

Supporting Information

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General Methods:

All reagents and buffer components were purchased from Sigma-Aldrich, Fisher Scientific, Alfa Aesar, TCI, or Wako Chemicals without further purification. TLC analysis (F-254) were performed with 60 Å silica gel from Merck. ¹H and ¹³C NMR spectra were measured on either a JEOL AL300 (300 MHz) or AL400 (400 MHz) instrument with the solvent peaks as internal standards. High-resolution mass spectroscopy (HRMS) was carried out on a Bruker MicroTOF-QIII electrospray ionization time-of-flight (ESI-TOF) mass spectrometer.

HPLC Methods:

Reverse-phase HPLC was used with a Shimadzu system (Kyoto, Japan), consisting of two LC-20AP pumps and a SPD-20AV photodiode array detector. Employed columns were the semi-preparative 10 × 250 mm Cosmosil 5C₁₈-Ar-300 and analytical 4.6 × 250 mm Cosmosil 5C₁₈-Ar-300 from Nacalai Tesque (Kyoto, Japan). Samples were eluted using a combination of mobile phases A (100% H₂O), B (100% acetonitrile), C (H₂O with 0.1% TFA), and D (acetonitrile with 0.1% TFA). For absorbance, the detector was typically set to 214 and 254 nm. For fluorometric HPLC analysis, EDANS-based fluorescence was set at wavelengths of $\lambda_{EX} = 340 \text{ nm}/\lambda_{EM} = 490 \text{ nm}$, while TAMRA-based fluorescence was set at wavelengths of $\lambda_{EX} = 560 \text{ nm}/\lambda_{EM} = 585 \text{ nm}$. The different elution methods that were employed for purification are shown in Table S1.

Table S1. Gradient profiles for HPLC analysis and purification

	Flow rate (ml/min)	Time (min)	%A (100% H ₂ O)	%B (100% ACN)	%C (H ₂ O with 0.1% TFA)	%D (ACN with 0.1% TFA)
<u>Method 1</u> (analytical)	1.0	0			95	5
		10			95	5
		40			30	70
		45			95	5
<u>Method 2</u> (semiprep)	3.5	0			95	5
		10			95	5
		40			30	70
		45			95	5
<u>Method 3</u> (analytical)	1.0	0			98	2
		10			98	2
		22			60	40
		27			10	90
		32			98	2
		35			98	2
<u>Method 4</u> (analytical)	1.0	0			99	1
		10			99	1
		50			60	40
		55			99	1
		60			99	1
<u>Method 5</u> (semiprep)	3.5	0			75	25
		8			75	25
		35			40	60
		40			75	25
<u>Method 6</u> (semiprep)	3.5	0	95	5		
		8	95	5		
		30	20	80		
		35	95	5		
		40	95	5		

Reaction Profiles of Probe 1 by HPLC:

The reaction profiles and major product yields of glycine propargyl ester **1** with various biological amines (putrescine, spermidine, spermine, L-lysine, and norepinephrine) were obtained. In each mixture, amine and ester (0.04 mmol each) were dissolved in 200 μ l of either 1:1 PBS buffer/dioxane (Figure S1 and S2), 1:1 DMEM media/dioxane (Figure S3), or 1:1 rat serum/dioxane (Figure S3). Normal rat serum and DMEM cell media was purchased from Wako Chemicals (Japan). Following room temperature incubation for 1 hour, an initial analytical run (HPLC Method 1) was carried out. A summary of the HPLC traces are shown in Figures S1, S2, and S3. Subsequent purification runs (HPLC Method 2) were then carried out to isolate each major peak. Accounting for the TFA salts, the yields of each major peak are displayed in Table 1 of the manuscript.

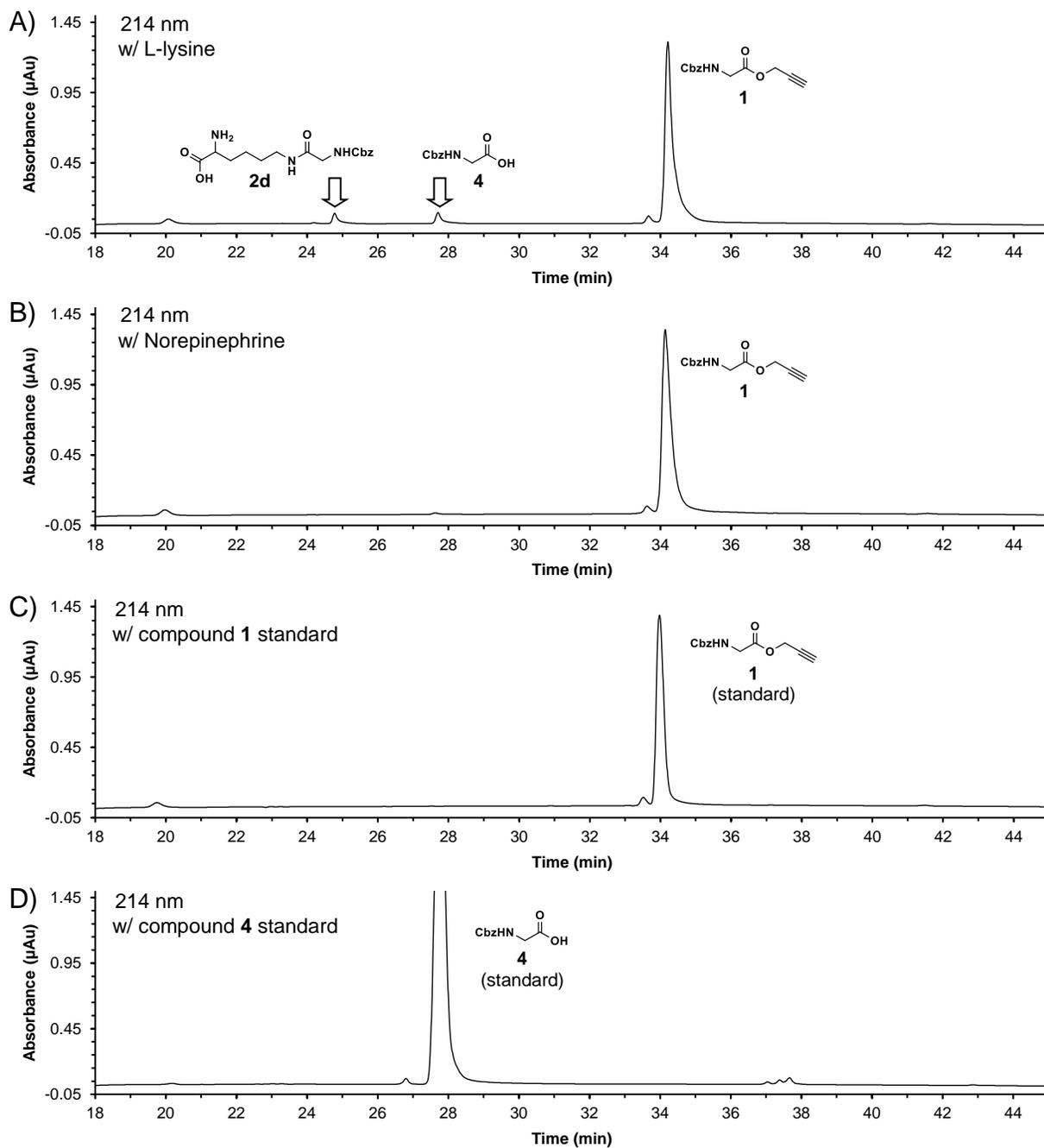


Figure S1. HPLC chromatograms (with method 1) of reaction mixtures in 1:1 PBS buffer/dioxane between probe **1** incubated with A) L-lysine, and B) Norepinephrine. Standard runs of C) probe **1**, and D) hydrolysis by-product **4** are also shown. Absorbance measured at 214 nm.

