# A Novel Small Molecule for Active Targeting of Metastatic Melanoma

## Supporting Information

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## Materials and Methods

*General Procedures*

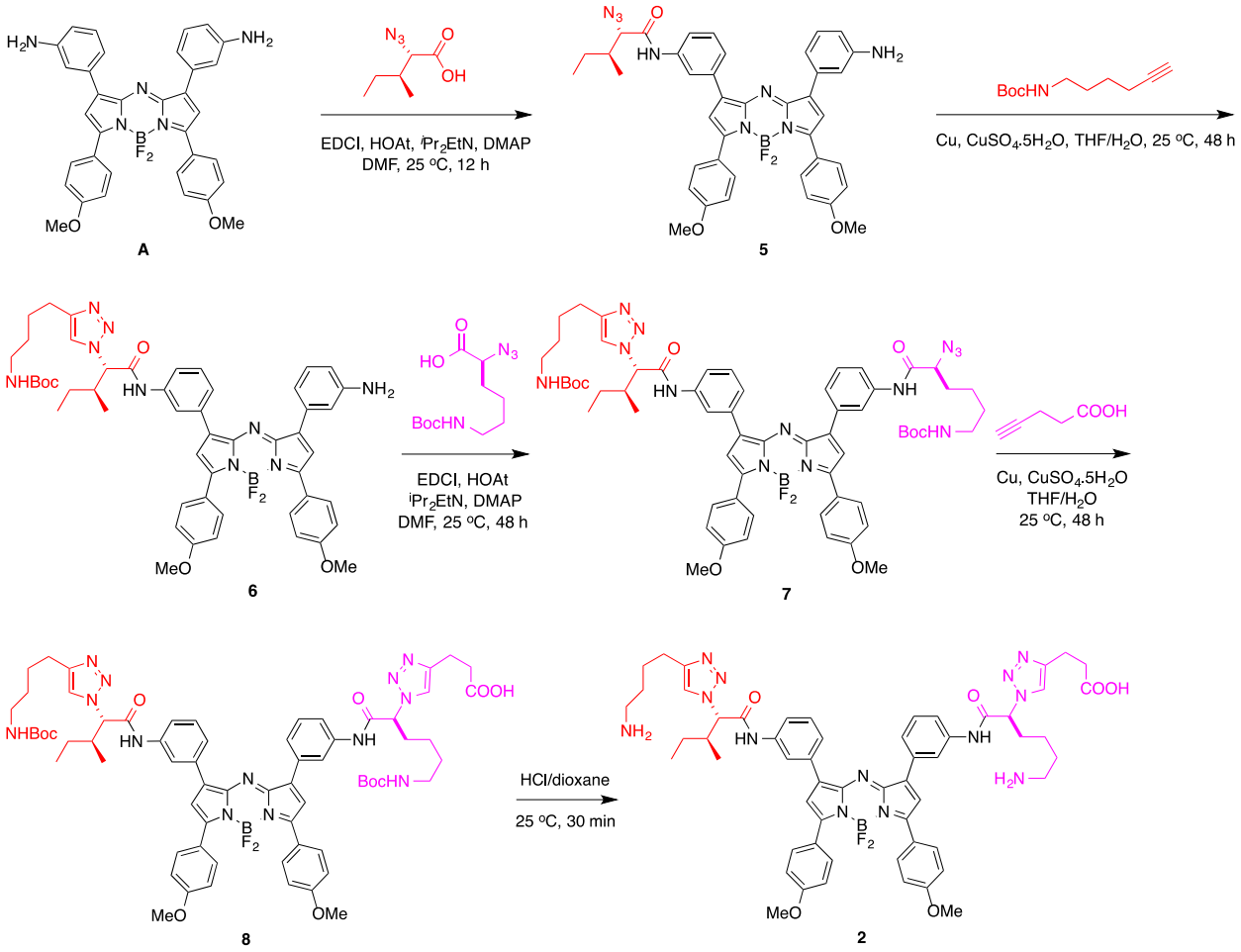
All reactions were carried out under an atmosphere of argon. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. All α-amino acids used were of the L-configuration. Dry DMF, (<50 ppm water) was purchased from Acros. Tetrahydrofuran (THF), Acetonitrile (MeCN), dichloromethane ($CH_2Cl_2$), and methanol (MeOH) were dried by Mbraun solvent drying system. Other solvents and reagents were used as received.

NMR spectra were recorded on a Bruker-400 MHz spectrometers ($^1H$ at 400 MHz and $^{13}C$ at 100 MHz) at room temperature unless other mentioned. Chemical shifts of $^1H$ NMR spectra were recorded and chemical shifts are reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, $CD_3OD$ 3.30 ppm, DMSO-$d_6$ 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants, and number of protons. Proton decoupled $^{13}C$ NMR spectra were also recorded in ppm from tetramethylsilane (TMS) resonance ($CDCl_3$ 77.0, $CD_3OD$ 49.1, DMSO-$d_6$ 39.5 ppm). Analytical thin layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica-gel 60-F plates, and visualized with UV light. Flash chromatography was performed using silica gel 60 (230–400 mesh). MS were measured under ESI or MALDI conditions.

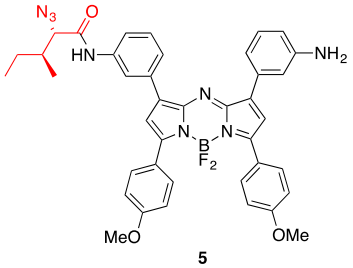
Analytical HPLC analyses were carried out on 150 x 4.6 mm C-18 column using gradient conditions (10 – 90% B, flow rate = 0.75 mL/min). Preparative HPLC was carried out on 100 x 21.2 mm C-18 column using gradient conditions (10 – 70% B, flow rate = 10.0 mL/min). The eluents used were: solvent A ($H_2O$ with 0.1% AcOH) and solvent B ($CH_3CN$ with 0.1% AcOH).

The purity of all biologically evaluated compounds is > 95% confirmed by analytical HPLC.

