Electronic Supplementary Material (ESI) for Chemical Science

Supporting Information

MegaStokes BODIPY-Triazoles as Environmentally-Sensitive Turn-on Fluorescent Dyes

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A. *In vitro* screening of BODIPY triazoles

Table S1. List of bio-molecules selected for unbiased *in vitro* screening. Buffer: HEPES, 10 mM, pH 7.4

Analyte class	Individual analyte molecules	Concentrations
Control	HEPES	10mM, pH 7.4
Viscosity	Glycerol	Volume %: 20%, 10%, 5%, 2.5%
pH	pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	
Nucleotides and nucleosides	ATP, CTP, GTP, UTP, cyclic AMP, cyclic GMP	100 µM, 50 µM, 25 µM, 12.5µM
Nucleic acids	Single strand DNA (ssDNA) double strand DNA (dsDNA) transfer RNA (t-RNA) total RNA	1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.12 mg/mL
Peptides	HA tag (YPYDVPDYA) HA12CA5 tag (CYPYDVPDYA) His tag (HHHHHH) HisG tag (HHHHHG) VSV-G tag (YTDIEMNRLGK) V5 tag (CGKPIPNPLLGLDST) IQ tag (IQSPHFF) AP tag (KKKGPGGLNDIFEAQKIEWH) FLAG tag (DYKDDDK) cMyc tag (EQKLISEEDL)	100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL
Protein	Histone, Myoglobin	0.5 mg/mL, 0.25 mg/mL, 0.12 mg/mL, 0.06 mg/mL
	Insulin, Ubiquitin	0.2 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL
	Human Immunoglobulin G (human IgG) Bovine Immunoglobulin G (bovine IgG) Human serum albumin (HSA) Bovine serum albumin (BSA) Holo-Transferrin Apo-Transferrin Concanavalin A Lysozyme Catalase Papapin Cytochrome C bovine heart Haemoglobin Fibrinogen	1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.12 mg/mL
Metal ions	NaCl, KCl	1 mM, 0.5 mM, 0.25 mM, 0.12mM
	$MgCl_2$, $ZnCl_2$, $FeCl_2$, $CaCl_2$	100 µM, 50 µM, 25 µM, 12.5 µM
Oxido-reduction related molecules	Ascorbic acid DL-dithiothreitol (DTT) L-Glutathione reduced form (GSH) L-Glutathione oxidized form (GSSG) Nicotinamide adenine dinucleotide (NAD)	2 mM, 1 mM, 0.5 mM, 0.25 mM

	Nicotinamide adenine dinucleotide reduced form (NADH) Nicotinamide adenine dinucleotide phosphate reduced form (NADPH) Sodium hypochlorite (NaOCl) Potassium dioxide (KO_2) Hydrogen peroxide (H_2O_2) Iron (II) with hydrogen peroxide (Fe^{2+} + H_2O_2) 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH)	
Miscellaneous molecules	Glycogen Dextran Chrontroitin Heparin Malachite green Sudan I Melamine	1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.12 mg/mL
	Glucose, Glucose phosphate Fructose, Fructose phosphate Caffeine Acetylcholine chloride Gamma.aminobutyric acid (GABA) Gamma butyrolactone (GHB) L-Glutamine Monosodium glutamate (MSG) Sarcosine, Histamine, Dopamine	2 mM, 1 mM, 0.5 mM, 0.25 mM

B. Supporting Experiments

i) Solvatochromic effects

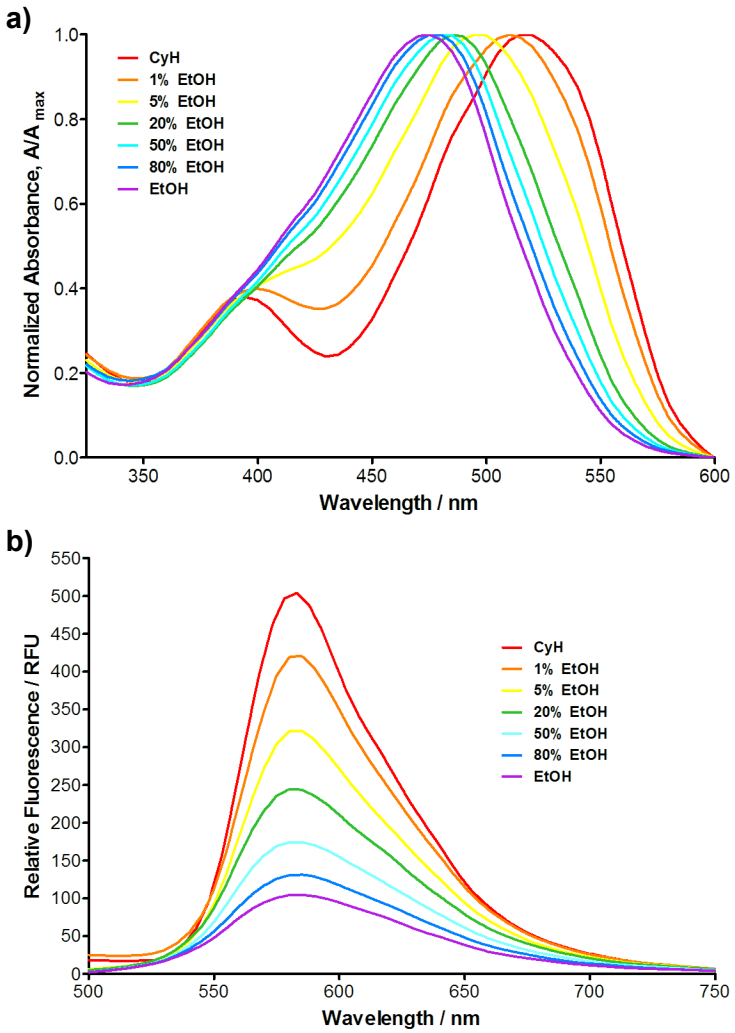


Fig. S1. Effect of solvent polarity on the absorbance and fluorescence of **BDC-9** (100 μM) at rt. (a) Normalized absorption spectra in different ratios of cyclohexane and ethanol. (b) Corresponding emission spectra of **BDC-9** in various ratios of cyclohexane and ethanol.

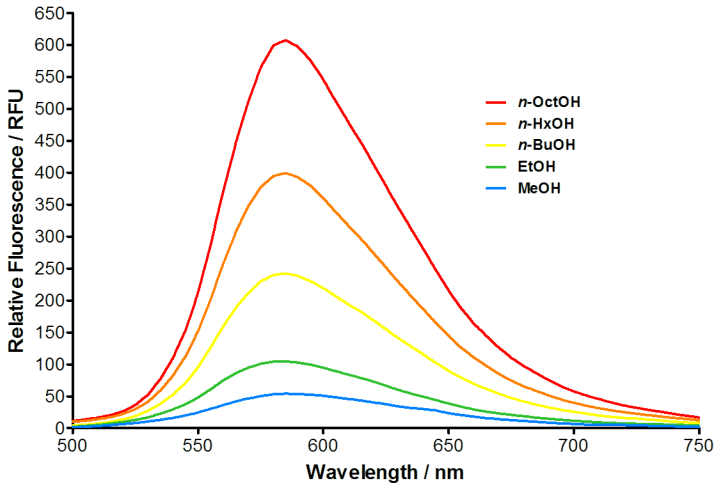


Fig. S2. Effect of solvent viscosity on the fluorescence intensity of **BDC-9** (100 μM) at rt. Intensity of **BDC-9** systematically increased in more viscous (and less polar) alcohols exceeding that of cyclohexane when in *n*-octanol and *n*-hexanol.

