

Electronic Supplemental Information for
Design of Oligothiophene-Based Tetrazoles for Laser-Triggered Photoclick Chemistry in Living Cells

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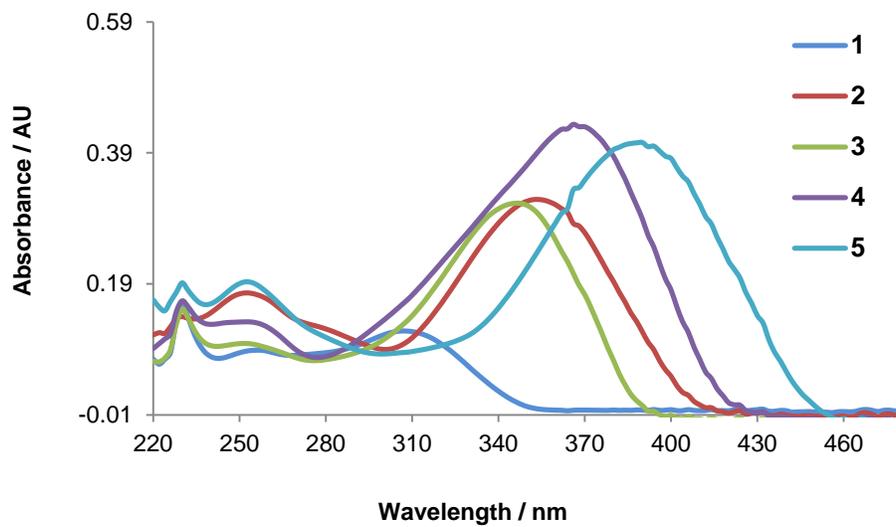


Figure S1. UV-Vis spectra of tetrazoles **1-5** dissolved in acetonitrile/PBS (2:1) at 15 μM concentration.

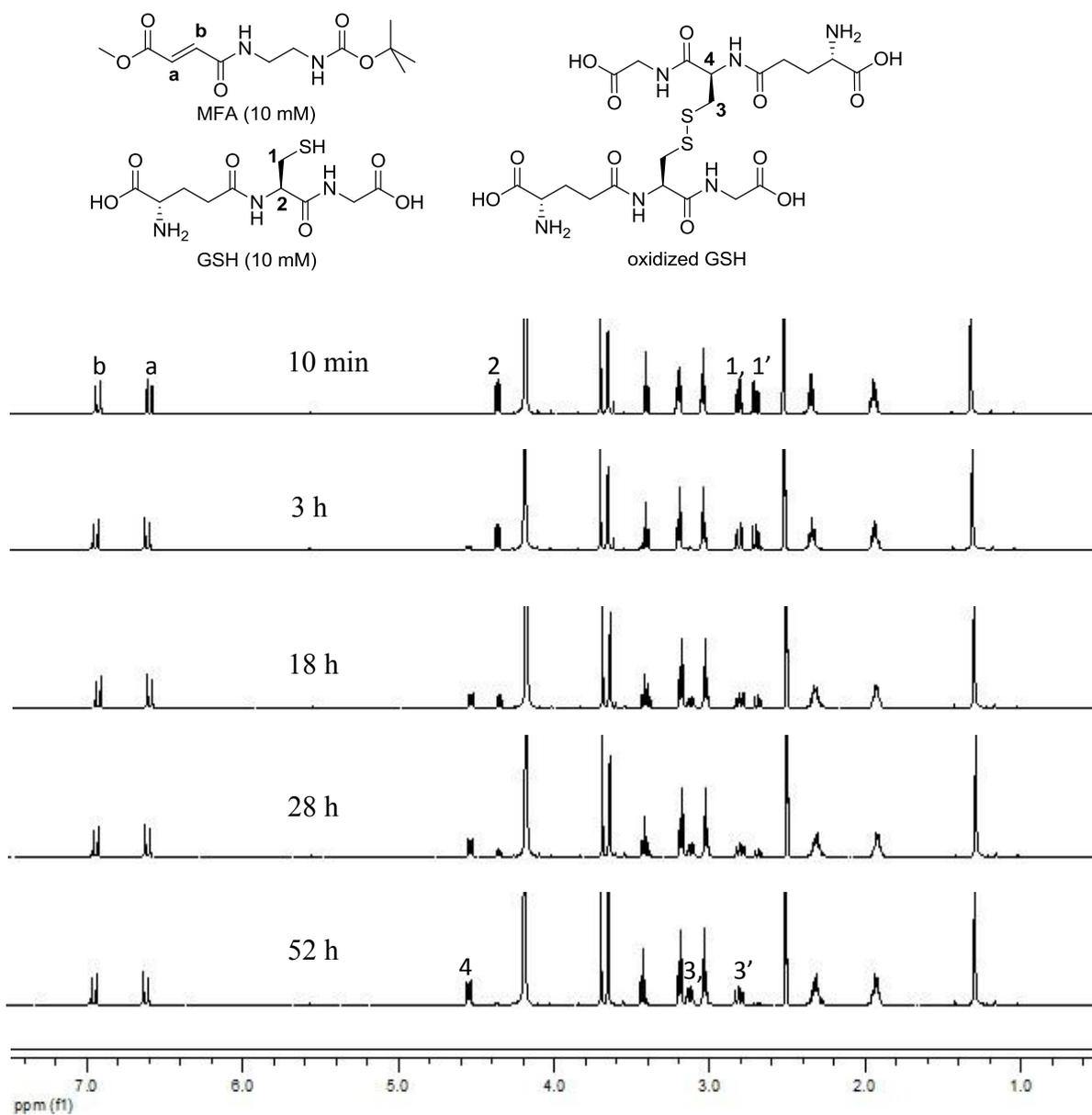
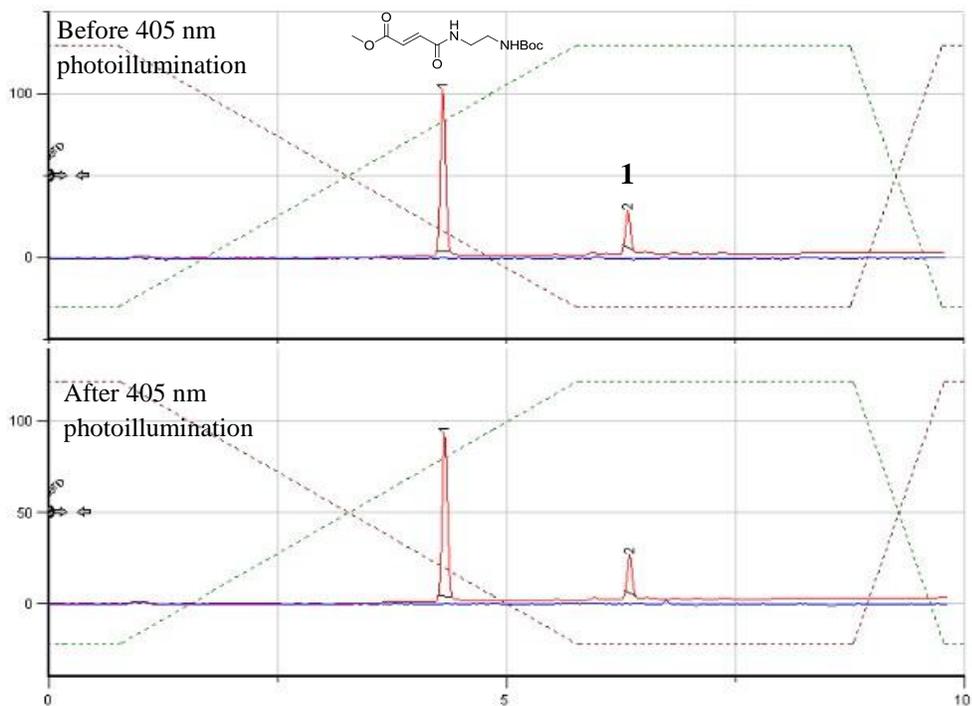
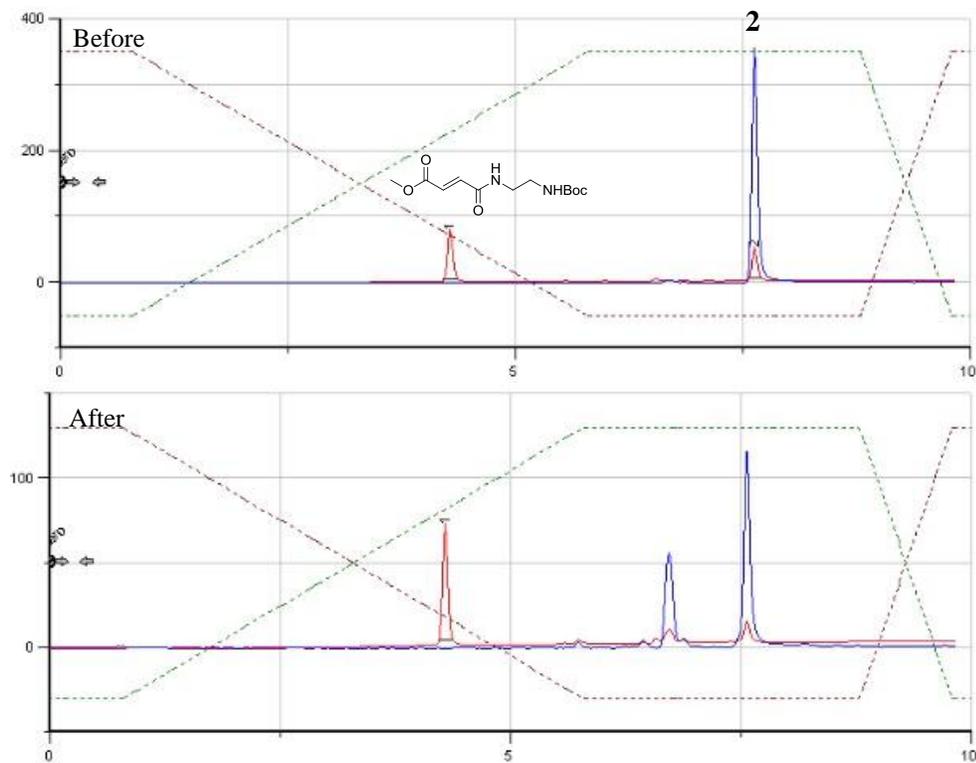


Figure S2. Stability of *mono*-methyl fumarate amide (MFA) towards glutathione in mixed DMSO-*d*₆/deuterated PBS buffer (7:3) as monitored by ¹H NMR. Condition: 10 mM MFA was mixed with 10 mM GSH (reduced form) in deuterated solvent. The formation of oxidized GSH dimer was confirmed by observation of the intact mass ion [M+H⁺] of 613.0 in LC-MS analysis.

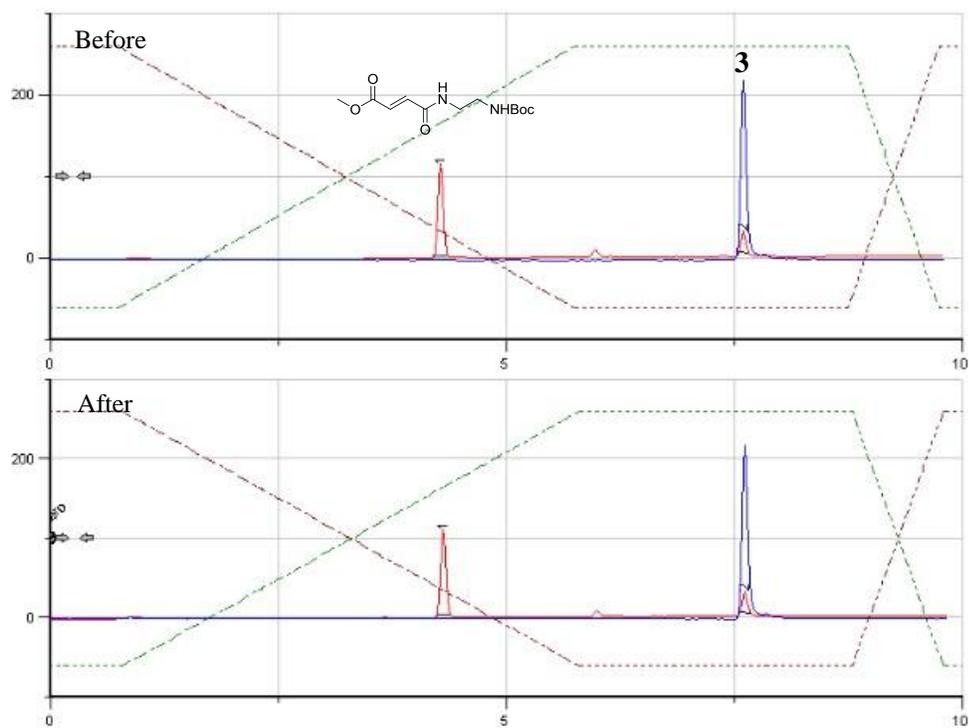
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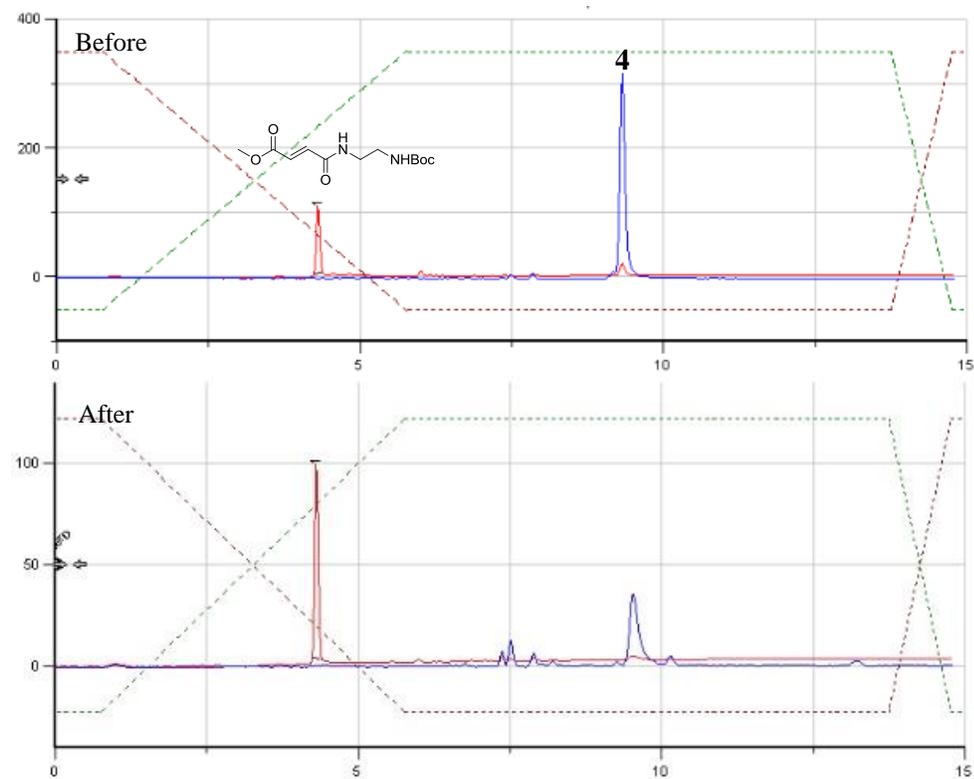
Tetrazole 2:



