Electronic Supplementary Material (ESI) for MedChemComm.

An Agent For Optical Imaging Of TrkC-Expressing, Breast Cancer

Supporting Information

Anyanee Kamkaew,[1,2] Feng Li,[3] Zheng Li,*[,3] and Kevin Burgess*[1]

[1] Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842, USA

[2] School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

[3] Center for Bioenergetics, Houston Methodist Research Institute, Houston, TX 77030, USA

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.

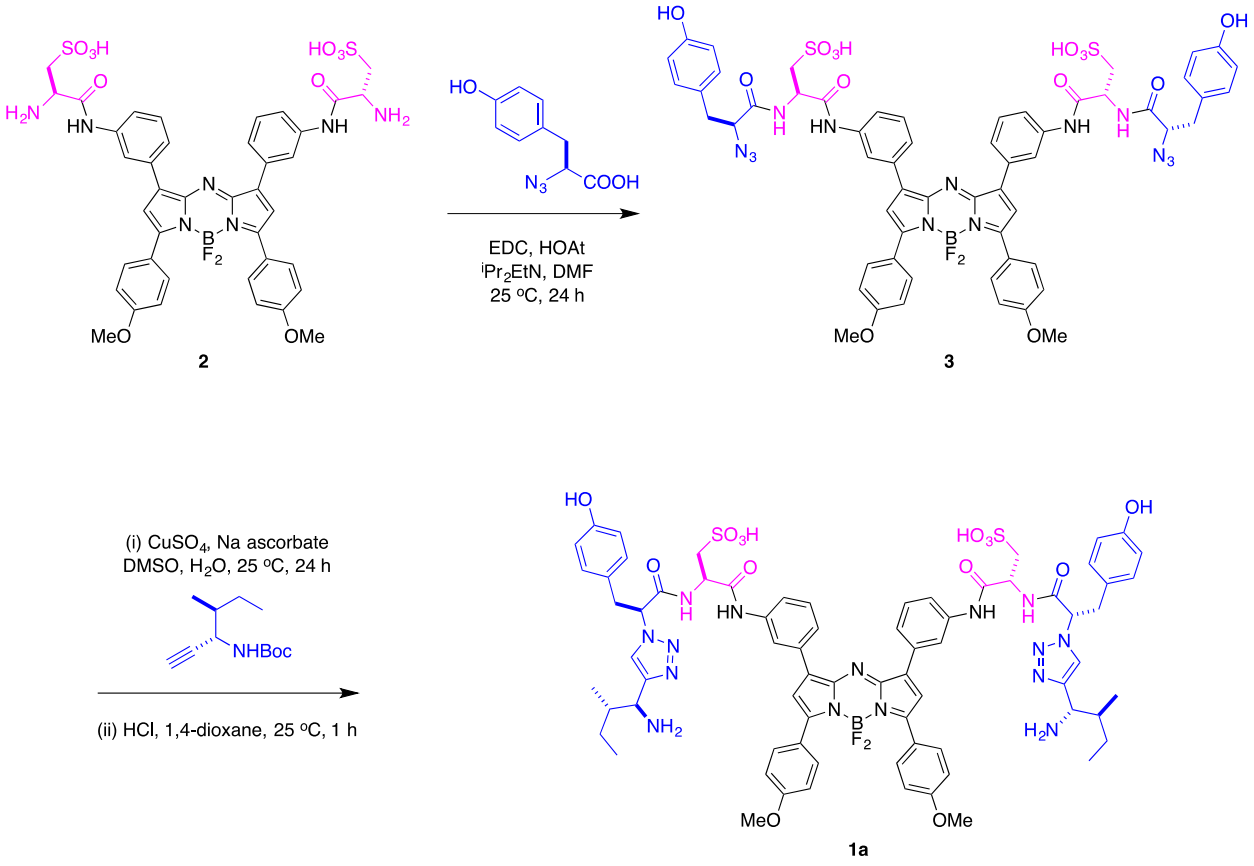
NMR spectra were recorded on a Bruker-400 MHz spectrometers (^1H at 400 MHz and ^{13}C at 100 MHz) at room temperature unless other mentioned. Chemical shifts of ^1H NMR spectra were recorded and chemical shifts are reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, CD_3OD 3.30 ppm, DMSO-d_6 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.

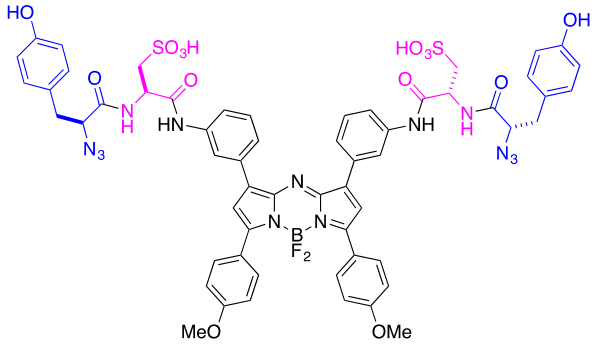
Throughout the confocal imaging studies, the laser used for excitation was a 633 nm HeNe, with an emission bandwidth of 700 – 750 nm.