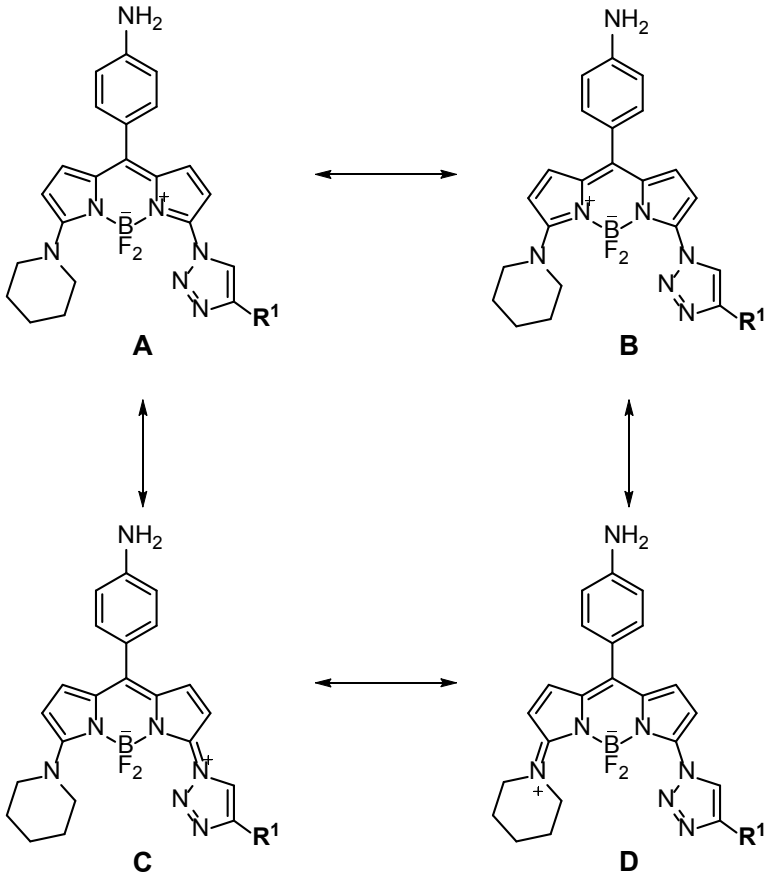


Fig. S3. Major resonance structures of **BDC** compounds

ii) Fluorescence and absorbance response of BDC-9 to HSA

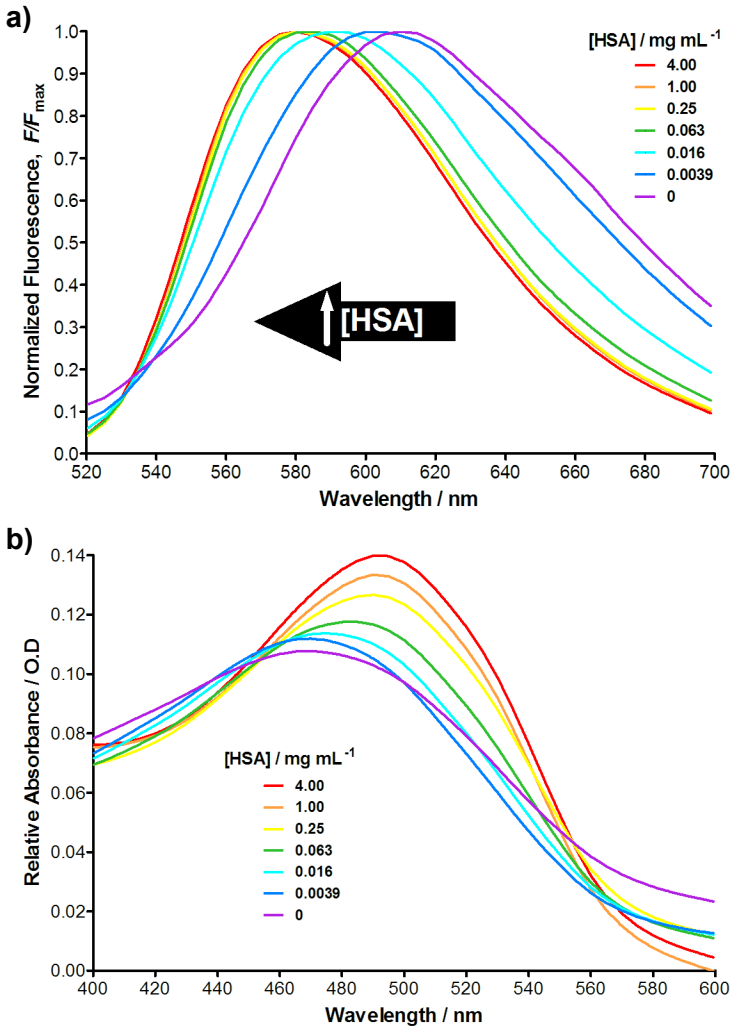


Fig. S4. Fluorescence and absorbance response of **BDC-9** (10 μM) upon incubation with 4.0, 1.0, 0.25, 0.063, 0.016, 0.0039 and 0 mg/mL HSA in 10 mM phosphate buffer (pH = 7.3). (a) Normalized emission spectra; $\lambda_{exc.}$: 460 nm. (b) Absorbance spectra. Values are represented as means (*n* = *3*). Measurements were taken at rt.

iii) Limit-of-detection and linear dynamic range of BDC-9

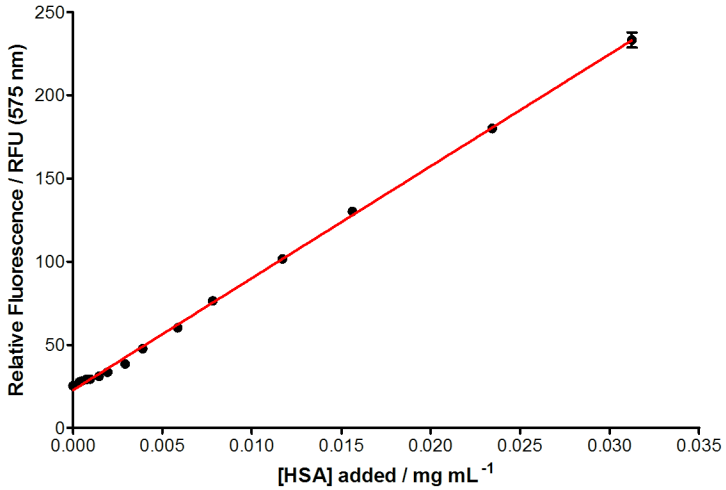


Fig. S5. Fluorescence emission response of **BDC-9** (10 μM) upon incubation with serial dilutions of HSA in 10 mM phosphate buffer (pH = 7.3); $\lambda_{exc.}$: 460 nm, $\lambda_{em.}$: 575 nm. Values are represented as means and error bars as standard deviations (n = 3). Measurements were taken at rt. LOD = 0.3 μg/mL; linear range = 0.37 to 31 μg/mL, R^2 = 0.999.

iv) Job plot analysis of BDC-9 with HSA

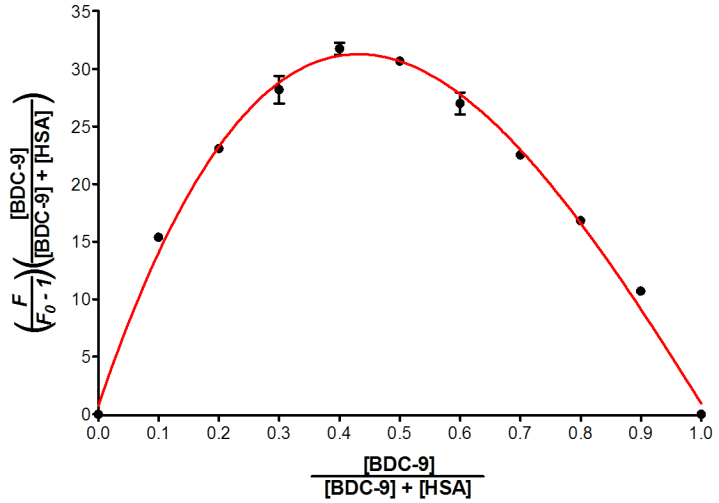


Fig. S6. Job plot analysis. **BDC-9** was mixed with HSA (fatty acid free) at different ratios in 10 mM phosphate buffer (pH = 7.3) while maintaining total concentration at 20 μM; $\lambda_{exc.}$: 460 nm, $\lambda_{em.}$: 575 nm. Values are represented as means and error bars as standard deviations (n = 3). Measurements were taken at rt.