Tetrazole 3:



Tetrazole 4:



Tetrazole **5**:

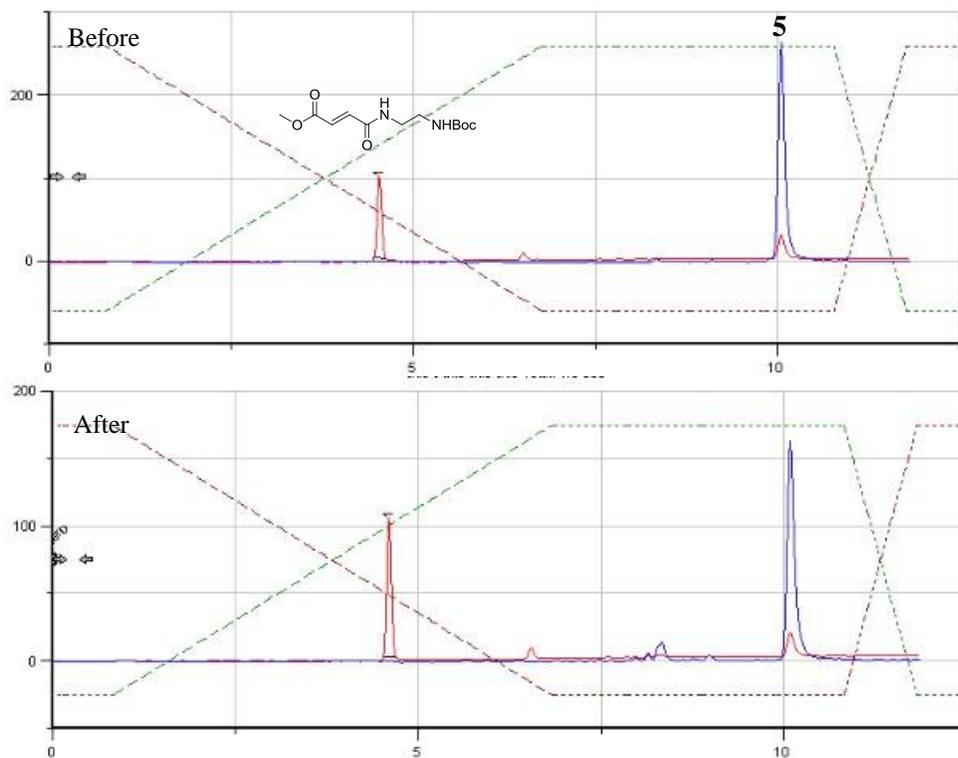


Figure S3. HPLC traces of the cycloaddition reactions for tetrazoles **1-5** before and after photoillumination with a 405-nm diode laser (24 mW). The reactions were set up by mixing 10 μM tetrazoles **1-5** with 50 μM MFA in acetonitrile/PBS (1:1) in a quartz tube, and the solutions were illuminated with 405-nm laser for 30 sec. The reaction mixtures were injected directly into HPLC column for analysis. Red line = 254 nm; blue line = 370 nm.

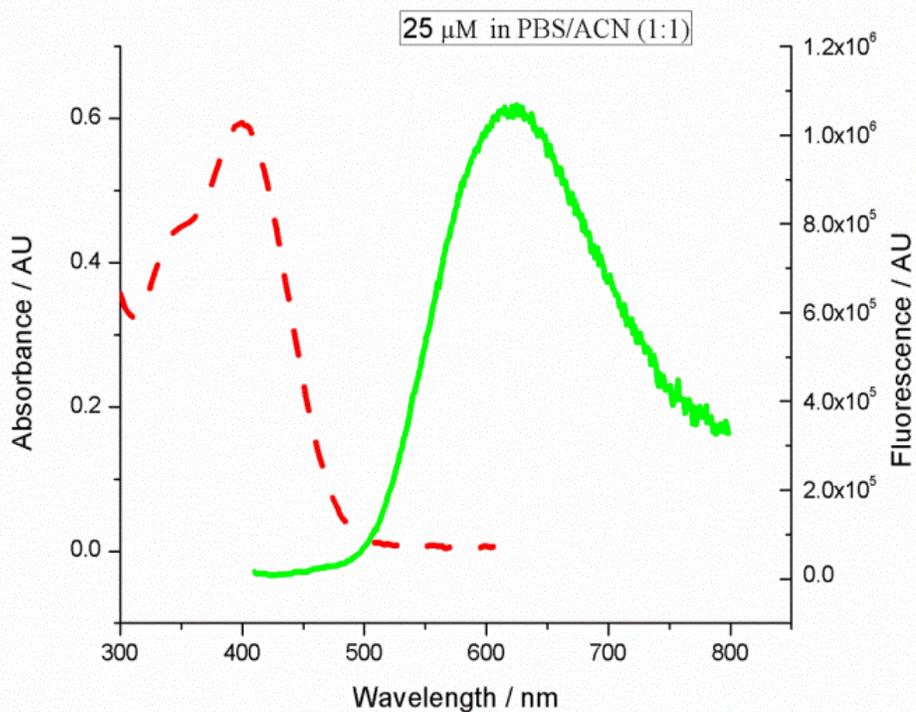
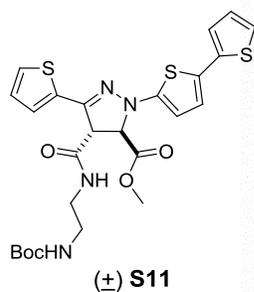
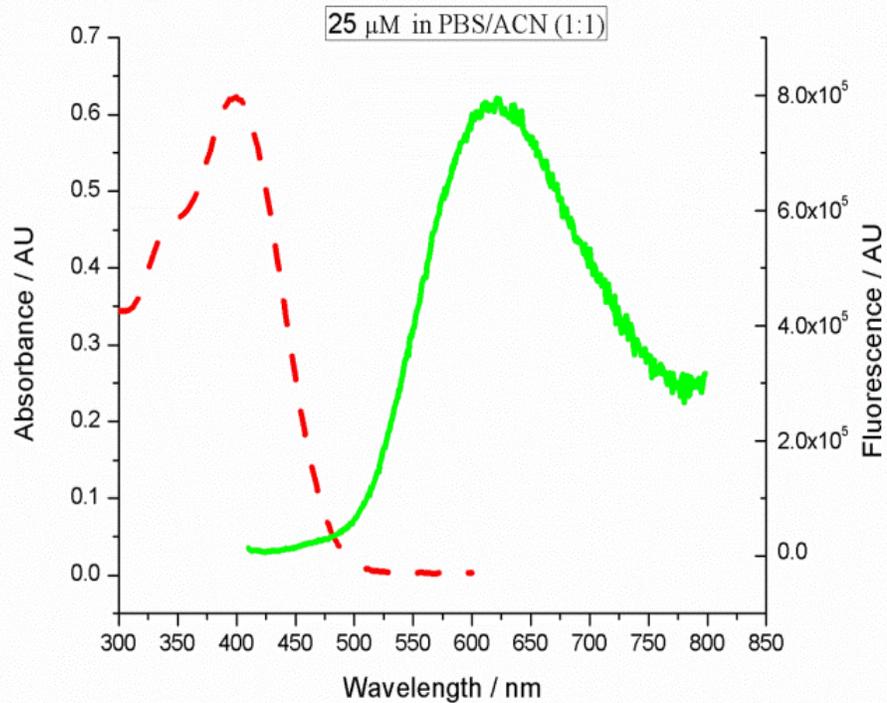
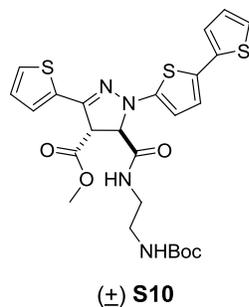


Figure S4. UV-vis (red dash line) and fluorescence (green solid line) spectra of pyrazolines (±)**S10** (top) and (±)**S11** (bottom). Pyrazolines were dissolved in acetonitrile/PBS (1:1) mixed solvent at 25 μ M concentration. For fluorescence measurement, $\lambda_{\text{ex}} = 405$ nm.

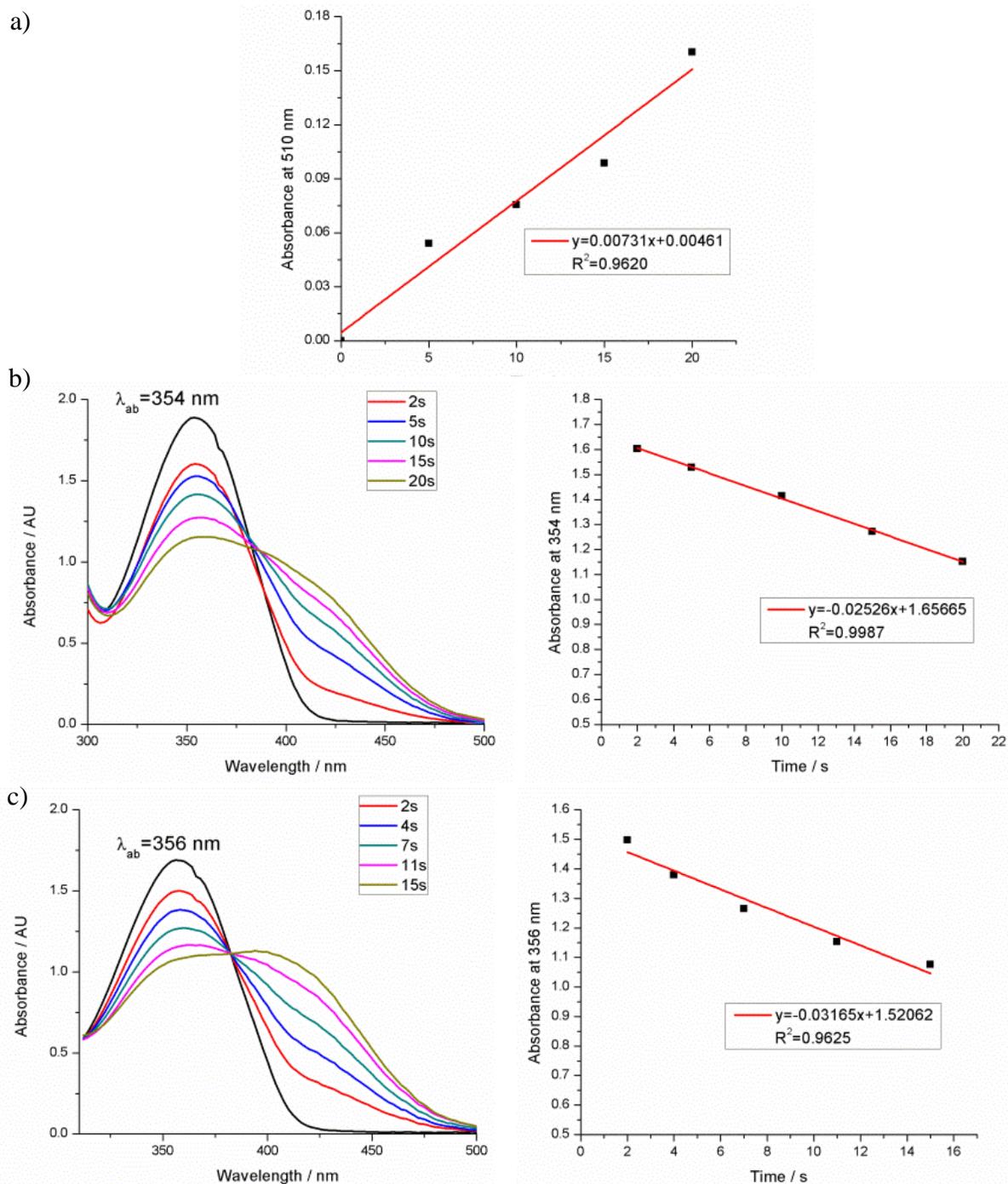


Figure S5. Determination of quantum yields of photoinduced ring rupture of tetrazoles **2** and **6** using potassium ferrioxalate-based chemical actinometer. (a) Time-course of absorbance change of Fe^{2+} -1,10-phenanthroline complex at 510 nm upon 405-nm laser irradiation. Absorption changes of tetrazole **2** (b) or **6** (c). A solution of 100 μM tetrazole **2** or **6** and 5 mM dimethyl fumarate in ACN/PBS (1:1) in quartz tubes was photoirradiated at 405 nm for a specified time before absorbance measurement.