*Syntheses of aza-BODIPY Derivatives* ***2-4***



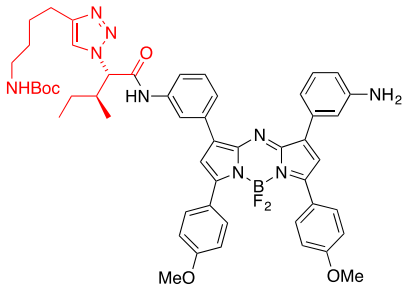
*Synthesis of compound* ***5***




**5**

EDCI (52.7 mg, 0.275 mmol) and HOAt (40.9 mg, 0.300 mmol) were added to a suspension of isoleucine azide (39.3 mg, 0.250 mmol) in 1 mL DMF at 0 °C. After stirring at 0 °C for 30 min, **A** (146.9 mg, 0.250 mmol) was added to the above suspension followed by DIPEA (139 μL, 103.4 mg, 0.800 mmol) and catalytic amount of DMAP. The resulting solution was stirred at 0 °C for 1 h and then warmed to 25 °C and continue stirring for 12 h. Ethyl acetate (*ca.* 20 ml) was added to the reaction mixture, and the organic layer was washed with sat. $NaHCO_3$ and brine then dried with anhydrous $Na_2SO_4$. Solvents were removed to afford green solid. The crude product was purified by column chromatography on silica gel, and eluted with a mixture of hexanes and ethyl acetate (2:1 to 1:2, v/v) afforded the desired **5** (93.9 mg, 34%) as a greenish solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.12 (s, 1H), 8.11 (dd, *J* = 9.0, 4.1 Hz, 4H), 7.86 (t, *J* = 8.9 Hz, 2H), 7.62 – 7.55 (m, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.10 (s, 2H), 7.04 (dd, *J* = 9.1 Hz, 1.2 Hz, 4H), 6.80 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 3.97 (d, *J* = 4.9 Hz, 1H), 3.92 (s, 6H), 3.78 (s, 2H), 2.30 – 2.12 (m, 1H), 1.67 – 1.56 (m, 1H), 1.42 – 1.32 (m, 1H), 1.11 (d, *J* = 6.9 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 167.7, 162.1, 161.9, 158.5, 157.6, 146.3, 145.5, 145.0, 143.3, 141.3, 137.2, 133.4, 131.7, 131.7, 131.6, 129.4, 129.1, 125.7, 124.1, 124.0, 121.9, 121.3, 119.6, 118.7, 116.7, 116.2, 114.3, 114.2, 69.8, 55.4 (2C), 29.7, 24.6, 16.1, 11.4. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.96 (t, *J* = 31.9 Hz).

*Synthesis of compound* ***6***

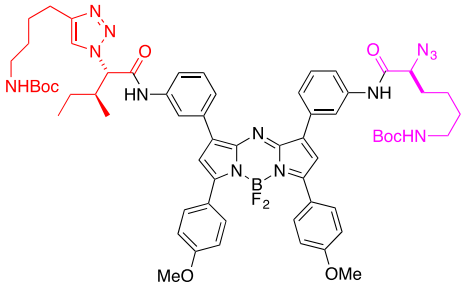



**6**

Copper powder (9.7 mg, 0.152 mmol) and $CuSO_4$ (0.015 mmol, 0.1 equiv, from 0.05 M aqueous solution) were added to a solution of **5** (110.3 mg, 0.152 mmol) and alkyne (29.9 mg, 0.152 mmol) in 7 mL dry THF at 25 °C. The mixture was stirred at 25 °C for 2 days (monitored by TLC). Then, solvents were removed under reduced pressure and the residue was acidified with 0.2 *N* HCl. After extracting with ethyl acetate three times, the combined organic phase was washed with 0.05M EDTA and brine. The organic layer was separated and dried over $Na_2SO_4$. Finally, the solvents were removed to afford green solid, which was purified by column chromatography on silica gel, and eluted with a mixture of $CH_2Cl_2$ and methanol (99:1 to 97:3, v/v) to afford the desired **6** (117.2 mg, 84 %) as a green solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.32 (s, 1H), 8.10 (dd, *J* = 12.2, 9.0 Hz, 4H), 8.03 – 7.95 (m, 1H), 7.76 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.12 (s, 1H), 7.09 – 6.99 (m, 6H), 5.09 (d, *J* = 10.7 Hz, 1H), 4.57 (s, 1H), 4.23 (s, 2H), 3.91 (s, 6H), 3.18 (d, *J* = 6.1 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.38 (dd, *J* = 9.3, 5.7 Hz, 1H), 1.77 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.61 (s, 2H), 1.46 (s, 9H), 1.28 (s, 2H), 1.24 – 1.09 (m, 1H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 166.8, 162.1, 161.9, 158.5, 157.6, 156.0, 148.3, 145.5, 145.3, 145.1, 143.3, 140.5, 137.3, 133.5, 133.1, 131.7, 131.6, 129.6, 129.1, 125.2, 124.0, 123.9, 122.5, 121.7, 120.1, 119.8, 118.6, 118.4, 118.1, 116.7, 114.3, 114.2, 79.1, 69.3, 55.4, 55.4, 40.3,

38.2, 29.7, 28.4, 26.5, 25.5, 24.8, 15.4, 10.3. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.91 (t, *J* = 32.0 Hz). MS-ESI $[M+H]^+$: cald 924.45, found 924.47.

*Synthesis of compound **7***

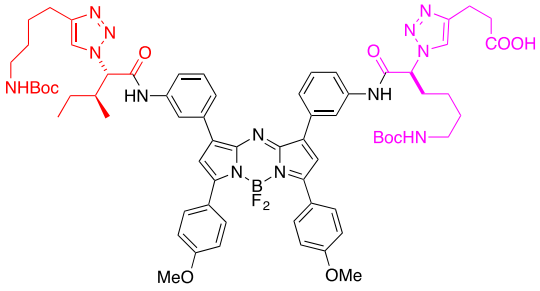



**7**

EDCI (133.9 mg, 0.70 mmol) and HOAt (96.8 mg, 0.71 mmol) were added to a suspension of Boc protected lysine azide (172.7 mg, 0.63 mmol) in 2 mL DMF at 0 °C. After stirring at 0 °C for 30 min, **6** (117.2 mg, 0.13 mmol) was added to the above suspension followed by DIPEA (354 μL, 262.6 mg, 2.03 mmol) and catalytic amount of DMAP. The resulting solution was stirred at 0 °C for 1 h and warmed to 25 °C and stirred for 12 h. Ethyl acetate (*ca*. 50 ml) was added to the reaction mixture, and the resulting suspension was washed with 5 % HCl, water, sat. $NaHCO_3$ and brine. The organic layer was separated, dried with $Na_2SO_4$, and solvent was removed to afford green solid. The crude product was purified by column chromatography on silica gel, and eluted with a mixture of $CH_2Cl_2$ and methanol (99:1 to 97:3, v/v) to obtained **7** (123.5 mg, 83%) as a green solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 8.37 (s, 1H), 8.14 (d, *J* = 9.4 Hz, 2H), 8.05 (ddd, *J* = 7.3, 4.7, 2.0 Hz, 4H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.75 (s, 2H), 7.64 (s, 2H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.31 – 7.21 (m, 1H), 7.00 (s, 2H), 6.98 (d, *J* = 2.6 Hz, 4H), 5.11 (d, *J* = 9.6 Hz, 1H), 4.83 (s, 1H), 4.68 (t, *J* = 5.6 Hz, 1H), 4.08 – 3.92 (m, 1H), 3.88 (s, 6H), 3.14 (d, *J* = 5.2 Hz, 4H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.40 (dd, *J* = 13.8, 7.4 Hz, 1H), 2.12 – 1.82 (m, 4H), 1.73 (dt, *J* = 15.3, 7.7 Hz, 2H), 1.63 – 1.49 (m, 6H), 1.45 (d, *J* =

