J. Braz. Chem. Soc., Vol. 26, No. 5, S1-S17, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Supplementary Information

Fluorescence Quenching of Two *meso*-Substituted Tetramethyl BODIPY Dyes by Fe(III) Cation

Lucas C. D. Rezende and Flavio S. Emery*

Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto-SP, Brazil

Compound **1**

Phosphoryl chloride ($POCl_3$, 92 µL, 1 mmol) was slowly added to a stirring solution of 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde (123 mg, 1 mmol) in dichloromethane at 0 ºC under inert atmosphere. After 3 h at room temperature, diisopropylethylamine (800 µL, ca. 5 mmol) and $BF_3.OEt_2$ (650 µL, ca. 5 mmol) were added to the reaction, and the mixture was stirred for 1 h, after which a green fluorescence was observed. The fluorescent solution was washed with water 3 times, dried with Na_2SO_4 and the solvent was removed by distillation under reduced pressure. Purification by flash column chromatography (230-400 mesh, hexane/ethyl acetate 9:1) yielded 53.9 mg (0.217 mmol) of **1** (43.5%).

5,5-Difluoro-1,3,7,9-tetramethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinine (**1**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.04 (s, 2H), 2.53 (s, 6H), 2.24 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.83, 141.34, 133.52, 120.21, 119.14, 77.16, 14.80, 11.41; EI-MS: $[M]^+$ *m/z* 248, $[M–F]^+$ *m/z* 228.

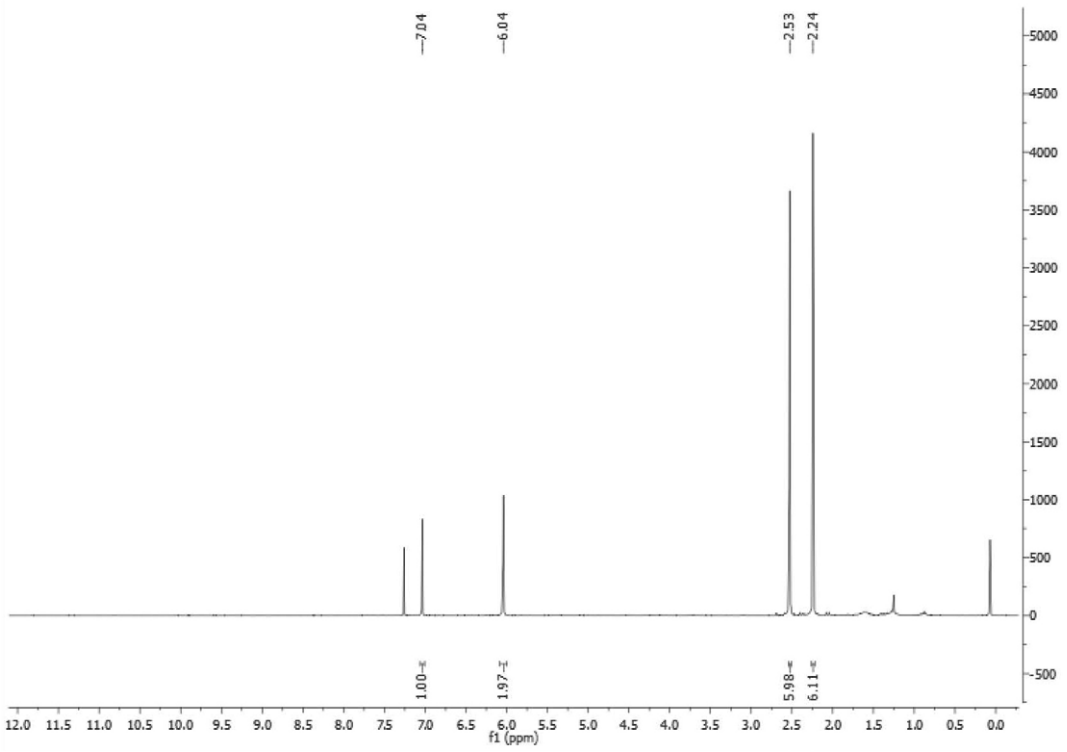


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1**.

*e-mail: flavioemery@fcfrp.usp.br

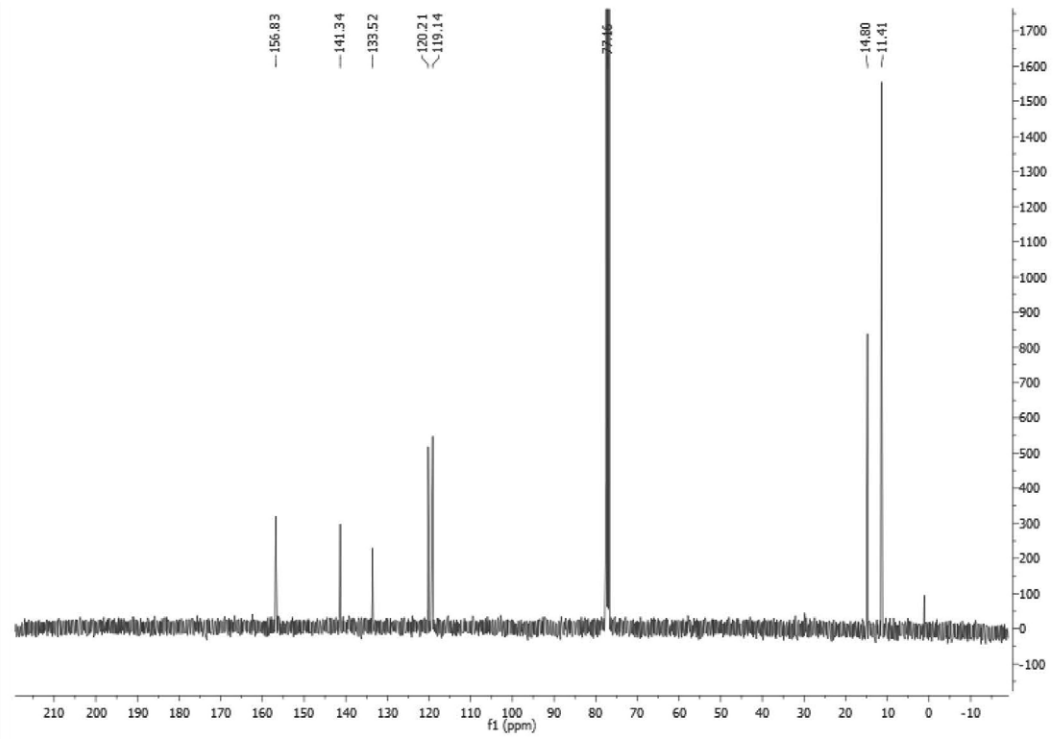


Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **1**.

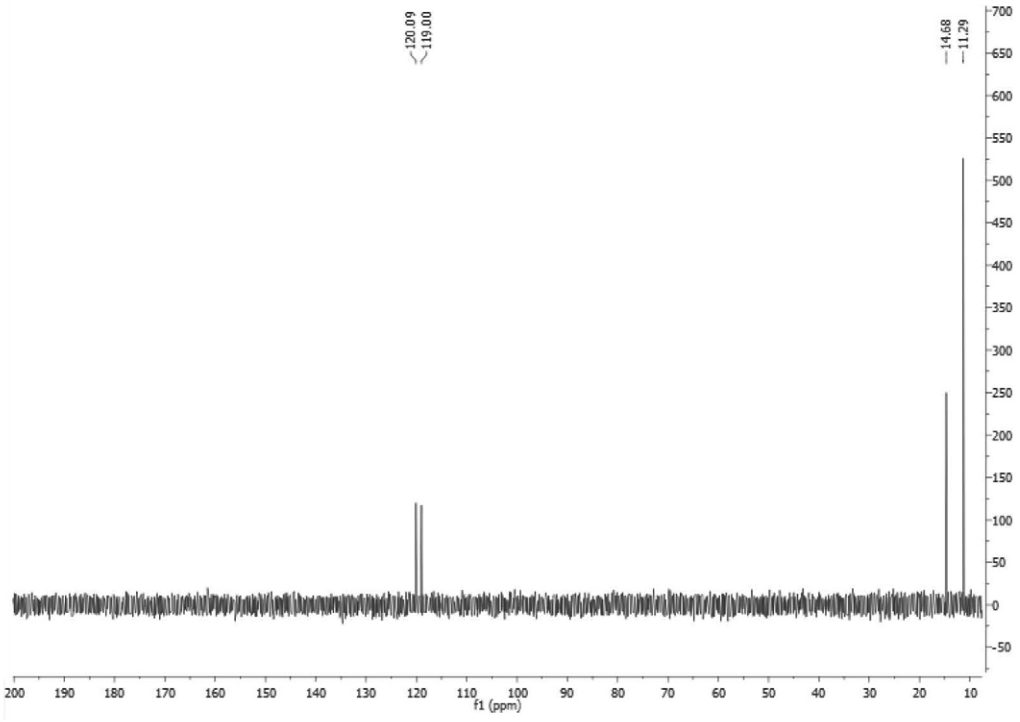


Figure S3. ^{13}C NMR-DEPT135 (100 MHz, $CDCl_3$) spectrum of compound **1**.