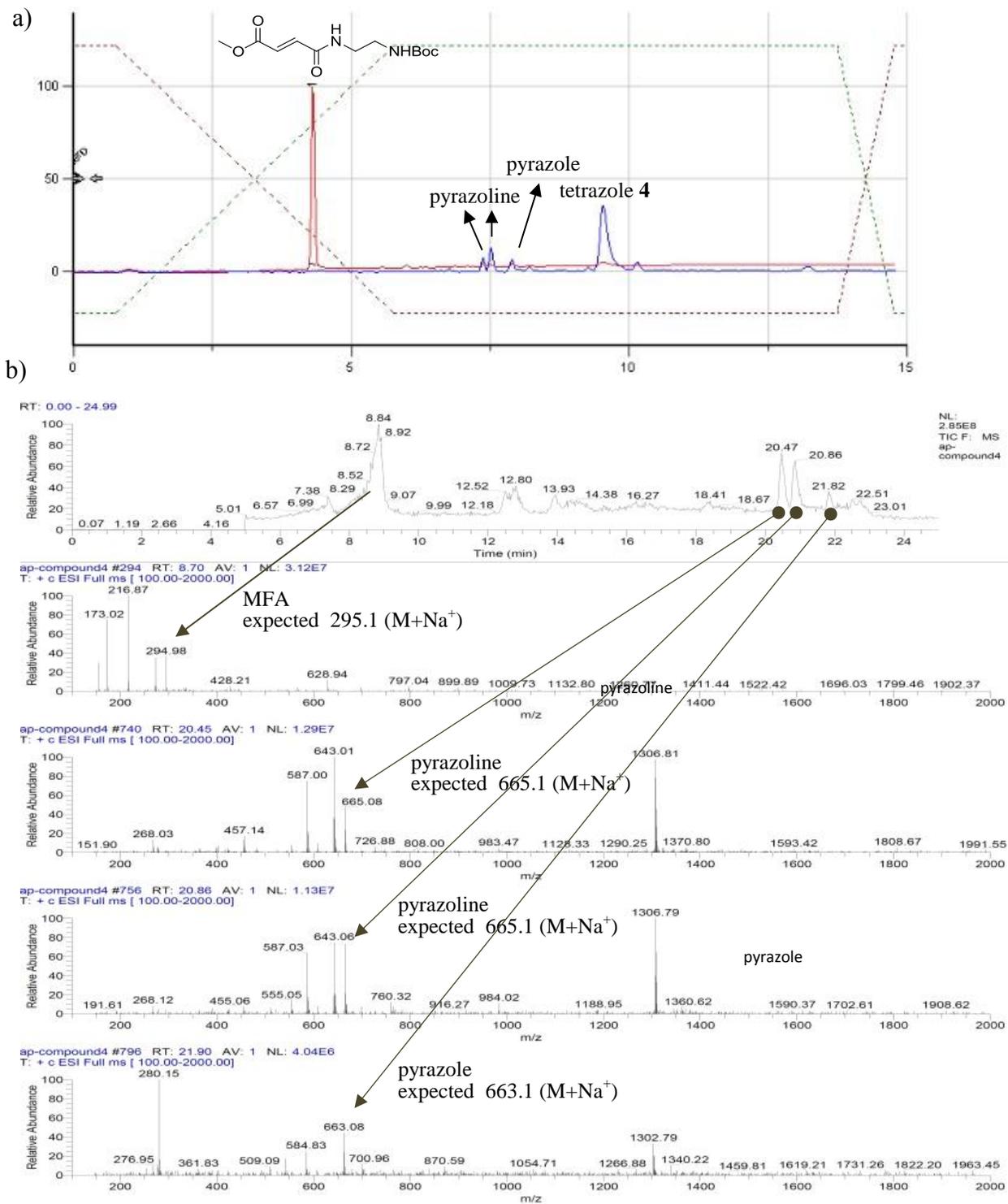


Figure S6. HPLC (a) and LC-MS (b) traces of the reaction mixture after illuminating the solution of 10 μ M tetrazole **4** and 50 μ M MFA in ACN/PBS (1:1) with a 405-nm diode laser for 30 sec.

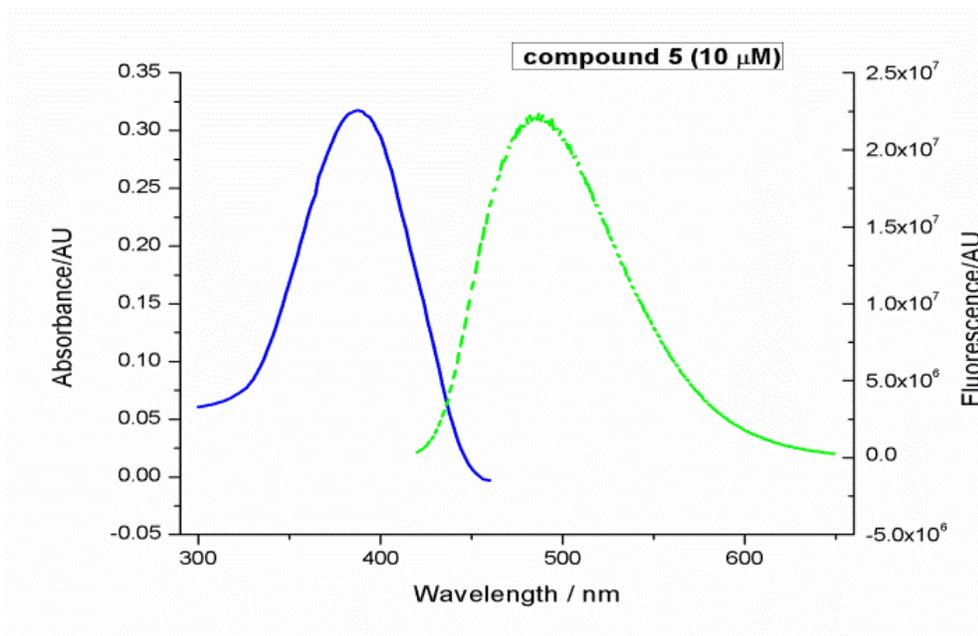
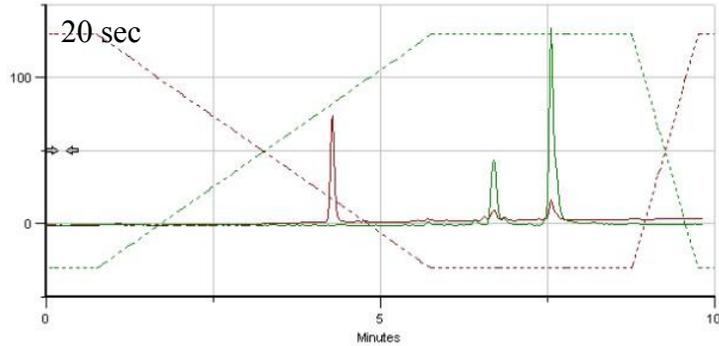
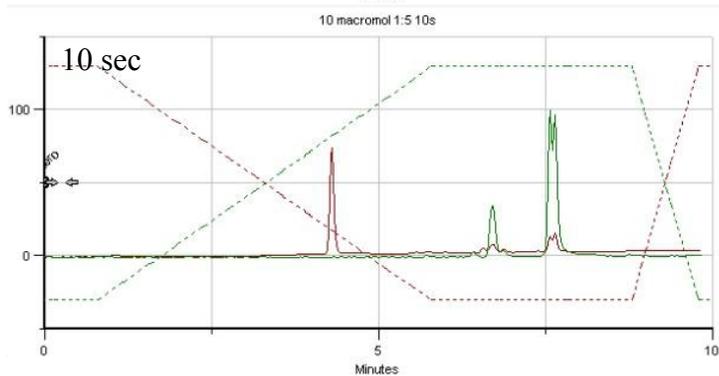
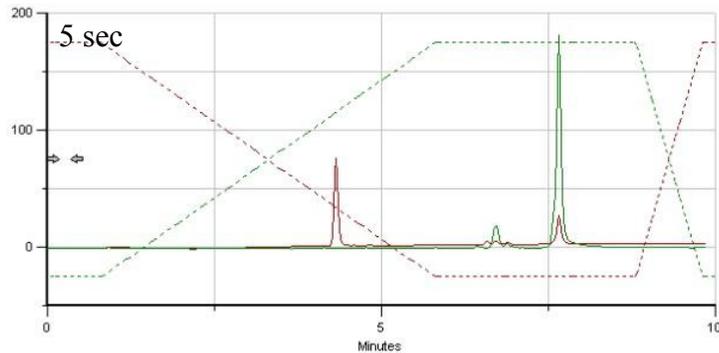
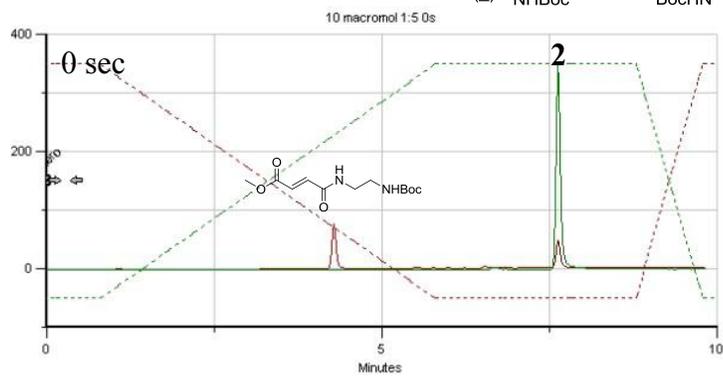
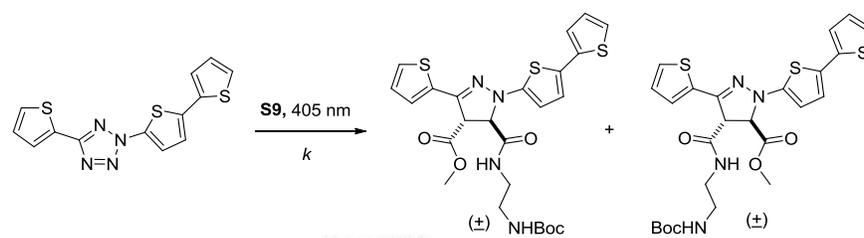


Figure S7. UV-vis (blue line) and fluorescence (green line) spectra of tetrazole **5** dissolved in acetonitrile.

a)



