Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Information

Molecular Engineering of B/N Substituents in Asymmetrical Salicylaldimine-Based Boranils for Tuning Solid-State Emission and Cellular Lipid-Droplet Imaging

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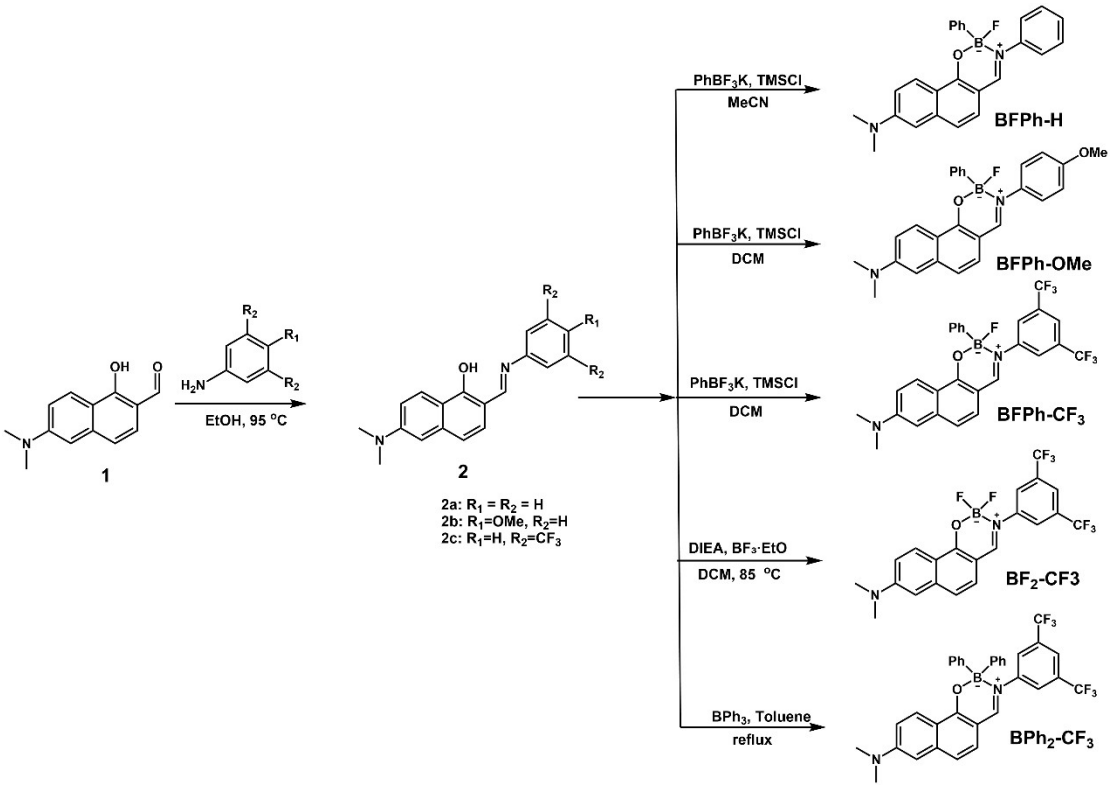
Materials and Methods

General Information

All chemicals were directly used as received without any purification unless otherwise specified. Anhydrous solvents were used for fluorescence property investigation. Deionized water was used throughout this study. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on a Bruker AVANCE III spectrometer using tetramethylsilane as the internal standard. High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies 6510 Q-TOF LC/MS instrument operated in the ESI mode. The UV-Vis absorption and fluorescence spectra were obtained on U2910 spectrophotometer and Hitachi F-2700 spectrofluorimeter, respectively. The absolute fluorescence quantum yield was determined using a calibrated integrating sphere. Two-photon excited fluorescence spectra were measured on a SpectroPro300i spectrameter using the pump laser beam came from a mode-locked Ti:sapphire laser system at the pulse duration of 130 fs, a repetition rate of 80 MHz. The wavelength of the output laser beam was tuned from 740 to 930 nm. Two-photon absorption cross sections were measured using the two-photon-induced fluorescence method with coumarin in methanol as the standard.1, 2 The 2PA cross sections of **BFPh-CF_3**, **BF_2-CF_3**, **BPh_2-CF_3** in toluene were calculated according to the equation[1]: $\delta_x = \delta_{st}[F_x\Phi_{st}\eta_{st}c_{st}]/[F_{st}\Phi_x\eta_x c_x]$, where δ was the 2PA cross section, F is the integrated intensity of two-photon excited fluorescence, Φ is the fluorescence quantum yield, η is the overall fluorescence collection efficiency of the experimental apparatus, and c is the concentration of the solution. The subscripts x and st refer to the unknown samples and standard material, respectively.

Synthetic Details



Scheme S1. Synthesis routes of boranils in this work.

Synthesis of Compound 2: Compound 1[2] (1 eq.) and substituted aniline derivatives with electron-donating/electron-withdrawing groups (1.2 eq.) were sequentially added to a Schlenk tube. Using anhydrous ethanol as the solvent, the mixture was stirred under reflux at 95 °C under a nitrogen atmosphere for 24 h. After the reaction, the mixture was cooled to room temperature, and the precipitate was collected, washed with ethanol and *n*-hexane, and dried under vacuum. This intermediate was used directly in the next step without further purification.

Synthesis of Compound BFPh-H: Compound 2a (2.2 eq.) and potassium phenyl trifluoroborate (2.2 eq.) were dissolved in anhydrous acetonitrile (15 ml), followed by the slow addition of chlorotrimethylsilane (TMSCl, 4.7 eq.). The mixture was stirred at room temperature under a nitrogen atmosphere for 5 h. The precipitate was collected and washed with acetonitrile and dried under vacuum to give compound **BFPh-H** as an orange solid (43 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 1.3 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.45 (dd, J = 12.7, 8.5 Hz, 3H), 7.38 – 7.30 (m, 4H), 7.29 – 7.24 (m, 1H), 7.18 (d, J = 8.7 Hz, 1H), 7.15 – 7.05 (m, 4H), 6.92 (d, J = 2.5 Hz, 1H), 3.11 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.77, 134.54, 131.97, 130.46, 130.27, 129.46, 129.40, 127.88, 127.80, 127.29, 127.07, 126.37, 124.40, 119.26, 118.90, 118.53, 116.47, 114.85, 112.39, 108.21, 106.18, 40.26. HRMS (ESI) m/z calcd for [$C_{25}H_{22}BF_2N_2O^+$] 397.1886 ([M+H] $^+$), found 397.1894.

Synthesis of Compounds BFPh-OMe: Compound 2b (2.2 eq.) and potassium phenyl trifluoroborate (2.2 eq.) were dissolved in anhydrous dichloromethane (15 ml), followed by the slow addition of chlorotrimethylsilane (TMSCl, 4.7 eq.). The mixture was stirred at room temperature under a nitrogen atmosphere for 5 h. The solvent was removed under reduced pressure, and the product was recrystallized using dichloromethane and *n*-hexane. The precipitate was collected and washed with hexane and dried under vacuum to give compound **BFPh-OMe** as yellow solid (50 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.07 (d, J = 9.3 Hz, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.9 Hz, 2H), 7.31 (d, J = 7.8

Hz, 2H), 7.17 (d, *J* = 8.7 Hz, 1H), 7.15 – 7.07 (m, 4H), 6.93 – 6.87 (m, 3H), 3.72 (s, 3H), 3.10 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.08, 158.79, 158.29, 152.31, 131.94, 127.80, 127.31, 127.04, 126.91, 126.24, 125.46, 125.08, 124.95, 120.92, 120.46, 118.22, 115.44, 115.27, 114.85, 114.49, 106.18, 55.91, 43.72, 40.29. HRMS (ESI) m/z calcd for [$C_{26}H_{24}BFN_2O_2^+$] 427.1992 ([M+H]$^+$), found 427.2001.

Synthesis of Compounds BFPh-CF$_3$: Compound 2c (2.2 eq.) and potassium phenyl trifluoroborate (2.2 eq.) were dissolved in anhydrous dichloromethane (15 ml), followed by the slow addition of chlorotrimethylsilane (TMSCl, 4.7 eq.). The mixture was stirred at room temperature under a nitrogen atmosphere for 5 h. The solvent was removed under reduced pressure, and the product was recrystallized using dichloromethane and *n*-hexane. The precipitate was collected and washed with hexane and dried under vacuum to give compound **BFPh-CF$_3$** as orange solid (46 %).^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.08 (d, J = 1.3 Hz, 1H), 8.14 – 8.08 (m, 3H), 8.00 (s, 1H), 7.46 (d, J = 8.8 Hz, 1H), 7.35 – 7.31 (m, 2H), 7.22 (d, J = 8.8 Hz, 1H), 7.16 – 7.09 (m, 4H), 6.94 (d, J = 2.5 Hz, 1H), 3.13 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.72, 160.93, 152.96, 146.18, 141.91, 134.54, 132.05, 131.21 (q, *J* = 33.3 Hz), 127.99, 127.49, 126.82, 125.28, 123.30 (q, *J* = 273.2 Hz),121.00 (m), 119.15, 115.39, 114.90, 108.38, 106.33, 40.25. HRMS (ESI) m/z calcd for [$C_{27}H_{20}BF_7N_2O^+$] 533.1634 ([M+H]$^+$), found 533.1660.

Synthesis of Compound BF$_2$-CF$_3$: This compound was re-synthesized following the literature[3] and checked by HNMR and HRMS. Compound 2c (1 eq.) was dissolved in anhydrous dichloromethaneq (15 ml), followed by the sequential slow addition of $BF_3{\cdot}OEt_2$ (3 eq.) and N, N-diisopropylethylamine (3 eq.). The mixture was stirred under reflux at 85 °C for 5 h. After cooling to room temperature, and a saturated sodium bicarbonate solution was added. The mixture was extracted with dichloromethane, and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the precipitate was purified by silica gel chromatography using *n*-hexane/ethyl acetate (from 40:1, 20:1, to 10:1, *v/v*) as the eluent to give compound **BF$_2$-CF$_3$** as a red solid (52 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.35 (d, J = 1.5 Hz, 2H), 8.26 – 8.18 (m, 2H), 7.45 (d, J = 8.8 Hz, 1H), 7.28 – 7.19 (m, 2H), 6.95 (d, J = 2.6 Hz, 1H), 3.16 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.44, 160.67, 153.22, 144.46, 141.95, 131.89 (q, *J* = 33.5 Hz), 127.70, 126.75, 124.62, 123.41 (q, *J* = 273.2 Hz), 121.68 (m), 119.90, 115.28, 106.32, 40.26. HRMS (ESI) m/z calcd for [$C_{21}H_{15}BF_8N_2O^+$] 475.1226 ([M+H]$^+$), found 475.1234.

Synthesis of Compound BPh$_2$-CF$_3$: Compound 2c (1 eq.) and triphenyl borane (1.2 eq.) were dissolved in anhydrous toluene (10 ml). The mixture was stirred under reflux at 115 °C under a nitrogen atmosphere for 5 h. After cooling to room temperature, and the solvent was removed under reduced pressure. The precipitate was purified by silica gel chromatography using *n*-hexane/ethyl acetate (20:1, *v/v*) as the eluent to give compound **BPh$_2$-CF$_3$** as an orange- red solid (52 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.12 (d, *J* = 9.2 Hz, 1H), 7.92 (s, 1H), 7.87 (s, 2H), 7.36 – 7.30 (m, 4H), 7.20 – 7.07 (m, 8H), 7.06 – 7.00 (m, 1H), 6.84 (s, 1H), 3.09 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.68, 163.53, 152.83, 147.10, 141.71, 133.58, 131.37, 130.71 (q, *J* = 33.3 Hz), 128.31, 127.39, 126.75, 125.57, 123.24 (q, *J* = 273.3 Hz), 120.43 (m), 118.28, 116.26, 114.75, 111.03, 106.24, 40.25. HRMS (ESI) m/z calcd for [$C_{33}H_{25}BF_6N_2O^+$] 591.2042 ([M+H]$^+$), found 591.2040.