v) Determination of dissociation constant for BDC-9 with HSA

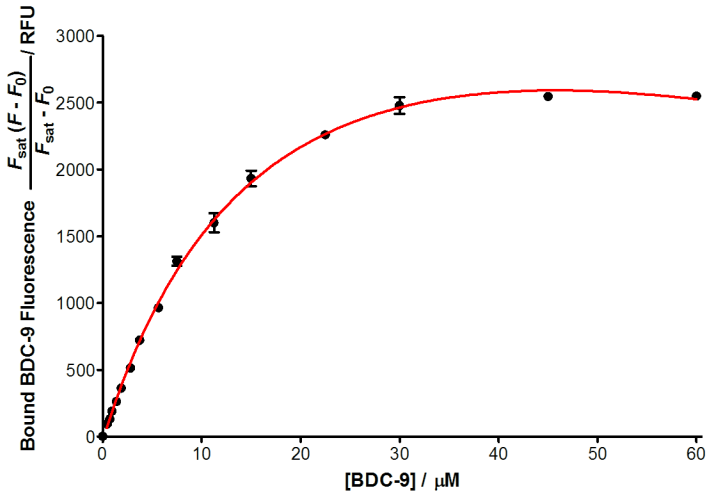


Fig. S7. Fluorescence emission of serial dilutions of **BDC-9** (0, 0.47 to 60 μM) upon incubation with HSA (10 μM) in 10 mM phosphate buffer (pH = 7.3); $\lambda_{exc.}$: 460 nm, $\lambda_{em.}$: 575 nm; values are represented as means and error bars as standard deviations (n = 3); Measurements were taken at rt; K_D = 12.7 ± 0.4 μM (one-site specific binding model).

C. Chemical Structures and Characterization Data for BODIPY triazoles

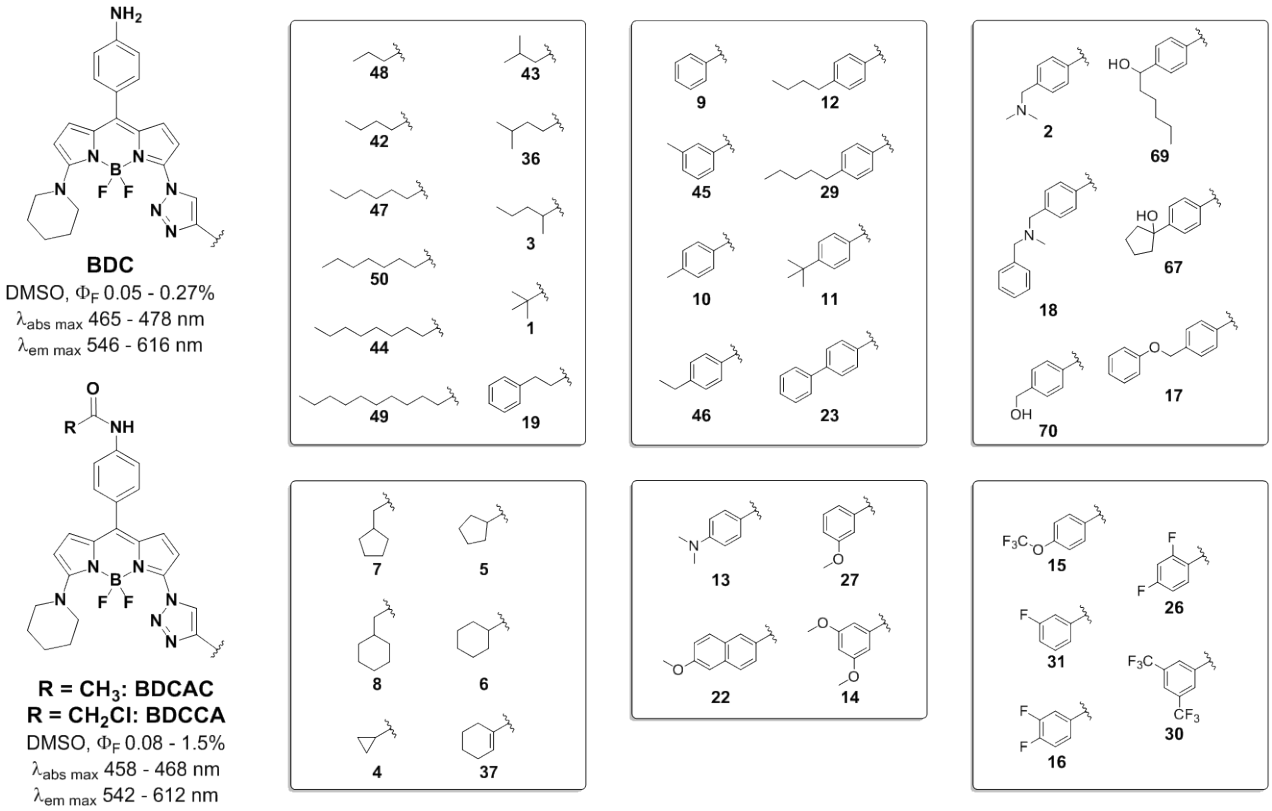


Fig. S8. Chemical structures of the **BDC, BDCAC** and **BDCCA** library.

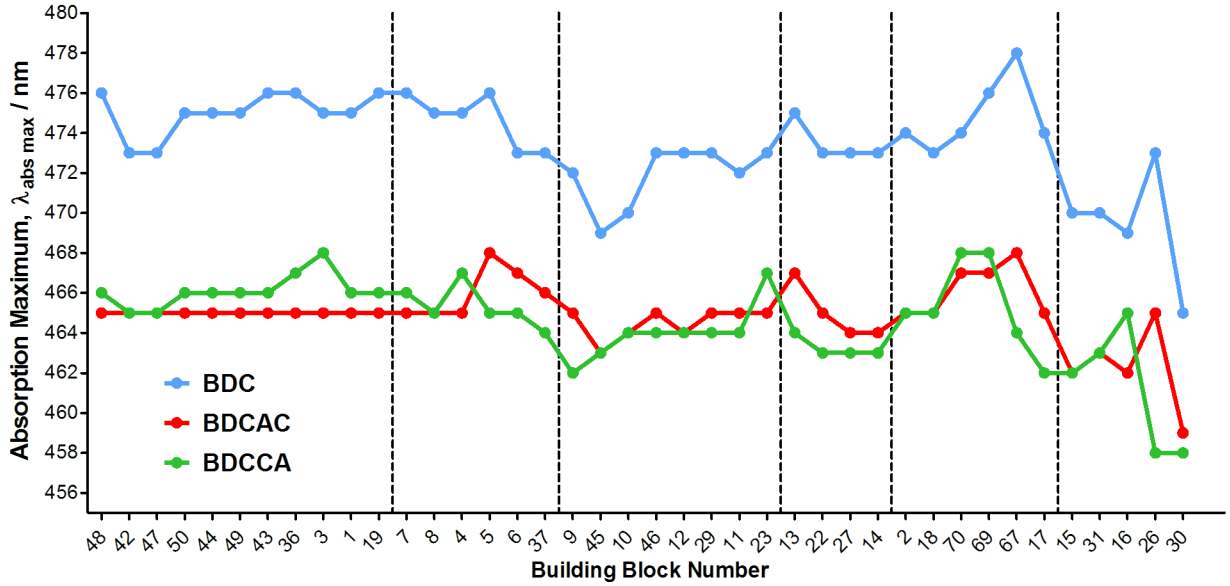


Fig. S9. Absorbance maximum trend of the **BDC, BDCAC** and **BDCCA** compounds. Concentration = 100 μM in DMSO.