7.1 Hz, 18H), 1.27 (d, *J* = 7.6 Hz, 1H), 1.24 – 1.14 (m, 1H), 1.09 (d, *J* = 6.7 Hz, 3H), 0.86 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 168.0, 167.9, 166.6, 162.1, 158.2, 158.1, 156.3, 156.1, 148.2, 145.3, 145.2, 142.3, 137.7, 137.3, 133.4, 133.2, 133.1, 131.8, 131.7, 129.1, 129.0, 126.1, 125.8, 123.9, 121.1, 121.0, 120.8, 120.6, 119.1, 114.3, 79.3, 79.1, 69.8, 64.0, 55.4 (2C), 40.2, 38.4, 31.7, 29.7, 29.6, 28.5, 28.4, 26.5, 25.4, 24.9, 22.7, 15.5, 10.4. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.92 (t, *J* = 31.9 Hz). MS-ESI $[M+H]^+$: cald 1178.59 found 1178.71.

*Synthesis of compound* ***8***

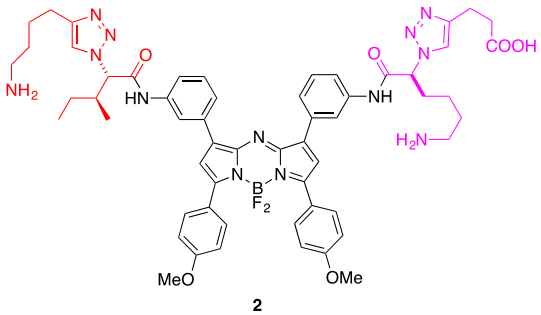



**8**

Copper powder (6.7 mg, 0.11 mmol) and $CuSO_4$ (0.1 equiv, from 0.05 M aqueous solution) were added to a solution of **7** (123.5 mg, 0.11 mmol) and alkyne (10.3 mg, 0.11 mmol) in 7 mL dry THF at 25 °C. The mixture was stirred at 25 °C for 2 days. Then, solvents were removed under reduced pressure and the residue was acidified with 0.2 *N* HCl. After extracting with ethyl acetate three times, the combined organic phase was washed with 0.05 M EDTA and brine. The organic layer was separated and dried over $Na_2SO_4$. Finally, the solvents were removed to afford green solid, which was purified by column chromatography on silica gel, and eluted with a mixture of $CH_2Cl_2$, and methanol (99:1 to 97:3, v/v) to afford the desired **8** (86.0 mg, 64 %) as a green solid. $^1$H NMR (400 MHz, $CD_3OD$) δ 8.00 (s, br, 4H), 7.88 (s, br, 4H), 7.72 (s, br, 1H), 7.56 (s, br, 1H), 7.40 (s, br, 2H), 7.03 (s, br, 2H), 6.80 (s, br, 6H), 5.38 (s, br, 1H), 5.19 (s, br, 1H), 3.71 (s, br, 6H), 3.01 (s, br, 6H), 2.69 (s, br, 4H), 2.39 (s, br, 1H), 2.17 (s,

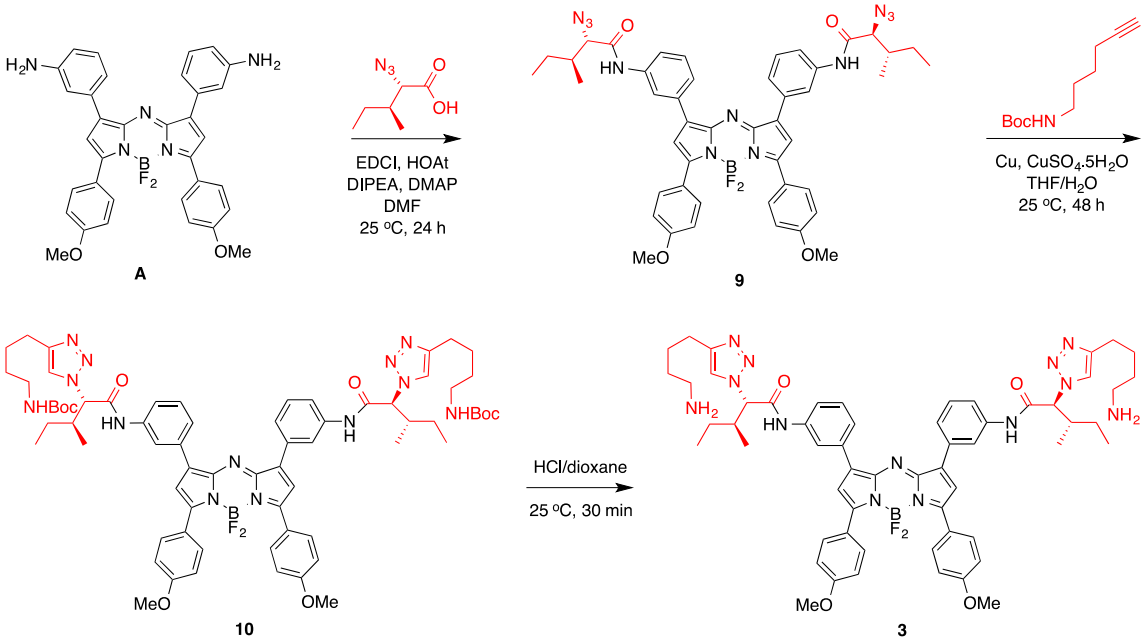
br, 2H), 1.65 (s, br, 2H), 1.48 (s, br, 4H), 1.39 (s, 18H), 1.28 (m, 4H), 1.04 (s, br, 3H), 0.83 (s, br, 3H). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 167.0, 162.0, 157.7, 157.1, 144.8, 141.6, 137.6, 137.5, 132.9, 131.6, 128.7, 126.2, 123.5, 120.7, 120.2, 118.7, 113.7, 78.6, 78.5, 68.9, 64.3, 54.6, 47.0, 46.5, 39.6, 37.8, 33.5, 32.3, 29.1, 28.9, 27.5, 26.3, 24.5, 22.7, 20.9, 14.6, 9.1, 7.8. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.86 (t, *J* = 32.3 Hz), MS-ESI $[M+H]^+$: cald 1276.63, found 1276.80.

