Electronic Supplementary Material (ESI) for RSC Advances.

Polypyridyl Substituted BODIPY Derivatives; Water Switchable Probes for Imaging Hydrophobic Domains in Cells that Exhibit Halogen Substituent Dependent Localisation.

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Supplementary Information

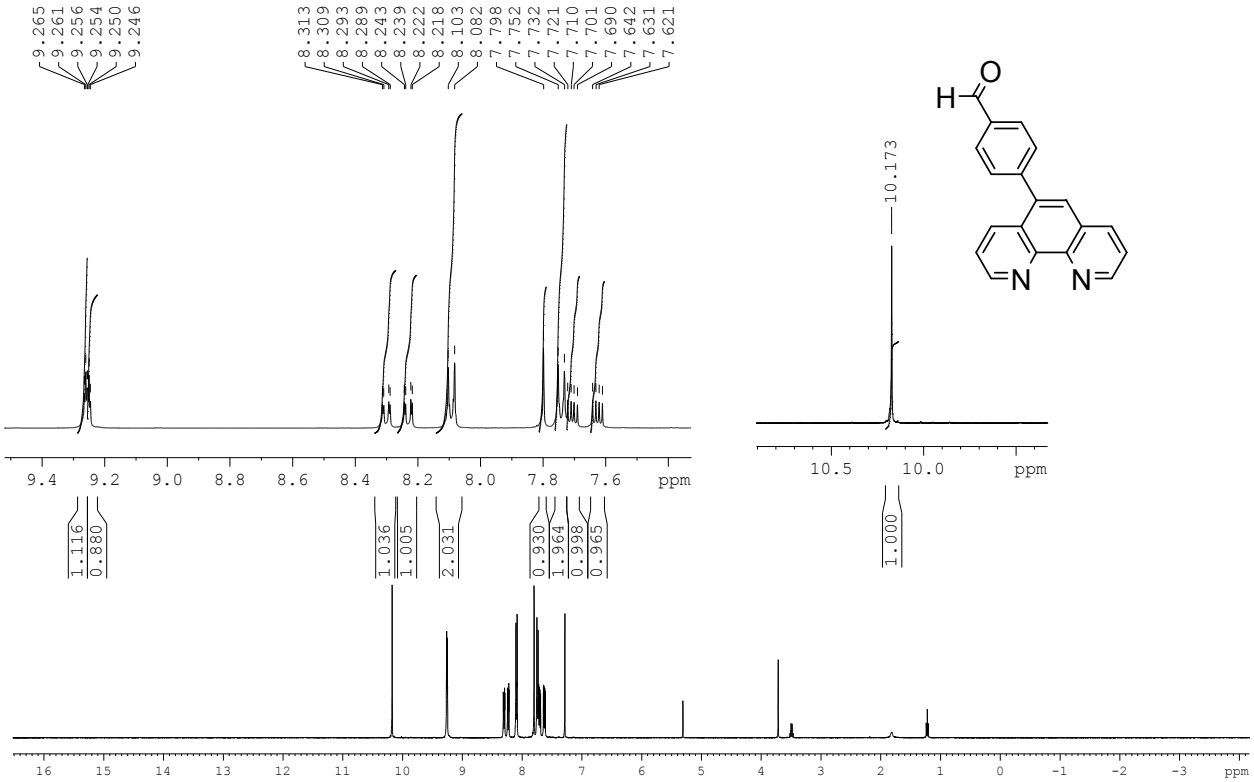


Figure S1: ^{1}H NMR spectra of **3** in $CDCl_3$.

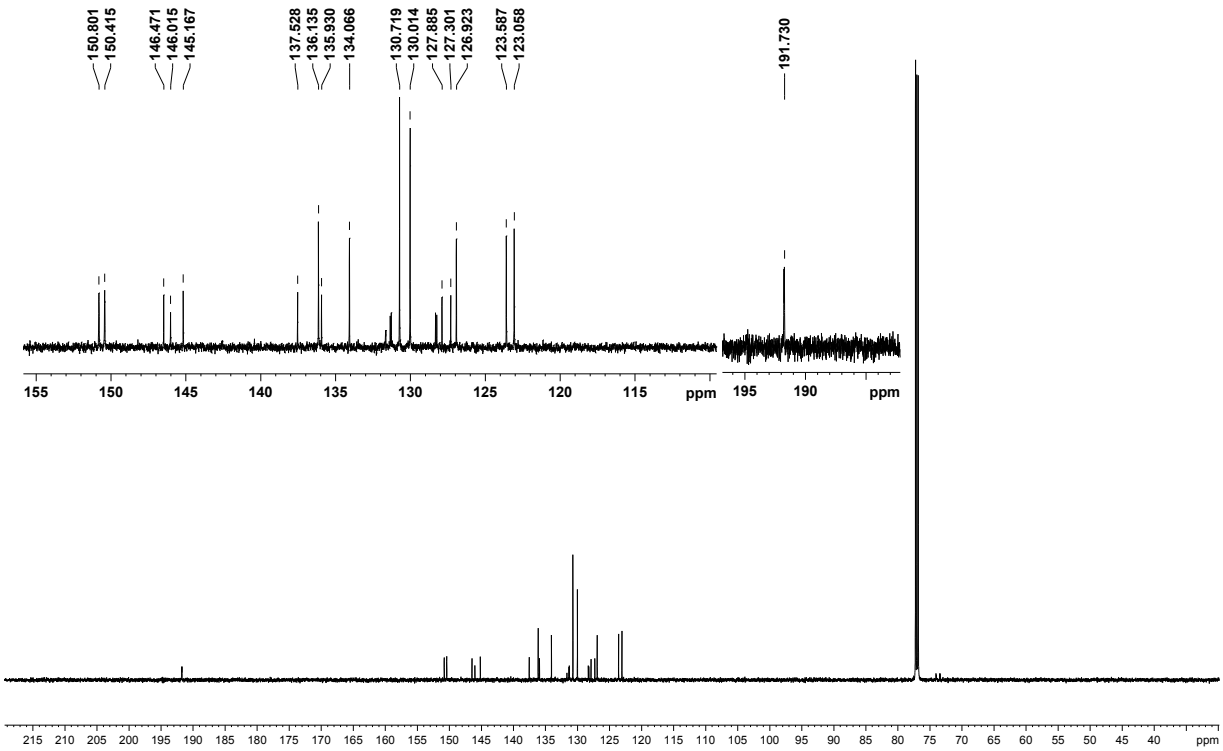


Figure S2: ^{13}C NMR spectra of **3** in $CDCl_3$.

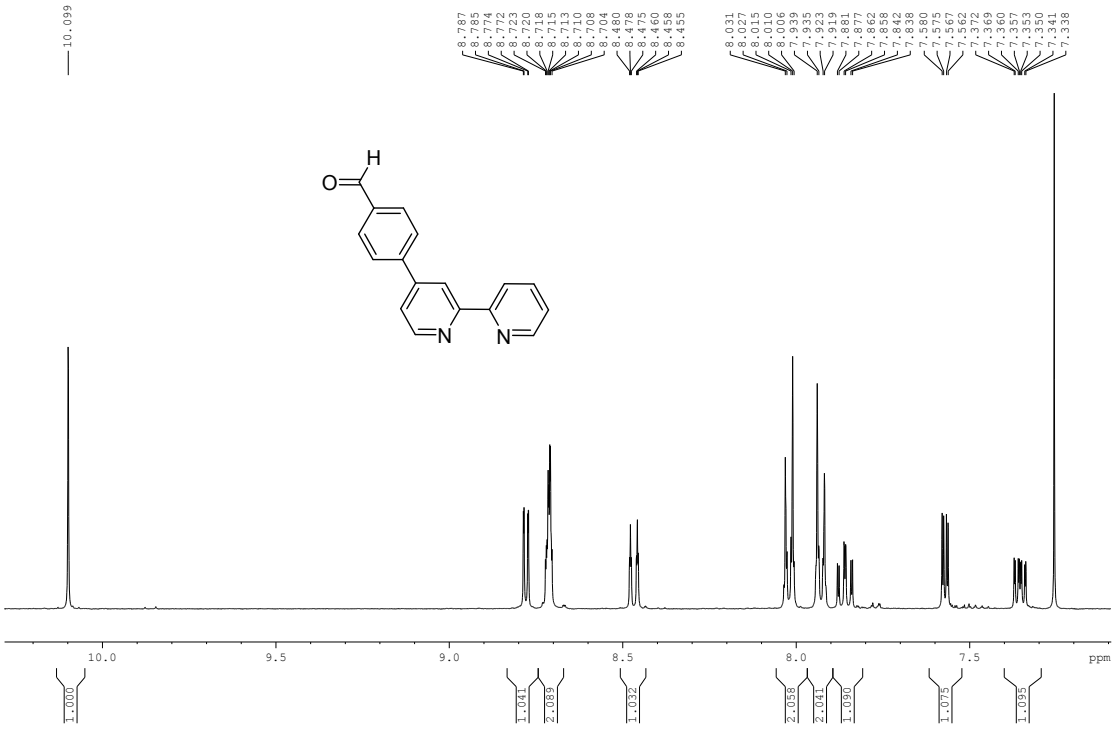


Figure S3: ^{1}H NMR spectra of **4** in $CDCl_3$.

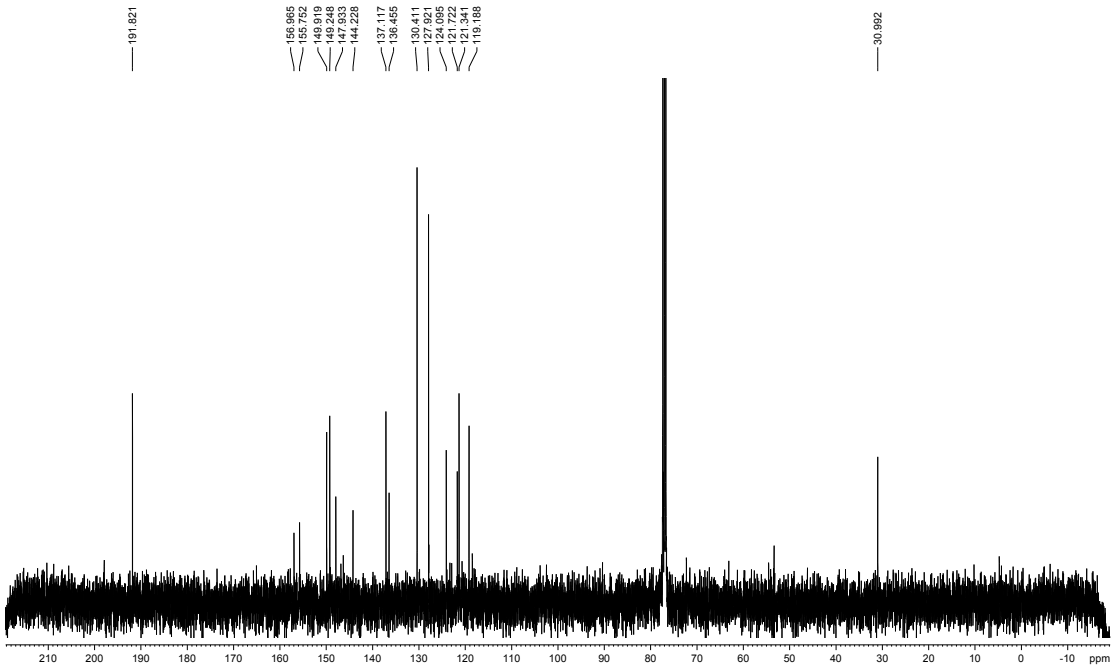


Figure S4: ^{13}C NMR spectra of **4** in $CDCl_3$.

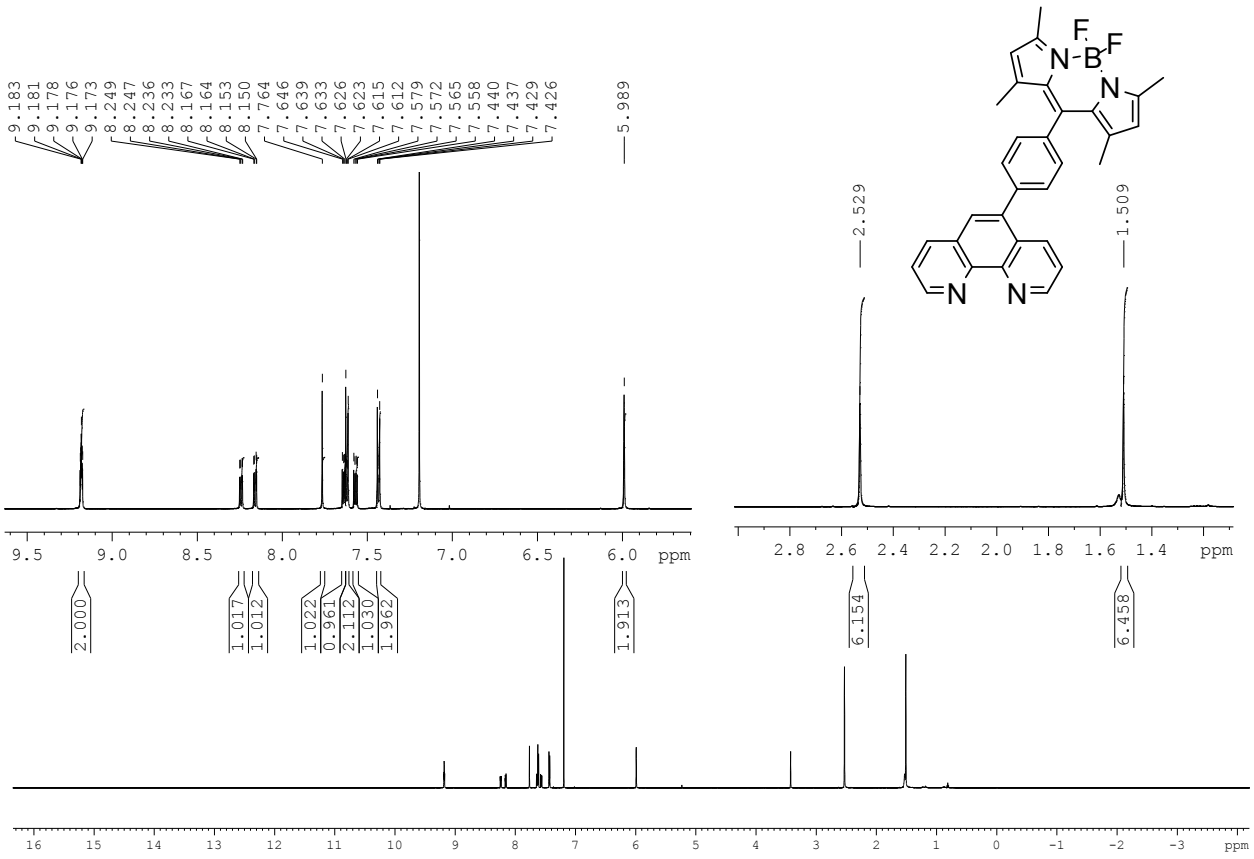


Figure S5: ^{1}H NMR spectra of **5** in $CDCl_3$.