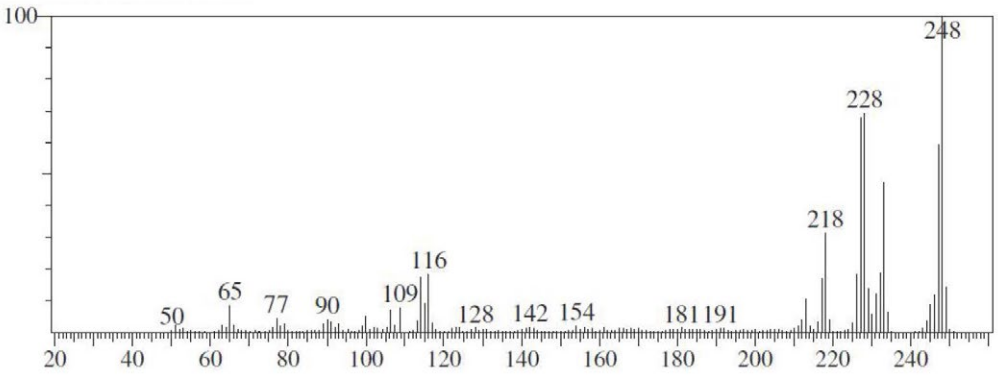
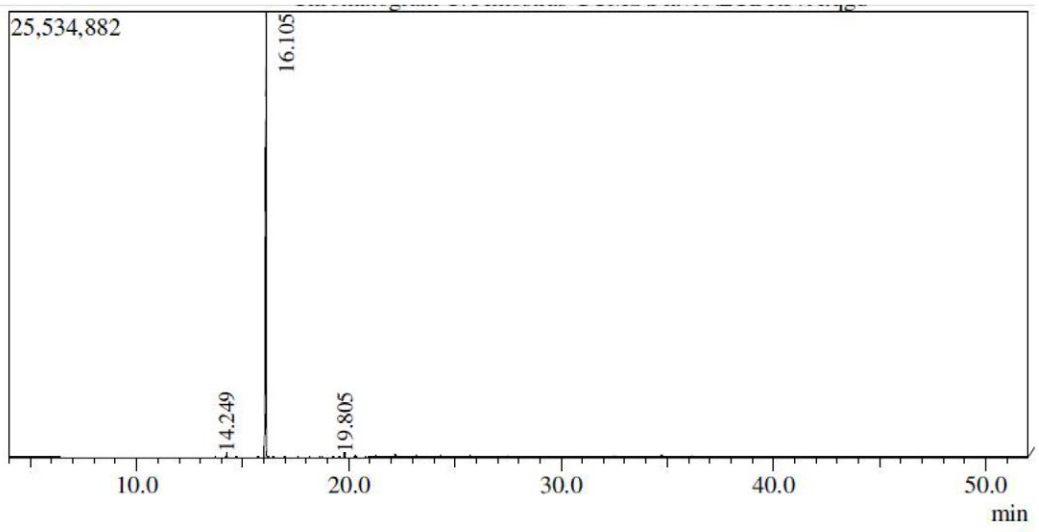


Figure S4. Electron ionization mass spectrum of compound **1**.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name	Base m/z
1	14.249	440615	0.57		220.10
2	16.105	76250661	98.89		248.15
3	19.805	418278	0.54		149.05
		77109554	100.00		

Figure S5. Gas chromatography of compound **1**.

Compound **2**

2,4-Dimethyl pyrrole (570 mg, 6 mmol) and thiophene-2-carbaldehyde (300 mg, ca. 2.7 mmol) were stirred for 5 min in dichloromethane at room temperature under inert atmosphere. Three drops of trifluoroacetic acid (TFA) were added to the reaction vessel, and after 1 h under the same conditions, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (613 mg, 2.7 mmol) was added, and the mixture was stirred for another 2 h, after which triethylamine (1.6 mL, 10 mmol) was added. The mixture was readily washed with 0.1 mol L^{-1} $NaOH_{(aq)}$, dried with Na_2SO_4 and filtered. Without any purification step, reaction cake was solubilized in 20 mL of dichloromethane. Triethylamine (3.2 mL, 20 mmol) and $BF_3.OEt_2$ (2.6 mL, 20 mmol) were added and stirred for 1 h at room temperature. The solution was washed with water and dried under Na_2SO_4. The solvent was removed by distillation under reduced pressure and the 135.7 mg (0.411 mmol) of **2** (15.2% yield) was obtained after flash column chromatography purification (230-400 mesh, hexane/ethyl acetate 3:1).

5,5-difluoro-1,3,7,9-tetramethyl-10-(thiophen-2-yl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinine (**2**): IR (KBr) ν_{max}/cm^{-1} 2923, 1544, 1303, 1243, 1172, 1078, 971, 806, 753, 474; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, 1H,

J 5.0 Hz), 7.13 (dd, 1H, J_1 5.0 Hz, J_2 3.5 Hz), 6.99 (d, 1H, *J* 3.5 Hz), 6.00 (s, 2H), 2.55 (s, 6H), 1.58 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.50, 143.89, 135.09, 132.82, 128.24, 127.98, 127.79, 125.91, 121.90, 15.01, 13.91; HRMS-ESI $[M+H]^+$ calcd. for $C_{17}H_{18}BF_2N_2S$: 331.1246; found: 331.1254.

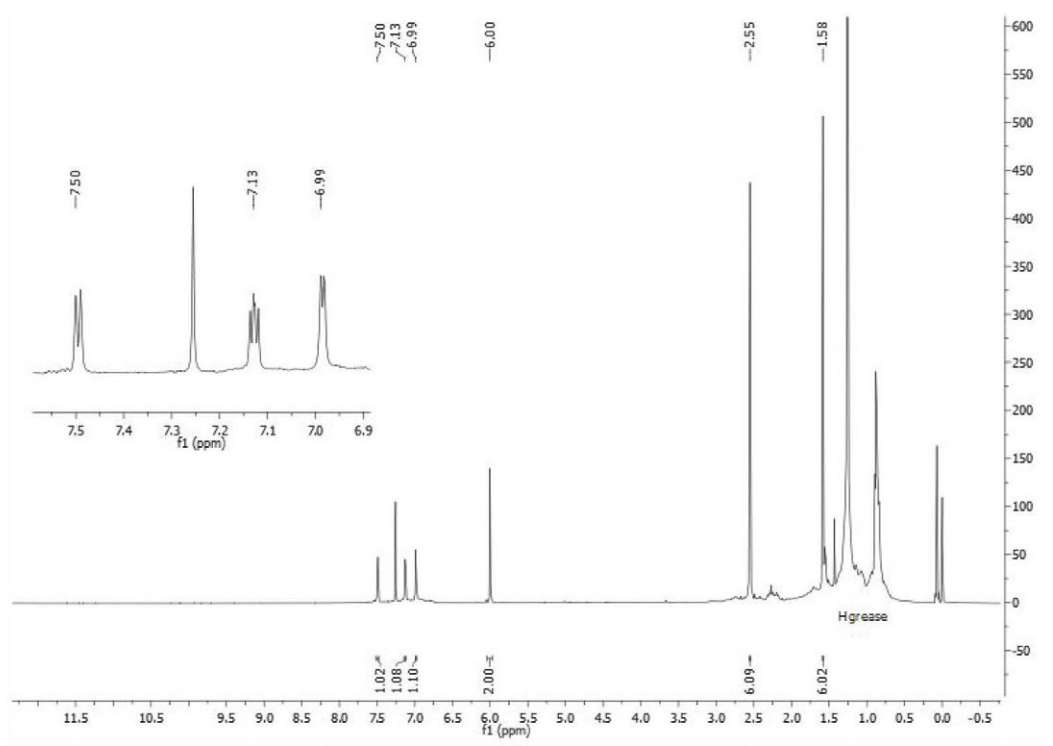


Figure S6. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **2**.

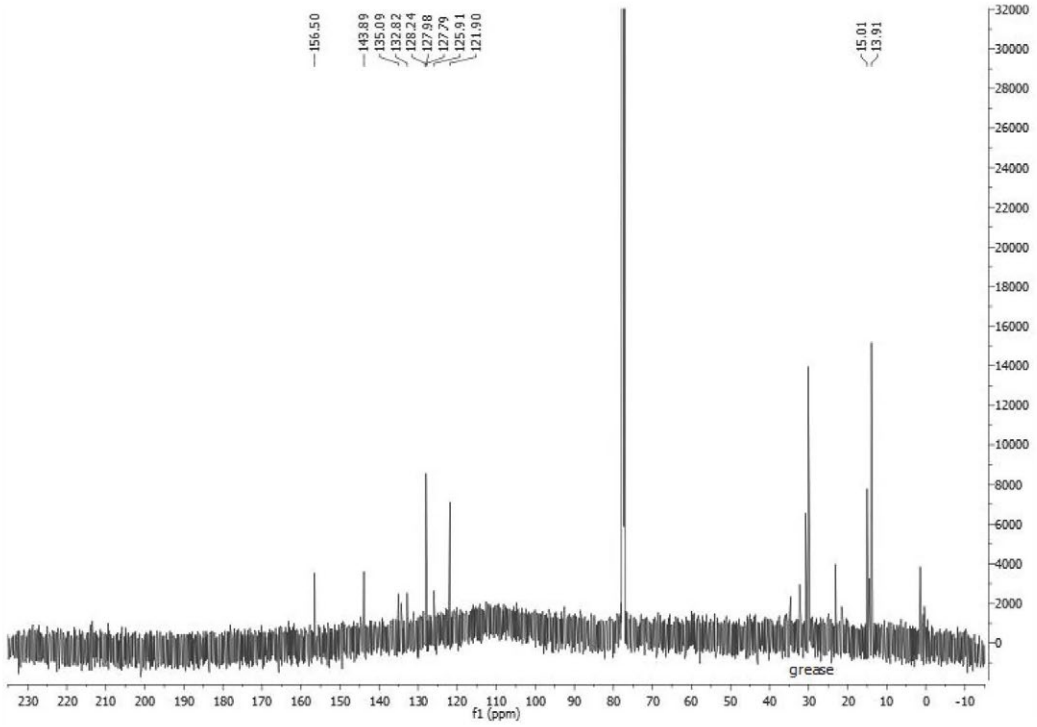


Figure S7. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **2**.