Crystal Preparation and Measurement

All samples were prepared using the diffusion method. A 3-mL solution of dichloromethane was added to a glass vial containing 2-3 mg of the sample. This vial was then placed inside a sealed 20-mL glass flask containing 5 mL of n-hexane. The vapors of the poor solvent slowly diffused into the smaller vial, altering its solvation environment. After about one week, the single crystals were obtained. Single crystals of **BFPh-H**, **BFPh-CF_3**, **BF_2-CF_3** and **BPh_2-CF_3** with appropriate dimensions were chosen under an optical microscope and quickly coated with high vacuum grease (Dow Corning Corporation) to prevent decomposition. Intensity data and cell parameters were recorded at 173 K for samples on a Bruker D8 VENTURE PHOTON III single crystal diffractometer, employing a Cu Kα radiation (λ =1.54184 Å) and a CCD area detector. CCDC: 2540294 (**BFPh-CF_3**), 2540295 (**BFPh-H**), and 2540296 (**BPh_2-CF_3**) contains the supplementary crystallographic data for this paper.

Cell Culture and Imaging

HeLa cells were cultured in confocal culture dishes using H-DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2/air for 24 hours. Fresh H-DMEM medium containing 1 μM **BFPh-H** was added into the culture medium of HeLa cells and incubated at 37 ℃ in 5% CO_2 for 15 min before imaging. Fresh H-DMEM medium containing 300 nM **BPh_2-CF_3** was added into the culture medium of HeLa cells and incubated at 37 ℃ in 5% CO_2 for 20 min before imaging. For co-stain imaging, the HeLa cells were incubated with 1 μM **BFPh-H** for 15 min and 800 nM Nile Red for 15 min, the HeLa cells were incubated with 300 nM **BPh_2-CF_3** for 15 min and 100 nM Lipid-Deep Red for 15 min (For **BFPh-H**, excitation = 488 nm, emission collection: 500-550 nm; for Nile Red, excitation = 543 nm, emission collection: 570-620 nm.)

NMR Spectra

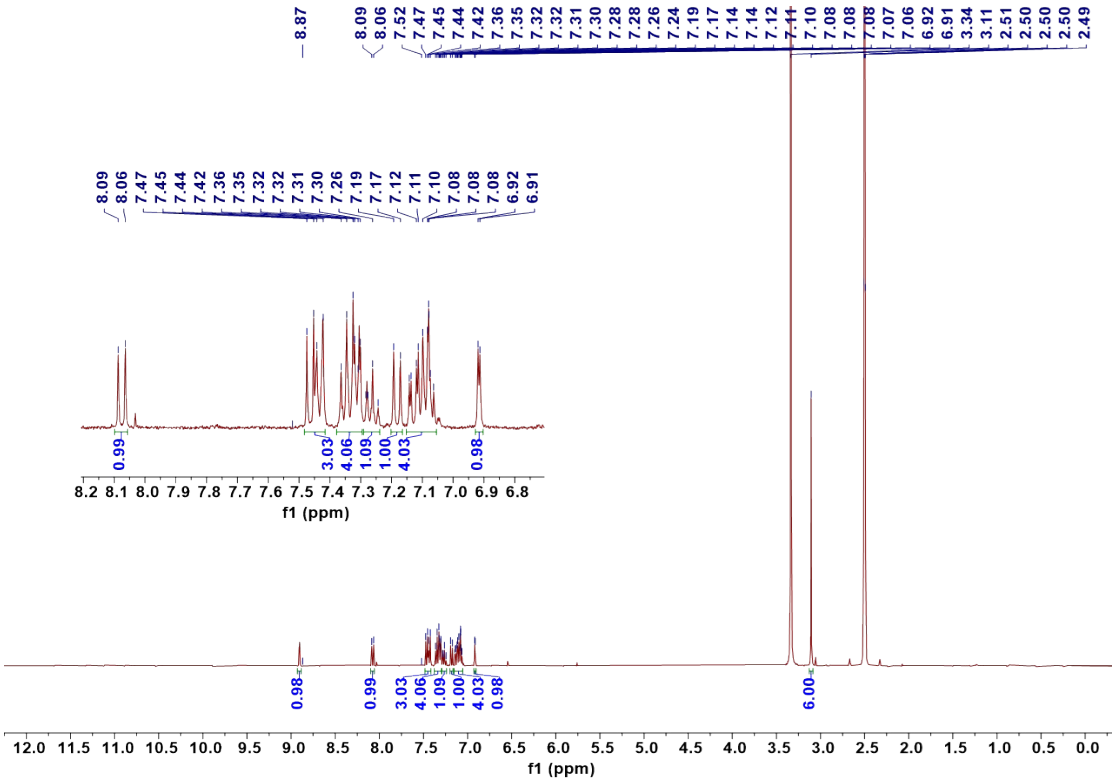


Figure S1. ^{1}H NMR spectrum (400 MHz) of **BFPh-H** in DMSO-*d6*.

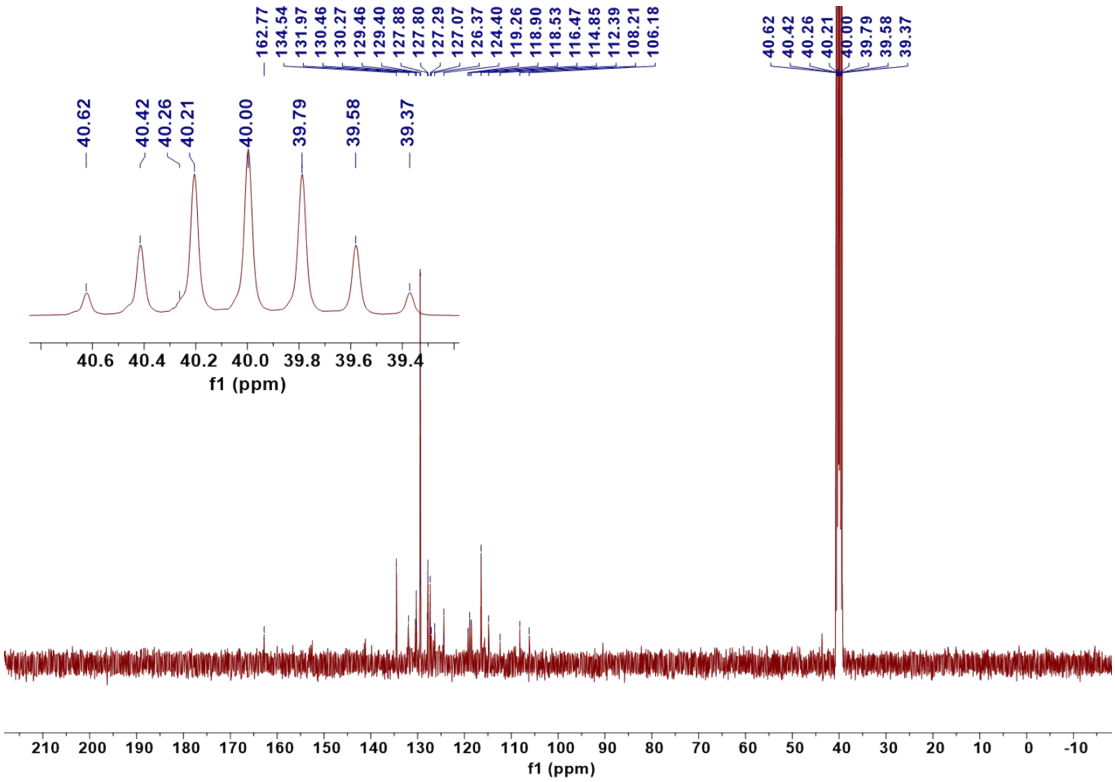


Figure S2. ^{13}C NMR spectrum (101 MHz) of **BFPh-H** in DMSO-*d6*.

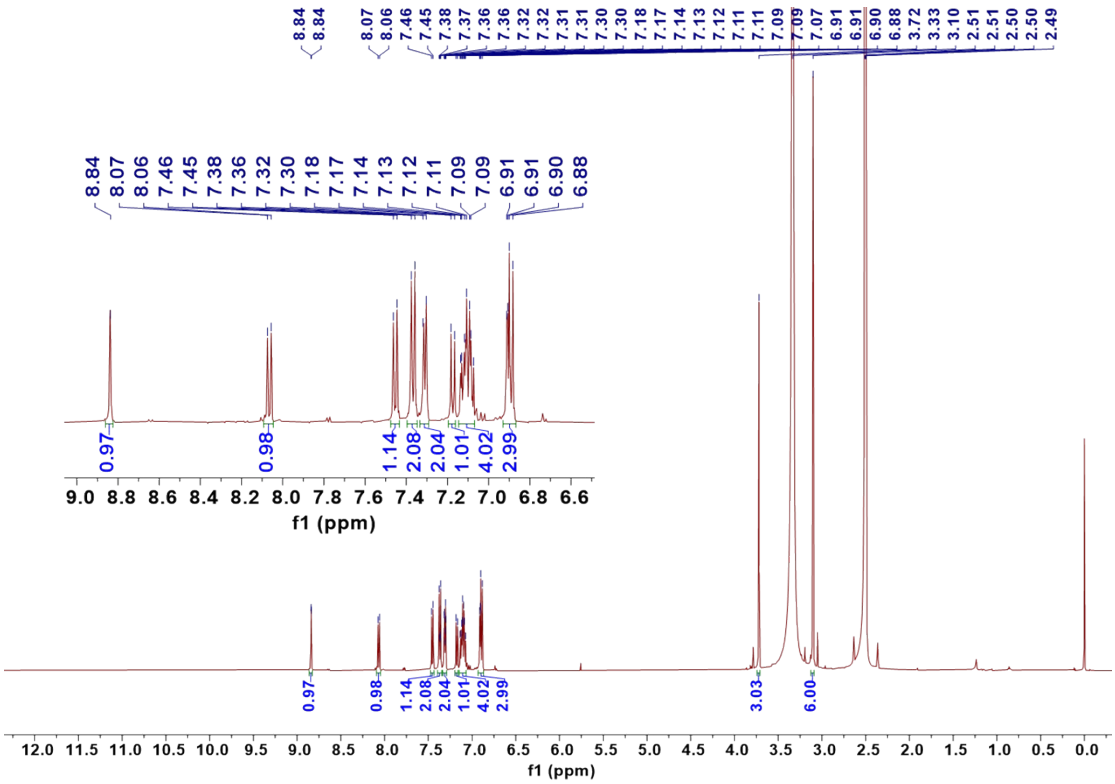


Figure S3. [1]H NMR spectrum (500 MHz) of **BFPh-OMe** in DMSO-*d6*.

