Supporting Information

Red-Emitting Fluorogenic BODIPY-Tetrazine Probes for Biological Imaging

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General Procedures

All reactions were performed using flame-dried round-bottomed flasks or reaction vessels unless otherwise stated. Where appropriate, reactions were carried out under an inert atmosphere of nitrogen with dry solvents, unless otherwise stated. Yields refer to chromatographically, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography carried out on 0.20-0.25 mm Qingdao Marine Chemical Plant silica gel plates (GF254) using ultraviolet light as visualizing agent and an acidic mixture of p-anisaldehyde or a basic mixture of potassium permanganate and heat as developing agents. NMR spectra were recorded on a Bruker 600 MHz, Bruker 400 MHz or JEOL 400 MHz spectrometer. High resolution mass spectra (HRMS) dates were recorded on Bruker Apex IV FTMS with ESI source and Bruker Maxis with EI source. Emission spectra were recorded on a Perkin Elmer LS-55 spectrometer.

Synthesis and characterization

Synthesis of compound 2

The synthesis of compound 2 was accomplished according to the reported procedure [1].

Synthesis of compound 3

In a 100 mL round-bottomed flask, 4-Aminobenzonitrile (1.0 g, 8.5 mmol) was dissolved in ethanol (15 mL), followed by addition of benzonitrile (2.6 mL, 25.4 mmol) and S8 (1.4 g, 42.3 mmol), then hydrazine hydrate (8.5 g, 169.2 mmol) was added in drops into the solution. The resulting solution was refluxed at 80 °C and stirred for 12 h. (Caution! This step generates a large amount of toxic nitrogenoxide gasses and should be performed in a well ventilated fume hood). After completion, the mixture was cooled. The solvent was concentrated in vacuo. The crude residue was purified via flash chromatography on silica gel (PE: EA = 3: 1) to give intermediates product as a pale brown solid. Then the solid was re-dissolved in acetone and exposed in air stir for 72 h. TLC analysis showed consumption of starting material. The solvent was concentrated in vacuo. The crude residue was purified via flash chromatography on silica gel (PE: EA = 15: 1) to give 3 (220.0 mg, 10.5%) as a red solid.

1H NMR (600 MHz, CDCl3) δ 8.59-8.61 (m, 2H), 8.46-8.48 (d, J = 8.5 Hz, 2H), 7.59-7.60 (d, J = 6.5 Hz, 3H), 6.82-6.84 (d, J = 8.5 Hz, 2H), 4.17 (s, 2H).

13C NMR (151 MHz, CDCl3) δ 163.7, 163.2, 150.8, 132.2, 132.1, 129.9, 129.2, 127.5, 121.4, 115.0.

HRMS (ESI) m/z: Calculated for C14H12N5 [M+H]+ 250.1087, found 250.1088.

Synthesis of compound 4
Under a \( \text{N}_2 \) atmosphere, 2 (11 mg, 0.04 mmol) and palladium acetate (0.5 mg, 0.002 mmol) was added. Then BINAP (2 mg, 0.003 mmol) was added, followed by cesium carbonate (18 mg, 0.056 mmol) and 3 (12 mg, 0.048 mmol) was added. The reaction was dissolved in anhydrous toluene (2 mL). The solution was stirred at 65 °C for 30 minutes. After which consumption of starting material was confirmed by TLC, the solution was concentrated in vacuo. The crude product was purified with thin layer chromatography on silica gel (PE: DCM = 1:1.5) to give 4 (16 mg, 52%) as an orange solid.

**1H NMR** (600 MHz, CDCl\(_3\)) \( \delta \) 8.64-8.67 (m, 4H), 7.94 (s, 1H), 7.62-7.63 (m, 3H), 7.41-7.42 (d, \( J = 8.8 \) Hz, 2H), 7.27-7.28 (d, \( J = 4.7 \) Hz, 1H), 6.52-6.53 (d, \( J = 4.6 \) Hz, 1H), 6.04 (s, 1H), 2.53 (s, 3H), 2.51 (s, 3H).

**13C NMR** (151 MHz, CDCl\(_3\)) \( \delta \) 163.7, 163.4, 152.8, 150.2, 143.2, 138.1, 135.3, 132.5, 131.9, 131.0, 129.6, 129.4, 128.8, 128.3, 127.9, 126.5, 119.6, 106.0, 29.7, 16.2, 16.1.

**HRMS** (ESI) m/z: Calculated for \( \text{C}_{26}\text{H}_{22}\text{BF}_2\text{N}_7\text{Na} [\text{M+Na}^+] \) 504.1895, found 504.1894.

**Synthesis of compound 5**

The synthesis of compound 5 was accomplished according to the reported procedure\(^2\).

1) In a 2.0 L round-bottomed flask, \( p \)-methoxybenzaldehyde (13.4 mL, 110 mmol) and pyrrole (23.4 mL, 330 mmol) were dissolved dilute hydrochloric acid (750 mL, 180 mmol), then the reaction mixture was stirred at room temperature for 12 h. TLC analysis showed consumption of starting material. The reaction was dissolved in ethyl acetate. The crude residue was purified via flash chromatography on silica gel (PE: EA = 10:1) to give desired product (20 g, 72%) as a light blue-green solid.

2) Under nitrogen atmosphere, \( N \)-bromosuccinimide (593 mg, 3.33 mmol) was added to a cooled (-78 °C) solution of the light blue-green solid (402 mg, 1.58 mmol) in anhydrous THF (30 mL), then the DDQ (396.7 mg, 1.75 mmol) was added. The reaction was stirred at room temperature for 30 minutes, after which TLC indicated consumption of starting material, the reaction was quenched with saturated ammonium chloride and extracted with ethyl acetate (50 mL × 3). The crude organic extracts were combined, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was then purified with flash chromatography on silica gel (PE: EA = 20:1) to give desired product (500 mg, 82%) as an orange solid.