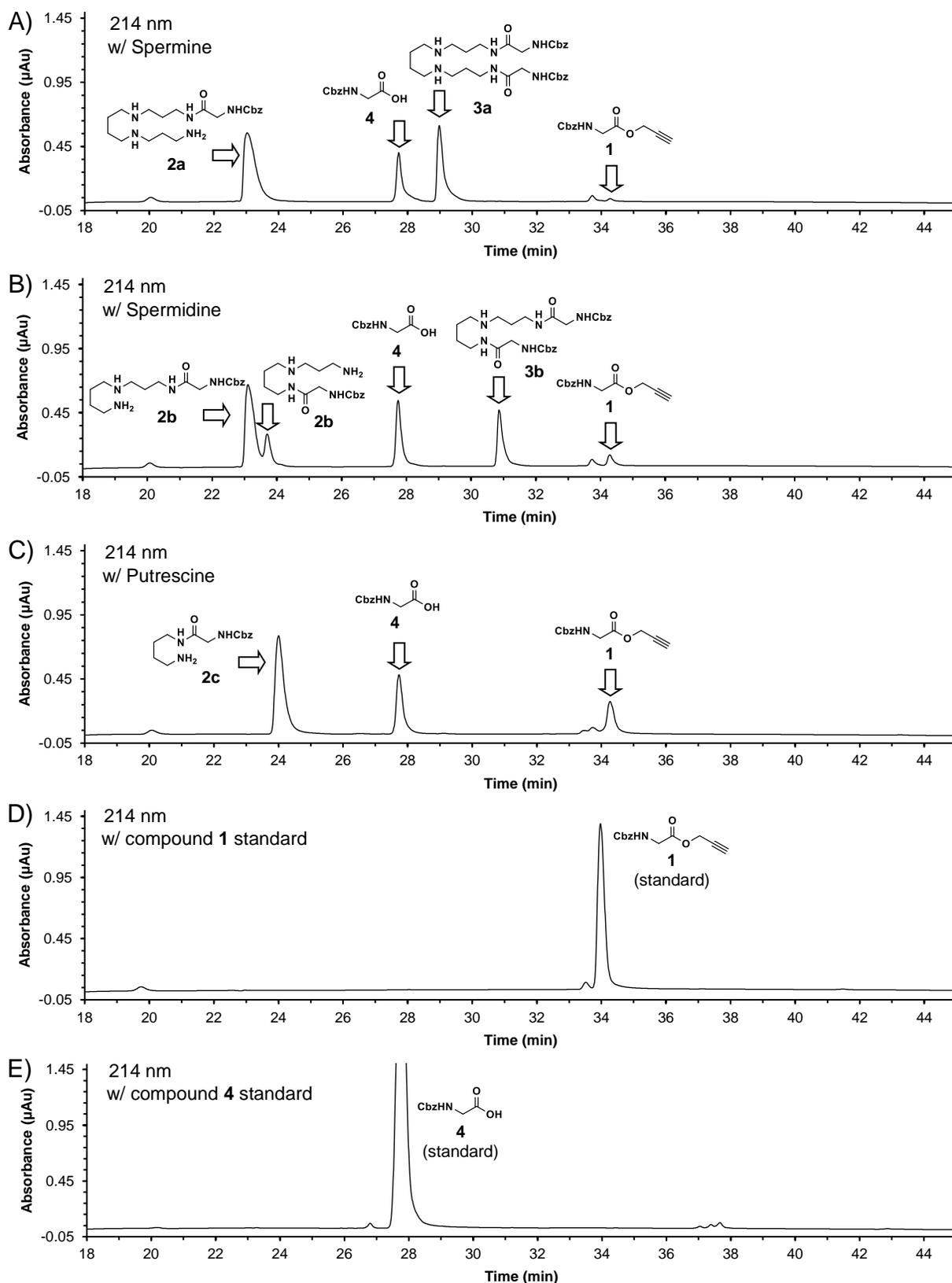


Figure S2. HPLC chromatograms (with method 1) of reaction mixtures in 1:1 PBS buffer/dioxane between probe 1 incubated with A) Spermine, B) Spermidine, and C) Putrescine. Standard runs of D) probe 1, and E) hydrolysis by-product 4 are also shown. Absorbance measured at 214 nm.

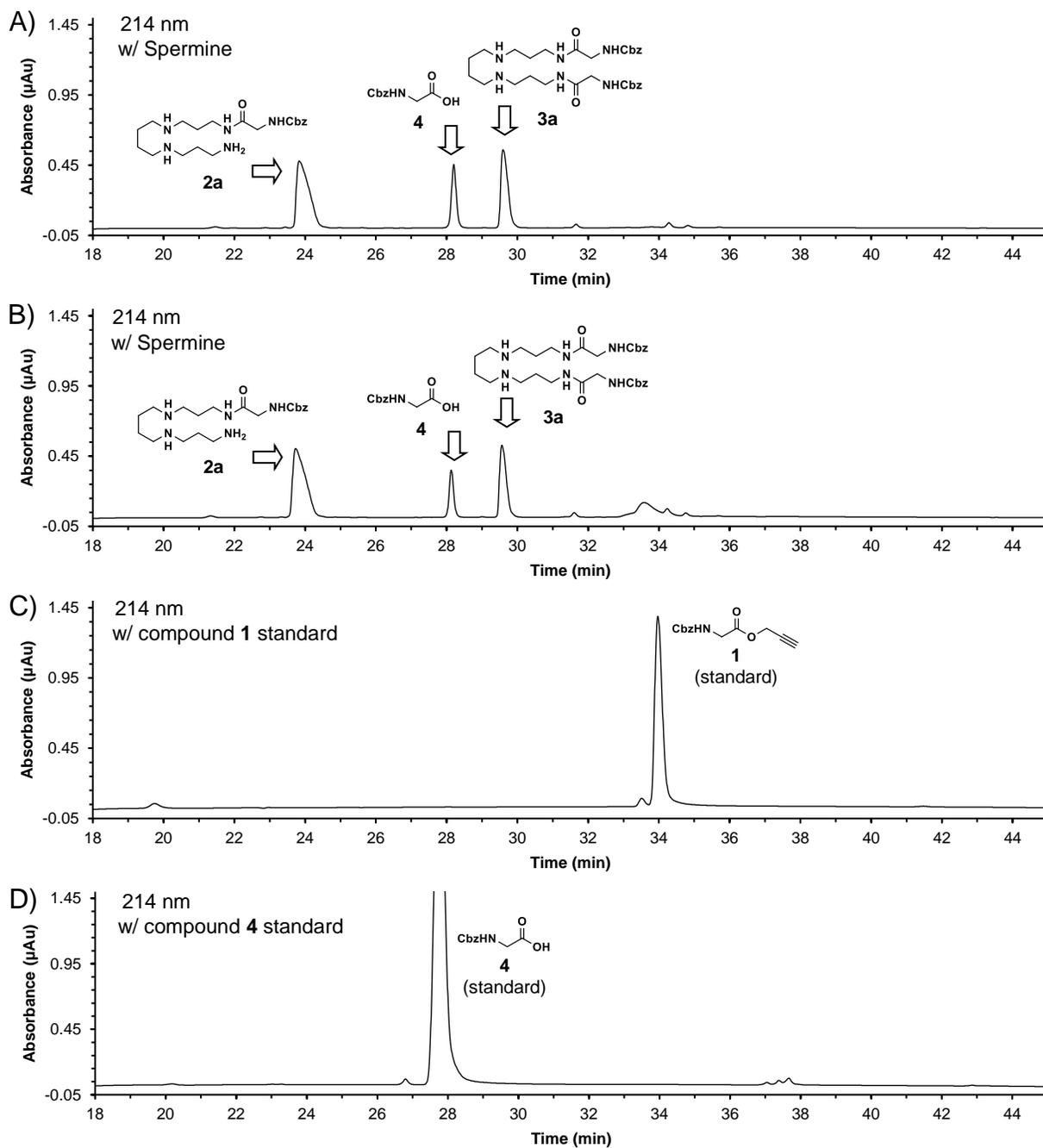


Figure S3. HPLC chromatograms (with method 1) of reaction mixtures between probe **1** incubated with Spermine in A) 1:1 DMEM cell culture media/dioxane, and B) 1:1 rat serum/dioxane. Standard runs of C) probe **1**, and D) hydrolysis by-product **4** are also shown. Absorbance measured at 214 nm.

Identification (by FRET assay) and Reaction Profiles (by HPLC) of Probe 9:

To identify the biological amine substrate scope of glycine propargyl esters, a FRET-based assay utilizing probe **9** was employed. The biological amines under study include commercial sources of human serum albumin, L-arginine, L-histidine, L-lysine, putrescine, spermidine, spermine, epinephrine, histamine, dopamine, phenethylamine, and sphingosine. The assay was performed using 96-well microtiter plates, where fluorescence ($\lambda_{\text{EX}} = 340 \text{ nm}$, $\lambda_{\text{EM}} = 490 \text{ nm}$) was monitored at specific time intervals (0, 15, 30, 60, 120, 180, and 360 min) using a JASCO FP-6500 Spectrofluorometer equipped with a JASCO FMP-963 Microplate reader. In each reaction well, 10 μl of FRET probe **9** and 10 μl of amine are dissolved in 80 μl of 1:1 PBS/DMSO buffer (100 μl total volume) at room temperature. In all cases, the final concentration of FRET probe **9** was 100 μM . The reaction was initiated with the addition of variable final concentrations of amine (0.5, 1.0, 2.0 mM). All data points are from triplicate experiments. Full results are shown in Figure S4.

Following the FRET-based amine reactivity assay, three reaction mixtures of interest (ones containing L-lysine, L-arginine, and spermine, respectively) were taken for fluorometric HPLC analysis in order to identify the generated reaction products. HPLC Method 3 was used and a summary of the acquire HPLC chromatograms are shown in Figure S5.

