Electronic Supplementary Information

Multicomponent Reactions for *de Novo* Synthesis of BODIPY Probes: *in vivo* Imaging of Phagocytic Macrophages

Ana M. Vázquez-Romero, Nicola Kielland, María J. Arévalo, Sara Preciado, Richard J. Mellanby, Yi Feng, Rodolfo Lavilla* and Marc Vendrell*

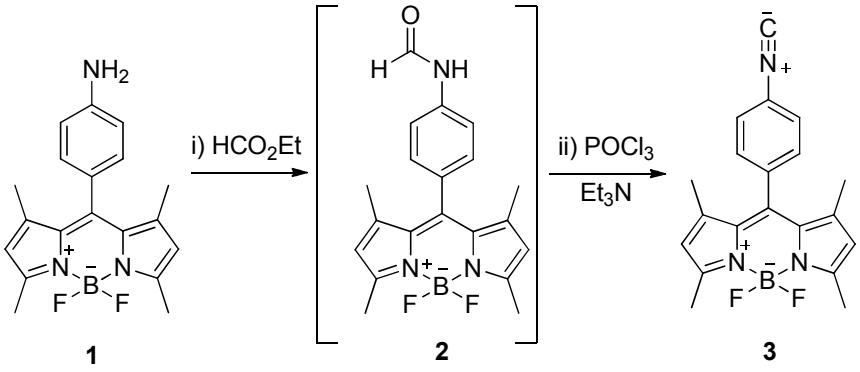
Table of contents:

1. General experimental information

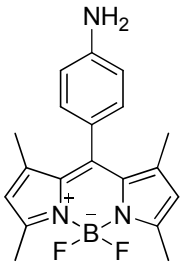
Unless stated otherwise, all reactions were carried out under argon in dried glassware. Commercially available reactants were used without further purification. Thin-layer chromatography was conducted on Merck silica gel 60 F254 sheets and visualized by UV (254 nm and 365 nm). Silica gel (particle size 35–70 μm) was used for flash column chromatography. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded in a Varian Mercury 400 spectrometer (at 400, 100 and 376 MHz, respectively). NMR spectra were recorded in $CDCl_3$ or DMSO-d6 solution with TMS as an internal reference. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ/ppm), multiplicity, coupling constant (Hz) and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift relative to the solvent peak of $CDCl_3$ set at δ = 77.0 ppm. ^{19}F NMR spectra are referenced to TFA (δ: -76.55 ppm). IR spectra were recorded in a Thermo Nicolet Nexus spectrometer and are reported in frequency of absorption (cm^{-1}). HPLC analysis were performed on a Waters Alliance 2695 separations module (Empower software) connected to a Waters PDA2996 photodiode array detection (PDA) system (190-800 nm) and using a *Symmetry* column (C_{18}, 5 μm, 4.6 × 150 mm).

2. Experimental procedures and characterization data.

2.1 Synthesis of isonitrile-BODIPY 3.



4,4-Difluoro-8-(4-aminophenyl)-3,5-dimethyl-4-bora-*3a,4a*-diaza-*s*-indacene (1) [1]



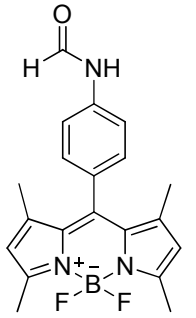
1
$C_{19}H_{20}BF_2N_3$
MW: 339,19

To a solution of 4,4-difluoro-8-(4-nitrophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (411 mg, 1.11 mmol) in 64 mL of degassed EtOH:CH_2Cl_2 (1:1), was added a suspension of Pd/C (41 mg, 10% mol) in EtOH under inert atmosphere. The resulting mixture was stirred at r. t. under H_2 (1 atm) for 12 h. When the reaction was completed, inorganic solids were removed by filtration through Celite® and washed with several portions of CH_2Cl_2. The solvent was removed under pressure affording aniline **1** in quantitative yields (370 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 5.97 (s, 2H), 4.37-3.93 (bs, 2H), 2.54 (s, 6H), 1.49 (s, 6H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.1, 147.1, 143.3, 142.8, 132.2, 129.1, 124.9, 121.1, 115.6, 14.8, 14.7 ppm.

4,4-Difluoro-8-(4-formamidophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (2)



2

$C_{20}H_{20}BF_2N_3O$

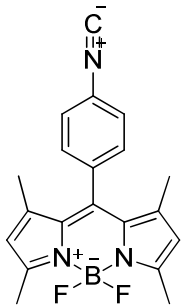
MW: 367,20

A solution of amine **1** (100 mg, 0.29 mmol) in ethyl formate (10 mL, 0.12 mol) was heated in a sealed tube at 60 °C for 4 days. When the reaction was completed, the solvent was removed under reduced pressure. The crude reaction mixture was dissolved in CH_2Cl_2 (15 mL) and washed with NH_4Cl (3 × 7 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane:CH_2Cl_2 5:95) to obtain 91 mg of the corresponding formamide **2** (90% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 – 8.38 (m, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.18 (m, 4H), 6.02 – 5.96 (m, 2H), 2.55 (s, 6H), 1.42 (s, 6H) ppm.

HRMS: calcd. for $C_{20}H_{21}BF_2N_3O$: 368.1746 ($M+H^+$); found 368.1749.

4,4-Difluoro-8-(4-isocyanophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (3)



3

$C_{20}H_{18}BF_2N_3$

MW: 349,18

To a solution of formamide **2** (380 g, 1.05 mmol) in 11 mL of $CHCl_3$ under inert atmosphere, 1 mL (7.37 mmol) of diisopropylamine was added. The resulting mixture was cooled at 0 °C and then $POCl_3$ (240 µL, 2.60 mmol) was added dropwise. The reaction was stirred in the cold for 2 h. Then, 10 mL of 2 M $NaHCO_3$ were added, and the mixture was stirred for a few minutes. The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (SiO_2, hexane:CH_2Cl_2 5:95) afforded 280 mg of isonitrile **3** as an orange solid (78% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 6.00 (s, 2H), 2.56 (s, 6H), 1.38 (s, 6H) ppm.

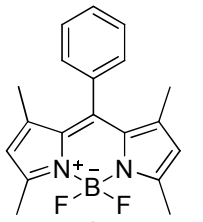
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 156.5, 142.8, 139.2, 136.7, 131.1, 129.8, 129.7, 127.4, 121.9, 121.8, 14.8, 14.8 ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = -146.30 (q, *J* = 32.8 Hz) ppm.

IR (film) υ_{max}: 2962.28, 2923.84, 2853.38, 1546.62, 1508.19, 1463.35, 1412.10, 1303.20, 1187.90, 149.47, 1085.41, 982.92, 809.96, 758.72 cm^{-1}.

HRMS: calcd. for $C_{20}H_{19}BF_2N_3$, 350.1641 ($M+H^+$); found: 350.1642.

5,5-Difluoro-1,3,7,9-tetramethyl-10-phenyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (9) [2]



9

$C_{19}H_{19}BF_2N_2$

MW: 324,18

2,4-Dimethylpyrrole (215 mg, 2.2 mmol) and benzaldehyde (106 mg, 1.1 mmol) were dissolved in CH_2Cl_2 with a catalytic amount of TFA (1-2 drops). The mixture was stirred for 16 h at r.t. Then a solution of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (227 mg, 1 mmol) in CH_2Cl_2 was added dropwise, and the mixture was stirred for 15 min. Finally, BF_3OEt_2 (2 mL, excess) and triethylamine (2 mL, excess) were added, and the mixture was stirred for 3 h at r.t. The crude mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (hexane:CH_2Cl_2 1:1) afforded 235 mg of compound **9** as an orange solid (72% yield).

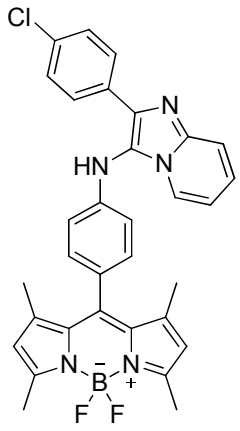
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 – 7.50 (m, 3H), 7.31 (dd, *J* = 6.2, 1.4 Hz, 2H), 6.01 (s, 2H), 2.59 (s, 6H), 1.41 (s, 6H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.5, 143.2, 141.8, 135.0, 129.1, 128.9, 127.9, 121.2, 121.2, 14.6, 14.3 ppm.

MS: calcd. for $C_{19}H_{19}BF_2N_2$, 324.1604; found: 324.1611.

2.2. Multicomponent reactions and characterization of BODIPY adducts.

10-(4-((2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (4).



4

$C_{32}H_{27}BClF_2N_5$

MW: 565,85

To a solution of 2-aminopyridine (41 mg, 0.44 mmol) in anhydrous MeOH (2 mL), were added *p*-chlorobenzaldehyde (41 mg, 0.29 mmol) and isonitrile **3** (100 mg, 0.39 mmol) in one portion. The mixture was purged and then TFA (0.5 mL) was added. The resulting mixture was stirred at 50 °C for 3 days. Then, the reaction was diluted and water (2 mL), and CH_2Cl_2 (9 mL) was added. The organic layer was washed with an aqueous saturated solution of $NaHCO_3$ (3 × 3 mL) and brine (1 × 9 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (CH_2Cl_2:MeOH 99:1) rendered 120 mg of compound **4** as an orange solid (77% yield).

^{1}H NMR (400 MHz $CDCl_3$): δ 7.97 – 7.92 (m, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.68 – 7.62 (m, 1H), 7.32 – 7.23 (m, 3H), 7.07 (d, *J* = 8.0 Hz, 2H), 6.86 (t, *J* = 6.8 Hz, 1H), 6.70 (d, *J* = 8.1 Hz, 2H), 5.97 (s, 2H), 5.89 (s, 1H), 2.53 (s, 6H), 1.46 (s, 6H) ppm.

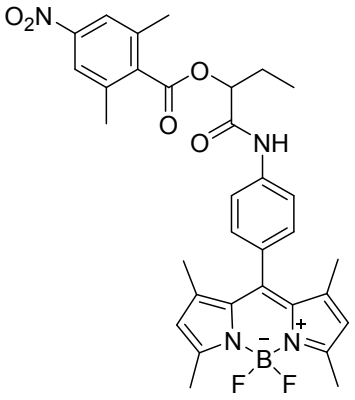
^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 155.4, 145.4, 143.0, 141.8, 134.0, 132.0, 129.8, 129.1, 128.8, 128.4, 126.7, 125.8, 122.6, 121.3, 120.2, 118.0, 117.9, 114.2, 112.9, 14.7, 14.6 ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = -146.23 (q, *J* = 32.4 Hz) ppm.

IR (film) υ_{max}: 3301.78, 3263.35, 2917.44, 2846.98, 1738.79, 1674.73, 1540.21, 1508.19, 1469.75, 1405.69, 1322.42, 1194.31, 1155.87, 1079.00, 976.51, 803.56, 752.31, 707.47 cm^{-1}.

HRMS: calcd. for $C_{32}H_{28}BClF_2N_5$, 566.2095 ($M+H^+$); found: 566.2090.

10-(4-(2-((2,6-dimethyl-4-nitrobenzoyl)oxy)butanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (5).



