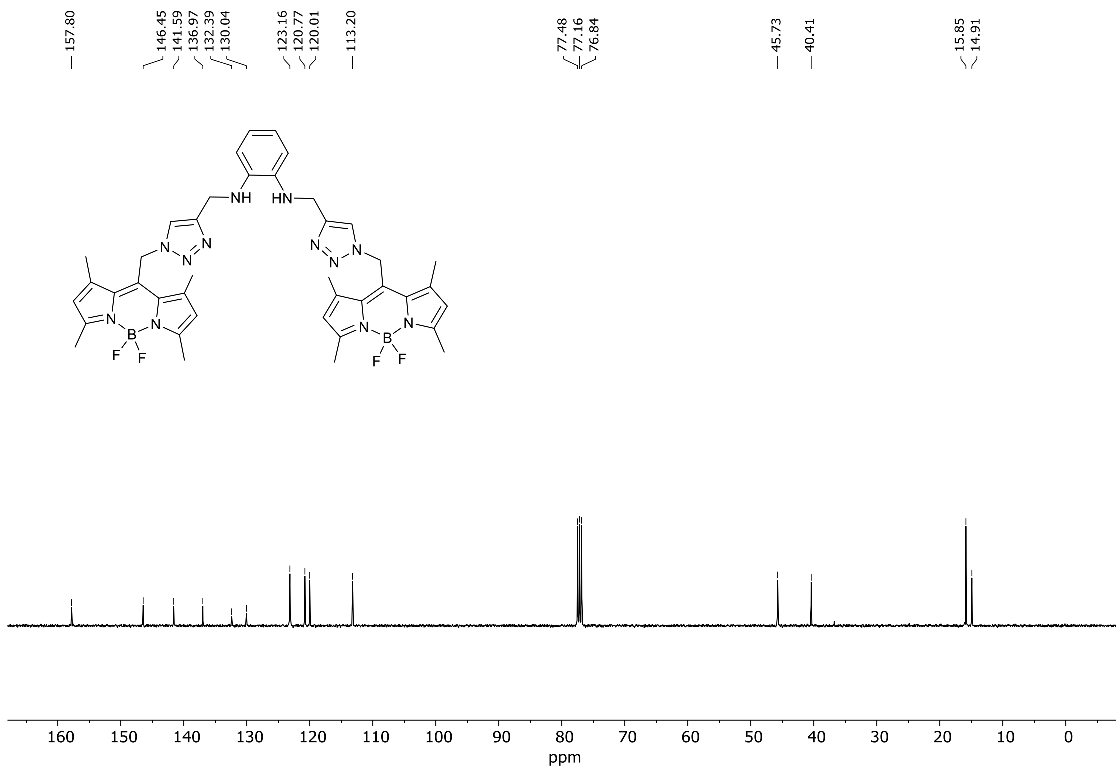


Figure S2. ^{13}C NMR spectrum (100 MHz) of **BODIPY**-**NN** in $CDCl_3$.

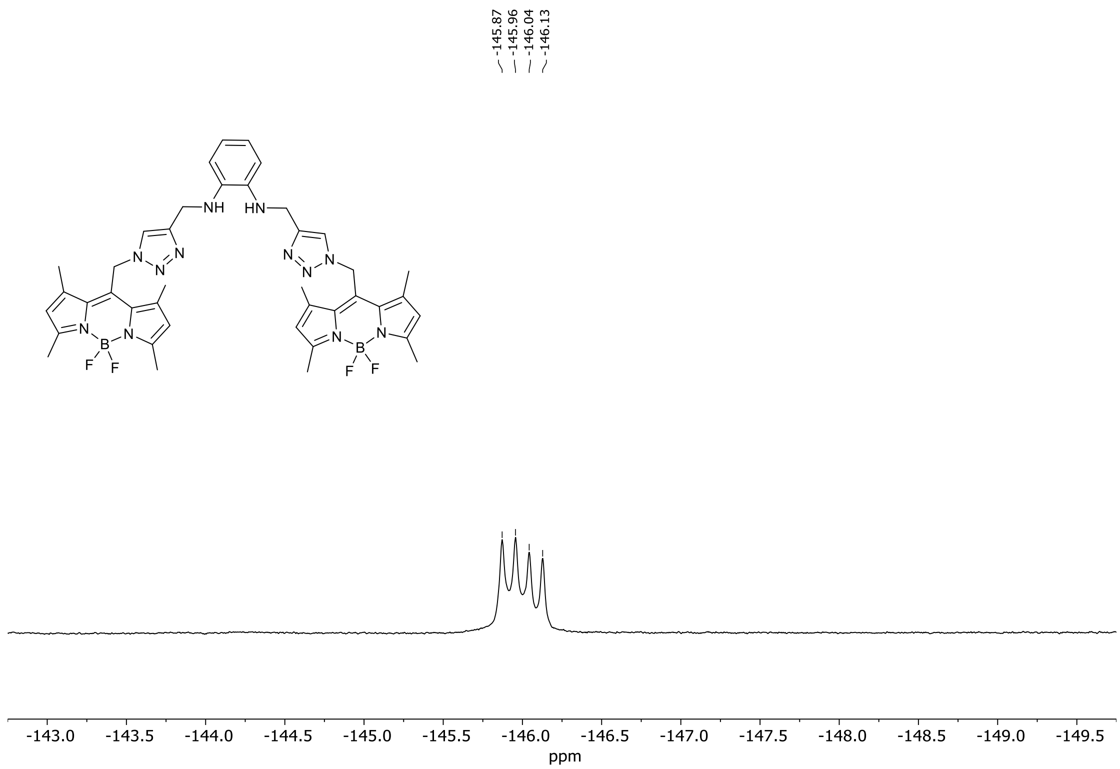


Figure S3. ^{19}F NMR spectrum (376 MHz) of **BODIPY**-**NN** in $CDCl_3$.