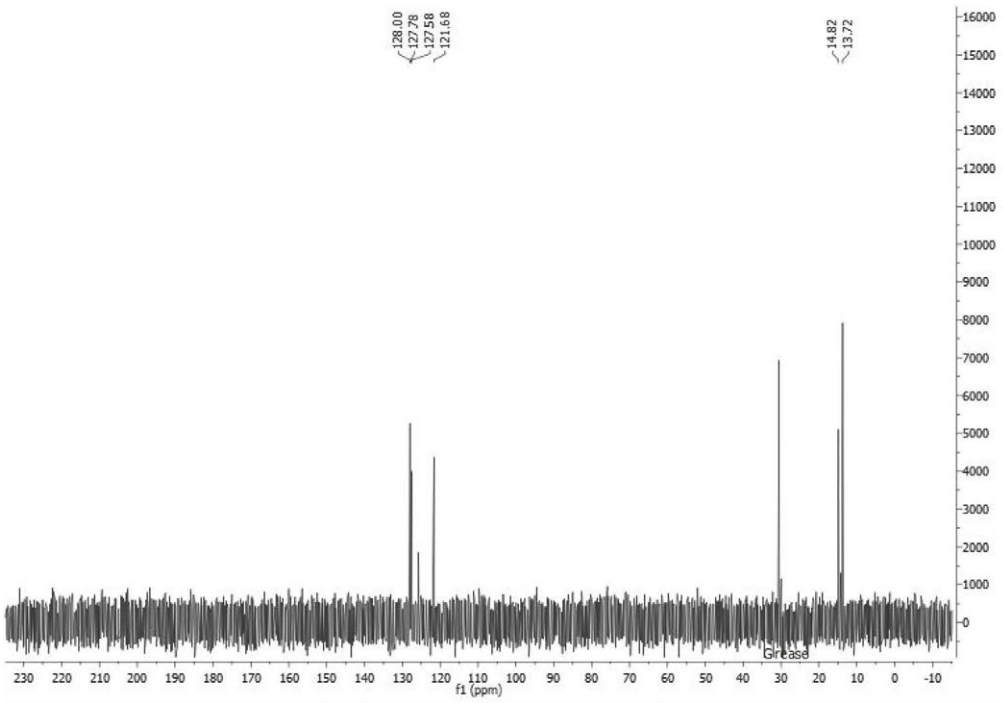


Figure S8. ^{13}C NMR-DEPT135 spectrum (125 MHz, $CDCl_3$) of compound **2**.

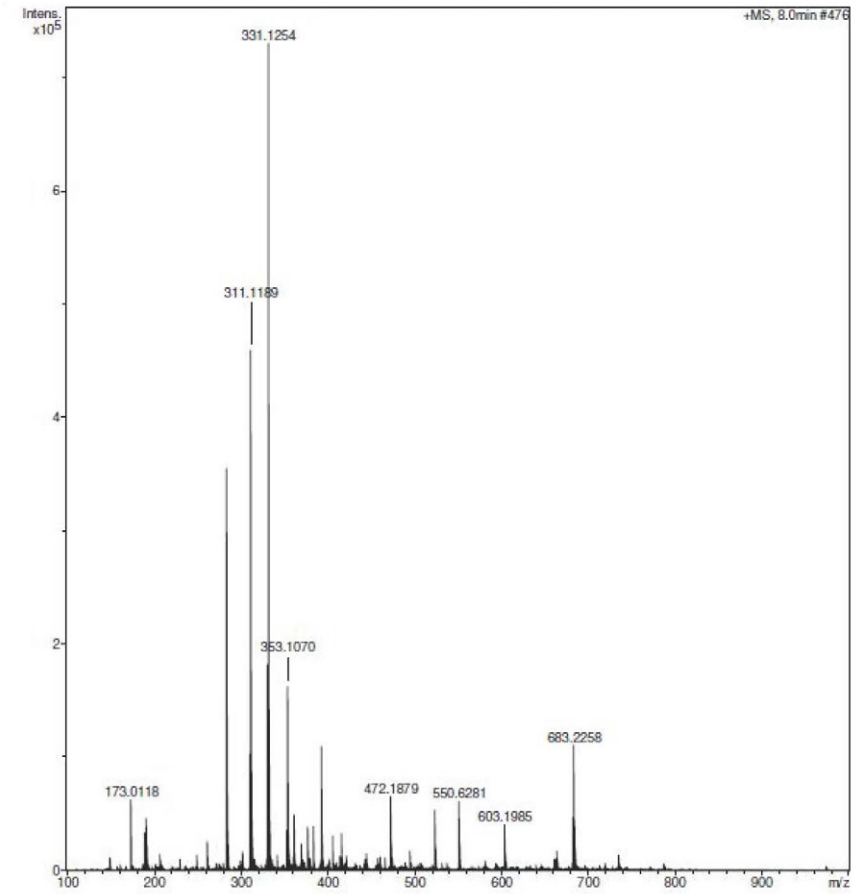


Figure S9. Electrospray ionization high-resolution mass spectrum of compound **2**.

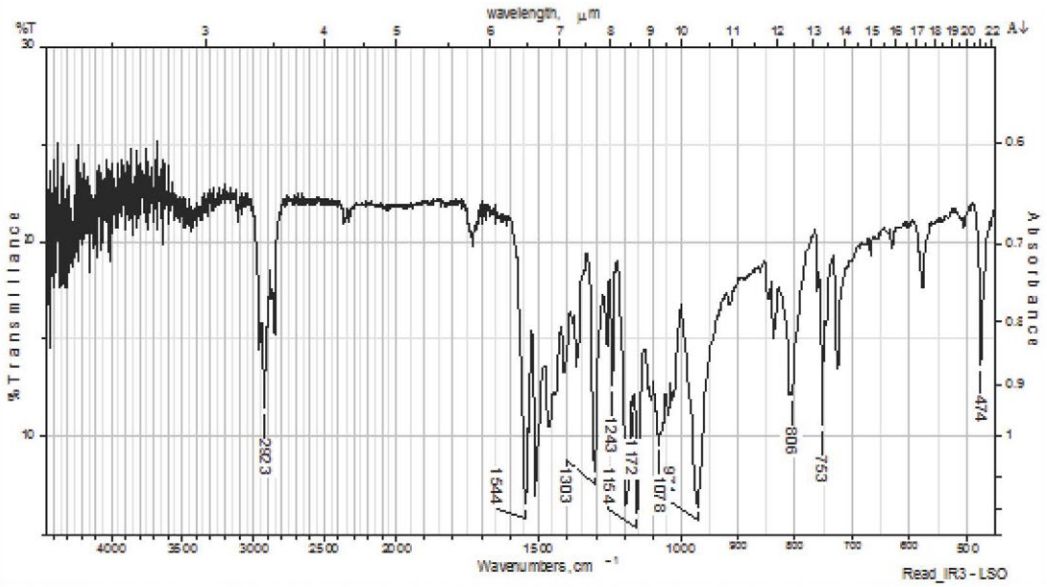
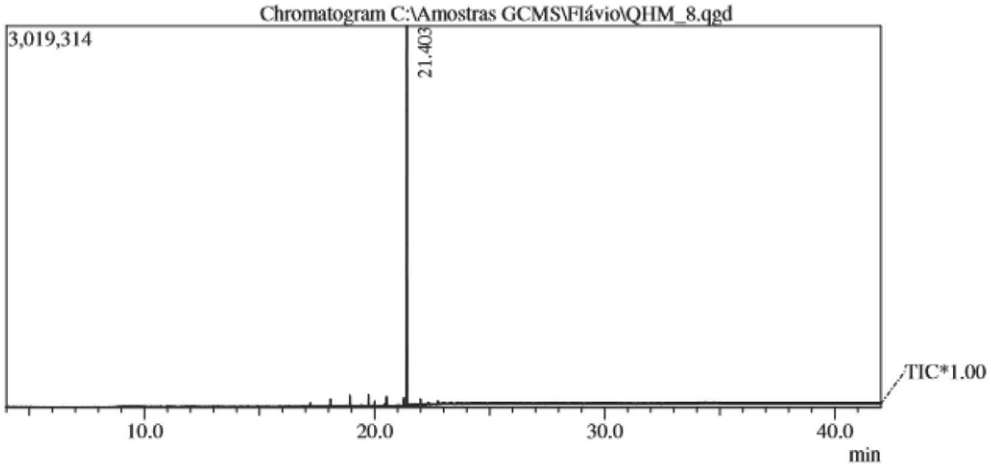


Figure S10. Infrared absorption spectrum of compound **2**.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name	Base m/z
1	21.403	4941860	100.00		330.05
		4941860	100.00		

Figure S11. Gas chromatography of compound **2**.

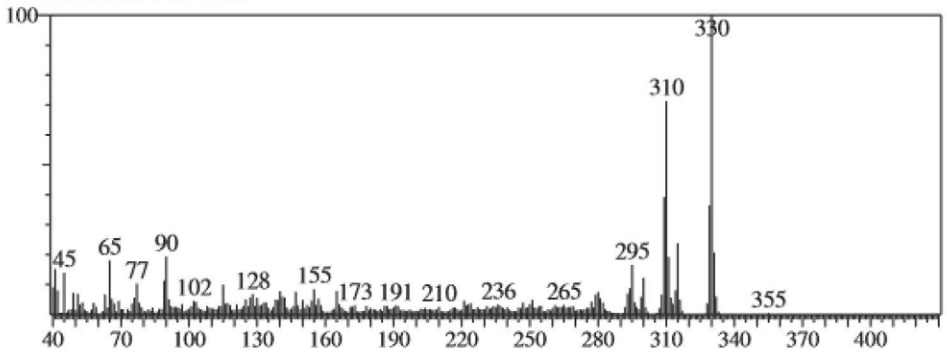


Figure S12. Electron ionization mass spectrum of compound **2**.