5

$C_{32}H_{33}BF_2N_4O_5$

MW: 602.4458

A solution of propionaldehyde (9.38 μL, 0.13 mmol), 2,6-dimethyl-4-nitrobenzoic acid (27.71 mg, 0.14 mmol) and isonitrile **3** (50 mg, 0.14 mmol) in CH_2Cl_2 (4 mL) was stirred at r.t. for 2 days. When the reaction was completed, the solvent was removed under pressure and the reaction crude was purified by flash chromatography (hexane:AcOEt 4:1) to obtain 80 mg of compound **5** as an orange solid (85% yield).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.90 (s, 2H), 7.69 (d, 2H), 7.23 (d, *J*= 8 Hz, 2H), 5.97 (s, 2H), 5.45 (t, *J*= 8Hz, 1H), 2.53 (s, 6H), 2.38 (s, 6H), 2.16 (m, 2H), 1.41 (s, 6H), 1.13 (t, *J*= 6 Hz, 3H), ppm.

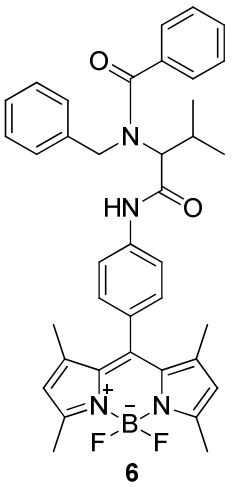
^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.5, 164.4, 155.5, 154.8, 142.9, 140.9, 137.7, 131.5, 130.3, 130.2, 130.1, 128.8, 121.2, 120.3, 76.4, 29.6, 25.1, 17.3, 14.6, 14.5, 9.6, ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = - 146.20 (q, *J* = 36.0 Hz) ppm.

IR (film) υ_{max}: 3385.05, 2962.28, 2923.84, 2853.38, 1732.38, 1693.95, 1604.27, 1546.62, 1501.78, 1463.35, 1309.61, 1187.90, 1155.87, 1085.41, 982.92, 835.59, 758.72, 694.66 cm^{-1}.

HRMS: calcd. for $C_{32}H_{34}BF_2N_4O_5$, 603.2591 ($M+H^+$); found: 603.2600.

10-(4-(2-(*N*-benzylbenzamido)-3-methylbutanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (6).



6

$C_{38}H_{39}BF_2N_4O_2$
MW: 632,55

Benzylamine (20 µL, 180 µmol) was added to a solution of isobutyraldehyde (16 µL, 180 µmol) in CH_2Cl_2 (2 mL) and stirred for 30 min in presence of molecular sieves 5Å (50 mg). The resulting mixture was filtered and dried *in vacuo*. The residue was dissolved in MeOH (2 mL), molecular sieves 5Å (50 mg) were added, followed by the addition of benzoic acid (20 mg, 161 µmol) and isonitrile **3** (50 mg, 143 µmol). After 18 h, the reaction mixture was quenched with a saturated solution of $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (15 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (hexane: methyl *tert*-Butyl ether (MTBE) 7:3) afforded 60 mg of compound **6** as an orange solid (67% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.68 (s, 1H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.51 – 7.44 (m, 2H), 7.41 (m, 3H), 7.22 – 7.15 (m, 2H), 7.13 – 6.92 (m, 5H), 5.99 (s, 2H), 4.70 – 4.56 (m, 2H), 4.25 (d, *J* = 9.8 Hz, 1H), 2.93 – 2.81 (m, 1H), 2.56 (s, 6H), 1.48 (s, 6H), 1.13 (dd, *J* = 11.3, 6.5 Hz, 6H) ppm.

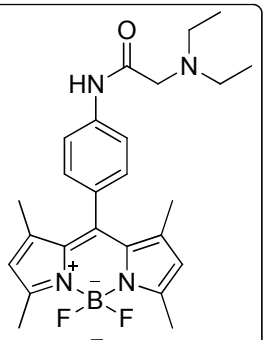
^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 168.7, 155.6, 143.2, 141.7, 139.1, 136.2, 135.9, 131.8, 130.7, 130.4, 128.9, 128.7, 128.5, 127.7, 127.6, 127.5, 121.3, 120.2, 29.9, 26.6, 20.4, 19.6, 14.9, 14.7 ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = -146.35 (q, *J* = 33.4 Hz) ppm.

IR (film) υ_{max}: 3301.78, 3256.94, 3096.80, 3045.55, 2955.87, 2923.84, 2872.60, 1693.695, 1617.08, 1553.02, 1508.19, 1463.35, 1405.69, 1309.61, 1194.31, 1155.87, 1079.00, 976.51, 829.18, 758.72, 694.66 cm^{-1}.

HRMS: calcd. for $C_{38}H_{40}BF_2N_4O_2$, 633.3213 ($M+H^+$); found: 633.3210.

10-(4-(2-(diethylamino)acetamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (7, PhagoGreen).



7

$C_{25}H_{31}BF_2N_4O$

MW: 452,35

Formaldehyde (30% in water, 34 µL, 341 µmol) was added to a stirring solution of isonitrile **3** (20 mg, 57 µmol), formic acid (13 µL, 171 µmol), and diethylamine (18 µL, 171 µmol) in 600 µL of *t*BuOH:$CHCl_3$ (3:2). After 16 h the solvent was removed under pressure, and the residue was carefully dried *in vacuo* to yield 25 mg of adduct **7** as a pure orange solid (99% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.58 (s, 1H), 7.73 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 5.98 (s, 2H), 3.18 (s, 2H), 2.73 – 2.62 (m, 4H), 2.55 (s, 6H), 1.44 (s, 6H), 1.12 (t, *J* = 7.1 Hz, 6H) ppm.

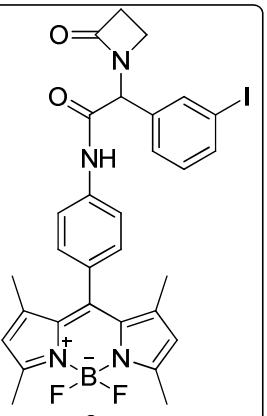
^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 155.6, 143.3, 141.5, 138.6, 131.8, 130.5, 128.9, 128.4, 125.4, 121.3, 119.7, 58.3, 48.9, 14.8, 14.7, 12.6 ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = -146.34 (q, *J* = 32.5 Hz) ppm.

IR (film) υ_{max}: 3250.53, 2975.09, 2923.84, 2840.57, 1738.79, 1681.14, 1521.00, 1508.19, 1456.94, 1405.69, 1303.20, 1187.90, 1149.47, 1091.81, 1046.98, 970.11, 829.18, 765.12 cm^{-1}.

HRMS: calcd. for $C_{25}H_{32}BF_2N_4O$, 453.2638 ($M+H^+$); found, 453.2635.

5,5-Difluoro-10-(4-(2-(3-iodophenyl)-2-(2-oxoazetidin-1-yl)acetamido)phenyl)-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (8).



8

$C_{30}H_{28}BF_2IN_4O_2$

MW: 652,28

β-alanine (8 mg, 96 µmol) and 3-iodobenzaldehyde (22 mg, 96 µmol) were disolved in MeOH (1 ml) and stirred at 50 °C for 1 h. After cooling down to r.t., isonitrile **3** (30 mg, 86 µmol) was added, and the reaction was stirred for 64 h under N_2 atmosphere. The solvent was removed under pressure, and the residue was purified by flash chromatography (hexane:MTBE 7:3) to yield 17 mg of compound **8** as an orange solid (30% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.83 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 7.17 (t, *J* = 7.8 Hz, 1H), 5.97 (s, 2H), 5.45 (s, 1H), 3.72 – 3.66 (m, 1H), 3.38 – 3.30 (m, 1H), 3.16 – 2.96 (m, 2H), 2.54 (s, 6H), 1.41 (s, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 166.5, 155.7, 143.2, 141.2, 138.4, 138.3, 137.1, 136.4, 131.7, 131.3, 131.1, 128.9, 127.3, 121.4, 120.4, 95.1, 61.9, 40.1, 36.3, 14.9, 14.7 ppm.

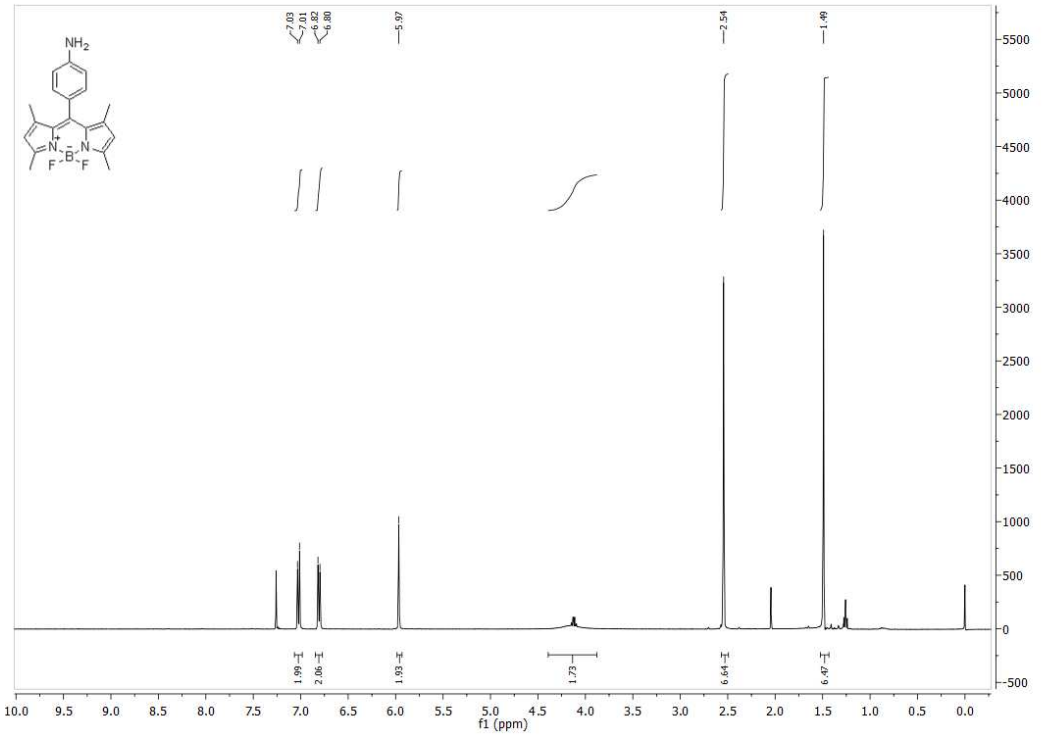
^{19}F-NMR (376 MHz, $CDCl_3$): δ = -146.34 (q, *J* = 33.0 Hz) ppm.