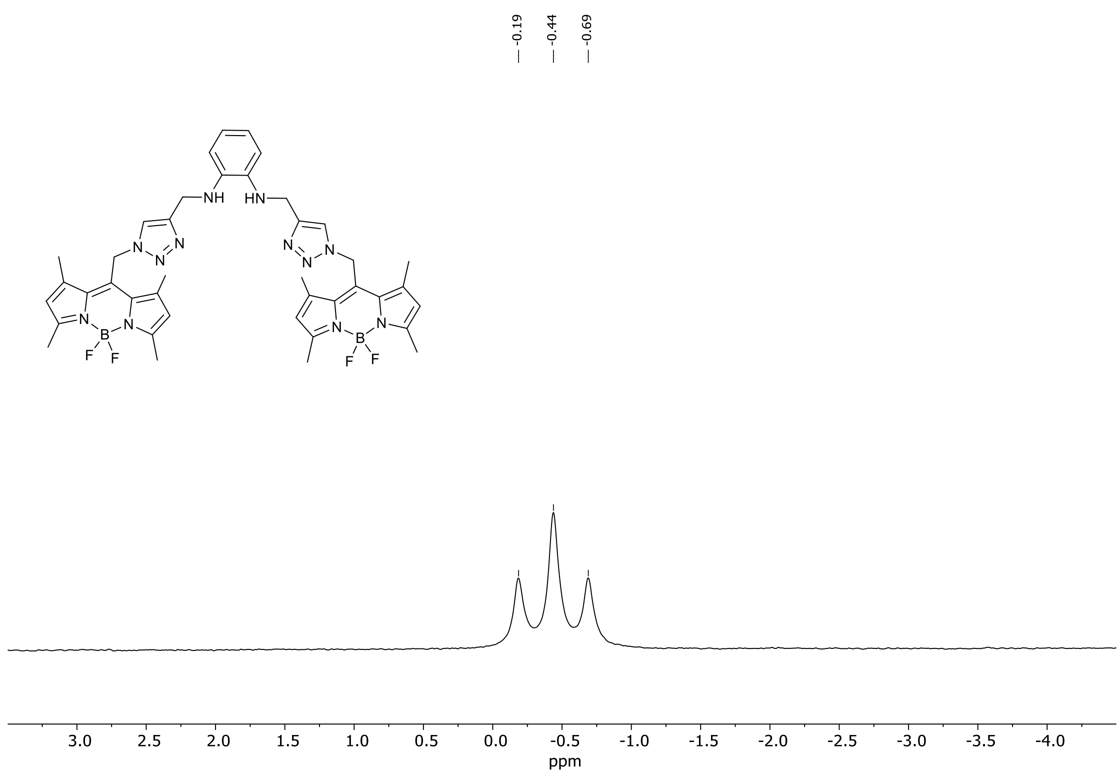


Figure S4. ^{11}B NMR spectrum (128 MHz) of **BODIPY**-**NN** in $CDCl_3$.

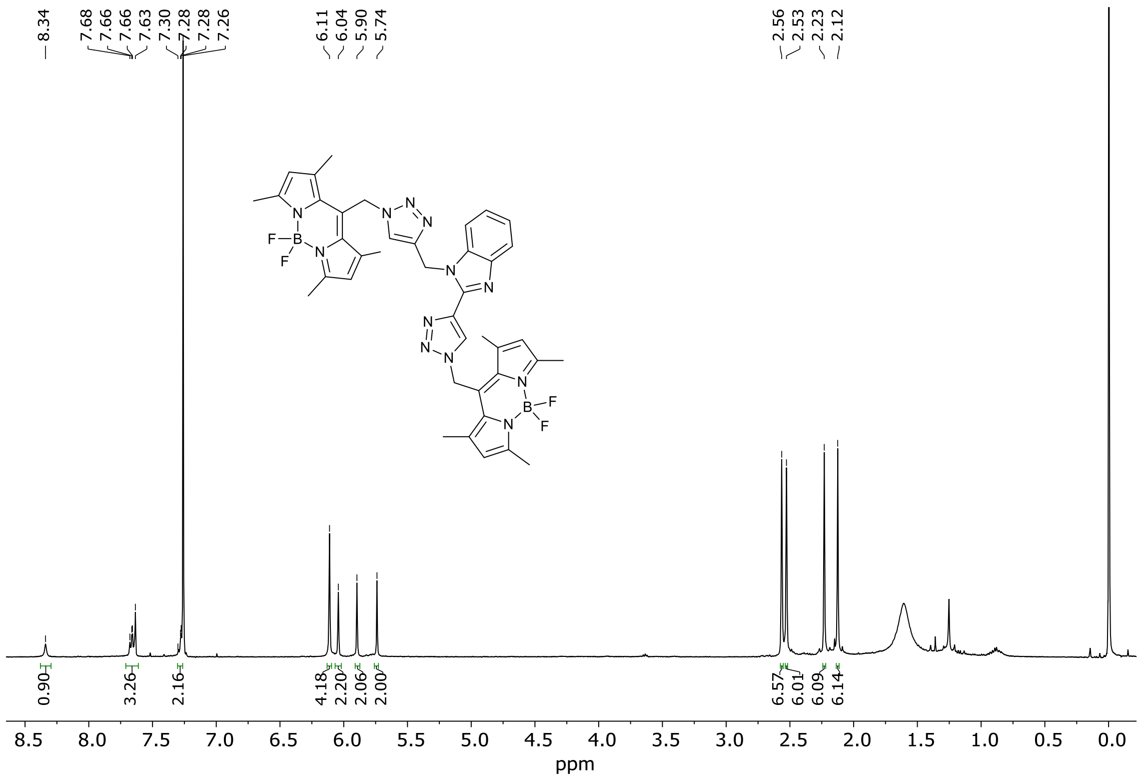


Figure S5. ^{1}H NMR spectrum (400 MHz) of **BTB** in $CDCl_3$.

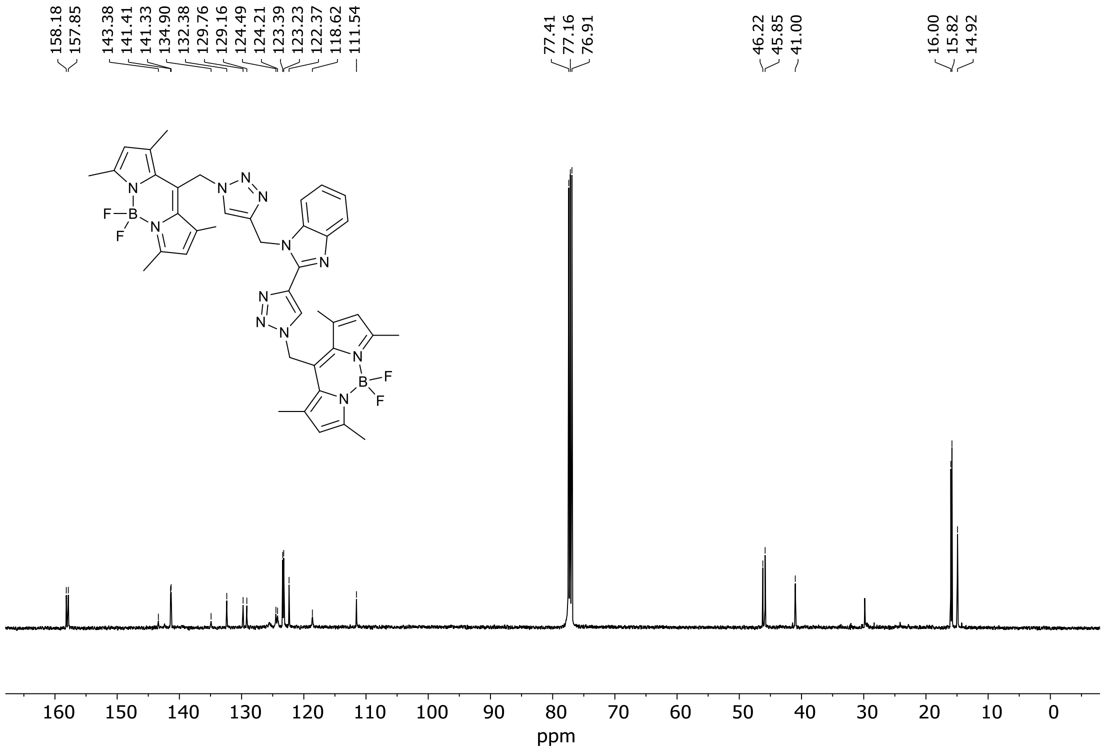


Figure S6. ^{13}C NMR spectrum (100 MHz) of **BTB** in $CDCl_3$.

