Electronic Supplementary Information

Bridging cells of three colors with two bio-orthogonal click reactions

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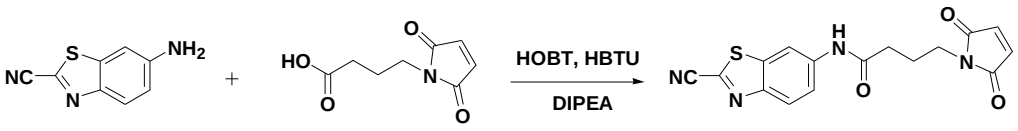


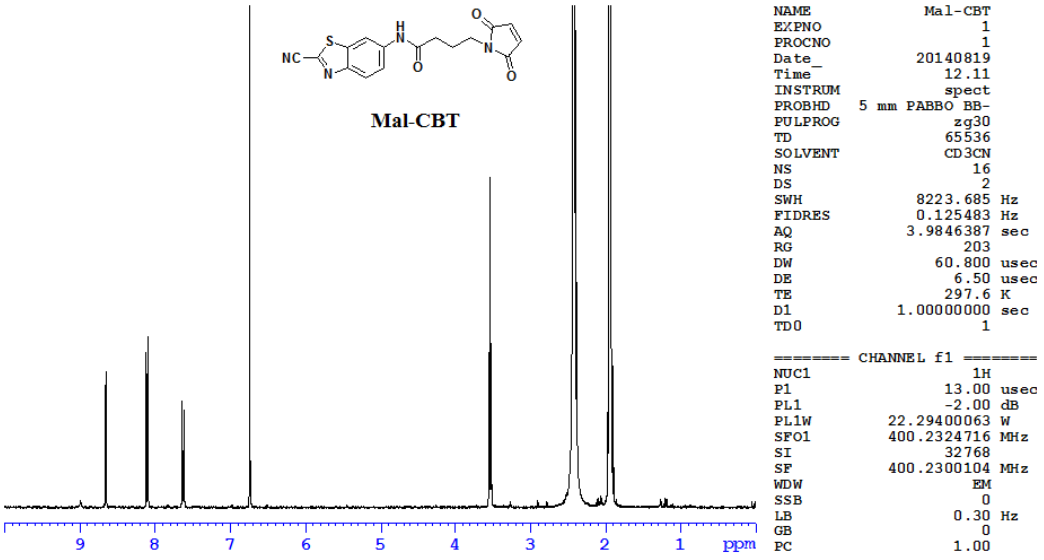


Figure S1. ^{1}HNMR spectrum of **Mal-CBT**.

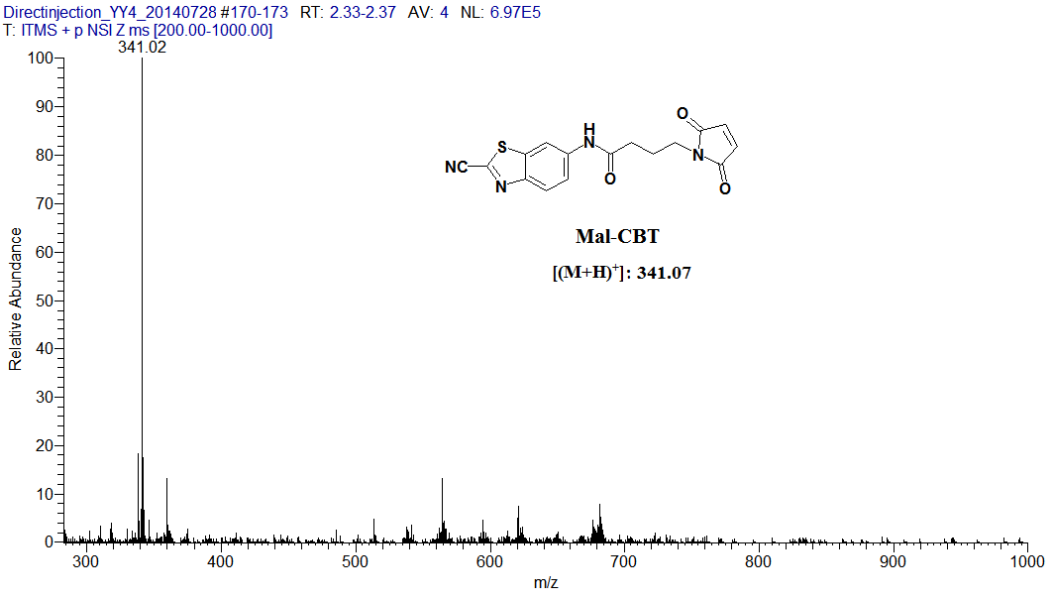
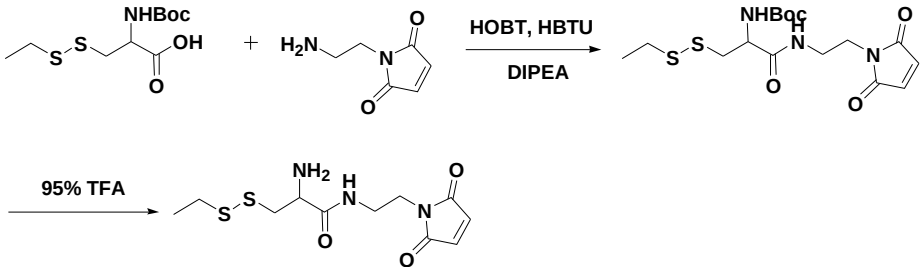