3) Under nitrogen atmosphere, the previous step product (292 mg, 0.72 mmol) was dissolved in...
anhydrous toluene, followed by addition of triethylamine (0.11 mL, 0.72 mmol) and BF$_3$·Et$_2$O (0.27 mL, 2.15 mmol). The reaction was heated to 100 °C and stirred for 1 h. Then the reaction was quenched with 0.1 M sodium hydroxide and extracted with dichloromethane (30 mL × 3). The crude organic extracts were combined, then washed with sat. NaCl (50 mL × 3) dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was then purified with flash chromatography on silica gel (PE: DCM = 3: 1) to give 5 (260 mg, 79%) as a red solid.

$^1$H NMR (600 MHz, CDCl$_3$) δ 7.45-7.46 (d, $J = 8.6$ Hz, 2H), 7.03-7.04 (d, $J = 8.6$ Hz, 2H), 6.83 (d, $J = 4.1$ Hz, 2H), 6.54 (d, $J = 4.1$ Hz, 2H), 3.90 (s, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) δ 162.15, 143.34, 135.47, 132.26, 131.89, 131.50, 124.87, 122.48, 114.20, 55.55.

Synthesis of compound 5b

Under nitrogen atmosphere, 5 (19.4 mg, 0.043 mmol)、4-Methoxyphenylboronic acid (6.6 mg, 0.043 mmol)、sodium carbonate (13.5 mg, 0.128 mmol) and Pd(PPh$_3$)$_4$ (1.6 mg, 0.001 mmol) was dissolved in ethylene glycol dimethyl ether. Then the reaction was heated to 80 °C for 45 minutes via microwave. The reaction solution was concentrated in vacuo. The crude product was purified with thin layer chromatography on silica gel (PE: EA = 3: 1) to give 5b (8.9 mg, 43%) as a purple-red solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.96-7.98 (m, 2H), 7.47-7.50 (m, 2H), 7.00-7.03 (m, 4H), 6.96-6.97 (d, $J = 4.5$ Hz, 1H), 6.70-6.73 (dd, $J = 8.0, 4.3$ Hz, 2H), 6.47-6.48 (d, $J = 4.1$ Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 161.6, 161.3, 160.9, 142.5, 137.2, 134.7, 132.8, 132.2, 131.3, 128.6, 127.2, 125.9, 124.4, 121.6, 120.9, 114.0, 113.9, 55.5, 55.3.

HRMS: (ESI) m/z: Calculated for C$_{23}$H$_{18}$BBrF$_2$N$_2$NaO$_2$ [M+Na]$^+$ 505.0509, found 505.0555.

Synthesis of compound 5c

Under nitrogen atmosphere, 5 (37.0 mg, 0.082 mmol)、phenylacetylene (10.0 mg, 0.098 mmol)、
cuprous iodide (1.0 mg, 0.004 mmol), palladium acetate (1.1 mg, 0.004 mmol) and triphenylphosphine (2.1 mg, 0.008 mmol) and Et₃N (34 μL, 0.246 mmol) was dissolved in anhydrous THF (2 mL). Then the reaction was remained 30 °C for 1.5 h. The reaction solution was concentrated in vacuo. The crude product was purified with thin layer chromatography on silica gel (PE: Ether = 2:1) to give 5c (5.8 mg, 15%) as a purple-red solid.

$^1$H NMR (400 MHz, CDCl₃) $\delta$ 7.67-7.69 (m, 2H), 7.47-7.49 (m, 2H), 7.38-7.40 (dd, $J = 5.2, 1.9$ Hz, 3H), 7.03-7.05 (d, $J = 8.7$ Hz, 2H), 6.90-6.92 (d, $J = 4.2$ Hz, 1H), 6.82-6.83 (d, $J = 4.3$ Hz, 1H), 6.72-6.73 (d, $J = 4.3$ Hz, 1H), 6.53-6.54 (d, $J = 4.3$ Hz, 1H), 3.91 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl₃) $\delta$ 161.9, 142.8, 137.7, 136.1, 135.8, 132.3, 131.4, 130.9, 130.7, 129.6, 129.0, 128.4, 128.2, 125.6, 123.9, 122.1, 114.1, 102.4, 82.8, 55.5.

HRMS: (ESI) m/z: Calculated for C₂₄H₁₆BBrF₂N₂NaO [M+Na]$^+$ 499.0404, found 499.0418.

General procedure for the synthesis of compounds 5a, 5d-5f

Compound 5 was dissolved in anhydrous acetonitrile to a final concentration of 0.02 M. The solution was placed under a N₂ atmosphere. Then, aniline (1.1 equiv.) or 2,4-dimethoxyaniline (1.1 equiv.) or methyl 4-aminobenzoate (1.1 equiv.) or methyl 5-aminomethoxybenzoate (1.1 equiv.) was added. The reaction mixture was stirred at room temperature for several hours. After which consumption of starting material was confirmed by TLC, the solution was concentrated in vacuo. The residue was purified with thin layer chromatography to afford the desired product.

Synthesis of compound 5a

![Chemical structure of 5](image)

340 mg (0.74 mmol) of 5 was converted to the 5a (226 mg, 65%), the reaction was stirred at room temperature for 12 h. The product (PE: EA = 10:1) as a red solid, via the general procedure.