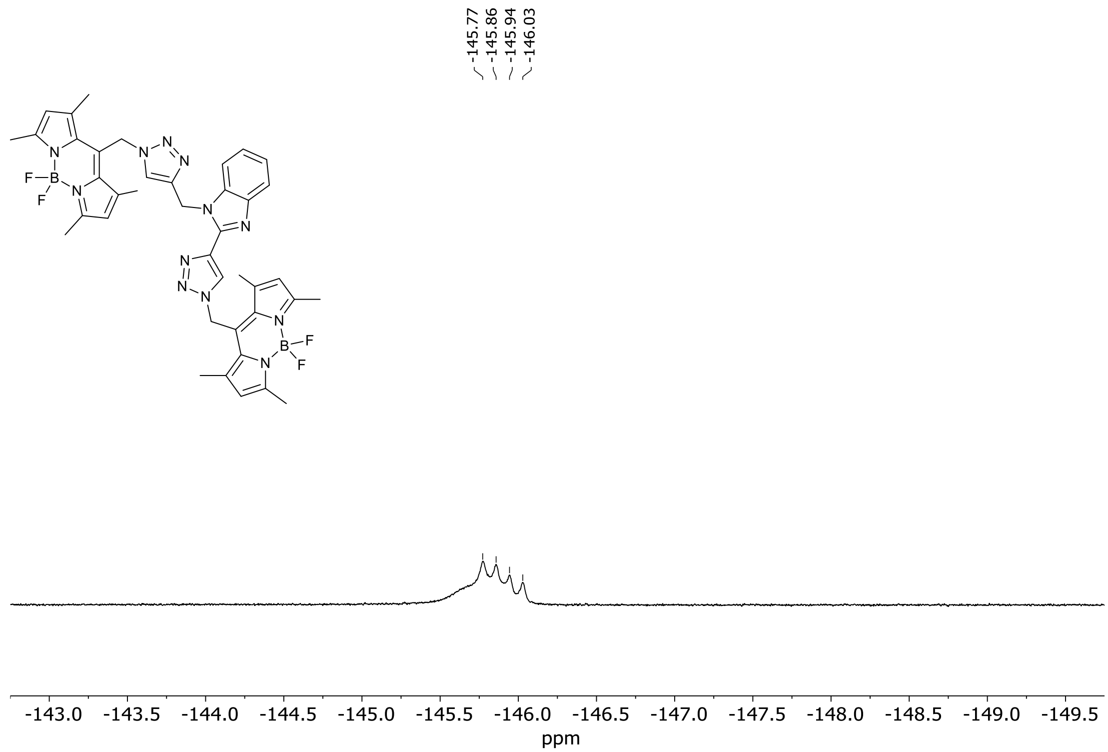


Figure S7. ^{19}F NMR spectrum (376 MHz) of **BTB** in $CDCl_3$.

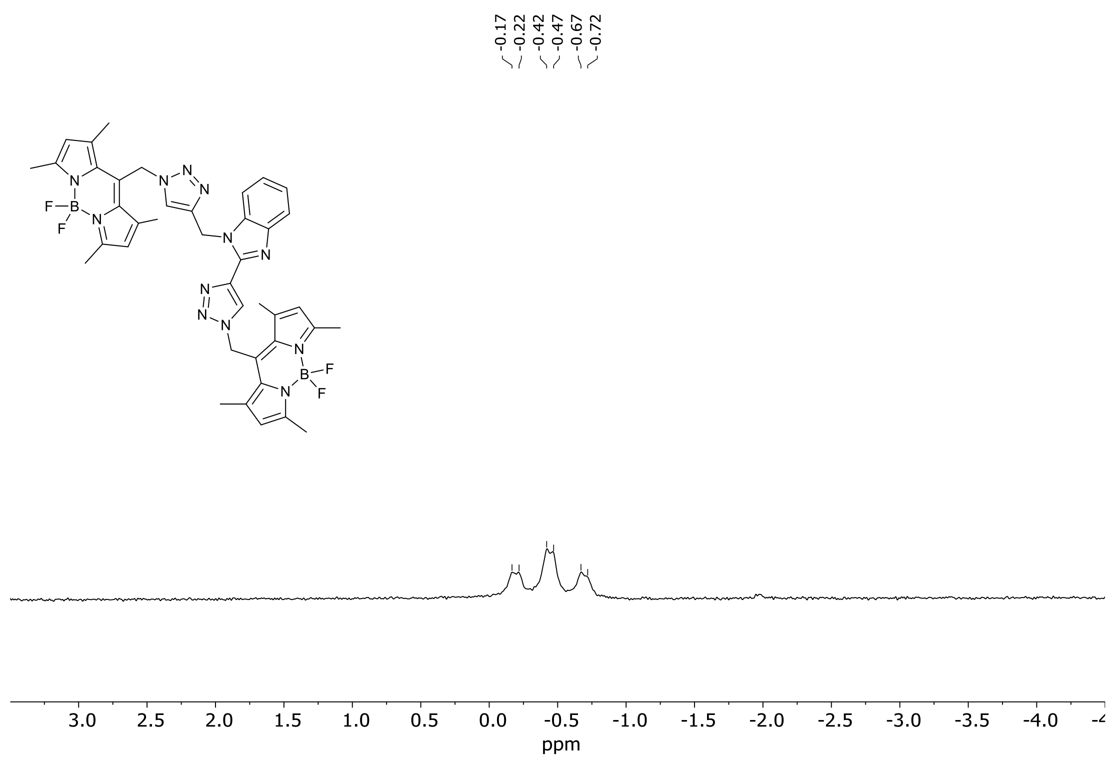


Figure S8. ^{11}B NMR spectrum (128 MHz) of **BTB** in $CDCl_3$.