Syntheses of Probes 1a and 1a-control



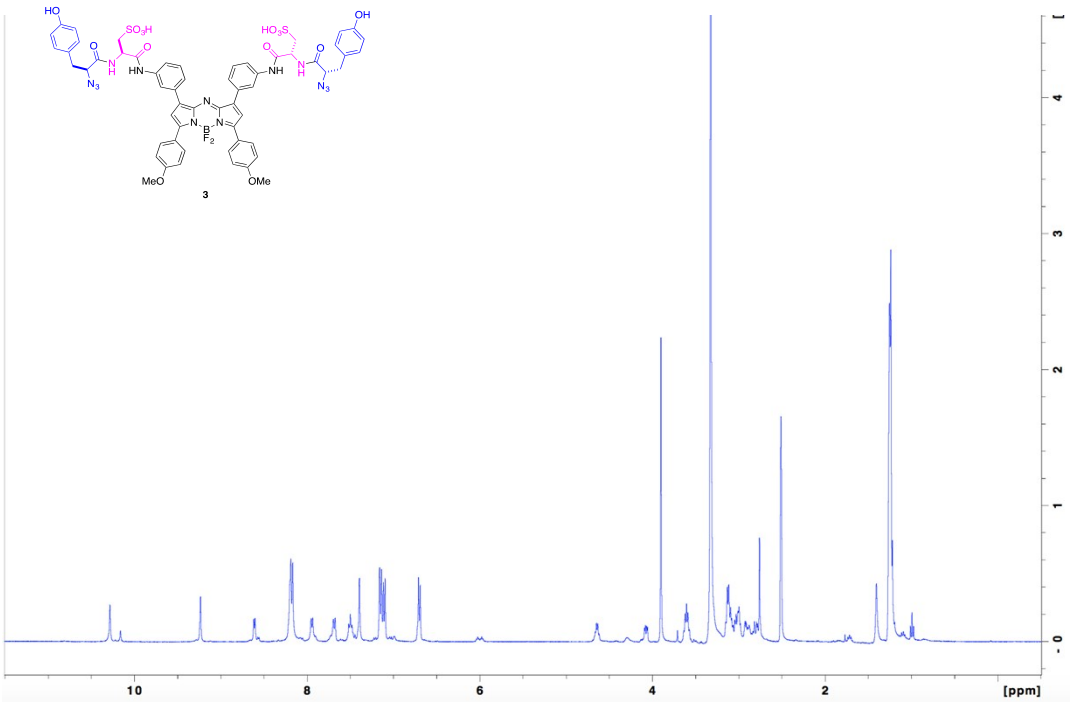
Scheme S1. Synthesis of **1a**.

*Synthesis of compound **3***

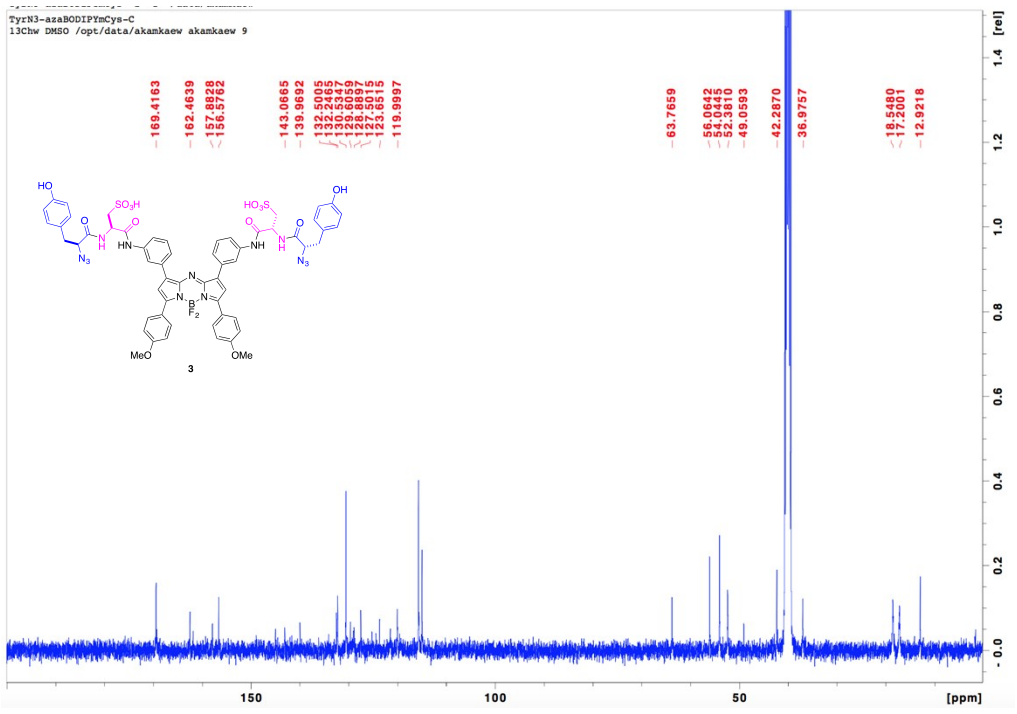


3

Tyrosine azide[1] (41 mg, 0.2 mmol) was dissolved in DMF (0.4 mL), then cooled to 0 °C. EDCI (41 mg, 0.22 mmol) and HOAt (20 mg, 0.21 mmol) were added to the solution. After stirring at 0 °C for 30 min, Compound **2**, synthesized according to previous report,[2] (35 mg, 0.04 mmol) was added to the above suspension followed by $^{i}Pr_2EtN$ (70 μL, 0.4 mmol). Then, the resulting solution was warmed to 25 °C and stirred for 24 h. Solvent was removed under reduced pressure. After that, the residue was purified by reverse phase MPLC using H_2O:CH_3CN (gradient) to afford **3** as a green powder (30 mg, 60 %). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 2H), 9.23 (s, 2H), 8.61 (d, J = 8.9 Hz, 2H), 8.61-8.60 (m, 6H), 8.17 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 7.6 Hz, 2H), 7.68 (t, J = 8.0 Hz, 2H), 7.51 (s, 2H), 7.16-7.09 (m, 6H), 6.69 (d, J = 8.8 Hz, 2H), 4.66-4.61 (m, 2H), 4.09-4.05 (m, 2H), 3.59 (s, 6H), 3.13-3.11 (m, 2H), 3.04-2.99 (m, 6H). ^{13}C (100 MHz, DMSO-d_6) δ 169.4, 162.5, 157.9, 156.6, 143.1, 140.0, 132.5, 132.3, 130.5, 129.6, 128.9, 127.5, 123.7, 120.0, 63.8, 56.1, 54.0, 52.4, 49.0, 42.3. ^{11}B NMR (128 MHz, $CDCl_3$) δ 0.88 (t, J = 32.3 Hz). HRMS (MALDI) calcd for $C_{58}H_{54}BF_2N_{13}NaO_{13}S_2$ $\{M+Na\}^+$ 1276.3364, found 1276.4642.