Compound **3**

2,4-Dimethyl pyrrole (199 mg, 2.1 mmol) and 4-pyridinecarboxaldehyde (188 μL, 2 mmol), were stirred for 5 min in 250 mL of tetrahydrofuran (THF) at room temperature and inert atmosphere. Three drops of TFA were added to the reaction vessel, and after 2 h under these conditions, a solution of DDQ (454 mg, 2 mmol) in 50 mL of THF was added to the reaction and stirred for 1 h, after which triethylamine (2 mL, 14 mmol) was added. THF was distilled by evaporation, the reaction cake was dissolved in dichloromethane, washed with 0.1 mol L^{-1} $NaOH_{(aq)}$, dried with Na_2SO_4 and filtered. Dipyrrin was purified by column chromatography with hexane/ethyl acetate 1:1 and solubilized in dichloromethane. Triethylamine (3.2 mL, 20 mmol) and $BF_3.OEt_2$ (2.6 mL, 20 mmol) were added and stirred for 1 h at room temperature. The solution was washed with water and dried under Na_2SO_4. The solvent was removed by distillation under reduced pressure and 153.7 mg (0.472 mmol) of **3** (23.7% yield) was obtained after flash column chromatography purification (230-400 mesh, hexane/ethyl acetate 3:1).

5,5-difluoro-1,3,7,9-tetramethyl-10-(pyridin-4-yl)-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinine (**3**): IR (KBr) ν_{max}/cm^{-1} 2918, 1654, 1508, 1466, 1410, 1306, 1156, 1120, 1076, 980, 812, 720; ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, 1H, *J* 5.3 Hz), 7.30 (d, 1H, *J* 5.3 Hz), 6.00 (s, 1H), 2.55 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.59, 150.70, 150.04, 143.78, 142.77, 137.71, 130.44, 123.45, 121.93, 113.83, 77.16, 14.77, 14.75; HRMS-ESI $[M+H]^+$ calcd. for $C_{18}H_{19}BF_2N_3$: 326.1635; found: 326.1643.

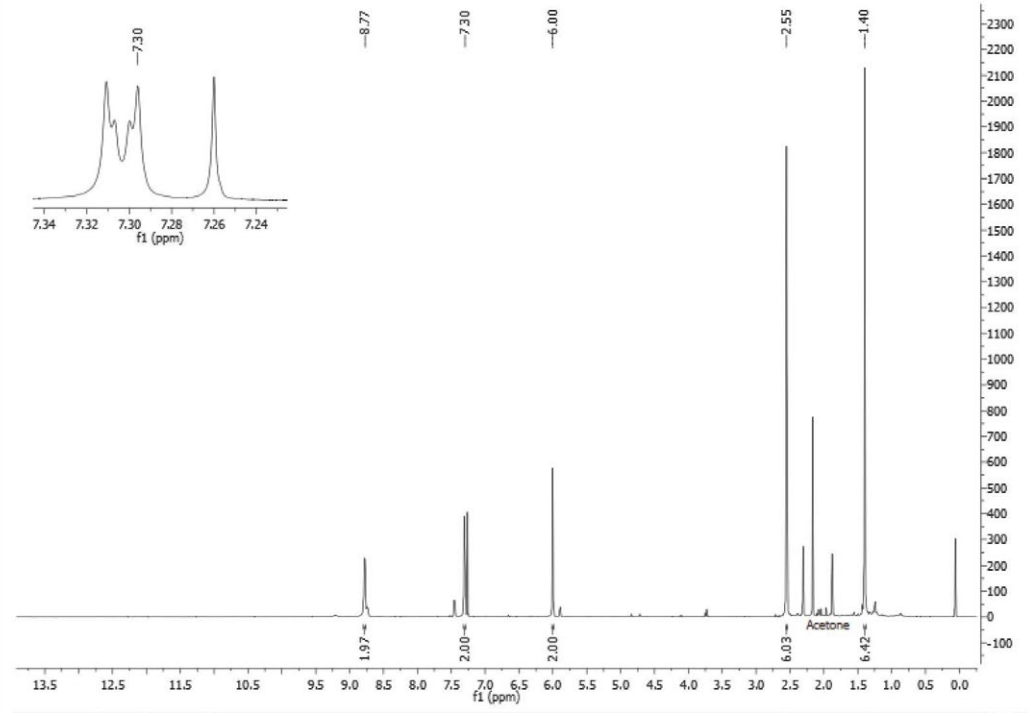


Figure S13. ^1H NMR spectrum (500 MHz, $CDCl_3$) of compound **3**.

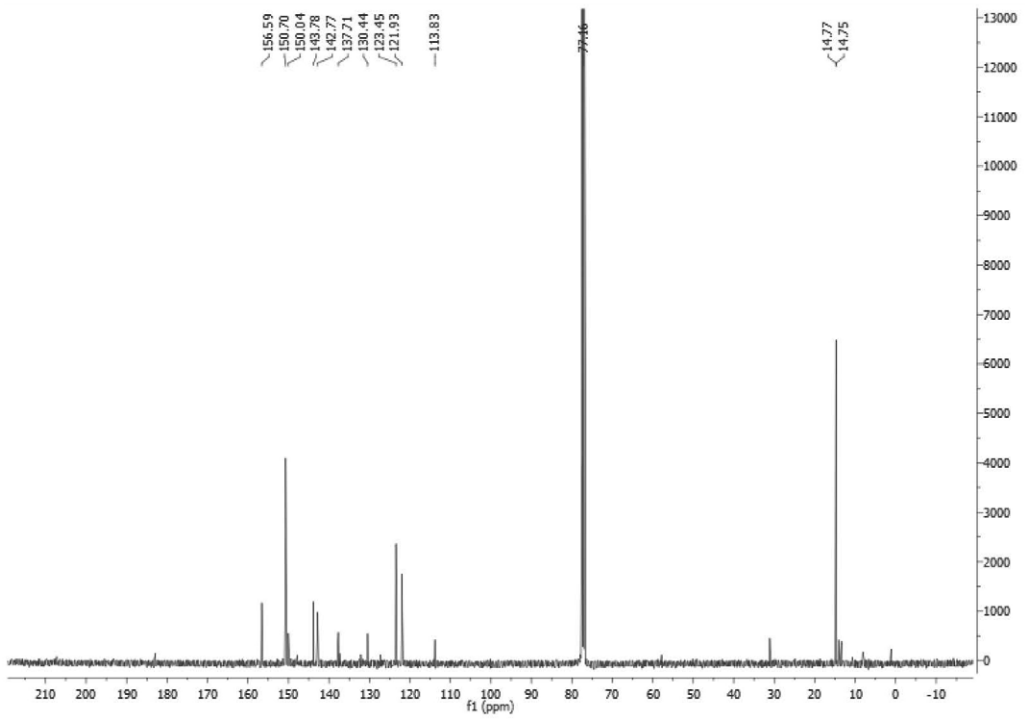


Figure S14. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **3**.

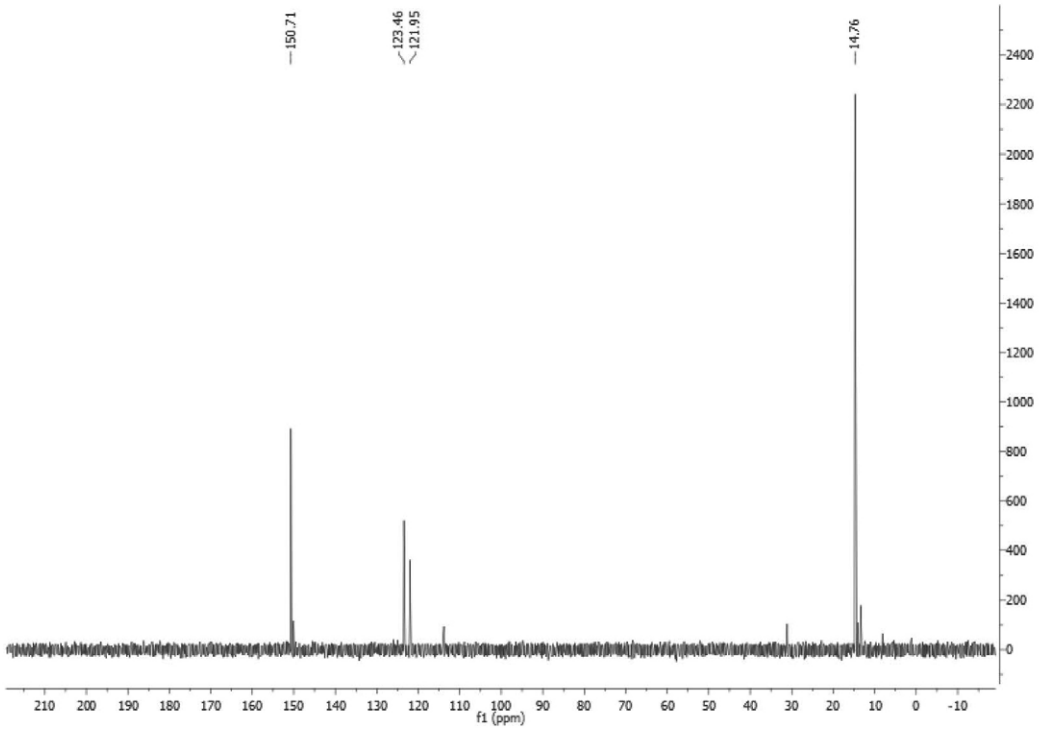


Figure S15. ^{13}C NMR-DEPT135 spectrum (100 MHz, $CDCl_3$) of compound **3**.