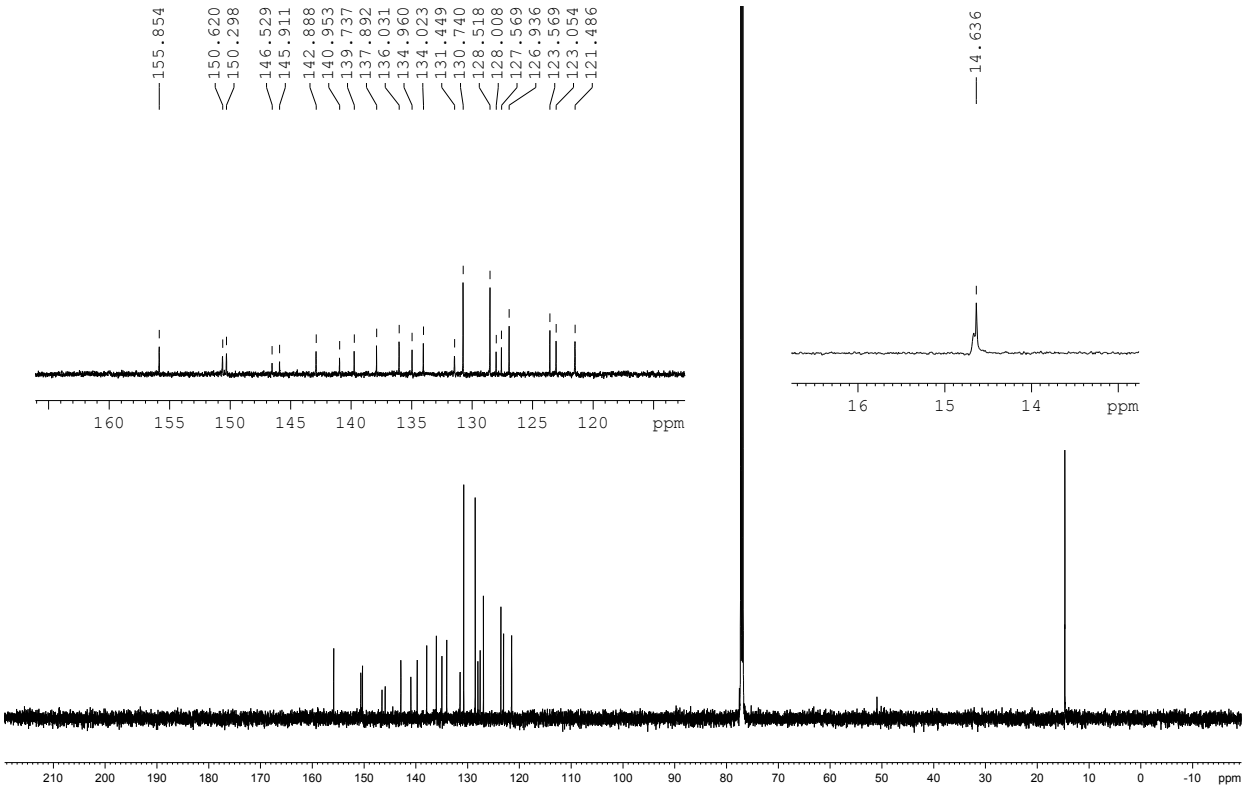


Figure S6: ^{13}C NMR spectra of **5** in $CDCl_3$.

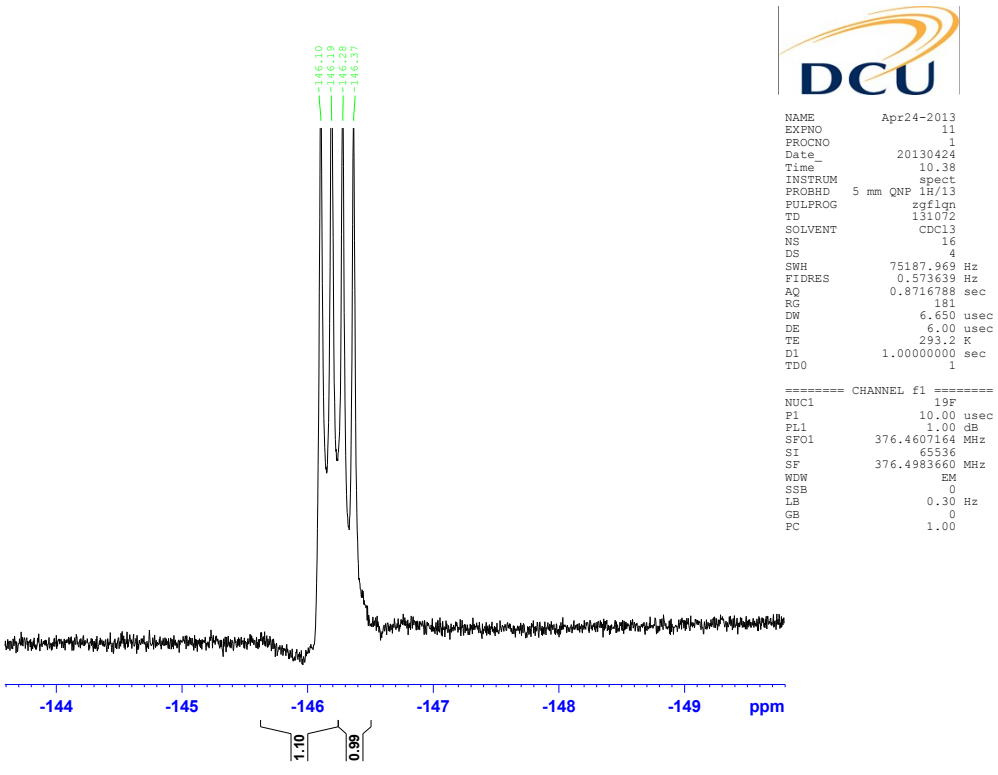
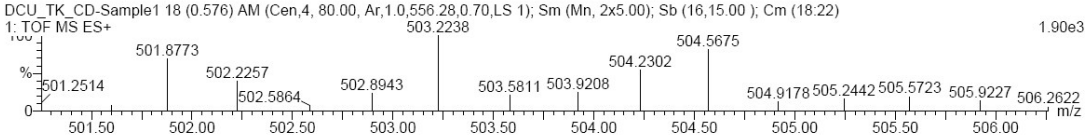


Figure S7: ^{19}F NMR spectra of **5** in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
80 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)



Minimum:		200.0	5.0	-1.5		
Maximum:				1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
503.2238	503.2219	1.9	3.9	20.5	1	C31 H26 N4 F2 B

Figure S8:. Mass spectrum of **5**.

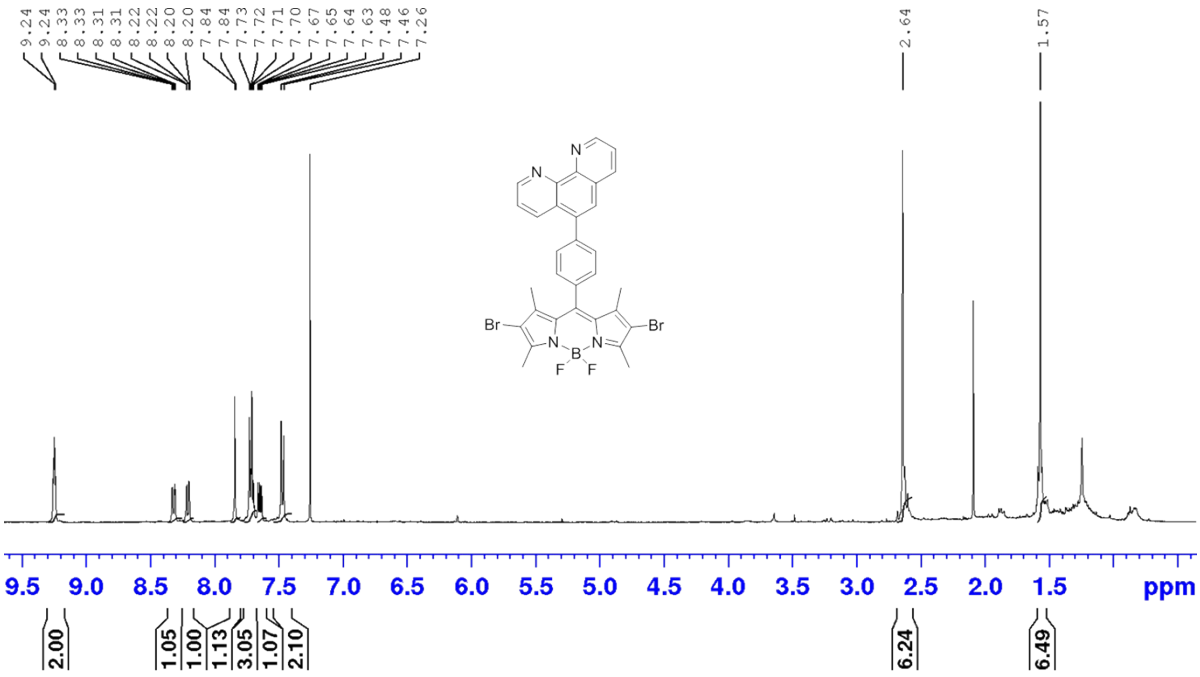


Figure S9: ^{1}H NMR spectra of **6** in $CDCl_3$.

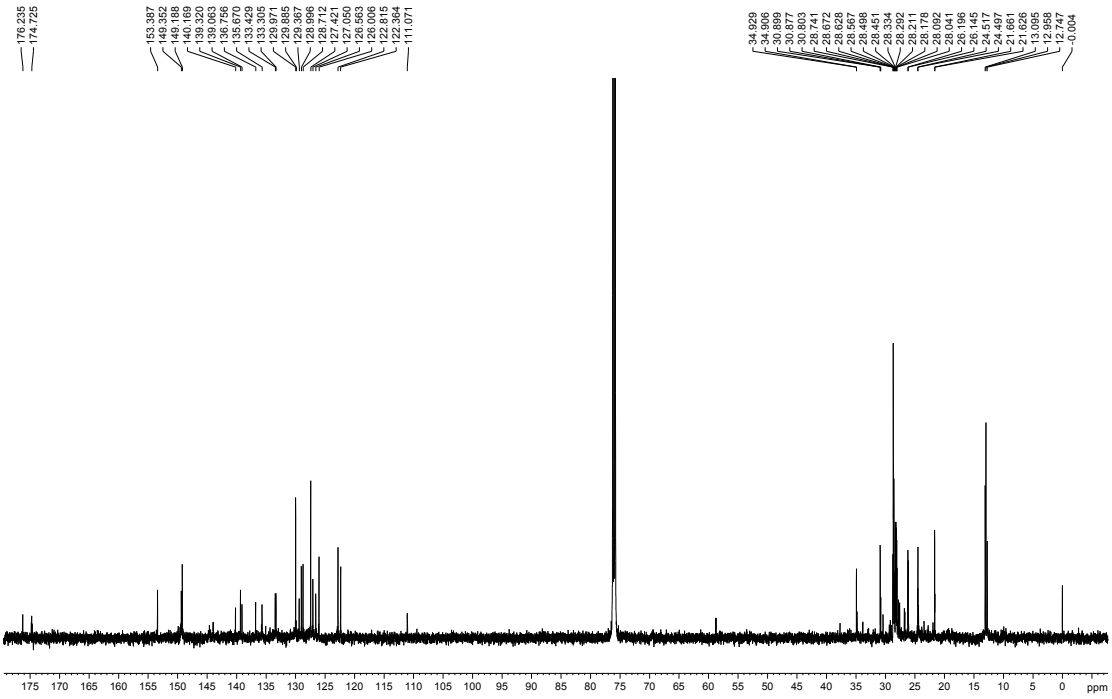


Figure S10: ^{13}C NMR spectra of **6** in $CDCl_3$.

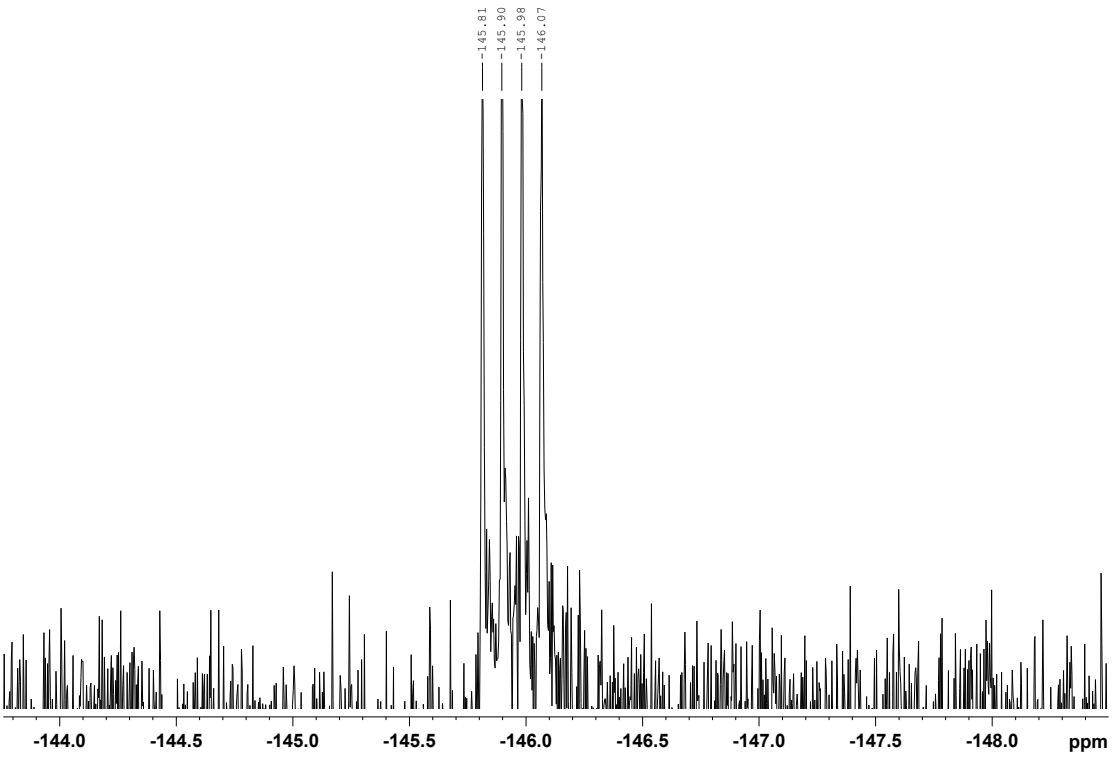


Figure S11: ^{19}F NMR spectra of **6** in $CDCl_3$.

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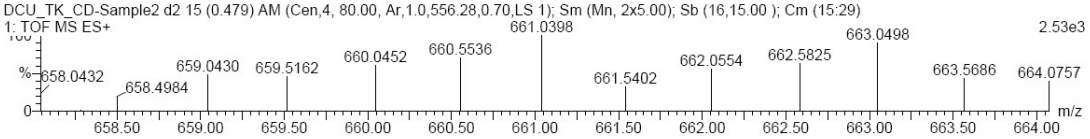
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
46 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

DCU_TK_CD-Sample2 d2 15 (0.479) AM (Cen,4, 80.00, Ar,1.0,556.28,0.70,LS 1); Sm (Mn, 2x5.00); Sb (16,15.00); Cm (15:29)
1: TOF MS ES+ 2.53e3



Minimum:				-1.5		
Maximum:		200.0	5.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
659.0430	659.0429	0.1	0.2	20.5	1	C31 H24 B N4 F2 Br2

Figure S12: Mass spectrum of **6**.