Figure S2. ESI-MS spectrum of **Mal-CBT**.

Scheme S2.The synthetic route for **Mal-Cys**.



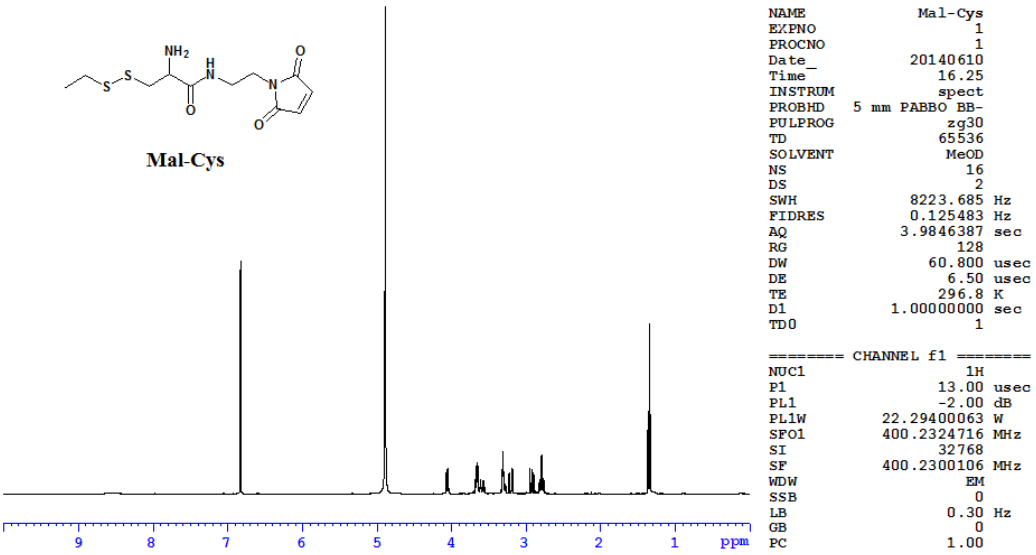


Figure S3. [1]HNMR spectrum of **Mal-Cys**.

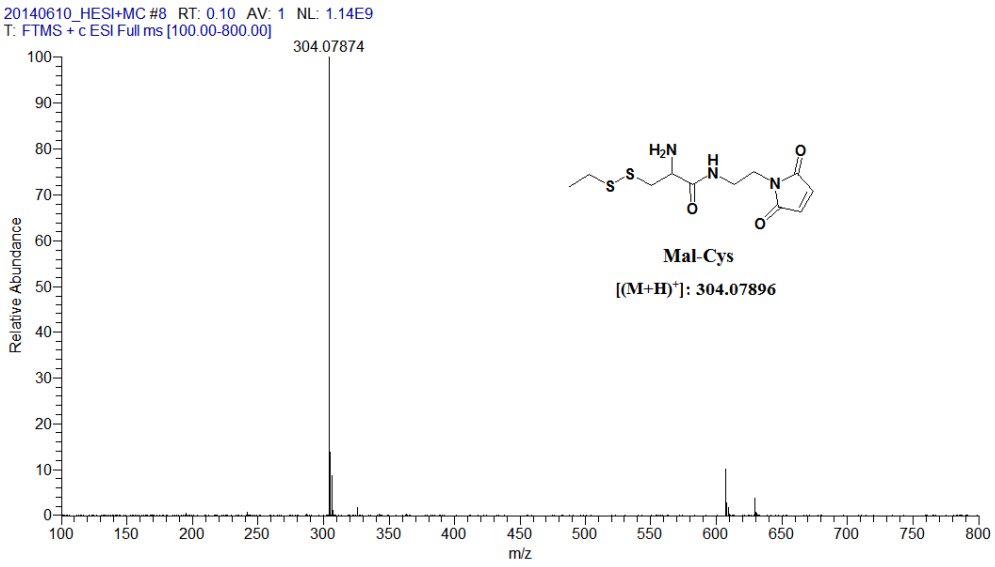
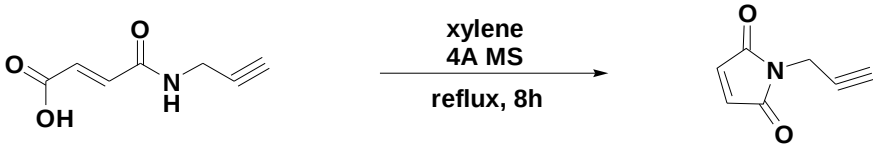


Figure S4. HR-GCT/MS spectrum of **Mal-Cys**.