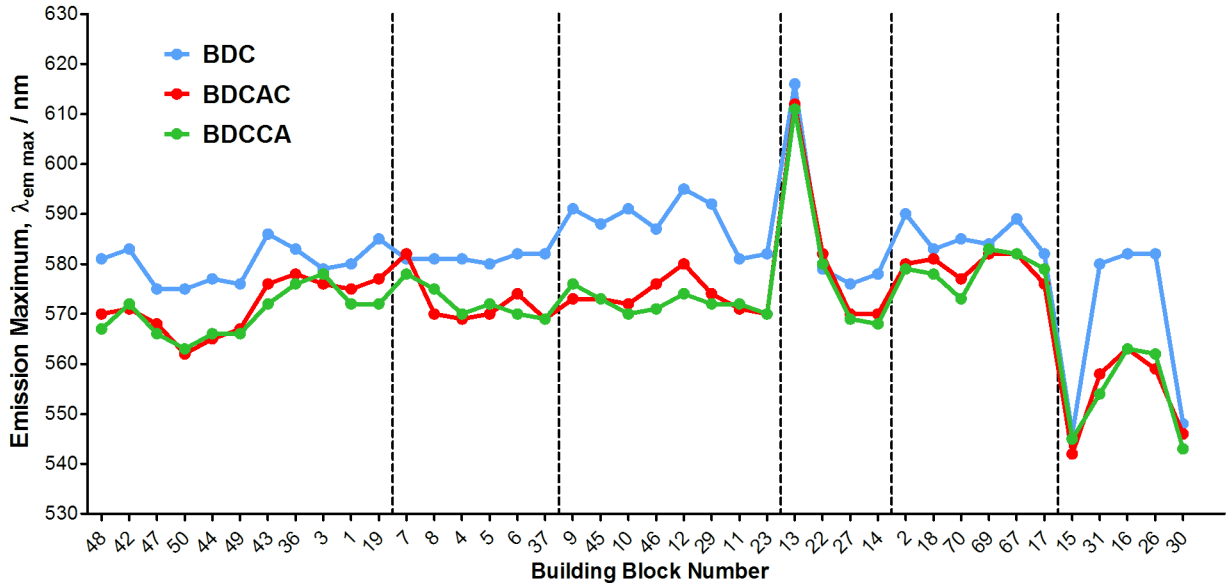


Fig. S10. Fluorescence emission trend of the **BDC, BDCAC** and **BDCCA** compounds. Concentration = 100 μM in DMSO.

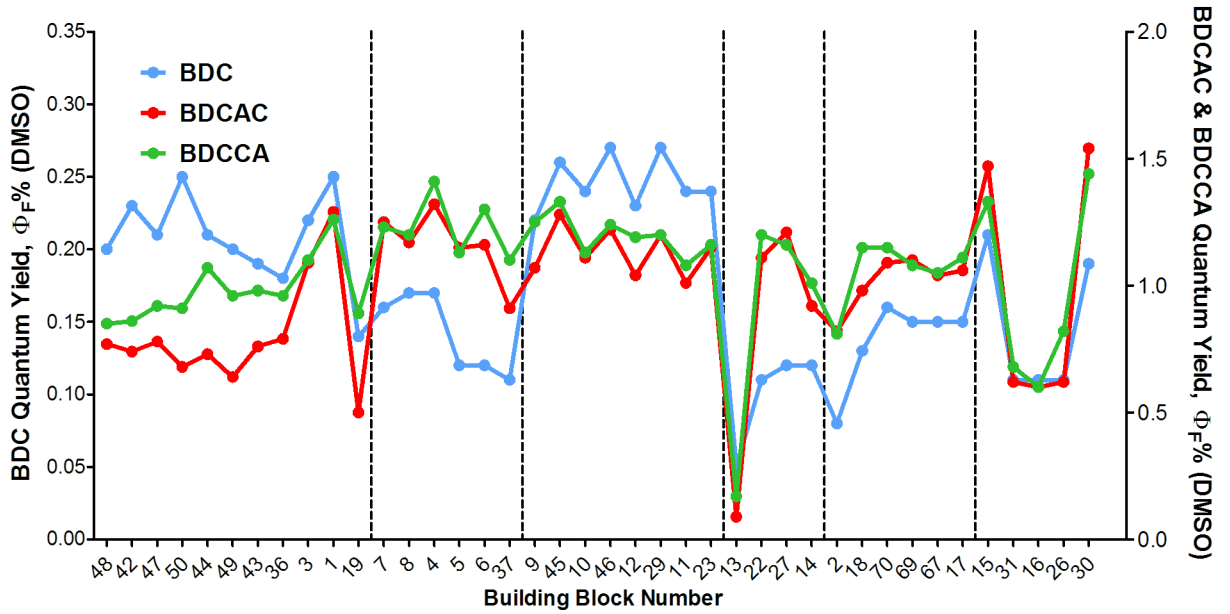
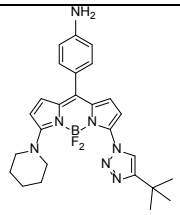
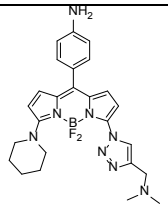
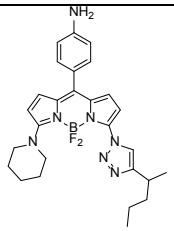
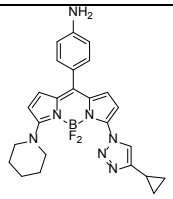
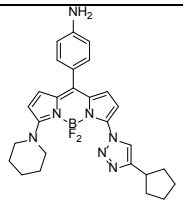
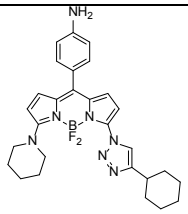
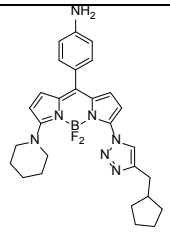
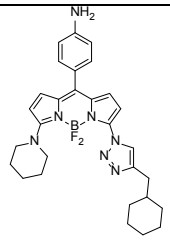
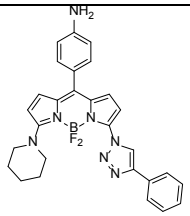
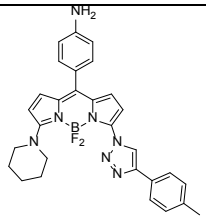
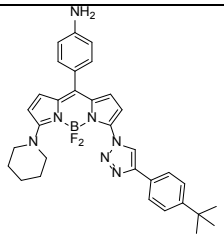
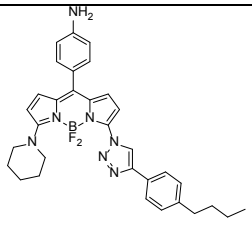
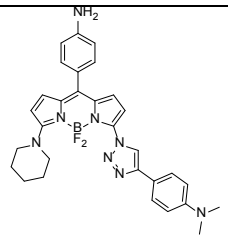
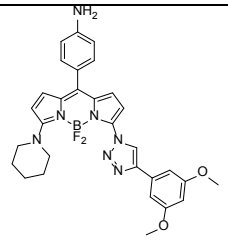
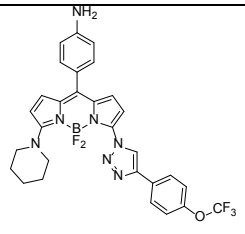
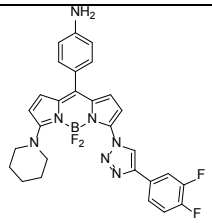
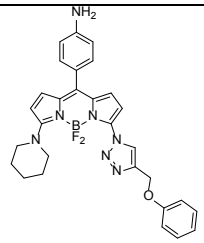
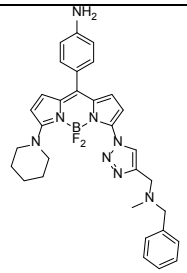


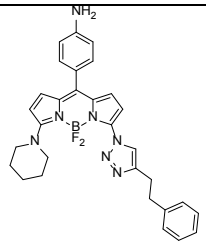
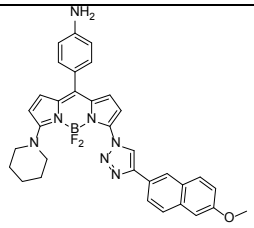
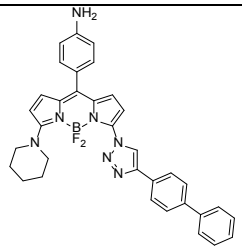
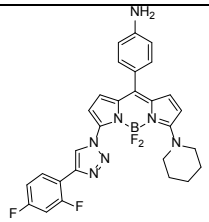
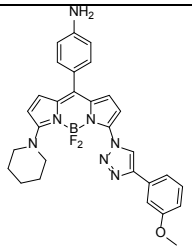
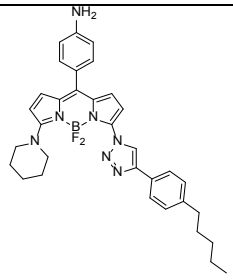
Fig. S11. Percentage quantum yield trend of the **BDC, BDCAC** and **BDCCA** compounds. Concentration = 100 μM in DMSO.