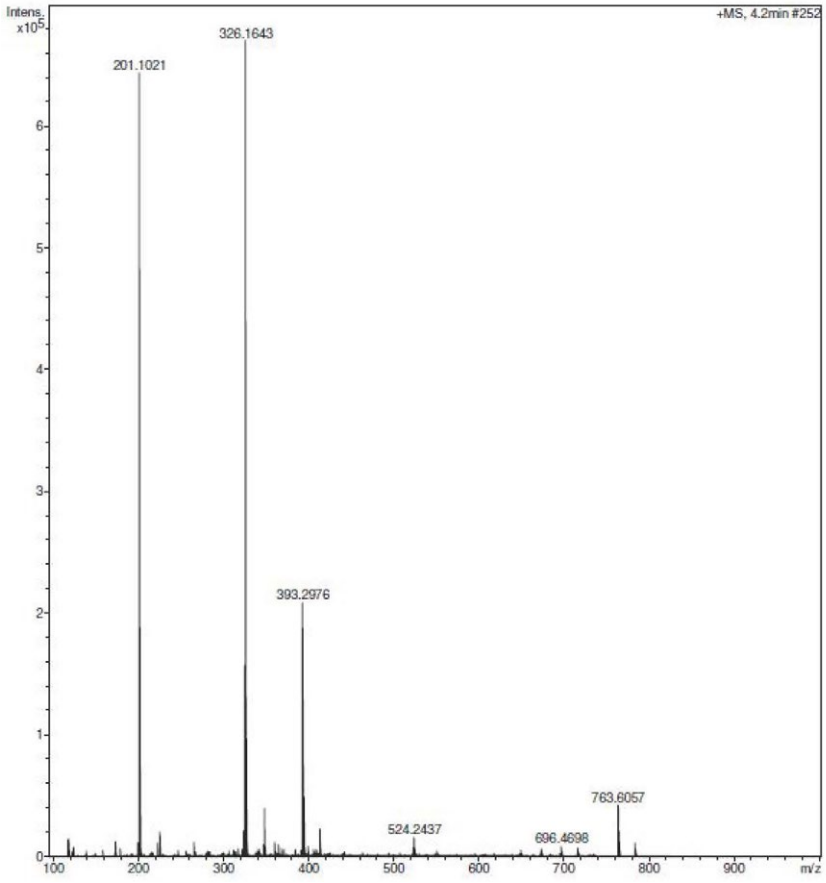


Figure S16. Electrospray ionization high-resolution mass spectrum of compound **3**.

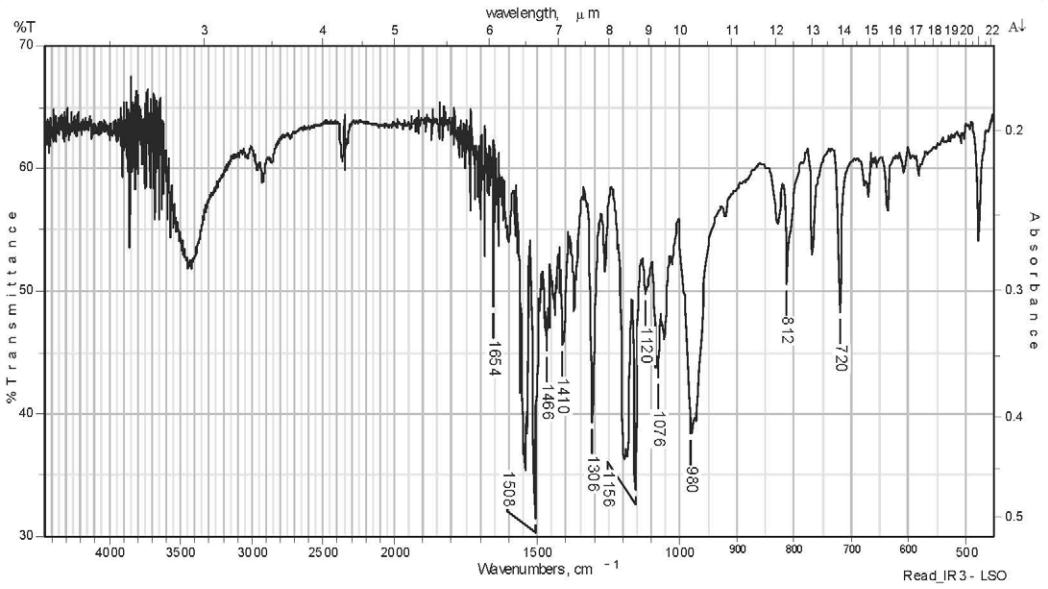


Figure S17. Infrared absorption spectrum of compound **3**.

Compound **4**

2,4-Dimethyl pyrrole (129 mg, 1.25 mmol) and 3,4-bis(benzyloxy)benzaldehyde (173 mg, 0.56 mmol), were stirred for 5 min in 250 mL THF at room temperature and inert atmosphere. Three drops of TFA were added to the reaction vessel, and after 2.5 h under these conditions, a solution of DDQ (153 mg, 0.68 mmol) in 50 mL of THF was added to the reaction and stirred for 3 h, after which triethylamine (2 mL, 14 mmol) was added. THF was distilled out under reduced pressure, the reaction cake was dissolved in dichloromethane, washed with 0.1 mol L^{-1} $NaOH_{(aq)}$, dried with Na_2SO_4 and filtered. Dipyrrin was purified by column chromatography with ethyl acetate and solubilized in dichloromethane. Triethylamine (1.4 mL, 8.7 mmol) and $BF_3.OEt_2$ (1.1 mL, 8.5 mmol) were added and stirred for 1 h at room temperature. The solution was washed with water and dried under Na_2SO_4, solvent was distilled under reduced pressure and 172.1 mg (56% yield) of the protected BODIPY **4** was purified by flash column chromatography (230-400 mesh, hexane/ethyl acetate 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48-7.27 (m, 10H), 7.01 (d, 1H, *J* 8.2 Hz), 6.80 (d, 1H, *J* 1.7 Hz), 6.74 (dd, 1H, J_1 8.1 Hz, J_2 1.7 Hz), 5.93 (s, 2H), 5.23 (s, 2H), 5.17 (s, 2H), 2.53 (s, 6H), 1.29 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.44, 149.36, 149.32, 143.37, 141.45, 136.81, 136.68, 131.72, 128.71, 128.65, 128.13, 128.09, 127.96, 127.61, 127.37, 121.16, 121.07, 115.68, 114.80, 77.16, 71.39, 71.01, 14.36, 14.33; HRMS-ESI $[M+H]^+$ calcd. for $C_{33}H_{32}BF_2N_2O_2$: 537.2519; found: 537.2520. 102 mg of the protected compound was dissolved in 20 mL of chloroform/methanol (2:3), and 120 mg of palladium supported in carbon was added. Hydrogen gas at 18 psi was added to the system and after 30 min total consumption of the reagent was observed. After filtration on selite and purification by flash column chromatography (230-400 mesh, hexane/ethyl acetate 1:1) compound **4** was obtained (46.2 mg, 68%) as a red solid.

4-(5,5-Difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-10-yl)benzene-1,2-diol (**4**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (d, 1H, *J* 8.0 Hz), 6.72 (d, 1H, *J* 1.9 Hz), 6.66 (dd, 1H, J_1 8.0 Hz, J_2 1.9 Hz), 5.96 (s, 2H), 2.53 (s, 6H), 1.48 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.36, 144.67, 144.64, 143.43, 141.70, 131.81, 129.26, 127.29, 127.15, 121.24, 120.67, 116.11, 115.02, 77.16, 14.71, 14.65; HRMS-ESI $[M + H]^+$ calcd for $C_{19}H_{20}BF_2N_2O_2$: 357.1580; found: 357.1599.

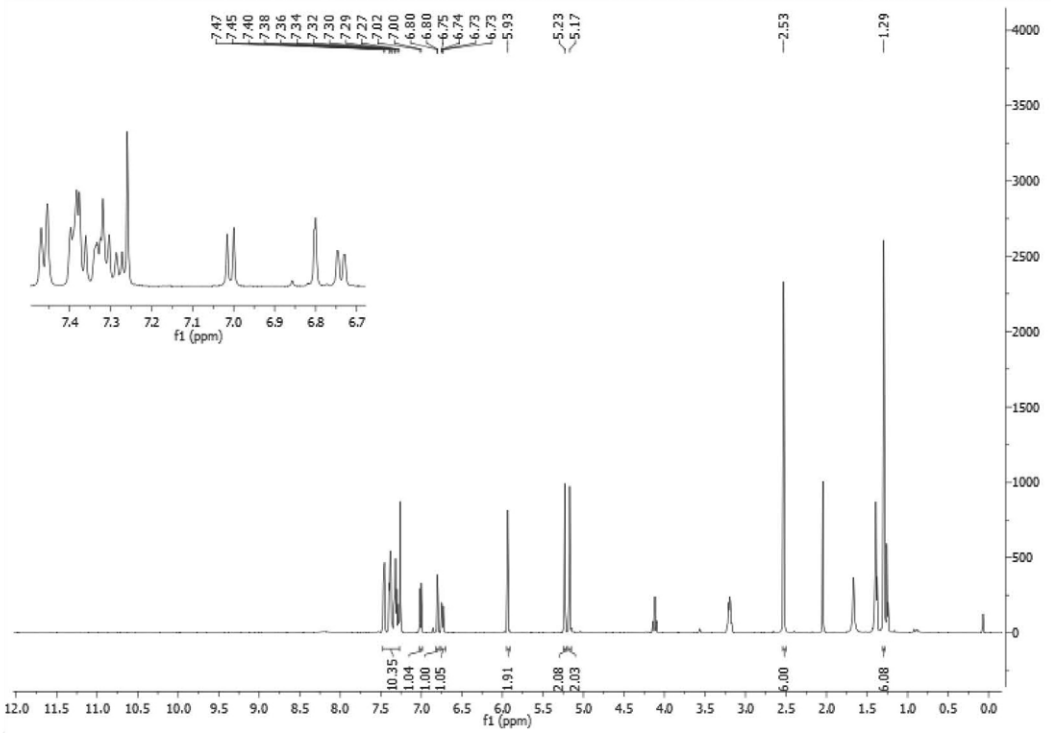


Figure S18. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4** before deprotection.