S11

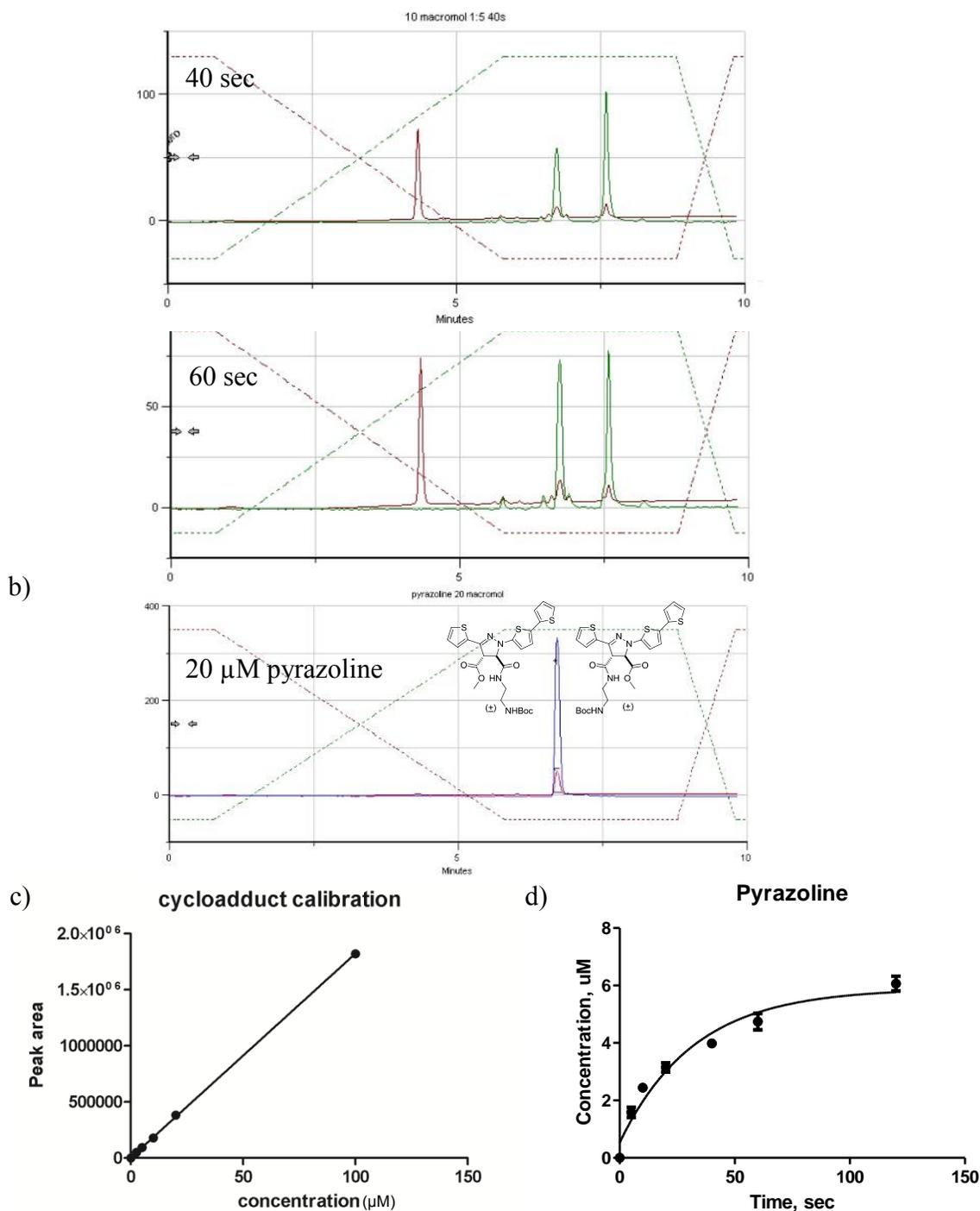
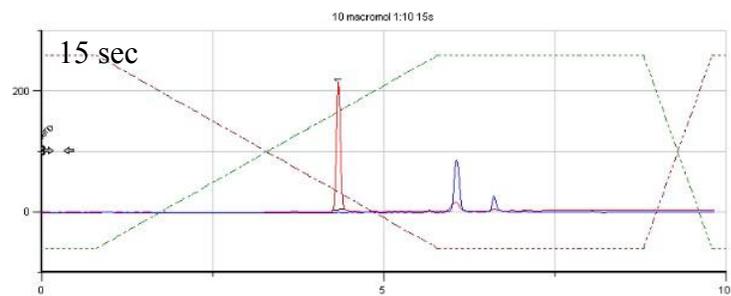
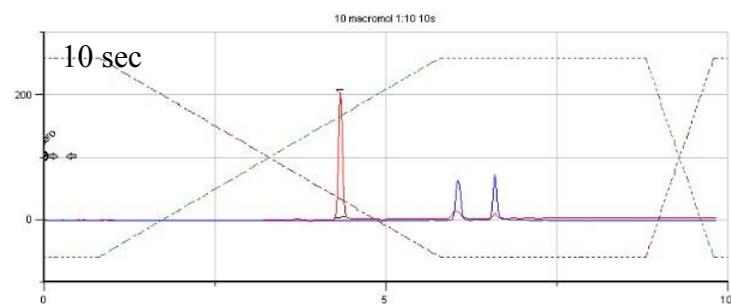
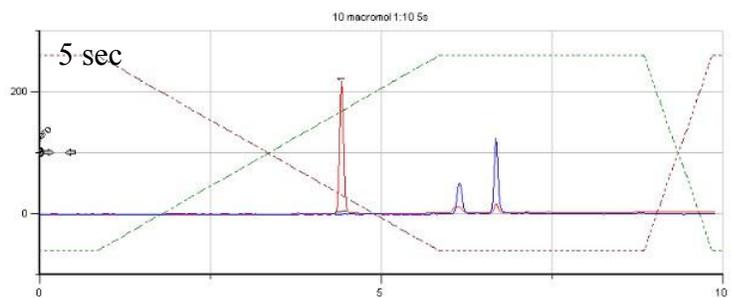
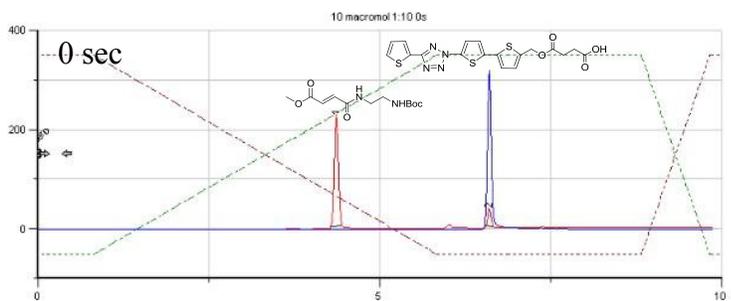
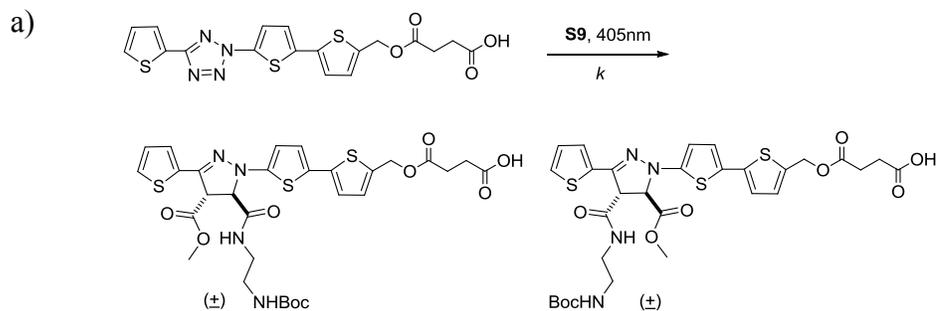


Figure S8. Kinetic characterization of tetrazole **2** in the laser-induced photoclick chemistry. (a) Time-course of the reaction as monitored by HPLC. Red traces = absorbance at 254 nm; green traces = absorbance at 370 nm. (b) HPLC trace of the purified pyrazoline. Red trace = absorbance at 254 nm; blue trace = absorbance at 370 nm. (c) Calibration curve. (d) Time course for the cycloadduct formation. The plot was fitted to an exponential rise to maximum equation: $y = (y_0 - a) e^{-kt} + a$. The concentrations of the pyrazoline were obtained by comparing their absorption peak areas at 254 nm to that of the standard curve.



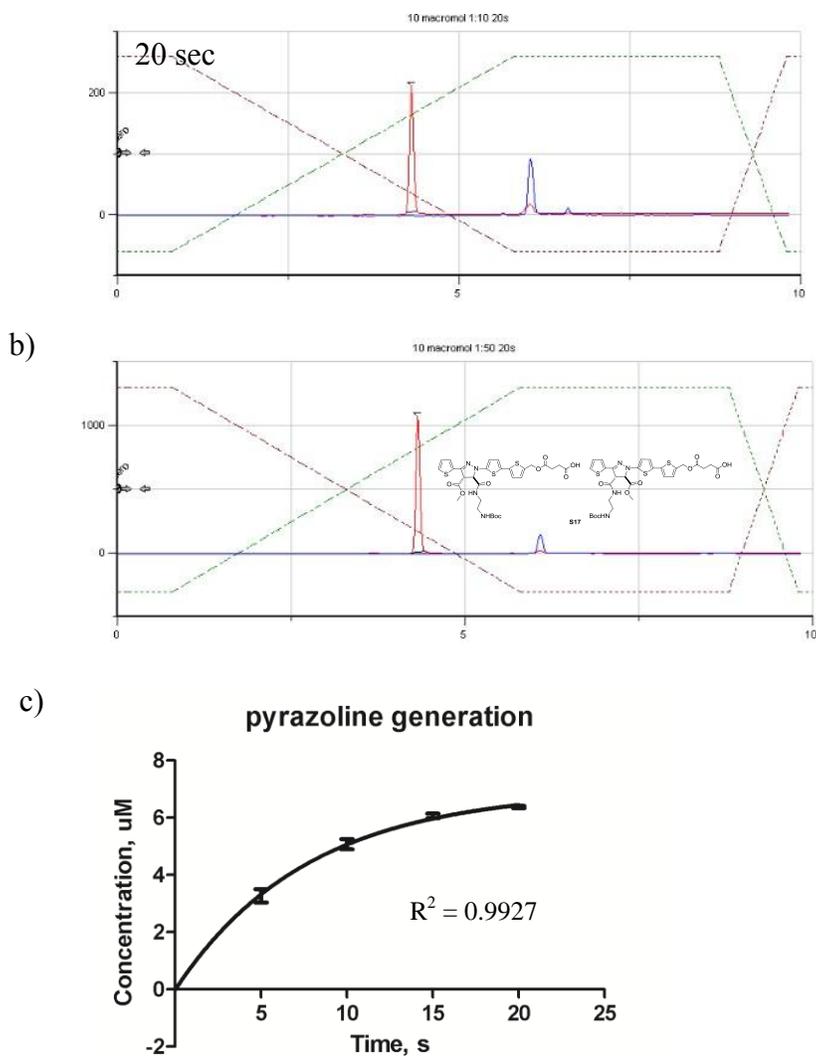


Figure S9. Kinetic characterization of tetrazole **6** in the laser-induced photoclick chemistry. (a) Time-course of the reaction as monitored by HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm. (b) HPLC trace of the reaction mixture after illuminating the solution of 10 μM tetrazole **6** and 500 μM MFA with 405 nm diode laser for 20 s to achieve 100% conversion (10 μM pyrazoline). Red trace = absorbance at 254 nm; blue trace = absorbance at 370 nm. (c) Time course for the pyrazoline generation. The plot was fitted to an exponential rise to maximum equation: $y = (y_0 - a) e^{-kt} + a$. The concentrations of the pyrazoline were obtained by comparing their absorption peak areas at 254 nm to the pyrazoline standard.

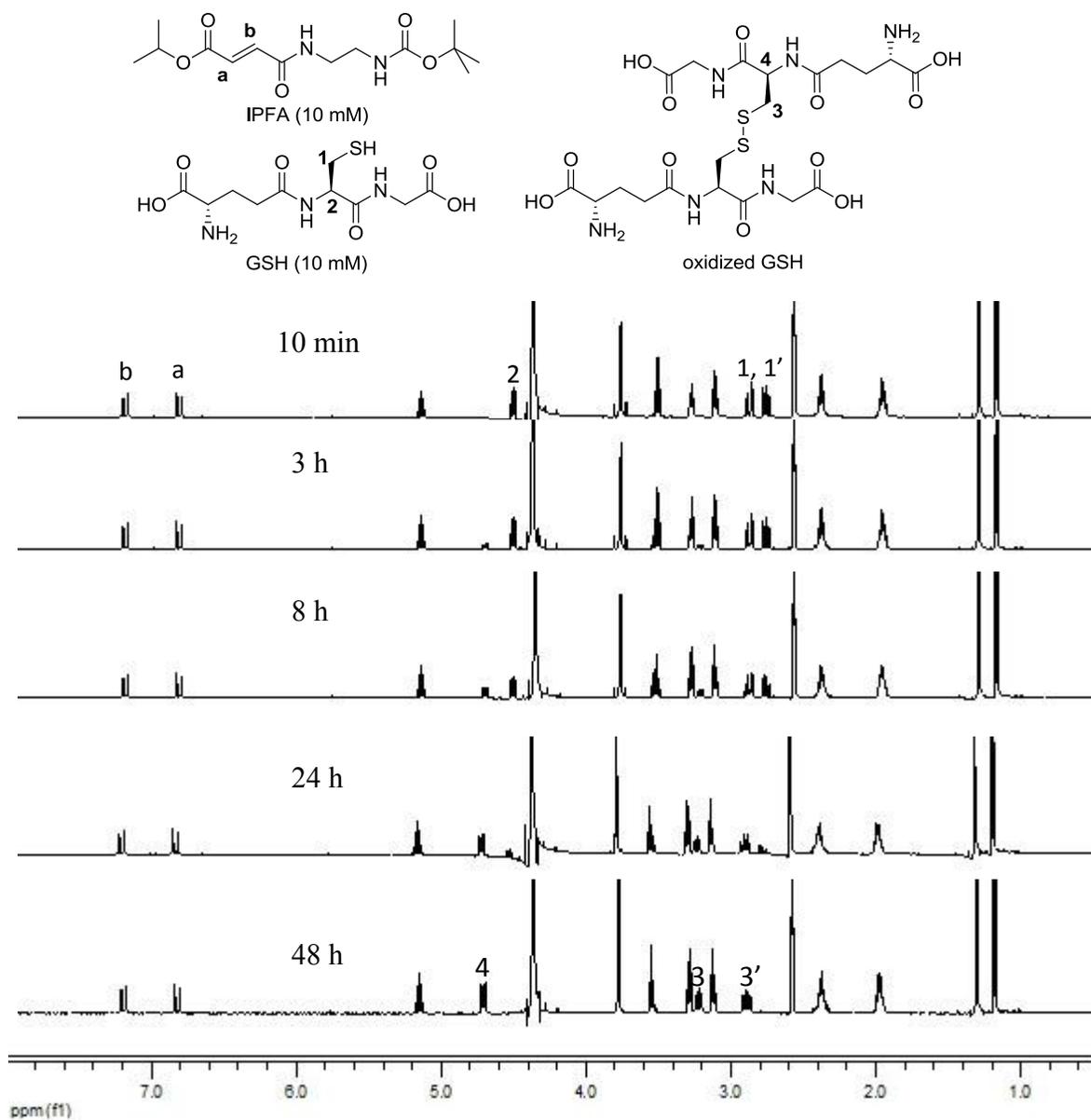


Figure S10. Stability of *mono*-isopropyl fumarate amide (IPFA) towards glutathione in mixed DMSO- d_6 /deuterated PBS buffer (7:3) as monitored by ^1H NMR. Condition: 10 mM IPFA was mixed with 10 mM GSH (reduced form) in deuterated solvent. The formation of oxidized GSH dimer was confirmed by observation of the intact mass ion $[\text{M}+\text{H}^+]$ of 613.0 in LC-MS analysis.