1c. Mass spectra

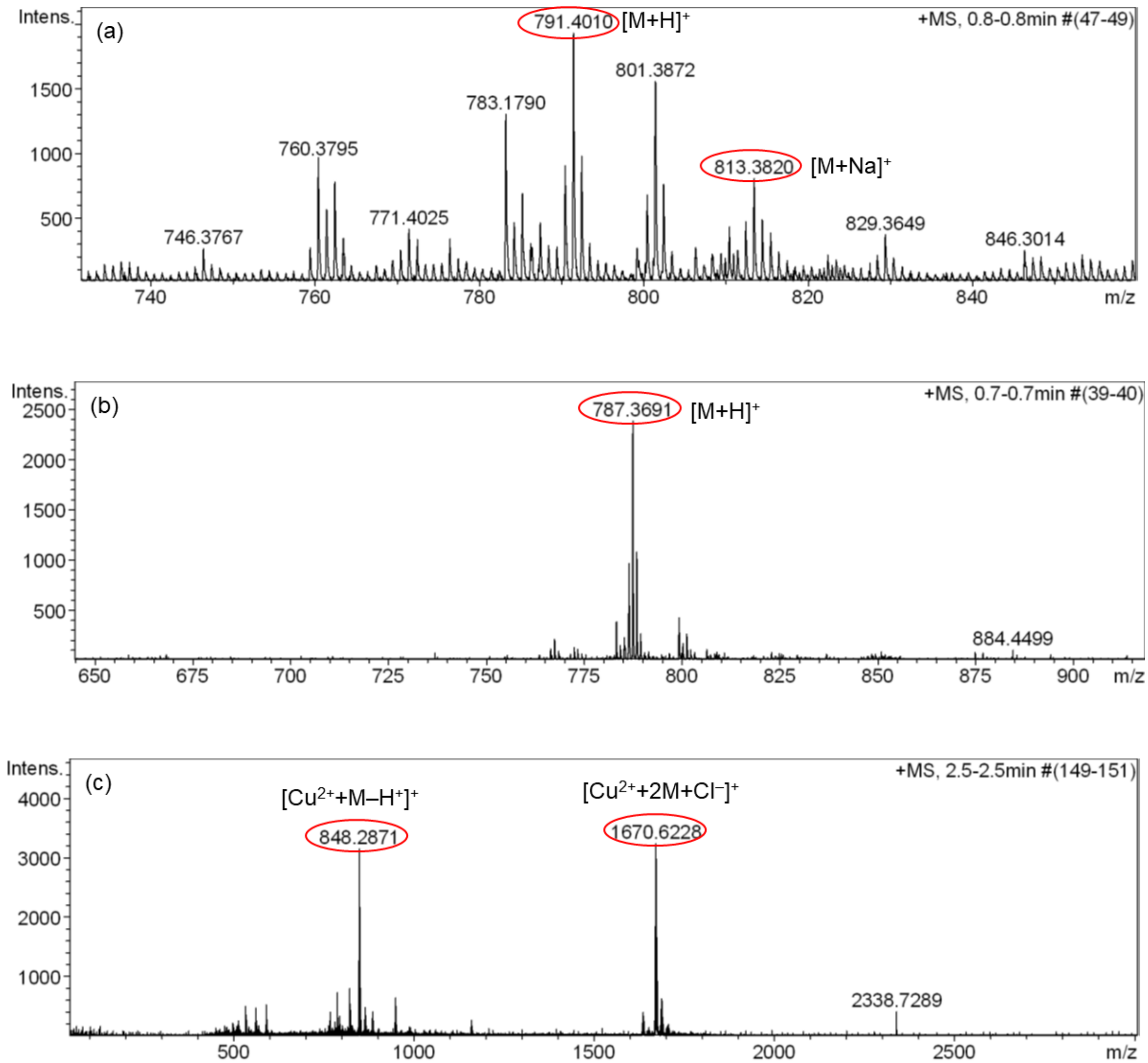


Figure S9. ESI-MS spectra of (a) **BODIPY**-**NN**, (b) **BTB**, and (c) **BODIPY**-**NN** + $CuCl_2$ (1 equiv.).

1d. ESR spectra

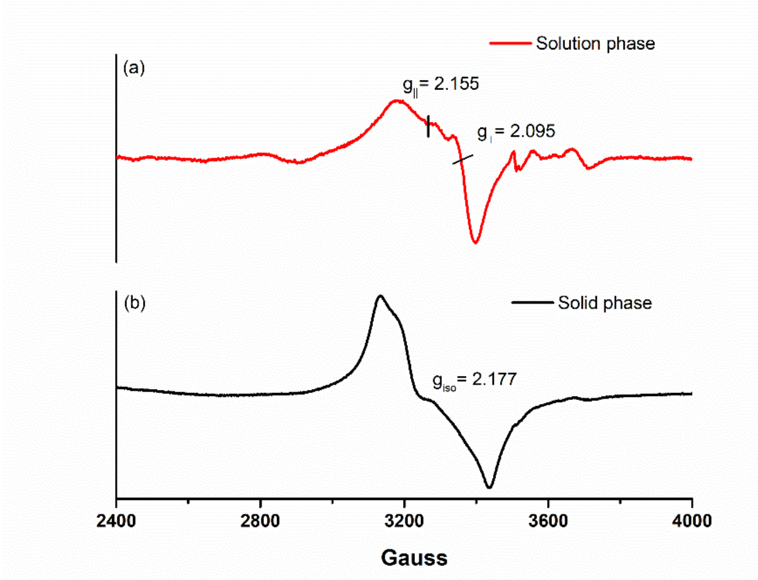


Figure S10. ESR spectra of Cu-**BTB** complex (a) in CH_3CN solution and (b) in solid form.

2. Photophysical and sensing studies

2a. Absorption studies of BODIPY-NN in the presence of different metal ions

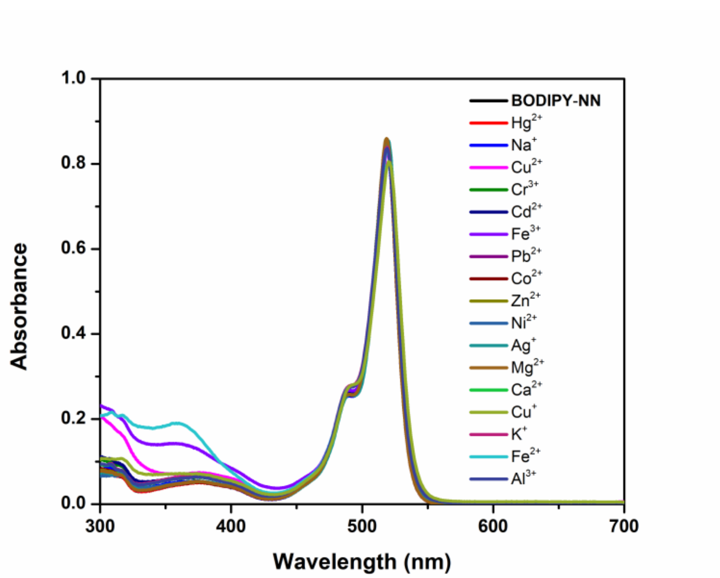


Figure S11. Absorption spectra of **BODIPY-NN** (5 μM) upon addition of different metal ions (10 equiv.) in THF/water (9:1).

2b. Determination of fluorescence quantum yield

Emission quantum yield was calculated by the following equation: $\Phi_x = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$

where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, Grad stands for the gradient from the plot of integrated fluorescence intensity vs absorbance, and η is the refractive index of the solvent. Fluorescein in 0.1 M NaOH (Φ = 0.95) was used as the standard.