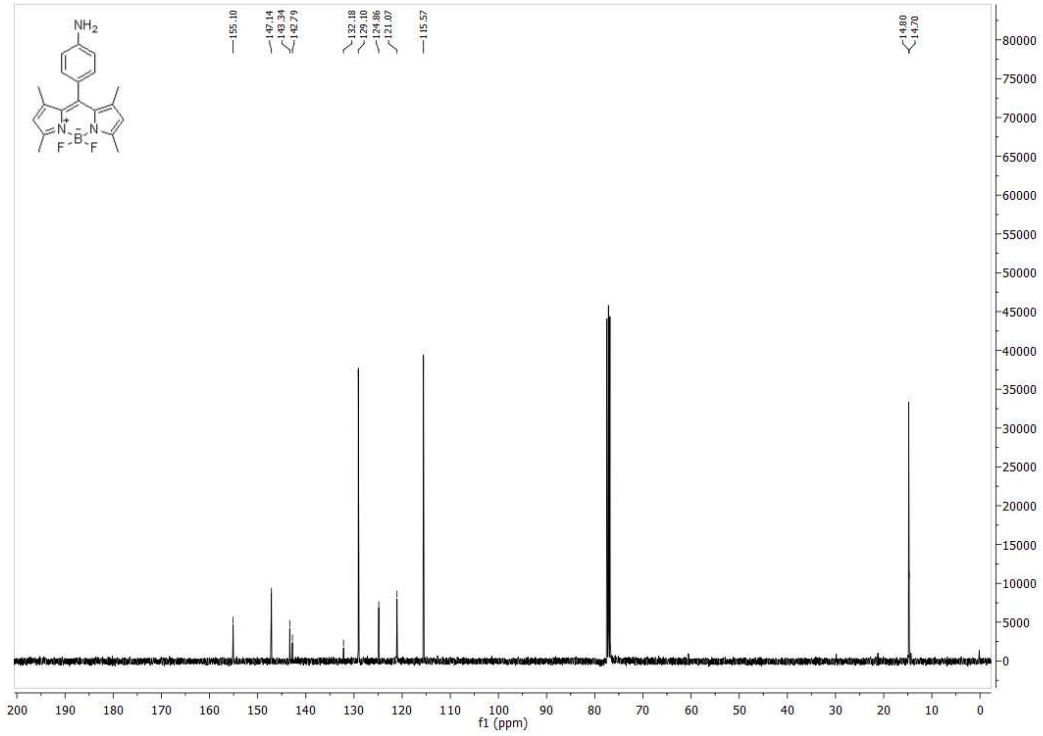
IR (film) υ_{max}: 3308.19, 2962.28, 2917.44, 2853.38, 1725.98, 1687.54, 1546.62, 1501.78, 1463.35, 1405.69, 1303.20, 1187.90, 1143.06, 1072.60, 982.90, 765.12, 694.66 cm^{-1}.

HRMS: calcd. for $C_{30}H_{29}BF_2IN_4O_2$, 653.1397 ($M+H^+$); found: 653.1392.

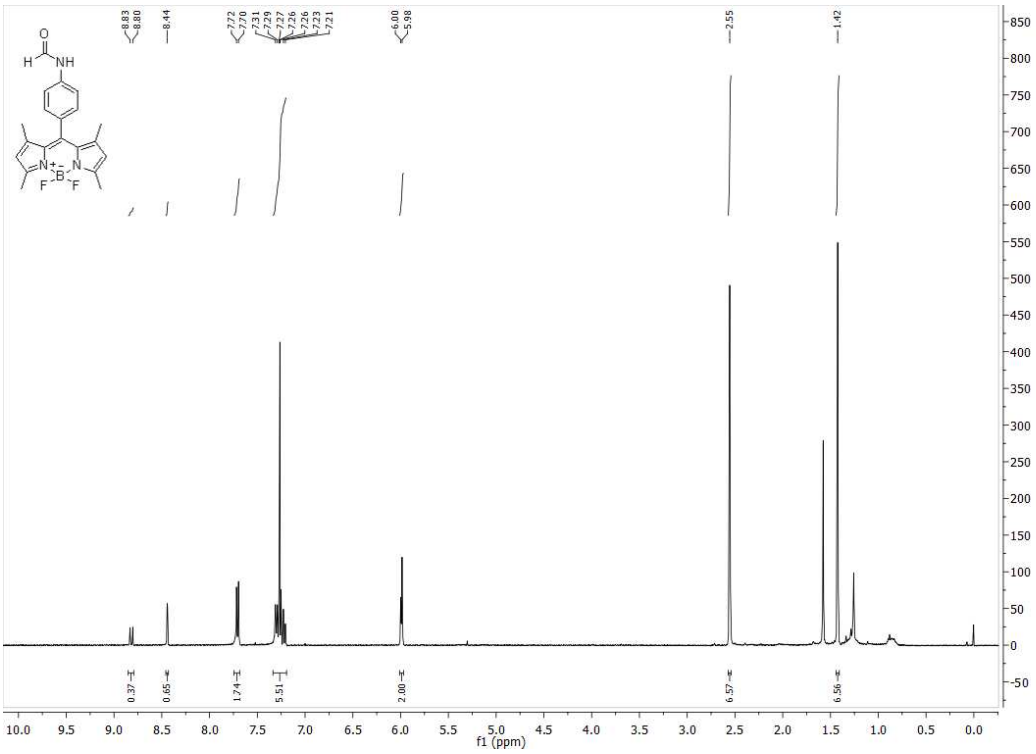
3. NMR data.

4,4-Difluoro-8-(4-aminophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (1).

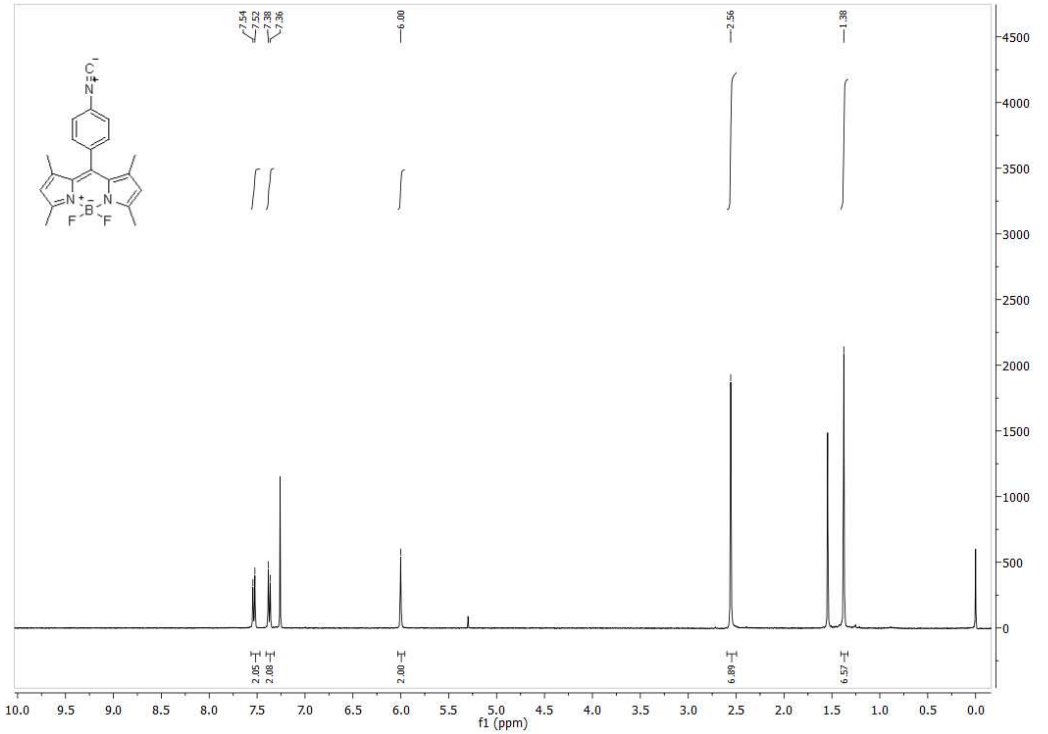


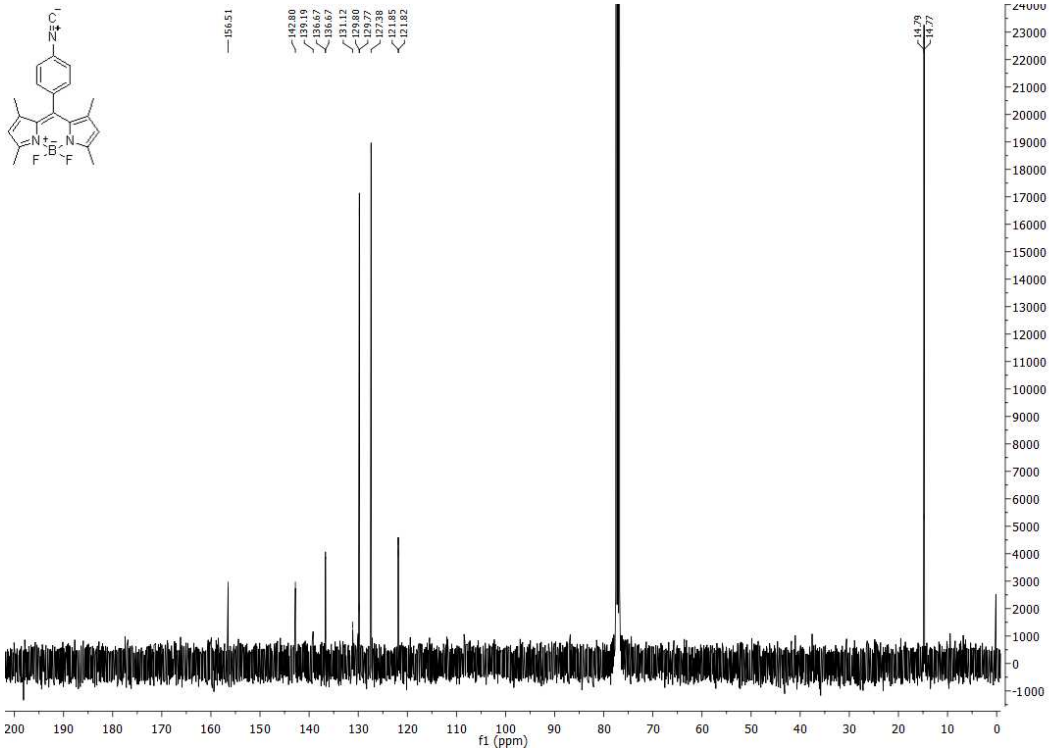


4,4-Difluoro-8-(4-formamidophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (2).