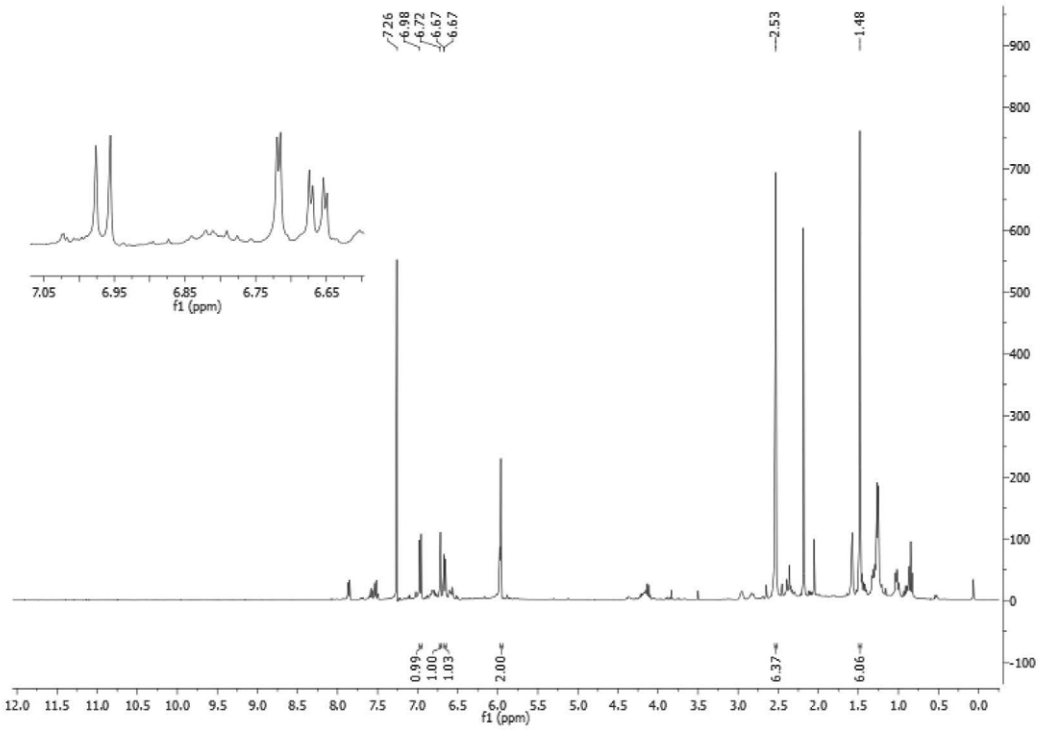


Figure S19. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **4**.

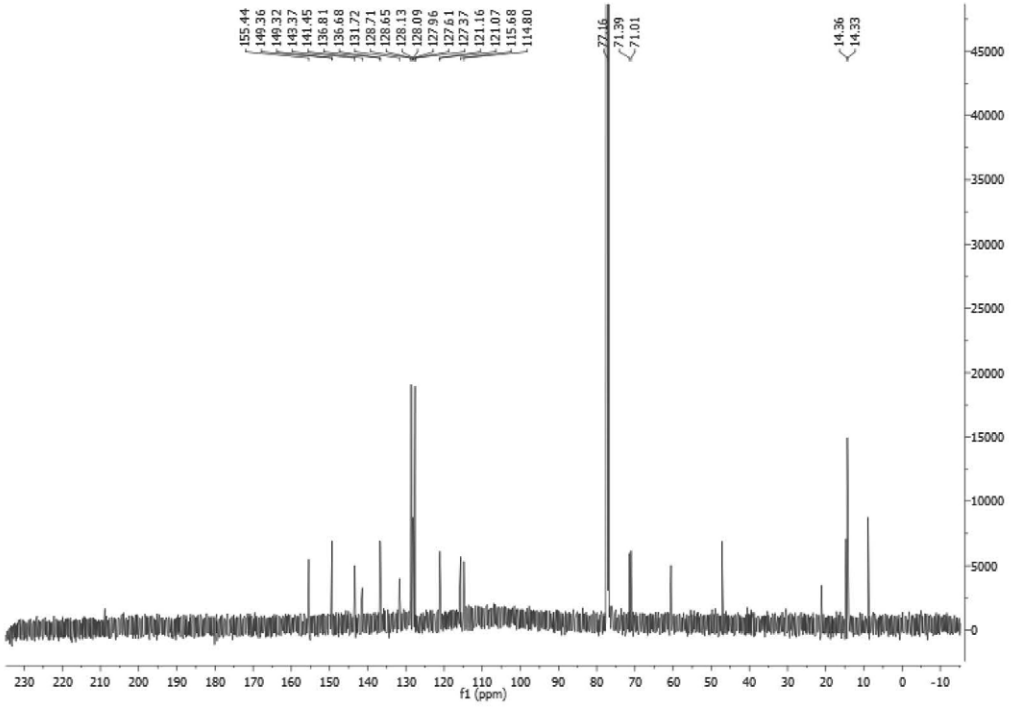


Figure S20. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **4** before deprotection.

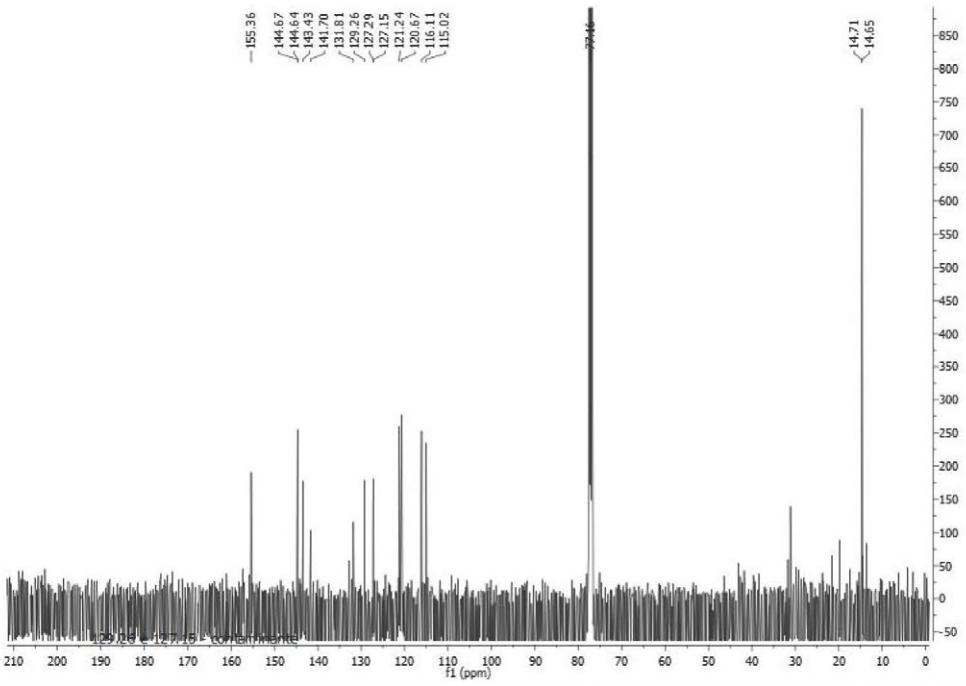


Figure S21. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **4**.

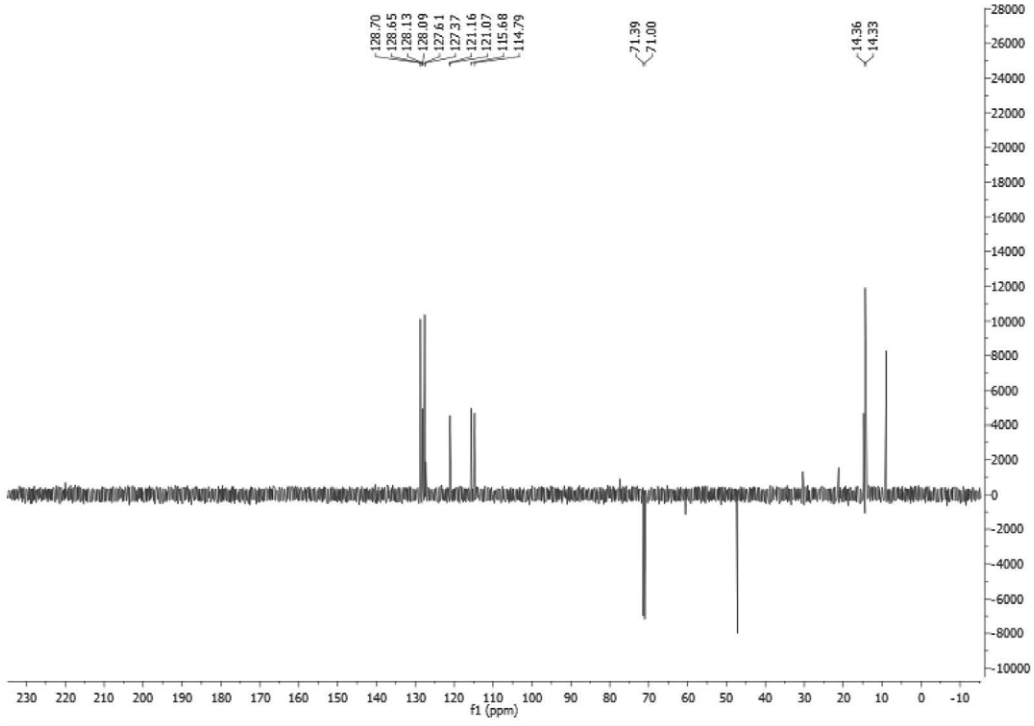


Figure S22. ^{13}C NMR-DEPT135 spectrum (125 MHz, $CDCl_3$) of compound **4** before deprotection.