Scheme S3.The synthetic route for **Mal-Alkyne**.



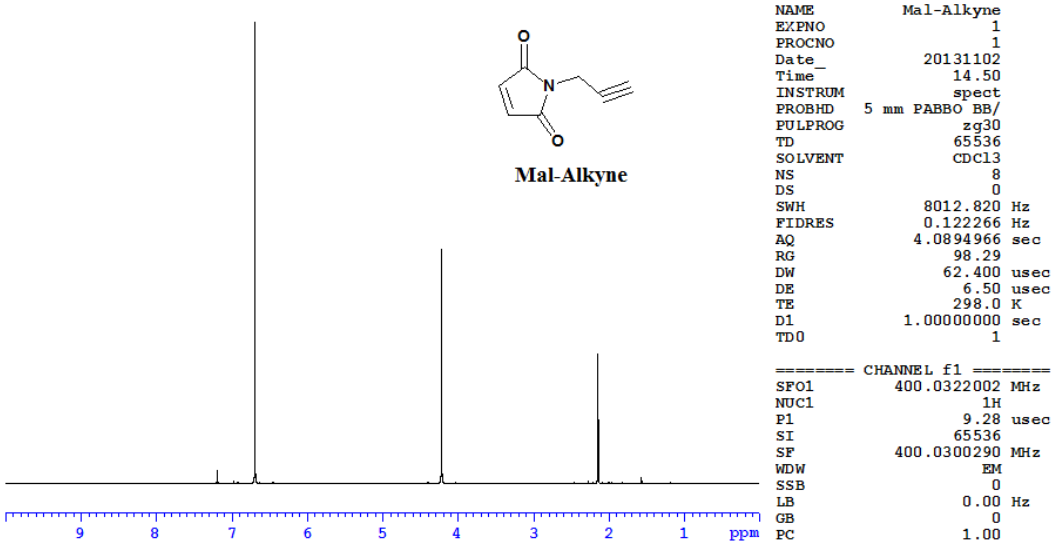
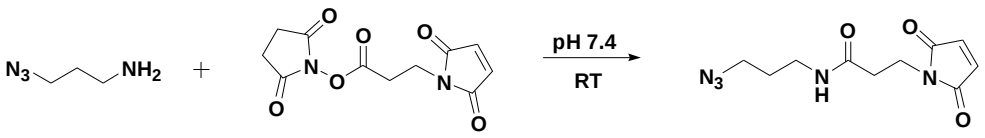


Figure S5. [1]HNMR spectrum of **Mal-Alkyne**.

Scheme S4. The synthetic route for **Mal-N_3**.



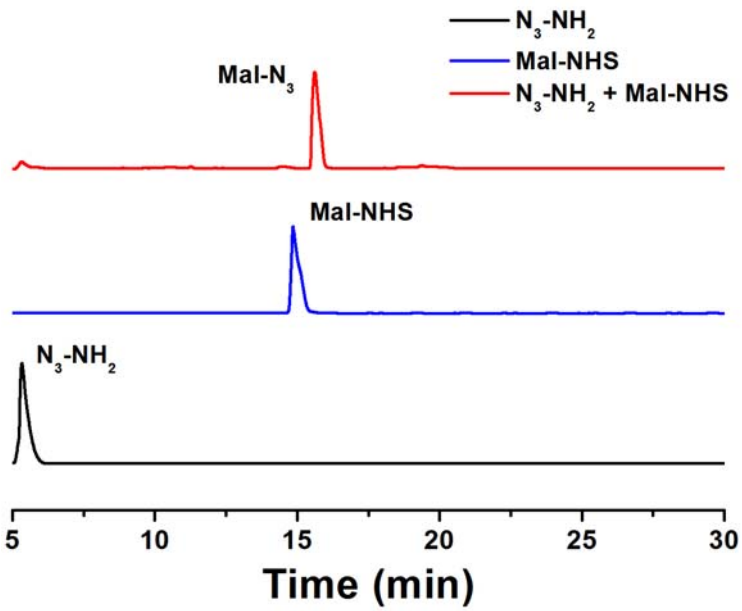


Figure S6. HPLC trace of **N_3-NH_2** (black), **Mal-NHS** (blue), and the mixture of **N_3-NH_2** and **Mal-NHS** in 500 μL PB (pH 7.4, 0.1 M) for 1 h at RT (red).