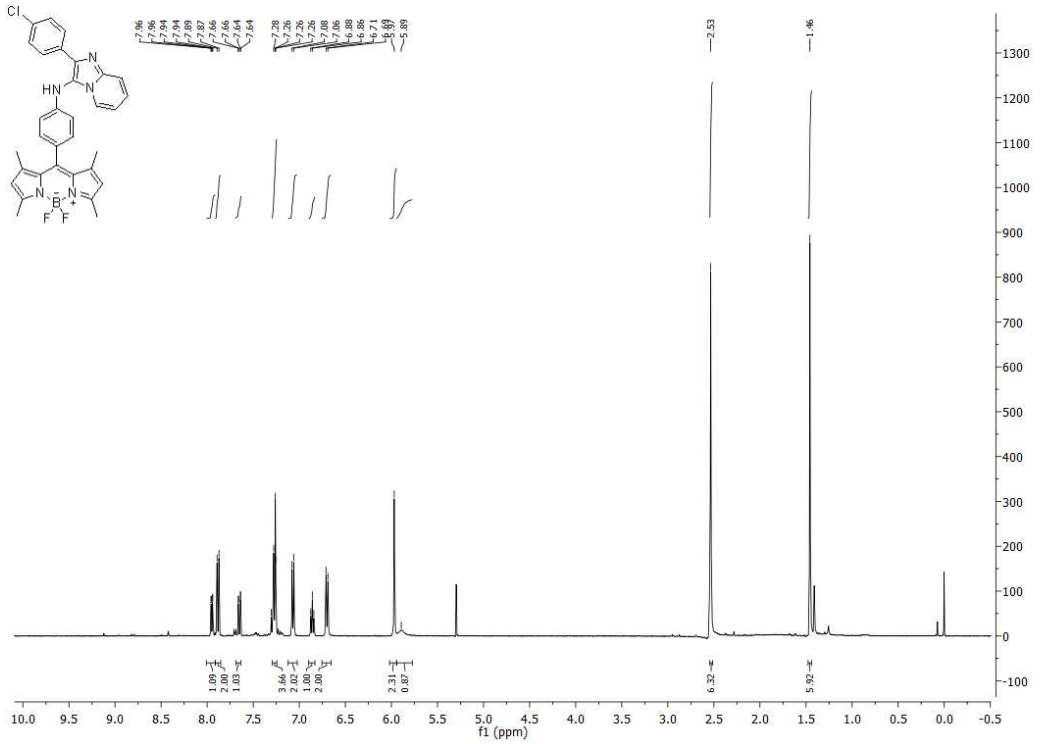


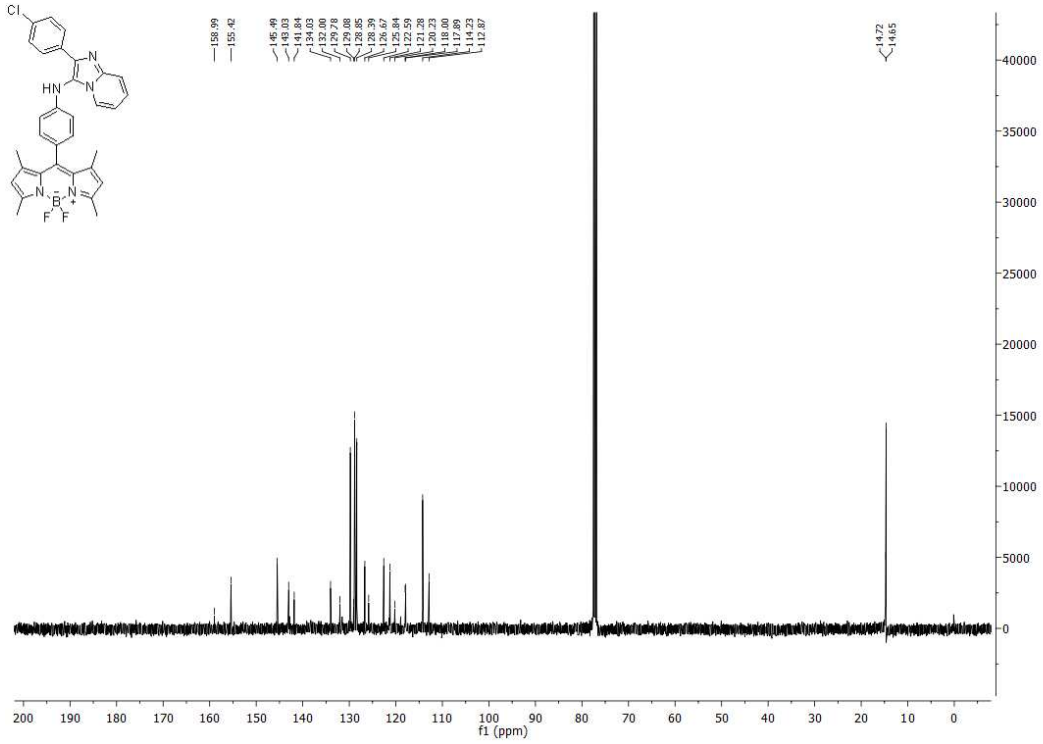
4,4-Difluoro-8-(4-isocyanophenyl)-3,5-dimethyl-4-bora-*3a*,*4a*-diaza-*s*-indacene (3).



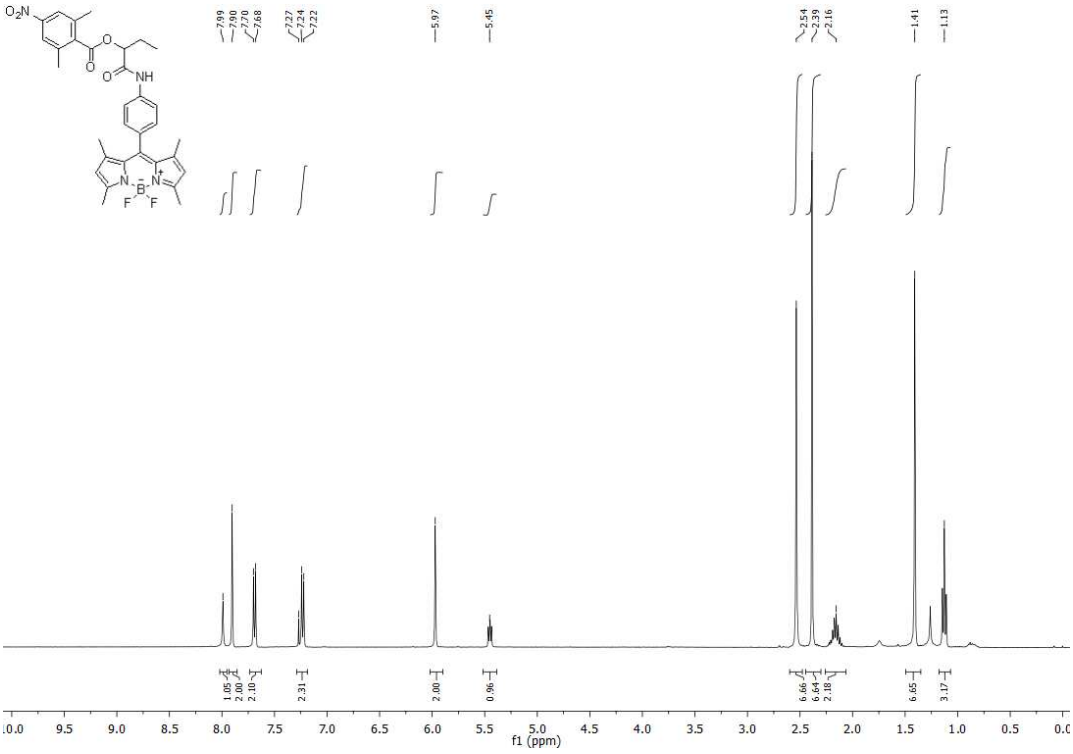


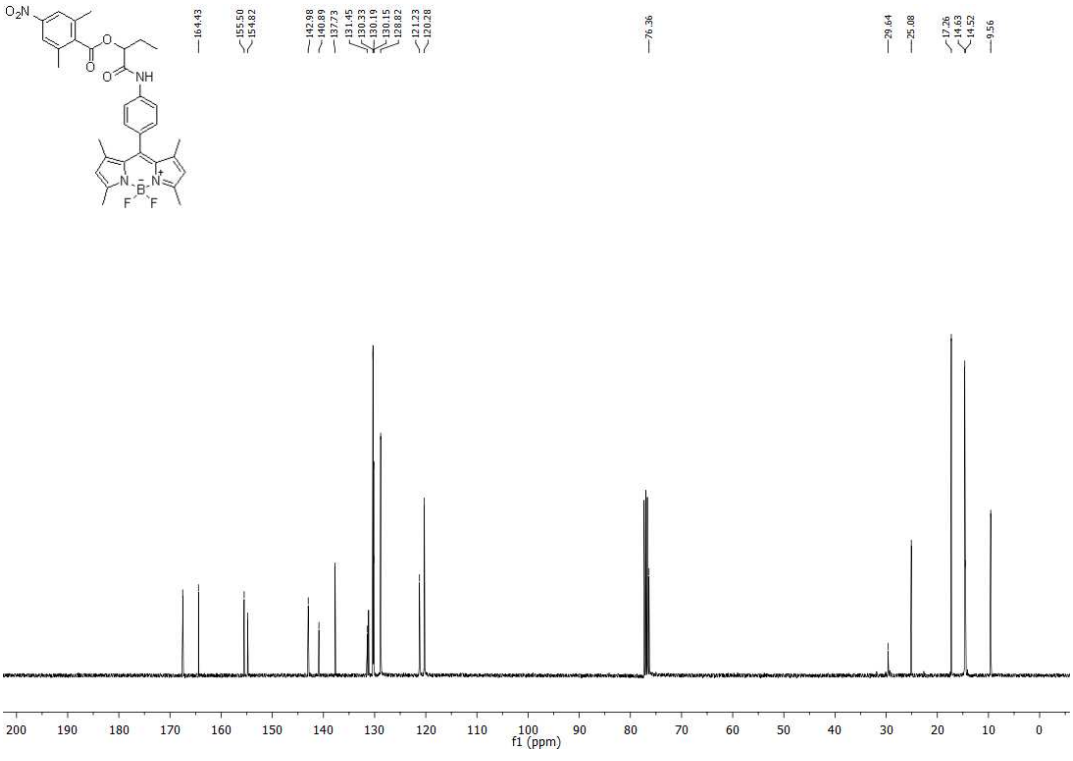
10-(4-((2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (4).