*Synthesis of the asymmetrical compound compound **2***

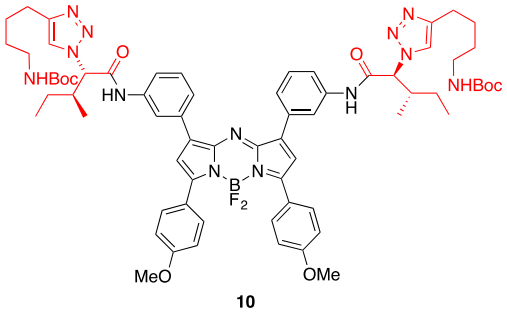



**2**

Compound **8** (12.7 mg, 0.01 mmol) was dissolved in 100 μL HCl in 1,4-dioxane (4 M), and the reaction mixture was stirred at 25 °C for 30 min. After the reaction was done, the solvent was removed under reduced pressure to give desired product **2** as a green solid quantitatively. The purity was confirmed by analytical HPLC (C18 column, $CH_3CN$-$H_2O$ 10-90 % with 0.1 % acetic acid), retention time was 13.1 min. $^1$H NMR (400 MHz, $CD_3OD$) δ 8.33 (s, 2H), 8.15 (d, *J* = 9.7 Hz, 2H), 8.07 (d, *J* = 7.3 Hz, 4H), 7.88 (s, 2H), 7.58 (t, *J* = 8.8 Hz, 2H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.17 (t, *J* = 6.6 Hz, 1H), 7.14 (s, 2H), 6.98 (dd, *J* = 8.7, 3.5 Hz, 4H), 5.70 (t, *J* = 7.2 Hz, 1H), 5.38 (d, *J* = 10.4 Hz, 1H), 3.86 (s, 6H), 3.11 (dd, *J* = 16.6, 10.0 Hz, 2H), 2.97 (d, *J* = 6.4 Hz, 4H), 2.85 (s, 2H), 2.78 (t, *J* = 7.0 Hz, 2H), 2.50 (s, 1H), 2.36 (s, 2H), 1.77 (s, 6H), 1.33 (dd, *J* = 14.3, 7.0 Hz, 4H), 1.19 – 1.09 (m, 3H), 0.90 (dd, *J* = 18.4, 11.1 Hz, 3H). $^{11}$B NMR (128 MHz, $CD_3OD$) δ 0.85 (t, *J* = 32.0 Hz). HRMS-MALDI $[M-H]^-$: cald 1074.5091, found 1074.5057.



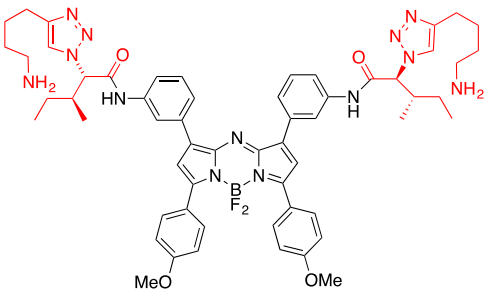

*Synthesis of compound **10***




To a solution of **9** (284.9 mg, 0.329 mmol) and alkyne (129.8 mg, 0.658 mmol) in 24 mL dry THF were added copper powder (41.8 mg, 0.658 mmol) and $CuSO_4$ (0.1 equiv, from 0.05 M aqueous solution) at 25 °C. The mixture was stirred at 25 °C for 2 days. The reaction was monitored by TLC. After the reaction was done, the solvent was removed under reduced pressure and 0.2 *N* HCl was added to the residue. After extracted with ethyl acetate three times, the combined organic phase was washed with 0.05 M EDTA and brine. The organic layer was

separated and dried over $Na_2SO_4$, and concentrated to afford green solid. The crude product was purified by column chromatography on silica gel, and eluted with a mixture of $CH_2Cl_2$ and methanol (100:2 to 100:4, v/v) afforded the desired **10** (197.1 mg, 48%) as green solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, br, 2H), 8.14 (s, 2H), 8.06 (d, *J* = 8.4 Hz, 4H), 7.80 (s, 2H), 7.59 (dd, *J* = 29.3, 8.5 Hz, 4H), 7.26 (m, 2H), 7.00 (q, *J* = 9.3 Hz, 6H), 5.25 (d, *J* = 8.3 Hz, 2H), 4.65 (s, 2H), 3.88 (s, 6H), 3.13 (s, 4H), 2.76 (t, *J* = 6.9 Hz, 4H), 2.42 (s, 2H), 1.93 (s, 2H), 1.71 (s, 4H), 1.63-1.47 (m, 4H), 1.44 (s, 18H), 1.33-1.25 (m, 4H), 1.21 (d, *J* = 6.8 Hz, 6H), 0.88 (t, *J* = 7.3 Hz, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 166.7, 162.1, 158.1, 156.0, 148.3, 145.3, 142.9, 137.2, 133.4, 131.7, 129.0, 126.0, 123.9, 121.7, 121.2, 119.4, 114.3, 79.1, 69.8, 55.4, 40.3, 38.3, 29.7, 28.4, 26.5, 25.4, 24.9, 15.4, 10.3. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.84 (t, *J* = 31.8 Hz). $^{19}$F NMR (376 MHz, $CDCl_3$) quartet at δ -132.16 HRMS-ESI $[M+Na]^+$: cald 1282.6527, found 1282.6589.

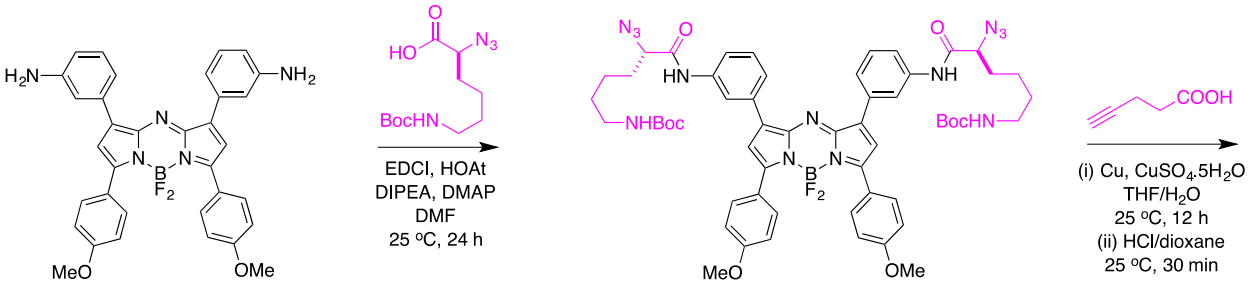
*Synthesis of the symmetrical targeting compound **3***