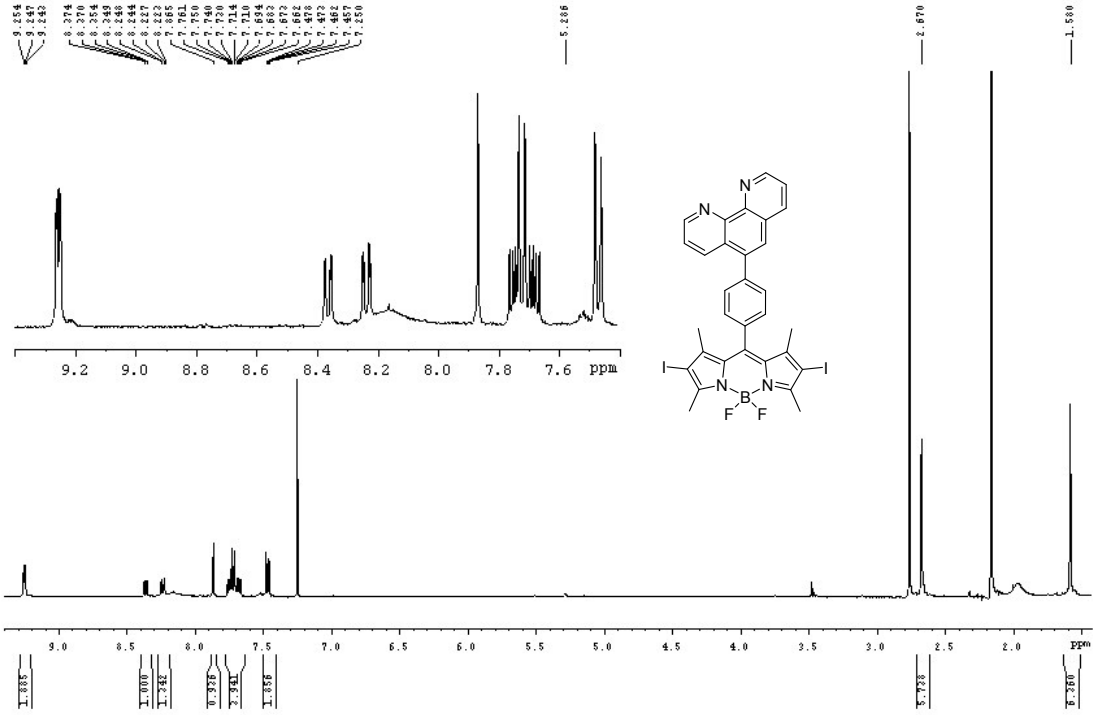


Figure S13: ^{1}H NMR spectra of **7** in $CDCl_3$.

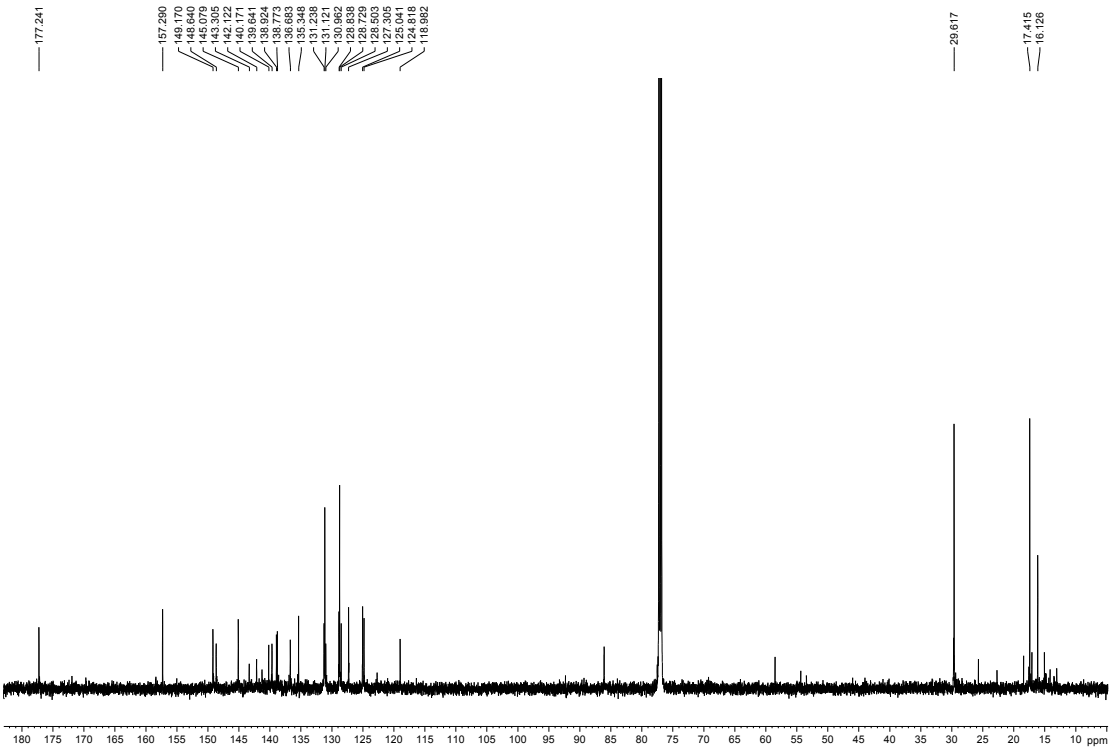


Figure S14: ^{13}C NMR spectra of **7** in $CDCl_3$.

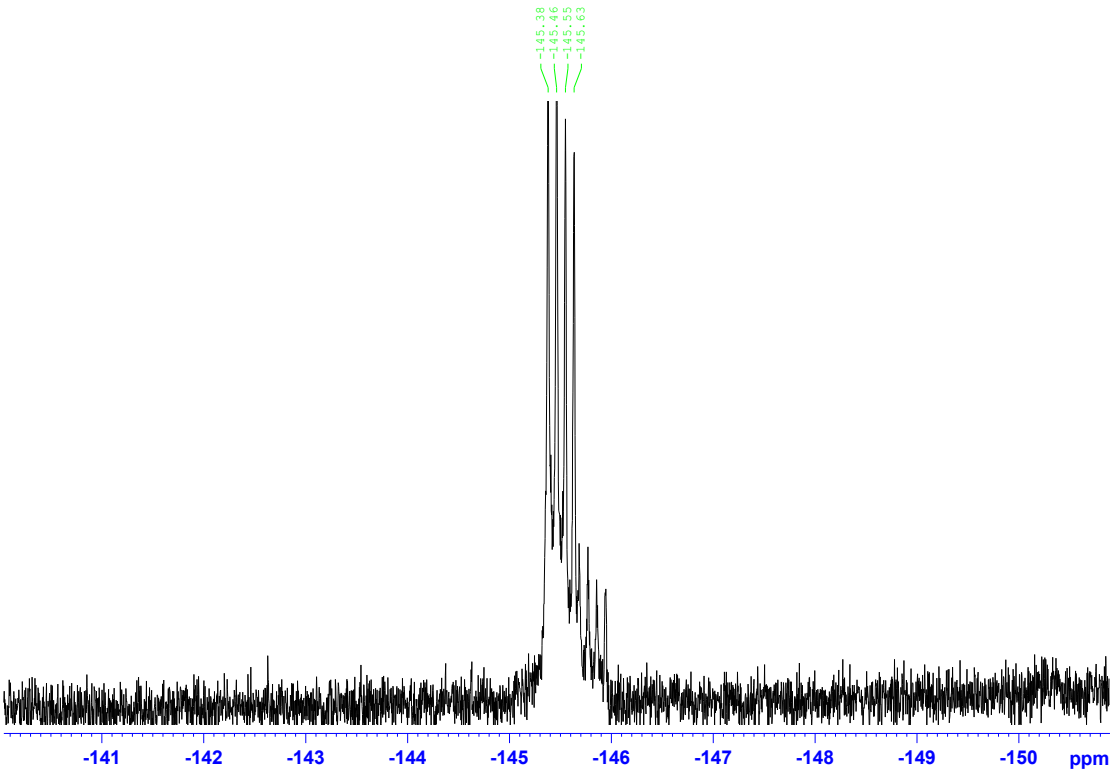


Figure S15: ^{19}F NMR spectra of **7** in $CDCl_3$.

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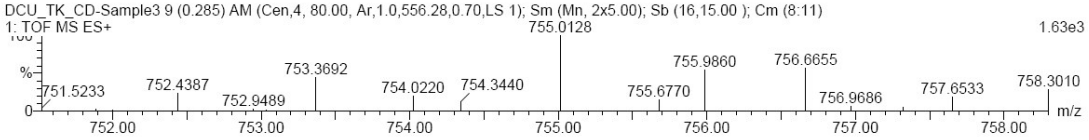
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
73 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

DCU_TK_CD-Sample3 9 (0.285) AM (Cen,4, 80.00, Ar,1.0,556.28,0.70,LS 1); Sm (Mn, 2x5.00); Sb (16,15.00); Cm (8:11)
1: TOF MS ES+ 1.63e3



Minimum:				-1.5		
Maximum:		200.0	5.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
755.0128	755.0152	-2.4	-3.1	20.5	1	C31 H24 B N4 F2 I2

Figure S16: Mass spectrum of **7**.

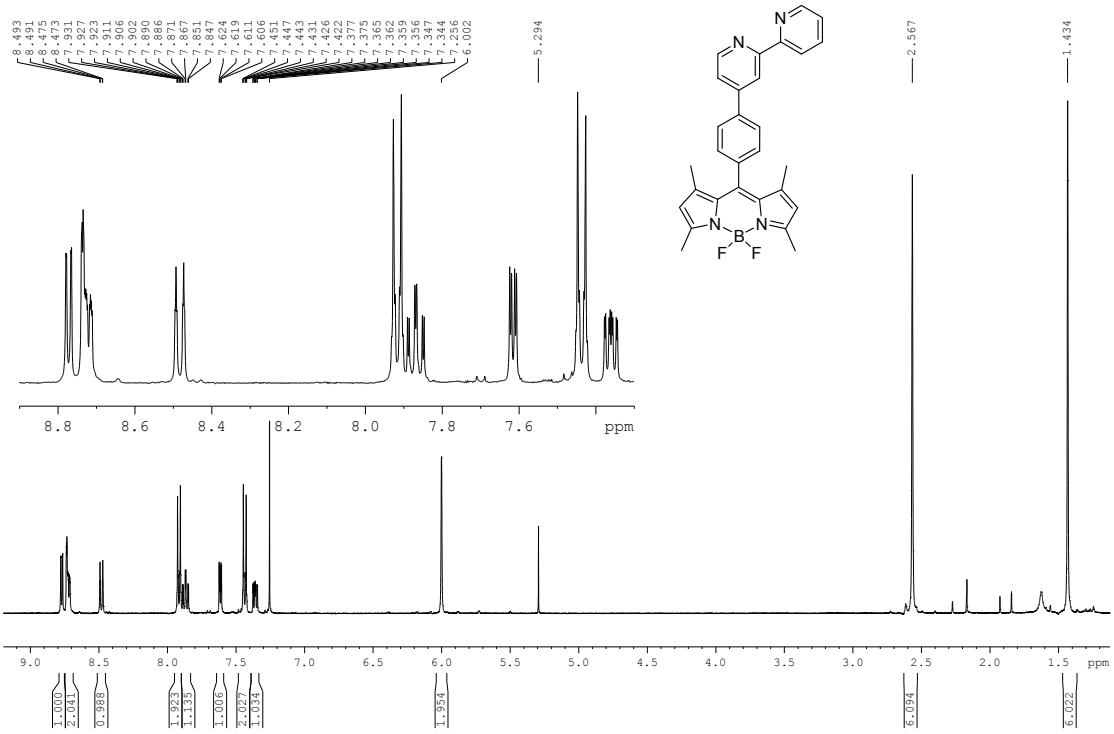


Figure S17: ^{1}H NMR spectra of **8** in $CDCl_3$.

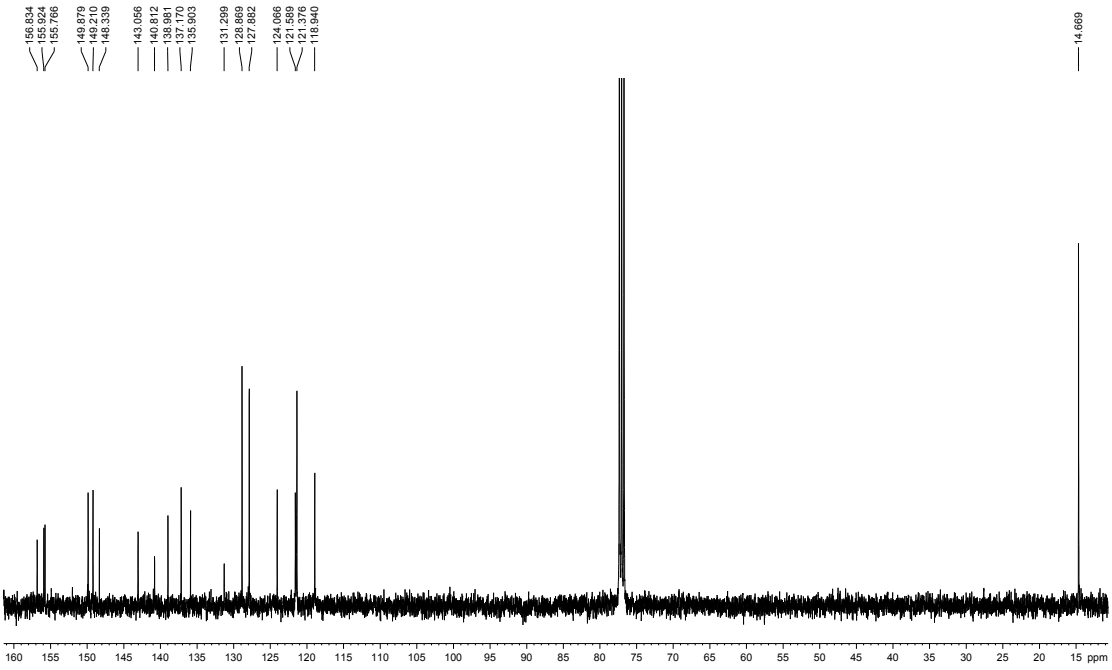


Figure S18: ^{13}C NMR spectra of **8** in $CDCl_3$.

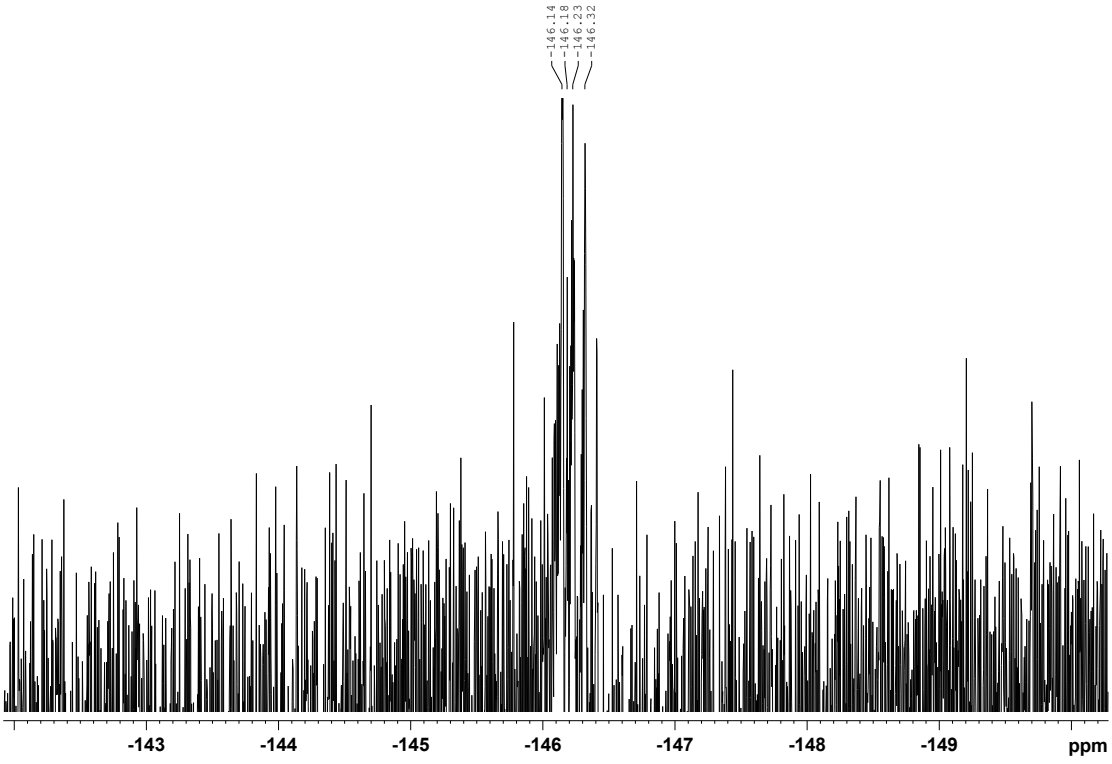


Figure S19: ^{19}F NMR spectra of **8** in $CDCl_3$.