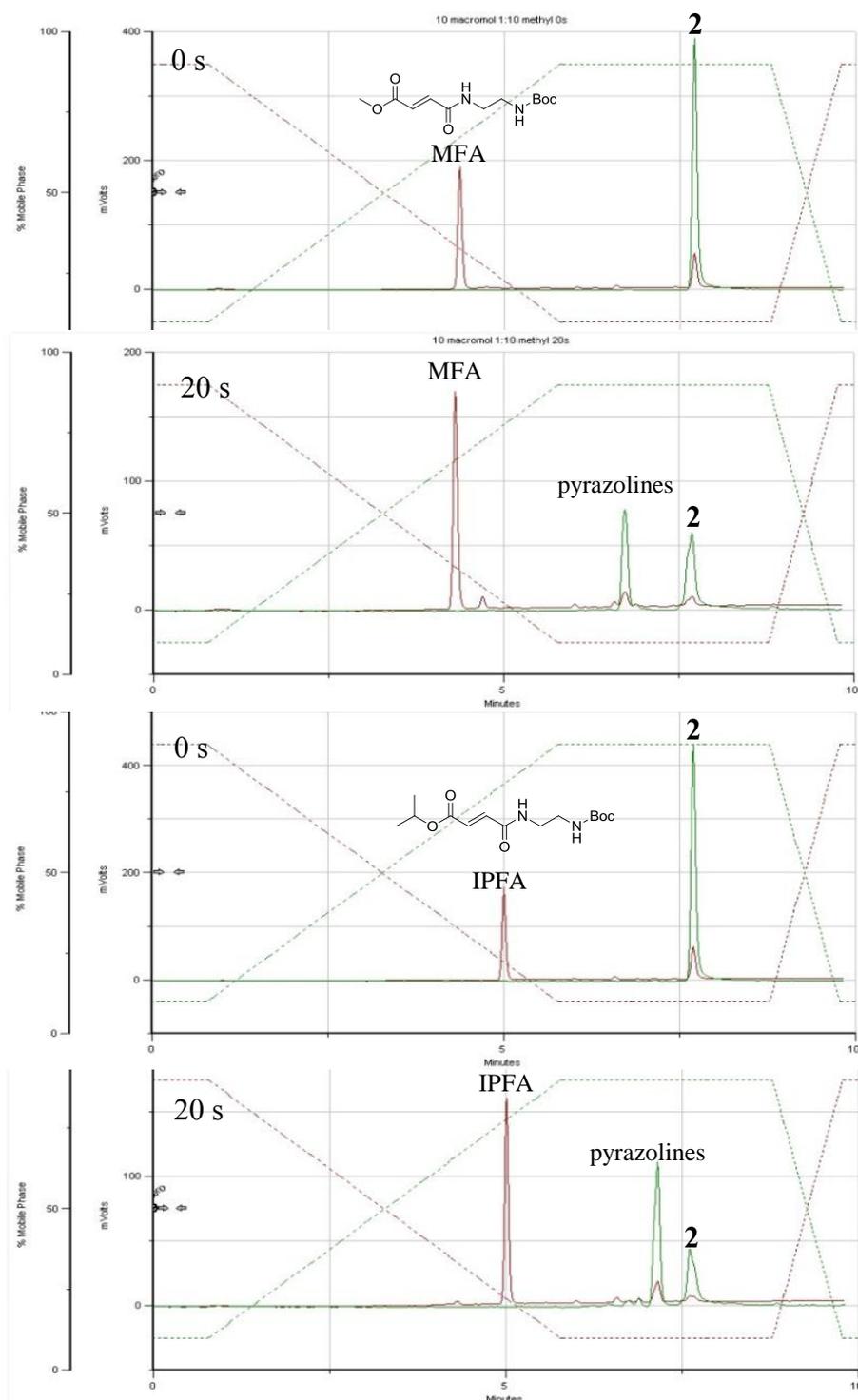


Figure S11. Reactivity comparison of MFA vs. IPFA in a laser-induced photoclick chemistry using tetrazole **2**. HPLC traces of the reaction mixtures before and after exposing the solution of 10 μM tetrazole **2** and 100 μM of MFA or IPFA in acetonitrile/PBS (1:1) to 405 nm photoillumination.

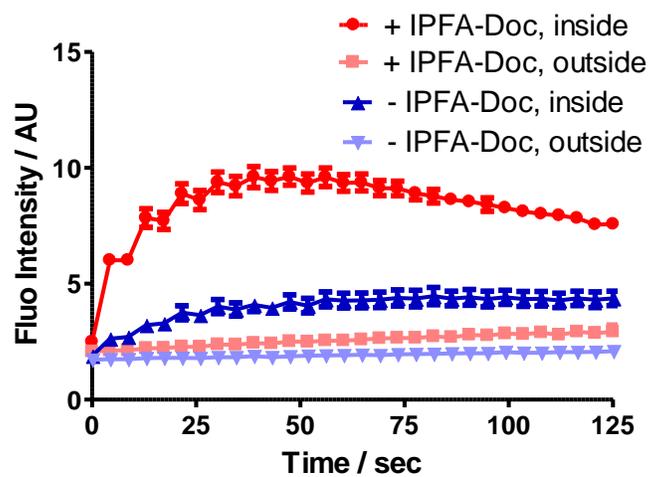
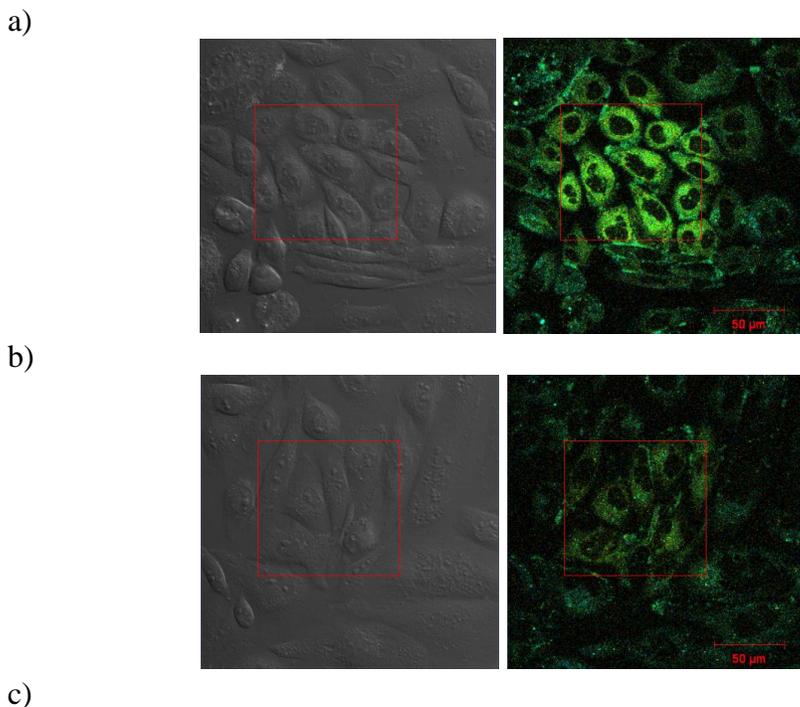


Figure S12. Spatiotemporally controlled imaging of microtubules *via* laser-triggered, docetaxel-directed photoclick chemistry. Plots of the time courses of the fluorescent intensities for inside and outside of the quadrangle areas for Fig. 3 in the main text.



c)

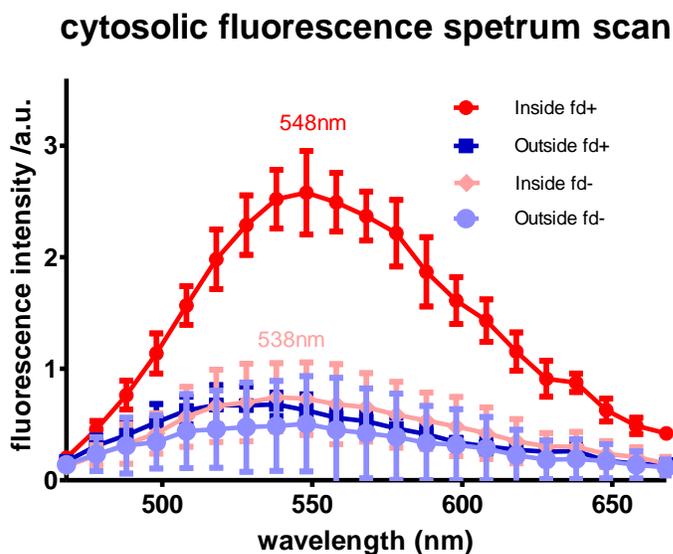
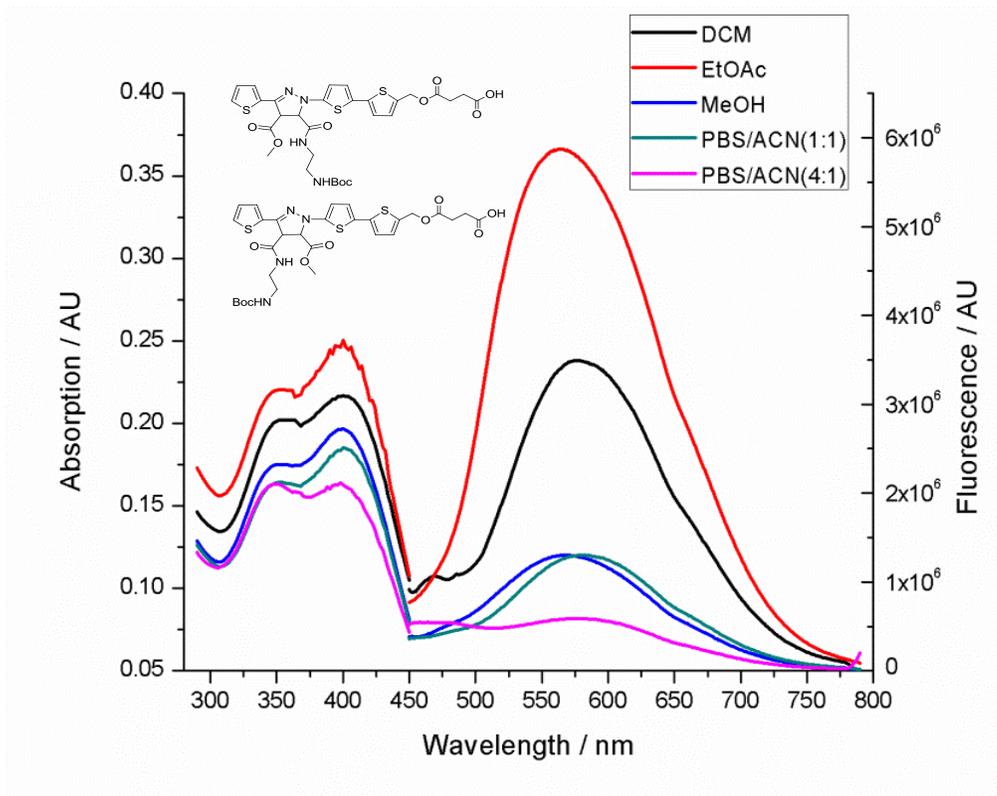


Figure S13. Confocal spectrum-scan micrographs of pyrazoline-docetaxel in live CHO cells. (a) Confocal fluorescent micrographs of live CHO cells and its corresponding DIC image. CHO cells were treated with 40 μM tetrazole **6** overnight and washed for 3 times with pre-warmed PBS. Then, 30 μM IPFA-docetaxel was added to cell culture for 30 min. Cells inside the quadrangle area were exposed to 405-nm laser at 0.15 mW for 15 s followed by fluorescence acquisition, $\lambda_{\text{ex}} = 458 \text{ nm}$. (b) Confocal micrographs of live CHO cells without treatment of IPFA-docetaxel (fd) and its corresponding DIC image. (c) Spectrum scans in the region of 468-668 nm with 10 nm window for each acquisition. The colors rendered in the fluorescence micrographs were λ -encoded (real color). Scale bar = 50 μm .

a)



b)

Solvent	EtOAc	MeOH	DCM	PBS/ACN (1:1)	PBS/ACN (4:1)
λ_{\max} (nm)	400	398	400	400	400
λ_{em} (nm)	560	568	577	581	585

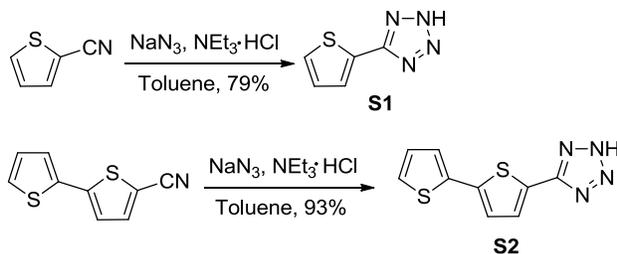
Figure S14. Solvent-dependency of the pyrazoline fluorophores. (a) UV-vis and fluorescence spectra of pyrazolines **S16** and **S17** in various solvents at 10 μM concentration. (b) Table of absorption and emission maxima of pyrazolines **S16** and **S17** in various solvents. For fluorescence measurement, $\lambda_{\text{ex}} = 405$ nm.