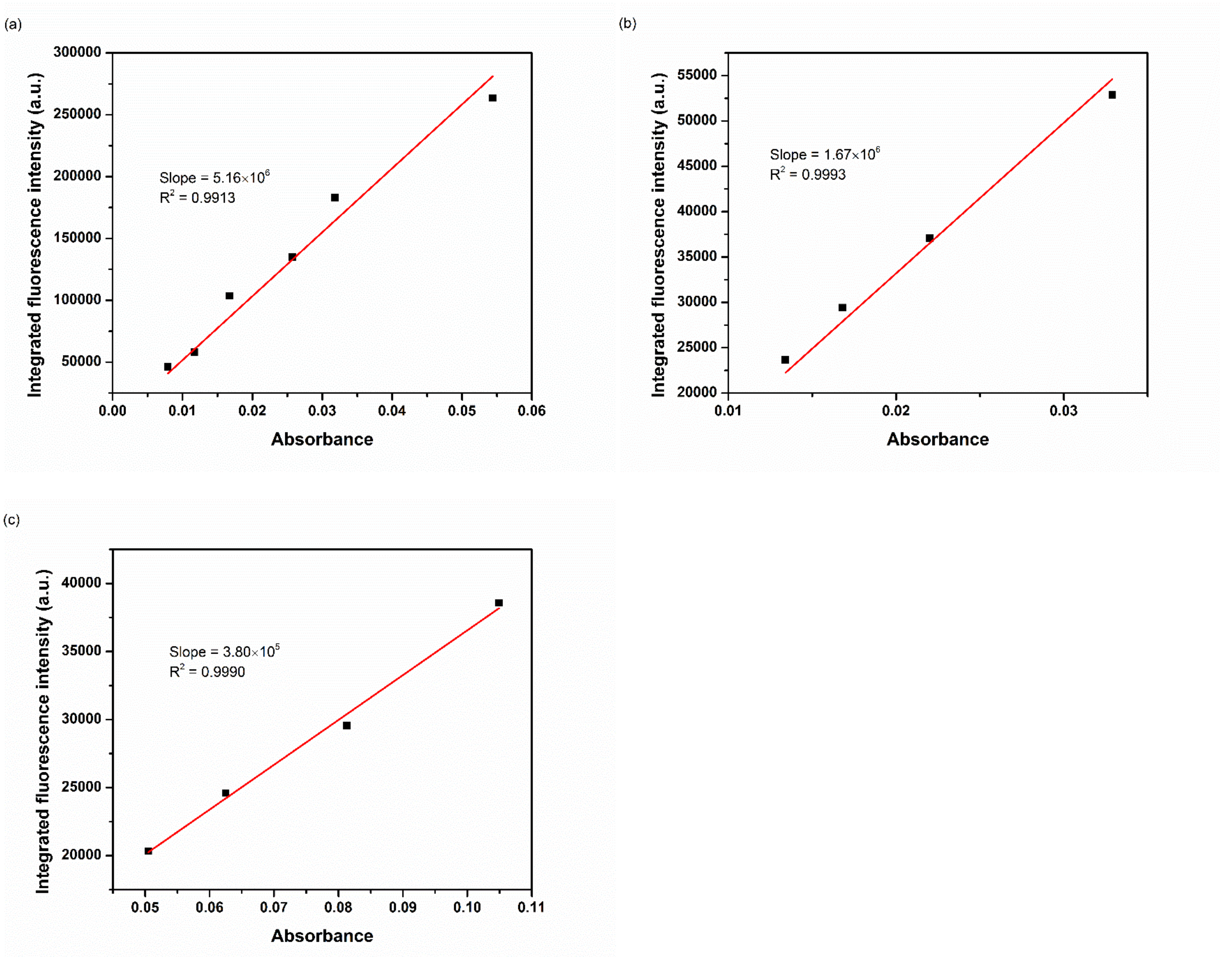


Figure S12. Plots of integrated fluorescence intensity of (a) fluorescein, (b) **BODIPY**-**NN** in the presence of 15 equiv. of Cu^{2+}, and (c) **BODIPY**-**NN** against absorbance.

2c. Calculation of limit of detection

The limit of detection (LOD) of the fluorescent probe **BODIPY**-**NN** for Cu^{2+} was determined from the following equation: LOD = $3\sigma/K$ where σ is the standard deviation of the blank solution and K is the slope of the calibration curve.

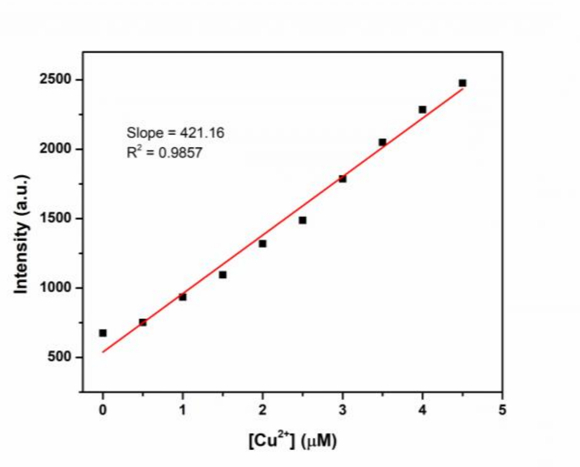


Figure S13. Linear relationship between fluorescence intensities of **BODIPY-NN** and concentrations of Cu^{2+}.

2d. Time-dependent fluorescence change studies

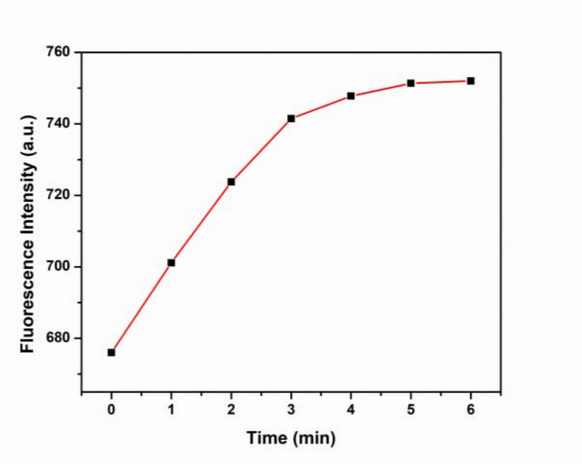


Figure S14. Fluorescence intensity changes of **BODIPY**-**NN** (0.5 μM) versus time in the presence of 1 equiv. of Cu^{2+}.

2e. DFT calculation

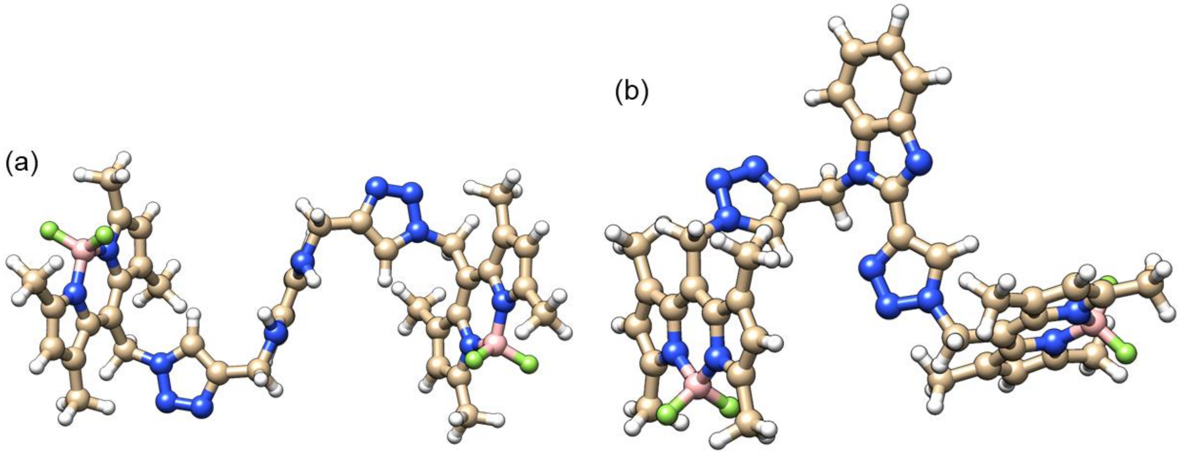
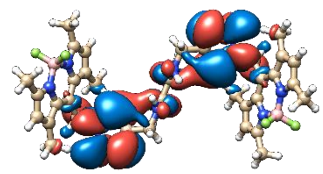
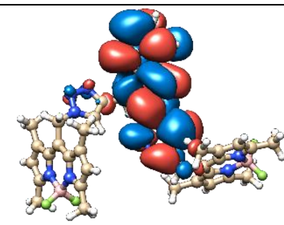
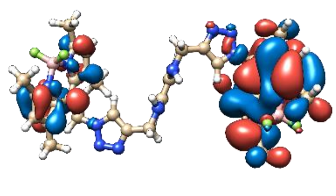
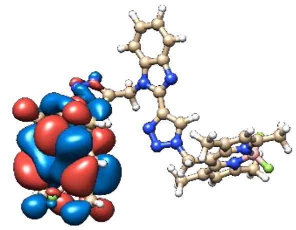
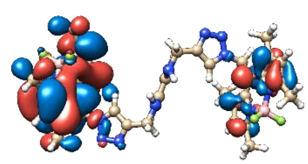
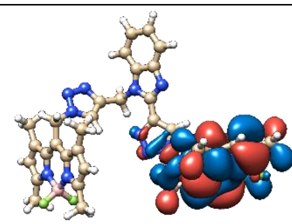
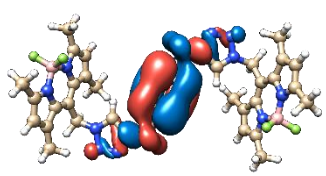
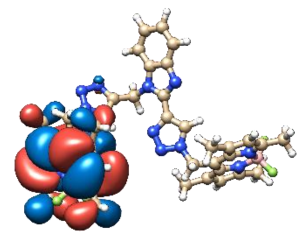
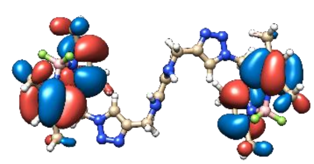
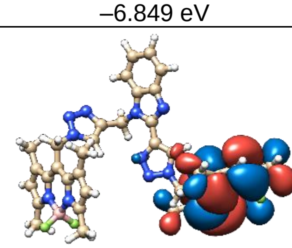
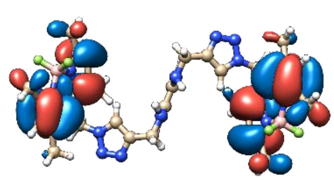
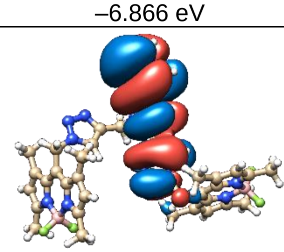