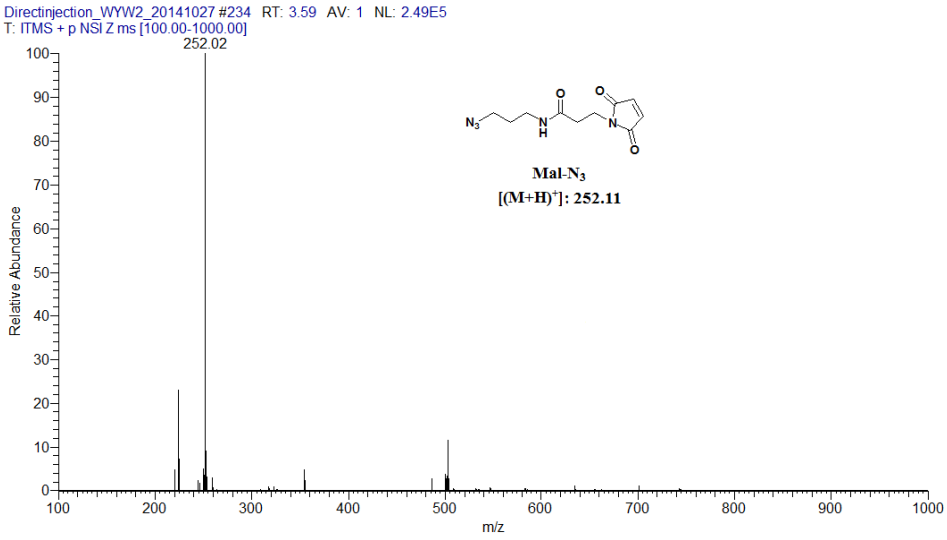
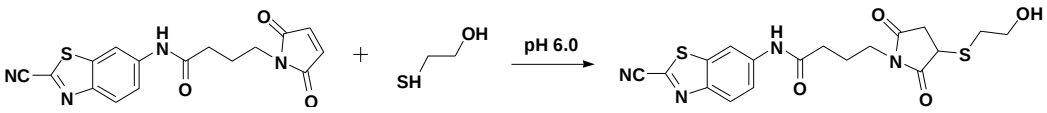


Figure S7. ESI-MS spectrum of **Mal-N_3**.

Scheme S5. The synthetic route for **1**.



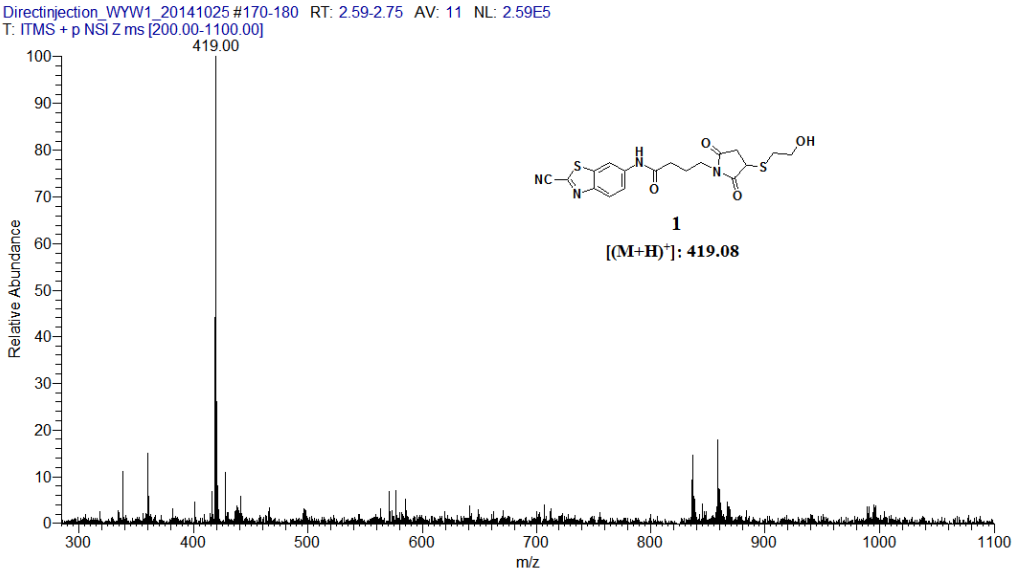
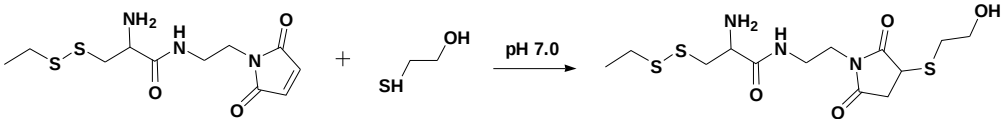


Figure S8. ESI-MS spectrum of **1**.