General Information

Solvents and chemicals were purchased from commercial sources and used directly without further purification. Flash chromatography was performed with SiliCycle P60 silica gel (40-63 μm , 60 \AA). ^1H NMR spectra were recorded with Inova-300, -400 or -500 MHz spectrometers and chemical shifts were reported in ppm using either TMS or deuterated solvents as internal standards (TMS, 0.00; CDCl_3 , 7.26; CD_3OD , 3.31; $\text{DMSO-}d_6$, 2.50). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra were recorded at 75.4 MHz, and chemical shifts were reported in ppm using deuterated solvents as internal standards (CDCl_3 , 77.0; $\text{DMSO-}d_6$, 39.5; CD_3OD , 49.05). UV-vis absorption spectra were recorded using 1-cm quartz cuvettes on a HP-8452 Diode Array Spectrophotometer. Fluorescence spectra were recorded using 1-cm plastic cuvettes on a JY Fluorolog Spectrofluorometer at 20 $^\circ\text{C}$. All fluorescence images were acquired with a Zeiss LSM-710 confocal microscope equipped with a continuous laser and fluorescence lifetime (FLIM) detector.

Experimental Procedures and Characterization Data

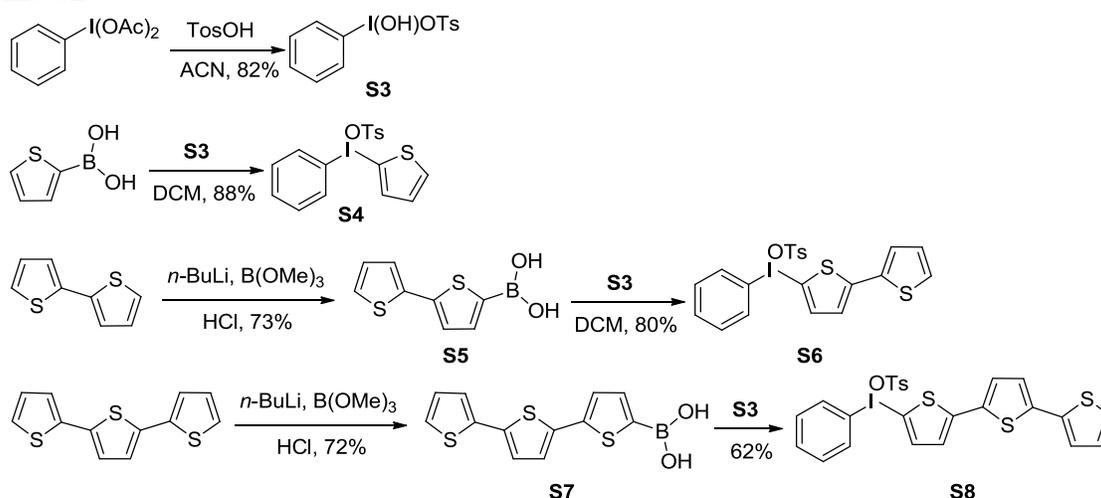
Scheme S1



5-(Thiophen-2-yl)-2H-tetrazole (S1): The compound was synthesized according to the published procedure^{S1} (79% yield): ^1H NMR (CDCl_3 , 300MHz) δ 7.91 (s, 1H), 7.54 (d, $J = 9.6$, 1H), 7.19 (t, $J = 4.2$, 1H); MS (ESI) calcd for $\text{C}_5\text{H}_4\text{N}_4\text{S}$ 153.0 [$\text{M}+\text{H}^+$], found 153.1.

5-([2,2'-Bithiophen]-5-yl)-2H-tetrazole (S2): The tetrazole S2 was synthesized using the same procedure as S1 to afford S2 as light brown solid (93% yield): ^1H NMR ($\text{DMSO-}d_6$, 300 MHz) δ 7.78 (d, $J = 3.6$ Hz, 1H), 7.52 (d, $J = 4.8$ Hz, 1H), 7.42 (d, $J = 3.0$ Hz, 1H), 7.38 (d, $J = 3.9$ Hz, 1H), 7.13 (dd, $J = 4.8, 3.6$ Hz, 1H); ^{13}C NMR δ ($\text{DMSO-}d_6$, 75 MHz) 151.6, 140.5, 135.6, 130.6, 129.1, 127.4, 126.1, 125.4, 124.2; MS (ESI) calcd for $\text{C}_5\text{H}_6\text{N}_4\text{S}_2$ 235.0 [$\text{M}+\text{H}^+$], found 235.0.

Scheme S2



Hydroxy(tosyloxy)iodobenzene (S3): The Koser's reagent **S3** was synthesized according to the literature procedure^{S2} (82% yield): ¹H NMR (DMSO-*d*₆, 300 MHz) δ 8.22 (d, *J* = 7.2 Hz, 2H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.64–7.59 (m, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 2.29 (s, 3H).

2-Thienyl-(phenyl) iodonium tosylate (S4): The diaryliodonium sulfonate **S4** was synthesized according to the literature procedure^{S3} (88% yield): ¹H NMR (CD₃OD, 300 MHz) δ 8.15 (d, *J* = 8.4 Hz, 2H), 8.00 (d, *J* = 3.0 Hz, 1H), 7.87 (d, *J* = 5.4 Hz, 1H), 7.70–7.65 (m, 3H), 7.54–7.49 (m, 2H), 7.23–7.16 (m, 3H), 2.36 (s, 3H).

(2,2'-Bithiophen)-5-yl-boronic acid (S5): The boronic acid **S5** was synthesized according to the literature procedure^{S4} (73% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.93 (d, *J* = 4.0 Hz, 1H), 7.39 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.33 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.28 (d, *J* = 4.5 Hz, 1H), 7.10 (dd, *J* = 5.0, 4.0 Hz, 1H).

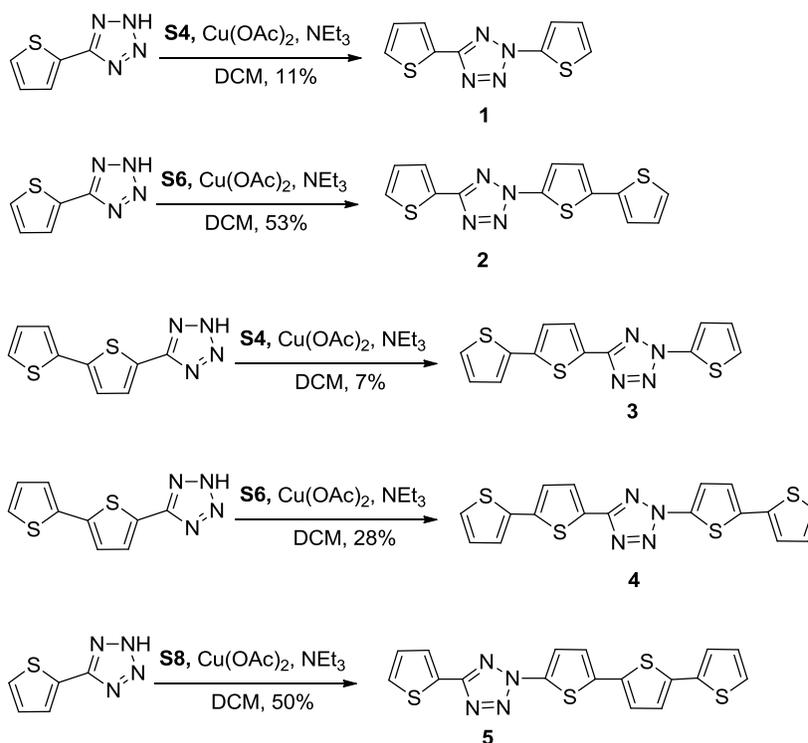
(2,2'-Bithiophen)-5-yl-(phenyl) iodonium tosylate (S6): To a stirred suspension of **S3** (681 mg, 1.74 mmol) in 25 mL DCM was added a solution of **S5** (365 mg, 1.74 mmol) in 15 mL DCM. The resulting mixture was stirred at room temperature overnight. The solvent was removed in reduced pressure and diethyl ether was added. The precipitate was collected to afford the product as a green solid after drying in vacuum (750 mg, 80% yield): ¹H NMR (DMSO-*d*₆, 300 MHz) δ 8.26 (d, *J* = 7.5 Hz, 2H), 8.01 (d, *J* = 3.3 Hz, 1H), 7.65–7.62 (m, 2H), 7.55–7.45 (m, 4H), 7.41 (d, *J* = 2.7 Hz, 1H), 7.32 (d, *J* = 4.2 Hz, 1H), 7.13–7.08 (m, 3H); ¹³C NMR δ (DMSO-*d*₆, 75 MHz) δ 147.0, 146.0, 142.1, 138.2, 135.1, 134.3, 132.6, 132.2, 129.2, 128.5, 128.3, 126.9, 126.1, 126.0, 120.0, 99.1, 21.2; MS (ESI) calcd for C₁₄H₁₀IS₂ 368.9 [M – (SO₃C₇H₇)]⁺, found 368.9.

[2,2':5',2''-Terthiophen]-5-ylboronic acid (S7): The boronic acid **S7** was synthesized using the same procedure as **S5** (72% yield): ¹H NMR δ (CD₃OD, 300 MHz) 7.55 (br, 1H), 7.34 (d, *J* = 5.1

Hz, 1H), 7.27 (br, 1H), 7.23 (d, $J = 3.6$ Hz, 1H), 7.19 (d, $J = 3.6$ Hz, 1H), 7.14 (d, $J = 3.6$ Hz, 1H), 7.04 (dd, $J = 5.1, 3.6$ Hz, 1H).

(2,2':5'2''-Terthiophen)-5-yl-(phenyl) iodonium tosylate (S8): The diaryliodonium sulfonate **S8** was synthesized using the same procedure as **S6** without further purification and used directly in the next-step synthesis (62% yield): ^1H NMR (CD_3OD , 500 MHz) δ 8.22 (d, $J = 7.5$ Hz, 2H), 7.95 (d, $J = 4.0$ Hz, 1H), 7.72 (m, 3H), 7.58 (t, $J = 7.5$ Hz, 2H), 7.41 (d, $J = 5.5$ Hz, 1H), 7.30 (d, $J = 4.0$ Hz, 2H), 7.24–7.26 (br, 3H), 7.21 (d, $J = 3.5$ Hz, 1H), 7.08 (dd, $J = 4.0, 5.5$ Hz, 1H), 2.38 (s, 3H); MS (ESI) calcd for $\text{C}_{18}\text{H}_{12}\text{IS}_3$ 450.9 $[\text{M} - (\text{SO}_3\text{C}_7\text{H}_7)]^+$, found 450.9.

Scheme S3



2,5-Di(thiophen-2-yl)-2H-tetrazole (1): To a solution of tetrazole **S1** (76 mg, 0.5 mmol) in 4 mL DCM was added iodonium salt **S4** (274 mg, 0.6 mmol), $\text{Cu}(\text{OAc})_2$ (90.5 mg, 0.5 mmol), and Et_3N (80 μL , 0.6 mmol). The resulting mixture was purged with argon and sealed. The reaction mixture was stirred at room temperature for 16 h. The mixture was diluted by adding 5 mL water and then extracted with DCM (5 mL \times 3). The organic layer was separated, washed sequentially with saturated NH_4Cl and brine, dried over anhydrous MgSO_4 , and concentrated in vacuum. The residue was purified by silica gel flash chromatography ($\text{EtOAc}/\text{hexanes} = 1:15$) to give the titled compound as a white solid (10 mg, 11% yield): ^1H NMR (CDCl_3 , 500 MHz) δ 7.90 (dd, $J = 4.0, 1.0$ Hz, 1H), 7.68 (dd, $J = 4.5, 1.5$ Hz, 1H), 7.50 (dd, $J = 4.8, 0.8$ Hz, 1H), 7.30 (dd, $J = 4.5, 1.5$ Hz, 1H), 7.19 (dd, $J = 5.2, 3.8$ Hz, 1H), 7.09 (dd, $J = 5.3, 3.8$ Hz, 1H); ^{13}C NMR (CDCl_3 , 75

MHz) δ 161.2, 137.7, 128.5, 128.5, 128.3, 128.0, 126.6, 123.7, 119.3; HRMS (EI) calcd for C₉H₆N₄S₂ 234.0028 [M⁺], found 234.0038.