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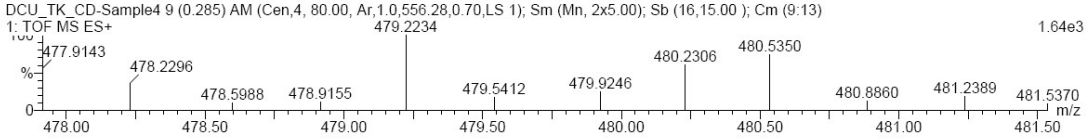
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
74 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

DCU_TK_CD-Sample4 9 (0.285) AM (Cen,4, 80.00, Ar,1.0,556.28,0.70,LS 1); Sm (Mn, 2x5.00); Sb (16,15.00); Cm (9:13)
1: TOF MS ES+ 1.64e3



Minimum:				-1.5		
Maximum:		200.0	5.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
479.2234	479.2219	1.5	3.2	18.5	1	C29 H26 B N4 F2

Figure S20: Mass spectrum of **8**.

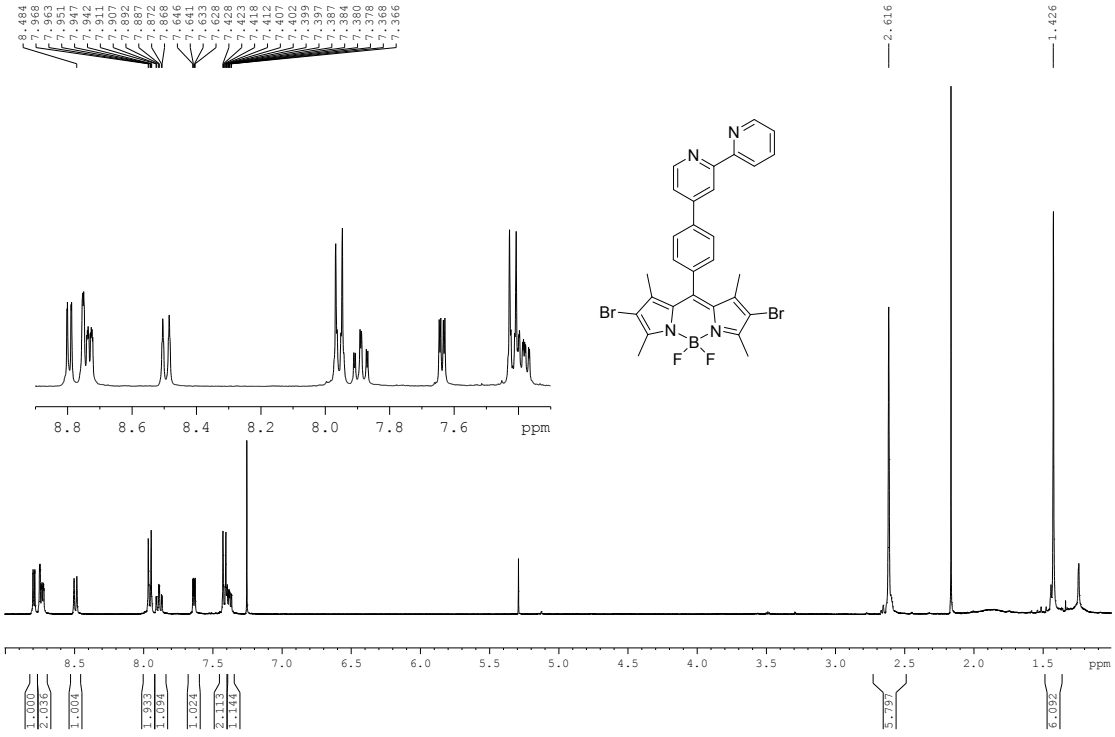


Figure S21: ^{1}H NMR spectra of **9** in $CDCl_3$.

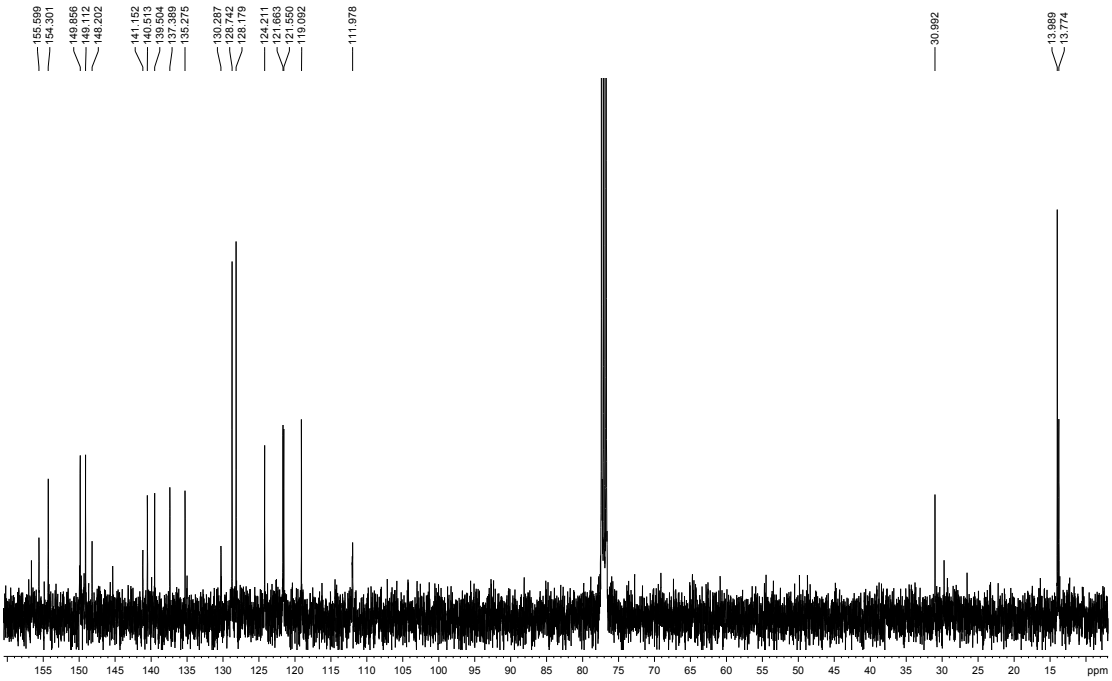


Figure S22: ^{13}C NMR spectra of **9** in $CDCl_3$.

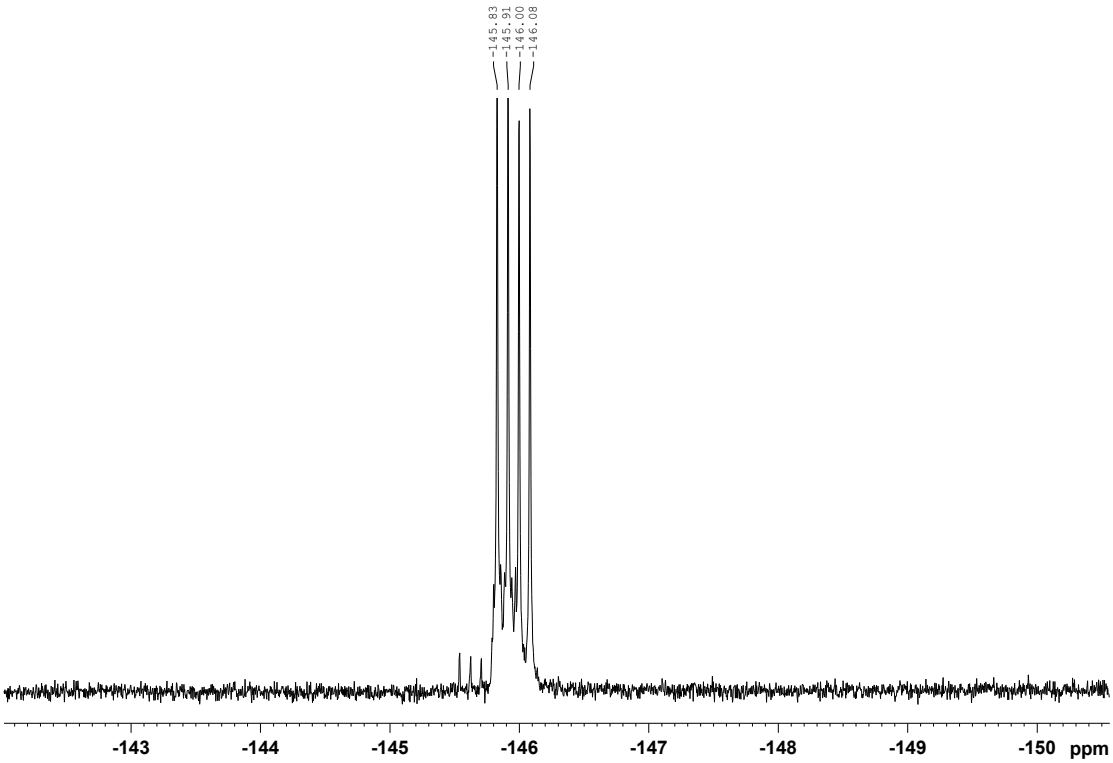
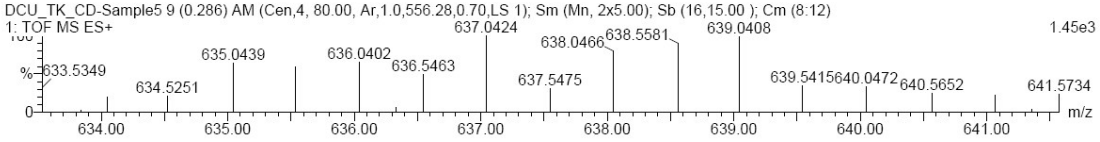


Figure S23: ^{19}F NMR spectra of **9** in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
45 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)



Minimum:				-1.5		
Maximum:		200.0	5.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
635.0439	635.0429	1.0	1.6	18.5	1	C29 H24 B N4 F2 Br2

Figure S24: Mass spectrum of **(9)**.

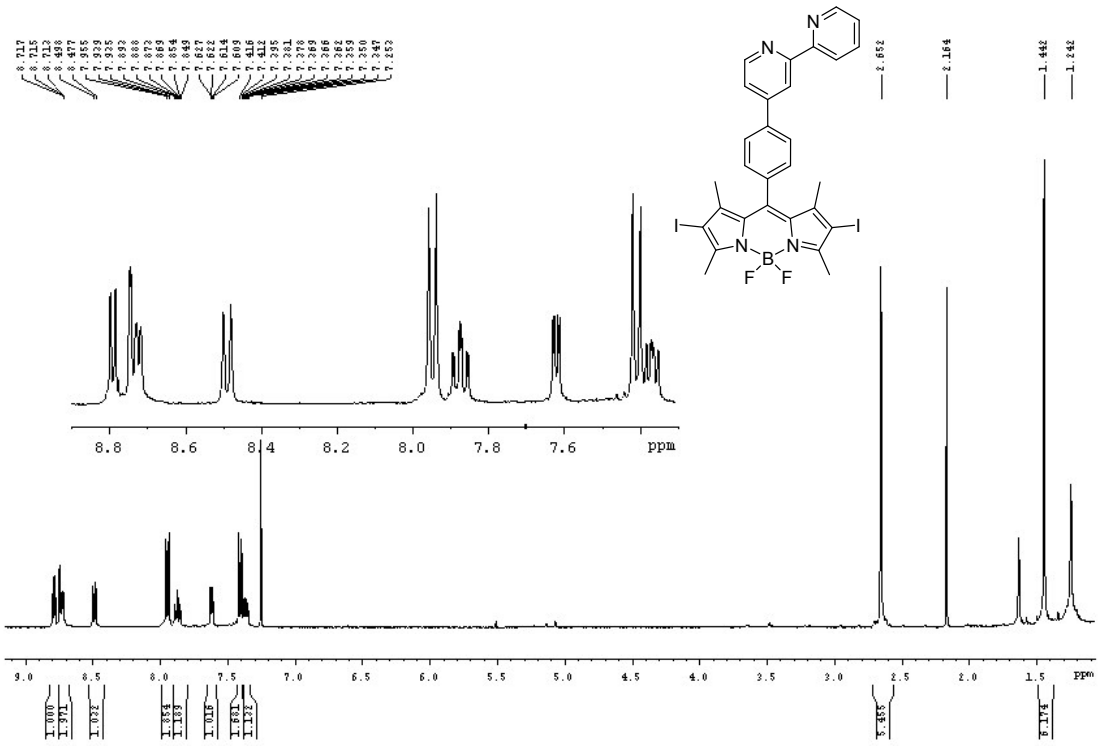


Figure S25: ^{1}H NMR spectra of **10** in $CDCl_3$.

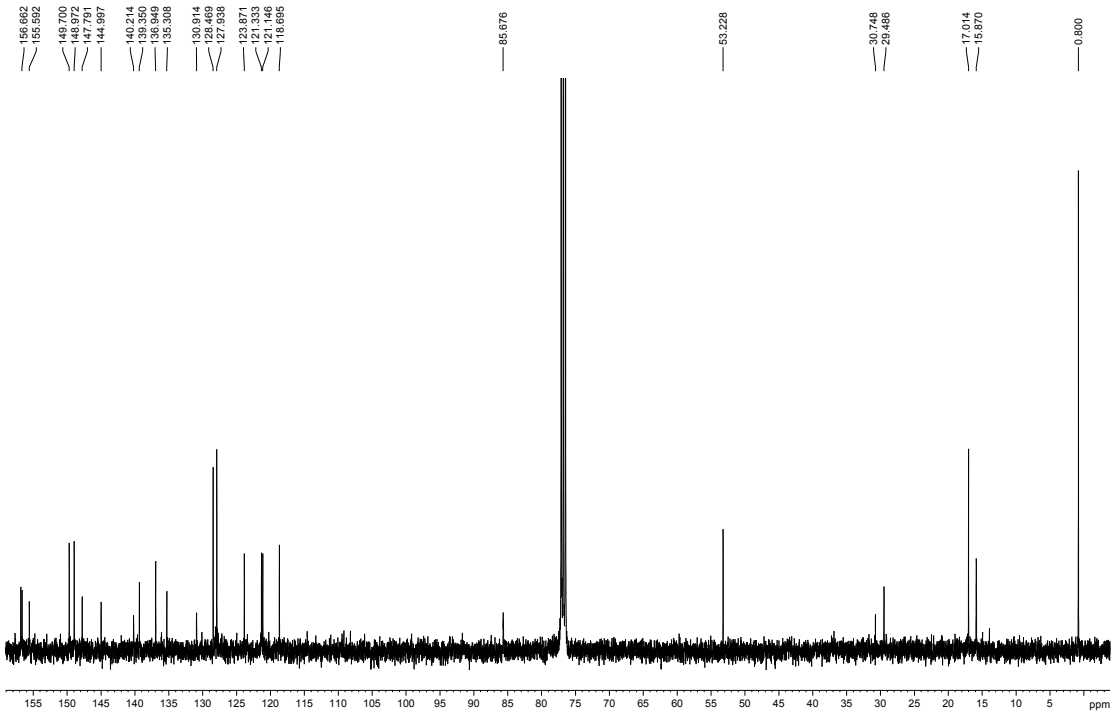


Figure S26: ^{13}C NMR spectra of **10** in $CDCl_3$.