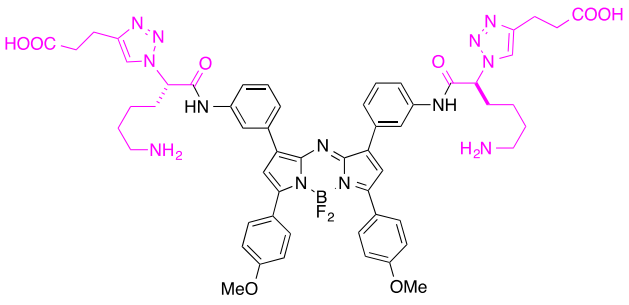

**3**

**10** (12.6 mg, 0.01 mmol) was dissolved in 100 μL HCl in 1,4-dioxane (4 M), and the reaction mixture was stirred at 25 °C for 30 min. After the reaction was done, the solvent was removed under reduced pressure to give desired product **3** as green solid quantitatively. The purity was confirmed by analytical HPLC (C18 column, $CH_3CN$-$H_2O$ 10-90% with 0.1% acetic acid), retention time was 13.5 min. $^1$H NMR (400 MHz, $CD_3OD$) δ 8.32 (s, 2H), 8.16 (s, 2H),

8.08 (d, *J* = 8.9 Hz, 4H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.23 (t, *J* = 8.0 Hz, 2H), 7.16 (s, 2H), 6.99 (d, *J* = 9.0 Hz, 4H), 5.37 (d, *J* = 10.5 Hz, 2H), 3.87 (s, 6H), 2.98 (t, *J* = 7.1 Hz, 4H), 2.86 (t, *J* = 7.2 Hz, 4H), 2.49 (dd, *J* = 9.5, 5.8 Hz, 2H), 1.92 – 1.67 (m, 8H), 1.22 (dd, *J* = 7.3, 3.2 Hz, 4H), 1.14 (d, *J* = 6.7 Hz, 6H), 0.92 (t, *J* = 7.4 Hz, 6H). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 166.4, 162.3, 157.9, 146.3, 144.9, 142.1, 137.7, 133.0, 131.6, 128.8, 126.1, 123.5, 122.6, 120. 8, 120.3, 119.0, 113.8, 69.5, 54.7, 39.0, 37.7, 26.5, 25.5, 24.5, 23.9, 14.3, 9.0. $^{11}$B NMR (128 MHz, $CD_3OD$) δ 0.87 (t, *J* = 31.8 Hz). HRMS-MALDI $[M+H]^+$: cald 1060.5651, found 1060.5679.

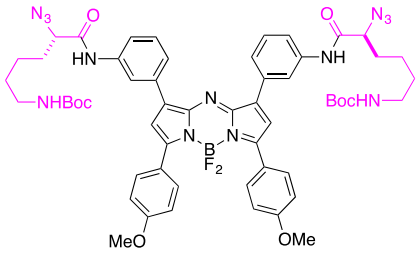



A 11



4

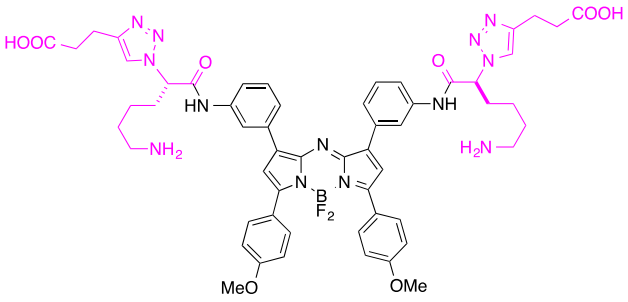
*Synthesis of compound **11***




**11**

EDCI (210.9 mg, 1.10 mmol) and HOAt (152.4 mg, 1.12 mmol) were added to a suspension of Boc protected lysine azide (272.3 mg, 1.00 mmol) in 4 mL DMF at 0 °C. After stirring at 0 °C for 30 min, **A** (117.2 mg, 0.127 mmol) was added to the above suspension followed by DIPEA (557 μL, 413.6 mg, 3.20 mmol) and trace amount of DMAP. The resulting solution was stirred at 0 °C for 1 h and warmed to 25 °C and stirred for overnight. Ethyl acetate (ca. 100 ml) was added to the reaction mixture, and the resulting suspension was washed with 5% HCl, $H_2O$, sat. $NaHCO_3$ and brine. The organic layer was separated, dried with $Na_2SO_4$, and concentrated to afford green solid. The crude product was purified by column chromatography on silica gel, and eluted with a mixture of $CH_2Cl_2$ and methanol (100:4, v/v) afforded the desired **11** (299.1 mg, 91%) as green solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H), 8.06 (d, *J* = 6.8 Hz, 6H), 7.80 (d, *J* = 7.4 Hz, 4H), 7.39 (td, *J* = 7.8, 3.7 Hz, 2H), 7.03 (d, *J* = 3.0 Hz, 2H), 7.01 – 6.93 (m, 4H), 4.70 (s, 2H), 4.05 (s, 2H), 3.89 (s, 6H), 3.14 (s, 4H), 2.06 – 1.90 (m, 4H), 1.64 – 1.48 (m, 8H), 1.45 (s, 18H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 167.7, 162.1, 158.1, 156.1, 145.2, 142.0, 137.5, 133.3, 131.8, 129.1, 125.7, 123.9, 120.6, 120.6, 119.0, 114.3, 79.2, 64.1, 55.4, 40.2, 31.8, 29.7, 28.4, 22.6. $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.98 (t, *J* = 31.7 Hz).

*Synthesis of the symmetrical compound 4*




**4**

To a solution of **11** (110.2 mg, 0.100 mmol) and alkyne (19.6 mg, 0.200 mmol) in 24 mL dry THF were added copper powder (12.7 mg, 0.200 mmol) and $CuSO_4$ (0.1 equiv, from 0.05 M aqueous solution) at 25 °C. The mixture was stirred at 25 °C for 2 days. The reactions were monitored by TLC. After the reaction was done, the solvent was removed under reduced pressure and 0.2 *N* HCl was added to the residue. After extracted with ethyl acetate three times, the combined organic phase was washed with 0.05 M EDTA and brine. The organic layer was separated and dried over $Na_2SO_4$, and concentrated to afford protected form of **4** as green solid. Then, the resulting solid was dissolved in 800 μL HCl in 1,4-dioxane (4 M), and the reaction mixture was stirred at 25 °C for 30 min. After the reaction was done, the solvent was removed under reduced pressure to give desired product **4** as green solid (91.7 mg, 84%). The purity was confirmed by analytical HPLC (C18 column, $CH_3CN$-$H_2O$ 10-90% with 0.1% acetic acid), retention time was 12.5 min. $^1$H NMR (400 MHz, $CD_3OD$) δ 8.32 (s, br, 2H), 8.14 (s, 2H), 8.06 (d, *J* = 8.3 Hz, 4H), 7.85 (d, *J* = 7.1 Hz, 2H), 7.63 (s, br, 2H), 7.23 (s, br, 2H), 7.12 (s, 2H), 6.98 (d, *J* = 8.2 Hz, 4H), 5.68 (s, 2H), 3.87 (s, 6H), 3.08 (s, br, 4H), 2.96 (s, br, 4H), 2.77 (s, br, 4H), 2.34 (s, br, 4H), 1.78 (s, br, 4H), 1.30 (s, br, 4H). $^{11}$B NMR (128 MHz, $CDCl_3$) δ 0.87 (t, *J* = 31.9 Hz). $^{19}$F NMR (376 MHz, $CDCl_3$) quartet at δ -131.76. $^{13}$C NMR could not be obtained due to the solubility of compound.