2-([2,2'-Bithiophen]-5-yl)-5-(thiophen-2-yl)-2H-tetrazole (2): To a solution of tetrazole **S1** (76 mg, 0.5 mmol) in 4 mL DCM was added iodonium salt **S6** (324 mg, 0.6 mmol), Cu(OAc)₂ (106 mg, 0.6 mmol), and Et₃N (80 μ L, 0.6 mmol). The resulting mixture was sealed with argon and stirred at room temperature for 18 h. The mixture was diluted by adding 5 mL water and then extracted with DCM (5 mL \times 3). The organic layer was separated, washed sequentially with saturated NH₄Cl and brine, dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography (DCM/hexanes = 1:4 then EtOAc/hexanes = 1:10) to give the titled compound as a light-yellow solid (84 mg, 53% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.92 (dd, *J* = 3.8, 1.3 Hz, 1H), 7.60 (d, *J* = 4.5 Hz, 1H), 7.53 (dd, *J* = 4.5, 1.0 Hz, 1H), 7.33 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.28 (dd, *J* = 3.5, 1.0 Hz, 1H), 7.21 (dd, *J* = 5.0, 4.0 Hz, 1H), 7.14 (d, *J* = 4.5 Hz, 1H), 7.09 (dd, *J* = 3.8, 5.3 Hz, 1H); ¹³C NMR (CDCl₃, 75 MHz) δ 161.2, 136.1, 135.7, 129.7, 128.6, 128.3, 128.1, 128.0, 125.8, 124.8, 122.6, 119.9, 119.7; HRMS (ESI) calcd for C₁₃H₉N₄S₃ 316.9984 [M+H⁺], found 316.9992.

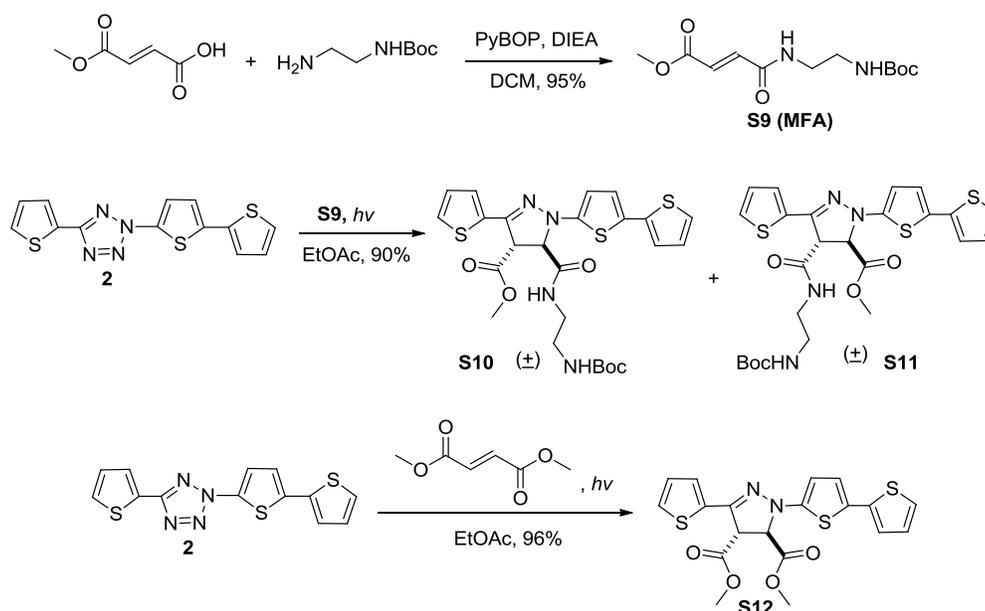
5-([2,2'-Bithiophen]-5-yl)-2-(thiophen-2-yl)-2H-tetrazole (3): To a solution of tetrazole **S2** (132 mg, 0.56 mmol) in 6 mL DCM was added iodonium salt **S4** (284 mg, 0.62 mmol), Cu(OAc)₂ (101 mg, 0.56 mmol), and Et₃N (95 μ L, 0.7 mmol). The resulting mixture was sealed with argon and stirred at room temperature for 18 h. The mixture was diluted by adding 5 mL water and then extracted with DCM (6 mL \times 3). The organic layer was separated, washed sequentially with saturated NH₄Cl aqueous and brine. The organic layer was dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography (EtOAc/hexanes = 1:15) to give the titled compound as a white solid (13 mg, 7% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.82 (d, *J* = 3.5 Hz, 1H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.33–7.31 (m, 3H), 7.26 (d, *J* = 3.5 Hz, 1H), 7.12 (t, *J* = 5.0 Hz, 1H), 7.09 (t, *J* = 5.0 Hz, 1H); ¹³C NMR (CDCl₃, 75 MHz) δ 160.9, 140.6, 137.7, 136.5, 129.2, 128.1, 126.6, 126.5, 125.5, 124.7, 124.3, 123.8, 119.3; HRMS (EI) calcd for C₁₃H₈N₄S₃ 315.9906 [M⁺], found 315.9906.

2,5-Di([2,2'-bithiophen]-5-yl)-2H-tetrazole (4): To a solution of tetrazole **S2** (117 mg, 0.5 mmol) in 6 mL DCM was added iodonium salt **S6** (270 mg, 0.5 mmol), Cu(OAc)₂ (90.5 mg, 0.5 mmol), and Et₃N (80 μ L, 0.6 mmol). The resulting mixture was sealed with argon and stirred at room temperature for 18 h. The mixture was diluted by adding 5 mL water and then extracted with DCM (5 mL \times 3). The organic layer was separated, washed sequentially with saturated NH₄Cl and brine, dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography (EtOAc/hexanes = 1:15) to give the titled compound as a light-yellow solid (55 mg, 28% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.80 (d, *J* = 4.0 Hz, 1H), 7.59 (d, *J* = 4.0 Hz, 1H), 7.32–7.26 (m, 4H), 7.24 (d, *J* = 4.0 Hz, 1H), 7.13 (d, *J* = 4.0 Hz, 1H), 7.09–7.06 (m, 2H); ¹³C NMR (CDCl₃, 75 MHz) δ 160.9, 140.6, 136.5, 135.7, 129.7, 129.3,

128.1, 128.0, 126.4, 125.8, 125.5, 124.8, 124.7, 124.3, 122.5, 119.8, 119.6; HRMS (ESI) calcd for $C_{17}H_{11}N_4S_4$ 398.9861 $[M+H^+]$, found 398.9874.

2-([2,2':5',2''-Terthiophen]-5-yl)-5-(thiophen-2-yl)-2H-tetrazole (5): To a solution of tetrazole **S1** (30.4 mg, 0.2 mmol) in 4 mL DCM was added iodonium salt **S8** (124 mg, 0.2 mmol), $Cu(OAc)_2$ (36.2 mg, 0.2 mmol), and Et_3N (35 μ L, 0.26 mmol). The resulting mixture was sealed with argon and stirred at room temperature for 18 h. The mixture was diluted by adding 5 mL water and then extracted with DCM (5 mL \times 3). The organic layer was separated, washed sequentially with saturated NH_4Cl and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuum. The residue was purified by silica gel flash chromatography (EtOAc/hexanes = 1:15) to give the titled compound as a light-yellow solid (52 mg, 50% yield): 1H NMR ($CDCl_3$, 500 MHz) δ 7.89 (dd, J = 3.8, 1.3 Hz, 1H), 7.58 (d, J = 4.0 Hz, 1H), 7.51 (dd, J = 5.0, 1.0 Hz, 1H), 7.26 (dd, J = 5.0, 1.0 Hz, 1H), 7.21–7.18 (m, 2H), 7.14 (d, J = 4.0 Hz, 1H), 7.12–7.10 (m, 2H), 7.04 (dd, J = 4.8, 3.8 Hz, 1H); ^{13}C NMR δ ($CDCl_3$, 75 MHz) 161.2, 137.8, 136.6, 135.7, 134.3, 128.6, 128.3, 128.1, 128.0, 125.4, 125.1, 124.5, 124.2, 122.4, 119.7; HRMS (ESI) calcd for $C_{17}H_{11}N_4S_4$ 398.9861 $[M+H^+]$, found 398.9874.

Scheme S4



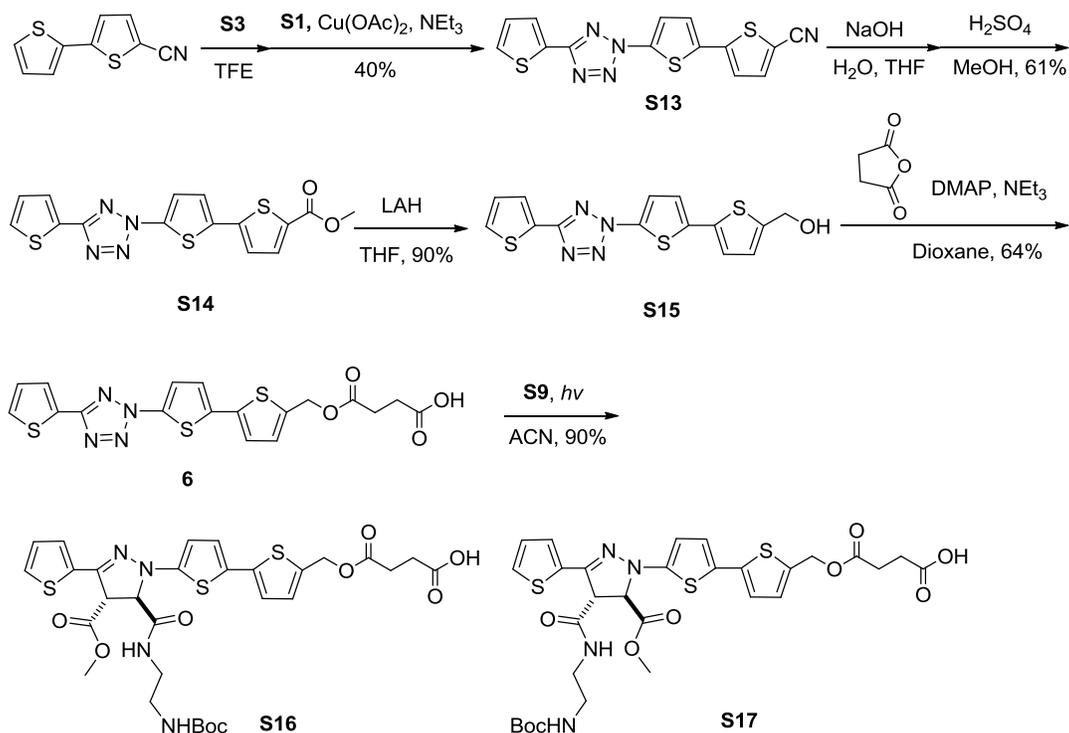
(*E*)-Methyl 4-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-4-oxobut-2-enoate (S9): To a suspended solution of *mono*-methyl fumarate acid (130 mg, 1 mmol) in 10 mL DCM was added DIEA (570 μ L, 3.3 mmol). After the acid was completely dissolved, PyBOP (572 mg, 1.1 mmol) and *N*-Boc-ethylenediamine (160 mg, 1.0 mmol) were added. The solution was stirred at room temperature for 1 h. Additional 10 mL DCM was added, and the organic layer was separated and washed successively with 5% citric acid in water and saturated $NaHCO_3$. The organic layer was

then dried over anhydrous MgSO_4 and concentrated in vacuum. The residue was purified by silica gel flash chromatography (EtOAc/hexanes = 1:1) to give the titled compound as a white solid (258 mg, 95% yield): ^1H NMR (CDCl_3 , 500 MHz) δ 7.00 (br, 1H), 6.92 (d, J = 15 Hz, 1H), 6.81 (d, J = 16 Hz, 1H), 5.02 (br, 1H), 3.80 (s, 3H), 3.47 (m, 2H), 3.29 (br, 2H), 1.44 (s, 9H); ^{13}C NMR δ (CDCl_3 , 75 MHz) δ 166.0, 164.1, 157.2, 136.5, 129.8, 80.1, 52.2, 41.5, 39.9, 28.3; ESI-MS calcd for $\text{C}_{12}\text{H}_{20}\text{N}_2\text{O}_5\text{Na}$ 295.1 [$\text{M}+\text{Na}^+$], found 295.1.

Methyl 1-([2,2'-bithiophen]-5-yl)-5-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-4-carboxylate (S10) and Methyl 1-([2,2'-bithiophen]-5-yl)-4-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-5-carboxylate (S11): To a solution of tetrazole **2** (10 mg, 0.03 mmol) in EtOAc was added **S9** (26 mg, 0.095 mmol), and the mixture was exposed to 365 nm UV lamp for 30 min with stirring at room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography to give a pair of regioisomers **S10** and **S11** as a yellow solid (16 mg, 90% yield) in roughly 1:1 ratio. The structures were assigned by comparing their chemical shifts to those of compound **S12**. **S10**: ^1H NMR (CDCl_3 , 500 MHz) δ 7.41 (d, J = 4.5 Hz, 1H), 7.39 (d, J = 4.0 Hz, 1H), 7.17 (dd, J = 5.5, 1.5 Hz, 1H), 7.08 (dd, J = 4.0, 1.5 Hz, 1H), 7.06 (dd, J = 5.5, 4.0 Hz, 1H), 7.01 (dd, J = 5.5, 4.0 Hz, 1H), 6.92 (d, J = 4.0 Hz, 1H), 6.16 (d, J = 4.0 Hz, 1H), 4.89 (d, J = 7.0 Hz, 1H), 4.57 (d, J = 6.5 Hz, 1H), 3.82 (s, 3H), 3.42-3.45 (br, 2H), 3.28-3.30 (br, 2H), 1.39 (s, 9H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 169.1, 168.9, 156.4, 148.9, 143.5, 137.6, 133.3, 128.6, 127.7, 127.6, 127.3, 123.4, 122.7, 122.5, 106.5, 79.8, 70.9, 58.3, 53.4, 40.5, 40.1, 28.2; ESI-MS calcd for $\text{C}_{25}\text{H}_{28}\text{N}_4\text{O}_5\text{S}_3$ 583.1 [$\text{M}+\text{Na}^+$], found 583.2. **S11**: ^1H NMR (CDCl_3 , 500 MHz) δ 7.39 (d, J = 5.5 Hz, 1H), 7.29 (d, J = 5.0 Hz, 1H), 7.14 (d, J = 5.0 Hz, 1H), 7.05-7.07 (m, 2H), 6.99 (dd, J = 5.0, 3.5 Hz, 1H), 6.90 (d, J = 3.5 Hz, 1H), 6.17 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 6.0 Hz, 1H), 4.44 (d, J = 6.0 Hz, 1H), 3.83 (s, 3H), 3.42-3.45 (br, 2H), 3.28-3.30 (br, 2H), 1.39 (s, 9H); ESI-MS calcd for $\text{C}_{25}\text{H}_{28}\text{N}_4\text{O}_5\text{S}_3\text{Na}$ 583.1 [$\text{M}+\text{Na}^+$], found 583.1.