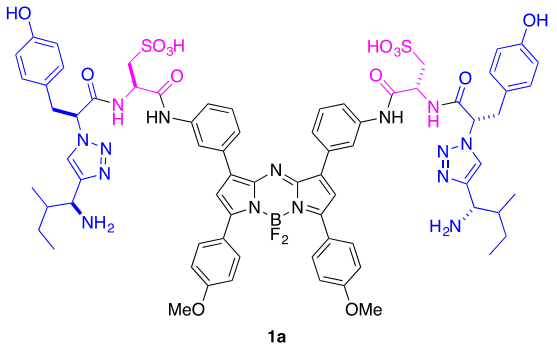


^{1}H-NMR of compound 3



^{13}C-NMR of compound 3

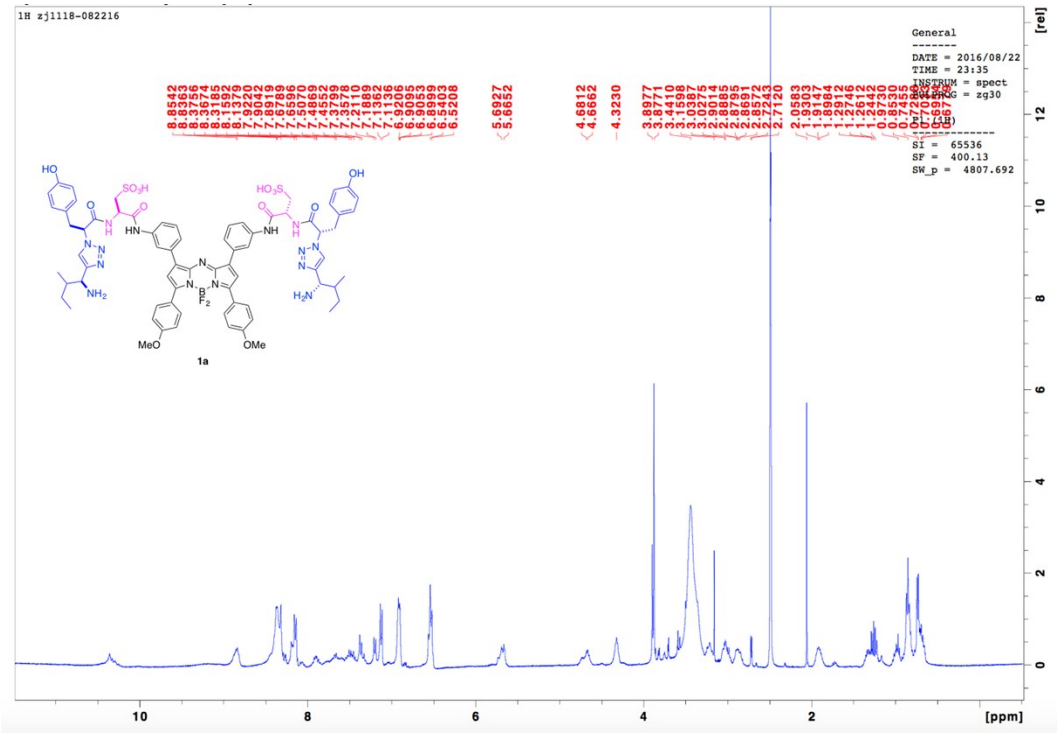
*Synthesis of targeting probe **1a***



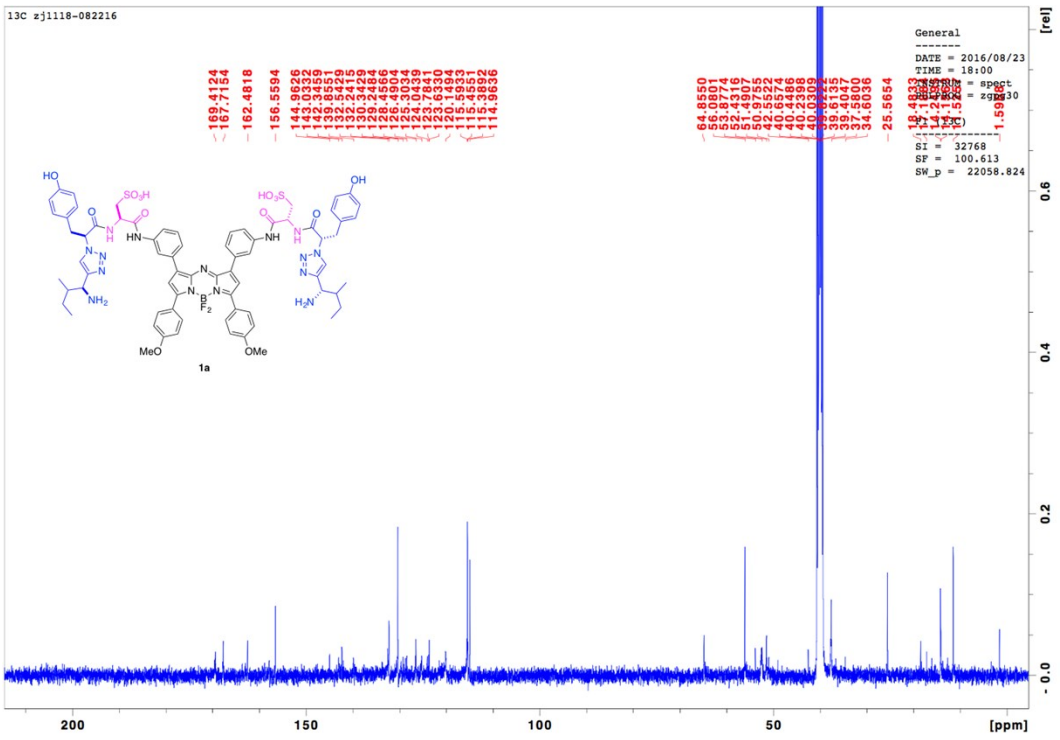
1a

3 (50 mg, 0.04 mmol) and Boc-Isoleucine alkyne[1] (20 mg, 0.09 mmol) were dissolved in DMSO (1 mL). Then, aqueous solution of $CuSO_4$ (0.1 M, 78 µL, 0.008 mmol) and sodium ascorbate (0.2 M, 156 µL, 0.032 mmol) were added to the mixture at 25 °C. The reaction was stirred at 25 °C for 24 h (monitored by C18-TLC using H_2O:CH_3CN (1:1) as solvents). Solvent was removed under reduced pressure. After that, the residue was purified by reverse phase MPLC using H_2O:CH_3CN (gradient) to afford **Boc-1a** as a green powder (39 mg, 58 %). Subsequently, **Boc-1a** (39 mg, 0.023 mmol) was dissolved in 1,4-dioxane (0.5 mL). Then HCl in 1,4-dioxane (2 M, 0.5 mL) was added into the solution. Reaction mixture was stirred at 25 °C for 1h, then solvent was removed under reduced pressure to give desired product **1a** as green solid quantitatively. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.32 (br, 2H), 8.85 (br, 2H), 8.37-8.33 (m, 6H), 8.15 (d, J = 8.5 Hz, 2H), 7.92-7.89 (m, 2H), 7.68-7.46 (m, 4H), 7.37 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 9.0 Hz, 2H), 6.91 (d, J = 7.2 Hz, 4H), 6.53 (d, J = 7.8 Hz, 4H), 5.69-5.67 (m, 2H), 4.68-4.67 (m, 2H), 4.32 (br, 2H), 3.89 (s, 6H), 3.44-3.31 (m, 2H), 3.04-2.90 (m, 2H), 2.89-2.86 (m, 2H), 1.93-1.90 (m, 2H), 1.29-1.24 (m, 4H), 1.05-0.90 (m, 2H), 0.89-0.75 (m, 6H), 0.74-0.68 (m, 6H). ^{13}C (100 MHz, DMSO-d_6) δ 169.4, 167.7, 162.5, 156.6, 145.0, 143.0, 142.3, 139.9, 132.5, 132.2, 130.3, 129.2, 128.5, 126.5, 125.3, 124.0, 123.8, 123.6, 120.1, 115.6, 115.5, 115.4, 115.0, 64.9, 56.1, 53.9, 52.4, 51.5, 51.0, 42.6, 37.6, 34.6, 25.6, 18.5, 17.2, 14.2, 14.1, 11.5.

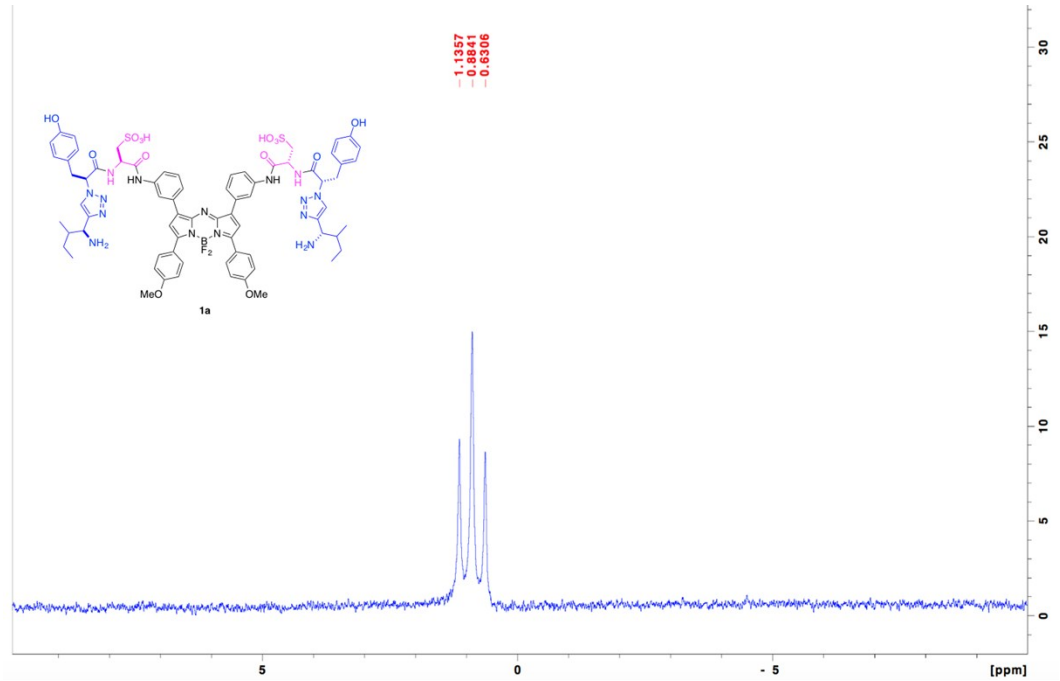
^{11}B NMR (128 MHz, $CDCl_3$) δ 0.88 (t, J = 32.3 Hz). HRMS (MALDI) calcd for $C_{72}H_{77}BF_2N_{15}O_{14}S_2$ {M-H}$^-$ 1488.5277, found 1488.6775.