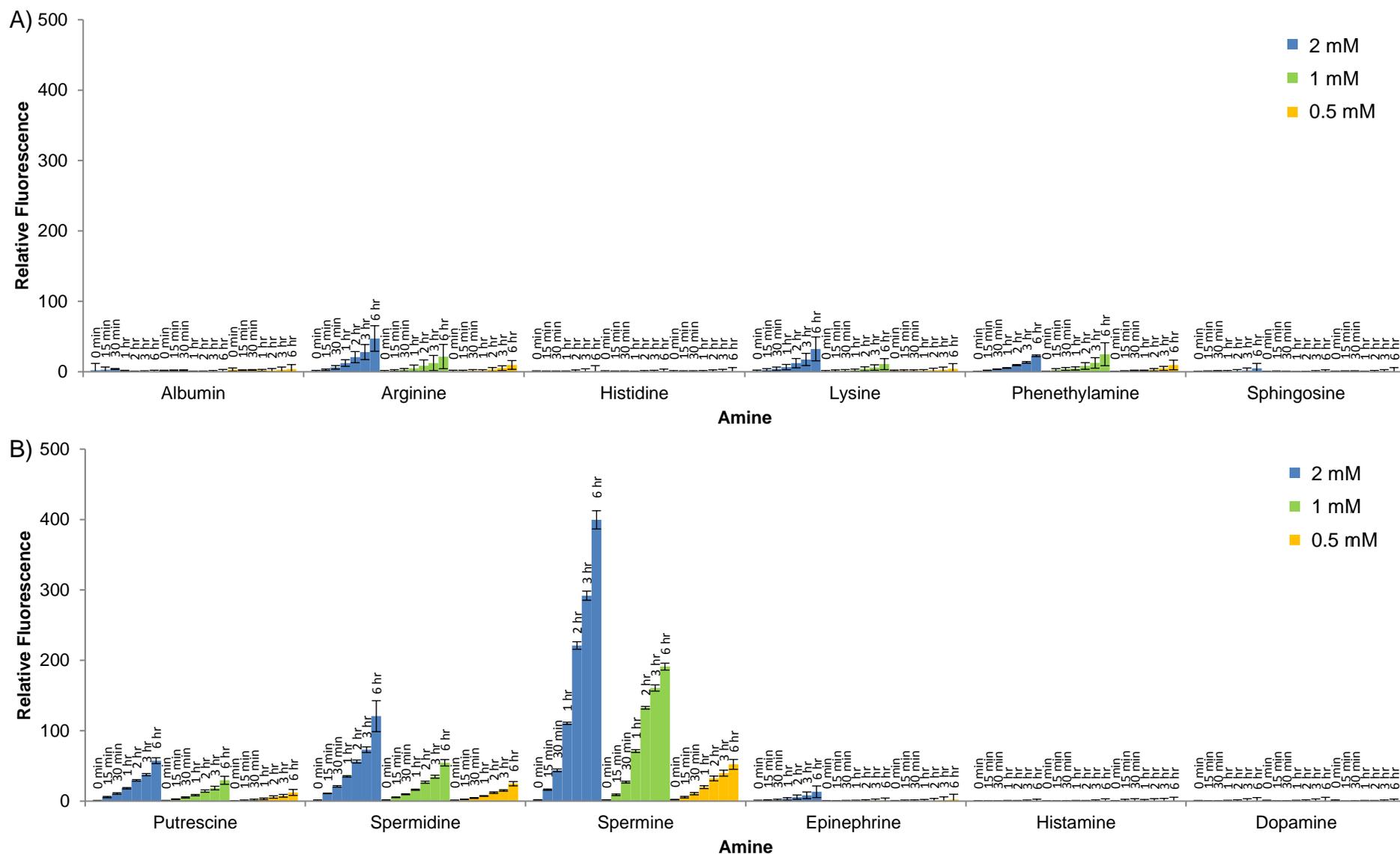


Figure S4. Full time profile of FRET-based probe **9** (100 μ M) reactivity with various biological amines through fluorescence observation. Amines used in this study include A) Human Serum Albumin, L-arginine, L-histidine, L-lysine, Phenethylamine, Sphingosine, and B) Putrescine, Spermidine, Spermine, Epinephrine, Histamine, Dopamine. Colours used to represent amine concentrations are shown as blue (2 mM), green (1 mM), and yellow (0.5 mM) bars.

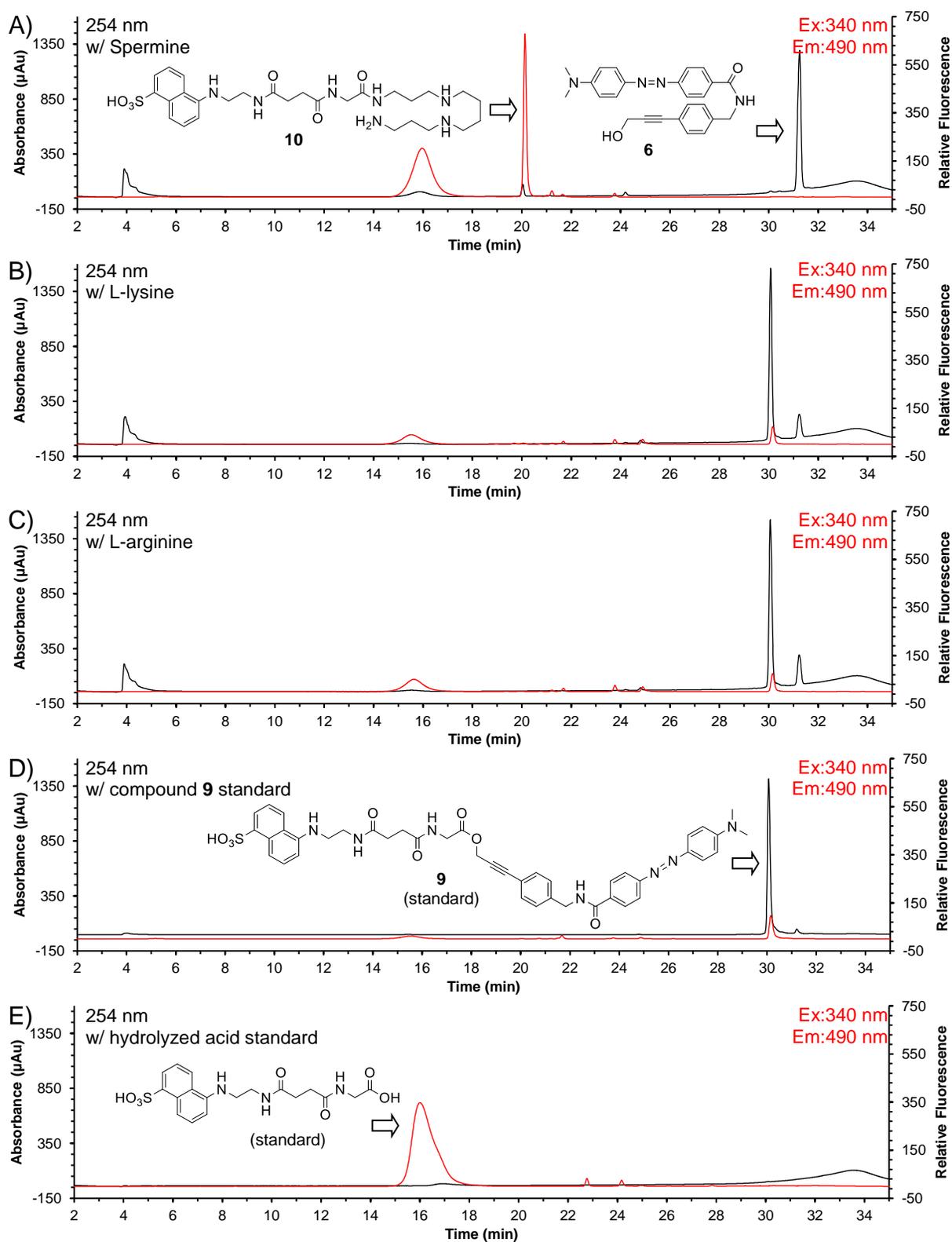
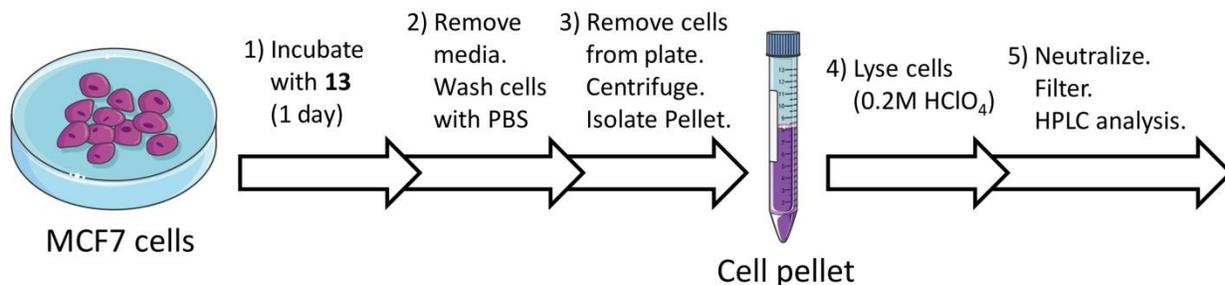


Figure S5. HPLC chromatograms (with method 3) of reaction mixtures between FRET-based compound **9** incubated with either A) Spermine, B) L-lysine, C) L-arginine, and D) PBS buffer as a control. E) Standard run of hydrolysis by-product is also shown. Absorbance (shown in black; left y-axis) is measured at 254 nm, while fluorescence (shown in red; right y-axis) is measured at $\lambda_{\text{EX}} = 340 \text{ nm}$, $\lambda_{\text{EM}} = 490 \text{ nm}$.

Metabolite Study of Probe 13 by HPLC:



MCF7 cells were grown on a 5 cm plate until 50-70% confluence. Fresh DMEM media (5 ml) containing 2.2 mM of compound **13** was then filtered and added. Following incubation overnight, the media was then removed and cells were washed with PBS Buffer pH 7.4 (2× 2 ml). Cells were removed by addition of trypsin (0.5 ml) and the cell pellet was obtained by centrifugation (10,000 rpm for 10 minutes). The cell pellet was then resuspended in perchloric acid (200 µl of 0.2M solution). Following centrifugation (10,000 rpm for 10 minutes), the supernatant was then neutralized with 1M K₂HPO₄ and filtered for HPLC analysis. Analytical runs were carried out with HPLC Method 4. A summary of HPLC traces with corresponding standards are shown in Figures S6 and S7.