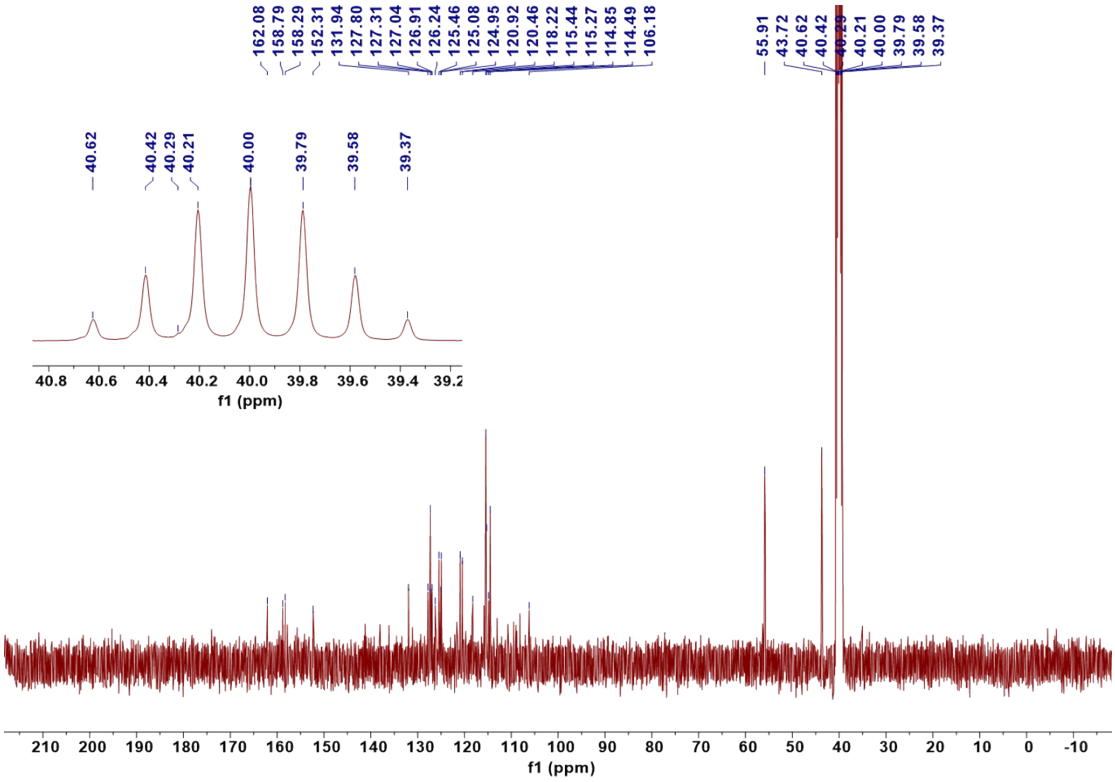


Figure S4. [13]C NMR spectrum (101 MHz) of **BFPh-OMe** in DMSO-*d6*.

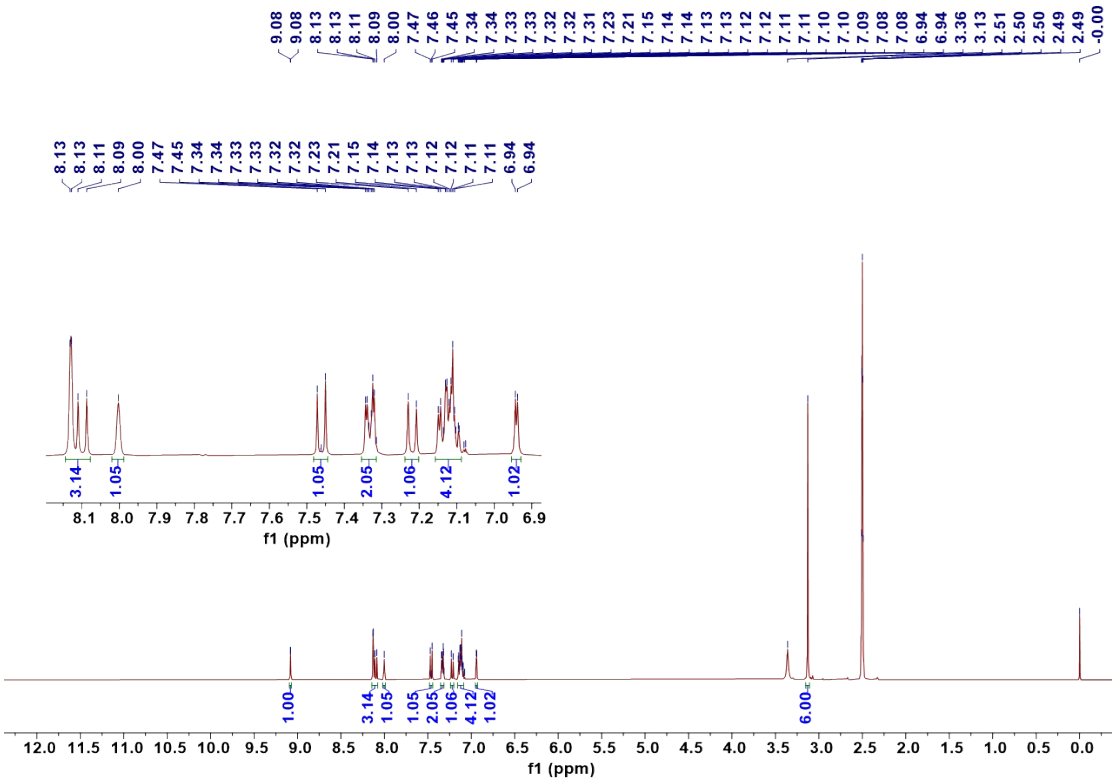


Figure S5. ^{1}H NMR spectrum (400 MHz) of **BFPh-CF$_3$** in DMSO-*d6*.

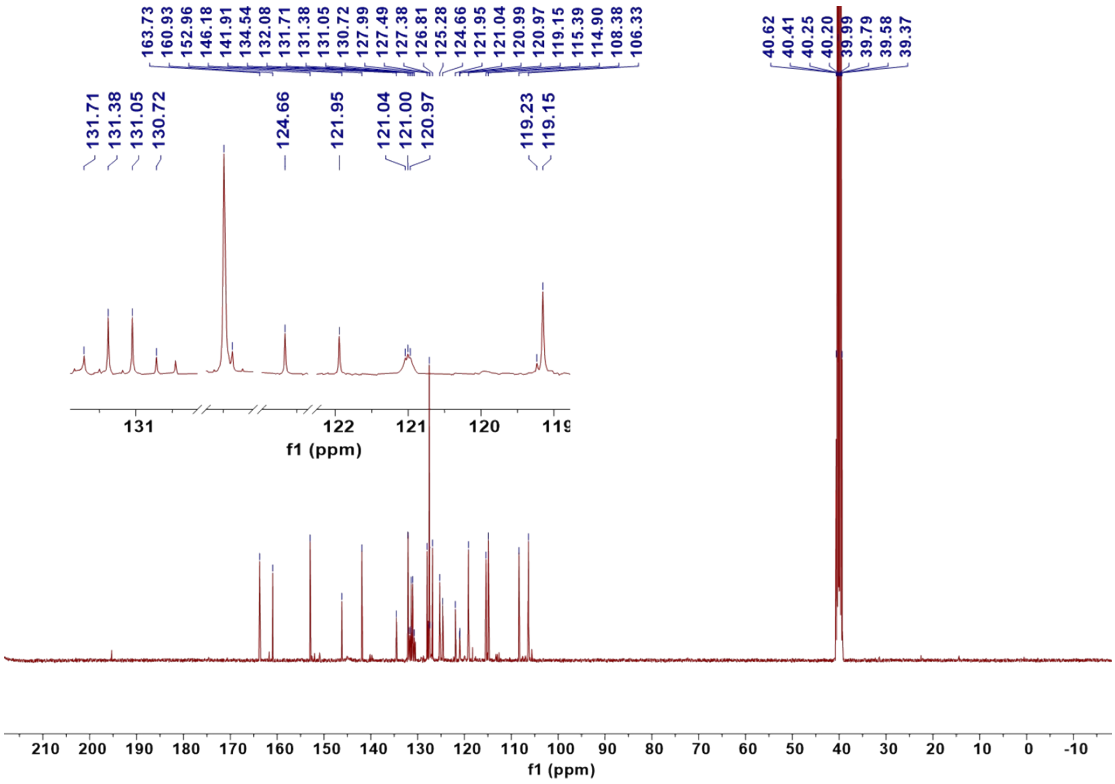


Figure S6. ^{13}C NMR spectrum (101 MHz) of **BFPh-CF$_3$** in DMSO-*d6*.

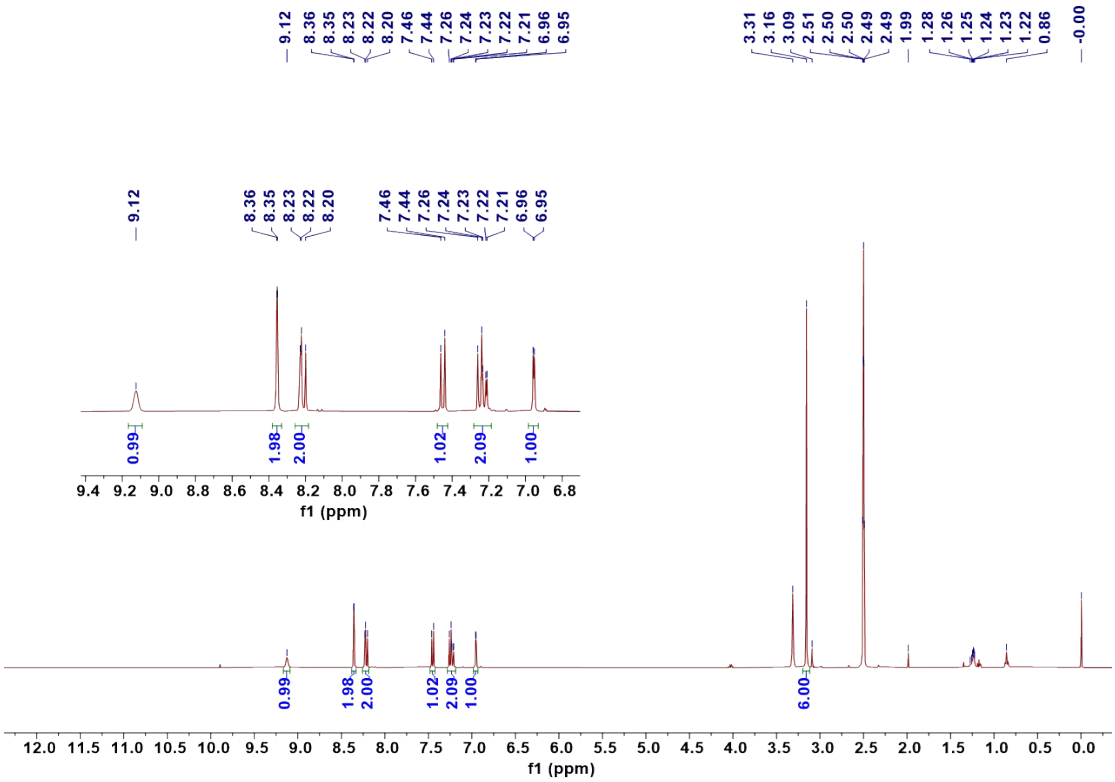


Figure S7. ^{1}H NMR spectrum (400 MHz) of **BF_2-CF_3** in DMSO-*d6*.