$^1$H NMR (400 MHz, CDCl₃) $\delta$ 8.12 (s, 1H), 7.39-7.44 (m, 5H), 7.27 (s, 1H), 7.24-7.25 (m, 1H), 6.98-7.01 (m, 2H), 6.94-6.95 (d, $J = 4.9$ Hz, 1H), 6.43-6.45 (d, $J = 4.8$ Hz, 2H), 6.35-6.36 (d, $J = 3.9$ Hz, 1H), 3.88 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl₃) $\delta$ 160.7, 158.7, 137.3, 135.5, 133.9, 133.3, 133.0, 131.7, 129.8, 126.1, 125.9, 122.5, 122.0, 117.2, 116.7, 113.8, 111.6, 55.4.

HRMS: (ESI) m/z: Calculated for C₂₂H₁₇BBrF₂N₂NaO [M+Na]$^+$ 490.0512, found 490.0529.

Synthesis of compound 5d

![Chemical structure of 5d](image)
10 mg (0.022 mmol) of 5 was converted to the 5d (7.5 mg, 65%), the reaction was stirred at room temperature for 12 hours. The product as a red solid, via the general procedure. The solid was purified with toluene.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1H), 7.38-7.41 (d, $J$ = 8.7 Hz, 2H), 7.21-7.23 (d, $J$ = 8.6 Hz, 1H), 7.17-7.18 (d, $J$ = 7.4 Hz, 1H), 6.97-6.99 (d, $J$ = 8.7 Hz, 2H), 6.90-6.91 (d, $J$ = 5.0 Hz, 1H), 6.54-6.55 (d, $J$ = 2.5 Hz, 1H), 6.48-6.51 (dd, $J$ = 8.7, 2.5 Hz, 1H), 6.32-6.39 (m, 2H), 3.87 (s, 6H), 3.83 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 160.6, 159.2, 158.9, 152.9, 135.3, 134.0, 133.3, 131.7, 129.0, 128.2, 126.2, 123.4, 121.0, 119.9, 116.8, 113.8, 112.1, 104.2, 99.4, 55.9, 55.6, 55.4.

HRMS: (ESI) m/z: Calculated for C$_{24}$H$_{22}$BBrF$_2$N$_3$O$_3$ [M+H]$^+$ 528.0904, found 528.0910.

Synthesis of compound 5e

55 mg (0.12 mmol) of 5 was converted to the 5e (44 mg, 69%), the reaction was stirred at room temperature for 5 h. The product as a red solid, via the general procedure. (PE: DCM = 1: 2)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.21 (s, 1H), 8.07-8.09 (d, $J$ = 8.6 Hz, 2H), 7.41-7.43 (d, $J$ = 8.6 Hz, 2H), 7.27-7.29 (d, $J$ = 8.6 Hz, 2H), 6.98-7.01 (m, 3H), 6.54-6.55 (d, $J$ = 4.9 Hz, 1H), 6.50-6.51 (d, $J$ = 3.9 Hz, 1H), 6.37-6.38 (d, $J$ = 3.9 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 166.1, 161.0, 157.0, 141.7, 135.5, 135.3, 134.0, 132.5, 131.8, 131.4, 126.7, 125.7, 123.5, 120.4, 118.6, 118.0, 113.9, 111.0, 55.4, 52.2.

HRMS: (ESI) m/z: Calculated for C$_{24}$H$_{19}$BBrF$_2$N$_3$NaO$_3$ [M+Na]$^+$ 548.0567, found 548.0582.

Synthesis of compound 5f
46 mg (0.10 mmol) of 5 was converted to the 5f (40 mg, 71%), the reaction was stirred at room temperature for 12 h. The product (PE: Acetone = 5: 1) as a red solid, via the general procedure. 

\[ ^1H \text{NMR (400 MHz, CDCl}_3 \delta 7.98 (s, 1H), 7.45-7.75 (d, J = 2.9 Hz, 1H), 7.36-7.41 (ddd, J = 8.8, 6.2, 2.4 Hz, 3H), 7.02-7.04 (d, J = 8.9 Hz, 1H), 6.97-6.99 (m, 2H), 6.92-6.93 (d, J = 4.9 Hz, 1H), 6.43 (d, J = 3.9 Hz, 1H), 6.34-6.35 (d, J = 3.9 Hz, 1H), 6.27-6.28 (d, J = 4.9 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H). ]

\[ ^{13}C \text{NMR (101 MHz, CDCl}_3 \delta 165.4, 160.8, 159.2, 157.5, 135.6, 133.9, 133.4, 133.0, 131.7, 129.7, 128.4, 126.8, 125.9, 122.0, 121.0, 117.2, 116.7, 113.8, 113.3, 111.2, 56.4, 55.4, 52.3. ]

\[ \text{HRMS (ESI) m/z: Calculated for } C_{25}H_{21}BrF_3N_3NaO_4 \text{ [M+Na]^+ 578.0673, found 578.0697.} \]

**General procedure for the synthesis of compounds 5g-5i**

The reaction was placed under a N\textsubscript{2} atmosphere. Next, 5 (1.0 equiv.) and palladium acetate (0.5 equiv.) was added. Then BINAP (0.75 equiv.) was added, followed by cesium carbonate (1.4 equiv.) and methyl-3-aminothiophene-2-carboxylate (1.2 equiv.) or 3-aminothiophene-2-carboxylic acid (1.2 equiv.) or 3-aminothiophene-2-carboxamide (1.2 equiv.) was added. The reaction was dissolved in anhydrous toluene to a final concentration of 0.02 M. The reaction was heated for several minutes. After which consumption of starting material was confirmed by TLC, the solution was concentrated in vacuo. The residue was purified with thin layer chromatography to afford the desired product.