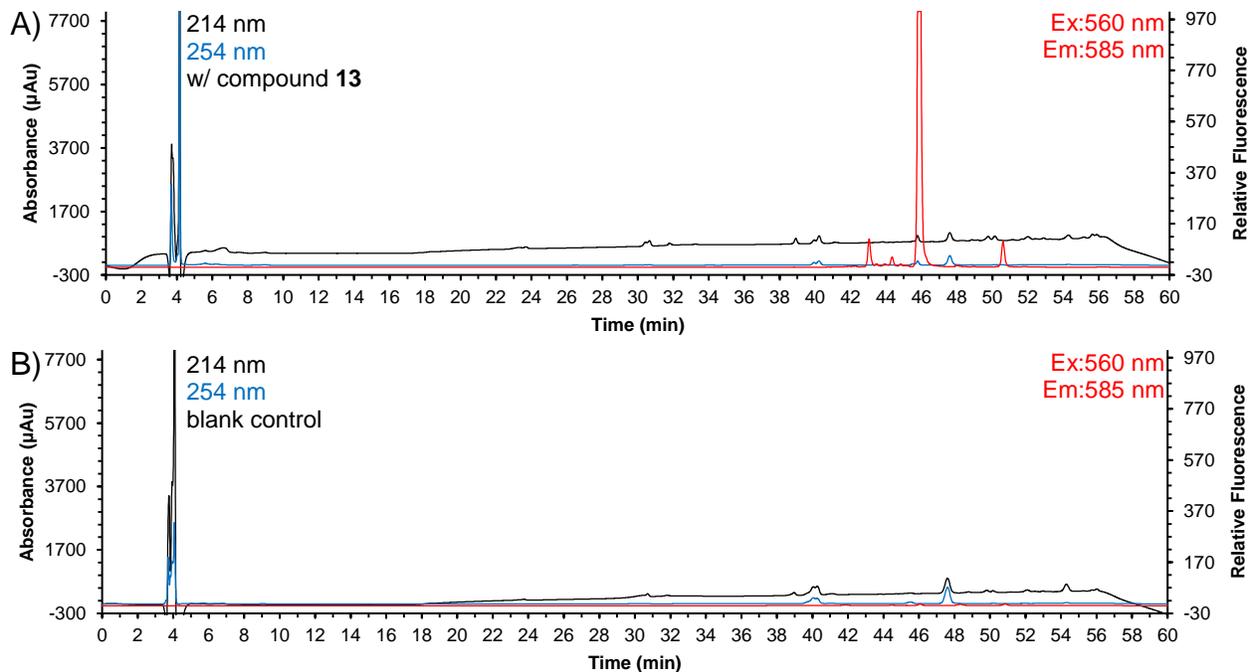


Figure S6. HPLC analysis of the intracellular metabolites extracted from MCF7 cells that were incubated with A) compound **13**, or B) DMEM media control. Cells were grown overnight before the metabolite extraction protocol was performed. On the HPLC chromatograms, relative absorbance (214nm shown in black, 254nm shown in blue) is displayed on the left Y-axis, while relative fluorescence ($\lambda_{\text{EX}}=560\text{nm}/\lambda_{\text{EM}}=585\text{nm}$; shown in red) is displayed on the right Y-axis.

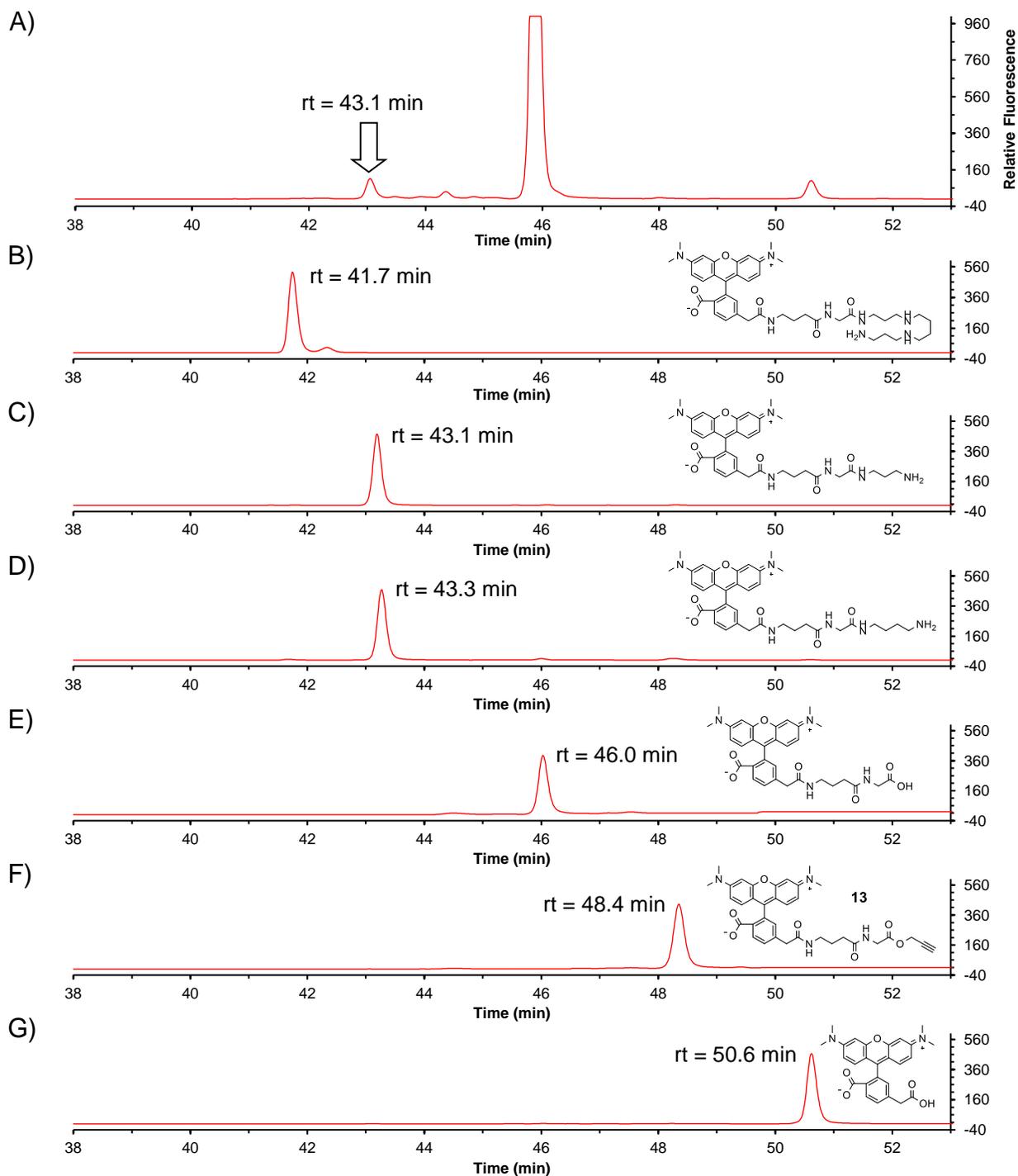


Figure S7. A) HPLC analysis (magnified view) of the intracellular metabolites extracted from MCF7 cells that were incubated with compound **13**. Product standards run in conjunction include B) Spermine-TAMRA product, C) trimethylenediamine-TAMRA product, and D) Putrescine-TAMRA product. Other standards used include E) hydrolysis by-product, F) compound **13**, and G) TAMRA by-product. On the HPLC chromatograms, relative fluorescence ($\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$; shown in red) is displayed on the right Y-axis.

Cell Culture:

The cell lines MCF7, MCF10A, MDA-MB231, and SK-BR-3 were purchased from American Type Culture Collection (ATCC), Manassas, VA, USA. The normal lymphocyte cell line was extracted from blood samples donated by Dr. Tomonori Tanei. All cells were grown under 5% CO₂ conditions at 37°C in DMEM (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Cellgro, Manassas, VA) and 1% penicillin/streptomycin.

Imaging Studies:

Cells under study were plated onto 4-well chamber slides at a density of 10⁵ cells per well and grown overnight. Cells were incubated for 10 minutes following addition of compound **13** (final concentrations of 10 or 30 μM diluted in media). For sample preparation, cells were then washed with PBS Buffer + 2% FBS (3 times), followed by DAPI staining, and formalin fixation. Imaging of cells under study were performed with a Laser Scanning Confocal Microscope LSM 710 at 400× magnification (Carl Zeiss MicroImaging Inc., Thornwood, NY). For TAMRA-dye observation, fluorescence was measured at $\lambda_{EX}=560\text{nm}$ / $\lambda_{EM}=585\text{nm}$, while for DAPI-dye observation, fluorescence was measured at $\lambda_{EX}=358\text{nm}$ / $\lambda_{EM}=461\text{nm}$. Images were obtained at 400× magnification. Full imaging results are shown in Figure S8, S9, S10, S11, and S12.