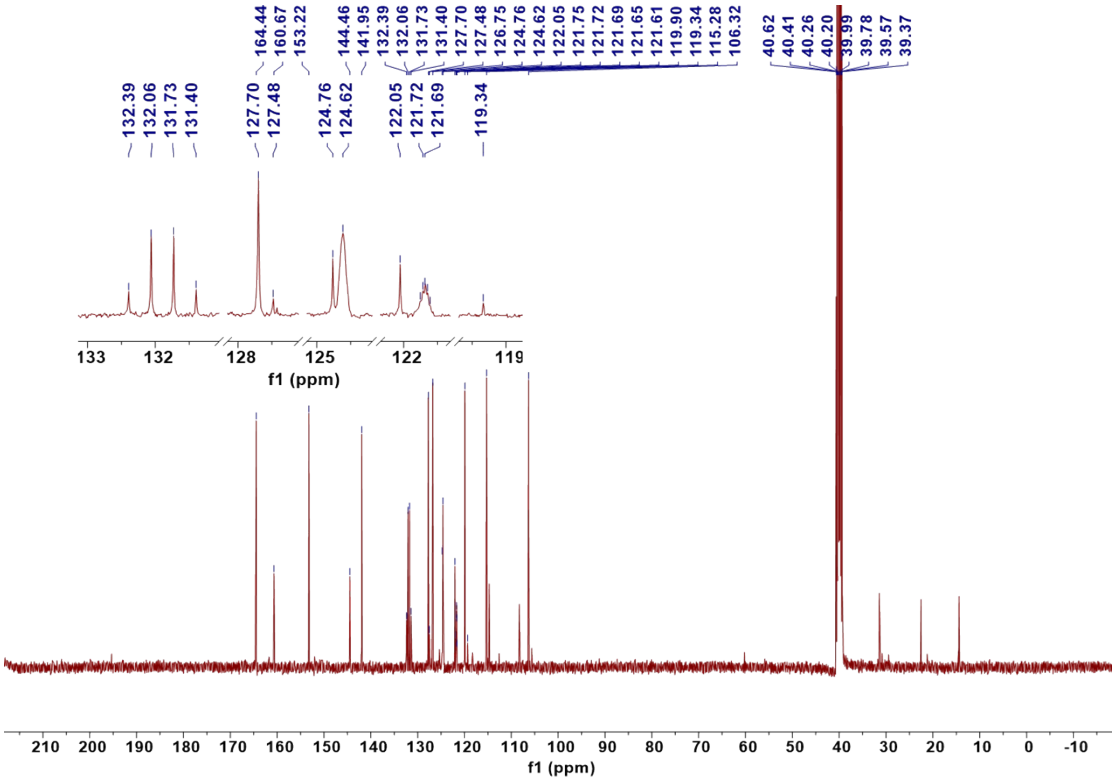


Figure S8. ^{13}C NMR spectrum (101 MHz) of **BF_2-CF_3** in DMSO-*d6*.

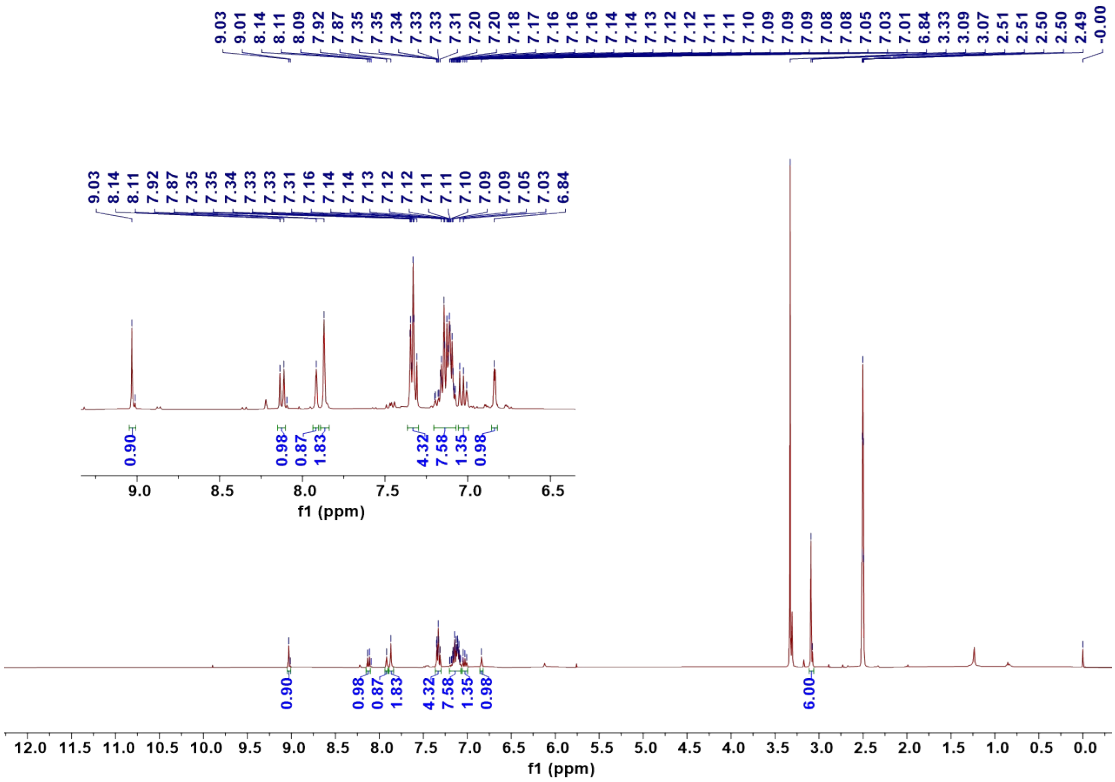


Figure S9. ^{1}H NMR spectrum (400 MHz) of **BPh_2-CF_3** in DMSO-*d6*.

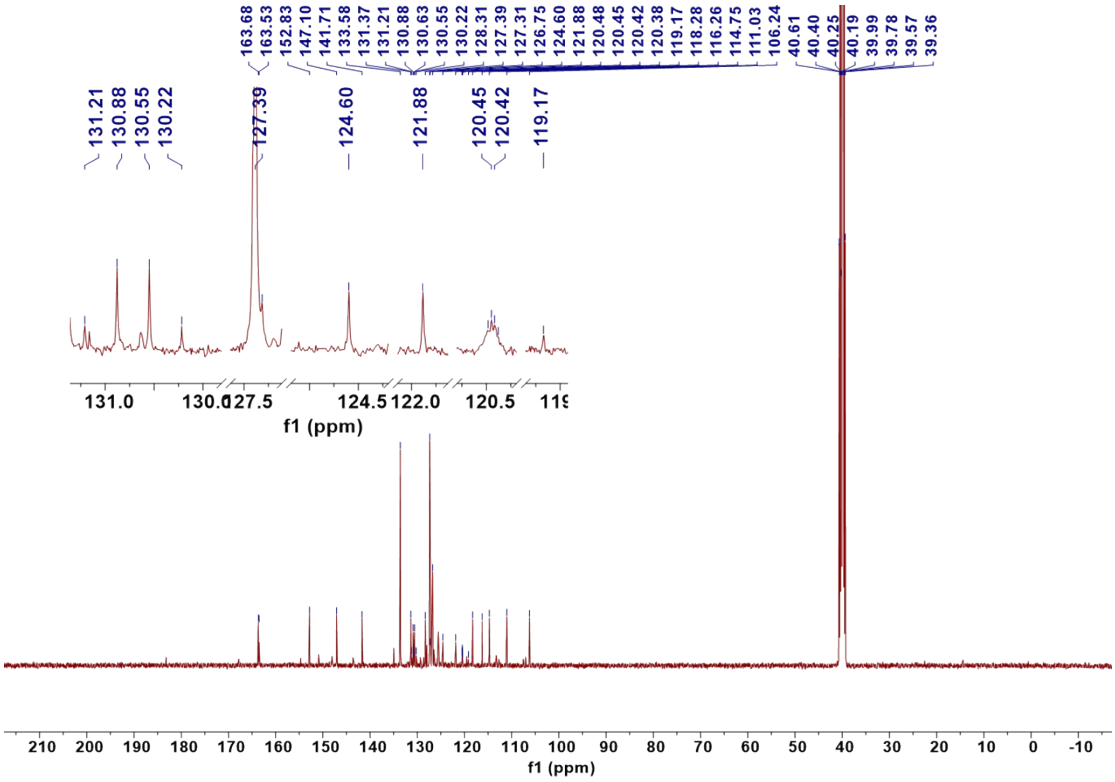


Figure S10. ^{13}C NMR spectrum (101 MHz) of **BPh_2-CF_3** in DMSO-*d6*.

HRMS Spectra

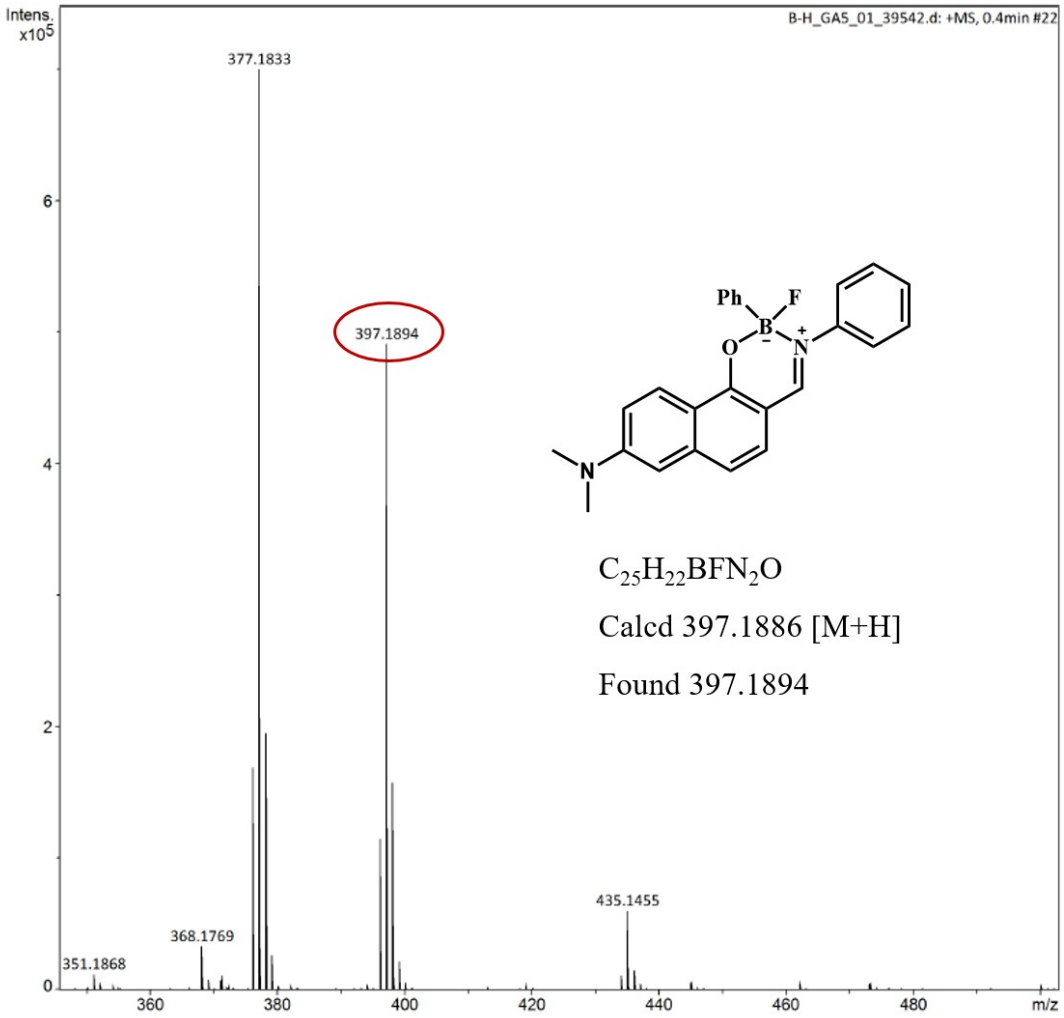


Figure S11. HRMS spectrum of **BFPh-H**.