Scheme S6. The synthetic route for **2**.



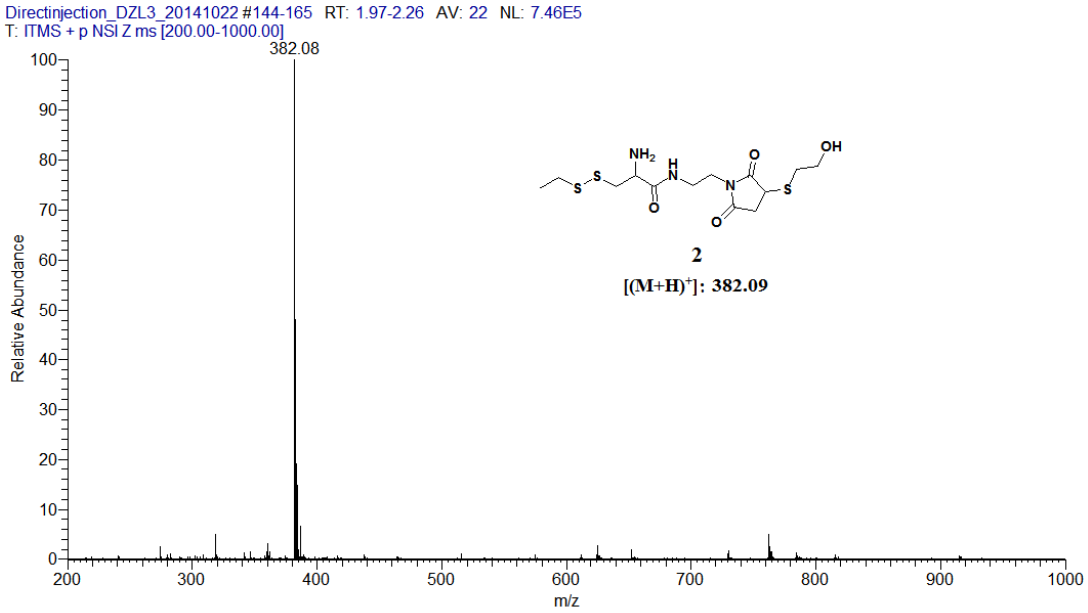
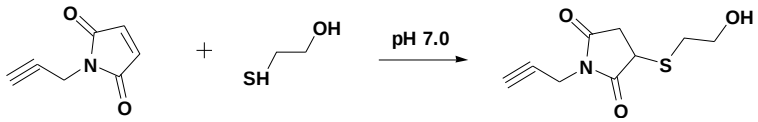


Figure S9. ESI-MS spectrum of **2**.

Scheme S7. The synthetic route for **3**.



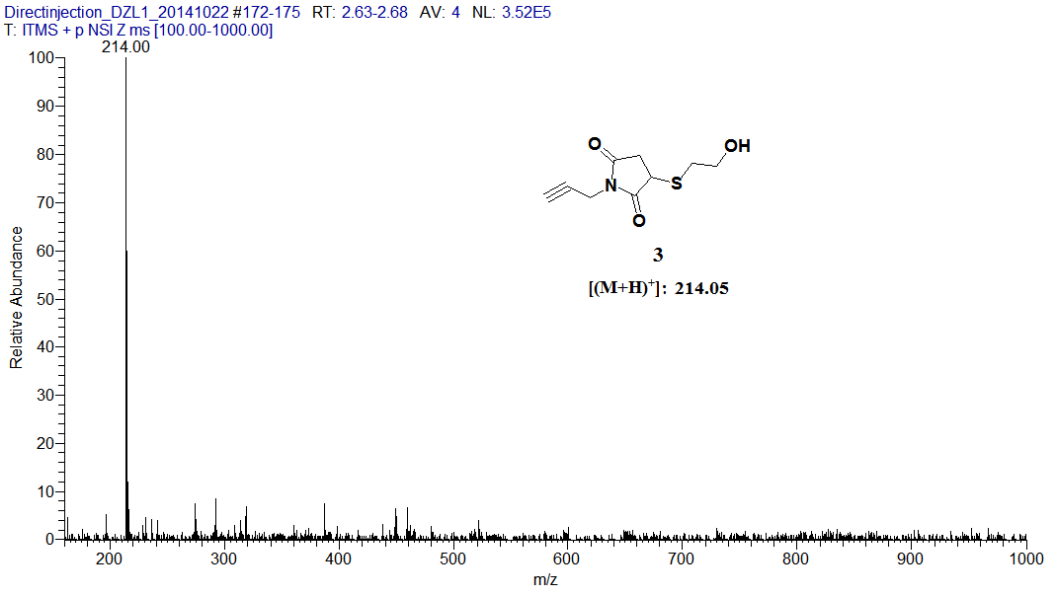


Figure S10. ESI-MS spectrum of **3**.