**Synthesis of compound 5g**

24 mg (0.05 mmol) of 5 was converted to the 5g (18 mg, 64%), the product as a red solid, via the general procedure. The reaction was heated to 65 °C for 5 minutes. The solid was purified with PE: Toluene = 1: 1 to toluene. 

\[ ^1H \text{NMR (600 MHz, CDCl}_3 \delta 10.51 (s, 1H), 7.54-7.55 (d, J = 5.4 Hz, 1H), 7.42-7.43 (m, 2H), 7.22-7.23 (d, J = 5.4 Hz, 1H), 6.99-7.01 (m, 3H), 6.57-6.58 (d, J = 4.8 Hz, 1H), 6.52 (d, J = 4.0 Hz, 1H), 6.38 (d, J = 4.0 Hz, 1H), 4.00 (s, 3H), 3.89 (s, 3H). ]

\[ ^{13}C \text{NMR (151 MHz, CDCl}_3 \delta 163.3, 161.0, 155.8, 143.1, 136.1, 135.2, 134.2, 132.4, 132.0, 131.8, ]


125.9, 124.1, 120.1, 120.0, 118.3, 113.9, 113.2, 110.9, 55.4, 52.5.

**HRMS:** (ESI) m/z: Calculated for C_{22}H_{17}BBrF_{2}N_{3}NaO_{3}S [M+Na]^+ 554.0131, found 554.0150.

Synthesis of compound 5h

![Chemical structure of 5h](image)

39 mg (0.086 mmol) of 5 was converted to the 5h (18 mg, 41%), the product (DCM: MeOH = 10: 1) as a red solid, via the general procedure. The reaction was heated to 70 °C for 30 minutes.

**1H NMR (400 MHz, DMSO)** δ 7.61-7.62 (d, J = 5.4 Hz, 1H), 7.41-7.43 (d, J = 8.5 Hz, 2H), 7.30-7.32 (d, J = 5.3 Hz, 1H), 7.06-7.08 (d, J = 8.6 Hz, 2H), 7.01-7.03 (d, J = 4.9 Hz, 1H), 6.91-6.92 (d, J = 5.0 Hz, 1H), 6.32-6.33 (d, J = 3.5 Hz, 1H), 6.23-6.24 (d, J = 3.4 Hz, 1H), 4.11 (s, 1H), 3.83 (s, 3H).

**13C NMR (101 MHz, DMSO)** δ 160.0, 158.1, 141.8, 135.5, 133.4, 133.3, 131.6, 128.4, 128.0, 125.9, 123.2, 120.4, 118.3, 116.0, 115.2, 114.0, 112.7, 112.0, 55.3.

**HRMS:** (ESI) m/z: Calculated for C_{21}H_{15}BBrF_{2}N_{3}NaO_{3}S [M+Na]^+ 539.9975, found 539.9962.

Synthesis of compound 5i

![Chemical structure of 5i](image)

20 mg (0.043 mmol) of 5 was converted to the 5i (14 mg, 62%), the product (DCM: MeOH = 50: 1) as a red solid, via the general procedure. The reaction was heated to 70 °C for 10 minutes.

**1H NMR (400 MHz, DMSO)** δ 11.82 (s, 1H), 7.86-7.87 (d, J = 5.4 Hz, 1H), 7.45-7.48 (dd, J = 6.9, 3.6 Hz, 3H), 7.09-7.13 (dd, J = 10.2, 6.9 Hz, 3H), 6.92-6.94 (d, J = 5.0 Hz, 1H), 6.44 (d, J = 2.4 Hz, 2H), 3.84 (s, 3H).

**13C NMR (101 MHz, DMSO)** δ 164.9, 160.5, 156.4, 141.4, 136.1, 133.2, 133.2, 132.3, 131.7, 130.1, 125.1, 121.9, 120.9, 117.3, 116.2, 115.4, 114.1, 113.7, 55.3.

**HRMS (ESI) m/z:** Calculated for C_{21}H_{15}BBrF_{2}N_{4}NaO_{2}S [M+Na]^+ 539.0134, found 539.0137.

**General procedure for the synthesis of compounds 6a-6i**

The reaction was placed under a N₂ atmosphere. Next, 5a-5i (1.0 equiv.) and palladium acetate (0.5 equiv.) was added. Then BINAP (0.75 equiv.) was added, followed by cesium carbonate (1.4 equiv.) and 2 (1.2 equiv.) was added. The reaction was dissolved in anhydrous toluene to a final
concentration of 0.02 M. The reaction was heated for several minutes. After which consumption of starting material was confirmed by TLC, the solution was concentrated in vacuo. The residue was purified with thin layer chromatography to afford the desired product.

Synthesis of compound 6a

62 mg (0.132 mmol) of 5a was converted to the 6a (22 mg, 26%), the product (PE: EA = 5: 1) as a blue-purple solid, via the general procedure. The reaction was heated to 65 °C for 30 minutes.

1H NMR (600 MHz, CDCl3) δ 8.63-8.65 (d, J = 8.0 Hz, 4H), 7.68 (s, 1H), 7.61-7.63 (t, J = 5.88 Hz, 4H), 7.46-7.47 (d, J = 8.3 Hz, 2H), 7.39-7.40 (m, 4H), 7.15-7.17 (t, J = 7.2 Hz, 1H), 7.00-7.02 (d, J = 8.3 Hz, 2H), 6.80-6.81 (d, J = 4.3 Hz, 1H), 6.71-6.72 (d, J = 4.0 Hz, 1H), 6.43-6.44 (d, J = 4.1 Hz, 1H), 6.32-6.33 (d, J = 4.3 Hz, 1H), 3.90 (s, 3H).