MCF7 (Estrogen receptor positive breast cancer cell line)

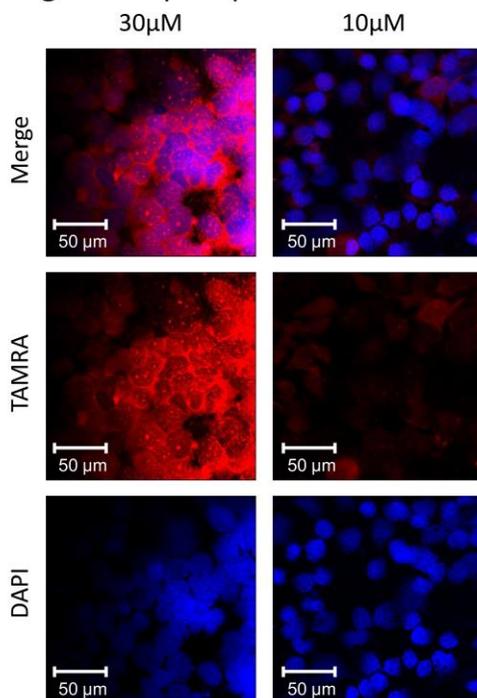


Figure S8. Imaging of MCF7 cancer cell line following incubation with various concentrations of compound **13** (10, 30 μ M). TAMRA-dye fluorescence was measured at $\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$. DAPI-dye fluorescence measured at $\lambda_{EX}=358\text{nm}/\lambda_{EM}=461\text{nm}$.

MDA-MB-231 (Triple negative breast cancer cell line)

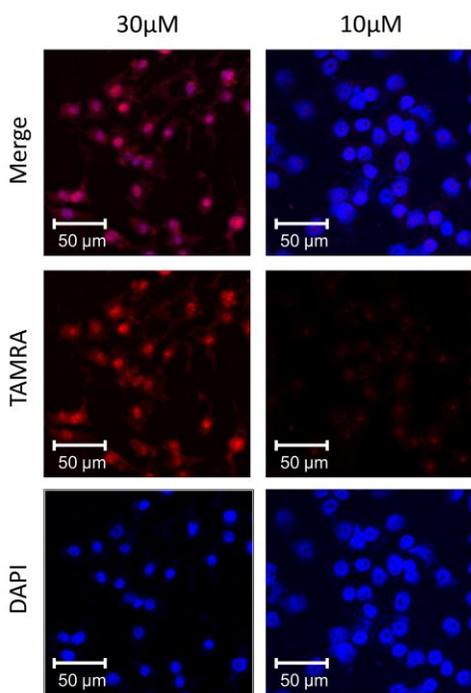


Figure S9. Imaging of MDA-MB-231 cancer cell line following incubation with various concentrations of compound **13** (10, 30 μ M). TAMRA-dye fluorescence was measured at $\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$. DAPI-dye fluorescence measured at $\lambda_{EX}=358\text{nm}/\lambda_{EM}=461\text{nm}$.

SK-BR-3 (HER2 positive breast cancer cell line)

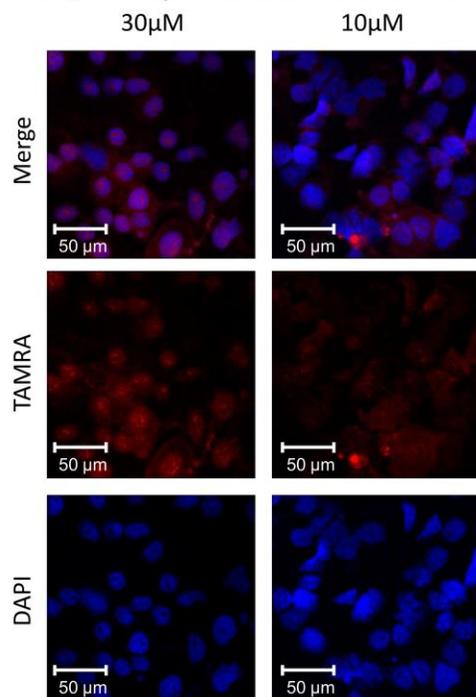


Figure S10. Imaging of SK-BR-3 cancer cell line following incubation with various concentrations of compound **13** (10, 30 μ M). TAMRA-dye fluorescence was measured at $\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$. DAPI-dye fluorescence measured at $\lambda_{EX}=358\text{nm}/\lambda_{EM}=461\text{nm}$.

MCF10A (Mammary epithelium cell line)

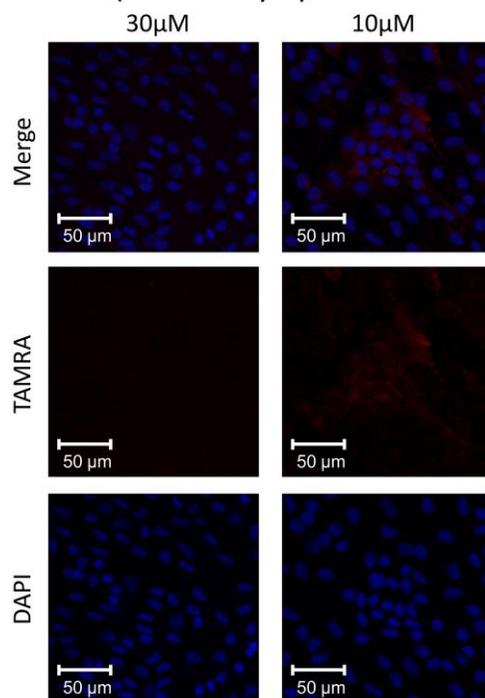


Figure S11. Imaging of MCF10A cell line following incubation with various concentrations of compound **13** (10, 30 μM). TAMRA-dye fluorescence was measured at $\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$. DAPI-dye fluorescence measured at $\lambda_{EX}=358\text{nm}/\lambda_{EM}=461\text{nm}$.

Lymphocyte

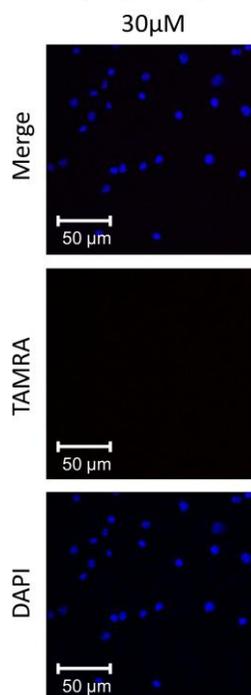
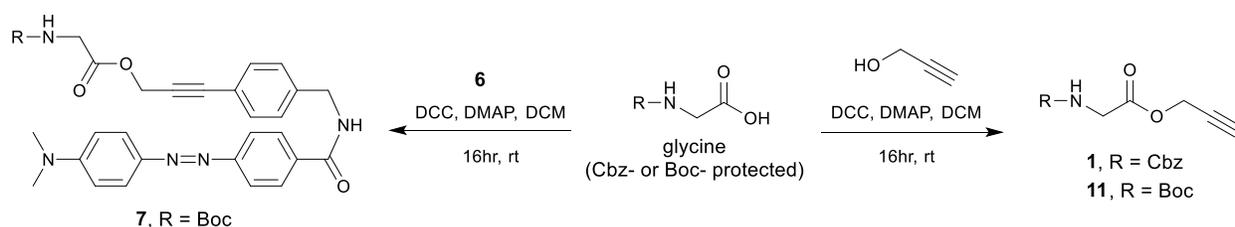


Figure S12. Imaging of normal lymphocyte cell line following incubation with 30 μM of compound **13**. TAMRA-dye fluorescence was measured at $\lambda_{EX}=560\text{nm}/\lambda_{EM}=585\text{nm}$. DAPI-dye fluorescence measured at $\lambda_{EX}=358\text{nm}/\lambda_{EM}=461\text{nm}$.

Synthetic Protocol:



General Protocol for Ester Coupling:

Unless stated otherwise, corresponding acid (1 equiv) and alcohol (1.1 equiv) were dissolved in anhydrous DCM (10 mL) with DCC (1.3 equiv) and DMAP (0.2 equiv). The reaction was stirred for 16 hr at room temperature under N₂. To workup, DCU was filtered off and washed with DCM (5 ml). The combined organic layers were concentrated under vacuum. Flash column chromatography using the indicated conditions were used to purify the desired compound.

Preparation of 1:

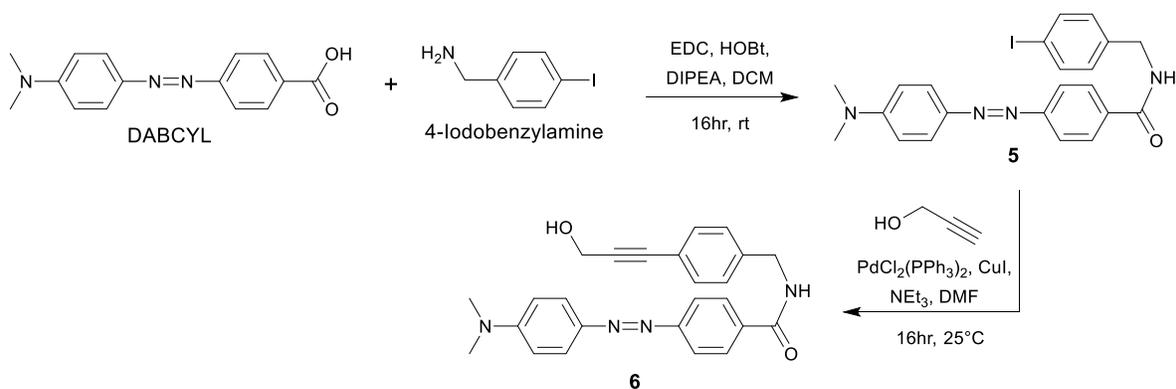
Cbz-glycine (238 mg, 1.14 mmol), Propargyl alcohol (73 mg, 1.3 mmol), DCC (309 mg, 1.5 mmol), DMAP (24 mg, 0.2 mmol) were used. Purified with isocratic 20% EtOAc in Hex. Yield: 279 mg, 99%. *R_f* = 0.63 (50% EtOAc/Hex); ¹H NMR (300 MHz, CDCl₃) δ 7.37-7.32 (m, 5H), 5.24 (s, 1H), 5.14 (s, 2H), 4.76 (s, 2H), 4.04 (d, *J* = 5.7 Hz, 2H), 2.51 (t, *J* = 2.4 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 169.4, 156.3, 136.2, 128.6(2), 128.3, 128.2(2), 75.5, 67.2, 52.7, 42.6, 0.1; HRMS for C₁₃H₁₃NO₄ [M+Na]⁺ calcd. 270.0737, found. 270.0732.