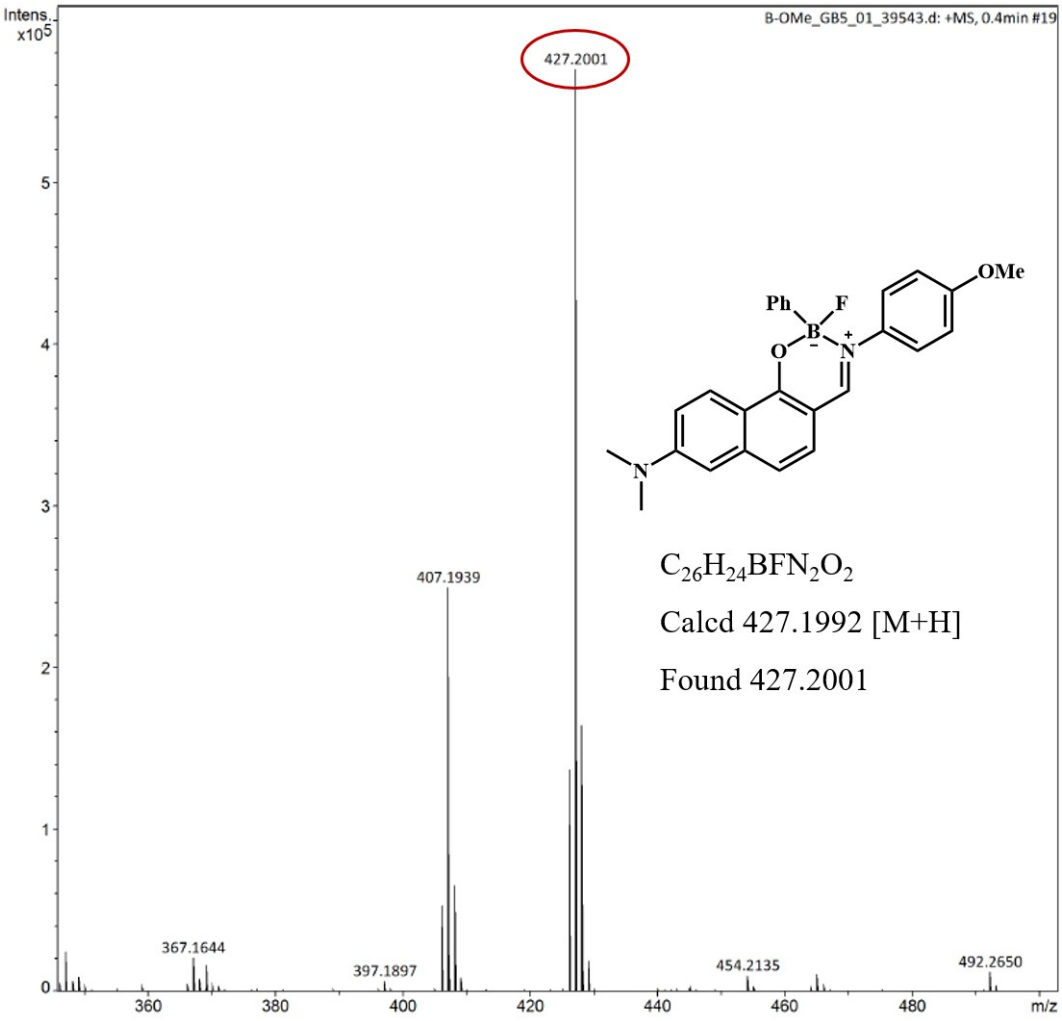


Figure S12. HRMS spectrum of **BFPh-OMe**.

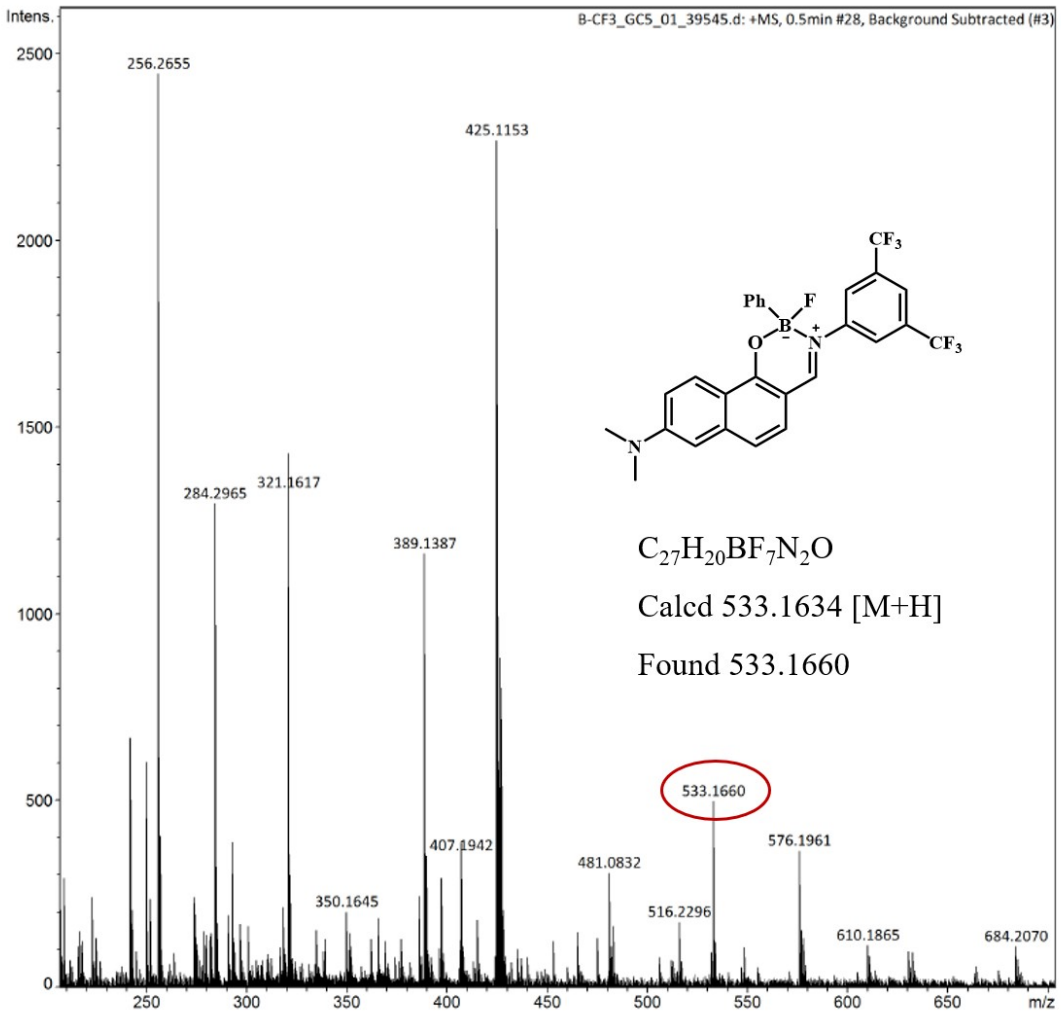


Figure S13. HRMS spectrum of **BFPh-CF_3**.

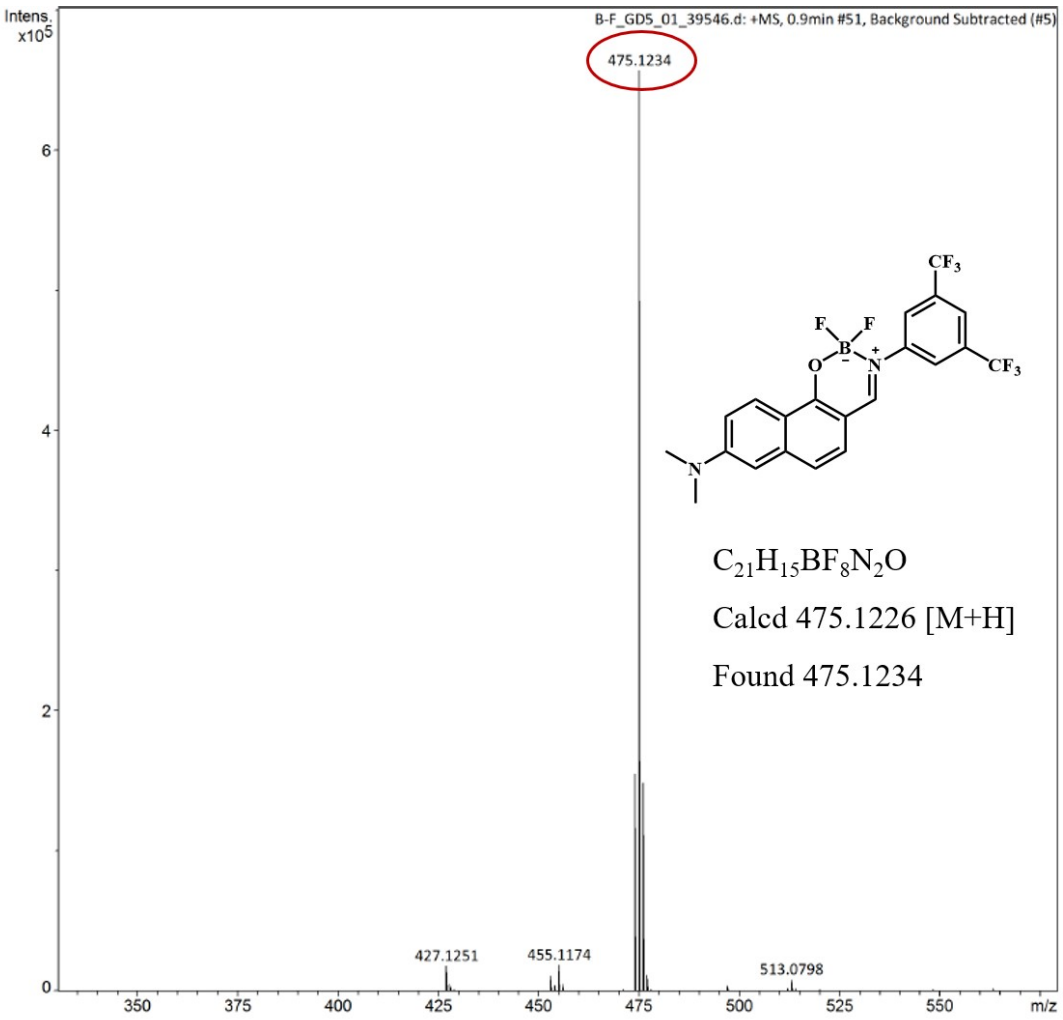


Figure S14. HRMS spectrum of **BF_2-CF_3**.