13C NMR (151 MHz, CDCl3) δ 163.5, 160.6, 156.9, 154.3, 148.2, 144.3, 138.9, 133.7, 132.4, 132.0, 131.1, 133.7, 130.1, 129.4, 128.9, 127.8, 127.2, 126.6, 125.0, 124.4, 123.6, 120.9, 118.1, 113.8, 110.6, 103.8, 55.4.

HRMS: (ESI) m/z: Calculated for C36H27BF2N8O [M+H]+ 636.2370, found 636.2396.

Synthesis of compound 6b

8 mg (0.016 mmol) of 5b was converted to the 6b (3.7 mg, 35%), the product (PE: DCM = 1.5: 1) as a blue-purple solid, via the general procedure. The was heated to 65 °C for 30 minutes.

1H NMR (400 MHz, CDCl3) δ 8.63-8.68 (m, 4H), 8.18 (s, 1H), 7.83-7.85 (d, J = 8.8 Hz, 2H), 7.62-7.65 (m, 3H), 7.49-7.51 (d, J = 8.6 Hz, 2H), 7.41-7.43 (d, J = 8.8 Hz, 2H), 6.99-7.04 (m, 5H), 6.69-6.70 (d, J = 4.0 Hz, 1H), 6.59-6.60 (d, J = 4.7 Hz, 1H), 6.48-6.49 (d, J = 4.0 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H).

13C NMR (101 MHz, CDCl3) δ 163.7, 163.3, 160.9, 160.3, 155.2, 151.5, 142.4, 136.9, 134.9, 133.7, 132.6, 131.9, 131.8, 131.6, 130.5, 129.6, 129.3, 127.9, 127.5, 126.8, 126.3, 125.5, 120.7, 116.9, 113.8, 113.6, 109.4, 55.4, 55.3.

HRMS: (ESI) m/z: Calculated for C37H28BF2N7NaO2 [M+Na]+ 674.2264, found 674.2247.

Synthesis of compound 6c
5 mg (0.010 mmol) of 5c was converted to the 6c (2.5 mg, 37%), the product (100% Toluene) as a blue-purple solid, via the general procedure. The reaction was heated to 65 °C for 30 minutes.

1H NMR (400 MHz, CDCl₃) δ 8.71-8.73 (d, J = 8.7 Hz, 2H), 8.64-8.67 (dd, J = 7.8, 1.8 Hz, 2H), 8.37 (s, 1H), 7.62-7.66 (m, 5H), 7.46-7.51 (dd, J = 12.9, 8.7 Hz, 4H), 7.35-7.37 (d, J = 7.1 Hz, 3H), 7.00-7.05 (t, J = 6.3 Hz, 3H), 6.64-6.67 (t, J = 4.6 Hz, 2H), 6.60-6.61 (d, J = 4.0 Hz, 1H), 3.90 (s, 3H).

13C NMR (101 MHz, CDCl₃) δ 163.8, 163.2, 160.9, 156.8, 141.8, 135.2, 135.1, 134.4, 133.3, 132.7, 131.8, 131.7, 129.6, 129.3, 128.5, 128.3, 128.2, 128.1, 127.9, 126.4, 123.1, 123.0, 121.3, 120.9, 113.9, 111.0, 96.6, 83.1, 77.2, 55.4 ppm.

HRMS: (ESI) m/z: Calculated for C₃₈H₂₇BF₂N₇O [M+H]+ 646.2403, found 646.2342.

Synthesis of compound 6d

11 mg (0.02 mmol) of 5d was converted to the 6d (5.4 mg, 37%), the product (Toluene: DCM = 2:1) as a blue solid, via the general procedure. The reaction was heated to 65 °C for 30 minutes.

1H NMR (400 MHz, CDCl₃) δ 8.61-8.65 (m, 4H), 7.82 (s, 1H), 7.66 (s, 1H), 7.61-7.62 (m, 4H), 7.44-7.47 (d, J = 8.7 Hz, 2H), 7.38-7.40 (d, J = 8.9 Hz, 2H), 6.99-7.01 (d, J = 8.7 Hz, 2H), 6.80-6.81 (d, J = 4.6 Hz, 1H), 6.55-6.66 (d, J = 4.2 Hz, 1H), 6.56 (d, J = 2.6 Hz, 1H), 6.49-6.52 (dd, J = 8.7, 2.6 Hz, 1H), 6.39-6.40 (d, J = 4.2 Hz, 1H), 6.27-6.28 (d, J = 4.7 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.83 (s, 3H).

13C NMR (101 MHz, CDCl₃) δ 163.4, 163.3, 160.4, 157.4, 155.1, 151.7, 147.1, 144.7, 132.7, 132.3, 132.0, 131.7, 130.6, 129.6, 129.2, 128.7, 127.7, 126.8, 125.8, 124.5, 121.6, 121.2, 117.8, 115.0, 113.7, 106.6, 104.1, 103.1, 99.4, 56.0, 55.6, 55.4.

HRMS: (ESI) m/z: Calculated for C₃₈H₅₁BF₂N₈O₃ [M+H]+ 696.2581, found 696.2607.

Synthesis of compound 6e
9 mg (0.017 mmol) of 5e was converted to the 6e (5.4 mg, 45%), the product (Toluene: DCM = 3:1) as a blue solid, via the general procedure. The reaction was heated to 70 °C for 30 minutes.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.63-8.67 (m, 5H), 8.04-8.06 (d, $J = 8.7$ Hz, 2H), 7.78 (s, 1H), 7.71 (s, 1H), 7.69-7.63 (m, 4H), 7.46-7.48 (d, $J = 8.7$ Hz, 2H), 7.41-7.43 (d, $J = 8.8$ Hz, 2H), 7.23 (s, 1H), 7.01-7.03 (d, $J = 8.7$ Hz, 1H), 6.80-6.82 (t, $J = 4.2$ Hz, 2H), 6.48-6.49 (d, $J = 4.4$ Hz, 1H), 6.41-6.42 (d, $J = 4.5$ Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H).