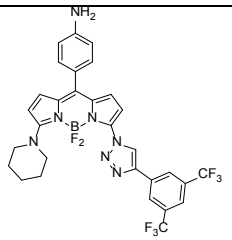
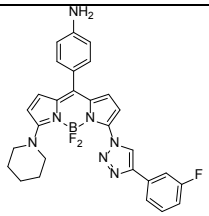
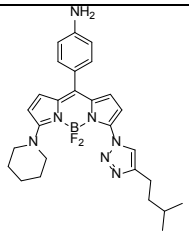
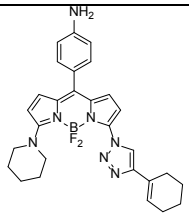
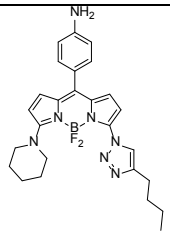
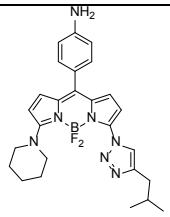
Table S2. Chemical structures and characterization data for the **BDC** library. Concentration = 100 μM in DMSO.

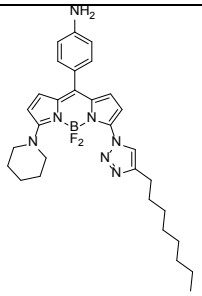
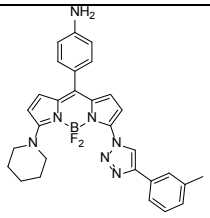
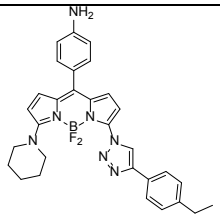
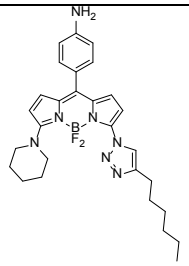
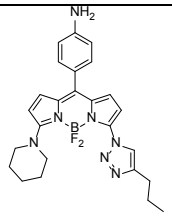
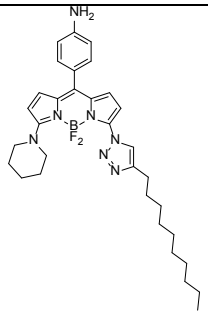
Code	Structure	Purity (254 nm)	m/z Calculated	m/z Experimental	λ_{max} Abs. (nm)	λ_{max} Em. (nm)	ϕ (%)
BDC-1		95%	489.3	490.0	475	580	0.25
BDC-2		95%	490.3	491.0	474	590	0.08
BDC-3		98%	503.3	504.3	475	579	0.22
BDC-4		95%	473.2	474.2	475	581	0.17
BDC-5		99%	501.3	502.2	476	580	0.12
BDC-6		86%	515.4	516.2	473	582	0.12

BDC-7		100%	515.3	516.2	476	581	0.16
BDC-8		88%	529.3	503.2	475	581	0.17
BDC-9		97%	509.2	510.2	472	591	0.22
BDC-10		92%	523.3	524.2	470	591	0.24
BDC-11		97%	565.3	566.3	472	581	0.24
BDC-12		90%	565.3	566.3	473	595	0.23

BDC-13		97%	552.3	553.2	475	616	0.05
BDC-14		99%	569.3	570.2	473	578	0.12
BDC-15		84%	593.2	594.0	470	546	0.21
BDC-16		95%	545.2	546.1	469	582	0.11
BDC-17		99%	539.2	540.2	474	582	0.15
BDC-18		87%	566.3	567.3	473	583	0.13

BDC-19		98%	537.3	538.2	476	585	0.14
BDC-22		99%	589.3	590.2	473	579	0.11
BDC-23		100%	585.5	586.3	473	582	0.24
BDC-26		91%	545.2	546.2	473	582	0.11
BDC-27		58%	539.2	540.2	473	576	0.12
BDC-29		93%	579.3	580.3	473	592	0.27

BDC-30		92%	645.2	646.1	465	548	0.19
BDC-31		96%	527.2	528.2	470	580	0.11
BDC-36		98%	503.3	504.1	476	583	0.18
BDC-37		96%	513.3	514.2	473	582	0.11
BDC-42		92%	489.3	490.2	473	583	0.23
BDC-43		95%	489.3	490.2	476	586	0.19

BDC-44		94%	545.3	546.1	475	577	0.21
BDC-45		84%	523.3	524.2	469	588	0.26
BDC-46		98%	537.3	538.2	473	587	0.27
BDC-47		90%	517.3	518.2	473	575	0.21
BDC-48		96%	475.3	476.2	476	581	0.20
BDC-49		92%	573.4	574.2	475	576	0.20

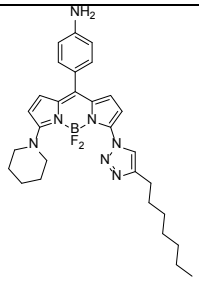
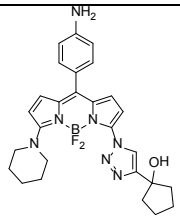
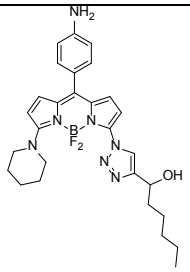
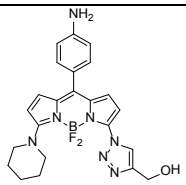
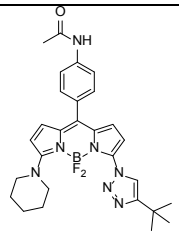
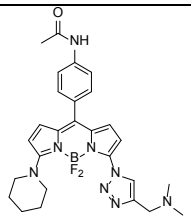
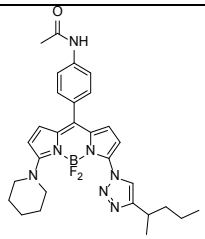
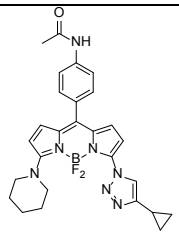
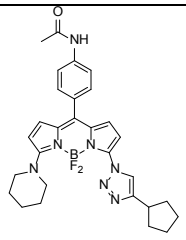
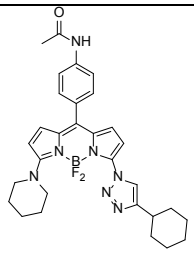
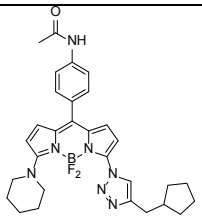
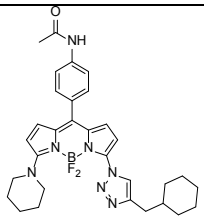
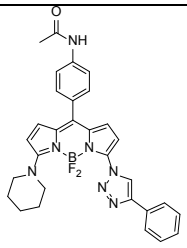
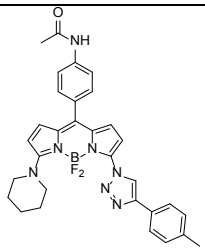
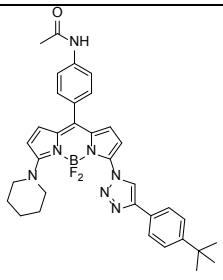
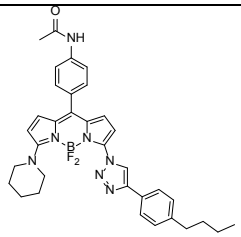
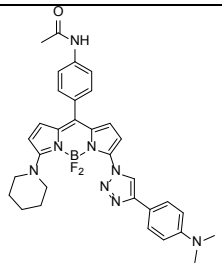
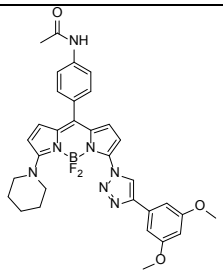
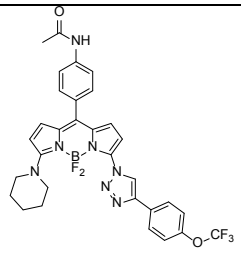
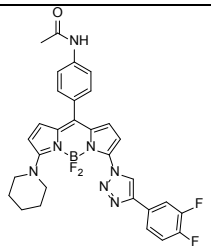
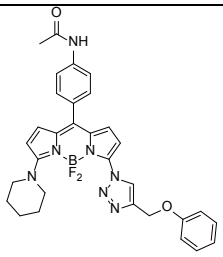
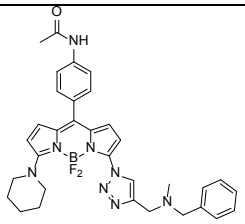
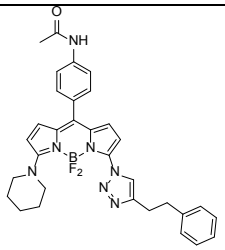
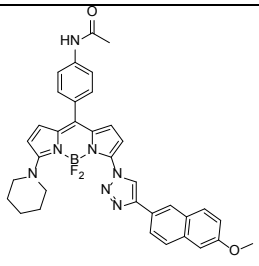
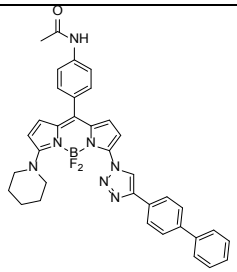
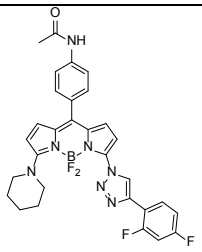
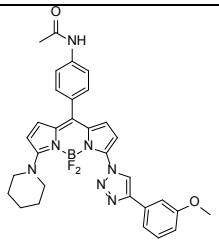
BDC-50		93%	531.3	532.2	475	575	0.25
BDC-67		100%	517.3	518.1	478	589	0.15
BDC-69		92%	533.3	534.2	476	584	0.15
BDC-70		100%	463.3	464.2	474	585	0.16