Figure S15. Optimized structures of (a) **BODIPY**-**NN** and (b) **BTB** by B3LYP/6-31G(d).

Table S1. Calculated excitation energies (in eV and nm), oscillator strengths (f), and molecular orbital (MO) compositions for the low-lying excited states of **BODIPY**-**NN** and **BTB** using CAM-B3LYP/6-31G(d).

Compound	**State**	**MO composition**	**Excitation energy (eV)**	**λ_{abs} (nm)**	f
BODIPY-**NN**	S1	H–2→LUMO (16%) H–2→L+1 (33%) H–1→ LUMO (33%) H–1→ L+1 (16%)	2.797	443.33	0.7041
	S2	H-2→LUMO (43%) H–2→L+1 (44%) H–1→ LUMO (6%) H–1→ L+1 (6%)	2.800	442.74	0.5210
BTB	S1	H–1→ LUMO (63%) HOMO→L+1 (35%)	2.783	445.46	0.1152
	S2	H–1→ LUMO (35%) HOMO→L+1 (63%)	2.800	442.80	1.1075

Table S2. Frontier molecular orbitals (MOs), MO energies of **BODIPY-NN** and **BTB**.

	BODIPY-NN	**BTB**
L+2		
	0.997 eV	0.111 eV
L+1		
	–1.780 eV	–1.791 eV
LUMO		
	–1.780 eV	–1.817 eV
HOMO		
	–6.579 eV	–6.849 eV
H–1		
	–6.846 eV	–6.866 eV
H–2		
	–6.846 eV	–7.354 eV

Cartesian coordinates of optimized geometries

BODIPY-NN

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BTB

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H	2.1510520000	-3.8925350000	-1.3674180000
H	2.1140340000	-3.4354940000	-3.0670070000
H	1.6607510000	-2.2603480000	-1.8269460000
C	7.2262050000	-0.3258870000	1.7513780000

C	6.3830370000	-0.1024520000	2.8586720000
H	6.6926210000	0.3311880000	3.8009360000
C	5.1022090000	-0.5312400000	2.5356350000
C	8.6832140000	-0.0262440000	1.6220520000
H	9.2473420000	-0.9426950000	1.4189240000
H	9.0620680000	0.4351130000	2.5373200000
H	8.8620460000	0.6468220000	0.7769870000
C	3.9452540000	-0.4632070000	3.4942940000
H	3.0843970000	0.0850590000	3.0952650000
H	4.2597090000	0.0534580000	4.4059520000
H	3.5947840000	-1.4587350000	3.7967210000
C	2.7834850000	-1.6874200000	0.7809090000
H	2.2800150000	-2.5496210000	0.3504580000
H	2.7322880000	-1.7894940000	1.8636780000
C	2.2636030000	0.7956960000	0.4491530000
H	3.2161630000	1.1913380000	0.7613710000
C	1.1052500000	1.4177860000	0.0158360000
C	0.8698240000	2.8523820000	-0.0678430000
N	1.6324680000	3.7220560000	0.5668400000
C	1.0946010000	4.9581730000	0.2632780000
C	1.5016080000	6.2394710000	0.6614870000
H	2.3679310000	6.3632430000	1.3042020000
C	0.7648030000	7.3271160000	0.2077090000
H	1.0572820000	8.3318510000	0.5004440000
C	-0.3638340000	7.1561890000	-0.6218470000
H	-0.9214240000	8.0308180000	-0.9452760000
C	-0.7885490000	5.8942770000	-1.0283230000
H	-1.6766600000	5.7613750000	-1.6380970000
C	-0.0348560000	4.8073280000	-0.5762720000
N	-0.1560430000	3.4419080000	-0.7889400000
C	-1.1140130000	2.8177360000	-1.7080970000
H	-0.7027880000	1.8565270000	-2.0141390000
H	-1.1893540000	3.4618030000	-2.5894160000
C	-2.4862760000	2.6361390000	-1.1316920000

C	-2.9874760000	1.6265960000	-0.3348600000
H	-2.5345140000	0.7283240000	0.0541860000
C	-5.3179580000	1.3424280000	0.6920690000
H	-6.2612320000	1.7561220000	0.3421670000
H	-5.1918650000	1.6617740000	1.7254010000
C	-5.3010130000	-0.1690870000	0.5924430000
C	-4.5807770000	-0.9078680000	1.5502280000
C	-5.9837950000	-0.7990280000	-0.4597250000
C	-6.7942770000	-0.2901990000	-1.5376270000
C	-7.1925910000	-1.3995640000	-2.2699920000
H	-7.8145280000	-1.3904530000	-3.1559320000
C	-6.6555320000	-2.5559410000	-1.6668310000
C	-6.8154170000	-3.9787920000	-2.0907220000
H	-5.8375630000	-4.4396580000	-2.2649180000
H	-7.4126240000	-4.0418650000	-3.0037020000
H	-7.3012050000	-4.5617220000	-1.3008170000
C	-7.2048980000	1.1160740000	-1.8676010000
H	-7.8879340000	1.5306710000	-1.1135800000
H	-7.7421520000	1.1245130000	-2.8207460000
H	-6.3613040000	1.8077720000	-1.9534780000
C	-3.8152120000	-0.5199380000	2.7069880000
C	-3.3394380000	-1.7005200000	3.2630580000
H	-2.7223570000	-1.7860120000	4.1483980000
C	-3.7928730000	-2.7817040000	2.4811930000
C	-3.5309230000	0.8384610000	3.2855830000
H	-3.0888630000	1.5299480000	2.5597720000
H	-2.8211420000	0.7402870000	4.1124560000
H	-4.4311480000	1.3162320000	3.6936190000
C	-3.5388500000	-4.2397690000	2.6800860000
H	-4.4837480000	-4.7821540000	2.7897590000
H	-2.9231620000	-4.4040170000	3.5679950000
H	-3.0334950000	-4.6632140000	1.8056550000
B	7.0695140000	-1.2661680000	-0.6410610000
B	-5.2003680000	-3.1810210000	0.3624430000