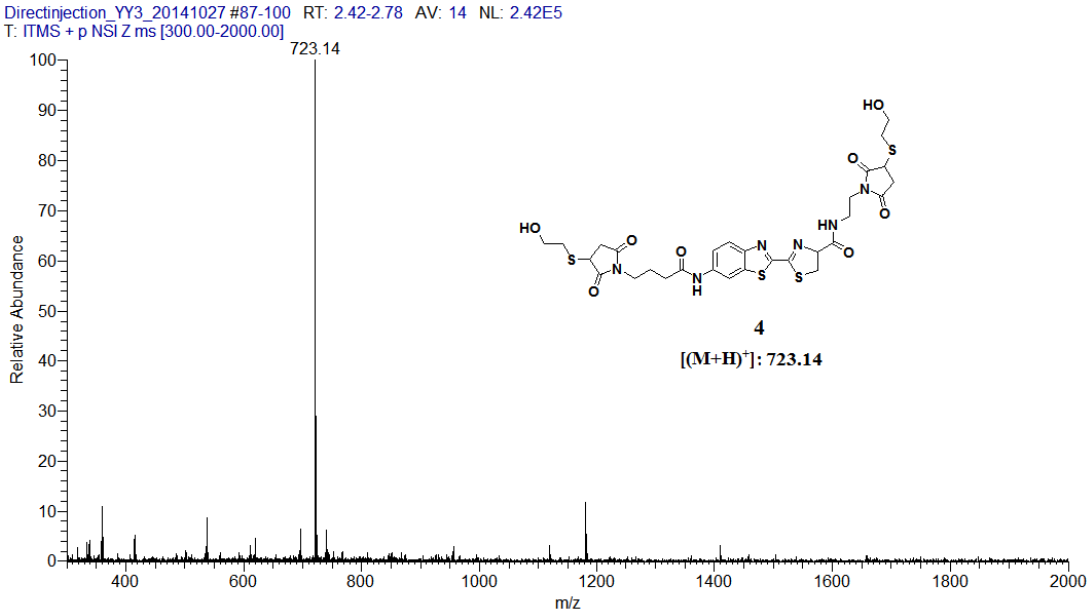
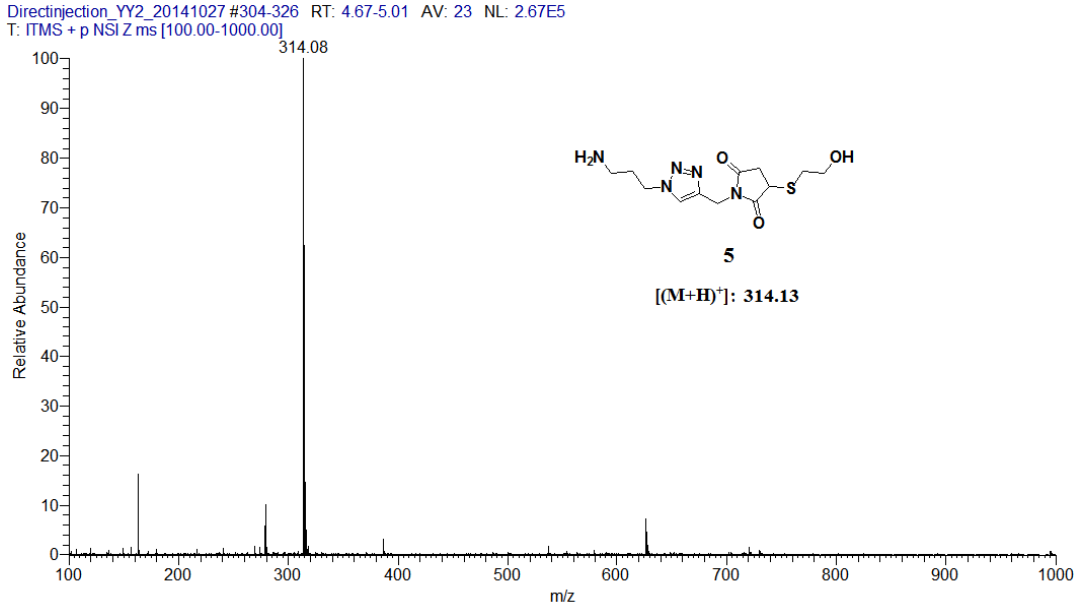


Figure S11. ESI-MS spectrum of **4**.