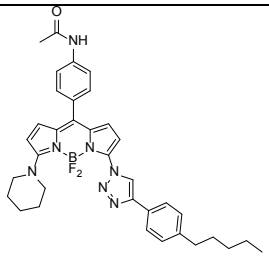
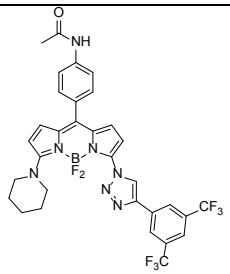
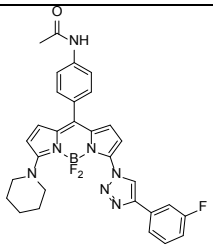
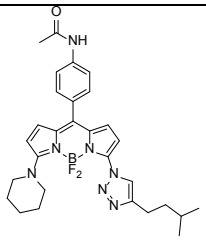
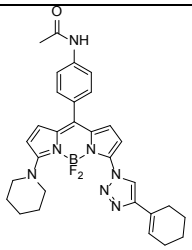
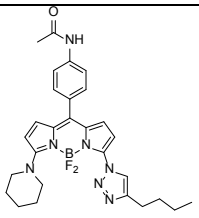
Table S3. Chemical structures and characterization data for the **BDCAC** library. Concentration = 100 μM in DMSO.

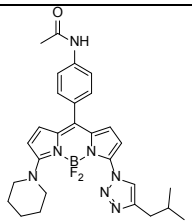
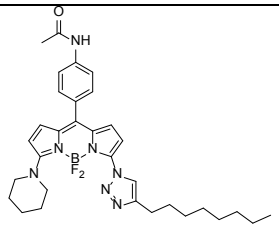
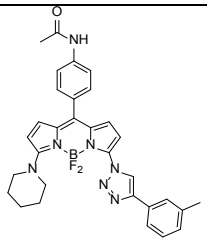
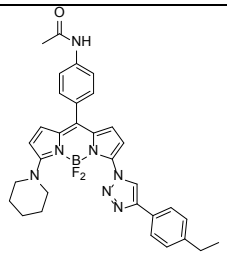
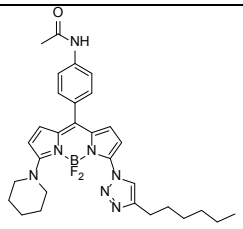
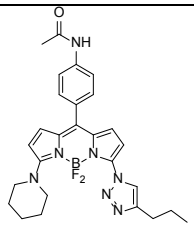
Code	Structure	Purity (254 nm)	m/z Calculated	m/z Experimental	λ_{max} Abs. (nm)	λ_{max} Em. (nm)	ϕ (%)
BDCAC-1		95%	531.4	532.3	465	575	1.29
BDCAC-2		98%	532.4	533.3	465	580	0.82
BDCAC-3		97%	545.4	546.3	465	576	1.09
BDCAC-4		97%	515.4	516.2	465	569	1.32
BDCAC-5		99%	543.4	544.3	468	570	1.15

BDCAC-6		93%	557.5	558.1	467	574	1.16
BDCAC-7		98%	557.5	558.3	465	582	1.25
BDCAC-8		98%	571.5	572.3	465	570	1.17
BDCAC-9		97%	551.4	552.1	465	573	1.07
BDCAC-10		91%	565.4	566.2	464	572	1.11
BDCAC-11		96%	607.5	608.3	465	571	1.01

BDCAC-12		89%	607.5	608.3	464	580	1.04
BDCAC-13		98%	594.5	595.2	467	612	0.09
BDCAC-14		98%	611.5	612.3	464	570	0.92
BDCAC-15		84%	635.4	636.2	462	542	1.47
BDCAC-16		93%	587.4	588.2	462	563	0.60
BDCAC-17		99%	581.4	582.2	465	576	1.06

BDCAC-18		88%	608.5	609.3	465	581	0.98
BDCAC-19		98%	579.5	580.3	465	577	0.50
BDCAC-22		98%	631.5	632.2	465	582	1.11
BDCAC-23		99%	627.4	628.3	465	570	1.15
BDCAC-26		91%	587.4	588.2	465	559	0.62
BDCAC-27		89%	581.4	582.2	464	570	1.21

BDCAC-29		95%	621.5	622.3	465	574	1.20
BDCAC-30		93%	687.4	688.2	459	546	1.54
BDCAC-31		97%	569.4	570.1	463	558	0.62
BDCAC-36		99%	545.4	546.2	465	578	0.79
BDCAC-37		95%	555.4	556.3	466	569	0.91
BDCAC-42		98%	531.4	532.2	465	571	0.74

BDCAC-43		95%	531.4	532.2	465	576	0.76
BDCAC-44		95%	587.5	588.4	465	565	0.73
BDCAC-45		87%	565.4	566.2	463	573	1.28
BDCAC-46		99%	579.5	580.3	465	576	1.22
BDCAC-47		81%	559.5	560.3	465	568	0.78
BDCAC-48		95%	517.4	518.1	465	570	0.77