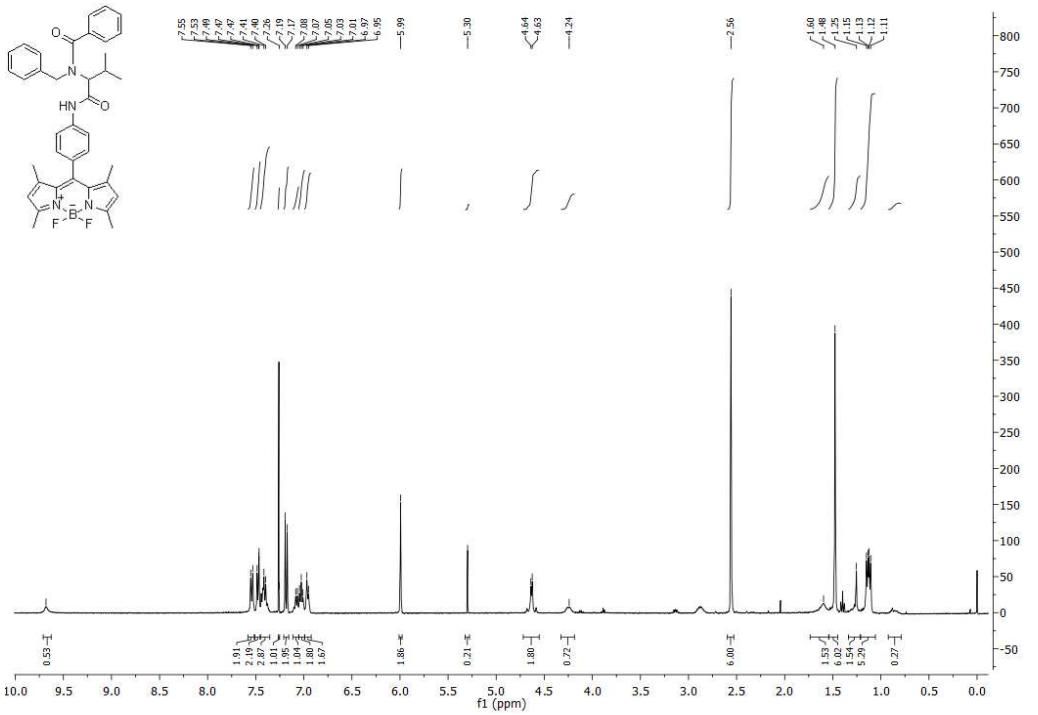


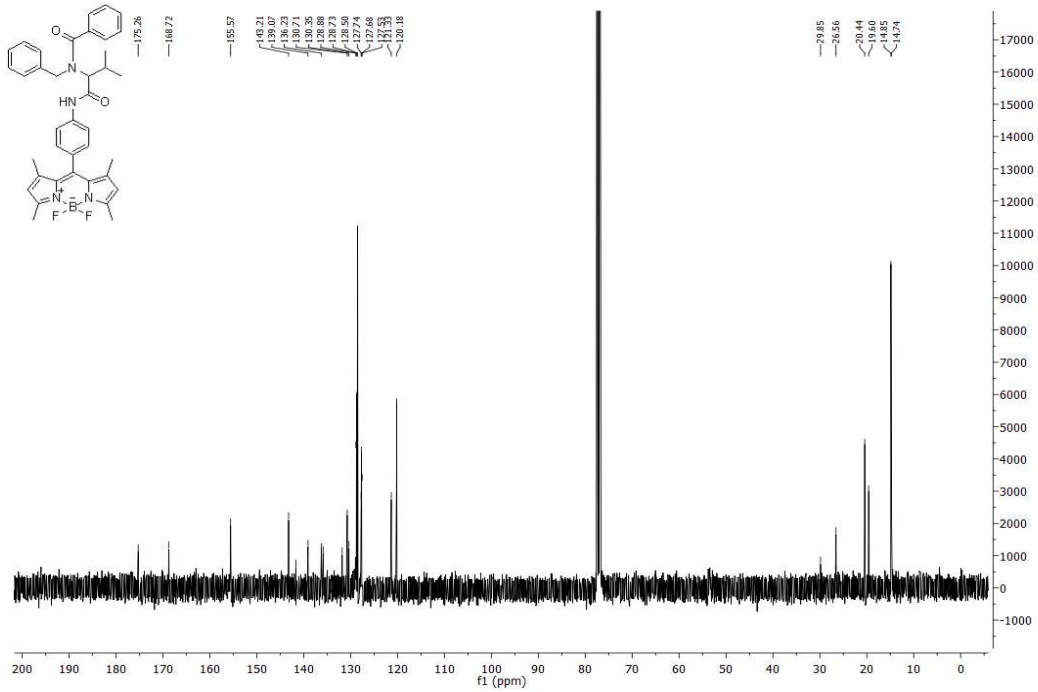
10-(4-(2-((2,6-dimethyl-4-nitrobenzoyl)oxy)butanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (5).



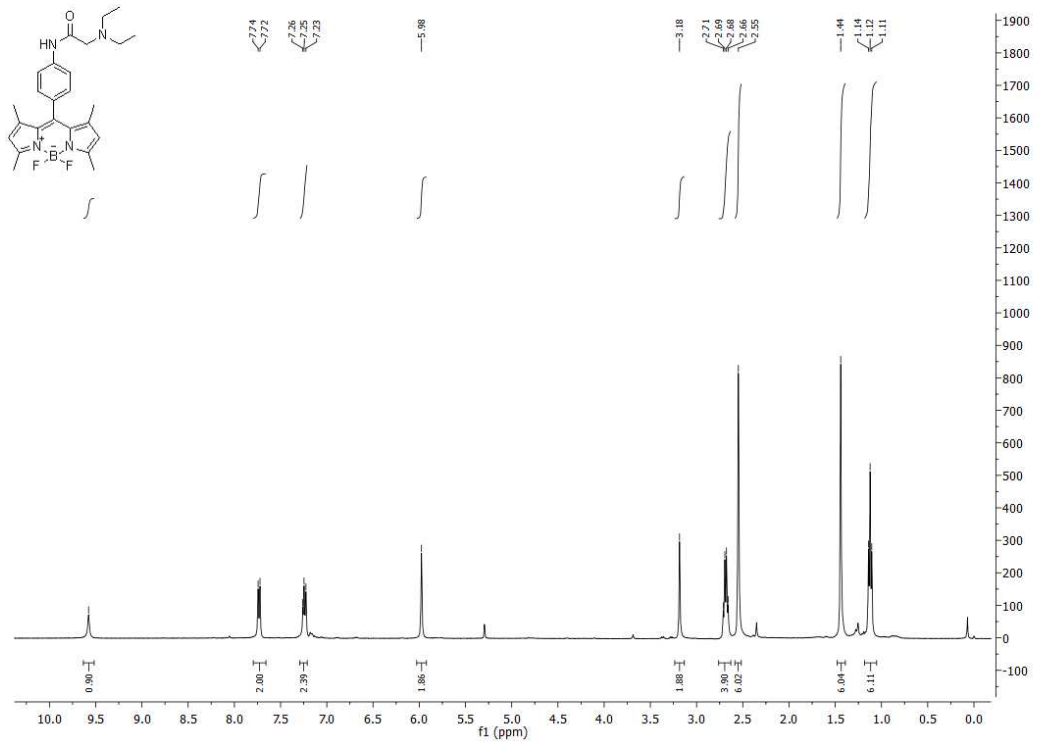


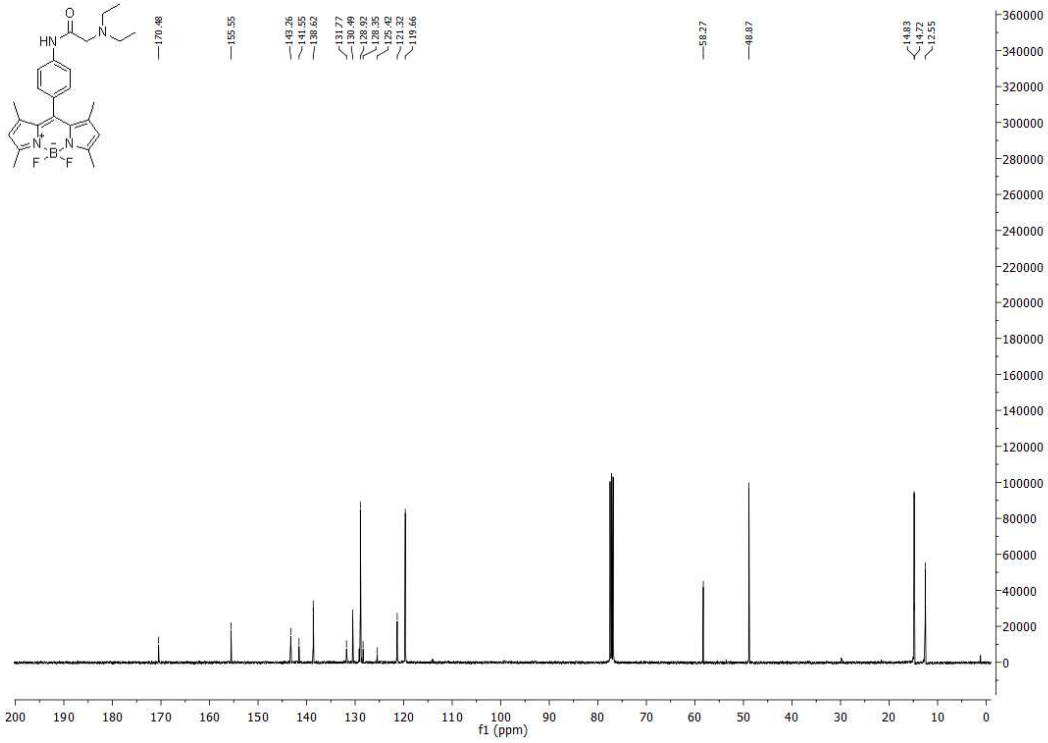
10-(4-(2-(*N*-benzylbenzamido)-3-methylbutanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (6).



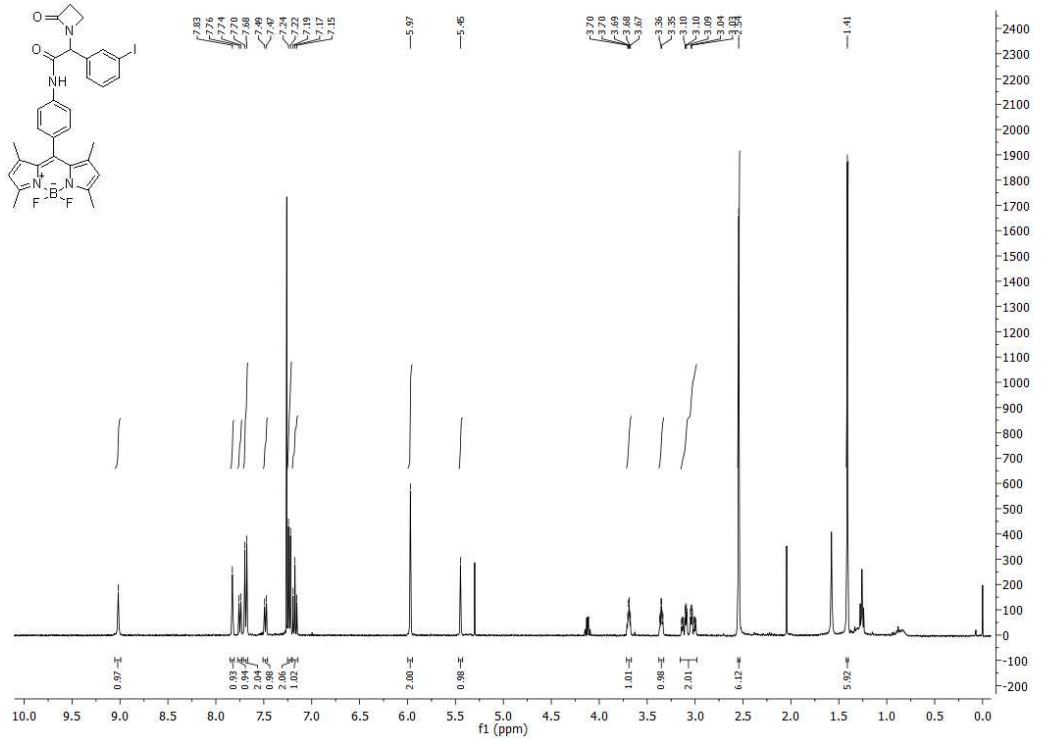


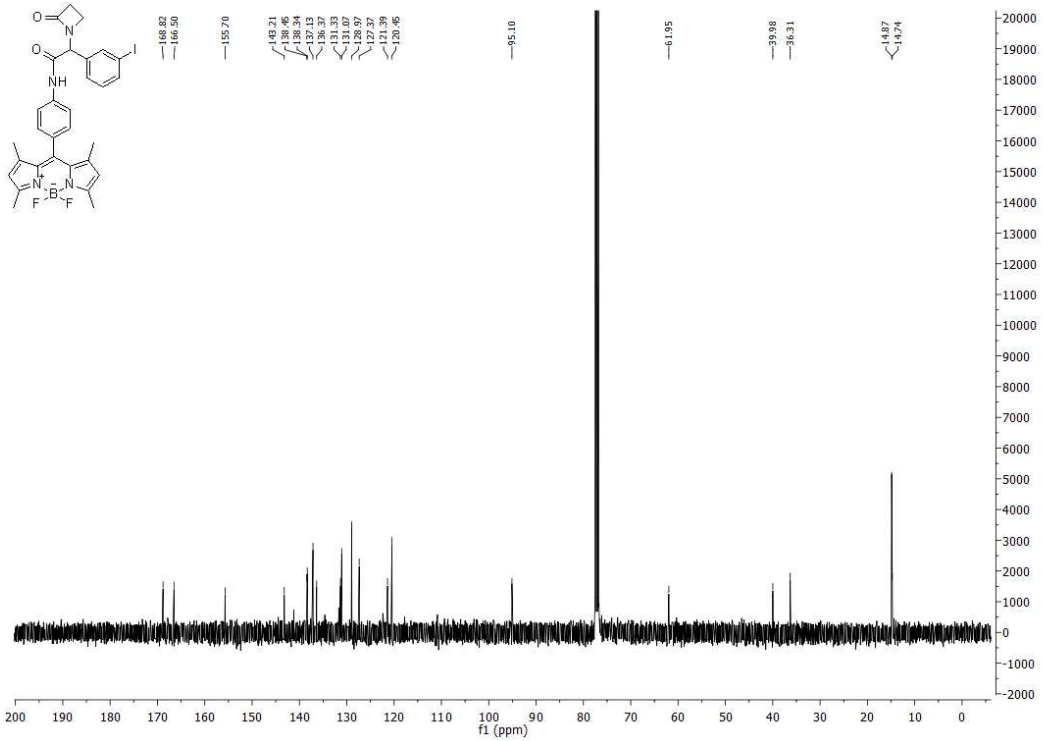
10-(4-(2-(diethylamino)acetamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (7).