^{1}H-NMR of compound 1a

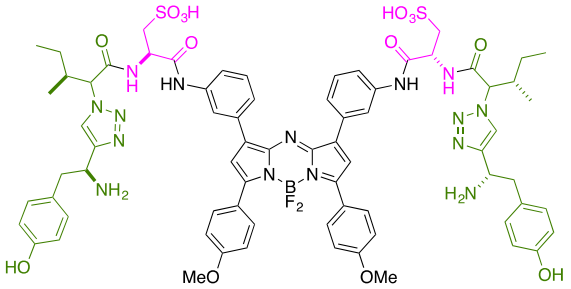


^{13}C-NMR of compound 1a



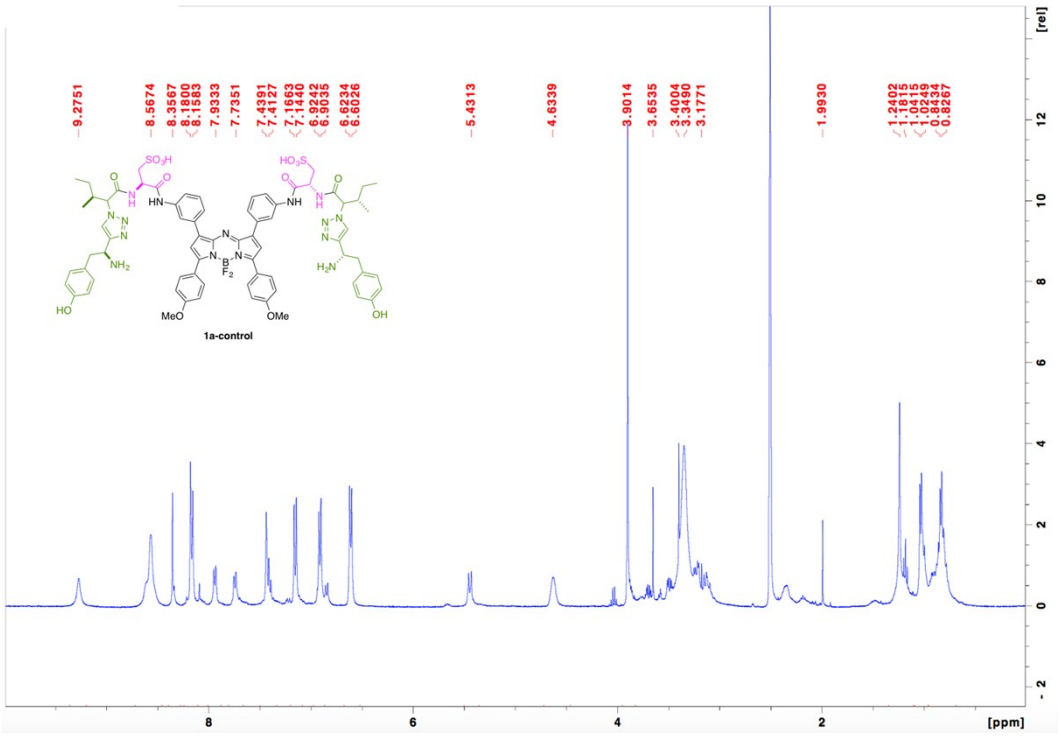
^{11}B-NMR of compound 1a

*Synthesis of **1a-control***

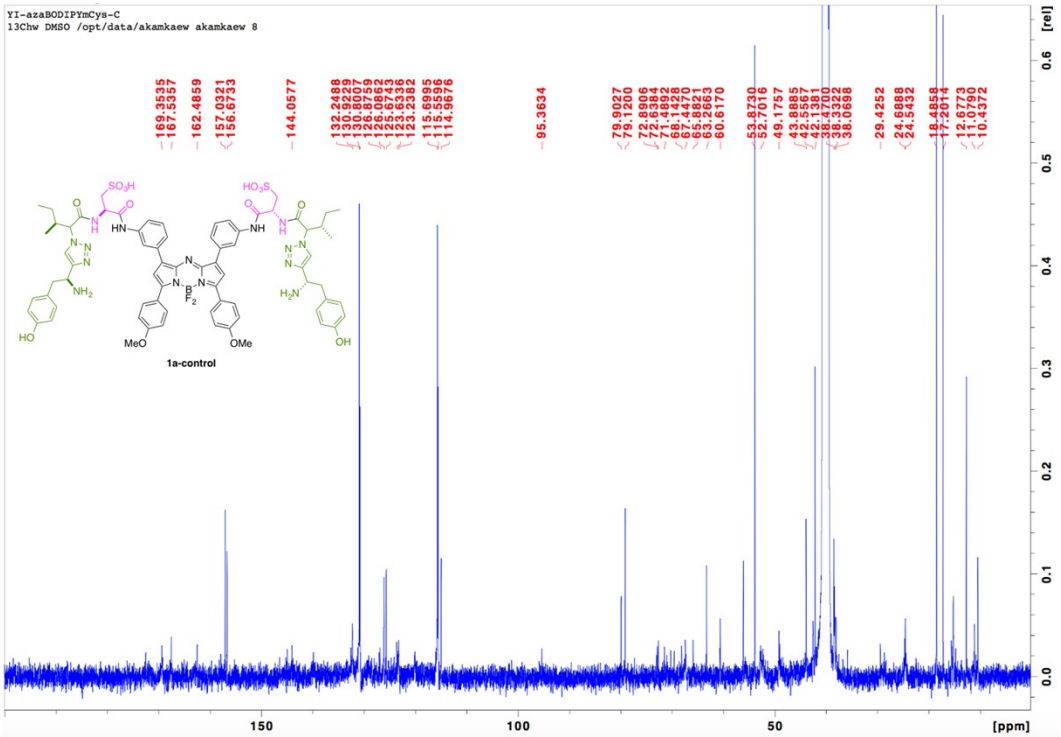


1a-control

1a-control was synthesized according the same procedure as **1a** by using isoleucine azide[1] and Boc-tyrosine alkyne[1] as precursors. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.28 (br, 2H), 8.57 (br, 6H), 8.36 (s, 2H), 8.17 (d, *J* = 8.7 Hz, 2H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 2H), 7.44-7.41 (m, 4H), 7.15 (d, *J* = 8.9 Hz, 4H), 6.91 (d, *J* = 8.3 Hz, 4H), 6.61 (d, *J* = 8.3 Hz, 4H), 5.43 (d, *J* = 7.8 Hz, 2H), 4.63 (br, 2H), 3.90 (s, 6H), 3.67-3.64 (m, 2H), 3.43-3.41 (m, 2H), 3.10-2.98 (m, 2H), 2.34-2.28 (m, 2H), 1.18-1.24 (m, 9H), 1.04-1.02 (m, 6H), 0.84-0.82 (m, 12H). ^{13}C (100 MHz, DMSO-d_6) δ 169.4, 167.5, 162.5, 157.0, 156.7, 145.2, 144.1, 132.3, 130.9, 130.8, 126.9, 126.1, 125.6, 123.6, 123.2, 120.1, 115.7, 115.6, 115.0, 95.4, 80.0, 79.1, 72.9, 72.6, 71.5, 68.1, 67.4, 65.9, 63.3, 60.6, 53.9, 52.7, 49.2, 43.9, 42.6, 42.1, 38.5, 38.3, 29.4, 24.7, 24.5, 18.5, 17.2, 12.7, 11.1, 10.4. ^{11}B NMR (128 MHz, $CDCl_3$) δ 0.90 (t, *J* = 32.0 Hz). HRMS (MALDI) calcd for $C_{72}H_{78}BF_2N_{15}NaO_{14}S_2$ $\{M+Na\}^+$ 1512.5253, found 1512.5082.