$^{13}$C NMR (151 MHz, CDCl$_3$) δ 166.5, 163.6, 163.4, 160.8, 150.9, 150.4, 143.7, 143.6, 134.9, 132.5, 132.0, 131.7, 131.5, 131.4, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 127.8, 126.4, 125.9, 124.5, 118.9, 118.1, 113.9, 105.4, 55.4, 52.0.

HRMS: (ESI) m/z: Calculated for C$_{38}$H$_{29}$BF$_2$N$_8$O$_3$ [M+H]$^+$ 694.2449, found 694.2449.

Synthesis of compound 6f

10.8 mg (0.019 mmol) of 5f was converted to the 6f (6.9 mg, 49%), the product (PE: DCM: ether = 1:1:0.1) as a blue solid, via the general procedure. The reaction was heated to 70 °C for 30 minutes.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.63-8.65 (d, $J = 8.7$ Hz, 4H), 7.75-7.76 (d, $J = 2.9$ Hz, 1H), 7.61-7.66 (m, 4H), 7.45-7.47 (d, $J = 8.7$ Hz, 2H), 7.38-7.40 (d, $J = 8.8$ Hz, 3H), 7.00-7.03 (dd, $J = 8.8$, 4.7 Hz, 4H), 6.78-6.79 (d, $J = 4.5$ Hz, 1H), 6.70-6.72 (d, $J = 4.4$ Hz, 1H), 6.42-6.43 (d, $J = 4.3$ Hz, 1H), 6.14-6.15 (d, $J = 4.5$ Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 165.8, 163.4, 160.6, 156.5, 154.9, 148.0, 144.3, 133.6, 132.4, 132.0, 131.7, 131.4, 131.3, 129.6, 129.5, 129.2, 128.8, 127.7, 127.2, 127.0, 126.6, 125.5, 124.9, 120.8, 118.0, 113.7, 113.3, 105.6, 103.7, 99.9, 56.4, 55.4, 52.3.

HRMS (ESI) m/z: Calculated for C$_{39}$H$_{32}$BF$_2$NaO$_4$[M+Na]$^+$ 747.2428, found 747.2435.

Synthesis of compound 6g
18 mg (0.033 mmol) of 5g was converted to the 6g (5.9 mg, 25%), the product (DCM: PE = 2: 1) as a blue-green solid, via the general procedure. The reaction was heated to 65 °C for 30 minutes.

\(^{1}\text{H NMR}\) (600 MHz, CDCl\(_3\)) \(\delta\) 10.26 (s, 1H), 8.64-8.67 (m, 4H), 7.95 (s, 1H), 7.62-7.63 (d, \(J = 7.8\) Hz, 3H), 7.49-7.50 (d, \(J = 5.5\) Hz, 1H), 7.43-7.47 (dd, \(J = 8.7, 3.5\) Hz, 4H), 7.28-7.29 (d, \(J = 5.5\) Hz, 2H), 7.01-7.03 (d, \(J = 8.7\) Hz, 2H), 6.82-6.83 (d, \(J = 4.5\) Hz, 1H), 6.77 (d, \(J = 4.4\) Hz, 1H), 6.49 (d, \(J = 4.4\) Hz, 1H), 6.35 (d, \(J = 4.4\) Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H).

\(^{13}\text{C NMR}\) (151 MHz, CDCl\(_3\)) \(\delta\) 167.3, 164.4, 163.3, 160.7, 152.1, 149.9, 146.1, 143.6, 132.5, 132.1, 132.0, 131.9, 131.8, 131.3, 129.6, 129.3, 129.0, 128.2, 128.0, 127.9, 126.6, 119.6, 119.5, 113.8, 112.3, 108.6, 106.0, 105.1, 55.4, 52.2.

HRMS: (ESI) m/z: Calculated for C\(_{36}\)H\(_{27}\)BF\(_2\)N\(_8\)O\(_3\)S [M+Na\(^+\)] 723.1886, found 723.1921.

Synthesis of compound 6h

7 mg (0.014 mmol) of 5h was converted to the 6h (2.7 mg, 29%), the product (DCM: MeOH = 10: 1) as a blue solid, via the general procedure. The reaction was heated to 65 °C for 30 minutes.

\(^{1}\text{H NMR}\) (400 MHz, DMSO) \(\delta\) 8.49-8.52 (m, 4H), 8.47 (s, 1H), 7.69 (d, \(J = 1.6\) Hz, 2H), 7.67-7.68 (d, \(J = 1.7\) Hz, 1H), 7.47-7.55 (dd, \(J = 22.2, 8.7\) Hz, 5H), 7.29-7.31 (d, \(J = 5.4\) Hz, 1H), 7.09-7.11 (d, \(J = 8.6\) Hz, 2H), 6.86-6.87 (d, \(J = 4.7\) Hz, 1H), 6.61-6.66 (dd, \(J = 14.2, 4.4\) Hz, 2H), 6.45-6.46 (d, \(J = 4.2\) Hz, 1H), 3.84 (s, 3H).

\(^{13}\text{C NMR}\) (151 MHz, DMSO) \(\delta\) 166.9, 164.7, 162.9, 160.4, 151.6, 149.1, 145.2, 144.3, 133.8, 133.1, 132.4, 132.0, 131.7, 131.6, 130.0, 129.5, 128.9, 128.7, 128.5, 127.4, 125.7, 120.4, 119.8, 114.1, 108.7, 108.2, 105.9, 71.2, 55.3.