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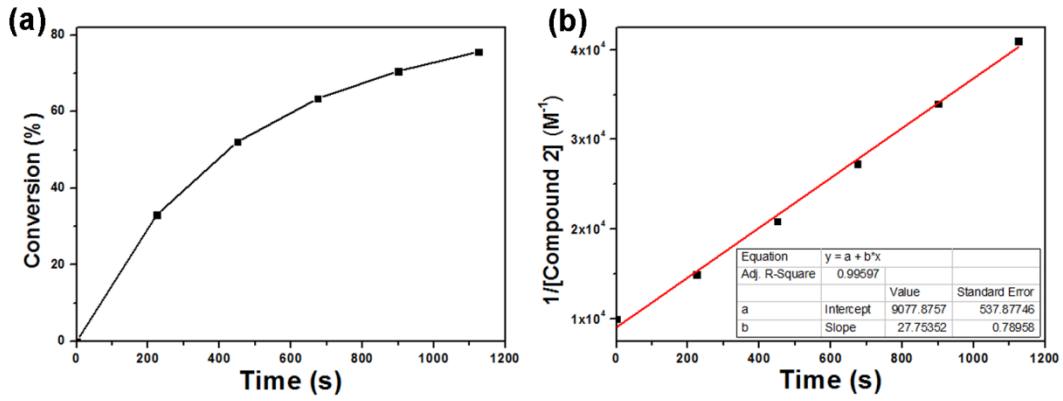

Figure S13. (a) The conversion rate of the condensation reaction vs. time. (b) Linear regression analysis of 1/[Compound **2**] vs. time of the Compound **2** condensation reaction to give formula: y = 9078 - 27.75 * x.

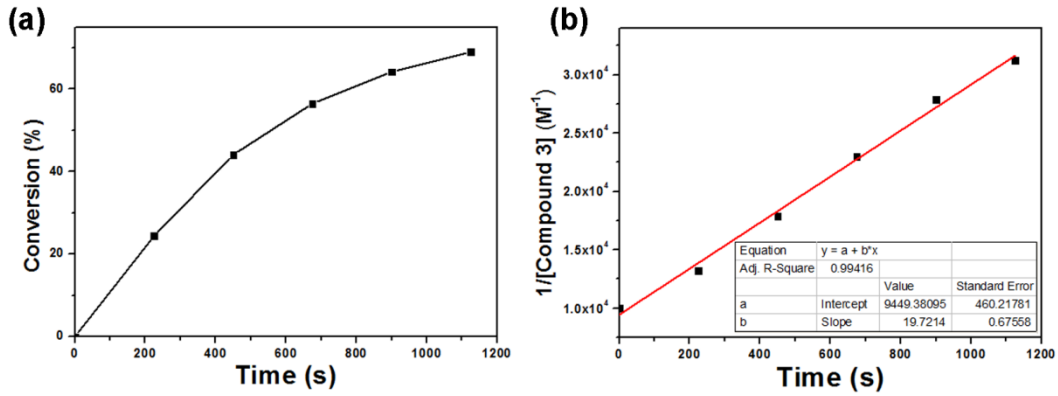

Figure S14. (a) The conversion rate of the condensation reaction vs. time. (b) Linear regression analysis of 1/[Compound **3**] vs. time of the Compound **3** condensation reaction to give formula: y = 9449 - 19.72 * x.

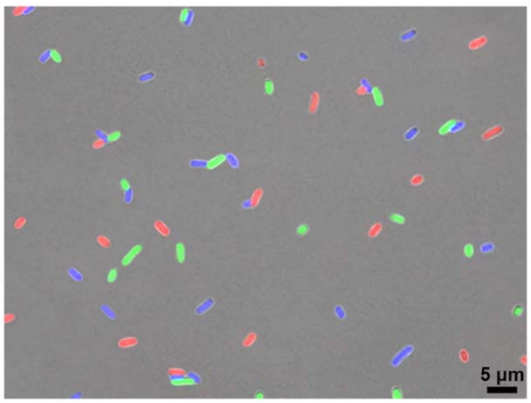

Figure S15. Microscopic image of the bridged prokaryotic cells at lower magnification with more cells.

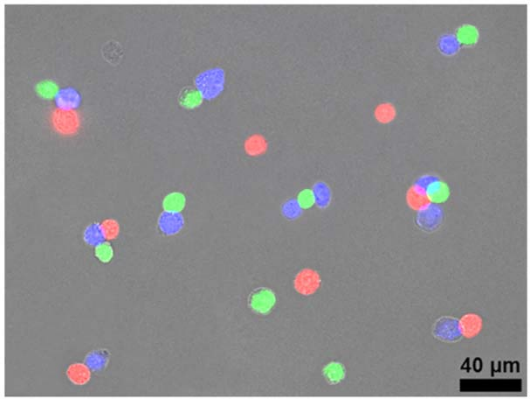

Figure S16. Microscopic image of the bridged eukaryotic cells at higher density with more cells.