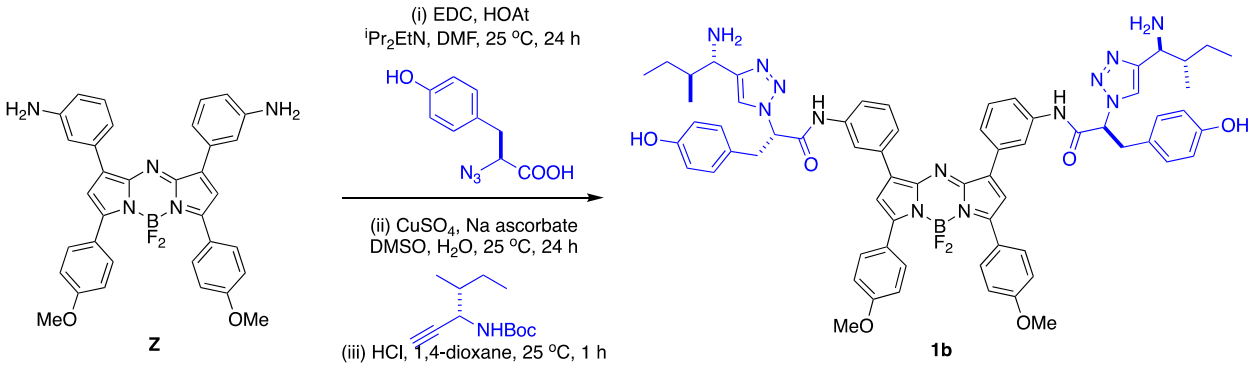


^{1}H-NMR of compound 1a-control



^{13}C-NMR of compound 1a-control

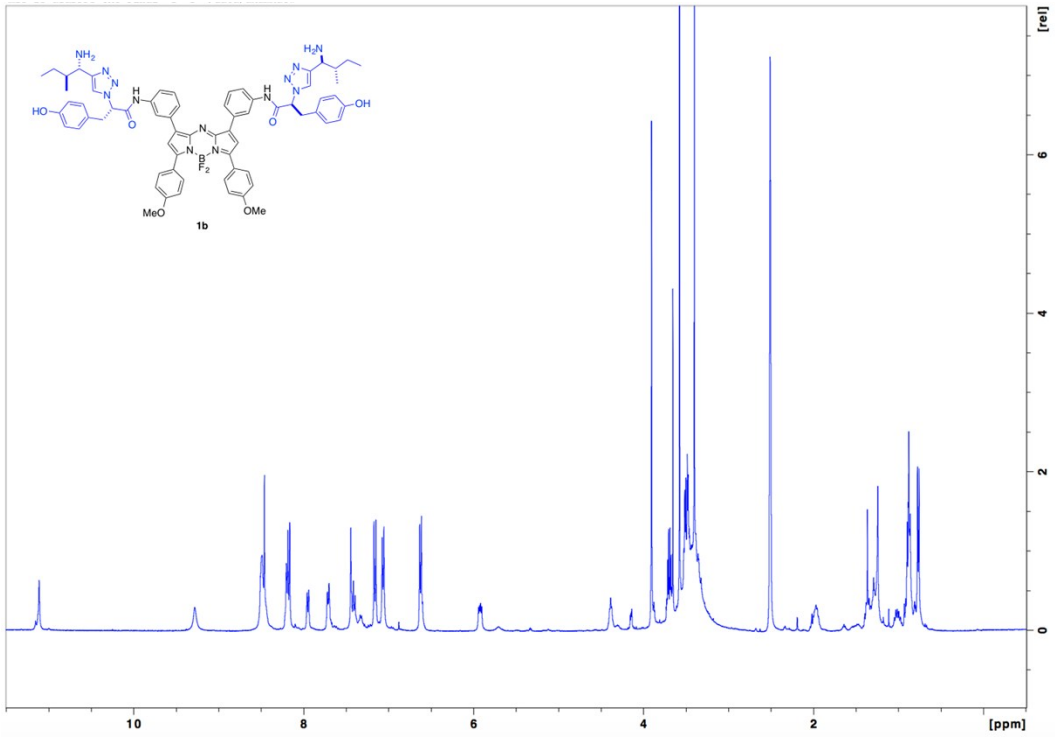
Synthesis of probe 1b



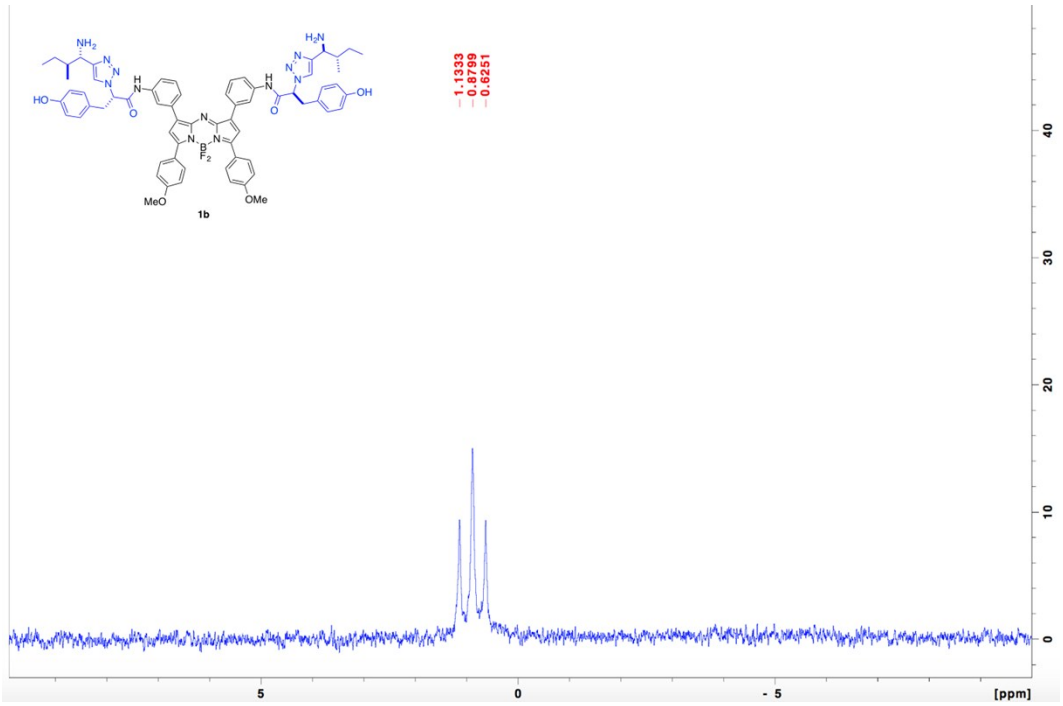
Scheme S2. Synthesis of **1b**.

EDCI (210.9 mg, 1.10 mmol) and HOAt (152.4 mg, 1.12 mmol) were added to a suspension of azido tyrosine[1] (207.2 mg, 1.00 mmol) in 4 mL DMF at 0 °C. After stirring at 0 °C for 30 min, **Z** (117.2 mg, 0.127 mmol) was added to the above suspension followed by $^{i}Pr_2EtN$ (557 µL, 413.6 mg, 3.20 mmol). 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 (98:2, v/v) afforded the desired azide compound (99.1 mg, 81%) as green solid. The resulting product (38.6 mg, 0.04 mmol) was then mixed with Boc-isoleucine alkyne[1] (40 mg, 0.18 mmol) in DMSO (1 mL). Then, aqueous solution of $CuSO_4$ (0.1 M, 78 µL, 0.008 mmol) and sodium ascorbate (0.2 M, 156 µL, 0.032 mmol) were added to the mixture at 25 °C. The reaction was stirred at 25 °C for 24 h (monitored by TLC). Solvent was removed under reduced

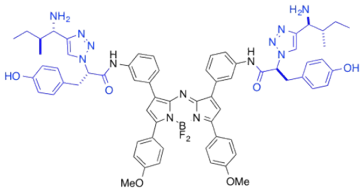
pressure. The residue was purified by flash silica chromatography eluting with CH_2Cl_2:MeOH (98:2) to yield 36.1 mg of **Boc-1b** (65 %) as a green powder. After removing a protecting group, **1b** was obtained quantitatively as a green solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 2H), 9.27 (s, 2H), 8.54-8.45 (m, 6H), 8.20 (s, 2H), 8.17 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 2H), 7.44-7.39 (m, 4H), 