**Cell Culture and Cell Imaging**

B16-F10 (mouse melanoma) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (Gibco) supplemented with 10 % fetal bovine serum (FBS, Gibco). Cultures were maintained at 37 ºC under a humidified atmosphere containing 5 % $CO_2$.

For cell imaging, cells were seeded on 18 mm glass cover slips ($1 \times 10^5$ cells per well) and cultured for 24 h. Agent **2**, **3** and **4** (2 μM in PBS with 1 % Tween-80) were added to a separated well and incubated for 3 h. Cells were then gently washed three times with PBS containing 1 % Tween 80 and fixed with cold acetone for 10- 15 min. The cells were washed again three times with PBS and mounted with DAPI mounting medium (VectaShield) before imaging with Nikon A1RS confocal microscope and imaging analysis was performed using the NIS-Elements Ar with Deconvolution package.

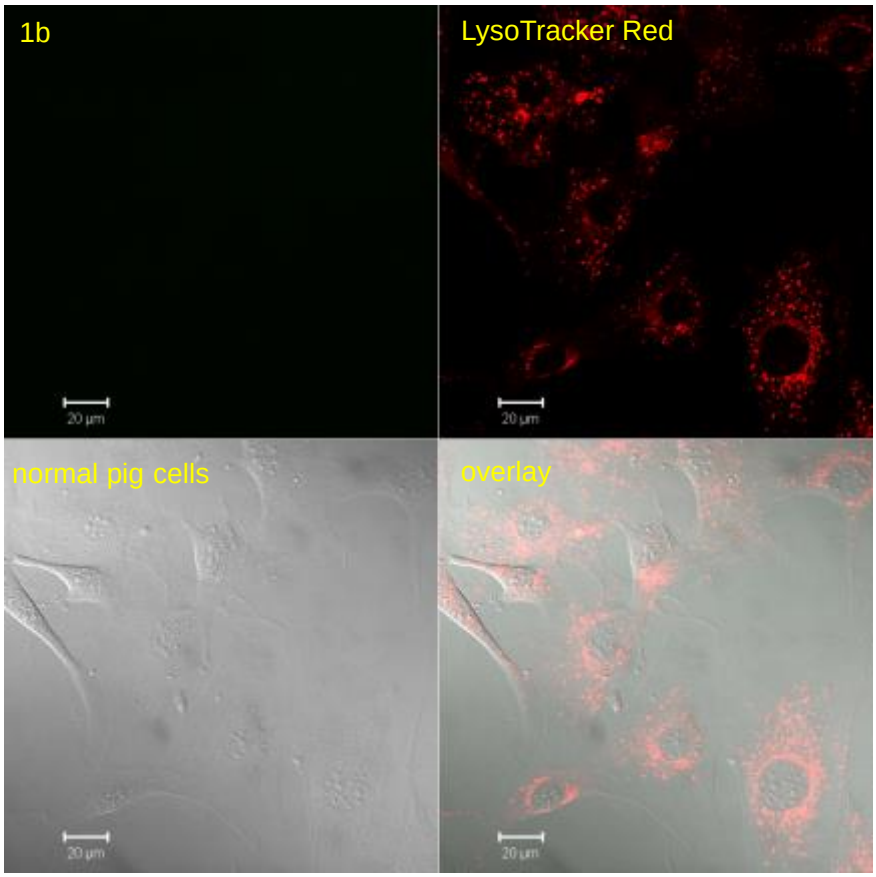



**Figure S1**. Confocal imaging of normal skin cells from Sinclair swine incubated with **1b**. No fluorescence signal is observed from **1b**, only red signal is detected from LysoTracker Red. Scale bar is 20 µm.