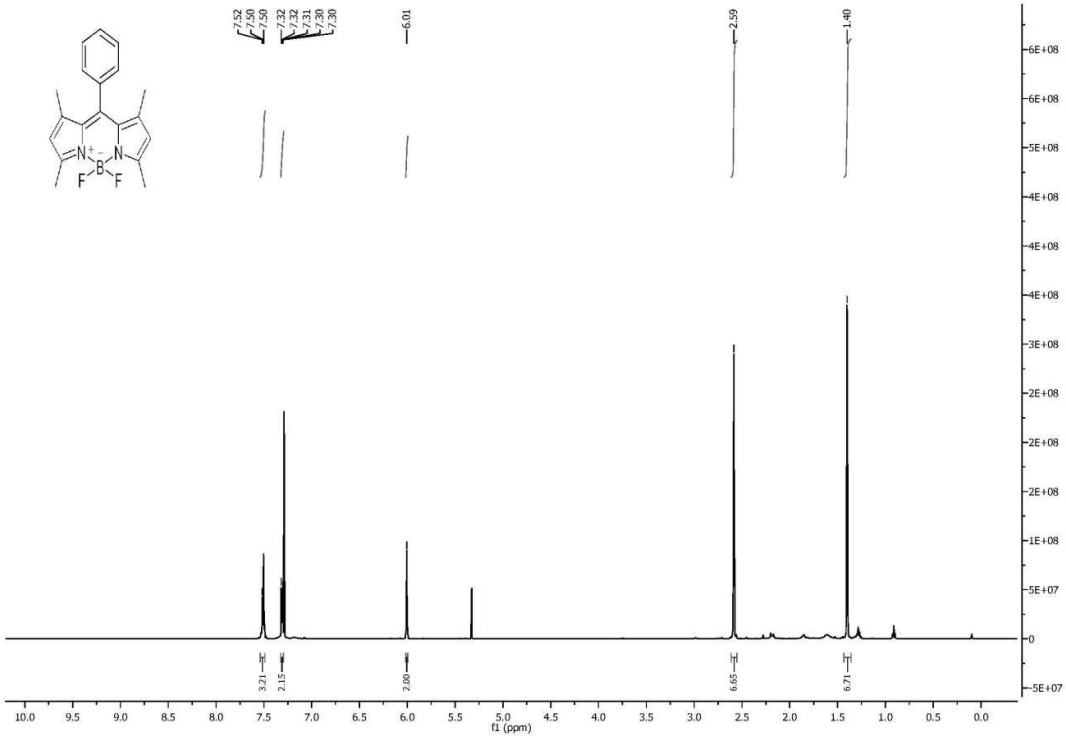


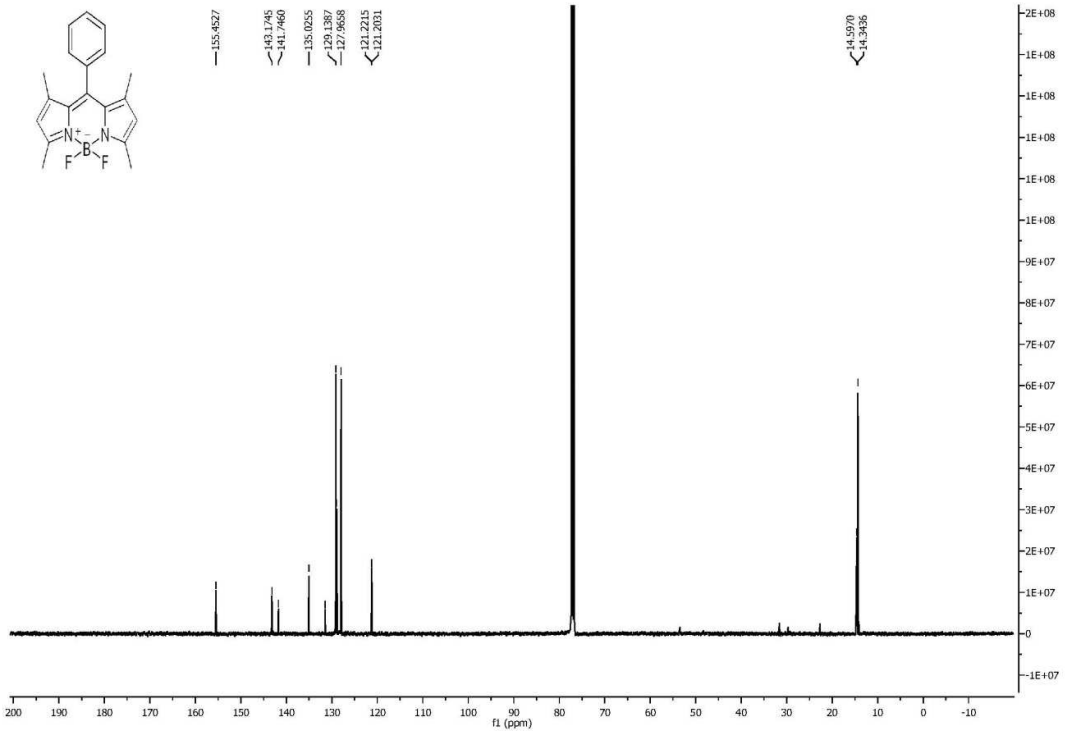
5,5-Difluoro-10-(4-(2-(3-iodophenyl)-2-(2-oxoazetidin-1-yl)acetamido)phenyl)-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (8).





5,5-Difluoro-1,3,7,9-tetramethyl-10-phenyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (9).





4. Absorbance and fluorescence spectra of BODIPY compounds (3-9).

Spectroscopic and quantum yield data were measured on a Synergy HT spectrophotometer (Biotek). Data analysis was performed using GraphPad Prism 5.0. Compounds **3**-**9** were dissolved in DMSO at 50 μM and spectra were recorded at room temperature.

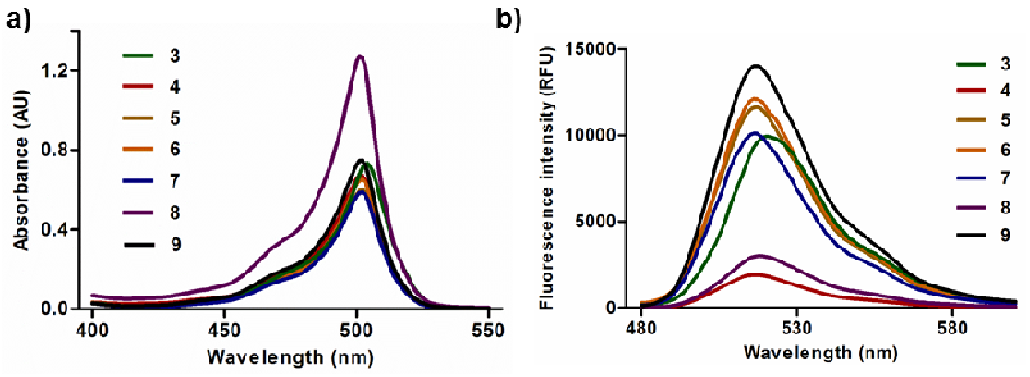


Figure S1. Spectral characterization of compounds 3-9. a) Absorbance and b) emission spectra (λexc.: 450 nm) of BODIPY compounds **3**-**9**.

Quantum yields were calculated by measuring the integrated emission area of the fluorescence spectra and comparing it to the area measured for fluorescein in 0.1 M NaOH (10 μM) when excited at 450 nm (QY: 0.92).[3] Quantum yields were calculated using the equation:

$$\Phi_{fluorescence}^{sample} = \Phi_{fluorescence}^{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{\eta^{sample}}{\eta^{reference}}\right)\left(\frac{Abs^{reference}}{Abs^{sample}}\right)$$

where *F* represents the area of fluorescent emission, *η* is the refractive index of the solvent, and *Abs* is absorbance at the excitation wavelength selected (i.e. 450 nm). Emission was integrated between 480 and 600 nm.

Table S1. Spectral properties for compounds 3-9 in DMSO.

compound	λabs	λem	QY
3	504	520	0.49
4	501	516	0.07
5	502	517	0.61
6	502	516	0.60
7	502	516	0.56
8	501	518	0.05
9	502	517	0.55

5. Co-localization experiments of compounds 3-8 and intracellular trackers.

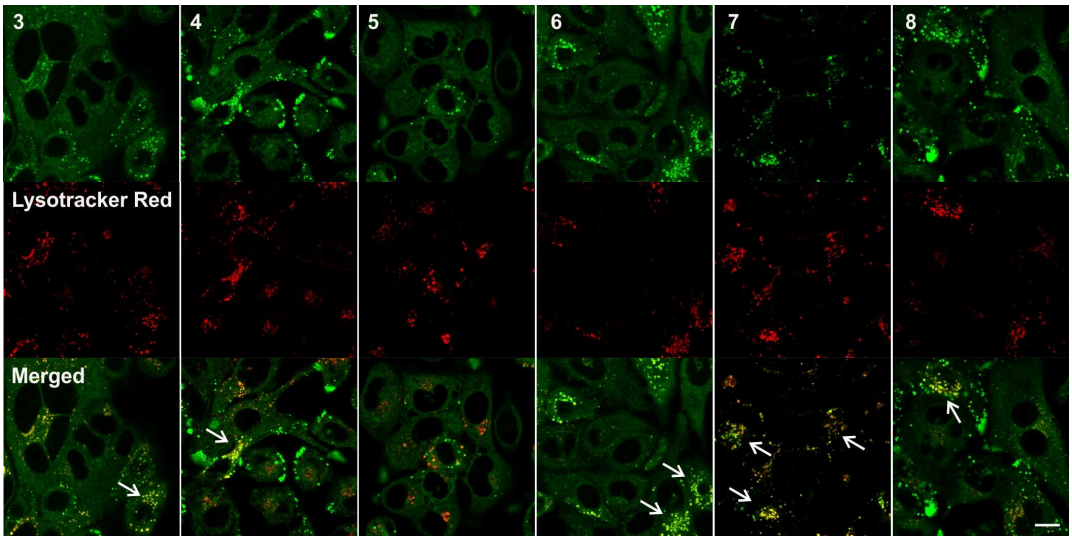


Figure S2. Co-localization of 3-8 and LysoTracker Red. A549 cells were incubated with compounds **3-8** (250 nM) and Lysotracker Red (100 nM) for 30 min and imaged under fluorescence microscopy. White arrows point at co-localization between adducts **3-8** and LysoTracker Red. Scale bar: 20 μm.