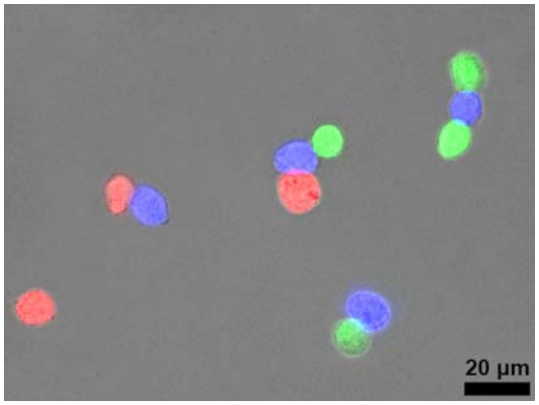

Figure S17. The **Mal-N_3**-treated RFP^+ HEK 293T cells after being shaken with bridged GFP^+ HEK 293T-HepG2 cells in PBS in the presence of 100 µM $CuSO_4$, and 700 µM NaVc at 37 °C for 1.5 h.

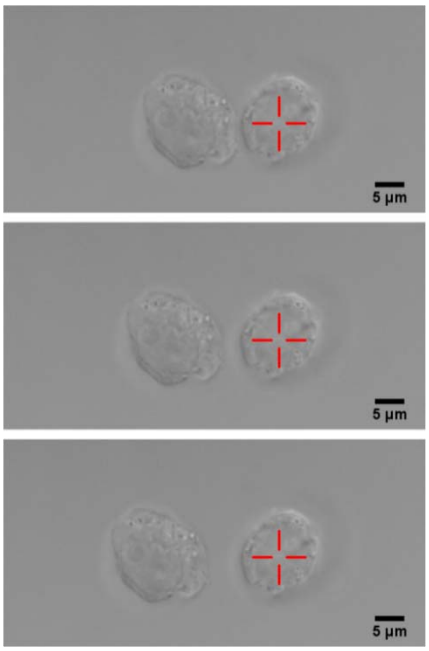

Figure S18. Typical microscopic images of GFP^+ cell and BFP^+ cell without interactions under optical tweezers.

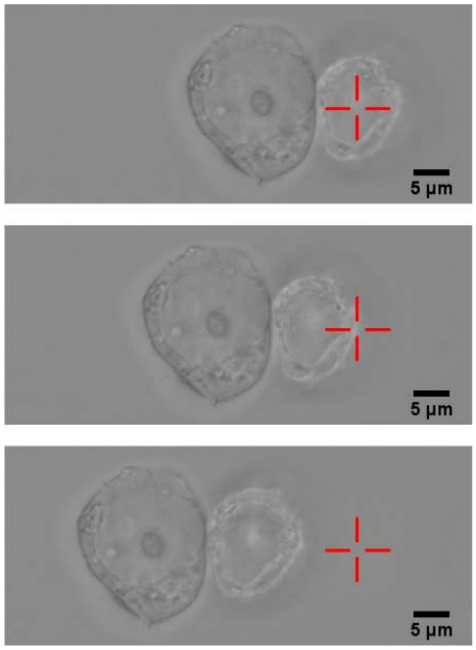

Figure S19. Typical microscopic images of bridged GFP^+-BFP^+ cells unable to be separated by optical tweezers.

2. Supporting Tables

Table S1. HPLC condition for the purification of the title compounds.

Time (minute)	Flow (mL/min.)	H_2O % (0.1%TFA)	CH_3CN % (0.1%TFA)
0	3.0	99	1
3	3.0	99	1
35	3.0	35	65
37	3.0	35	65
38	3.0	99	1
40	3.0	99	1

Table S2. Probability of different stretching phenomenons in optical tweezers experiments.

	Without interactions between GFP^+ cells and BFP^+ cells	Bridged cells can be separated by optical tweezers	Bridged cells can not be separated by optical tweezers
With TCEP	33.90%	25.42%	40.68%
Without TCEP	80.85%	12.77%	6.38%

3. Supporting Videos

Video S1. Typical video of GFP^+ cell and BFP^+ cell without interaction under optical tweezers.

Video S2. Typical video of **Mal-Cys**-treated GFP^+ HEK 293T cells and **Mal-CBT**-treated BFP^+ HEK 293T cells with interaction under optical tweezers.

Video S3. Typical video of bridged GFP^+-BFP^+ cells unable to be separated by optical tweezers.