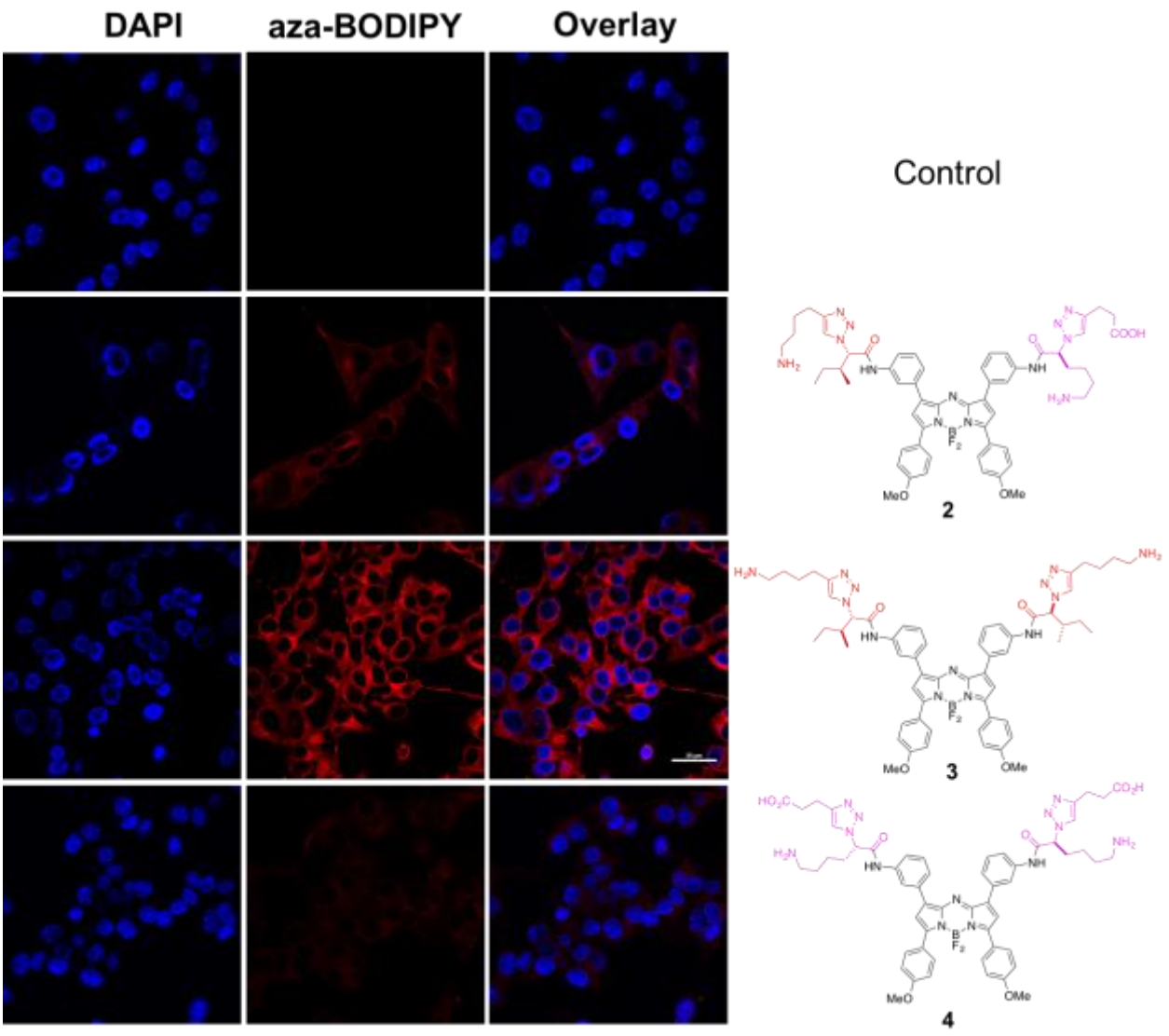



**Figure S2**. B16-F10 cells stain with agent **2**, **3** and **4** (2 μM in PBS with 1 % Tween-80). Bright red fluorescence signal was clearly observed from cells incubated with agent **3**. Little signal was observed from cells incubated with agent **2** whereas no signal was observed from cells incubated with agent **4** and control cells with no fluorescence agents. Scale bar is 20 μm.

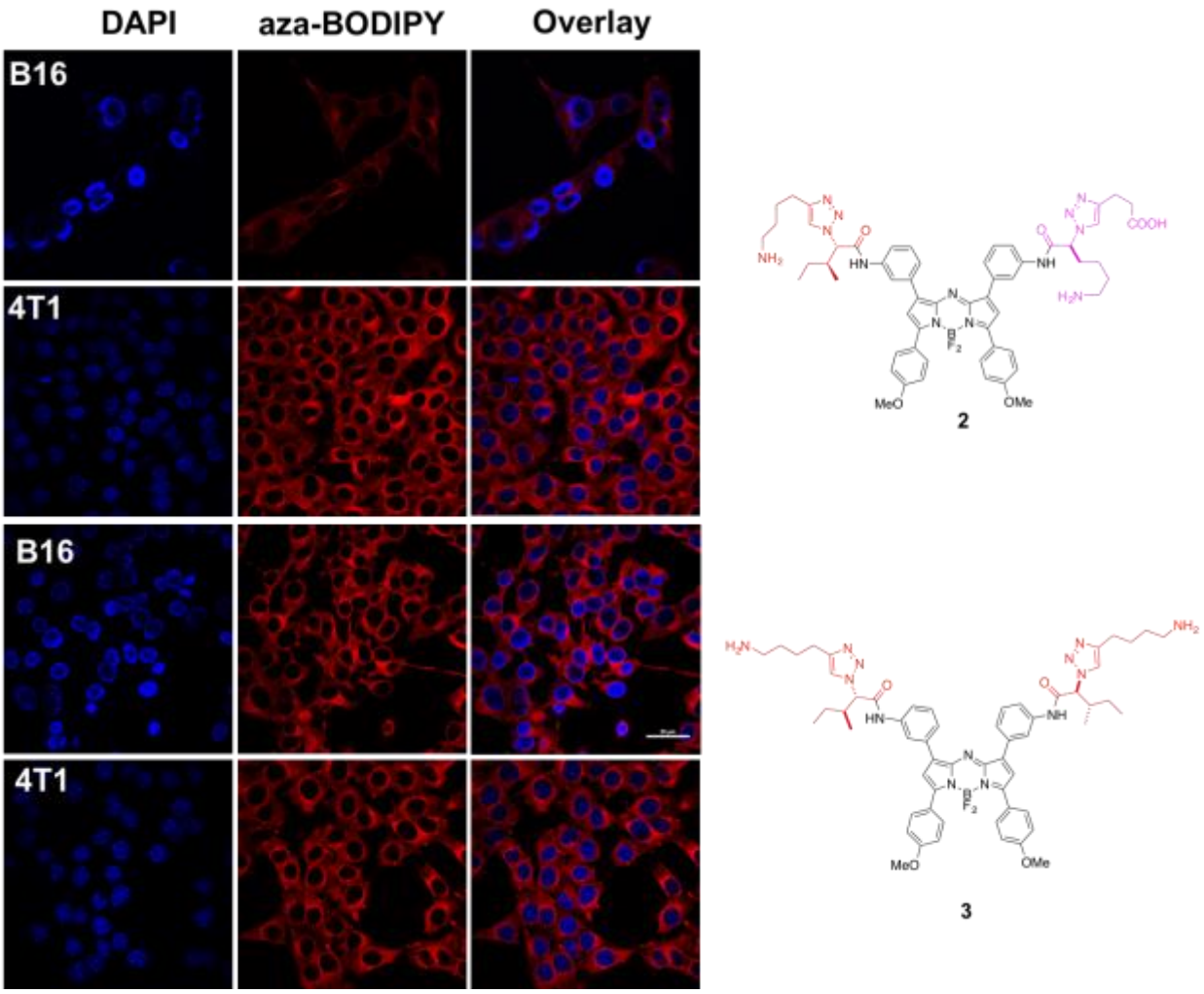



**Figure S3**. B16-F10 and 4T1 cells stain with agent **2** and **3** (2 μM in PBS with 1 % Tween-80). There is no different when 4T1 cells stained with agents **2** or **3**, implying that there is no selectivity on 4T1 cells between two probes. On the other hand, significant staining was observed in case of B16-F10 incubated with **3** not with **2**. Scale bar is 20 μm.

**Tumor Implantation and *In Vivo* Imaging**

All animal studies were conducted under an IACUC protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. B16-F10 cells were harvested using 0.05% trypsin-EDTA when they were 80-90% confluence. Cells were pelleted by centrifugation

at 3000 rpm for 4 min and resuspended in a mixture of 1:1 PBS and Matrigel (BD Biosciences, San Jose, CA). The cells ($5 \times 10^6$ cells per tumor site) were implanted subcutaneously into the hind leg of 4-5 week-old female athymic nude mice (Envigo, Cambridgeshire, UK). When the tumor reached 4-6 mm in diameter (7-10 days after implantation) and, the mice were utilized for *in vivo* studies. The body weights of mice used in experiments ranged from 20-23 g, and the average weight was ~21 g.

*In vivo* and *ex vivo* fluorescence imaging were performed with an IVIS spectrum imaging system (excitation: 670 ± 15 nm filter; emission collected with 720 ± 10 nm filter).