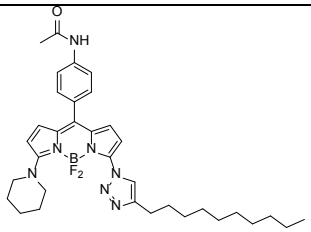
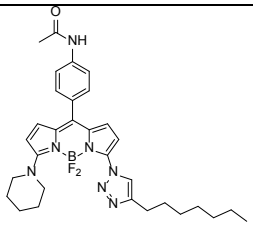
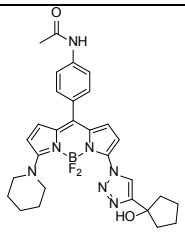
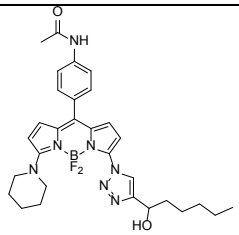
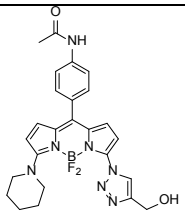
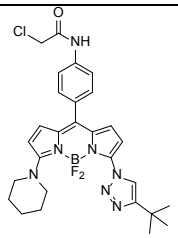
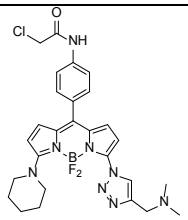
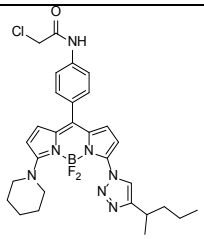
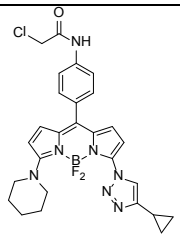
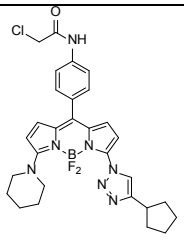
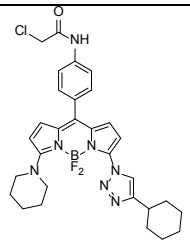
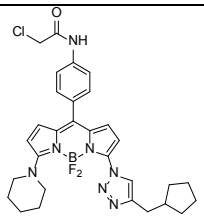
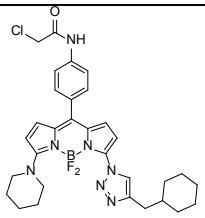
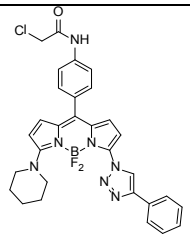
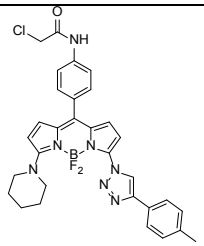
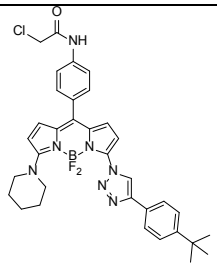
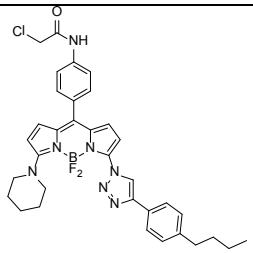
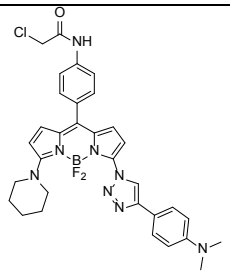
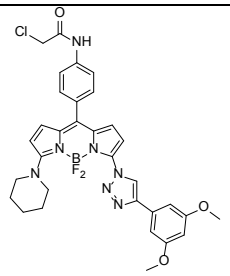
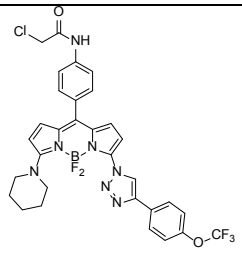
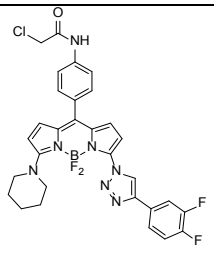
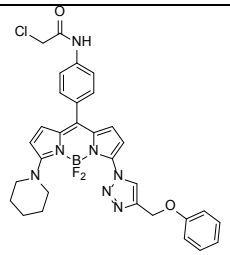
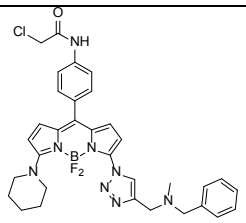
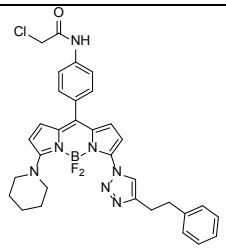
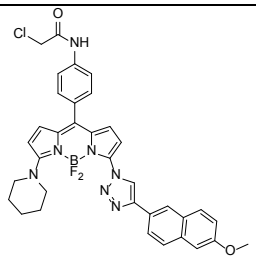
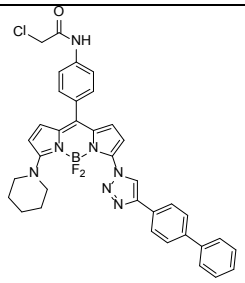
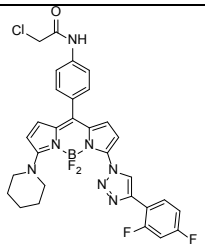
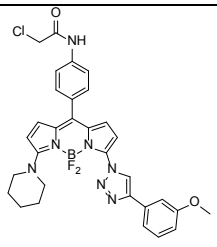
BDCAC-49		96%	615.6	616.4	465	567	0.64
BDCAC-50		94%	573.5	574.3	465	562	0.68
BDCAC-67		97%	559.4	560.3	468	582	1.04
BDCAC-69		92%	575.5	576.3	467	582	1.10
BDCAC-70		99%	505.3	506.0	467	577	1.09

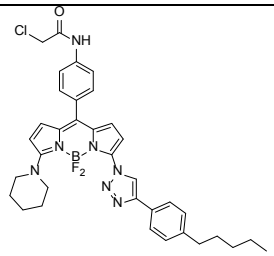
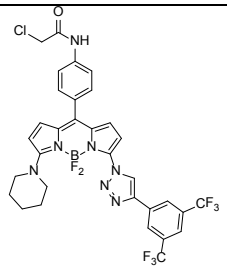
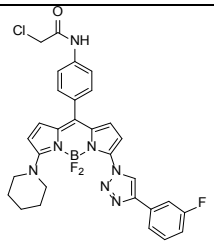
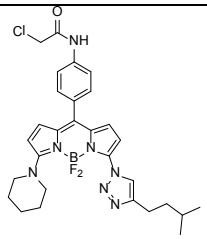
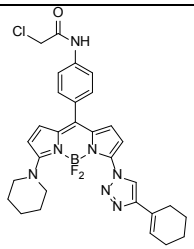
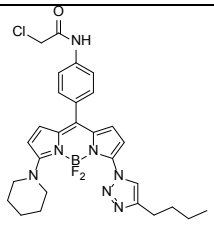
Table S4. Chemical structures and characterization data for the **BDCCA** library. Concentration = 100 μM in DMSO.

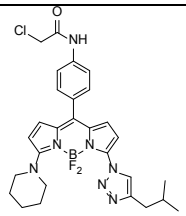
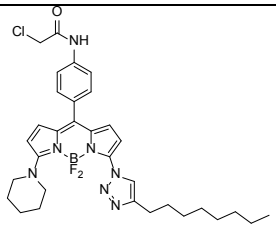
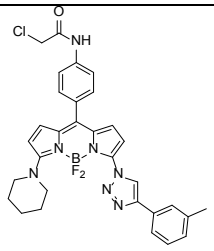
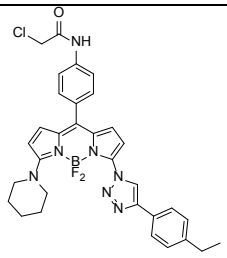
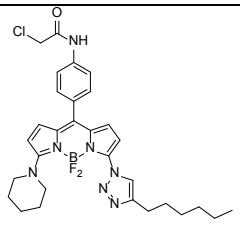
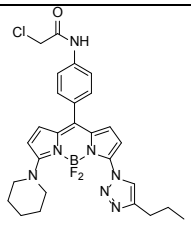
Code	Structure	Purity (254 nm)	m/z Calculated	m/z Experimental	λ_{max} Abs. (nm)	λ_{max} Em. (nm)	ϕ (%)
BDCCA-1		93%	565.9	566.2	468	572	1.26
BDCCA-2		52%	566.8	567.1	463	579	0.81
BDCCA-3		97%	579.9	580.2	467	578	1.10
BDCCA-4		92%	549.8	550.1	465	570	1.41
BDCCA-5		98%	577.9	578.3	467	572	1.13