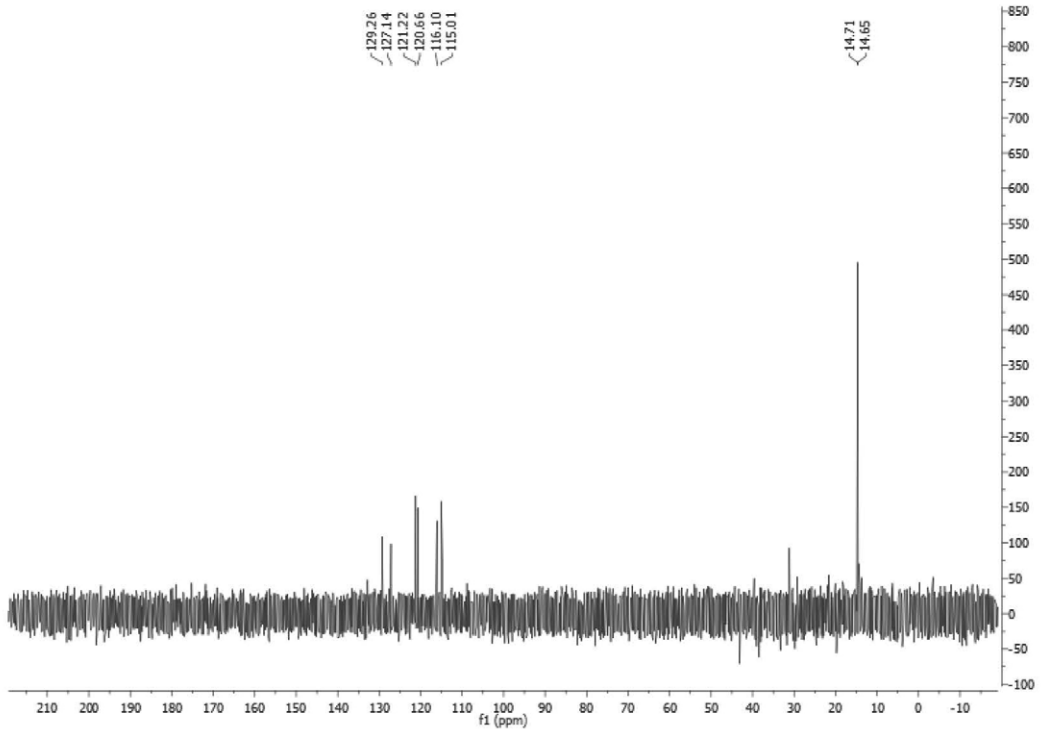


Figure S23. ^{13}C NMR-DEPT135 spectrum (100 MHz, $CDCl_3$) of compound **4**.

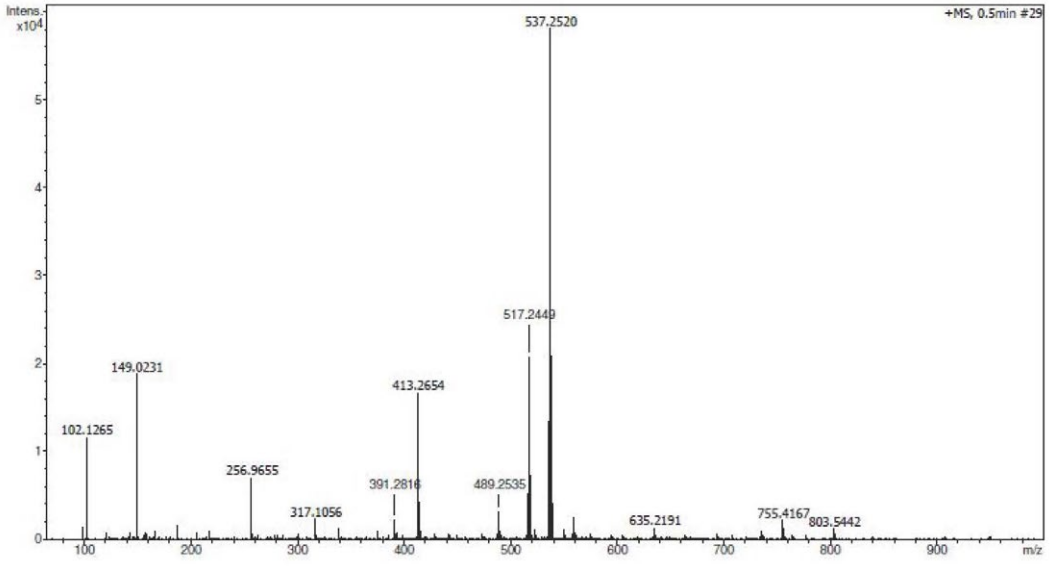


Figure S24. Electrospray ionization high-resolution mass spectrum of compound **4** before deprotection.

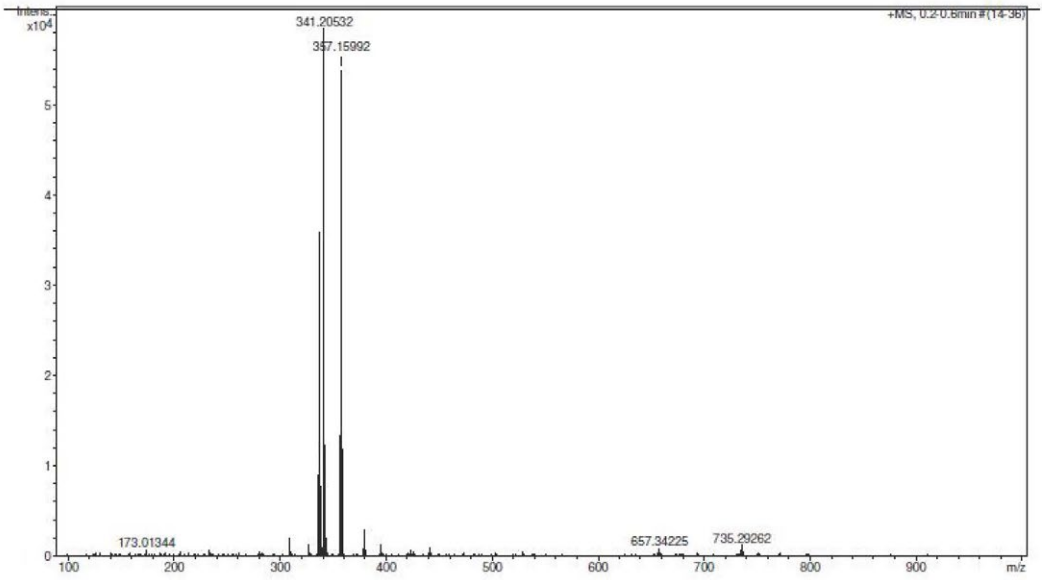


Figure S25. Electrospray ionization high-resolution mass spectrum of compound **4** before deprotection.

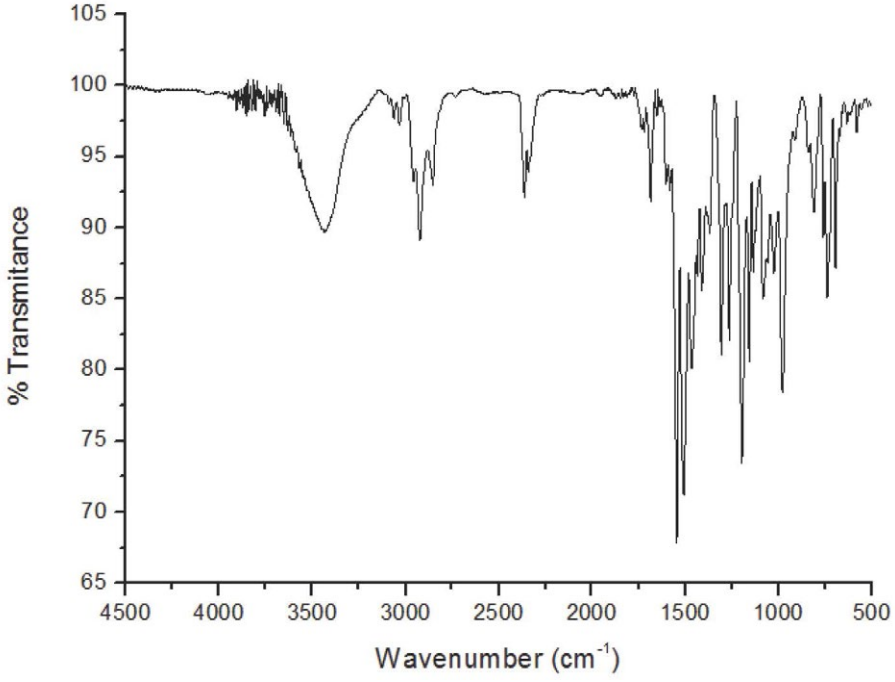


Figure S26. Infrared absorption spectrum of compound **4** before deprotection.

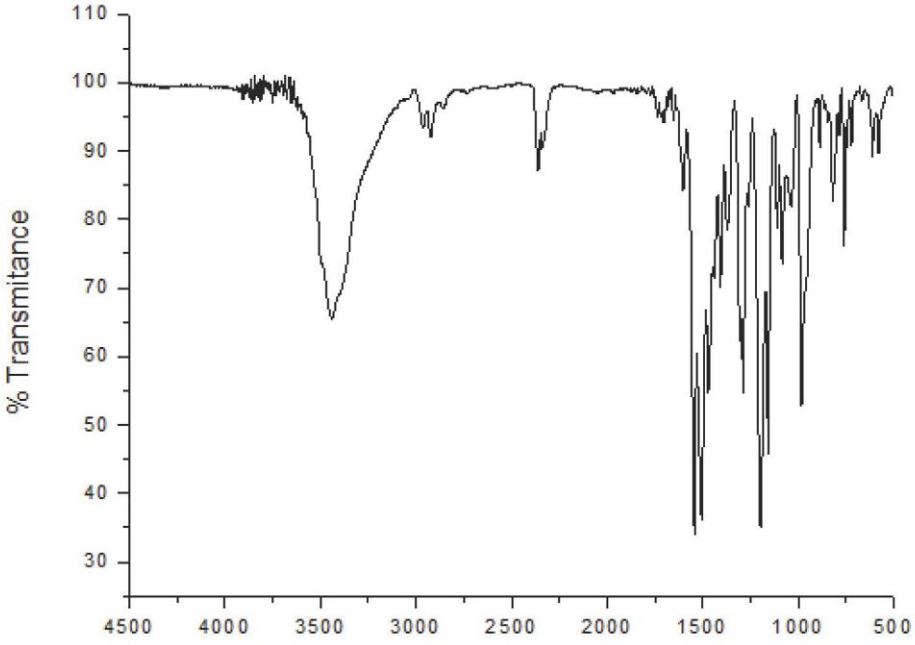


Figure S27. Infrared absorption spectrum of compound **4** before deprotection.