7.16 (d, *J* = 8.9 Hz, 4H), 7.06 (d, *J* = 8.3 Hz, 4H), 6.62 (d, *J* = 8.3 Hz, 4H), 5.95-5.90 (m, 2H), 4.39-4.37 (m, 2H), 3.90 (s, 6H), 3.73-3.70 (m, 4H), 2.0-1.8 (m, 2H), 1.36-1.24 (m, 9H), 0.94-0.90 (m, 2H), 0.85-0.89 (m, 6H), 0.76 (d, *J* = 7.2 Hz, 6H). ^{11}B NMR (128 MHz, $CDCl_3$) δ 0.88 (t, *J* = 32.5 Hz). HRMS (MALDI) calcd for $C_{66}H_{67}BF_2N_{13}O_6$ {M-H}$^-$ 1186.5398, found 1186.1261.

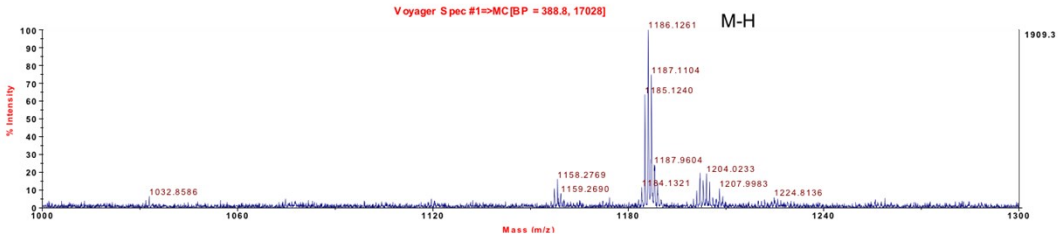


^{1}H-NMR of compound 1b



^{11}B-NMR of compound 1b





HRMS-MALDI of compound 1b

MTT Cell Viability Assays with 1a in 4T1 cells

4T1 cells (5,000-7,000 cells/well, 50 μL in completed Dulbecco's Modified Eagle Medium (DMEM, Sigma Chemical, St. Louis, MO) were plated on 96-well plates and allowed to adhere at 37 °C in 5 % CO_2 and 95 % air for 24 h. Thereafter, the cells were treated with 50 μL aliquot of each test compounds in cell medium at different concentrations, ranging from 0-25 μM. The cells were then incubated for 24 h. The cell's viability was assessed through an MTT conversion assay.[3] Briefly, 20 μL of MTT (5mg/mL, in Hank's balanced salt solution, HBSS) were added and the cells were incubated for an additional 3 h. Afterwards, the media were replaced with 100 μL dimethyl sulfoxide (DMSO) to solubilize the blue crystals. The plate was shaken for 15 min at room temperature before measuring the optical density (OD) at 570 nm using SpectraMax Plus 384 microplate reader. Cell viability (%) = (mean of OD of treatment group/mean of OD of control group) × 100.