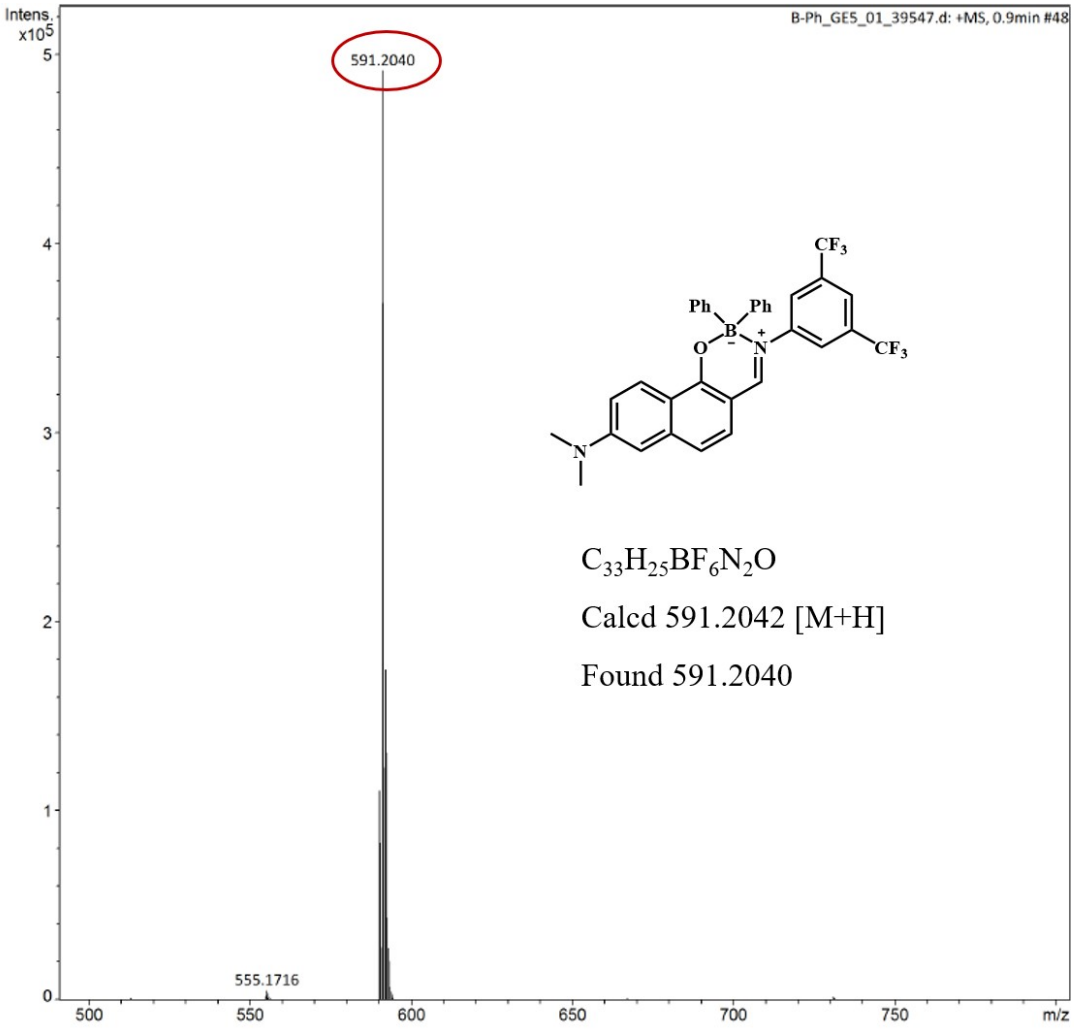


Figure S15. HRMS spectrum of **BPh_2-CF_3**.

Crystallographic Data

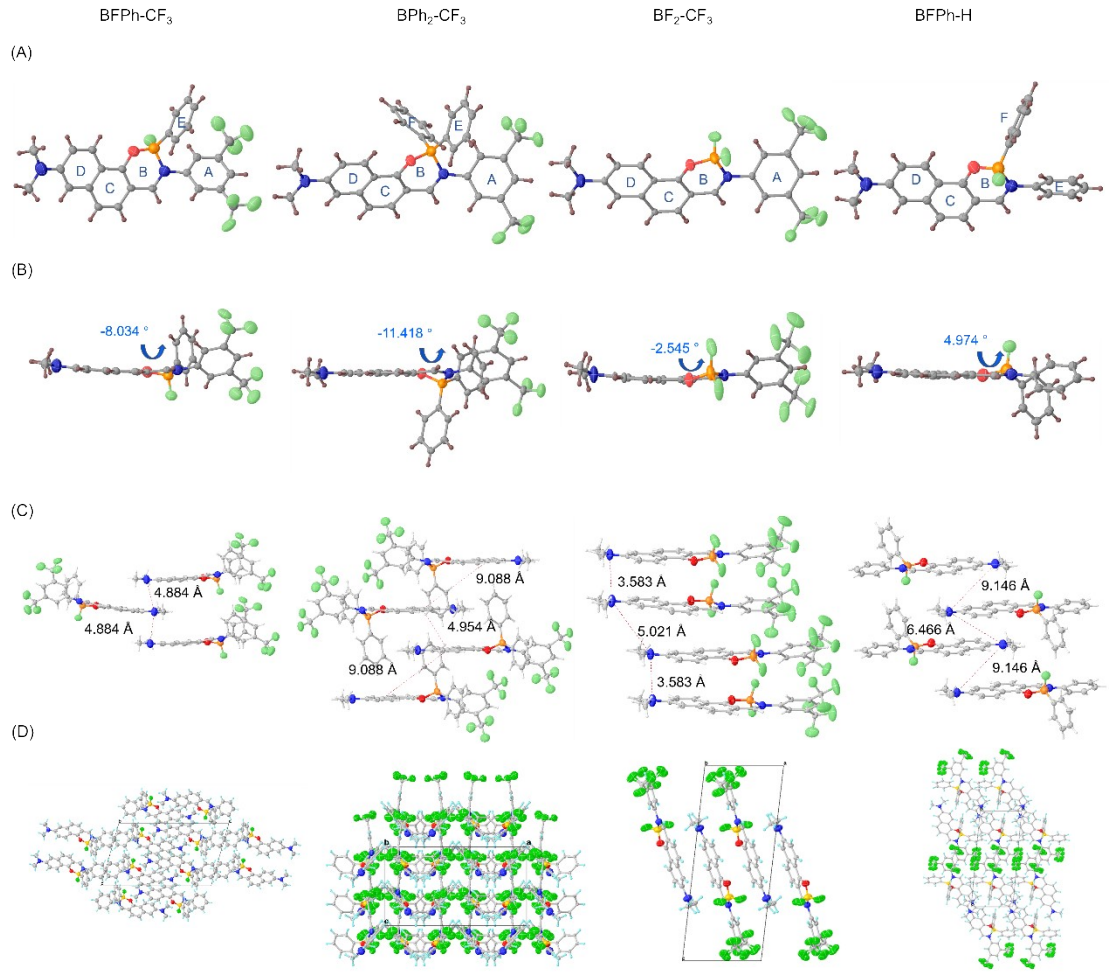


Figure S16. Single-crystal X-ray structures of **BFPh-CF$_3$**, **BPh$_2$-CF$_3$**, **BF$_2$-CF$_3$**, and **BFPh-H** viewed from different directions. (A) Top view; (B) Side view; (C) and (D) Intermolecular packing interactions in the crystal.

Table S1. Dihedral angle (°).

Plane	A-B	B-C	A-E	B-E
BFPh-H	31.702	4.253	71.209	85.028
BFPh-CF$_3$	45.325	4.924	116.376	91.549
BF$_2$-CF$_3$	30.800	6.010	-	-
BPh$_2$-CF$_3$	40.074	10.577	68.094	91.077

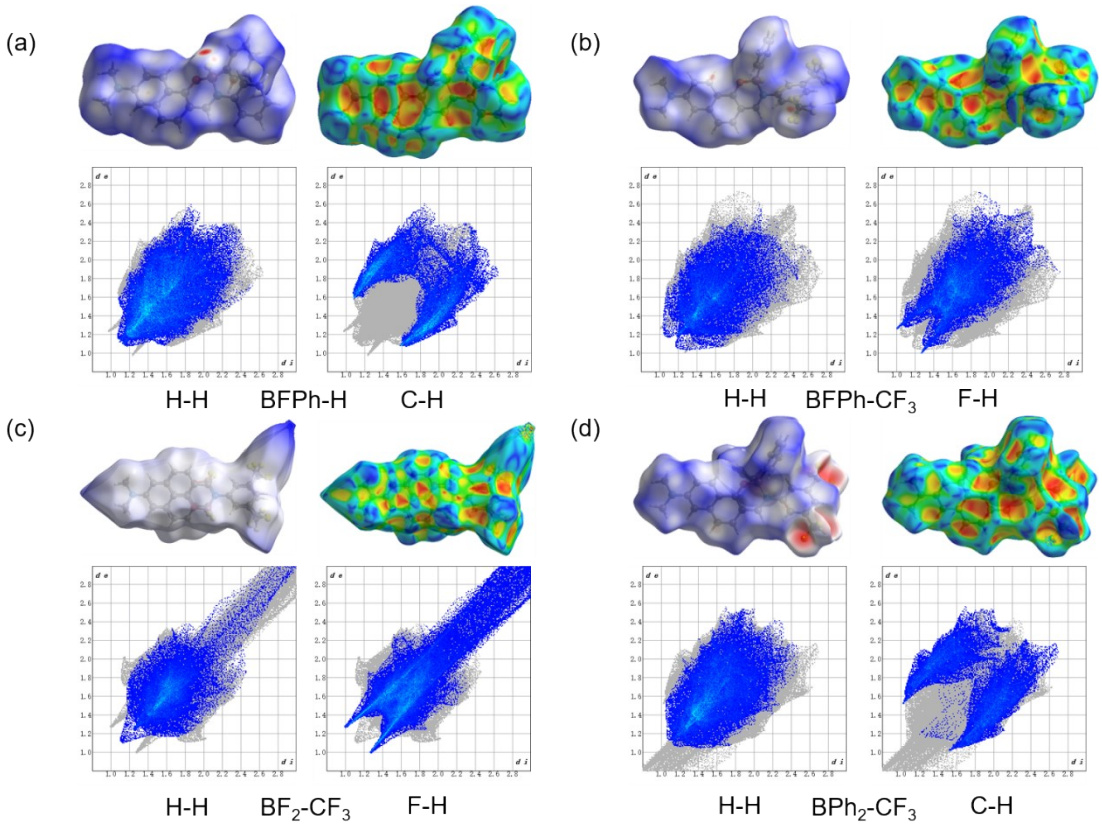


Figure S17. Hirshfeld surface mapped with dnorm, shape index and the 2D fingerprint plots.

Table S2. 2D fingerprint plots data.