HRMS: (ESI) m/z: Calculated for C\(_{35}\)H\(_{25}\)BF\(_2\)N\(_8\)O\(_3\) [M+H\(^+\)] 685.1995, found 685.1995.

Synthesis of compound 6i
14 mg (0.027 mmol) of 5i was converted to the 6i (5.0 mg, 27%), the product (DCM: Acetone = 25:1) as a blue-green solid, via the general procedure. The reaction was heated to 65 °C for 20 minutes.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 11.09 (s, 1H), 8.63-8.65 (m, 4H), 7.97 (s, 1H), 7.61-7.63 (dd, \(J = 4.1, 3.3\) Hz, 3H), 7.43-7.47 (dd, \(J = 5.2, 3.5\) Hz, 4H), 7.40-7.41 (d, \(J = 5.5\) Hz, 1H), 7.00-7.02 (d, \(J = 8.8\) Hz, 2H), 6.75-6.80 (dd, \(J = 12.5, 4.2\) Hz, 2H), 6.46-6.47 (d, \(J = 4.5\) Hz, 1H), 6.32-6.33 (d, \(J = 5.5\) Hz, 1H), 5.50 (s, 2H), 3.89 (s, 3H).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 165.7, 163.5, 163.4, 160.6, 151.1, 148.9, 145.9, 143.7, 135.2, 134.7, 132.4, 132.0, 131.7, 129.6, 129.5, 129.3, 129.1, 128.3, 127.8, 126.6, 125.9, 120.4, 119.5, 113.8, 111.4, 109.2, 105.8, 105.1, 55.5.

HRMS (ESI) m/z: Calculated for C\(_{35}\)H\(_{26}\)BF\(_2\)N\(_9\)NaO\(_2\)S [M+Na]\(^+\) 708.1890, found 708.1897.

**Measurement of fluorescence quantum yields**

Fluorescence quantum yields were measured by creating 2 mM stocks of freshly-purified tetrazine-BODIPY dyes in DMF and reacted with 10-fold excess trans-cyclooctenol (TCO) or 100-fold excess cyclopropene at the final concentrations of 2 mM tetrazine-BODIPY dye and 20 mM trans-cyclooctenol (TCO) or 200 mM cyclopropane in DMF/H\(_2\)O (V/V 9:1) solution, the reaction mixture was kept at 37 °C, after the reaction completed, then further diluted in DMF/PBS (V/V 1:1) solution to various final concentration. The absorbance and emission spectra of five different concentrations of tetrazine-BODIPY dyes were measured while keeping maximum absorbance under 0.2. The solution of tetrazine-BODIPY dye were excited at 600 nm (5.0 nm slit width,\(_x\)) and emission signal were tracked over the 620-750 nm range (8.0 nm slit width). Emission were measured compared against a control sample lacking trans-cyclooctenol (TCO) or cyclopropane. Plotting emission peak height vs. absorbance at 600 nm yielded a line, whose slope corresponds to the fluorescence quantum yield. The same strategy was used for compound 3 (\(\lambda_{ex} = 530\) nm, \(\lambda_{em} = 530-700\) nm). Absolute quantum yields were determined by comparison with the slope of the line measured for cresyl violet in MeOH (\(\Phi_l = 0.54\)). The reported quantum yields are the average of three sets of measurements. Absorbance spectra were recorded on a TU-1901 spectrometer. Emission spectra were recorded on a Perkin Elmer LS-55 spectrometer. Measurements were made in 1 cm x 1 cm quartz cuvettes with a total sample volume of 2.5 mL.
Figure S1. The fluorescence quantum yields of 6i measurement experiment. a) Emission spectra of cresyl violet in MeOH at different absorbances and plot peak height vs. absorbance. b) Emission spectra of 6i in DMF/PBS(V/V=1:1) at different absorbance and plot peak height vs. absorbance.

Table S1. Photophysical Properties of BODIPY-Tetrazine 6a-f, and Products of 6a-f +7, 6a-f +8

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<th>λ_{abs} [nm]</th>
<th>λ_{em} [nm]</th>
<th>Φ_f [×10^{-3}]</th>
<th>Turn-on</th>
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<tr>
<td>6a</td>
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<td>624</td>
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<td>110</td>
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*Measured in DMF/PBS (pH=7.4) (1:1 v/v) at 37 °C.
Scheme S1. Cycloaddition products of 6d and 6e reaction with 7.

The stability of compound 6i

The compound 6i was equally divided into 5 parts and warped with silver paper and stored at 4 °C. While measure the stability, take a part created 1 mM stocks of compound 6i in DMF. Then to a 1 cm x 1 cm quartz cuvette was added 2480 μL DMF/PBS (V/V 1:1) solution, next added 20 μL 1 mM compound 6i in DMF and the solution mixed with a pipette, then measured the emission spectra. To the same 1 cm x 1 cm quartz cuvettes was added 20 μL 1 mM compound 6i in DMF, then added 2 μL 100 mM TCO in DMF, after the reaction completed, added 2478 μL DMF/PBS (V/V 1:1) to the quartz cuvettes with a total volume of 2.5 mL, the solution mixed with a pipette, next measured the emission spectra. The measure time at 0, 7, 15, 22, 30 days. The excitation wavelength at 600 nm, and emission signal was tracked over the 620-750 nm range.

Figure S2. The stability of 6i within a month. a) Emission spectra of compound 6i within a month. b) Emission spectra of compound 6i+TCO within one month.