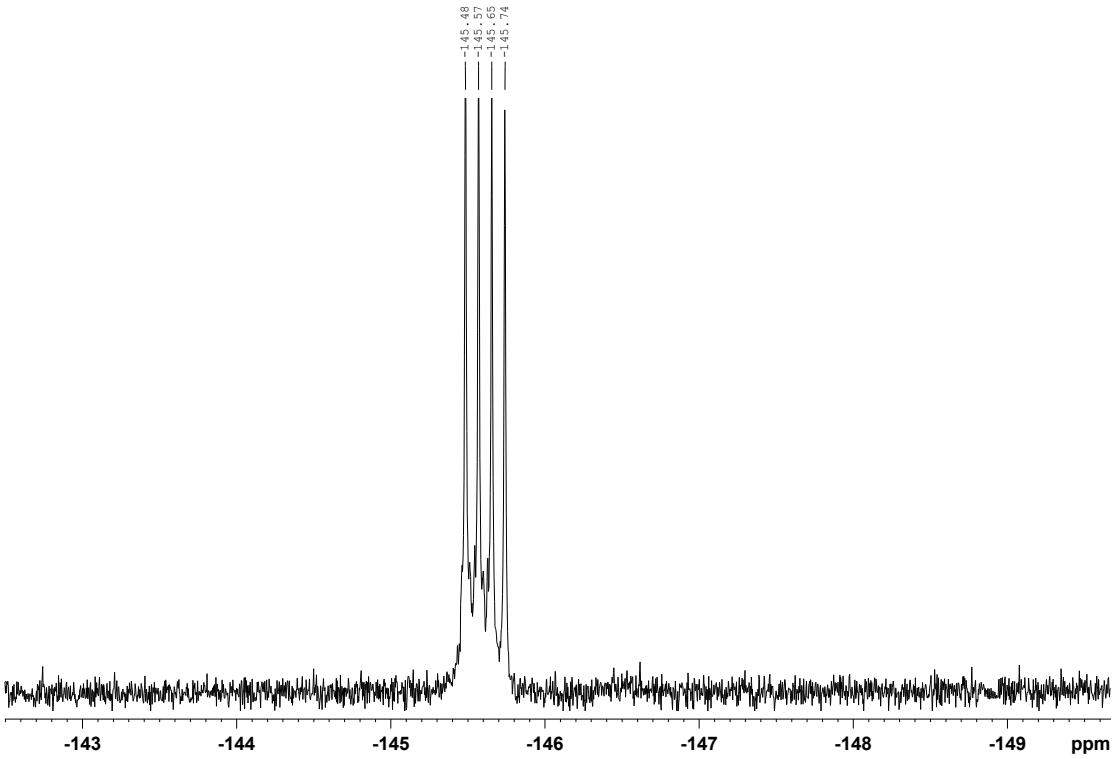


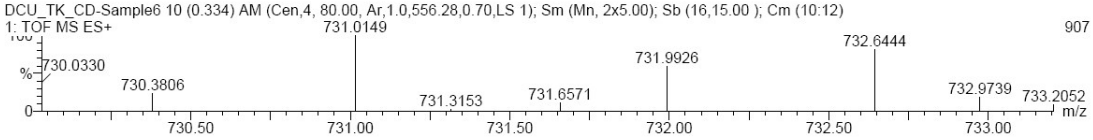
Figure S27: ^{19}F NMR spectra of **10** in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
134 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

DCU_TK_CD-Sample6 10 (0.334) AM (Cen,4, 80.00, Ar,1.0,556.28,0.70,LS 1); Sm (Mn, 2x5.00); Sb (16,15.00); Cm (10:12)
1: TOF MS ES+



Minimum:				-1.5		
Maximum:		200.0	5.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
731.0149	731.0152	-0.3	-0.4	18.5	1	C29 H24 B N4 F2 I2

Figure S28: Mass spectrum of **10**.

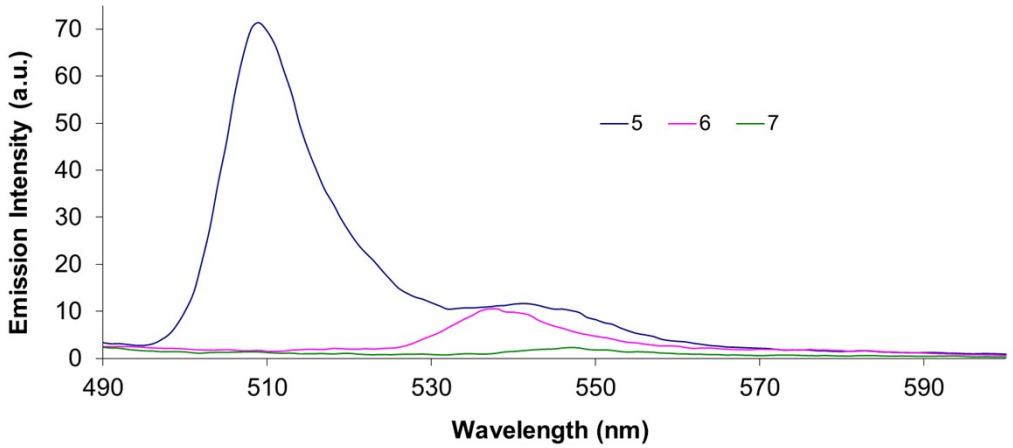


Figure S29: Overlaid low temperature (77K) emission spectra of 1,10-phenanthrolyl BODIPY-based derivatives (**5-7**) in butyronitrile:propionitrile (5:4 v/v).

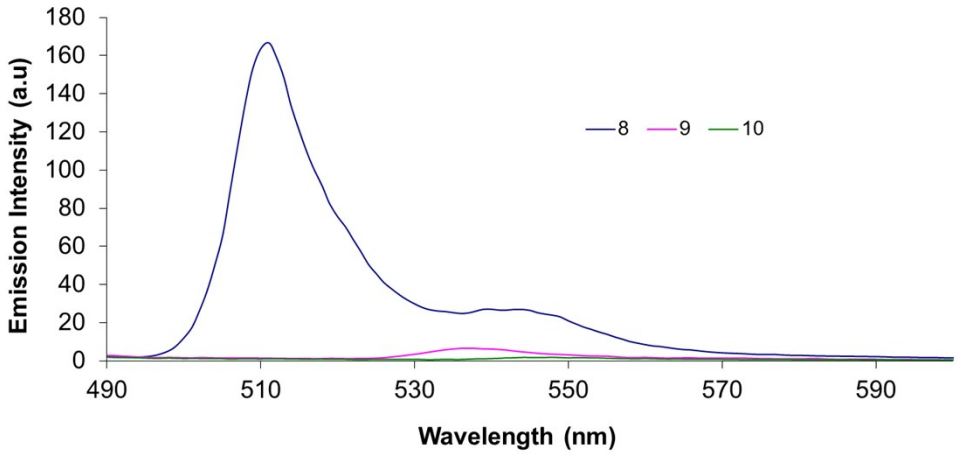


Figure S30: Overlaid low temperature (77K) emission spectra of 2,2'-bipyridyl BODIPY-based derivatives (**8-10**) in butyronitrile:propionitrile (5:4 v/v).

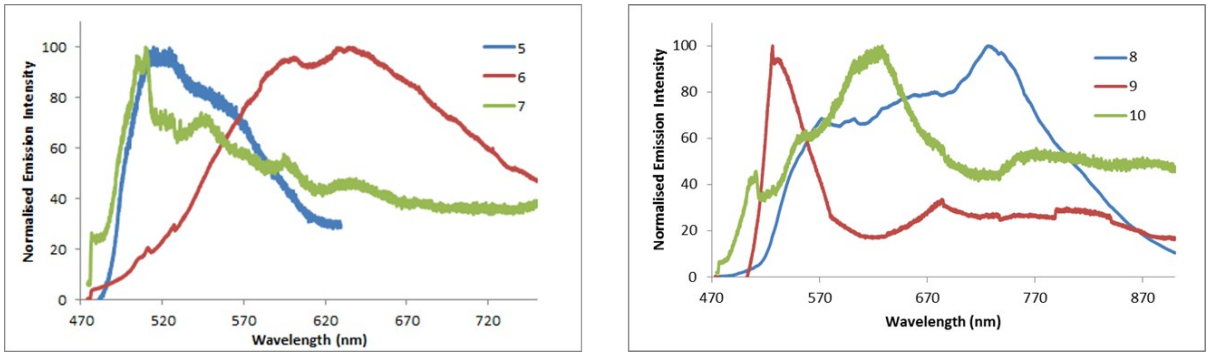


Figure S31: Normalised solid state emission spectra of (left) **5-7** and (right) **8-10** after 473 nm excitation using Raman spectroscopy. Solid state samples were simply deposited on a glass microscope slide and resulting fluorescence spectra were recorded.

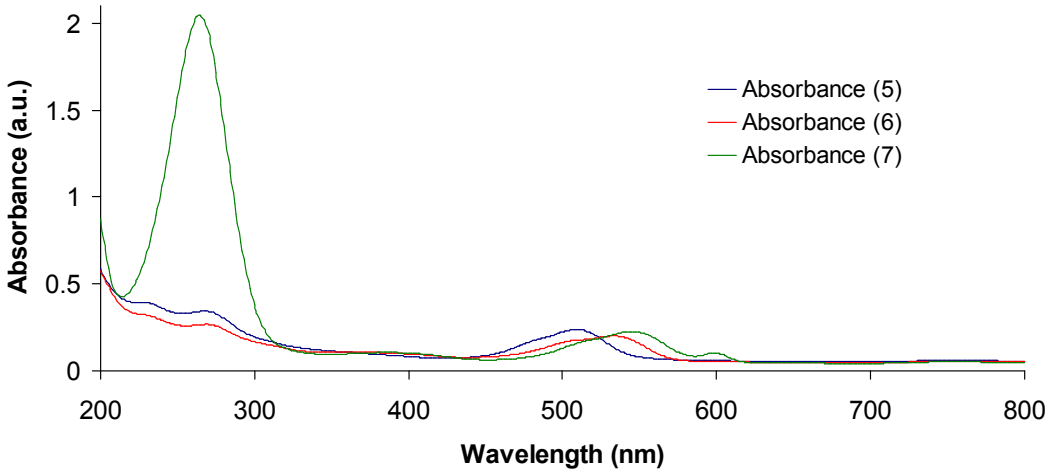


Figure S32: Overlaid absorbance spectra of 1,10-phenanthrolyl BODIPY-based derivatives (**5-7**) in H_2O (10 µM concentrations).

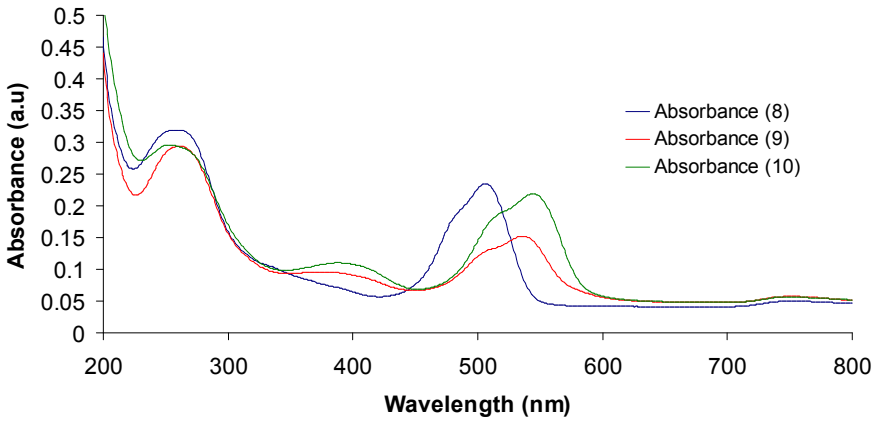


Figure S33: Overlaid absorbance spectra of 2,2'-bipyridyl BODIPY-based derivatives (**8-10**) in H_2O (10 µM concentrations).