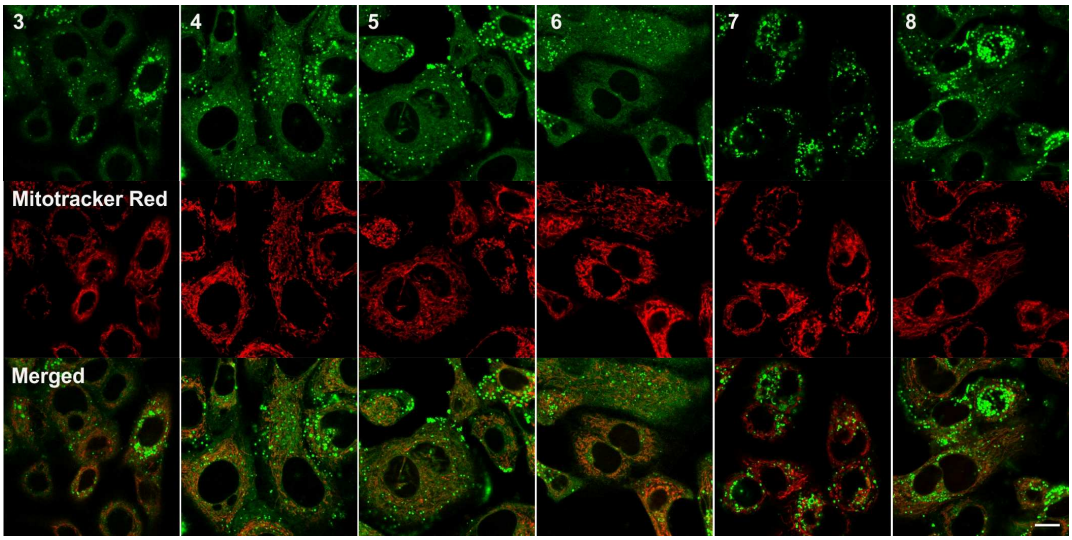


Figure S3. Co-localization of 3-8 and MitoTracker Red. A549 cells were incubated with compounds **3-8** (250 nM) and Mitotracker Red (100 nM) for 30 min and imaged under fluorescence microscopy. Scale bar: 20 μm.

6. Co-localization experiments of compound 7 and LysoTracker Red.

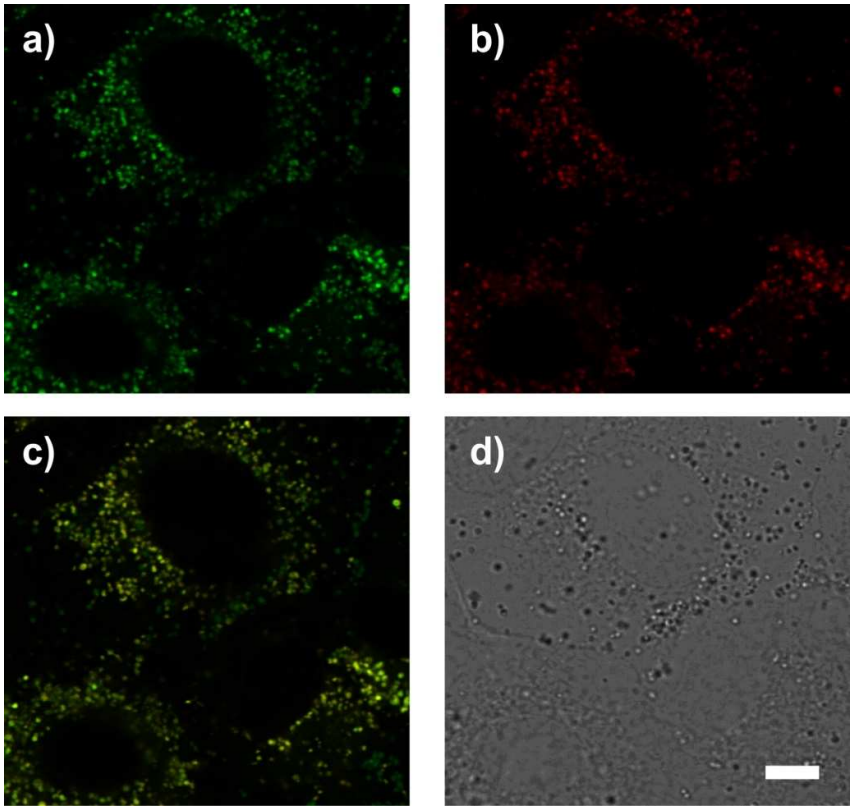


Figure S4. Co-localization of 7 and LysoTracker Red. A549 cells were incubated with **7** (100 nM) and Lysotracker Red (100 nM) for 30 min and imaged under fluorescence microscopy. a) Fluorescence staining of **7**, b) fluorescence staining of LysoTracker Red, c) merged a and b images, d) bright field image. Scale bar: 5 μm.

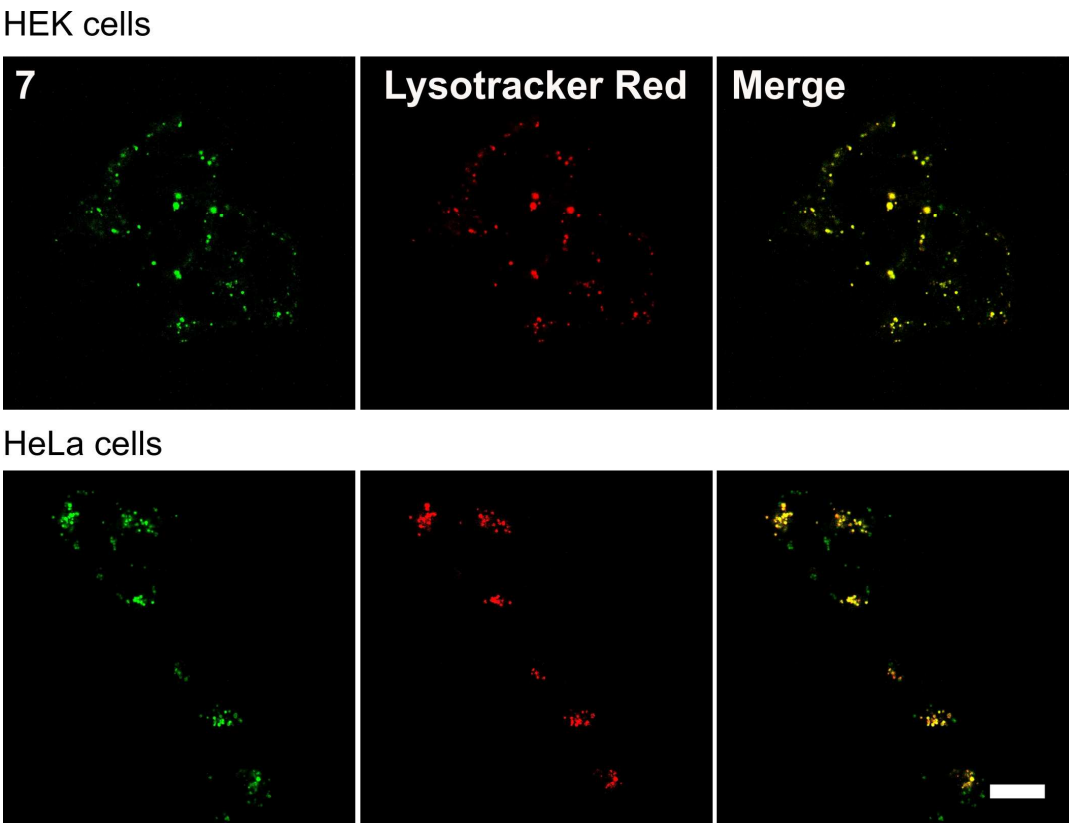
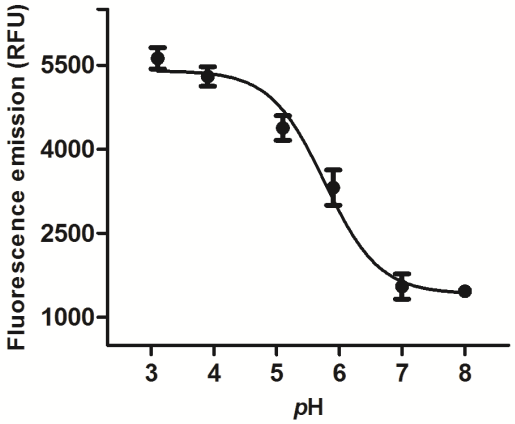


Figure S5. Co-localization of 7 and LysoTracker Red. HEK and HeLa cells were incubated with **7** (100 nM) and Lysotracker Red (100 nM) for 30 min and imaged under fluorescence microscopy. *top*) Fluorescence staining in HEK293 cells; *bottom*) Fluorescence staining in HeLa cells. Scale bar: 20 μm.

7. *p*Ka determination.

Compound **7** and LysoTracker Red (10 μM) were dissolved in buffered solutions with *p*H values between 3 and 8. Fluorescence intensities (**7**: λexc.: 440 nm, λem.: 510 nm; LysoTracker Red: λexc.: 530 nm, λem.: 595 nm) were measured in a Synergy HT spectrophotometer (Biotek) and fitted to a non-linear regression. Data analysis was performed using GraphPrism 5.0.



*p*H	QY
3	0.42±0.02
4	0.39±0.02
5	0.34±0.01
6	0.27±0.03
7	0.16±0.01
8	0.10±0.01

Figure S6. *p*Ka determination of compound 7. Values are represented as means (*n*=4) and error bars as SD. *p*Ka: 5.76 ± 0.07, R^2: 0.97. Quantum yields were determined using fluorescein in 0.1 M NaOH as standard (QY: 0.92).[3]

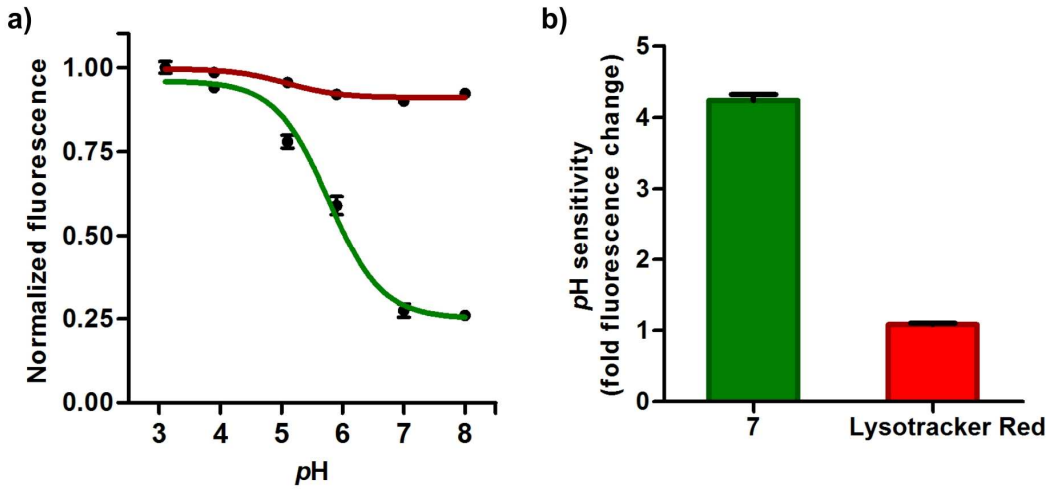


Figure S7. Comparative *p*H dependence of compound 7 and Lysotracker Red. a) Normalized fluorescence emission of **7** and Lysotracker Red at different *p*H values; b) *p*H sensitivity (as fold in fluorescence emission between low *p*H and high *p*H) of compound **7** and Lysotracker Red. Values are represented as means (*n*=4) and error bars as SD.