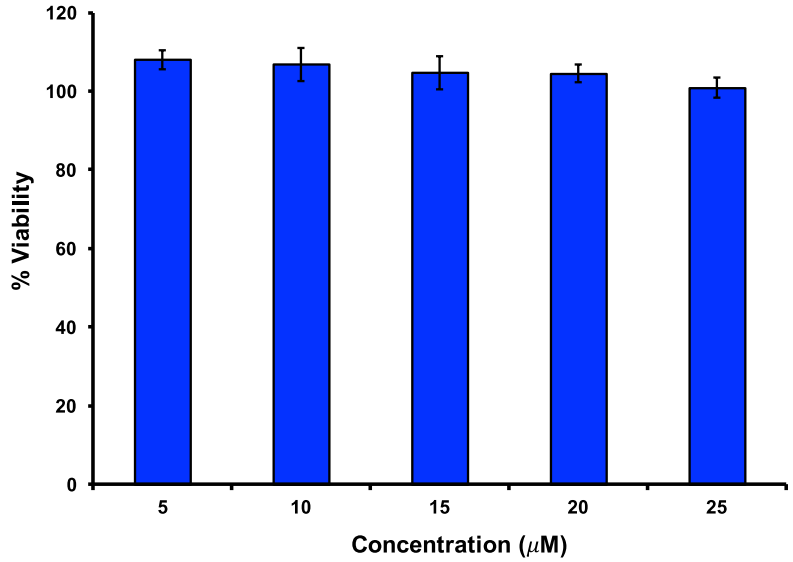


Figure S1. Cell viability of **1a** in 4T1 cells. Targeting probe **1a** does not cause toxicity to 4T1 cells up to concentration at 25 μM.

Histochemistry of anti-TrkC on human breast tissue array

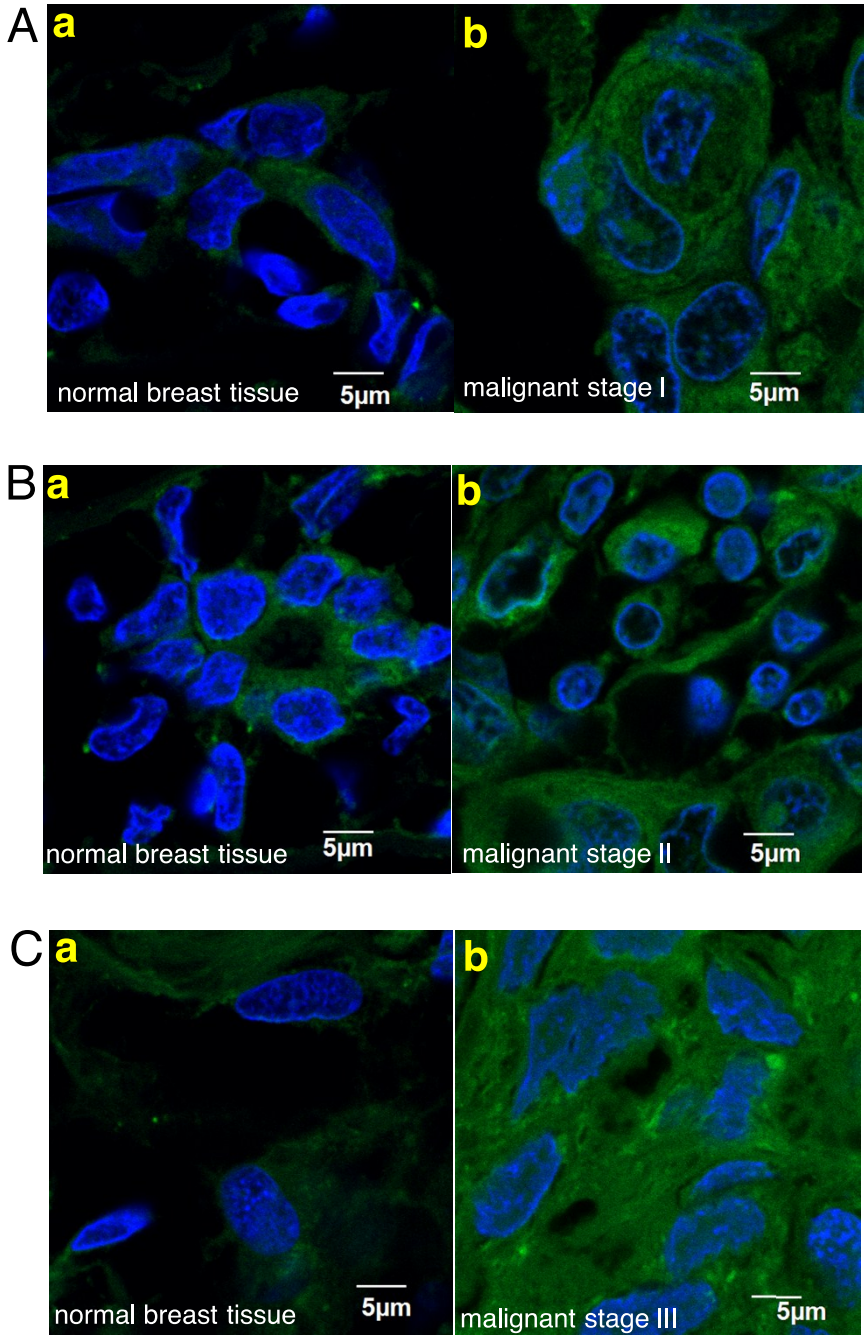


Figure S2. Histochemistry on human breast tissue array. Fluorescently labeled TrkC mAb also stained the normal tissue (**a**); but much brighter staining was observed on malignant breast cancer tissue (**b**) (example from 3 cases).

References

1. Angell, Y.; Chen, D.; Brahimi, F.; Saragovi, H. U.; Burgess, K. A Combinatorial Method for Solution-Phase Synthesis of Labeled Bivalent β-Turn Mimics. *J. Am. Chem. Soc.* **2008,** *130*, 556-565

2. Kamkaew, A.; Burgess, K. Aza-BODIPY Dyes with Enhanced Hydrophilicity. *Chem. Comm.* **2015,** *51*, 10664-10667.

3. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65* (1-2), 55-63.