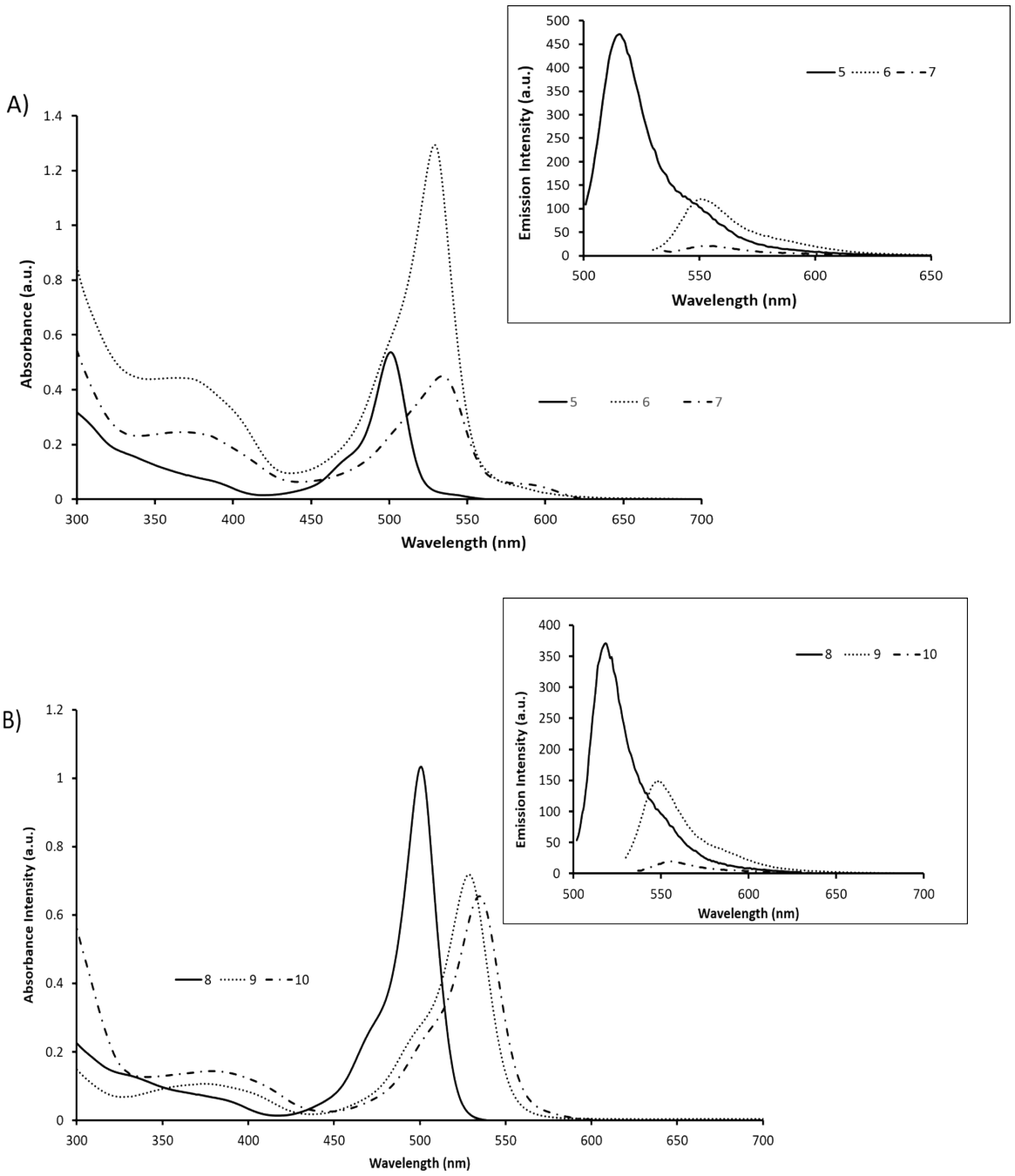
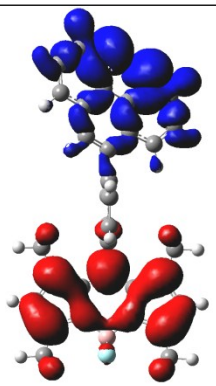
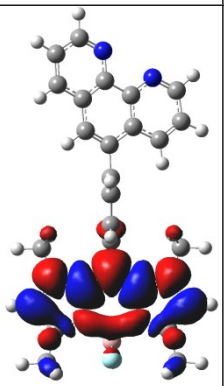
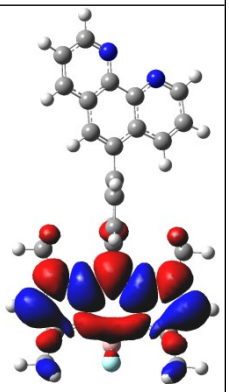
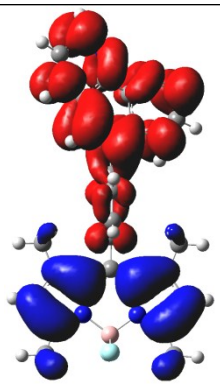
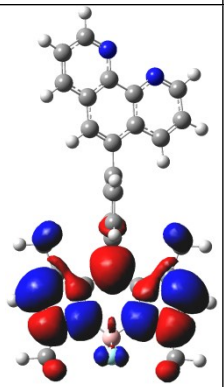
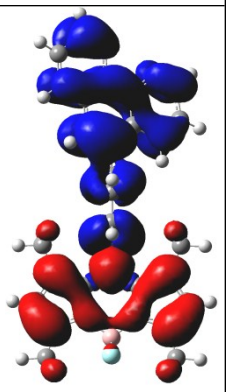
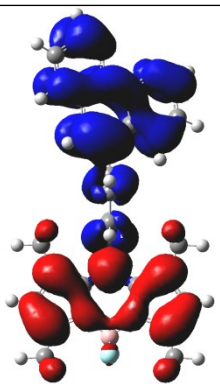
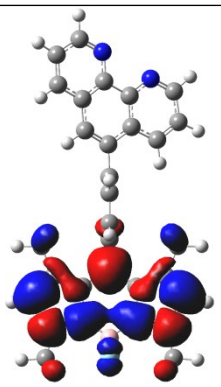
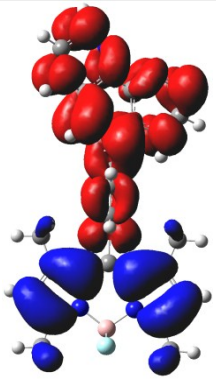
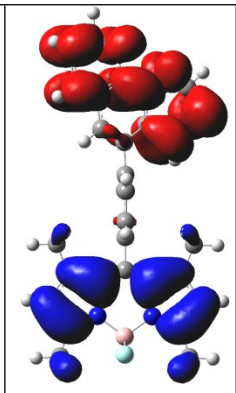
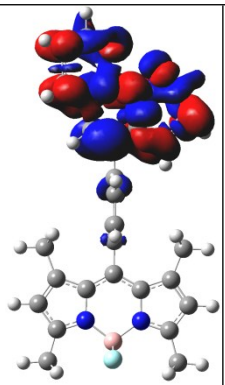
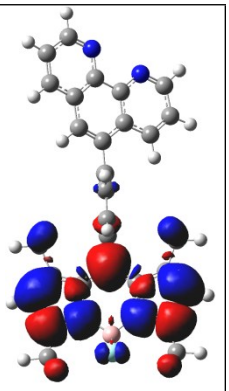
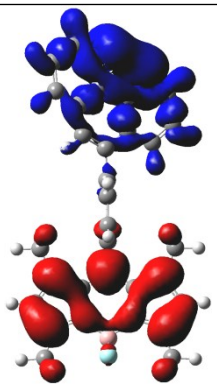
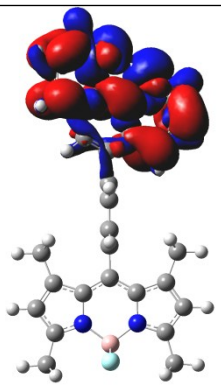
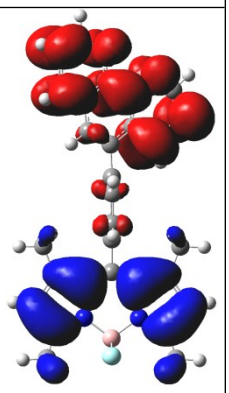
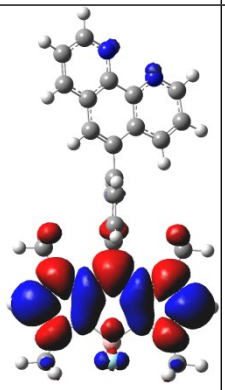
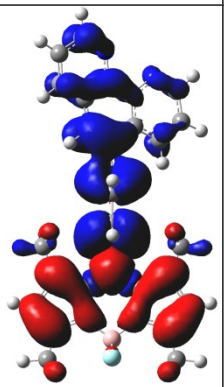
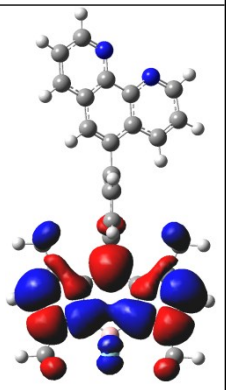
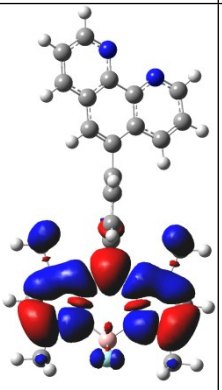
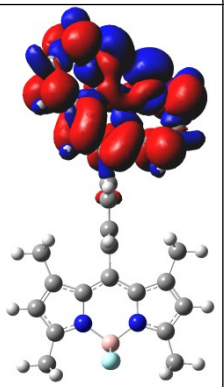
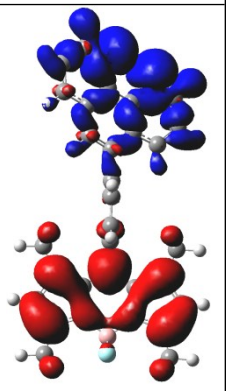
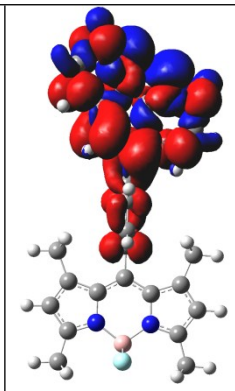
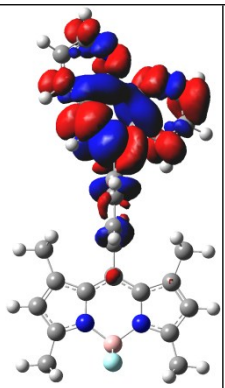
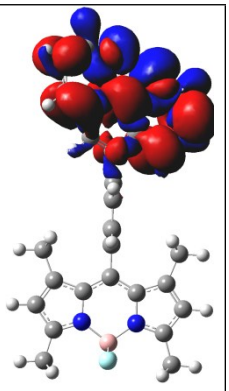
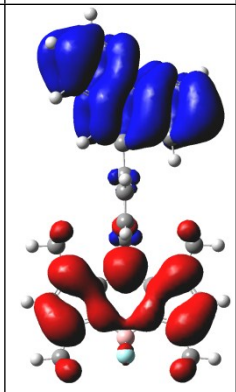
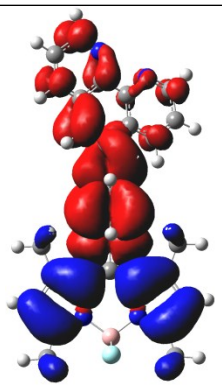
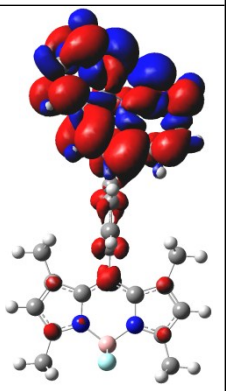
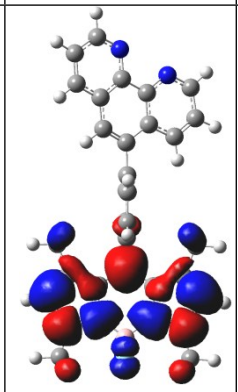
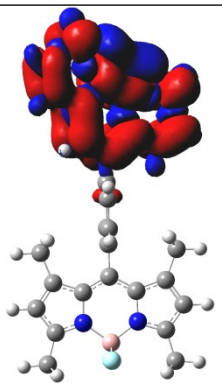
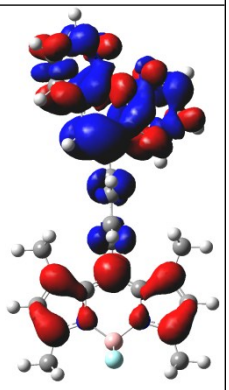


Figure S34: Absorbance and emission spectra (inset) of (A) 1,10-phenanthrolyl BODIPY-based derivatives (**5-7**) and (B) 2,2'-bipyridyl BODIPY-based derivatives (**8-10**) in DCM (10 µM). Emission spectra were recorded by exciting each sample at its maximum visible absorbance and slit widths were kept constant at 2.5 nm.

ES	BLYP	Electron Density Maps BLYP	Cam-B3LYP	Electron Density Maps cam-B3LYP	B3LYP	Electron Density Maps B3LYP
1	2.3945 eV 517.78 nm f=0.0000		3.0172 eV 410.92 nm f=0.5103		2.9942 eV 414.08 nm f=0.4605	
2	2.5038 eV 495.18 nm f=0.0005		3.9221 eV 316.12 nm f=0.0437		3.3768 eV 367.17 nm f=0.0023	
3	2.5883 eV 479.01 nm f=0.0019		4.2066 eV 294.74 nm f=0.0511		3.3999 eV 364.67 nm f=0.0001	

4	2.6259 eV 472.16 nm f=0.0005		4.4032 eV 281.58 nm f=0.0025		3.4568 eV 358.67 nm f=0.0526	
5	2.8223 eV 439.31 nm f=0.0026		4.4147 eV 280.84 nm f=0.0004		3.5955 eV 344.83 nm f=0.0011	
6	2.8541 eV 434.41 nm f=0.2304		4.4834 eV 276.54 nm f=0.0126		3.6899 eV 336.01 nm f=0.0302	
7	3.0137 eV 411.40 nm f=0.2596		4.5151 eV 274.60 nm f=0.0038		3.8701 eV 320.36 nm f=0.0000	

8	3.0399 eV 407.86 nm f=0.0005		4.5957 eV 269.79 nm f=0.2361		3.9221 eV 316.12 nm f=0.0001	
9	3.0430 eV 407.45 nm f=0.0009		4.6861 eV 264.58 nm f=0.0005		3.9336 eV 315.19 nm f=0.0010	
10	3.1493 eV 393.69 nm f=0.0220		4.9758 eV 249.17 nm f=0.0000		3.9537 eV 313.59 nm f=0.0013	
11	3.2290 eV 383.97 nm f=0.0000		5.0118 eV 247.38 nm f=0.0020			
12	3.2682 eV 379.37 nm f=0.0005		5.0206 eV 246.95 nm f=0.0410			

13	3.2999 eV 375.72 nm f=0.0001		5.1848 eV 239.13 nm f=0.0011			
14	3.3755 eV 367.30 nm f=0.0157		5.2469 eV 236.30 nm f=0.9817			
15	3.4211 eV 362.41 nm f=0.0004		5.2796 eV 234.84 nm f=0.1222			
16	3.4656 eV 357.76 nm f=0.0003		5.3199 eV 233.06 nm f=0.1927			
17	3.4704 eV 357.26 nm f=0.0007		5.3264 eV 232.77 nm f=0.1830			
18	3.4799 eV 356.29 nm f=0.0018		5.3602 eV 231.31 nm f=0.0255			
19	3.4982 eV 354.42 nm		5.3943 eV 229.84 nm			

	f=0.0028		f=0.2288			
20	3.5653 eV 347.75 nm f=0.0011		5.4116 eV 229.11 nm f=0.0399			

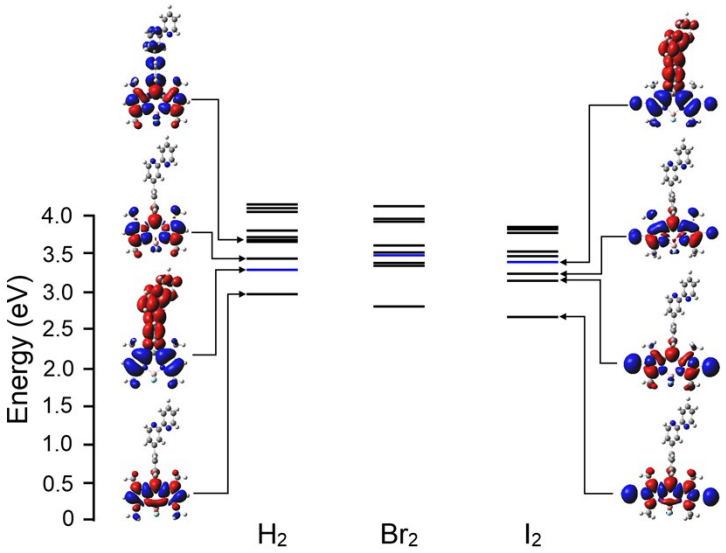


Figure S35 Singlet excited state energies for **8** (H_2), **9** (Br_2) and **10** (I_2) and the electron density difference maps for 8 (left) and 10 (right); levels indicated in blue are BODIPY to bipyridyl charge-transfer in character while black indicates BODIPY-based states.