Plane	H-H	C-H	F-H	F-F
BFPh-H	52.6%	33.2%	6.7%	-
BFPh-CF_3	27.2%	24.2%	29.4%	10.4%
BF_2-CF_3	21.0%	7.5%	47.2%	14.8%
BPh_2-CF_3	35.9%	25.1%	13.9%	15.9%

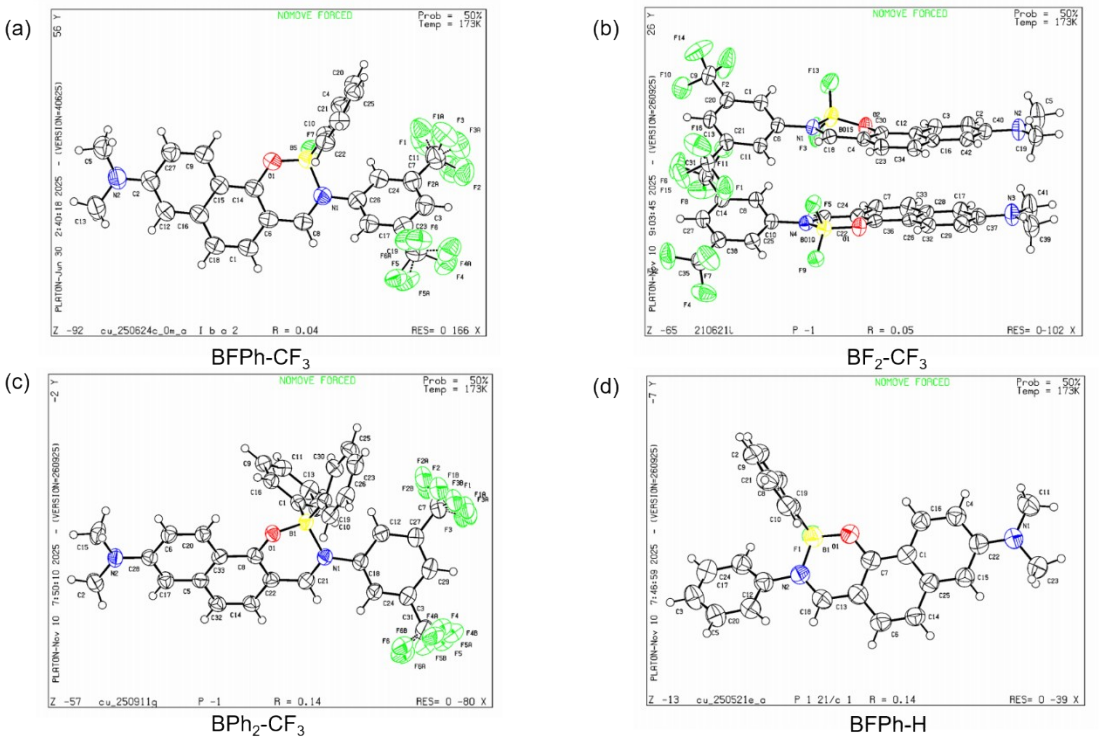


Figure S18. Thermal ellipsoid plot of the X-ray structure of boranils at the 50% probability level.

Table S3. Crystal data and structure refinement for **BFPh-H**

Bond precision:	C-C = 0.0107 Å	Wavelength = 1.54178	
Cell:	a = 20.859(7)	b = 8.111(3)	c = 12.247(5)
	alpha = 90	beta = 105.946(17)	gamma = 90
Temperature:	173 K		
	Calculated	Reported	
Volume	1992.3(13)	1992.4(12)	
Space group	P 21/c	P 1 21/c 1	
Hall group	-P 2ybc	-P 2ybc	
Moiety formula	C25 H22 B F N2 O	C25 H22 B F N2 O	
Sum formula	C25 H22 B F N2 O	C25 H22 B F N2 O	
Mr	396.26	396.25	
Dx, g cm-3	1.321	1.321	
z	4	4	
Mu (mm-1)	0.693	0.693	
F000	832.0	832.0	
F000’	834.45		
h, k, l max	25, 9, 14	25, 9, 14	
Nref	3614	3549	
Tmin, Tmax	0.920, 0.966	0.038, 0.164	
Tmin’	0.871		

Correction method = # Reported T Limits: Tmin = 0.038 Tmax = 0.164	
AbsCorr = MULTI - SCAN	
Data completeness = 0.982	Theta(max) = 67.745
R(reflections) = 0.1407(1722)	wR2(reflections) = 0.4264(3549)
S =1.203	Npar = 273

Table S4. Crystal data and structure refinement for **BFPh-CF_3**

Bond precision:	C-C = 0.0053 Å	Wavelength = 1.54184	
Cell:	a = 17.4864(9)	b = 29.1844(15)	c = 9.6824(5)
	alpha = 90	beta = 90	gamma = 90
Temperature:	173 K		
	Calculated	Reported	
Volume	4941.2(4)	4941.2(4)	
Space group	I b a 2	I b a 2	
Hall group	I 2 -2c	I 2 -2c	
Moiety formula	C27 H20 B F7 N2 O	C27 H20 B F7 N2 O	
Sum formula	C27 H20 B F7 N2 O	C27 H20 B F7 N2 O	
Mr	532.26	532.26	
Dx, g cm-3	1.431	1.431	
z	8	8	
Mu (mm-1)	1.071	1.071	
F000	2176.0	2176.0	
F000’	2184.63		
h, k, l max	20, 34, 11	20, 34, 11	
Nref	4402[2350]	4294	
Tmin, Tmax	0.898, 0.898	0.064, 0.164	
Tmin’	0.898		
Correction method = # Reported T Limits: Tmin = 0.064 Tmax = 0.164			
AbsCorr = MULTI - SCAN			
Data completeness = 1.83/0.98		Theta(max) = 66.930	
R(reflections) = 0.0418(3344)		wR2(reflections) = 0.1123(4294)	
S =1.080		Npar = 401	

Table S5. Crystal data and structure refinement for **BPh_2-CF_3**

Bond precision:	C-C = 0.0117 Å	Wavelength = 1.54178	
Cell:	a = 9.0883(17)	b = 9.1361(17)	c = 19.103(3)
	alpha = 99.015(7)	beta = 90.806(7)	gamma = 115.810(7)
Temperature:	173 K		
	Calculated	Reported	
Volume	1404.2(4)	1404.3(4)	
Space group	P -1	P -1	
Hall group	-P 1	-P 1	
Moiety formula	C33 H25 B F6 N2 O	C33 H25 B F6 N2 O	
Sum formula	C33 H25 B F6 N2 O	C33 H25 B F6 N2 O	
Mr	590.30	590.36	
Dx, g cm-3	1.396	1.396	
z	2	2	
Mu (mm-1)	0.949	0.949	
F000	607.9	608.0	
F000’	610.17		
h, k, l max	10, 10, 22	10, 10, 22	
Nref	4635	4544	
Tmin, Tmax		0.062, 0.164	
Tmin’			
Correction method = # Reported T Limits: Tmin = 0.062 Tmax = 0.164			
AbsCorr = MULTI - SCAN			
Data completeness = 0.980		Theta(max) = 63.685	
R(reflections) = 0.1352(3825)		wR2(reflections) = 0.4305(4544)	
S =1.126		Npar = 504	

Photophysical Data

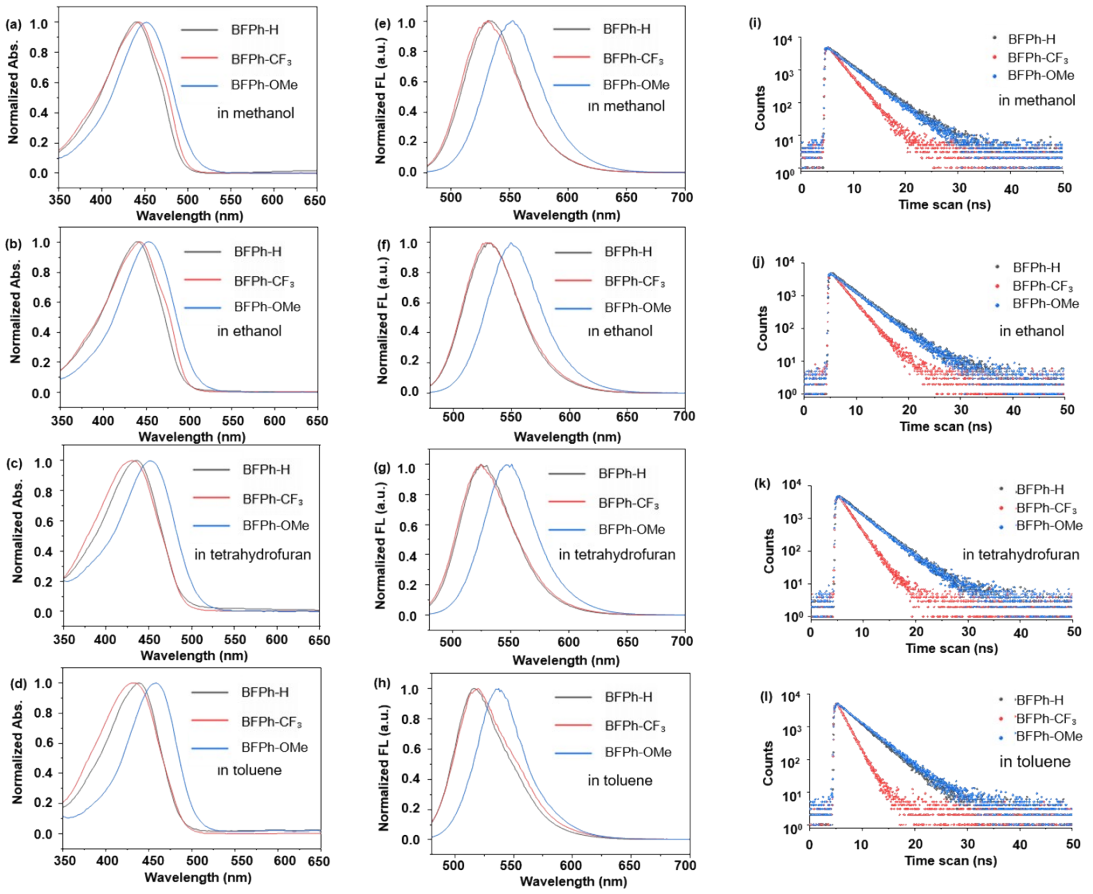


Figure S19. The absorption spectra of **BFPh-H**, **BFPh-CF₃** and **BFPh-OMe** in different solvents are shown in (a), (b), (c), (d). The normalized emission spectra of **BFPh-H**, **BFPh-CF₃** and **BFPh-OMe** in different solvents are shown in (e), (f), (g), (h). Fluorescence lifetime decay curve of **BFPh-H**, **BFPh-CF₃** and **BFPh-OMe** are shown in (i), (j), (k), (l). Concentration: 10 μM.