Preparation of 11:

Boc-glycine (200 mg, 1.14 mmol), Propargyl alcohol (73 mg, 1.3 mmol), DCC (309 mg, 1.5 mmol), DMAP (24 mg, 0.2 mmol) were used. Purified with isocratic 20% EtOAc in Hex. Yield: 153 mg, 63%. *R_f* = 0.59 (50% EtOAc/Hex); ¹H NMR (300 MHz, CDCl₃) δ 4.97 (s, 1H), 4.72 (d, *J* = 2.4 Hz, 2H), 3.94 (d, *J* = 5.7 Hz, 2H), 2.47 (t, *J* = 2.5 Hz, 1H), 1.42 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 170.0, 155.9, 80.4, 75.6, 52.8, 42.4, 28.4(3), 0.01; HRMS for C₁₀H₁₅NO₄ [M+Na]⁺ calcd. 236.0893, found. 236.0901.

Preparation of 7:

Boc-glycine (57 mg, 0.33 mmol), Compound **6** (90 mg, 0.22 mmol), DCC (135 mg, 0.66 mmol), DMAP (2 mg, 0.02 mmol) were dissolved in anhydrous DMF (5 ml) and the reaction was stirred for 2 days at 40°C under N₂. To workup, DCM (50 ml) was added and the organic layer was washed with H₂O (3 x 50 ml), dried over sodium sulfate, and concentrated under vacuum. Flash column chromatography using isocratic 50% EtOAc in Hex was used to purify the desired compound. Yield: 124 mg, 99%. R_f = 0.26 (50% EtOAc/Hex); ¹H NMR (400 MHz, CDCl₃) δ 7.92-7.87 (m, 6H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 6.77 (d, *J* = 9.4 Hz, 2H), 6.51 (s, 1H), 5.03 (s, 1H), 4.99 (s, 2H), 4.68 (d, *J* = 5.8 Hz, 2H), 4.00 (d, *J* = 5.7 Hz, 2H), 3.12 (s, 6H), 1.46 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 167.2, 155.4, 153.0, 143.8, 139.4, 134.3, 132.5, 128.1, 128.0, 125.6, 122.5, 121.4, 111.6, 86.8, 82.6, 53.7, 49.2, 43.9, 40.4, 34.0, 28.4, 25.7, 25.0; HRMS for C₃₂H₃₅N₅O₅ [M+H]⁺ calcd. 570.2711, found. 570.2727.

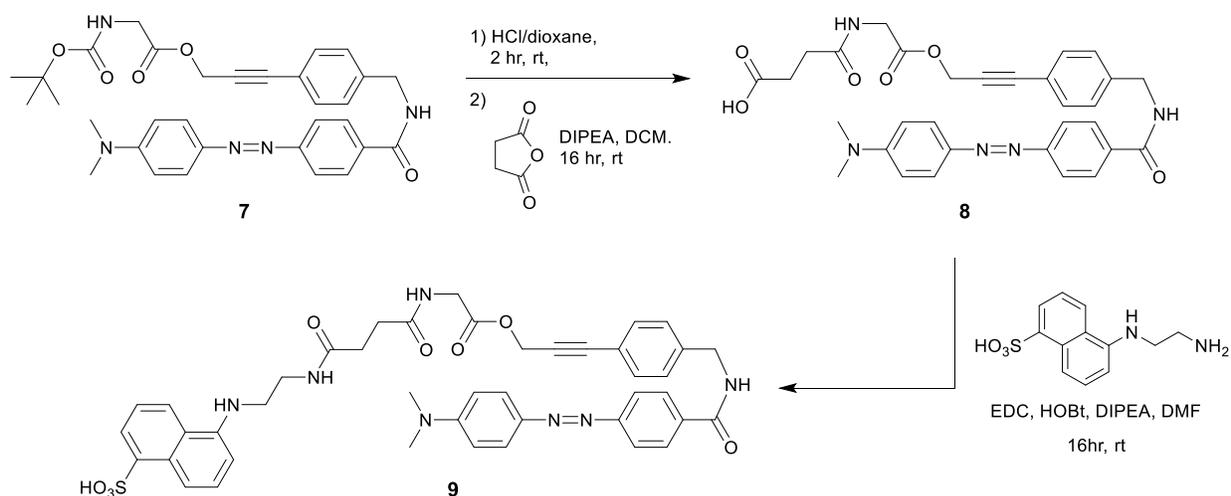


Preparation of 5:

DABCYL (150 mg, 0.56 mmol), 4-Iodobenzylamine (130 mg, 0.56 mmol), EDC (173 mg, 1.11 mmol), HOBT (150 mg, 1.11 mmol) were dissolved in anhydrous 1:1 DCM/DMF (20 ml). DIPEA (388 μ l, 2.23 mmol) was then added to the mixture and the reaction was stirred for 16 hr at room temperature under N₂. To workup, DCM (10 ml) was added and the organic layer was washed with H₂O (3 x 10 ml), dried over sodium sulfate, and concentrated under vacuum. Flash column chromatography using isocratic 50% EtOAc in Hex was used to purify the desired compound. Yield: 175 mg, 65%. R_f = 0.53 (50% EtOAc/Hex); ¹H NMR (400 MHz, DMSO) δ 9.16 (t, *J* = 5.9 Hz, 1H), 8.03(d, *J* = 8.5 Hz, 2H), 7.83 (dd, *J* = 8.6 Hz, *J* = 2.0 Hz, 4H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 6.85 (d, *J* = 9.2 Hz, 2H), 4.46 (d, *J* = 5.9 Hz, 2H), 3.08 (s, 6H); ¹³C NMR (100 MHz, DMSO) δ 165.8, 154.2, 152.9, 142.7, 139.6, 137.1, 134.5, 129.8, 128.5, 125.2, 121.6, 111.6, 92.5, 42.3, 28.2; HRMS for C₂₂H₂₁IN₄O [M+H]⁺ calcd. 485.0833, found. 485.0849.

Preparation of 6:

Compound **5** (130 mg, 0.27 mmol) and propargyl alcohol (31 μ g, 0.54 mmol) were dissolved in NEt₃ (2 ml) and DMF (2 ml) and degassed. To this solution, PdCl₂(PPh₃)₂ (1%, 1.8 mg, 0.0027 mmol) and CuI (2%, 1 mg, 0.0054 mmol) were added and the mixture was degassed again. The reaction was stirred for 2 days at 25°C under N₂. To workup, DCM (50 ml) was added and the organic layer was washed with H₂O (3 x 25 ml), dried over sodium sulfate, and concentrated under vacuum. Flash column chromatography using isocratic 75% EtOAc in Hex was used to purify the desired compound. Yield: 109 mg, 99%. R_f = 0.10 (50% EtOAc/Hex); ¹H NMR (400 MHz, DMSO) δ 9.19 (t, *J* = 5.9 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 2H), 7.85 (dd, *J* = 2.7 Hz, 8.6 Hz, 4H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 6.87 (d, *J* = 9.2 Hz, 2H), 5.33 (t, *J* = 5.9 Hz, 1H), 4.53 (d, *J* = 5.6 Hz, 2H), 4.31 (d, *J* = 6.0 Hz, 2H), 3.10 (s, 6H); ¹³C NMR (100 MHz, DMSO) δ 165.8, 154.2, 153.0, 152.3, 142.7, 140.2, 134.5, 131.3, 128.5, 127.6, 125.2, 121.6, 120.9, 111.6, 89.7, 83.6, 49.4, 42.5; HRMS for C₂₅H₂₄N₄O₂ [M+H]⁺ calcd. 413.1972, found. 413.1988.