2f. Application in real water samples

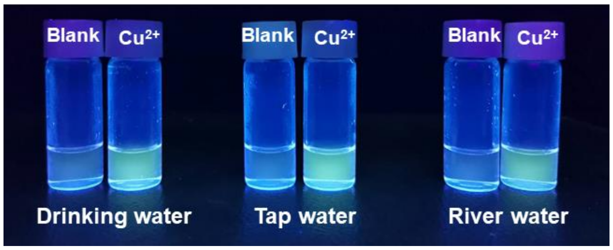


Figure S16. Visual tests were performed on **BODIPY-NN**. Blank solution contains 0.5 mL of **BODIPY-NN** (5 µM) in THF and 0.5 mL of real water samples. Test solution contains 0.5 mL of **BODIPY-NN** (5 µM) in THF and 0.5 mL of copper(II)chloride in real water samples (31.5 µM).

2g. Cell imaging studies

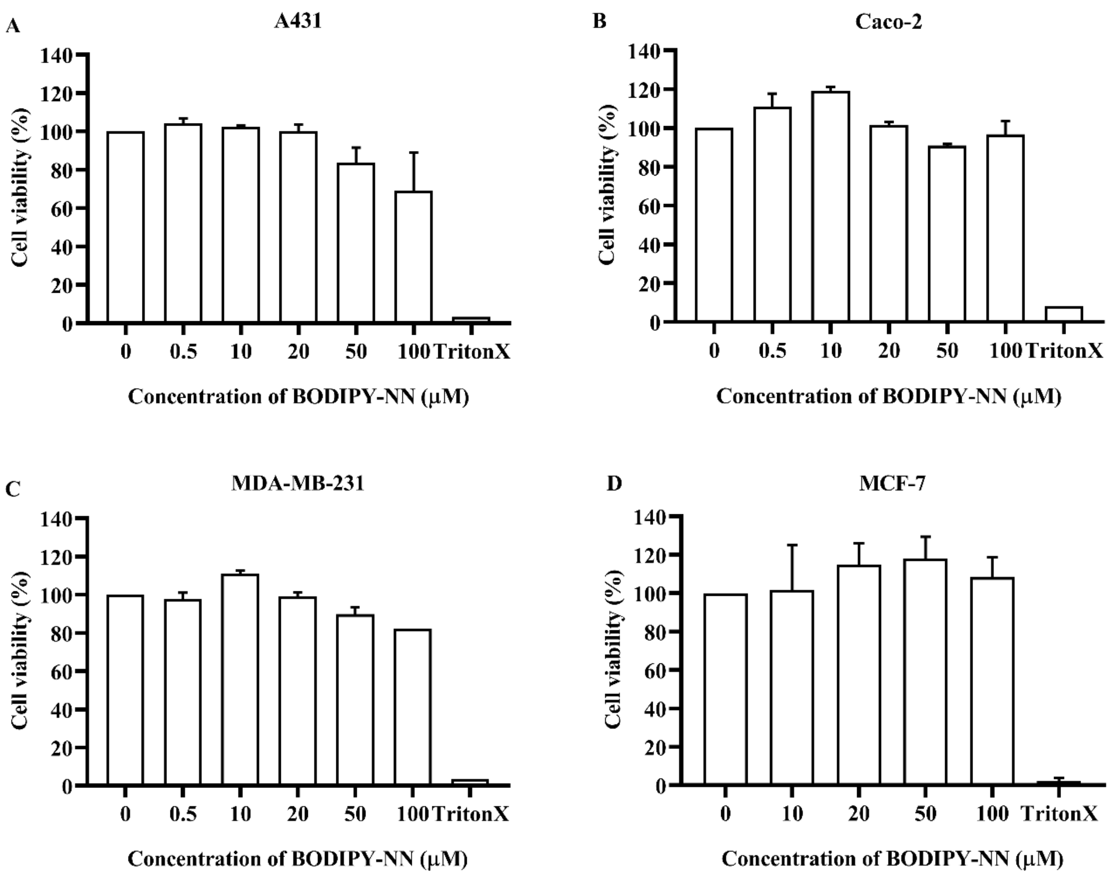


Figure S17. Viability of cells after incubation with different concentrations of **BODIPY-NN** for 24 h. (A) human epidermoid squamous carcinoma cell line (A431 cells), (B) human colorectal adenocarcinoma cell line (Caco-2 cells), (C) human triple-negative breast cancer cell line (MDA-MB-231 cells) and (D) human breast cancer cell line (MCF-7 cells). Data are represented as mean ± standard deviation (SD). N = 2.

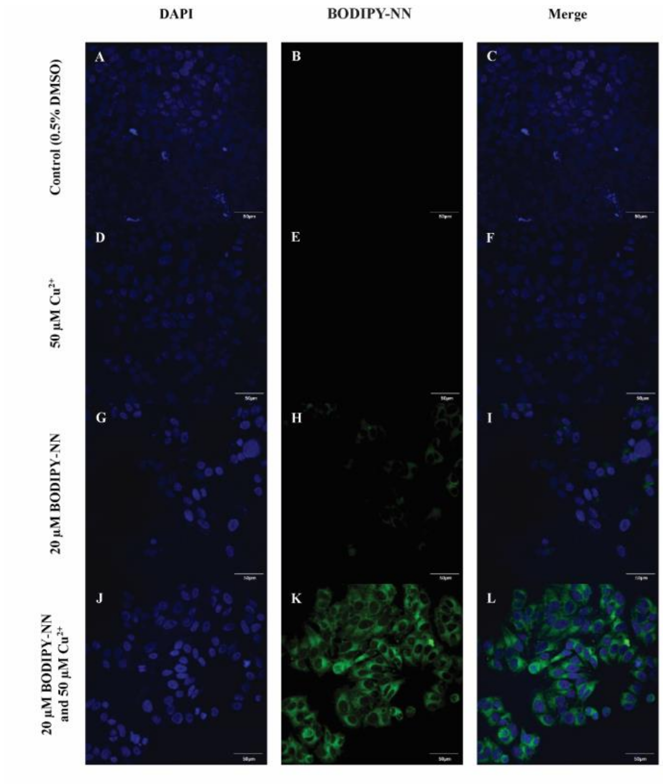


Figure S18. Confocal Laser Scanning Microscope (FV10i-DOC) images of MCF-7 cells: (A-C) images of cells after 2 h incubation with 0.5% DMSO; (D-F) images of cells after 2 h incubation with **50 µM Cu^{2+}**; (G-I) images of cells after 2 h incubation with **20 µM BODIPY**-**NN**; and (J-L) images of cells after 2 h incubation with **20 µM BODIPY**-**NN** followed by the addition of **50 µM Cu^{2+}**. (A, D, G, J) are blue fluorescence of MCF-7 cells nuclei stained with DAPI. (B, E, H, K) are green fluorescence of **BODIPY**-**NN**. (C, F, I, L) are combined images of DAPI and **BODIPY**-**NN**. Scale bars represent 50 µm.