Dimethyl 1-([2,2'-bithiophen]-5-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-4,5-dicarboxylate (S12): To a solution of tetrazole **2** (10 mg, 0.03 mmol) in EtOAc was added dimethyl fumarate (13.7 mg, 0.09 mmol), and the mixture was exposed to 365 nm UV lamp for 30 min with stirring at room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography to give the titled compound **S12** as a yellow solid (12.6 mg, 92% yield): ^1H NMR (CDCl_3 , 500 MHz) δ 7.38 (dd, J = 5.0, 1.0 Hz, 1H), 7.35 (dd, J = 3.5, 1.0 Hz, 1H), 7.14 (dd, J = 5.0, 1.0 Hz, 1H), 7.05-7.07 (m, 2H), 6.99 (dd, J = 5.5, 3.5 Hz, 1H), 6.91 (d, J = 4.0 Hz, 1H), 6.17 (d, J = 4.0 Hz, 1H), 5.08 (d, J = 6.0 Hz, 1H), 4.65 (d, J = 6.0 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 169.1, 168.5, 148.2, 141.0, 138.0, 133.7, 128.0, 127.7, 127.6, 127.5, 126.5, 123.1, 122.6, 122.2, 106.2, 68.4, 57.1, 53.4, 53.3; ESI-MS calcd for $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_4\text{S}_3\text{Na}$ 455.0 [$\text{M}+\text{Na}^+$], found 455.1.

Scheme S5



5'-(5-(Thiophen-2-yl)-2H-tetrazol-2-yl)-[2,2'-bithiophene]-5-carbonitrile (S13): To a solution of (2,2'-bithiophene)-5-carbonitrile (120 mg, 0.6 mmol) in 10 mL 2,2,2-trifluoroethanol at 0 °C was added hydroxy(tosyloxy)iodobenzene (245 mg, 0.6 mmol) in one portion, and the solution changed to a dark color. The mixture was stirred at 0 °C for 4 h before 5 mL MeOH was added, and the stirring was allowed to continue for 5 min. The solvent was removed under reduced pressure, and the crude iodonium salt was used directly for the next coupling. To the crude iodonium salt in 20 mL DCM was added tetrazole **S1** (45 mg, 0.3 mmol), Cu(OTf)₂ (108 mg, 0.3 mmol) and Et₃N (262 μL, 1.88 mmol), and the mixture was stirred under argon at room temperature for 20 h. The mixture was then diluted by adding 10 mL water and extracted with DCM (10 mL × 3). The organic layer was separated, washed successively with saturated NH₄Cl and brine, dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography to give the titled compound as a light yellow solid (40 mg, 40% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.91 (dd, *J* = 4.0, 1.5 Hz, 1H), 7.64 (d, *J* = 4.0 Hz, 1H), 7.58 (d, *J* = 4.0 Hz, 1H), 7.52 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.25 (d, *J* = 4.0 Hz, 1H), 7.22 (d, *J* = 4.0 Hz), 7.20 (dd, *J* = 5.0, 3.5 Hz, 1H); ¹³C NMR (CDCl₃, 75 MHz) δ 161.5, 142.7, 138.3, 133.0, 128.9, 128.1, 127.9, 125.1, 124.4, 119.7, 113.7, 109.0. ESI-MS calcd for C₁₄H₈N₅S₃ 342.0 [M+H⁺], found 342.1.

Methyl 5'-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)-[2,2'-bithiophene]-5-carboxylate (S14): To a solution of 1 M NaOH in mixed THF/H₂O (1:4) solvent was added **S13** (34 mg, 0.1 mmol), and

the stirred mixture was heated to 80 °C under argon until the starting materials disappeared. After adjusting pH to 4.0 with 4 M HCl, the solution was extracted with EtOAc. The solvents were removed under reduced pressure. The residue was added 50 mL methanol and 0.5 mL concentrated H₂SO₄, and the solution was refluxed for 12 h. After removing 30 mL of organic solvent, the solution was extracted with EtOAc. The organic layer was separated, dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography to give the titled compound as a light yellow solid (30 mg, 80% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.93 (dd, *J* = 3.5, 1.0 Hz, 1H), 7.76 (d, *J* = 4.0 Hz, 1H), 7.65 (d, *J* = 4.0 Hz, 1H), 7.54 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.26 (d, *J* = 4.0 Hz, 1H), 7.24 (d, *J* = 4.0 Hz, 1H), 7.22 (dd, *J* = 5.0, 1.5 Hz, 1H), 3.94 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 162.2, 161.3, 142.1, 138.3, 136.8, 134.7, 134.3, 132.8, 128.9, 128.8, 128.1, 124.9, 124.2, 119.7, 52.4; ESI-MS calcd for C₁₅H₁₁N₄O₂S₃ 375.0 [M+H⁺], found 375.3.

(5'-(5-(Thiophen-2-yl)-2H-tetrazol-2-yl)-[2,2'-bithiophen]-5-yl)methanol (S15): To a solution of compound **S14** (30 mg, 0.08 mmol) in 5 mL THF at 0 °C was add LAH (6 mg, 0.16 mmol). One mL methanol was added to the reaction mixture after the starting materials disappeared. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography to give the titled compound as a light-yellow solid (20 mg, 72% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.92 (dd, *J* = 4.0, 1.0 Hz, 1H), 7.61 (d, *J* = 3.5 Hz, 1H), 7.53 (dd, *J* = 5.5, 1.5 Hz, 1H), 7.21 (dd, *J* = 5.0, 4.0 Hz, 1H), 7.14 (d, *J* = 3.5 Hz, 1H), 7.12 (d, *J* = 4.0 Hz, 1H), 6.99 (d, *J* = 3.0 Hz, 1H), 4.87 (s, 1H); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 160.5, 147.9, 135.8, 133.8, 133.3, 130.1, 128.9, 128.7, 127.2, 125.2, 125.1, 122.9, 120.9, 58.3; ESI-MS calcd for C₁₄H₁₁N₄OS₃ 347.0 [M+H⁺], found 347.2.

4-Oxo-4-((5'-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)-[2,2'-bithiophen]-5-yl)methoxy)butanoic acid (6): To a solution of **S15** (15 mg, 0.04 mmol) in dioxane was added succinic anhydride (21.6 mg, 0.22 mmol), 4-dimethylaminopyridine (1.3 mg, 0.01 mmol), and Et₃N (60 μL, 0.43 mmol). The mixture was stirred at 80 °C under argon for 5 h. After evaporating the solvent, 3 M HCl was added to adjust the pH to 6.0. The solution was then diluted by adding 5 mL water and extracted with DCM (5mL × 3). The organic layer was separated, dried over anhydrous MgSO₄, and concentrated in vacuum. The residue was purified by silica gel flash chromatography (EtOAc/MeOH = 5:1) to give the titled compound as a light-yellow solid (16 mg, 83% yield): ¹H NMR (CDCl₃, 500 MHz) δ 7.91 (dd, *J* = 3.5, 1.0 Hz, 1H), 7.59 (d, *J* = 4.0 Hz, 1H), 7.52 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.20 (dd, *J* = 5.0, 3.5 Hz, 1H), 7.12 (t, 2H), 7.04 (d, *J* = 3.0 Hz, 1H), 5.28 (s, 2H), 2.69-2.71 (m, 4H); ¹³C NMR (CDCl₃, 75 MHz) δ 171.8, 161.2, 138.1, 137.2, 135.6, 129.2, 128.6, 128.2, 128.1, 124.3, 122.8, 119.7, 60.8, 28.9, 28.6; ESI-MS calcd for C₁₈H₁₅N₄O₄S₃ 447.0 [M+H⁺], found 447.1.

Pyrazolines S16 and S17: To a solution of tetrazole **6** (10 mg, 0.022 mmol) in 30 mL acetonitrile was added **S9** (61 mg, 0.22 mmol), and the mixture was exposed to 365 nm UV lamp for 10 min with stirring at room temperature. Afterwards, the solvent was evaporated and the

residue was purified by silica gel flash chromatography to give a pair of regioisomers **S16** and **S17** in 1:1 ratio: ^1H NMR (CD_3OD , 500 MHz) δ 7.41-7.44 (m, 2H; **S17**, **S18**), 7.29 (d, $J = 3.0$ Hz, 1H; **S17**), 7.22 (d, $J = 3.5$ Hz, 1H; **S18**), 6.99-7.01 (m, 2H; **S17**, **S18**), 6.91-6.92 (m, 2H; **S17**, **S18**), 6.82-6.86 (m, 4H; **S17**, **S18**), 6.03 (d, $J = 4.0$ Hz, 1H; **S18**), 6.00 (d, $J = 4.0$ Hz, 1H; **S17**), 5.17 (s, 4H; **S17**, **S18**), 4.83 (d, $J = 7.0$ Hz, 1H; **S18**), 4.79 (d, $J = 7.0$ Hz, 1H; **S17**), 4.60 (d, $J = 7.0$ Hz, 1H; **S17**), 4.51 (d, $J = 7.0$ Hz, 1H; **S18**), 3.72 (s, 3H; **S18**), 3.71 (s, 3H; **S17**), 3.26 (m, 4H; **S17**, **S18**), 3.13 (m, 4H; **S17**, **S18**), 1.35-1.36 (br, 18H; **S17**, **S18**); ESI-MS calcd for $\text{C}_{30}\text{H}_{34}\text{N}_4\text{O}_9\text{S}_3\text{Na}$ 713.1 [$\text{M}+\text{Na}^+$], found 712.8.

Determination of quantum yield

The quantum yields of tetrazoles **2** and **6** were determined using potassium ferrioxalate-based chemical actinometer.^{S5} In brief, a fresh 500 μL solution of 6 mM potassium ferrioxalate in 0.1 N H_2SO_4 was irradiated with 405-nm laser light in a quartz tube for specified times before quenching by addition of 4.5 mL of NaOAc/HOAc buffer (pH = 4.6) and 5 mL of 0.1% 1,10-phenanthroline solution in water. The mixture was stirred for 30 min before UV-vis measurement. All the work was carried out in the dark and the samples were protected from light with aluminum foil during handling. The quantum yield for a test compound was calculated based on the following equation^{S6}: $\Phi_t = (\varepsilon_c / \varepsilon_t)(k_t / k_c)(c_c / c_t)\Phi_c$, where ε_c and ε_t are extinction coefficients of the standard and the test compound, respectively; k_t and k_c are slopes for the test compound and the standard, respectively; and c_c and c_t are concentrations of the standard and the test compound, respectively. The quantum yields of 405 nm light-induced ring rupture for tetrazoles **2** and **6** were determined to be 0.16 and 0.15, respectively.

Confocal fluorescence microscopy

CHO cells were allowed to grow to about 70-80% confluency in DMEM medium supplemented with 10% FBS on 35-mm culture dishes pre-installed with a glass cover-slip at the bottom. The cells were treated with 40 μM of tetrazole **6** overnight before washing three times with pre-warmed PBS. The cells were treated with 30 μM of IPFA-docetaxel for 30 min in OPTI-MEM. The sample was then placed underneath the confocal microscope for photoactivation followed by imaging intermittently (405 nm laser dwell 1.27 $\mu\text{s}/\text{pixel}$; 458 nm excitation, 6.30 $\mu\text{s}/\text{pixel}$, repeatedly). The imaging acquisitions were carried out using a Zeiss LSM-710 confocal microscope equipped with a continuous laser and fluorescence lifetime (FLIM) detector; InVis: f-MBS 405/505c Plate; DBS1: Mirror; FW1: rear with specific filter window (531-623 nm); and Plan-Apochromat 40x/1.3 oil objective. The data quantifications were carried out using the Zeiss ZEN 2009 light edition, LSM image browser, or NIH ImageJ program. For turn-on fluorescence quantification, at each time point the fluorescence intensities of the cytosols of 10 individual cells with unsaturated fluorescence inside and outside of the rectangular area were used to derive the average intensities along with standard deviations. For spectrum scan, the average intensities

of the unsaturated fluorescence-labeled cytoskeleton areas in 10 selected cells were plotted to give the in-cell fluorescence spectrum along with standard deviations.

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^1H and ^{13}C NMR Spectra