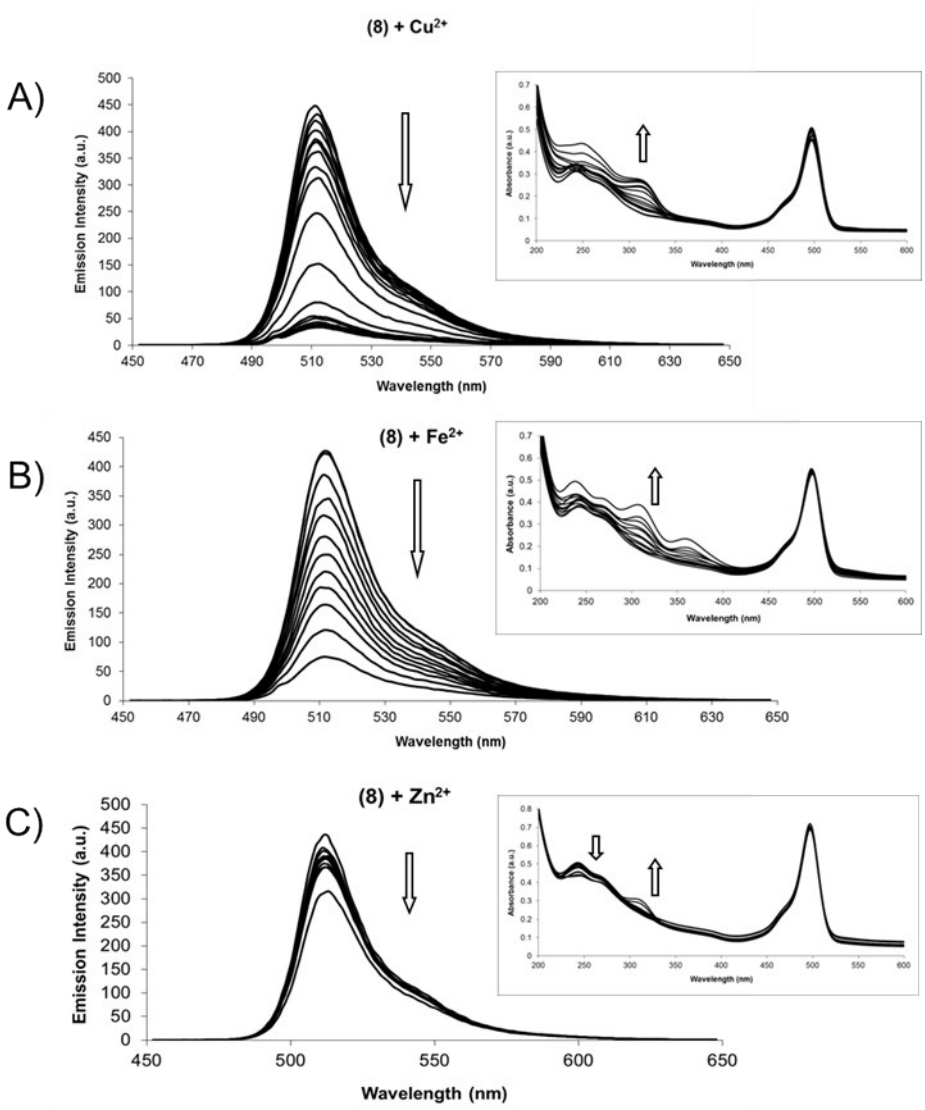


Figure 36: Representative fluorescence and absorbance response of 1,3,5,7-tetramethyl-4-bipyridyl-4,4'-difluoroboradiazaindacene **(8)** (10 µM concentration) on titration with A) copper(II) acetate, B) iron(II) chloride and C) zinc(II) acetate in acetonitrile, up to 3 molar equivalence. Excitation wavelength was 497 nm and emission slit widths were 2.5 nm. Insets shown the corresponding absorbance spectra.

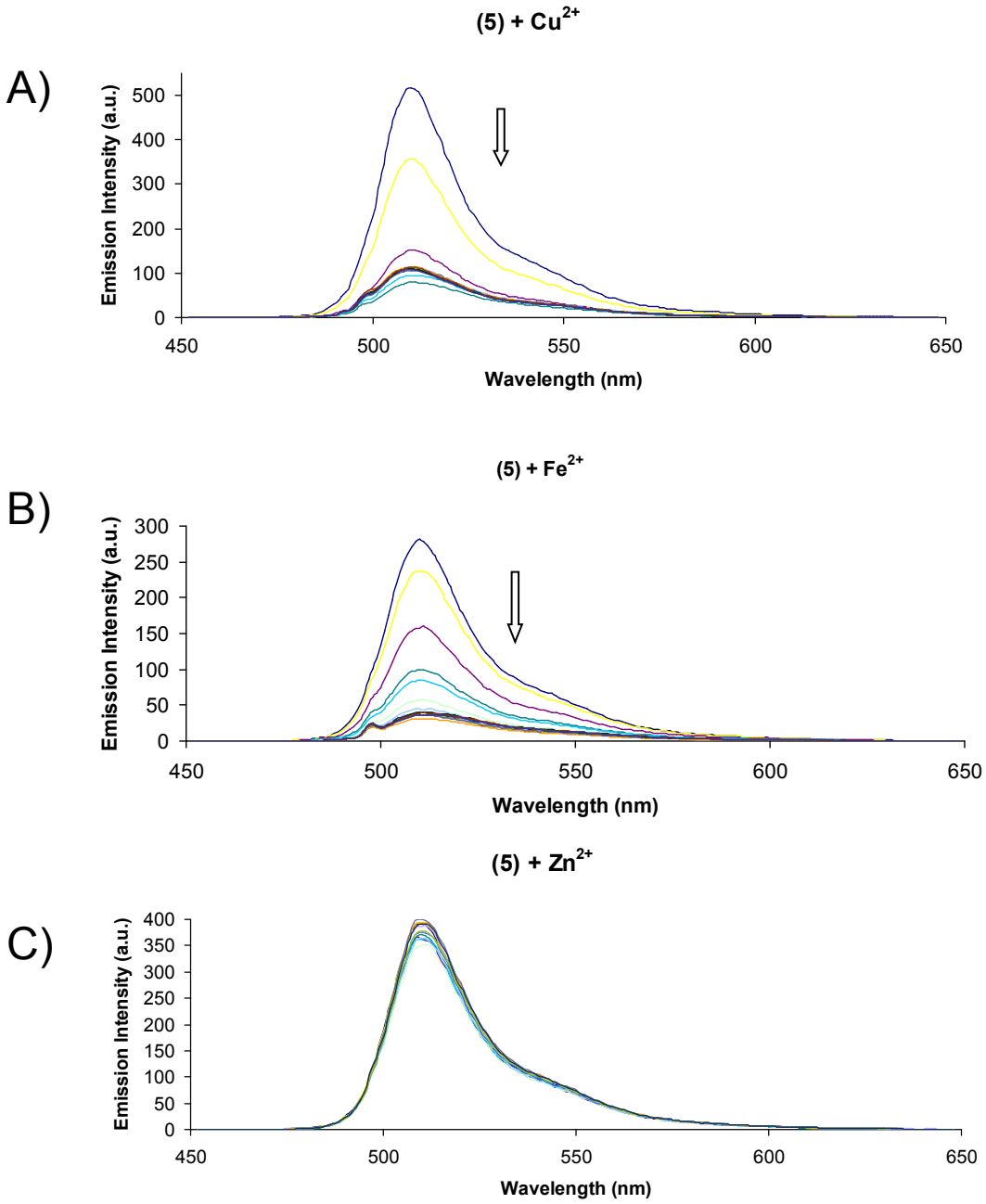


Figure S37: Overlaid fluorescent emission response of **5** (10 µM concentration) to A) Cu^{2+} metal ions, B) Fe^{2+} metal ions and C) Zn^{2+} metal ions in acetonitrile. Excitation wavelength was 497 nm and emission slit widths were 2.5 nm. Molar equivalence of each metal ion to BODIPY molecule ranged from 0 to a maximum of 3 equivalences.

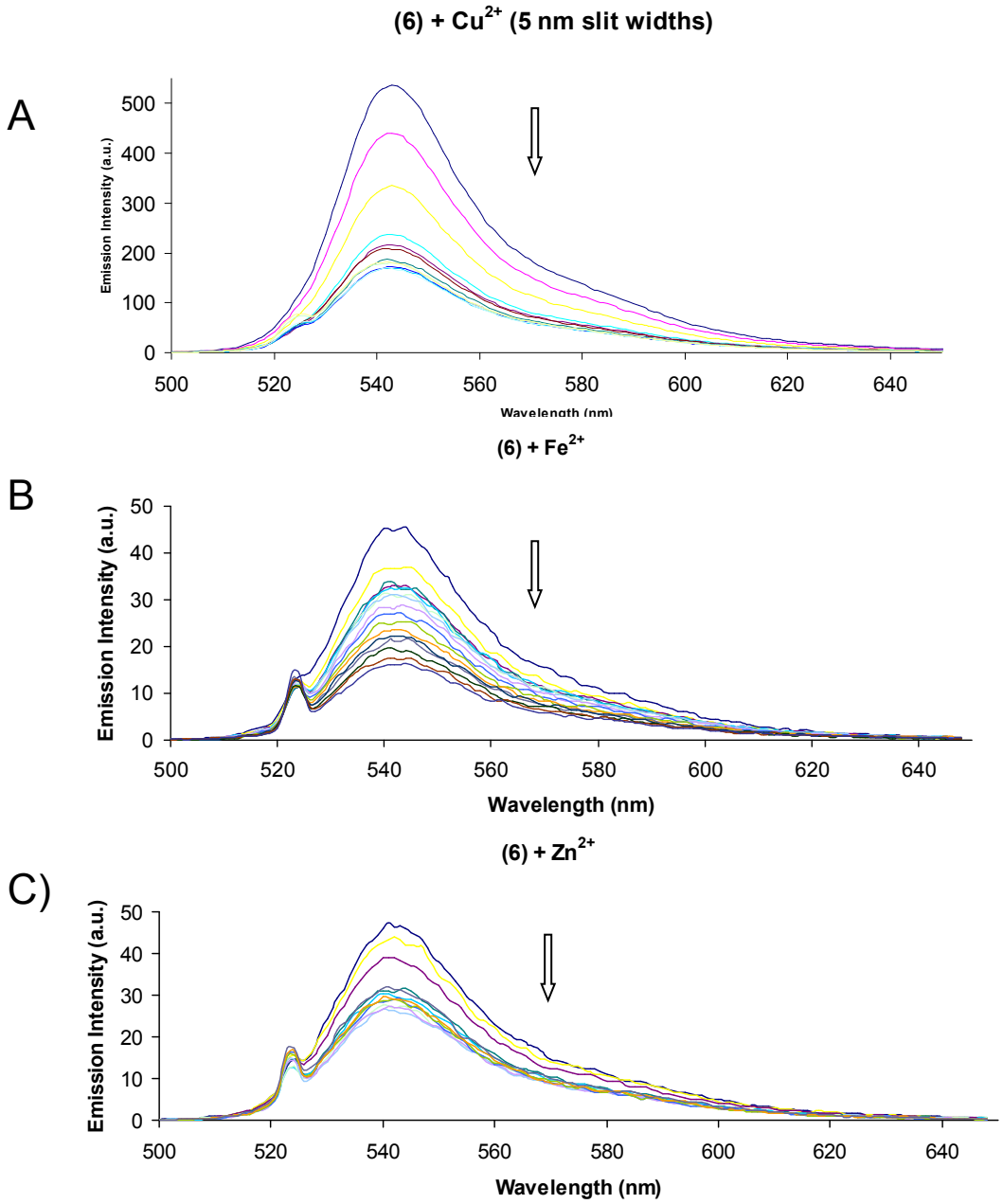


Figure S38: Overlaid fluorescent emission response of **6** (10 µM concentration) to A) Cu^{2+} metal ions (5 nm slit widths), B) Fe^{2+} metal ions and C) Zn^{2+} metal ions in acetonitrile. Excitation wavelength was 523 nm and emission slit widths were 2.5 nm. Molar equivalence of each metal ion to BODIPY molecule ranged from 0 to a maximum of 3 equivalences.

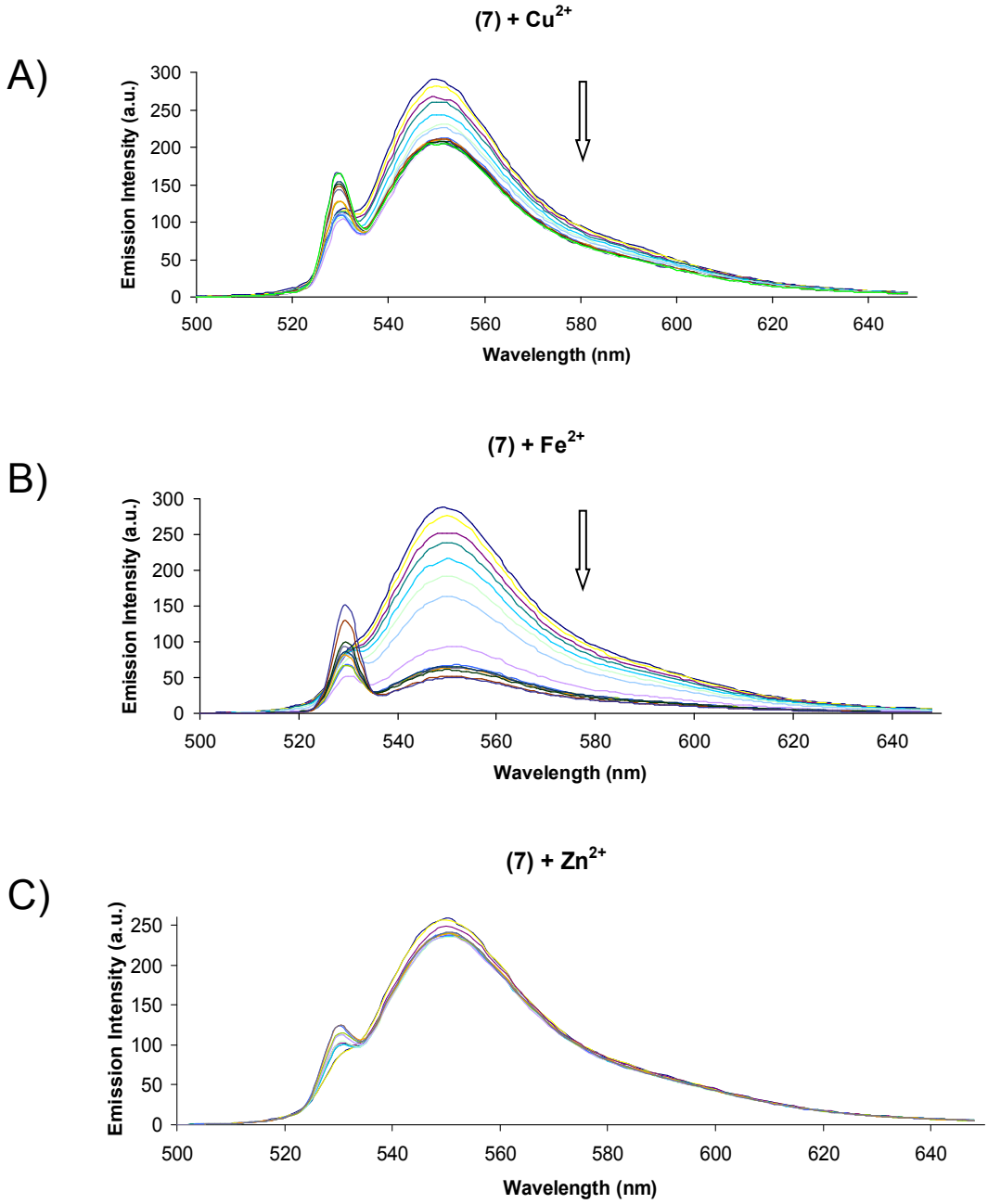


Figure S39: Overlaid fluorescent emission response of **7** (10 µM concentration) to A) Cu^{2+} metal ions, B) Fe^{2+} metal ions and C) Zn^{2+} metal ions in acetonitrile. Excitation wavelength was 529 nm and emission slit widths were 5 nm. Molar equivalence of each metal ion to BODIPY molecule ranged from 0 to a maximum of 3 equivalences.