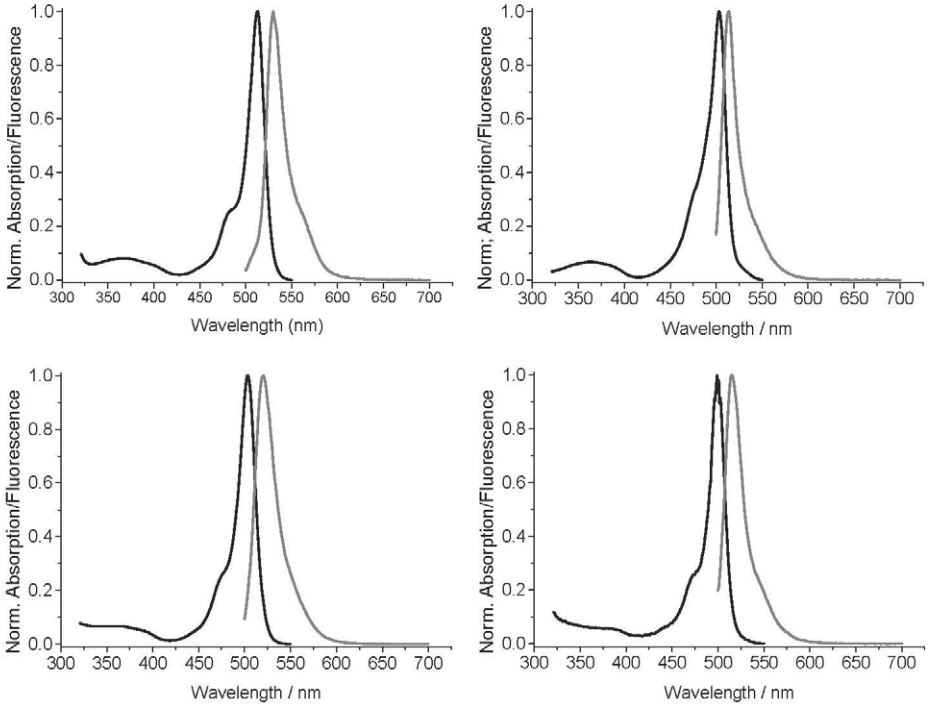


Figure S28. Absorption (black) and emission (grey) spectra of compounds **1** (upper-left), **2** (upper-right), **3** (lower-left) and **4** (lower-right) showing sharp absorption and emission with a small Stokes shift and significant spectral overlap. Aqueous DMSO (30% v/v) was used as solvent.

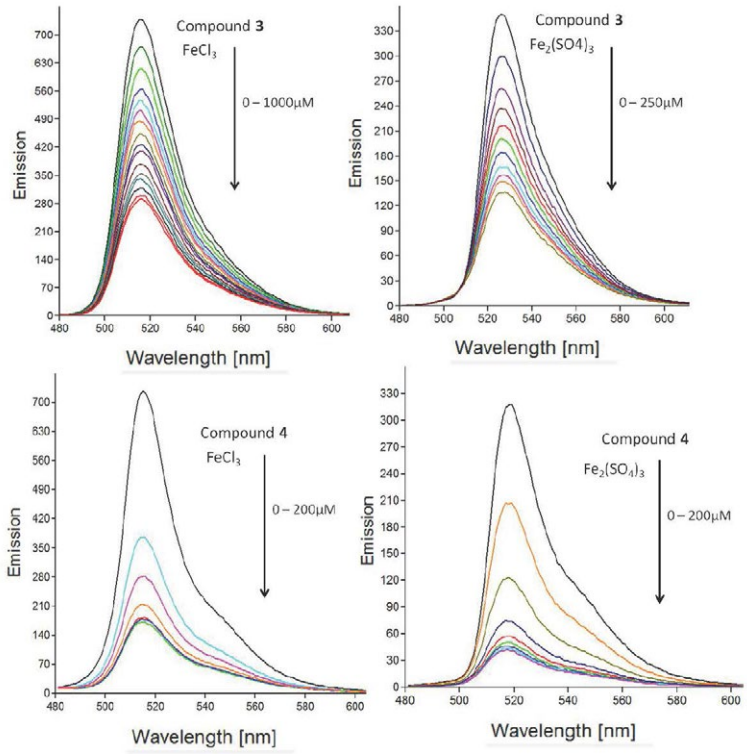


Figure S29. Fluorescence quenching of compounds **3** and **4** by Fe(III). Top left: Fluorescence quenching of **3** by $FeCl_3$. Top right: Fluorescence quenching of **3** by $Fe_2(SO_4)_3$. Bottom left: Fluorescence quenching of **4** by $FeCl_3$. Bottom right: Fluorescence quenching of **4** by $Fe_2(SO_4)_3$.

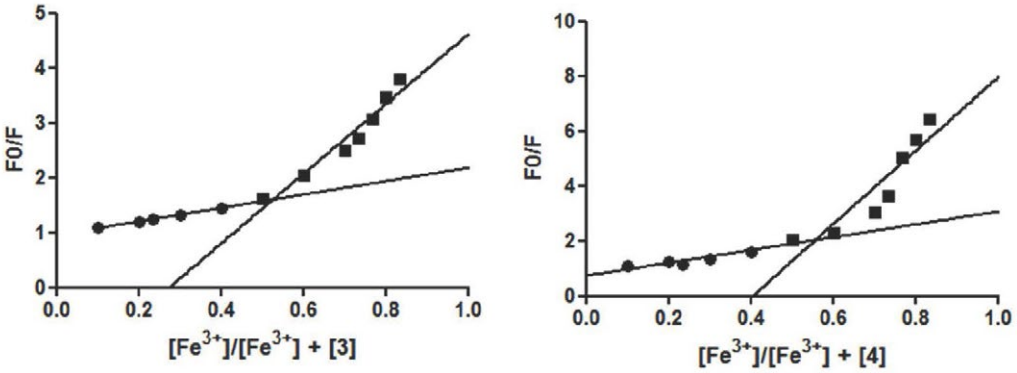


Figure S30. Jobs plot of fluorescence quenching of compounds **3** (left) and **4** (right) by $FeCl_3$. Intersection near 0.5 in both cases suggests stoichiometry of 1:1 between $FeCl_3$ and the BODIPYs.

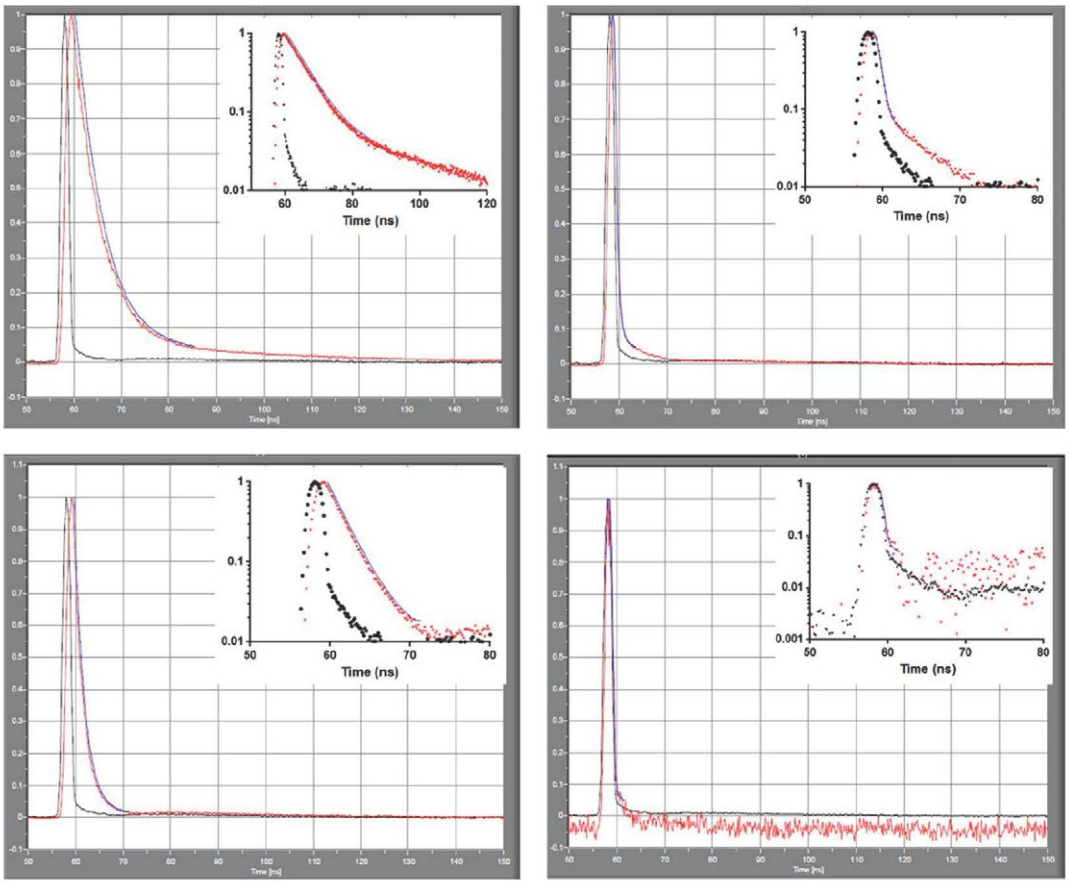


Figure S31. Time-resolved fluorescence of compounds **1** (top left), **2** (top right), **3** (bottom left) and **4** (bottom right). Only approximate fluorescence lifetime were calculated for compounds **2** and **4** due to their weak fluorescence, short-lived excited state and also due to the equipment's detection limit.