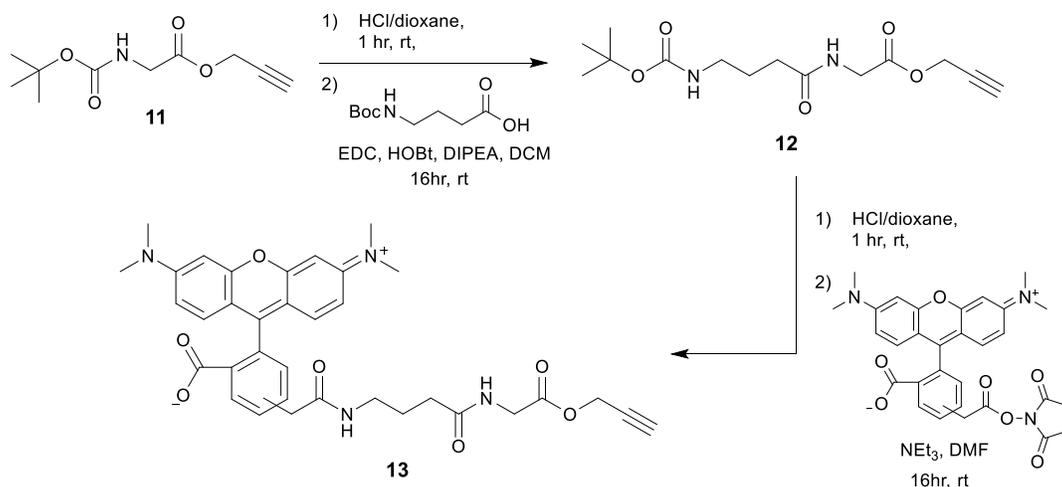


Preparation of 8:

Compound **7** (124 mg, 0.22 mmol) was dissolved in 4M HCl:dioxane (5 ml) and stirred for 1 hr at room temperature. Following TLC (50% EtOAc/Hex) confirmation for the disappearance of starting material, the reaction was evaporated under vacuum to an oil. The crude residue was then suspended in DCM (10 ml) with Succinic anhydride (44 mg, 0.44 mmol). DIPEA (76 μ l, 0.44 mmol) was then added to the mixture and the reaction was stirred for 16 hr at room temperature under N₂. To workup, the solvent was removed under vacuum. Flash column chromatography using isocratic 3% MeOH in DCM was used to purify the desired compound. Yield: 33 mg, 26%. R_f = 0.20 (10% MeOH/DCM); ¹H NMR (400 MHz, DMSO) δ 9.18 (t, *J* = 6.0 Hz, 1H), 8.40 (t, *J* = 5.9 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 7.83 (dd, *J* = 2.7 Hz, *J* = 8.6 Hz, 4H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 9.3 Hz, 2H), 4.97 (s, 2H), 4.52 (d, *J* = 5.9 Hz, 2H), 3.90 (d, *J* = 6.0 Hz, 2H), 3.08 (s, 6H), 2.41-2.39 (m, 4H), ¹³C NMR (75 MHz, DMSO) δ 174.0, 171.9, 169.7, 165.9, 154.3, 153.0, 142.8, 141.1, 134.5, 131.7, 128.6, 127.7, 125.3, 121.7, 119.9, 111.6, 86.0, 83.7, 80.4, 79.3, 78.1, 52.8, 29.8, 29.2; HRMS for C₃₁H₃₁N₅O₆ [M+H]⁺ calcd. 570.2347, found. 570.2344

Preparation of 9:

Compound **8** (27 mg, 0.05 mmol), EDANS (14 mg, 0.05 mmol), EDC (15 mg, 0.10 mmol), and HOBT (13 mg, 0.10 mmol) were dissolved in anhydrous DMF (2 ml). DIPEA (33 μ l, 0.20 mmol) was then added to the mixture and the reaction was stirred for 16 hr at room temperature under N₂. The resulting mixture was directly purified by reverse phase HPLC using method 5. Yield: 17.5 mg, 45%; ¹H NMR (400 MHz, DMSO) δ 9.19 (t, J = 6.2 Hz, 1H), 8.37 (t, J = 6.1 Hz, 1H), 8.11-8.02 (m, 5H), 7.90 (d, J = 7.2 Hz, 1H), 7.83-7.80 (m, 4H), 7.44-7.21 (m, 7H), 6.85 (d, J = 9.3 Hz, 2H), 6.54 (d, J = 7.7 Hz, 2H), 4.96 (s, 2H), 4.50 (d, J = 5.8 Hz, 2H), 3.88 (d, J = 5.8 Hz, 2H), 3.35 (t, J = 6.2 Hz, 2H), 3.23 (t, J = 6.4 Hz, 2H), 3.07 (s, 6H), 2.40-2.34 (m, 4H), ¹³C NMR (75 MHz, DMSO) δ 172.3, 172.1, 169.7, 168.3, 167.4, 165.9, 158.5, 158.0, 154.3, 153.2, 153.1, 144.2, 143.8, 142.8, 141.1, 139.3, 138.4, 134.5, 131.7, 130.3, 128.6, 127.7, 125.3, 123.7, 122.9, 122.4, 121.7, 119.8, 118.7, 118.6, 111.8, 88.8, 85.9, 51.0, 30.7, 30.4; HRMS for C₄₃H₄₃N₇O₈S [M+Na]⁺ calcd. 840.2786, found. 840.2792.



Preparation of 12:

Compound **11** (191 mg, 0.90 mmol) was dissolved in 4M HCl:dioxane (5 ml) and stirred for 1 hr at room temperature. Following TLC (50% EtOAc/Hex) confirmation for the disappearance of starting material, the reaction was evaporated under vacuum. The crude residue was then suspended in DCM (10 ml) with *N*-(*tert*-butoxycarbonyl)-4-aminobutyric acid (182 mg, 0.90 mmol), EDC (278 mg, 1.79 mmol), and HOBT (243 mg, 1.79 mmol). DIPEA (625 μ l, 3.58 mmol) was then added to the mixture and the reaction was stirred for 16 hr at room temperature under N₂. To workup, DCM (50 ml) was added and the organic layer was washed with H₂O (3 x 25 ml), dried over sodium sulfate, and concentrated under vacuum. Flash column chromatography using isocratic 10% MeOH in CHCl₃ was used to purify the desired compound. Yield: 261 mg, 98% in 2 steps. R_f = 0.41 (10% MeOH/DCM); ¹H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 5.02 (s, 1H), 4.75 (d, J = 2.4 Hz, 2H), 4.07 (d, J = 6.0 Hz, 2H), 3.96 (d, J = 6.4 Hz, 2H), 3.20 (m, 2H), 2.54 (t, J = 2.4 Hz, 1H), 2.31 (t, J = 6.8 Hz, 2H), 1.82 (quin, J = 6.8 Hz, 2H), 1.44 (s, 9H), ¹³C NMR (100 MHz, CDCl₃) δ 173.4, 169.3, 156.5, 79.2, 75.4, 52.6, 41.1, 39.4, 38.5, 33.0, 28.3, 26.3; HRMS for C₁₄H₂₂N₂O₅ [M+H]⁺ calcd. 299.1601, found. 299.1605.

Preparation of 13:

Compound **12** (58 mg, 0.19 mmol) was dissolved in 4M HCl:dioxane (5 mL) and stirred for 1 hr at room temperature. Following TLC (10% MeOH/DCM) confirmation for the disappearance of starting material, the reaction was evaporated under vacuum. The crude residue was then suspended in dry DMF (0.1 mL) with 5-(and-6)-Carboxytetramethylrhodamine Succinimidyl Ester (10.2 mg, 19.5 μ mol). Triethylamine (14 μ l, 0.10 mmol) was then added to the mixture and the reaction was stirred for 16 hr at room temperature under N₂. The resulting mixture was directly purified by reverse phase HPLC using method 6. Yield: 5.8 mg, 48%. ¹H NMR (500 MHz, DMSO) δ 8.82 (t, J = 5.5 Hz, 1H), 8.45 (d, J = 1.0 Hz, 1H), 8.36 (t, J = 5.5 Hz, 1H), 8.23 (dd, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 6.61 (s, 1H), 6.53-6.47 (m, 5H), 4.73 (d, J = 3.0 Hz, 2H), 3.88 (d, J = 6.0 Hz, 2H), 3.57 (t, J = 3.0 Hz, 1H), 3.34 (m, 2H), 2.94 (s, 12H), 2.24 (t, J = 8.0 Hz, 2H), 1.80 (quin, J = 7.5 Hz, 2H), ¹³C NMR (125 MHz, DMSO) δ 172.4, 169.4, 168.4, 164.7, 154.8, 152.1, 152.0, 136.2, 134.5, 128.4, 126.8, 124.1, 123.1, 109.0, 105.6, 98.0, 86.7, 78.2, 77.9, 52.1, 40.4, 32.6, 25.1; HRMS for C₃₄H₃₅N₄O₇ [M+H]⁺ calcd. 611.2500, found. 611.2547.

Photophysical characterization (compound 9):

Extinction coefficients were determined by plotting a standard Beer-Lambert plot. The reported values and standard deviations were determined from measurement of three different concentrations.