The biorthogonal reaction kinetic measurements

A 1 mM stock solution of compound 6i was prepared in DMF was used for the kinetic measurement, and the change of the probe absorption intensity was measured over time. Measurements were immediately started upon the addition of excess TCO 7 (final concentrations of 0.04, 0.06, 0.08
mM) or cycloproene 8 (final concentrations of 0.8, 1.0, 1.2 mM) to reaction solution. Compound 6i solutions at 4 μM final concentration in DMF/PBS (V/V 1:1). The peak height of the compound 6i reacted with TCO 7 at 635 nm and with cyclopropane 8 at 636 nm was tracked over the reaction timeframe by measuring the emission spectra every 25 s for TCO 7 and 5 min for cyclopropane 8. Reaction rates were obtained by fitting the exponential increase of TCO 7 or cyclopropane 8 as a pseudo first order reaction. According to the time and peak height can obtain the $t_{1/2}$, then get the observed reaction rates ($K_{\text{obs}}$), next Plotted $K_{\text{obs}}$ vs. TCO concentrations yielded a line, the slope of the resulting line was used to determine the second-order rate constant.

![Chemical structures](image)

**Figure S3.** Reaction kinetic measurement of compound 6i with TCO 7 (three separate concentrations in DMF/PBS = 1:1 at 37 °C).
Figure S4. Reaction kinetic measurement of compound 6i with cyclopropane 8 (three separate concentrations in DMF/PBS = 1:1 at 37 °C).

HPLC characterization of the bioorthogonal reaction between compound 6i with trans-cyclooctenol 7 or cyclopropene 8

Characterization of the reaction between probe 6i and TCO 7
**Figure S5.** HPLC traces of biorthogonal product Bodipy probe 6i with TCO (red) and probe 6i (blue) at $\lambda = 370$ nm and associated mass traces of the reaction solution between 6i (0.08 mM) and 7 (1 mM) after 3 min. at r.t. in DMF/H$_2$O = 1:1. MS trace of the reaction solution, selected ion monitoring at m/z 806 (i.e. [M+Na]$^+$ peak) (black).

**Characterization of the reaction between probe 6i and TCO 8**
Figure S6. HPLC traces of biorthogonal product Bodipy probe 6i with CYC. (red) and probe 6i (blue) at $\lambda = 370$ nm and associated mass traces of the reaction solution between 6i (0.08 mM) and 8 (1 mM) after 10 min. at r.t. in DMF/H$_2$O = 1:1. MS trace of the reaction solution, selected ion monitoring at m/z 851 (i.e. [M+Na]$^+$ peak) (black).

Experimental procedures for fluorescence imaging

HEK293T cells culture

For labeling experiments, HEK293T cells were cultured in high-glucose DMEM, supplemented with 10% fetal bovine serum (FBS). Cells were maintained in 100-mm culture dishes, in a 5% CO$_2$ atmosphere at 37 °C. Cells were allowed to grow at 80% confluency every 2-4 days.

Mannosamine labeling in HEK239T cell

For live-cell imaging, cells were seeded on a 2 mL confocal culture dish, next added 2 $\mu$L 100 mM peracetylated N-(4-CYCP)-Mannosamine 10 or unmodified peracetylated N-acetylmannosamine in DMSO cultured for 72 h. The cells were then washed with 3 x 1 mL PBS and added DMEM, next added 2 $\mu$L 10 mM freshly 6i in DMSO and incubated in a 5% CO$_2$ atmosphere at 37 °C. then after 1 h, the cells directly subjected to confocal microscopy no-wash labeling imaging.

A549 cells culture

For labeling experiments, A549 cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS). Cells were maintained in 100-mm culture dishes, in a 5% CO$_2$ atmosphere at 37 °C. Cells were allowed to grow at 80% confluency every 2-4 days.

Phospholipid labeling in A549

For live-cell imaging, cells were seeded on a 2 mL confocal culture dish, next added 2 $\mu$L 100 mM trans-cyclooctene-DOPE 9 in DMSO cultured for 24 h, the cells were then washed with 3 x 1 mL PBS and the RPMI-1640 medium, next added 2 $\mu$L 10 mM freshly 6i in DMSO and incubated in a 5% CO$_2$ atmosphere at 37 °C. after 5 min, the cells directly subjected to confocal microscopy no-wash labeling imaging.
NMR spectra

Compound 3 $^1\text{H}$ NMR

Compound 3 $^{13}\text{C}$ NMR
Compound 4 $^1$H NMR

Compound 4 $^{13}$C NMR
Compound 5 $^1$H NMR

Compound 5 $^{13}$C NMR
Compound 5a $^1$H NMR

Compound 5a $^{13}$C NMR
Compound 5b $^1$H NMR

Compound 5b $^{13}$C NMR
Compound 5c $^1$H NMR

Compound 5c $^{13}$C NMR
Compound 5e $^1$H NMR

Compound 5e $^{13}$C NMR
Compound 5f $^1$H NMR

Compound 5f $^{13}$C NMR
Compound 5g $^1$H NMR

Compound 5g $^{13}$C NMR
Compound 5h $^1$H NMR

Compound 5h $^{13}$C NMR
Compound 5i $^1$H NMR

Compound 5i $^{13}$C NMR
Compound 6b $^1$H NMR

Compound 6b $^{13}$C NMR
Compound 6c $^1$H NMR

[Image of $^1$H NMR spectrum for Compound 6c]

Compound 6c $^{13}$C NMR

[Image of $^{13}$C NMR spectrum for Compound 6c]
Compound 6d $^1$H NMR

Compound 6d $^{13}$C NMR
Compound 6e $^1$H NMR

Compound 6e $^{13}$C NMR
Compound 6f $^1$H NMR

Compound 6f $^{13}$C NMR
Compound 6g $^1$H NMR

Compound 6g $^{13}$C NMR
Compound 6h $^1$H NMR

Compound 6h $^{13}$C NMR
Compound 6i $^1$H NMR

Compound 6i $^{13}$C NMR
References