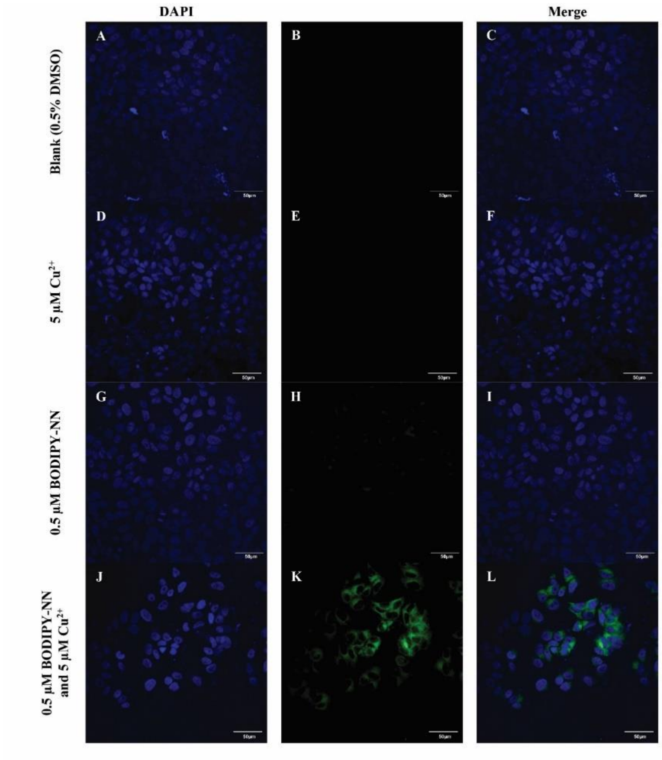
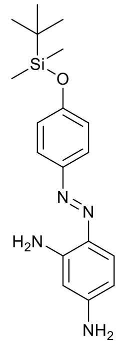
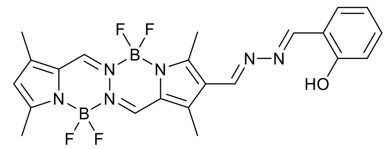


Figure S19. Confocal Laser Scanning Microscope images of MCF-7 cells: (A-C) images of cells after 2 h incubation with 0.5% DMSO; (D-F) images of cells after 2 h incubation with **5 µM Cu^{2+}**; (G-I) images of cells after 2 h incubation with **0.5 µM BODIPY-NN**; and (J-L) images of cells after 2 h incubation with **0.5 µM BODIPY-NN** followed by the addition of **5 µM Cu^{2+}** for 30 min. Notes: **BODIPY-NN** is green, while nuclei of the cells were blue stained using DAPI. (A, D, G, J) are blue fluorescence of MCF-7 nuclei. (B, E, H, K) are green fluorescence of **BODIPY-NN**. (C, F, I, L) are combined images of blue and green fluorescence. Scale bars represent 50 µm.

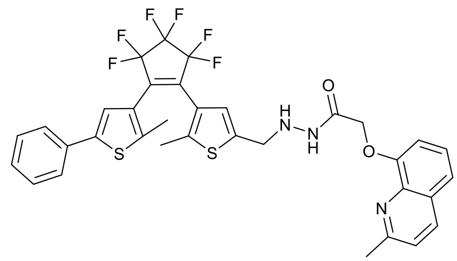
3. Comparison of BODIPY-NN to other recently reported fluorescent Cu(II) probes



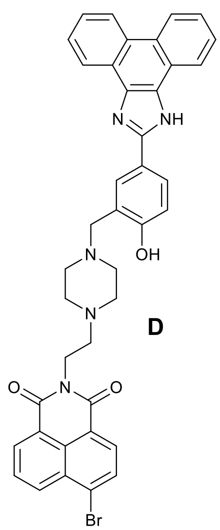
A



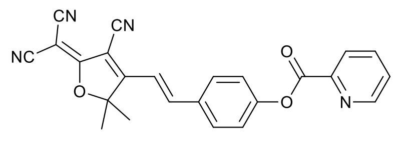
B



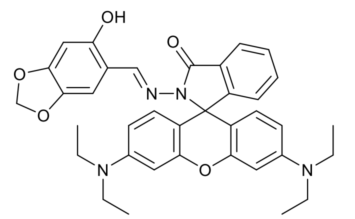
C



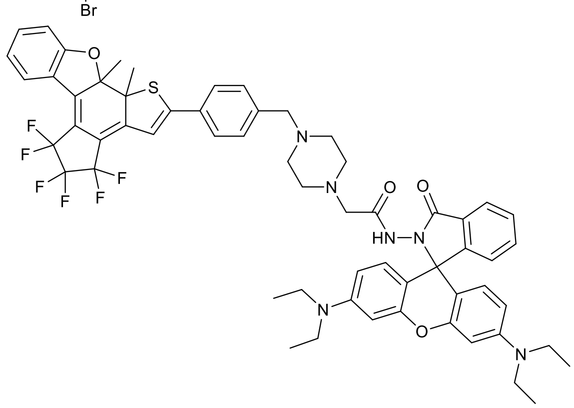
D



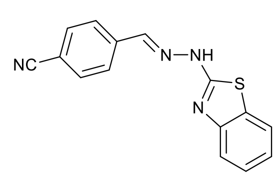
E



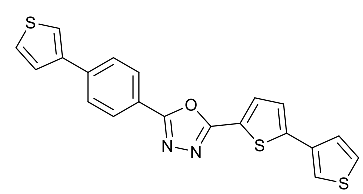
F



G



H



I

Table S3. Comparison of **BODIPY**-**NN** to other recently reported fluorescent probes for Cu(II) detection.

Probe	$\lambda_{Ex}/\lambda_{Em}$ **(nm)**	**Media**	**Roles of** Cu^{2+}	**LOD (μM)**	**Ref.**
A	350/484	DMSO/HEPES, (0.2/9.8, v/v)	catalyst for cyclization	100	[1]
B	452/482	MeCN/phosphate buffer, (5/5, v/v)	catalyst for hydrolysis	0.050	[2]
C	350/510	MeCN	chelating agent	0.370	[3]
D	405/455	MeCN/HEPES, (0.6/9.4, v/v)	chelating agent	0.650	[4]
E	580/610	DMSO/H_2O, (1/9, v/v)	catalyst for C-O cleavage	0.054	[5]
F	515/585	MeCN/HEPES, (2/3, v/v)	chelating agent /ring-opening catalyst	0.110	[6]
G	520/617	MeCN/H_2O, (4/1, v/v)	chelating agent	0.280	[7]
H	375/ 455	MeCN/H_2O, (3/1 v/v)	chelating agent	0.640	[8]
I	325/403	MeCN/H_2O, (1/1 v/v)	chelating agent	0.593	[9]
BODIPY-**NN**	470/529	THF/H_2O, (9/1, v/v)	catalyst for cyclization/ chelating agent	0.085	This work

4. References

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