Note: absorbance (A), extinction coefficient (ϵ), concentration (c), path length (l).

$$A = \epsilon c l$$

Relative fluorescence quantum yields were determined according to the method of Fery-Forgues at al.¹ The reported values and standard deviations were determined from triplicate emission spectrums. Literature values used were the refractive index of 1:1 DMSO/H₂O ($n_X = 1.4071$),² the refractive index of 1N H₂SO₄ ($n_S = 1.346$),³ and the quantum yield of quinine sulfate ($\Phi_{F(S)} = 0.546$ in 1N H₂SO₄).³

Note: quantum yield (Φ_F), absorbance (A), area under the emission spectrum (F), refractive index of solvent (n). Subscripts used were in relation to the unknown (X) and standard (S).

$$\Phi_{F(X)} = \left(\frac{A_S}{A_X}\right) \left(\frac{F_X}{F_S}\right) \left(\frac{n_X}{n_S}\right)^2 \Phi_{F(S)}$$

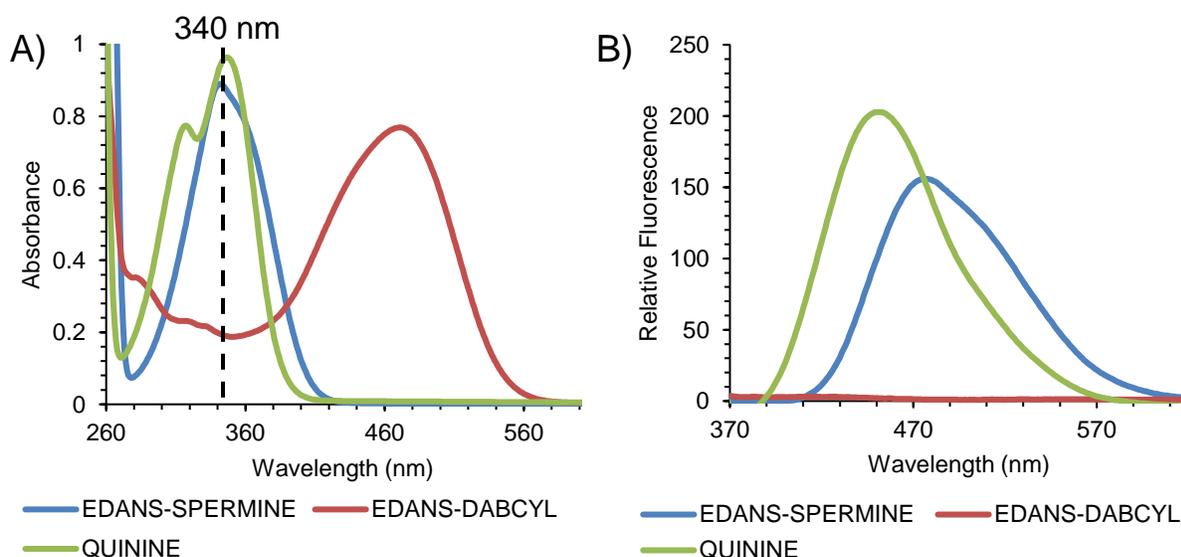


Figure S13. Photophysical characterization. A) Overlay of absorbance spectra. B) Overlay of emission spectra with fixed excitation (340 nm).

Kinetics (between spermine and compound **9**):

Serial dilutions of compound **9** (20, 50, and 160 μM) were made from a stock solution of 400 μM in DMSO, along with serial dilutions of spermine (0, 1, 2, 5, 10 and 20 mM) from a stock solution of 20 mM in DMSO. For analysis, aliquots of compound **9** (100 μl) and spermine (100 μl) were mixed to give final concentrations of 10, 25, 80 μM for compound **9** and final concentrations of 0, 0.5, 1, 2.5, 5 and 10 mM for spermine. Fluorescence intensities ($\lambda_{\text{ex}} = 340$ nm, $\lambda_{\text{em}} = 490$ nm) were recorded at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360, 420, 480, 540, and 600 minutes after mixing. The concentration of **10** in the reaction mixture was determined by interpolation of a standard curve.

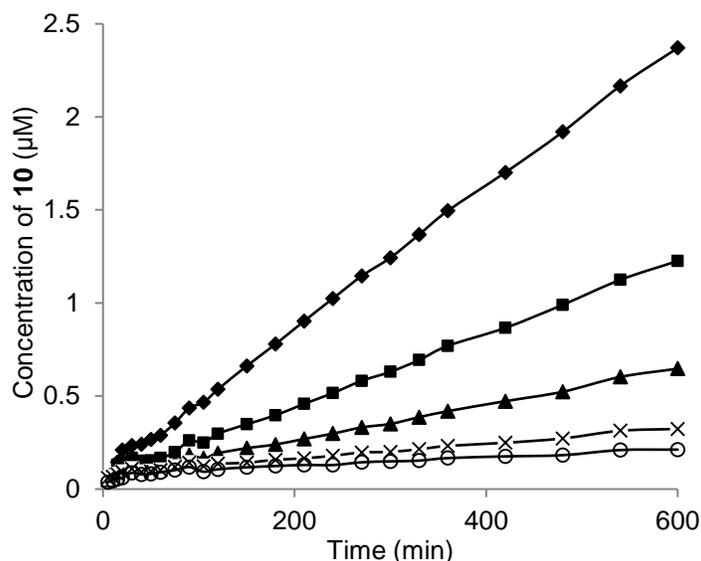


Figure S14. Concentrations of **10** in relation to time where an initial **9** concentration of 25 μM is used. Shown are different concentrations of spermine: 0.5(○), 1(×), 2.5(▲), 5(■) and 10 (◆) mM.

The principle complexity of measuring a second order reaction rate is the need to simultaneously monitor multiple reactants, in our case, reactants "9" and "spermine".

$$\text{rate} = k[\mathbf{9}][\text{spermine}]$$

Note: rate constant (k)

Under pseudo first-order approximation, one reactant is applied in excess to assume the relative concentration remains constant.

$$\text{rate} = \frac{d[\mathbf{9}]}{dt} = k'[\mathbf{9}]$$

Note: pseudo first order rate constant (k')

Through integrating and plotting the above formula, the pseudo first-order rate constant k' can be determined from the slope.

$$\ln \frac{[\mathbf{9}]_t}{[\mathbf{9}]_0} = -k't$$

Note: concentration of $\mathbf{9}$ at any time ($[\mathbf{9}]_t$), initial concentration of $\mathbf{9}$ ($[\mathbf{9}]_0$)

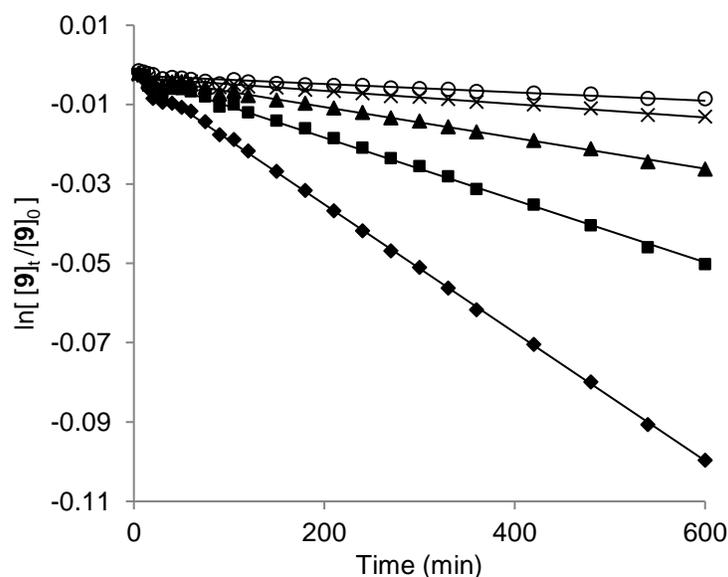


Figure S15. Pseudo first-order kinetics of the reaction between $\mathbf{9}$ ($[\mathbf{9}]_0 = 25 \mu\text{M}$) and various concentrations of excess spermine. Shown are different concentrations of spermine: 0.5(○), 1(×), 2.5(▲), 5(■) and 10 (◆) mM.

By plotting the pseudo first-order rate constant k' with the concentration of [spermine], which is a constant, the apparent second-order rate constant k ($\text{mM}^{-1}\text{min}^{-1}$) for the bimolecular reaction can be determined.

$$k' = k[\text{spermine}]$$

Note: pseudo first order rate constant (k'), apparent second-order rate constant (k)

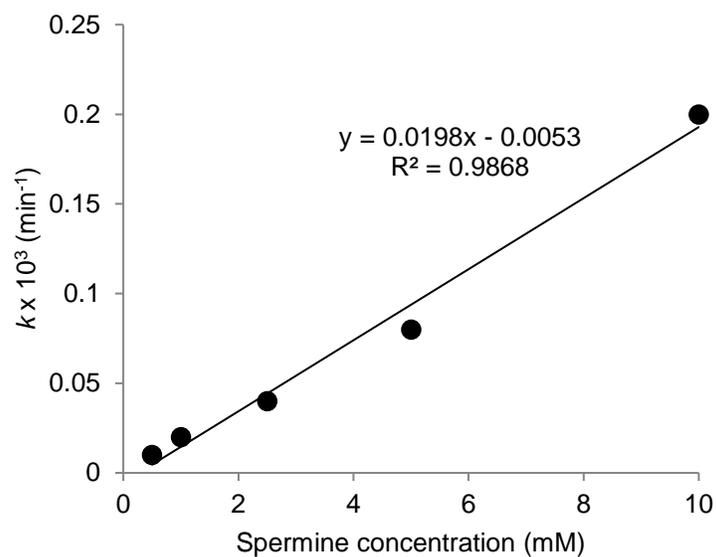


Figure S16. Determination of the apparent second-order rate constant k . Plot of the pseudo first-order rate constant k' versus the concentration of spermine.

References:

- (1) Fery-Forgues, S.; Lavabre, D. Are fluorescence quantum yields so tricky to measure? a demonstration using familiar stationary products. *J. Chem. Educ.* **1999**, *76*, 1260-1264.
- (2) LeBel, R. G.; Goring, D. A. I. Density, viscosity, refractive index, and hygroscopicity of mixtures of water and dimethyl sulfoxide. *J. Chem. Eng. Data* **1962**, *7*, 100-101.
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