**Histology**

*Histology on patient's tissues*. Slide of human malignant melanoma tissue microarray (T382A) was purchased from US Biomax, Inc., the array includes 10 cases of malignant melanoma and 2 cases of normal in duplicates. The slide was transferred to a xylene bath for 10 min and then rehydrated in two changes of fresh absolute ethanol for 7 min each. Excess liquid was shaken off and the slide was incubated in fresh 90%, 70 % ethanol then water for 7 min each. The slide was washed in two changed of PBS for 5 min each, then incubated with PBS containing 4 % BSA for 30 min. The tissues were rinsed with PBS and incubated again in two changed of PBS for 5 min each. Compound **3** solution in 4 % PBS/BSA was added to the slide and incubated overnight at 4 $^{o}$C. The slide was rinsed twice in PBS, then in water (10 min each). Then the slide contained **3** was mounted in permanent mounting media with DAPI (Vector) and incubated at 4 $^{o}$C for 4 h. The tissues were imaged with an Olympus FV1000 Confocol Microscope. Throughout, digital images were captured with a 100x / 1.4 oil objective with the excitation filter 633 nm for aza-BODIPY **3** and the 405 nm filter for DAPI.

*Histology mice tumors.* Tumor-bearing mice were euthanized 2 h after injection with agents **2**, **3** and **4** (50 μM in PBS containing 3 % Tween-80). The tumor tissues were frozen in optimal cutting temperature (OCT) medium, cryosectioned at -20 °C into 5 μm slices, and the tissue were mounted with DAPI mounting medium to visualize cell nucleus. All images were taken with a Nikon A1RS Confocal Microscope and imaging analysis was performed using the NIS-Elements Ar with Deconvolution package.

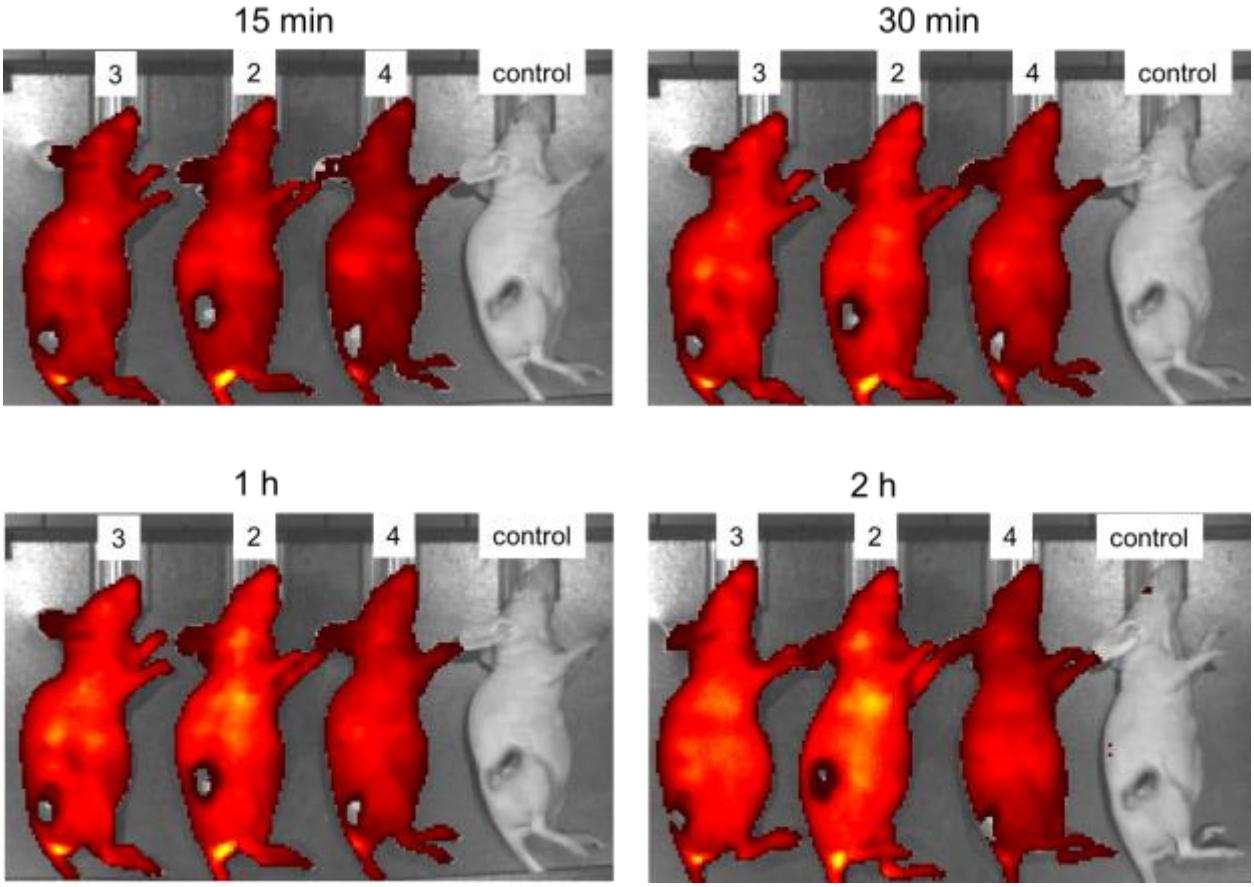



**Figure S4**. In vivo fluorescence imaging of B16-F10 tumor-bearing mice. Time-dependent fluorescence imaging of B16-F10 tumor-bearing mouse intravenously injected with agents **2**, **3** and **4** (50 μM in PBS containing 3 % Tween-80) at 15 min, 30 min, 1 h and 2 h p.i.

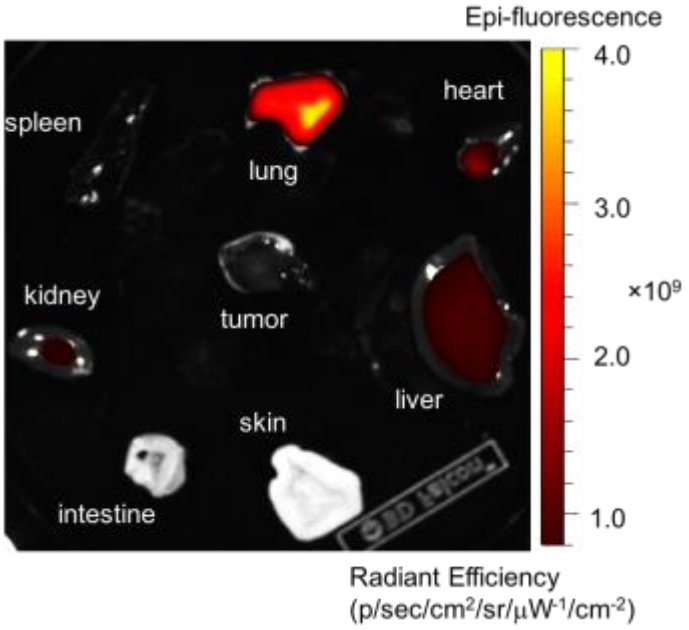



**Figure S5**. Ex vivo fluorescence imaging of organs excised from the mice injected with agent **3**.