8. Time dependent studies of zymosan treatment in RAW264.7 cells.

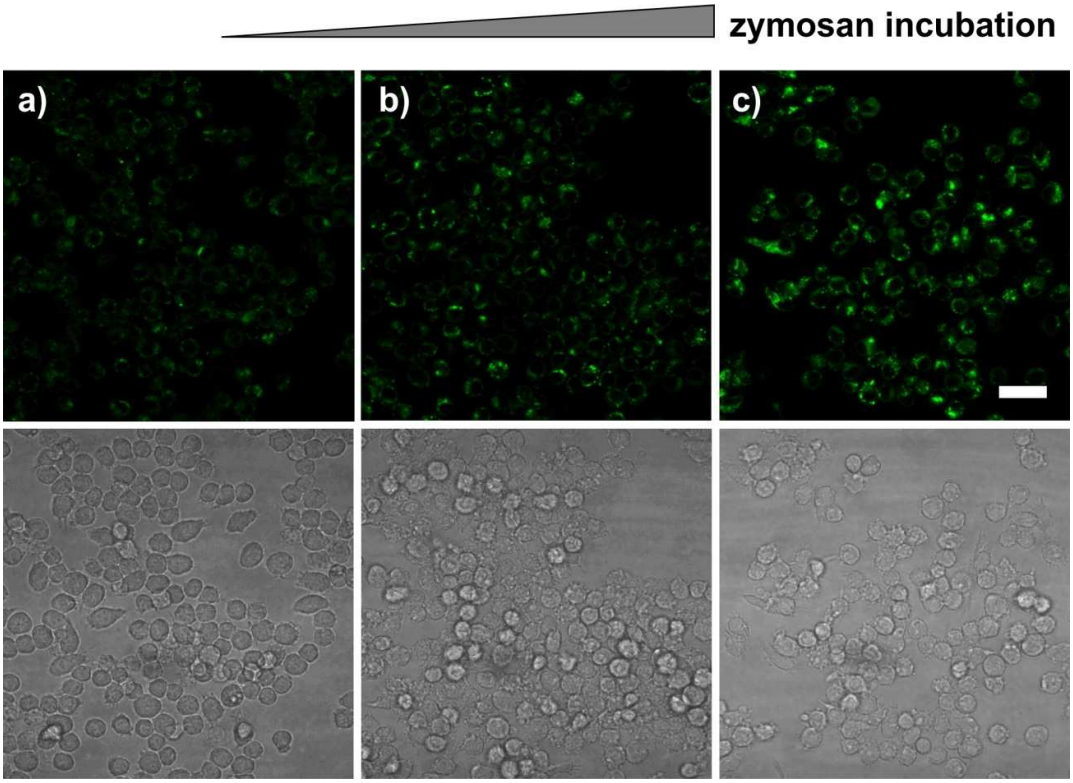


Figure S8. Compound 7 progressively stains phagosomal acidification in zymosan-activated macrophages. RAW264.7 macrophages were treated without (a) and with zymosan (0.5 mg/mL) for 15 min (b) or 60 min (c). All cells were washed and incubated with **7** (100 nM) for 15 min. The staining of **7** is proportional to the length of the treatment with zymosan and proves that its fluorescence emission correlates with the degree of activation of macrophages. Scale bar: 50 μm.

9. LysoTracker Red imaging in RAW264.7 cells.

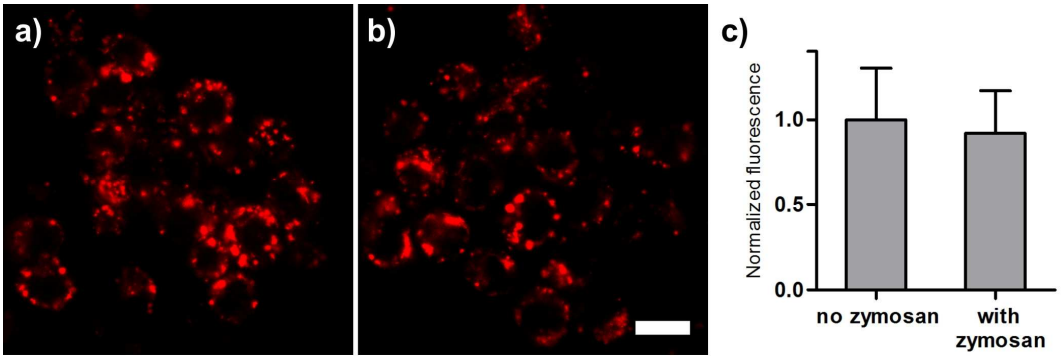


Figure S9. RAW264.7 cells (pre-incubated or not with zymosan 0.5 mg/mL) were treated with LysoTracker Red (100 nM) for 15 min and imaged by confocal microscopy. Fluorescence staining of LysoTracker Red in: a) non-activated macrophages, b) zymosan-activated macrophages, c) quantification of fluorescence emission represented as means (*n*=3) and error bars as SD, no significance observed at $p < 0.05$. Scale bar: 20 μm.

10. Cell viability assays in RAW264.7 cells.

Cell viability was determined with a TACS® MTT Cell Proliferation assay (Trevigen) according to the manufacturer's instructions. Briefly, RAW264.7 cells were plated on 96-well plates the day before the experiment, reaching 90-95% confluence on the day of the experiment. Compound **7** was added to the cells at different concentrations (0, 250, 500 and 1000 nM) and incubated at 37 °C for 4 h. After 4 h, cells were washed, treated according to the manufacturer's instructions and their absorbance values (570 nm) were measured in a Synergy HT spectrophotometer (Biotek). Data analysis was performed using GraphPrism 5.0. Cell viability data was normalized to the proliferation of RAW264.7 cells without addition of compound **7**.

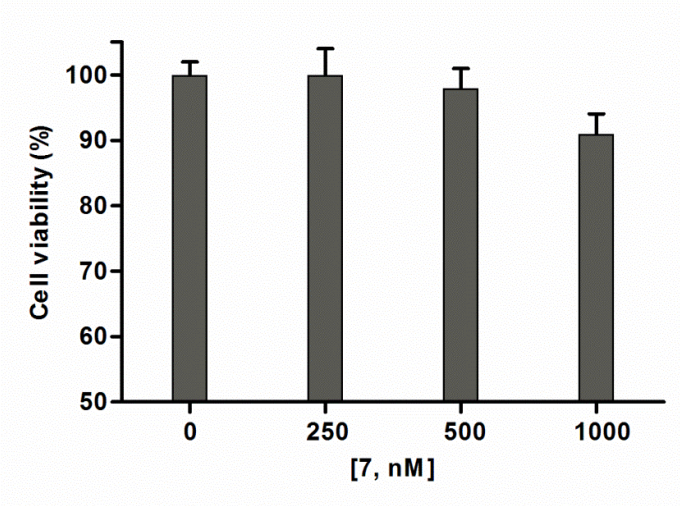


Figure S10. Cell proliferation assays. Viability of RAW264.7 cells after incubation with different concentrations of compound **7**. Values are represented as means (*n=4*) and error bars as SD.

11. Procedures for cell culture, imaging and cytokine release analysis.

A549 and RAW264.7 cell lines were grown using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), antibiotics (100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin) and 2 mM L-glutamine in a humidified atmosphere at 37 $^{\circ}C$ with 5% CO_2. Cells were regularly passaged in T-75 cell culture flasks, and plated on glass chamber slides Lab-Tek™ II (Nunc) the day before imaging, reaching 75% to 90% confluence on the day of the experiment. Cells were incubated at 37 $^{\circ}C$ with fluorescent probes at the concentration and time indicated, gently washed with PBS, and imaged in phenol red-free DMEM under Leica SP5-AOBS or Zeiss LSM 510 Meta fluorescent confocal microscopes equipped with live cell imaging stages. Cell images were acquired using 40X or 63X oil objectives. Fluorescent probes were excited with suitable lasers: 405 nm (DAPI), 488 nm (compounds **3**-**8**), 543 nm (LysoTracker Red) and 594 nm (mCherry). Images were acquired with the corresponding microscope software (LAS AF, LSM 510 META), and processed and analyzed with ImageJ.

For cytokine release assays, RAW264.7 cells were grown to 75-90% confluence and then incubated with either vehicle or **PhagoGreen** at the stated concentrations for 30 min. The adherent cells were then washed twice with PBS before a 18 h stimulation with or without LPS (100 ng/mL) in RPMI media. TNF-α and IL-6 concentrations were measured in the supernatants by ELISA (ebioscience) following manufacturer's instructions.

12. Procedures and videos for *in vivo* zebrafish imaging.

For zebrafish *in vivo* imaging studies, 3 day post fertilization (dpf) cfms:mCherry+ larvae were pre-treated with **PhagoGreen** (600 nM) for 30 min. Larvae were rinsed in clean Daneau's solution for 10 min, then mounted on their sides in 1.5% low melting Agarose in a glass bottomed dish, filled with 0.3% Daneau's solution containing 0.01 mg mL^{-1} tricaine. Images were collected using a Leica SP5-AOBS Confocal Laser Scanning Microscope with a 63X water immersion lens. Video S1 was taken at 60s/frame and was exported from Volocity 6.1.1 as Quicktime movie using the Sornson3 video compressor at 6 frame/s. Video 2 was taken at 19s/frame with 2.5 X Zoom and processed using ImageJ.

Video S1 (Supporting movie for Figures 4a and b). Time-lapse movie of the flank region of a 3 dpf Tg(cfms:mCherry) larva incubated with **PhagoGreen**. The movies show the active engulfing behaviour of macrophages. *Left panel*: yellow arrows point at macrophages (strong red fluorescence) containing mature phagosome (strong green fluorescence), and red arrows point at pigment cells (weak red fluorescent). *Right panel*: Green fluorescence channel to highlight the strong signal of **PhagoGreen** in macrophage phagosomes (yellow arrows). Scale bar: 20 μm.

Video S2 (Supporting movie for Figure 4c). Time-lapse movie of a single macrophage in the flank region of a 3 dpf Tg(cfms:mCherry) larva after incubation with **PhagoGreen**. The movie shows the strong green signal of **PhagoGreen** only in mature phagosomes (i.e. phagosomes upon acidification) compared to the non-stained newly formed phagosomes (yellow arrows). Scale bar: 20 μm.

13. References

(1) Matsumoto, T.; Urano, Y.; Shoda, T.; Kojima, H.; Nagano, T. *Org. Lett*. **2007**, *9,* 3375.

(2) Gabe, Y.; Urano, Y.; Kikuchi, K.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2004,** *126,* 3357.

(3) Magde, D.; Wong, R.; Seybold, P. G. *Photochem. Photobiol.* **2002**, *75*, 327.