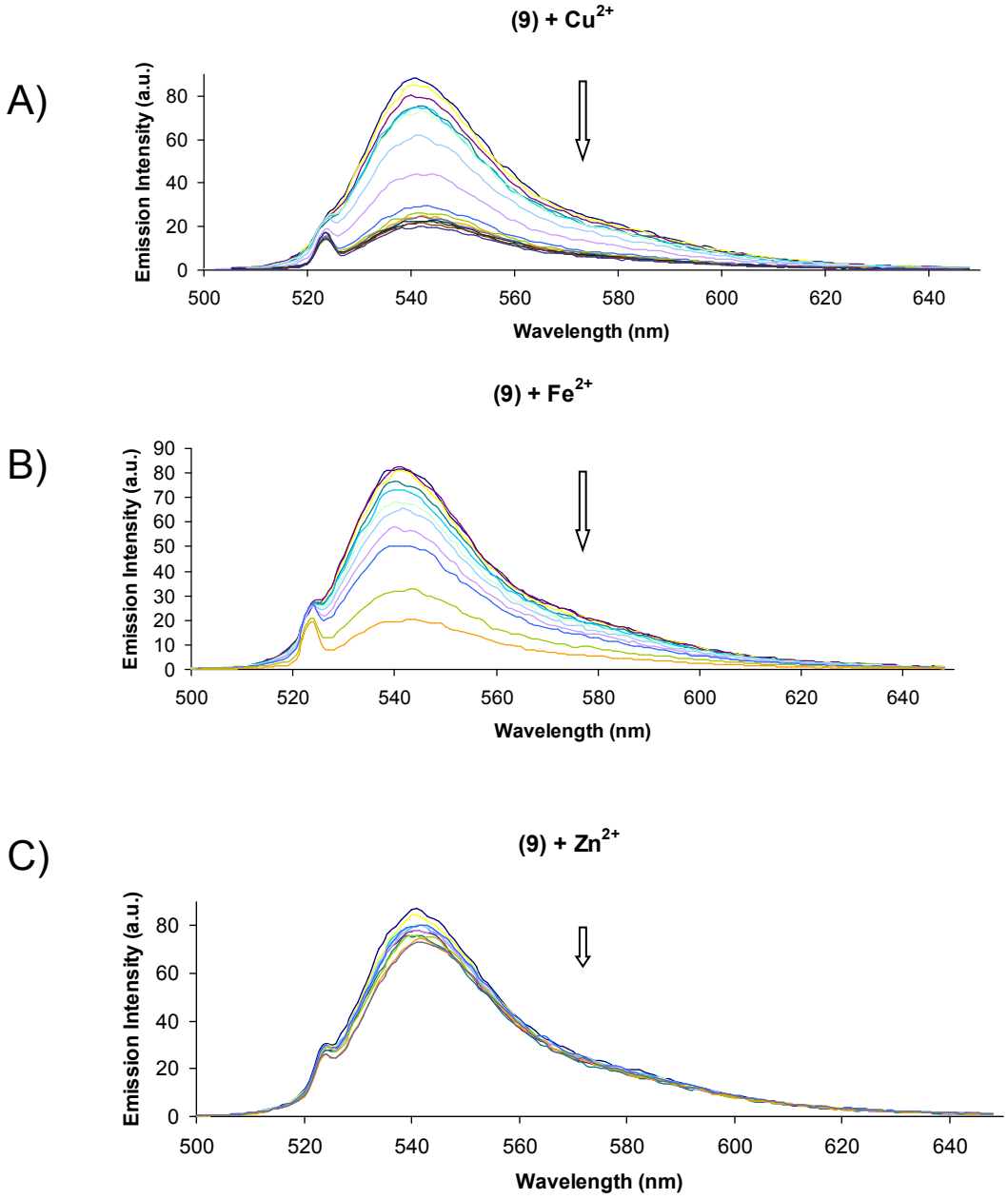


Figure S40: Overlaid fluorescent emission response of **9** (10 µM concentration) to A) Cu^{2+} metal ions, B) Fe^{2+} metal ions and C) Zn^{2+} metal ions in acetonitrile. Excitation wavelength was 523 nm and emission slit widths were 2.5 nm. Molar equivalence of each metal ion to BODIPY molecule ranged from 0 to a maximum of 3 equivalences.

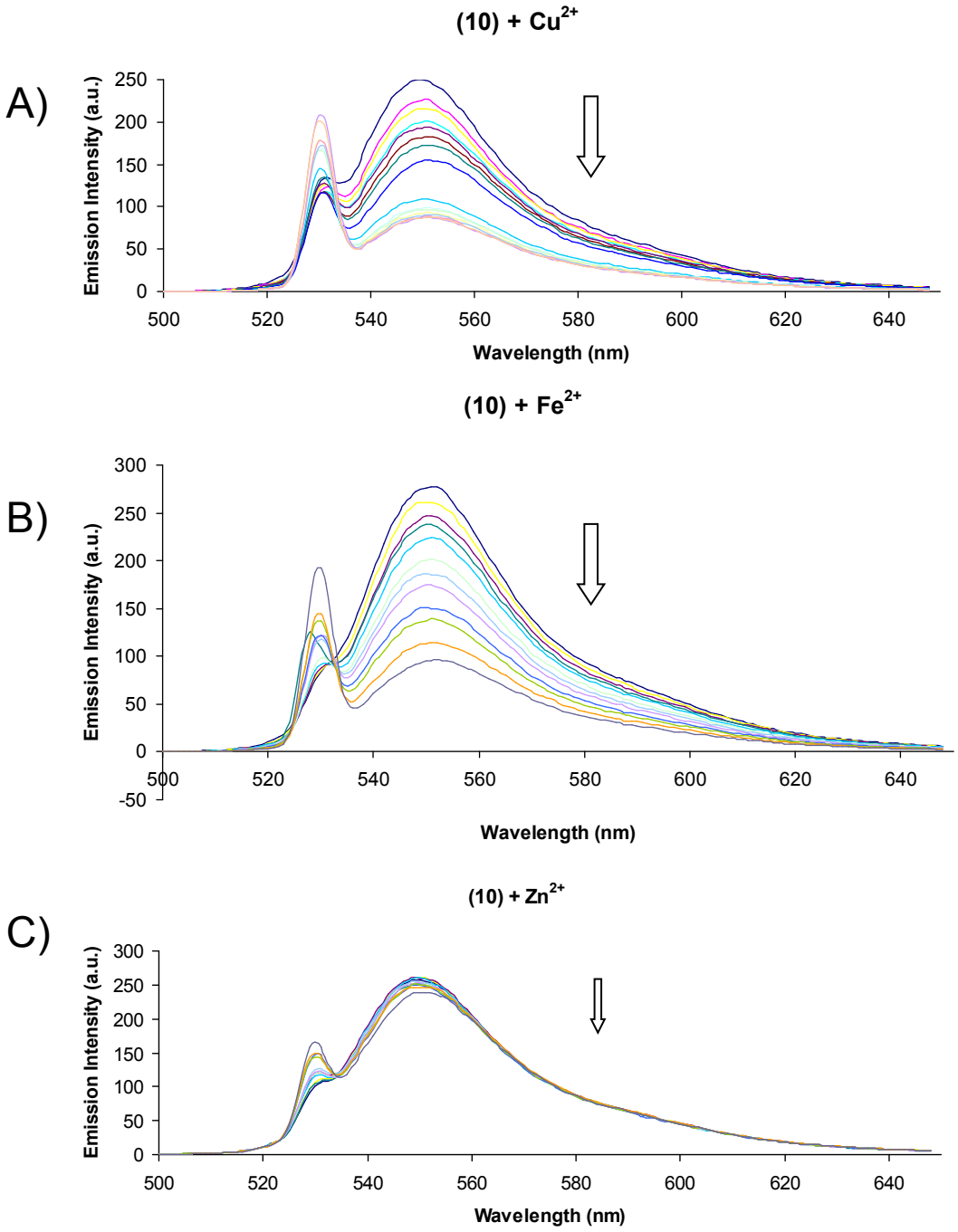


Figure S41: Overlaid fluorescent emission response of **10** (10 µM concentration) to A) Cu^{2+} metal ions, B) Fe^{2+} metal ions and C) Zn^{2+} metal ions in acetonitrile. Excitation wavelength was 530 nm and emission slit widths were 5 nm. Molar equivalence of each metal ion to BODIPY molecule ranged from 0 to a maximum of 3 equivalences.

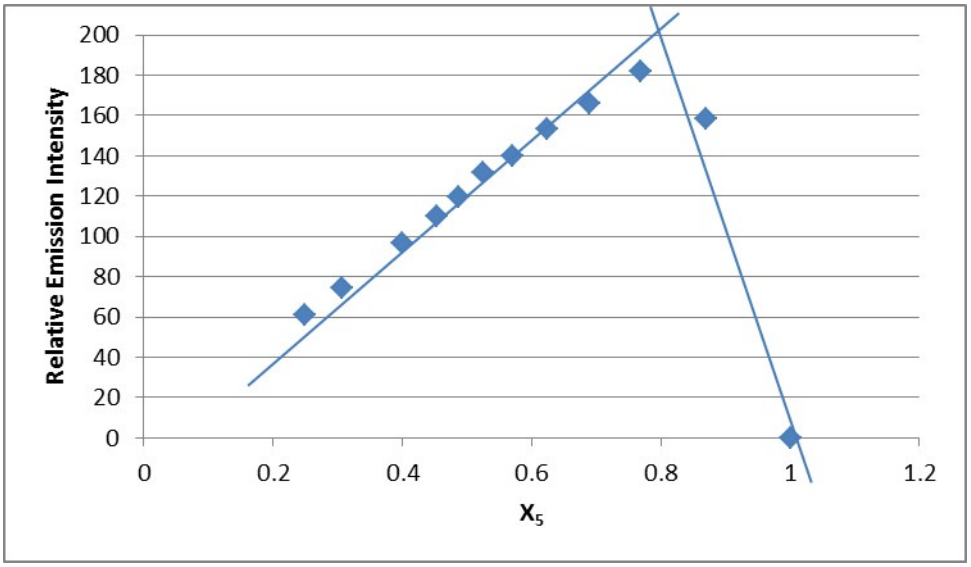


Figure S42: Representative Job plot of emission data, for compound **5** (data Fig S34 B) on addition of Fe^{2+}, X is mole fraction of **5** (I is taken from emission intensity at 511 nm) at a constant BODIPY concentration of 10 µM (1 equiv.) in CH_3CN solution at room temperature.

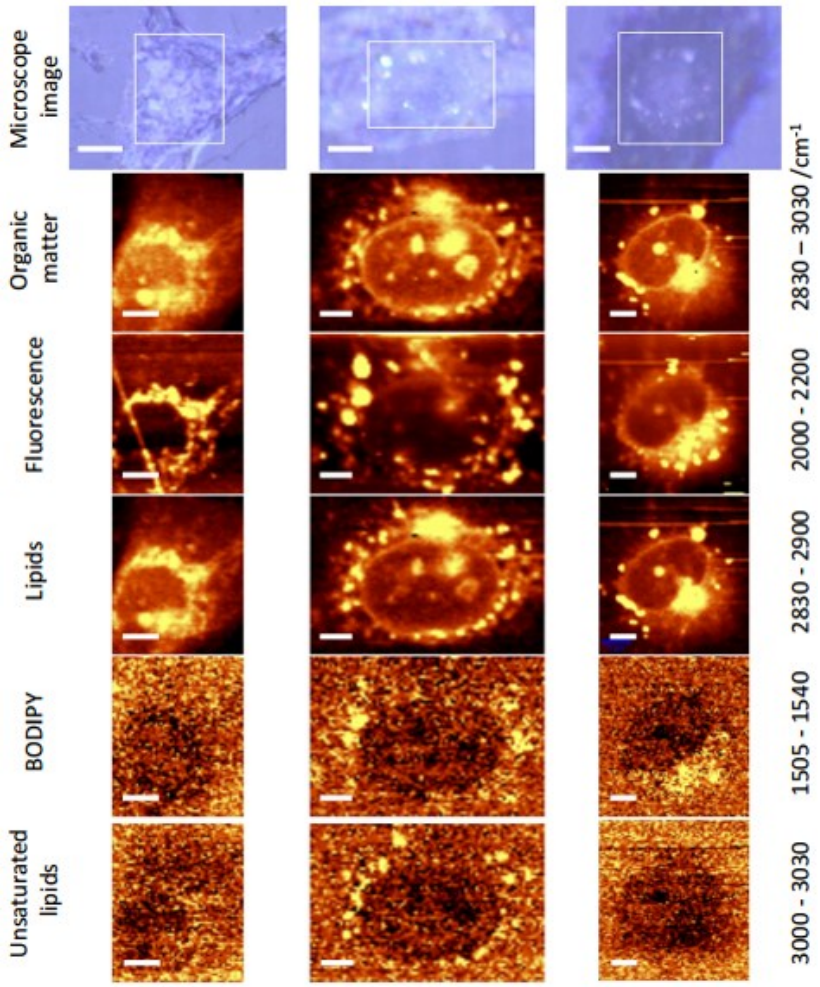


Figure S43: Fluorescence (background) images and Raman intensity images of live HMEC-1 cells. Shown are results obtained for control cells and BODIPY (**5** and **7**) stained cells. Cells were incubated with 10 µM of each dye for 24 h prior to obtaining Raman spectra. A 532 nm laser line was used to excite the compounds. A sampling step of 0.4 µm and integration time of 0.7-1.0 s was used to obtain the Raman spectra.

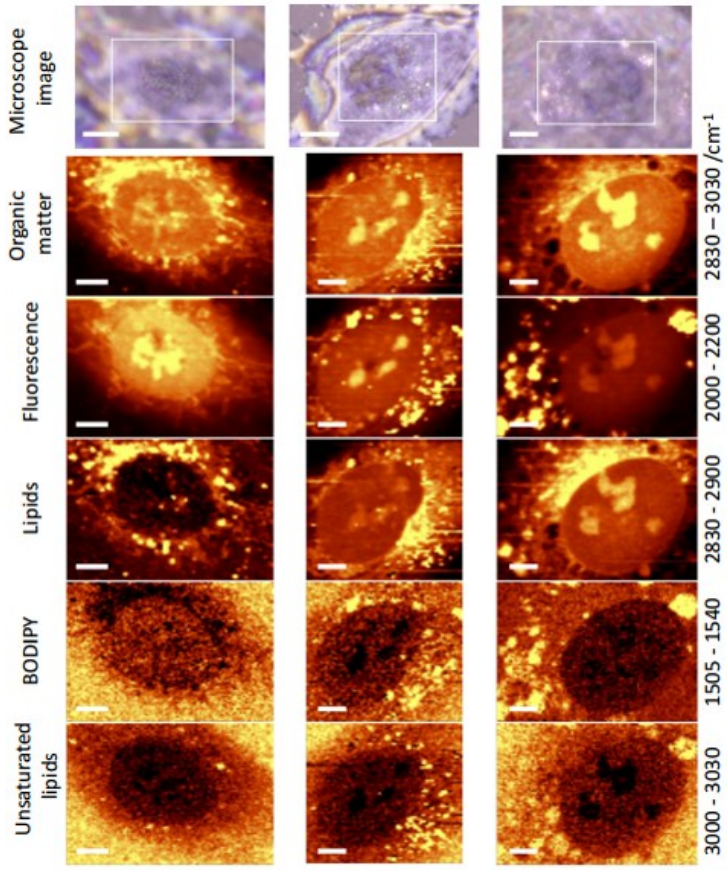


Figure S44: Fluorescence background images and Raman generated intensity images of fixed HMEC-1 cells. Shown are results obtained for control cells and BODIPY (**5** and **7**) stained cells. Cells were incubated with 10 µM of each dye for 24 h and were fixed using 2.5 % glutaraldehyde prior to obtaining Raman spectra. A 532 nm laser line was used to excite the compounds. A sampling step of 0.4 µm and integration time of 0.7-1.0 s was used to obtain the Raman spectra.