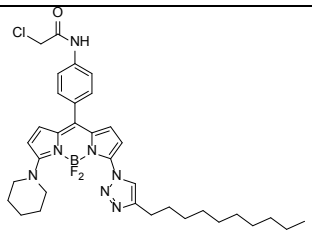
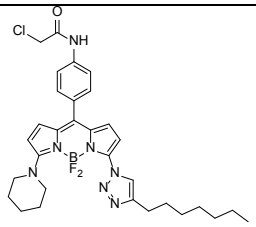
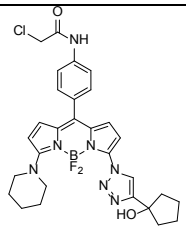
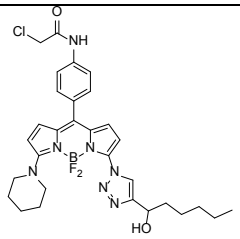
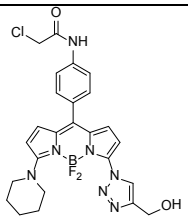
BDCCA-6		80%	591.9	592.3	465	570	1.30
BDCCA-7		99%	591.9	592.3	466	578	1.23
BDCCA-8		79%	605.9	606.3	466	575	1.20
BDCCA-9		97%	585.8	586.1	464	576	1.25
BDCCA-10		90%	599.9	600.2	463	570	1.13
BDCCA-11		90%	642.0	642.3	464	572	1.08

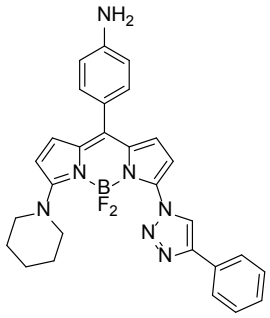
BDCCA-12		91%	642.0	642.3	464	574	1.19
BDCCA-13		98%	628.9	629.3	467	611	0.17
BDCCA-14		97%	645.9	646.2	463	568	1.01
BDCCA-15		82%	669.8	670.2	462	545	1.33
BDCCA-16		93%	621.8	622.1	463	563	0.60
BDCCA-17		98%	615.9	616.2	464	579	1.11

BDCCA-18		87%	642.9	643.3	465	578	1.15
BDCCA-19		96%	613.9	614.2	466	572	0.89
BDCCA-22		97%	665.9	666.3	464	580	1.20
BDCCA-23		96%	661.9	662.2	464	570	1.16
BDCCA-26		89%	621.8	622.2	465	562	0.82
BDCCA-27		81%	615.9	616.2	463	569	1.16

BDCCA-29		90%	656.0	656.3	464	572	1.20
BDCCA-30		87%	721.8	722.2	458	543	1.44
BDCCA-31		94%	603.8	604.2	462	554	0.68
BDCCA-36		99%	579.9	580.3	466	576	0.96
BDCCA-37		95%	589.9	590.2	465	569	1.10
BDCCA-42		89%	565.9	566.1	466	572	0.86

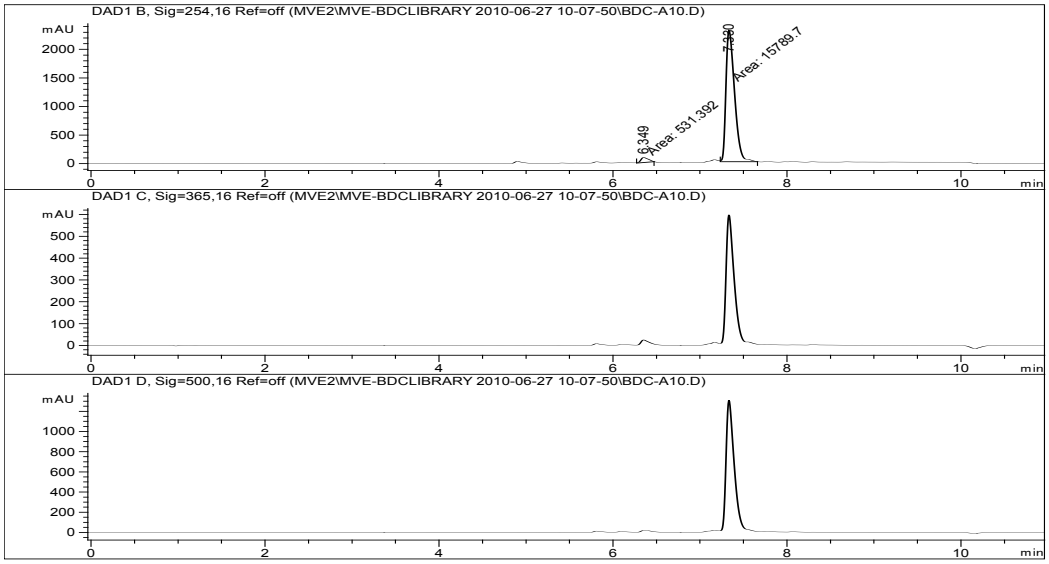
BDCCA-43		94%	565.9	566.2	466	572	0.98
BDCCA-44		95%	621.3	622.3	466	566	1.07
BDCCA-45		81%	599.9	600.2	462	573	1.33
BDCCA-46		99%	613.9	614.1	464	571	1.24
BDCCA-47		89%	593.9	594.3	465	566	0.92
BDCCA-48		95%	551.8	552.2	467	567	0.85

BDCCA-49		90%	649.3	650.3	466	566	0.96
BDCCA-50		87%	607.9	608.3	465	563	0.91
BDCCA-67		87%	593.2	594.2	468	582	1.05
BDCCA-69		89%	609.9	610.3	468	583	1.08
BDCCA-70		93%	539.8	540.0	465	573	1.15

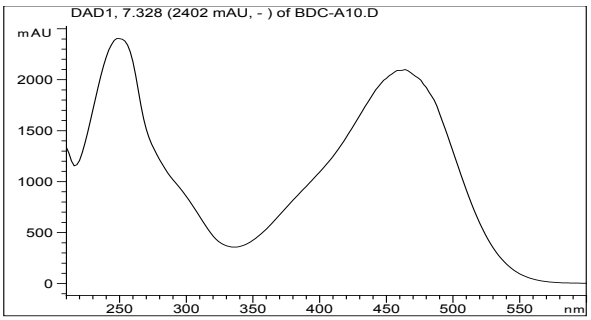


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.349	MM	0.0983	531.39209	90.08173	3.2559
2	7.330	MM	0.1141	1.57897e4	2306.55444	96.7441
Totals :				1.63211e4	2396.63618	

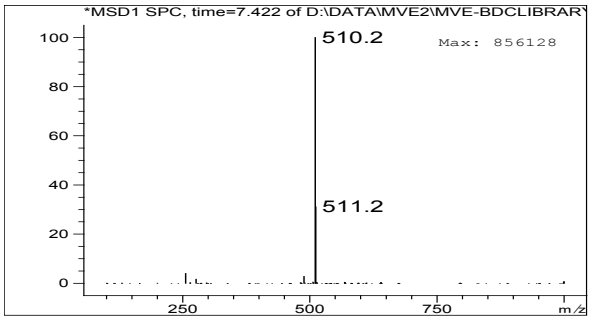
a)



b)



c)



d)

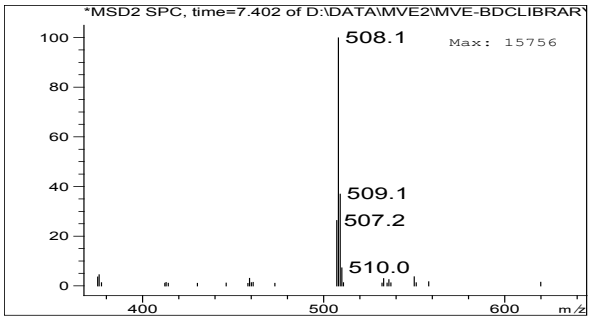


Fig. S12 HPLC-MS characterization of **BDC-9** a) chromatograms at 254, 365 and 500 nm; HPLC conditions: A: H_2O (0.1% HCOOH), B: CH_3CN (0.1% HCOOH); gradient 5% B to 95% B (10 min), isocratic 95% B (2 min). Reverse-phase Phenomenex C_{18} Luna column (4.6 x 50 mm^2, 3.5 µm particle size), flow rate: 1 mL/min. b) absorbance spectrum (200 – 600 nm); c) ESI-MS positive spectrum; d) ESI-MS negative spectrum.

D. NMR Spectra