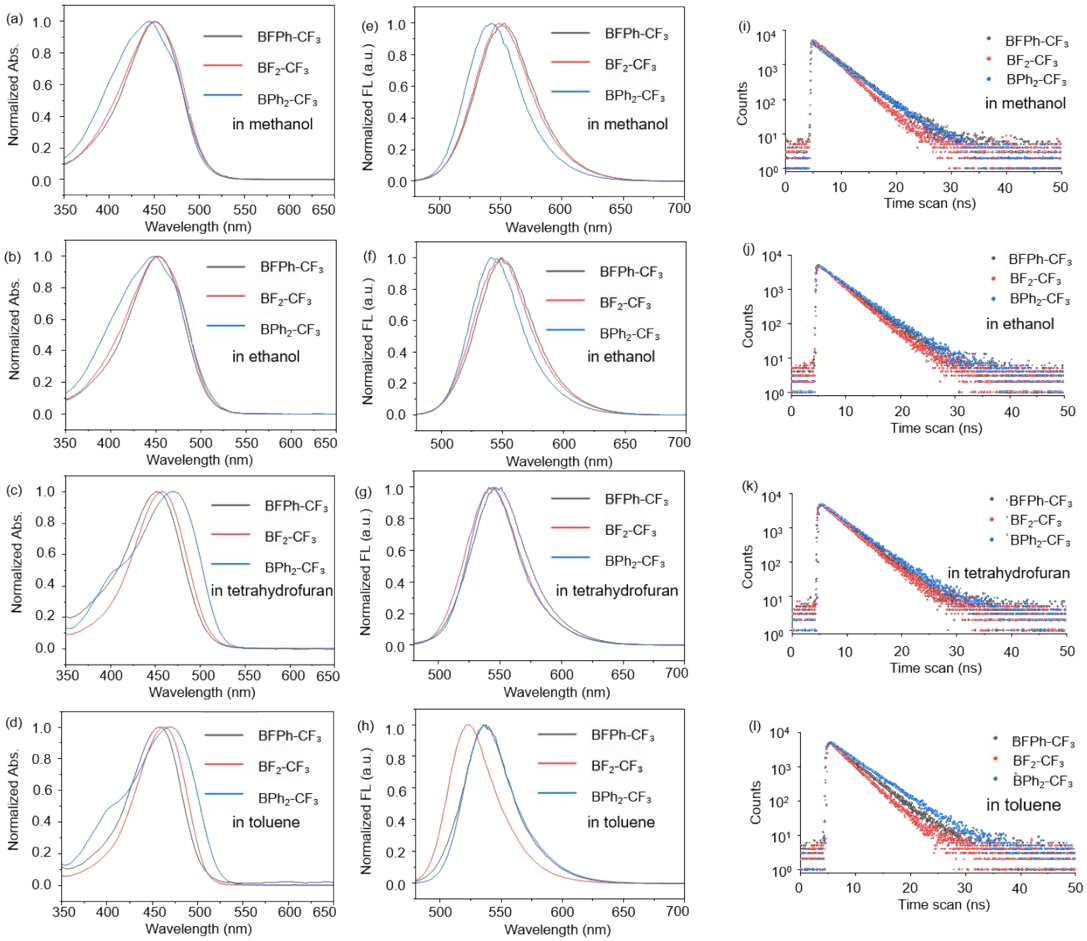


Figure S20. The absorption spectra of **BFPh-CF₃**, **BF₂-CF₃**, and **BPh₂-CF₃** in different solvents are shown in (a), (b), (c), (d). The normalized emission spectra of **BFPh-CF₃**, **BF₂-CF₃**, and **BPh₂-CF₃** in different solvents are shown in (e), (f), (g), (h). Fluorescence lifetime decay curve of **BFPh-CF₃**, **BF₂-CF₃**, and **BPh₂-CF₃** are shown in (i), (j), (k), (l). Concentration: 10 μM.

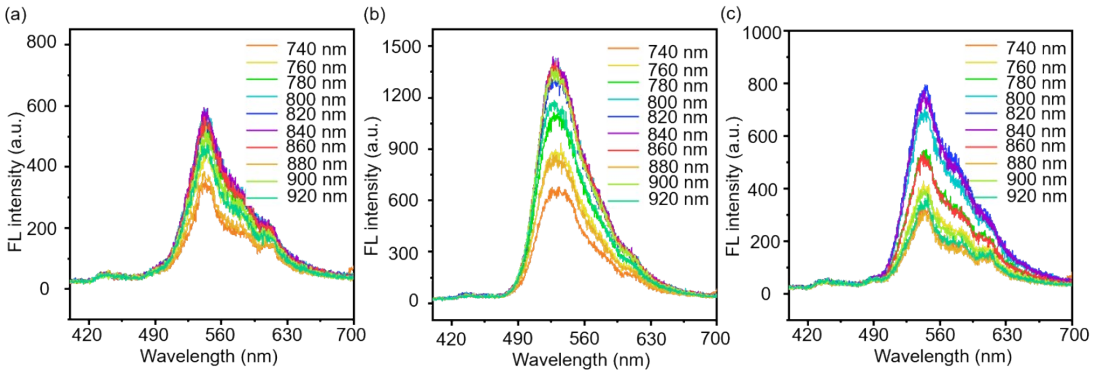


Figure S21. Two-photon excited fluorescence spectra of (a) **BFPh-CF₃**, (b) **BF₂-CF₃** and (c) **BPh₂-CF₃** in Toluene under different pulse lasers. The concentration was 10 μM.

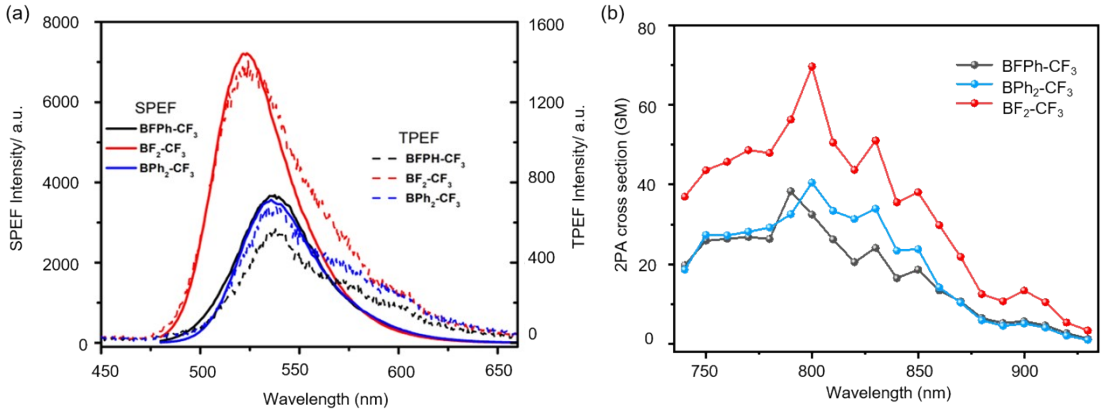


Figure S22. (a) One-photon and Two-photon (800 nm) excited fluorescence spectra of **BFPh-CF₃**, **BF₂-CF₃** and **BPh₂-CF₃** in Toluene; (b)Two-photon absorption cross sections of **BFPh-CF₃**, **BF₂-CF₃**, and **BPh₂-CF₃** in Toluene. 1 GM = 10^{-50} cm^4 s/photon. The concentration was 10 μM.

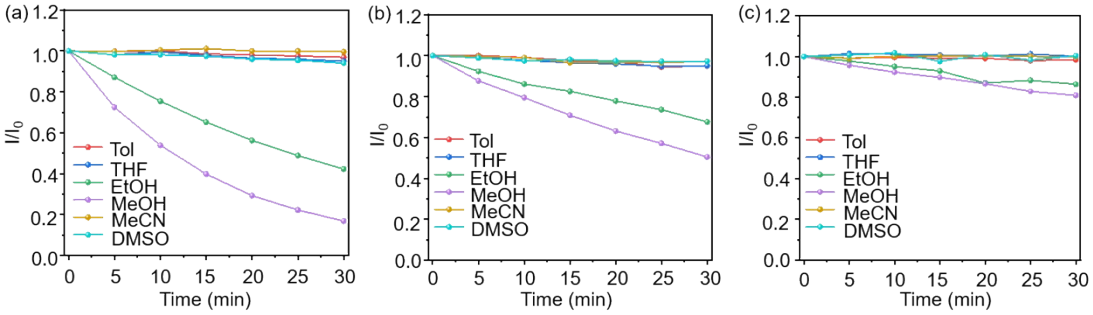


Figure S23. (a), (b), and (c) show the changes in photostability of **BFPh-CF₃**, **BPh₂-CF₃**, and **BF₂-CF₃**, respectively, in different solutions over time under white light irradiation. (λ_{1em} = 450 nm; λ_{2em} = 460 nm; λ_{3em} = 470 nm). The concentration was 10 μM.

Imaging Data

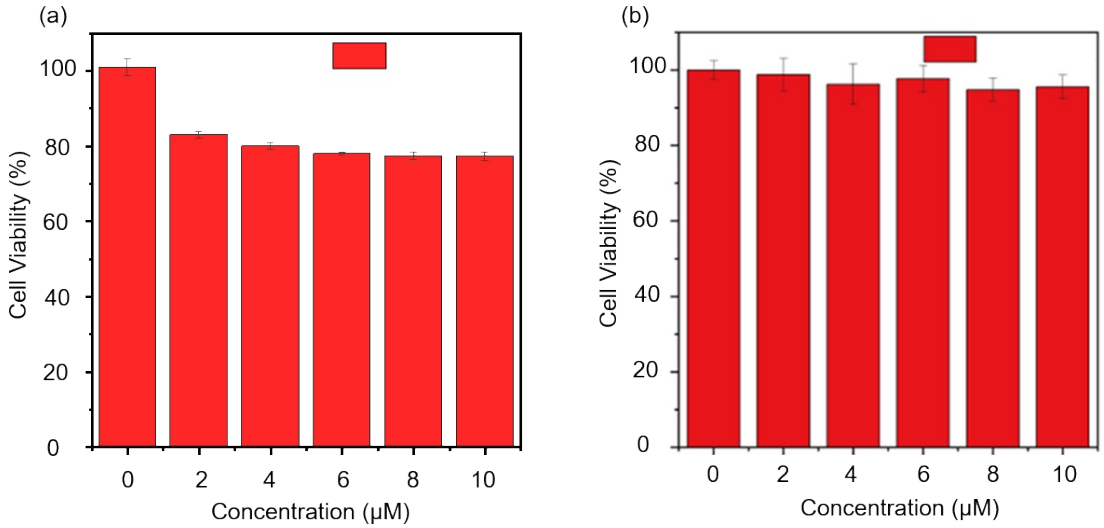


Figure S24. Cytotoxicity in HeLa cells at different concentrations. (a) **BFPh-H,** (b) **BPh_2-CF_3**.

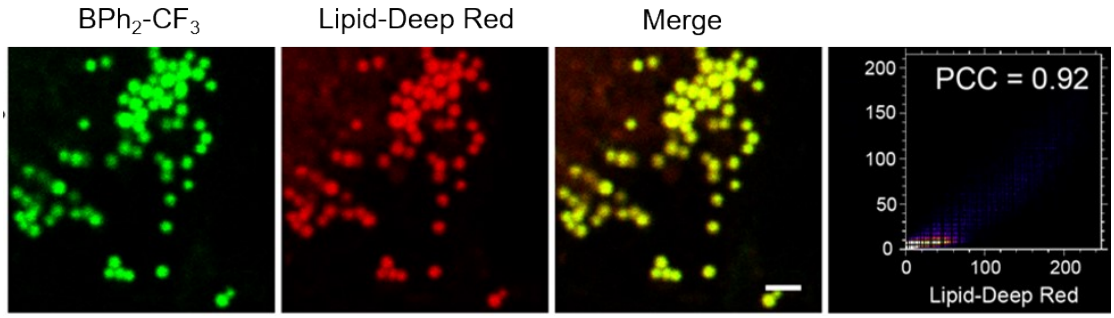


Figure S25. (c) CLSM images of HeLa cells incubated with **BPh_2-CF_3** and Lipid-Deep Red. PCC: Pearson's correlation coefficient. Scale bar: 2 μm. Concentration: 300 nM (**BPh_2-CF_3** probes), 100 nM (Lipid-Deep Red).

Table S6. The ClogP Values of boranils.

Compound	BFPh-H	BFPh-OMe	BFPh-CF_3	BF_2-CF_3	BPh_2-CF_3
ClogP	6.30	6.22	8.07	5.76	10.38

Reference

1. N. S. Makarov, M. Drobizhev and A. Rebane, *Opt. Express*, 2008, **16**, 4029–4047.
2. G. Niu, W. Liu, H. Xiao, H. Zhang, J. Chen, Q. Dai, J. Ge, J. Wu and P. Wang, *CHEM-ASIAN J*, 2016, **11**, 498–504.
3. M. Cao, T. Zhu, M. Zhao, F. Meng, Z. Liu, J. Wang, G. Niu and X. Yu, *Anal. Chem.*, 2022, **94**, 10676–10684.